p 155, at end of section 4.4.3 add section as follows:

Section 4.4.3.1 Effect of intervention on clinical isolates of MRSA

In addition to determination of the effect of the intervention on new MRSA colonisations, its effect on clinical isolates was also determined. This was done by calculating the number of new MRSA clinical isolates per 1000 ICU admissions per month. Information on clinical isolates had been collected since January 2001, which was approximately half way through the first screening period (July 31st 2000 to May 20th 2001). Only one isolate per patient was recorded and patients were excluded if they had been in the ICU for <48 hours or had a previous isolate of MRSA.

Interrupted time series with segmental regression analysis was used to compare the number of MRSA clinical isolates per 1000 ICU admissions per month in three time periods (Wagner *et al.*, 2002; Ansari *et al.*, 2003; Weinberg *et al.*, 2001). Time period 1 was from 1st January 2001 to May 31st 2001, time period 2 from 1st June 2001 to 31st December 2002 and time period 3 from 1st January 2003 to 30th September 2003 inclusive. Because time period 1 finished on 20th May and time period 2 finished on 15th December, these periods were rounded to the end of the month to ensure that all time points were equivalent, that is, in months. Time period 1 included half of the first screening period. During time period 2, there was no MRSA screening. Time period 3 encompassed the time of the hand hygiene intervention (aside from the first two weeks) when screening was also performed. The Durbin-Watson statistic was used to check for serial correlation.

A graphic representation of the regression analysis is shown in Figure 4.2a. The Durbin-Watson statistic was 2.00, almost exactly equal to its expected value under the null hypothesis of no serial correlation. This means that there was no evidence of correlation in numbers of MRSA isolates from one month to the next. Hence serial correlation was not included in the final segmented regression analysis (Figure 4.2b). Within each period, there is no evidence of a change in the rate of clinical isolates per 1000 ICU admissions over time (Figure 4.2a: p-value for difference in slope between periods 1 and 2 = 0.91 and p-value for difference in slope between period 1 and 3 = 0.49). On this basis, trend over time within periods was removed from the analysis. When comparing overall rate between periods (Figure 4.2b), there was a downward shift of 29.9 isolates per 1000 ICU admissions between periods 2 and 3, that is after the intervention (p-value = 0.002, 95%CI 12.4-47.5).

p 174, paragraph 3, delete sentence commencing with "However, with a 95% confidence interval for the proportion of ....."

p 176, at end of section 4.5.5.1, add the following:
Comparison of the number of clinical isolates per 1000 ICU admissions using interrupted time series with segmental regression analysis during the first screening period, the intervening period and the second screening period, during the time of the hand hygiene intervention was also performed. Although isolates from the first half of the first screening period were not available, there was no evidence of a change in rate between the first and second time period. This would be expected, as during these time periods, there were no specific interventions in place to reduce MRSA, aside from the usual infection control measures. There was a significant drop in the rate of new clinical isolates, however, in the third time period after the introduction of the intervention. As described, a statistical comparison of the two screening periods (Period 1 and Period 3) was not performed for the outcome of colonisation because of the heterogeneity of the two time periods (described earlier in this section). However, because clinical isolates had been recorded throughout the three time periods, it was reasonable to perform tests of significance to compare directly before and after the intervention. Although there may still have been some differences between time periods 2 and 3 (for example, the immunocompromised patients were moved to a separate ICU in April 2002), there was no evidence of a change in rate during time period 2. Thus, the dramatic, significant drop in the rate of MRSA clinical isolates between time periods 2 and 3 is strong evidence that the intervention was successful.

p 184, Section 4.6 Conclusions, replace first paragraph with:
This study has demonstrated success of a multifaceted program (primarily based on introduction of a new hand hygiene agent) in reducing MRSA. This was demonstrated by a reduction in new MRSA colonisations, especially in trauma patients, between the two screening periods and a significant reduction in new clinical isolates in the time periods before and after the intervention was introduced. Analysis of clinical isolates directly before and after the intervention has overcome some of the methodological difficulties of comparing the two screening periods which were separated by a considerable gap in time. This reduction is also supported by results of the Stewhart control charts in the ICU, with a possible flow-on effect to other wards.

p 232, Section 7.3 Improving hand hygiene in the ICU, first paragraph, replace from "The major outcome assessed ...... between the two time periods" with the following:
The proportion of patients who became newly MRSA colonised was reduced compared with that in the initial screening study, especially amongst trauma patients. There was a significant reduction in the rate of new MRSA clinical isolates per 1000 ICU admissions between the time periods before and after the intervention was introduced. Despite some methodological limitations, all of these data are strong evidence for the success of the intervention.

Figure 4-2a Number of clinical MRSA isolates per 1000 ICU admissions per month: segmented regression lines with period-specific intercepts and slopes

Period 1 – 2nd half of first screening period, Period 2 – no screening or intervention, Period 3 – after introduction of hand hygiene intervention
Month 1 = January 2001, Month 33 = September 2003
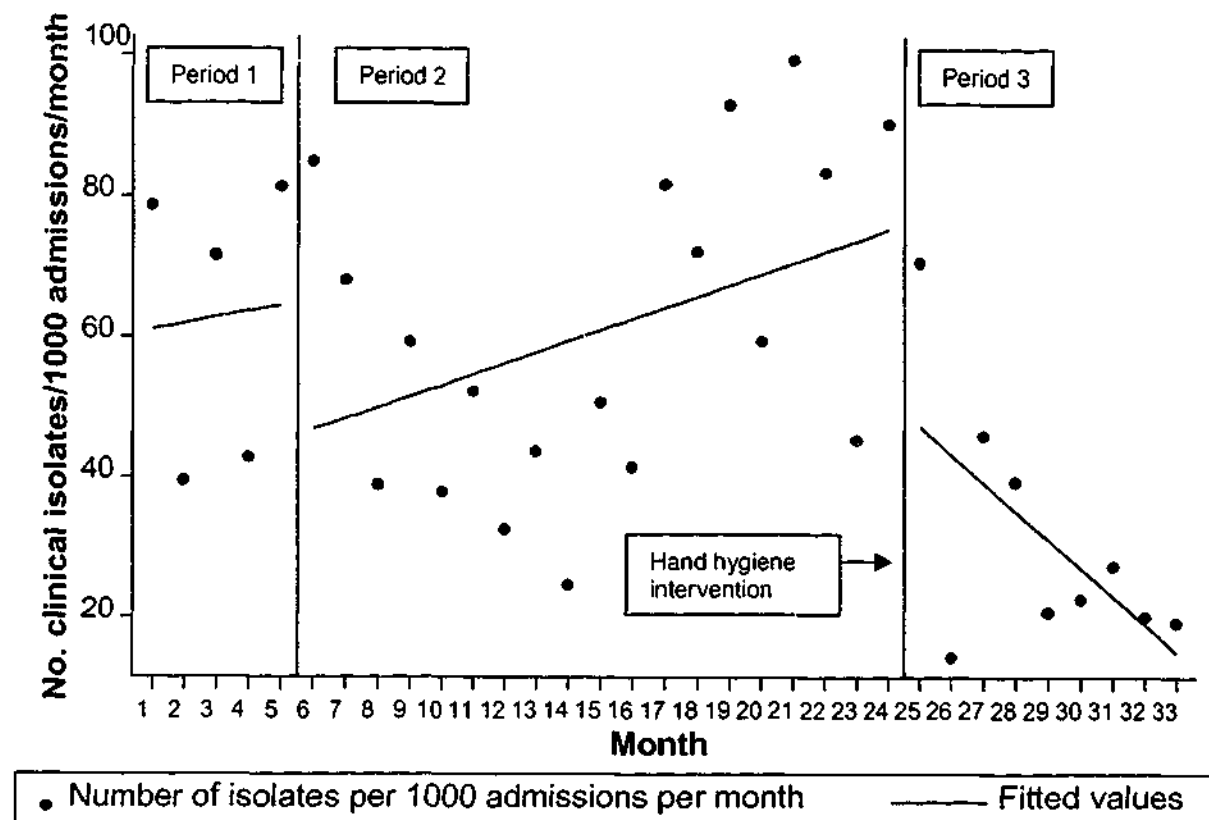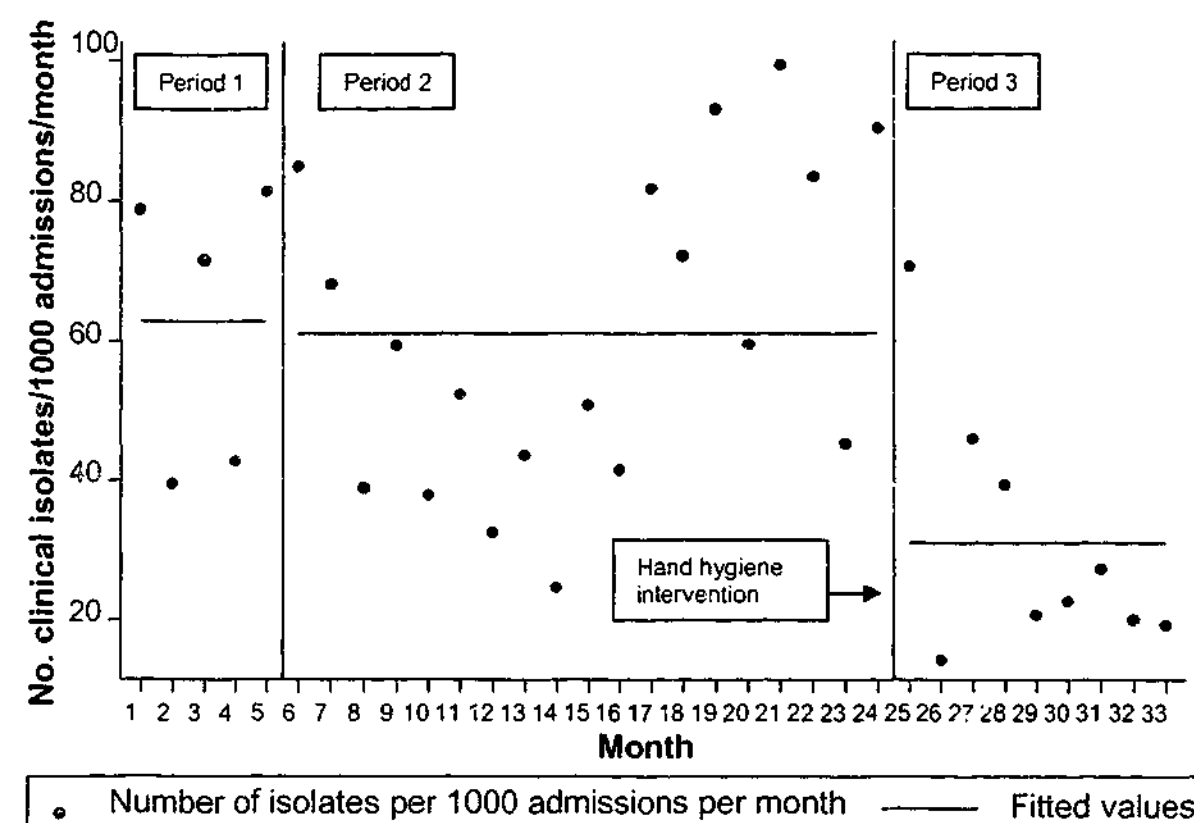Hand hygiene intervention introduced 16th December 2002

Figure 4-2b Number of clinical MRSA isolates per 1000 ICU admissions per month: segmented regression lines with period-specific intercepts but all slopes equal to zero (i.e. assuming no underlying month-to-month change in rate within a period)

Period 1 – 2nd half of first screening period, Period 2 – no screening or intervention, Period 3 – after introduction of hand hygiene intervention
Month 1 = January 2001, Month 33 = September 2003
Hand hygiene intervention introduced 16th December 2002

p 260, include in References section the following:

Wagner AK, Soumerai SB, Zhang F, Ross-Degnan D. (2002). Segmented regression analysis of interrupted time series studies in medication use research. *J Clin Pharm Ther* 27:299-309.

Ansari F, Gray K, Nathwani D, et al. (2003). Outcomes of an intervention to improve hospital antibiotic prescribing: interrupted time series with segmented regression analysis. *J Antimicrob Chemother* 52:842-8.

Weinberg M, Fuentes JM, Ruiz AI, et al. (2001). Reducing infections among women undergoing cesarean section in Colombia by means of continuous quality improvement methods. *Arch Intern Med* 161:2357-65.

<u>ERRATA</u>

Cover page line 7: "FRACP" for "FRACS"
p 234 paragraph 1, line 2: "may be more important" for "may more important"

ENDEMIC

METHICILLIN-RESISTANT

STAPHYLOCOCCUS AUREUS

IN THE

INTENSIVE CARE UNIT

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MB BS, FRACS,

Graduate Diploma of Clinical Epidemiology

A thesis submitted for the degree of

Doctor of Philosophy

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April 2004

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SUMMARY

Control of methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the greatest medical challenges we face. Despite an extensive literature, our knowledge remains incomplete and generally, we have failed to make a significant impact on endemic levels. The objective of this thesis was to add to the evidence base regarding MRSA containment by gaining a greater understanding of its epidemiology.

The initial aim was to quantify the extent of MRSA in the intensive care unit (ICU) at the Alfred Hospital using active surveillance. 6.8% of patients were colonised at admission. Risk factors included a previous stay in ICU or the trauma/orthopaedic ward and increasing length of stay prior to ICU admission. 11.4% of patients acquired MRSA in the ICU, with length of stay and trauma strongly associated.

MRSA isolates were subtyped using antibiogram, pulsed-field gel electrophoresis (PFGE) and the RiboPrinter® in order to determine their relative utility. PFGE is time-consuming and labour intensive, whilst the RiboPrinter® is fully automated. The RiboPrinter® had similar discriminatory power to PFGE, but issues concerning interpretation of PFGE gels using the "Tenover criteria" limited whether firm conclusions could be reasonably drawn from these results.

Waterless, alcohol based hand disinfectants have been associated with sustained reductions in MRSA transmission in association with other infection control measures. These comprehensive interventions are labour and cost intensive, yet it is not known whether all components are necessary. In a three-pronged approach, an alcohol-chlorhexidine based hand disinfectant was introduced to the ICU. 8.6% of patients acquired MRSA compared with 11.4% in the previous study. There was good acceptance of the product, with overall use of hand hygiene products increasing.
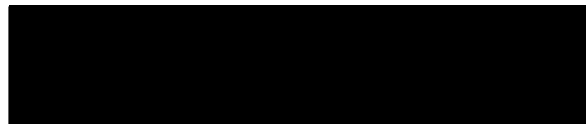
Analysis of screening swabs showed nose and throat swabs to be strongly associated and groin swabs to be of underestimated importance in MRSA screening. Patients frequently lost and re-acquired colonisation at different sites, raising questions about sensitivity of skin and mucosal swabs to detect MRSA.

In the initial screening study, one third of trauma patients acquired MRSA. Two cohort studies were performed to examine risk factors. The first found that length of stay, laparotomy, receipt of ticarcillin-clavulanic acid or vancomycin and road traffic trauma were associated. The second larger study found presence of a tracheostomy or gastric tube to be protective and a central venous catheter and penicillin or amoxycillin administration to be risk factors, taking timing of MRSA acquisition into account in the analysis. MRSA acquisition was substantially reduced in trauma patients during the time of the second cohort study, probably as a result of the new hand hygiene agent.

This thesis generated local data regarding MRSA transmission as a first step to raising awareness of the problem and therefore had a great impact at a practical level. The results allowed generation of further questions which were examined in subsequent studies, producing novel findings. Further methodologically sound studies are required before we can say that recommended control measures are truly evidence based. MRSA has now been "put back on the agenda" in the local and the global context, with many new initiatives being developed in order to combat this large and ever increasing problem.

STATEMENT OF AUTHORSHIP

I certify that the material presented in this thesis has not been submitted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

Caroline Marshall

ACKNOWLEDGEMENTS

PUBLICATIONS

C Marshall, G Harrington, R Wolfe, CK Fairley, S Wesselingh, D Spelman. Acquisition of methicillin-resistant *Staphylococcus aureus* in a large intensive care unit. *Infection Control and Hospital Epidemiology* 2003;24:322-6

C Marshall, S Wesselingh, M McDonald, D Spelman. Control of endemic MRSA – what is the evidence? A personal view. *Journal of Hospital Infection* 2004; 56:253-68

C Marshall, T Kossmann, S Wesselingh, D Spelman. MRSA and beyond: What's new in the world of the golden staph? *Australian and New Zealand Journal of Surgery* 2004: 74: In Press

C Marshall, R Wolfe, , T Kossmann, S Wesselingh, G Harrington, D Spelman. Risk factors for acquisition of methicillin-resistant *Staphylococcus aureus* (MRSA) by trauma patients in the intensive care unit. *Journal of Hospital Infection* 2004: In Press

LIST OF ABBREVIATIONS

ACI   allergy and clinical immunology

AIDS   acquired immune deficiency syndrome

APACHE   acute physiology and chronic health evaluation

ASID   Australasian Society for Infectious Diseases

BES   breast endocrine surgery

CCD   charge-coupled device

CCU   coronary care unit

CDC   Centers for Disease Control and Prevention

CI   confidence interval

CRS   colorectal surgery

CT scan   computerised tomography scan

CT surgery   cardiothoracic surgery

Derm   dermatology

DNA   deoxyribonucleic acid

(EA)MRSA   Eastern Australian methicillin-resistant *Staphylococcus aureus*

EMRSA   epidemic methicillin-resistant *Staphylococcus aureus*

Endo   endocrinology

ENT	ear, nose and throat
EPIC	European Prevalence of Infection in Intensive Care
Gastro	gastroenterology
GCS	Glasgow Coma Scale
Gen Surg	general surgery
GI	gastrointestinal
Haematol	haematology
HBA	horse blood agar
HIV	human immunodeficiency virus
HR	hazard ratio
ICU	intensive care unit
ISS	Injury Severity Score
LOS	length of stay
Med	general medicine
μg	micrograms
MIC	minimum inhibitory concentration
MIDG	Melbourne Infectious Diseases Group
ml	millilitre
MLEE	multilocus enzyme electrophoresis

MLST	multilocus sequence typing
MRSA	methicillin-resistant *Staphylococcus aureus*
MSSA	methicillin-sensitive *Staphylococcus aureus*
Neurol	neurology
Neurosurg	neurosurgery
NIAID	National Institute of Allergy and Infectious Diseases
NISS	New Injury Severity Score
NNIS	National Nosocomial Infection Surveillance
Onc	oncology
Ophthalmol	ophthalmology
OR	odds ratio
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
Plas	plastic surgery
RAPD	randomly amplified polymorphic DNA
REA	restriction enzyme analysis of chromosomal DNA
Rheum	rheumatology
RNA	ribonucleic acid
RTS	Revised Trauma Score

S. aureus *Staphylococcus aureus*

SDD selective decontamination of the digestive tract

SHEA Society for Healthcare Epidemiology of America

SSI surgical site infection

TRISS Trauma Injury Severity Score

Urol urology

Vasc vascular surgery

VISA vancomycin-intermediate *Staphylococcus aureus*

VRE vancomycin-resistant enterococcus

VRSA vancomycin-resistant *Staphylococcus aureus*

vs. versus

(WA)MRSA Western Australian methicillin-resistant *Staphylococcus aureus*

WSPP Western Samoan phage pattern

1 CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The use of BRL 1241, a new antibiotic, was first described in the September 3, 1960 edition of the British Medical Journal, (Douthwaite *et al.*, 1960; Knudsen *et al.*, 1960). Its generic name was methicillin and commercially it was known as "Celbenin". It was the first available agent active against penicillinase-producing staphylococci. The development of this novel antibiotic at a time when penicillin-resistant *Staphylococcus aureus* (*S. aureus*) was rife in hospitals was acclaimed as "an outstanding achievement" (Anon, 1960) and it was widely believed that "the penicillin resistant staphylcoccus ha(d) finally been conquered" (Chain, 1960).

One author, however, cautioned that perhaps it was "a little early to take comfort from the idea that staphylococci cannot achieve resistance to it" (Barber, 1960). This warning was borne out when the first isolates of methicillin-resistant *S. aureus* (MRSA) were reported from the United Kingdom (Barber, 1961; Jevons, 1961) and at least one author was forced to "eat some if not all of (his) own words" (Stewart *et al.*, 1963). At this time, it was believed that "since naturally occurring Celbenin-resistant organisms are not only rare but of doubtful clinical significance, it has been suggested that (they) are not likely to be of

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clinical importance" although the same author cautioned that "it is unwise to assume that the staphylcoccus has met its match" (Barber, 1961).

Soon after these initial reports, the pathogenic potential of MRSA was confirmed when it caused numerous outbreaks of nosocomial infection. Initial attempts at control involved a "seek and destroy" attitude, although it was soon realised that eradication from the hospital environment was unlikely, except in exceptional circumstances (Pearman *et al.*, 1985; Rosdahl *et al.*, 1991). We have subsequently witnessed its entrenchment into the resident hospital flora as one of the most significant causes of hospital acquired infection, with attendant serious morbidity and mortality.

The need for active control of MRSA has been questioned (Teare *et al.*, 1997), with some suggesting that attempting to control MRSA "causes more problems than it solves" (Barrett *et al.*, 1998) while others have even stated that "staphylococcal infection is staphylococcal infection and ...the title MRSA makes no difference" (McManus *et al.*, 1989). Despite these opinions, it is now well accepted that MRSA containment *is* worthwhile (Herwaldt, 1999; Muto *et al.*, 2003; Wenzel *et al.*, 1991).

Despite the legion of material published on MRSA, there still remain many unanswered questions about its transmission dynamics and control. Much of the literature refers to epidemic MRSA and consists of retrospective, often uncontrolled or historically controlled descriptive studies. Measures which are effective for outbreak containment may not be

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generalisable to control of endemic disease. In addition, the MRSA story continues to progress, with increasing problems with community acquired MRSA and development of vancomycin resistance. This literature review aims to critically analyse studies particularly pertaining to endemic MRSA in order to elucidate how our approach to MRSA has evolved and what are the best strategies to implement for its control.

1.2 MRSA: The past and the present

1.2.1 The origins of MRSA

In September 1959, Beecham Research Laboratories reported the isolation of 6-aminopenicillanic acid, the penicillin nucleus to which various side chains could be added to produce new agents with expanded properties (Batchelor *et al.*, 1959). This paved the way for the development of methicillin. In September 1960 it was marketed as "Celbenin", with confirmation of its efficacy against penicillin-resistant *S. aureus* soon following (Douthwaite *et al.*, 1960; Knudsen *et al.*, 1960).

Less than one year later, the first isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) were reported from the United Kingdom (Barber, 1961; Jevons, 1961) with other reports following from around the world (Borowski *et al.*, 1964; Bulger, 1967; Cetin *et al.*, 1962). Many of these initial isolates were found in patients who had not received methicillin or were from hospitals and countries where methicillin had not been used.

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Whilst initially believed to be "of doubtful clinical significance", it was soon realised that these organisms were pathogenic and of epidemic potential (Cafferkey, 1988; Rountree *et al.*, 1973; Stewart *et al.*, 1963). Outbreaks began to be reported, initially from the United Kingdom (Colley *et al.*, 1965; Turner *et al.*, 1967) and subsequently from other countries (Barrett *et al.*, 1968; Klimek *et al.*, 1976; Rountree *et al.*, 1968). It was soon realised that methicillin was ineffective for treatment of these organisms (Rountree *et al.*, 1968) and that cross-resistance also extended to cephalosporins (Kind *et al.*, 1968; Klimek *et al.*, 1976). After reports of failures of treatment with several other antibiotics, vancomycin was found to have reasonable activity (Sorrell *et al.*, 1982).

1.2.2 United Kingdom

In the United Kingdom, the proportion of *S. aureus* isolates resistant to methicillin rose from <0.1% in 1960 to 4% in 1969, 10% in 1984 and 75% in 1999 (Aucken *et al.*, 2002). The proportion of *S. aureus* bacteraemias that were methicillin resistant rose from 1-2% in 1989 to >40% in 1999-2000 (PHLS, 2000; Reacher *et al.*, 2000; Woodford *et al.*, 2001), coinciding with the appearance of epidemic strains 15 and 16 (EMRSA-15 and EMRSA-16) (Johnson *et al.*, 2001). An epidemic strain was defined as two or more isolates from two or more hospitals. They can be differentiated using phenotypic and molecular methods. The first epidemic UK strain (EMRSA-1) was recognised in the early 1980s (Marples *et al.*, 1986). Epidemic strains 2-14 were subsequently recognised (Kerr *et al.*, 1990), but only EMRSA -3 and -12 were found in more than ten hospitals (Aucken *et al.*, 2002). EMRSA-15 was described in 1993 (Richardson *et al.*, 1993) and EMRSA-16 in

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1995 when it caused a large outbreak (Cox *et al.*, 1995). These two strains subsequently spread widely in the United Kingdom (Cookson, 1999) and by 2000 accounted for 95.6% of MRSA bacteraemias (Johnson *et al.*, 2001). Most recently, EMRSA-17 has been described (Aucken *et al.*, 2002). It has been found across the United Kingdom and of note is its borderline resistance to teicoplanin.

1.2.3 Australia

The first Australian report of MRSA was from the Royal Prince Alfred Hospital in Sydney, where a single isolate was found in 1965 (Rountree *et al.*, 1968). By 1967, 5.7% of the *S. aureus* in that hospital were methicillin resistant, increasing to 30% in 1973 (Rountree *et al.*, 1973). In Victoria, prior to 1975, less than 2% of *S. aureus* were resistant to methicillin but by 1979, this had risen to 20–40% in some hospitals (McDonald *et al.*, 1981; Pavillard *et al.*, 1982). Around this time, other outbreaks were recorded in Melbourne (Gilbert *et al.*, 1982) and Sydney (King *et al.*, 1982) with subsequent spread to Adelaide, Hobart and Brisbane (Gedney *et al.*, 1982). In Western Australia, although MRSA had been first isolated in 1966 from Royal Perth Hospital, apart from occasional sporadic cases over the next 15 years, MRSA did not become established as an endemic pathogen (Pearman *et al.*, 1985). In 1982, an outbreak at this hospital was eventually brought under control. Subsequent cases of MRSA were introduced from the eastern states, however large outbreaks were avoided by strict infection control programs (Pearman *et al.*, 1985) and in 1986-7, the prevalence of methicillin resistance was only 0.4% in Western Australia (Turnidge *et al.*, 1989). Between 1986 and 1994, the proportion

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of *S. aureus* that was resistant to methicillin remained fairly constant with levels of 13-27% in Melbourne and Sydney, 25-42% in Brisbane, less than 13% in Hobart, Adelaide and Canberra and less than 5% in Perth (Turnidge *et al.*, 1996). However, by 1996, the prevalence in Adelaide and Canberra had risen strikingly to 28% and 15% respectively and to 9-12% in Perth, largely as a result of increasing community acquired strains (Turnidge *et al.*, 2000).

The "eastern Australian" (EA)MRSA found in the eastern states of Australia is the classic multi-resistant MRSA, characterised by resistance to β-lactams, erythromycin, clindamycin, tetracycline, gentamicin, trimethoprim-sulfamethoxazole and variable resistance to ciprofloxacin, rifampicin and fusidic acid (Townsend *et al.*, 1985; Turnidge *et al.*, 2000). In contrast, the Western Australian (WA)MRSA, first appearing in the late 1980s in the Kimberley region and more recently in some remote communities in the Northern Territory and South Australia, is a non-multiresistant community-acquired strain (Udo *et al.*, 1993; Maguire *et al.*, 1996; Turnidge *et al.*, 2000). It has caused nosocomial outbreaks and, with a prevalence of up to 42% colonisation in some remote communities, (O'Brien *et al.*, 1999) it now threatens the ability of Western Australian hospitals to remain essentially MRSA free (Riley *et al.*, 1995). Since the late 1990s, other unrelated community-acquired strains of MRSA have been reported in eastern Australia, predominantly in people of Polynesian or South Pacific backgrounds (Collignon *et al.*, 1998; Munckhof *et al.*, 2002). These belong to the Western Samoan phage patterns (WSPP-1 and WSPP-2) that were reported in New Zealand in the mid 1990s (Nimmo *et*

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al., 2000). Many of these strains contain the Panton-Valentine leukocidin gene, coding for a staphylococcal toxin associated with cases of severe soft tissue infection and necrotising pneumonia, which have been a common clinical manifestation in these reports (Collignon *et al.*, 1998; Collins *et al.*, 2002; Nimmo *et al.*, 2003).

1.2.4 United States

In the United States, MRSA was isolated in the early 1960s (Boyce *et al.*, 1982) with occasional clinical infections reported over the next few years (Benner *et al.*, 1967; Bulger, 1967; Seligman, 1967). Although there were reports of some hospital outbreaks in the late 1960s and early 1970s (Barrett *et al.*, 1968; O'Toole *et al.*, 1970), MRSA infection still remained uncommon (Boyce *et al.*, 1982). However, by the mid to late 1970s, American hospitals were experiencing the same problems that had afflicted British hospitals several years earlier (Craven *et al.*, 1981; Crossley *et al.*, 1979; Klimek *et al.*, 1976; Linnemann Jr *et al.*, 1982; Locksley *et al.*, 1982; Peacock *et al.*, 1980). Initially, only larger, university-affiliated hospitals were affected (Haley *et al.*, 1982) with subsequent spread to other smaller institutions (McGowan Jr, 1988; Panlilio *et al.*, 1992). Over the next decade, MRSA spread to become an established nosocomial pathogen in American hospitals, with the prevalence of methicillin resistance rising from 2.4% among isolates of *S. aureus* in 1975 to 29% in 1991 (Panlilio *et al.*, 1992). By 1996-1997, 35.2% of *S. aureus* isolates from the intensive care unit (ICU), 31.9% from non-ICU inpatient and 17.7% from outpatient areas were methicillin resistant (Fridkin *et al.*, 1999). These figures had risen to

51.3%, 41.4% and 25.7% respectively by 1998-2002 (National Nosocomial Infections Surveillance (NNIS) System, 2002).

1.2.5 Europe

A similar picture has emerged from around the world, with several exceptions. Although the general trend is of an increasing prevalence of MRSA in most countries, it is important to note that reporting of prevalence of MRSA from many countries has involved variable numbers of institutions with differing numbers of isolates tested and therefore reported rates may depend on the type of hospital from where isolates were derived and the number of participating institutions in that study. Numbers also vary according to whether tested isolates were from surveillance or clinical specimens. In France, MRSA was reported in the early 1960s where it caused a significant nosocomial problem (Bulger, 1967). The proportion of *S. aureus* resistant to methicillin rose from 23% in 1990 to 31% in 1998 (Lepelletier *et al.*, 2001) and in 1992, the prevalence of methicillin resistance in intensive care units was 78.4% (Vincent *et al.*, 1995). In Spain, the first outbreak of MRSA was reported in 1981, with subsequent rates remaining low until the early 1990s, when outbreaks affected several hospitals (Coello *et al.*, 1994; Romero-Vivas *et al.*, 1995) and 30.3% of *S. aureus* were methicillin resistant (Voss *et al.*, 1994). In the Republic of Ireland, sporadic infections with MRSA were reported from 1971 with it rapidly becoming endemic after 1976 (Cafferkey *et al.*, 1985). Between 1979 and 1982, 30% of *S. aureus* blood culture isolates were methicillin resistant (Cafferkey, 1988) and in 1998-1999, 34-36% were resistant (McDonald *et al.*, 2002; O'Connell *et al.*, 1999). In Italy, resistance in

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clinical isolates rose from 6% in 1981 to 26% in 1986 (Schito *et al.*, 1988), 34.4% in 1991

(Voss *et al.*, 1994) and 50.5% in all isolates in 1997-1999 (Diekema *et al.*, 2001).

Although Poland reported MRSA in the early 1960s (Borowski *et al.*, 1964), by 1986 only

17.1% of *S. aureus* isolates were methicillin-resistant (Borowski, 1988) rising to 25.8% in

1997-1998 (Diekema *et al.*, 2001). In Athens in 1986, 17.6% and in 1997-1998 34.4% of

S. aureus were resistant to methicillin (Kosmidis *et al.*, 1988; Diekema *et al.*, 2001).

Between 1997 and 1999, other countries were found to have a prevalence ranging from

2% in Switzerland and The Netherlands, 4.9% in Germany, 9.4% in Austria and 54.4% in

Portugal (Diekema *et al.*, 2001).

Of great interest are several European countries that, despite early problems with MRSA,

have managed to virtually eliminate it from their nosocomial repertoire. In Denmark, for

example, the prevalence of methicillin resistance in *S. aureus* rose from 4% in 1966 to

18% in 1968 where it remained for the next few years. At one stage, the proportion in

blood stream isolates was 45%. After 1971, however, the prevalence steadily decreased

and after 1978, it has remained below 1% (Jepsen, 1986) falling to 0.2% in 1982-1988

(Rosdahl *et al.*, 1991). The reason for this dramatic decline is not entirely clear, but may

be related to strict antibiotic policies, surveillance and infection control practices which

have identified any imported MRSA and prevented its spread (Sørensen *et al.*, 2000;

Rosdahl *et al.*, 1991).

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Other countries have also managed to maintain low levels of MRSA, despite repeated introduction of new cases from outside. These include a prevalence of 0% in Iceland in 1998-1999, 1% in Sweden, 4% in Finland, 7% in Germany and <1% in The Netherlands (Vandenbroucke-Grauls, 1996; Veldhuijzen *et al.*, 2000). Although outbreaks have occurred in these countries, strict infection control policies have managed to virtually eliminate the organisms (Kotilainen *et al.*, 2003; Rosdahl *et al.*, 1991; Vandenbroucke-Grauls, 1996).

1.2.6 Other countries

Between 1998 and 1999, the prevalence of methicillin resistance in *S. aureus* was reported by the SENTRY Antimicrobial Surveillance Program in several countries in south-east Asia and South Africa as follows: Japan – 69.5%, Mainland China – 27.8%, Hong Kong – 69.8%, Philippines – 5%, Singapore – 62.3% and South Africa – 41.5% (Bell *et al.*, 2002). In 1986 in Malaysia, the prevalence of MRSA was between 10 and 25% in some hospitals (Lim, 1988). At the National Taiwan University Hospital, the prevalence in blood stream isolates rose from 4.3% in 1981-1986 to 58.9% in 1993-1998 to 69.2% in 1999 (Hsueh *et al.*, 2002). The SENTRY study also reported a prevalence of methicillin resistance of 42.7% in Argentina, 33.7% in Brazil, 45.3% in Chile, 8.6% in Colombia and 11.4% in Mexico between 1997 and 1999 (Diekema *et al.*, 2001).

1.2.7 Community acquired MRSA

In the United States and Canada, emergence of community-acquired MRSA has become an increasing problem. Initial outbreaks were reported in intravenous drug users (Saravolatz *et al.*, 1982). Other reports have found increasing prevalence of MRSA carriage on admission to hospital, but this was often associated with previous hospitalisation or hospital contact (Boyce, 1998; Troillet *et al.*, 1998; Warshawsky *et al.*, 2000) or in indigenous populations (Embil *et al.*, 1994). However, some patients had no risk factors (Moreno *et al.*, 1995) and most isolates were resistant only to β-lactams (Anon, 1999; Herold *et al.*, 1998). More recently, there have been several outbreaks of community acquired skin and soft tissue infections in several settings, including correctional facilities, athletic teams and men who have sex with men (Anon, 2003; Culpepper *et al.*, 2001). It has been suggested that the changing epidemiology of MRSA from sporadic reports to endemicity in hospitals to increasing community carriage parallels that of the emergence of penicillin-resistant *S. aureus* (Chambers, 2001).

1.2.8 Vancomycin resistance

The 40-year history of MRSA is generally one of an increasing problem geographically and numerically, to the point where, in some countries, it is firmly entrenched in the hospital, and more recently, in the community flora. We are now witnessing the ability of *S. aureus* to alter its profile again with the emergence of MRSA resistant to vancomycin. Isolates with decreased susceptibility to vancomycin were first reported from Japan in 1996 and the term vancomycin-intermediate *S. aureus* (VISA) was coined (Hiramatsu,

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1997). The minimum inhibitory concentration (MIC) of these organisms to vancomycin was defined as 8-16 µg/ml. Other cases were reported from around the world in subsequent years (Khurshid *et al.*, 2000; Martin *et al.*, 1997; Martin *et al.*, 1997; Ploy *et al.*, 1998; Trakulsomboon *et al.*, 2001). The first case in Australia was reported from Melbourne in 2001 (Ward *et al.*, 2001). Most of these reports have arisen from MRSA isolates in patients who have received prolonged courses of vancomycin (Fridkin, 2001; Rotun *et al.*, 1999). Fridkin *et al* confirmed these findings in a case-control study where risk factors for *S. aureus* with reduced susceptibility to vancomycin (MIC ≥ 4 µg/ml) that remained significant in a multivariate analysis were receipt of vancomycin and oxacillin-resistant *S. aureus* infection in the preceding 2-3 months (Fridkin *et al.*, 2003). The mechanism of reduced susceptibility to vancomycin has not been fully elucidated, but is believed to be related changes in the thickness and composition of the bacterial cell wall (Naimi *et al.*, 2003). After the demonstration of *in vitro* transfer of the *van*A vancomycin resistance gene from *Enterococcus faecalis* to *S. aureus*, it was believed that it was only a matter of time before *S. aureus* fully resistant to vancomycin was reported (Miller *et al.*, 2002). This finally occurred in 2002 (Centers for Disease Control and Prevention, 2002; Miller *et al.*, 2002). Both of these isolates contained the *van*A gene and in one, it was believed to have possibly been acquired from a concurrent vancomycin-resistant enterococcus (VRE) strain harboured by the patient.

1.3 Epidemiology of MRSA

1.3.1 Reservoirs and transmission

The major reservoir of MRSA consists of infected and colonised patients who may serve as a source of transmission to other patients (Thompson *et al.*, 1982), with health-care workers and the environment serving as occasional reservoirs. Spread between patients has been documented numerous times using epidemiological studies (Crossley *et al.*, 1979; Klimek *et al.*, 1976; Peacock *et al.*, 1980) and has been supported by subtyping (Kumari *et al.*, 1998; Peacock *et al.*, 1980). There are many examples of introduction of a new MRSA strain into an institution and sometimes into a country by a patient who is MRSA colonised or infected (Embil *et al.*, 2001; Farrington *et al.*, 1990; O'Toole *et al.*, 1970; Roman *et al.*, 1997). The nose is the most commonly colonised site, although many other sites including groin/perineum, throat, hairline, axilla and rectum may also be colonised (Sanford *et al.*, 1994; Rimland *et al.*, 1986; Manian *et al.*, 2002; Coello *et al.*, 1994; Cox *et al.*, 1995). Studies of *S. aureus* colonisation show that carriage may be transient or long term (Kluytmans *et al.*, 1997; VandenBergh *et al.*, 1999). Similarly, Sanford *et al* found that carriage of MRSA may also be prolonged (Sanford *et al.*, 1994). These authors found that of 36 patients previously known to carry MRSA who were readmitted to hospital, 33% were still colonised after 12 months and 38% after 48 months. 5/12 with persistent carriage were of the same plasmid type as original strains (Sanford *et al.*, 1994). This knowledge impacts on screening policies for MRSA surveillance, as patients who have previously been colonised or infected with MRSA may well be positive on a subsequent admission.

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Transmission between patients occurs largely on the contaminated hands of health-care workers. Some studies of dubious ethical quality performed more than 40 years ago, demonstrate that transmission of *S. aureus* to neonates was most common via the deliberately unwashed hands of nurses (Mortimer *et al.*, 1962; Mortimer *et al.*, 1966; Wolinsky *et al.*, 1960). There are many studies showing transient carriage of *S. aureus* or MRSA on health-care workers' hands which may be eradicated by hand washing or disinfection (Mortimer *et al.*, 1962; Mortimer *et al.*, 1966; Thompson *et al.*, 1982). Hands are most commonly contaminated during direct patient contact (Crossley *et al.*, 1979), but some have shown that contact with the patient's contaminated environment or equipment may also contaminate health-care workers' gloves or hands (Boyce *et al.*, 1997; Crossley *et al.*, 1979). In one study, 58% of nurses' gloves were contaminated after routine care of patients with MRSA in wounds or urine and 42% after touching inanimate objects in the patient's room, without actually touching the patient (Boyce *et al.*, 1997). Pittet *et al* showed hand contamination increased linearly with time spent on patient care and independent risk factors for higher levels of contamination were direct patient contact, respiratory care, handling of body fluids and disruption in the sequence of patient care (Pittet *et al.*, 1999).

There are several studies showing that health-care workers also may acquire nasal carriage after caring for an MRSA colonised patient (Opal *et al.*, 1990; Shanson *et al.*, 1985; Ward *et al.*, 1981). This may occur especially after close contact, including wound care, bathing a colonised patient or urethral catheterisation but not after walking into a contaminated

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environment without patient contact (Cookson *et al.*, 1989). Carriage may be transient, short term or, less commonly, persistent and this may impact on reported prevalence in health-care workers, depending on the timing of the screening in relationship to their shift (Cookson *et al.*, 1989; Farrington *et al.*, 1990). There are several reports of outbreaks which have been traced to a colonised health-care worker. This has usually been related to colonised eczema (Shanson *et al.*, 1980; Wang *et al.*, 2001) or to upper respiratory tract colonisation or infection and may be related to increased dispersal during an upper respiratory tract infection, the so called "cloud adult" (Belani *et al.*, 1986; Boyce *et al.*, 1993; Sherertz *et al.*, 1996; Ward *et al.*, 1981). Apart from these examples, however, the role of the colonised health-care worker as an MRSA reservoir is believed to be limited. Generally, the yield from staff screening has not been found to be helpful. Staff may be found to be nasally colonised, but either the prevalence is low (Boyce *et al.*, 1981; Crossley *et al.*, 1979; Kumari *et al.*, 1998; Layton *et al.*, 1993; McNeil *et al.*, 1984; Peacock *et al.*, 1980; Rampling *et al.*, 2001; Thompson *et al.*, 1982), there is no suggestion of a causal role in the outbreak (Klimek *et al.*, 1976; Thompson *et al.*, 1982) or the subtypes have been different from those causing patient infections (Peacock *et al.*, 1980). The implied mode of transmission from the nasally colonised health-care worker to the patient is via the airborne route or via auto-contamination of the health-care worker's hands (Cookson *et al.*, 1989). Reagan *et al* found that elimination of nasal carriage of *S. aureus* also significantly reduced hand carriage in health-care workers (Reagan *et al.*, 1991).

Boyce *et al* demonstrated that environmental contamination occurred in the rooms of 73% and 69% of MRSA infected and colonised patients respectively and was six times more likely if wounds and urine were colonised compared with other body sites. 65% of nurses' gowns or uniforms were contaminated after routine care of patients with wound or urine colonisation (Boyce *et al.*, 1997). In addition, MRSA has been shown to survive on fabrics for prolonged periods of up to several weeks (Neely *et al.*, 2000), however, the role of clothes in the direct transmission of MRSA to patients or their role in contamination of health-care workers hands and subsequent patient transmission has not been determined. Others have not found evidence of contamination of nurses' clothes or aprons (Cookson *et al.*, 1989).

Screening of the environment has found MRSA in many instances, particularly in burns units (Crossley *et al.*, 1979). Contamination of multiple surfaces has been reported, including doorhandles (Oie *et al.*, 2002), ward-based computer terminals (Bures *et al.*, 2000; Devine *et al.*, 2001), doctors' and nurses' pens (French *et al.*, 1998), hospital television sets (Stacey *et al.*, 1998), paper towel holders (Marshall *et al.*, 1998), blood pressure cuffs and a shower recess (Layton *et al.*, 1993), hospital hairdresser's equipment (Ruddy *et al.*, 2001) and multiple other pieces of patient equipment and furniture (Barakate *et al.*, 1999; Bartzokas *et al.*, 1984; Bitar *et al.*, 1987; Blythe *et al.*, 1998; Embil *et al.*, 2001; Kumari *et al.*, 1998). Some of these studies have attempted to correlate environmental contaminants and clinical isolates using subtyping methods, such as pulsed-field gel electrophoresis (PFGE) (Bures *et al.*, 2000; Embil *et al.*, 2001; Kumari *et al.*,

1998), antibiogram (French *et al.*, 1998) and arbitrary-primed polymerase chain reaction (AP-PCR) (Boyce *et al.*, 1997). Others have not found MRSA in the environment, although this may depend on how many sites are cultured and whether this is after cleaning processes (Klimek *et al.*, 1976; Rimland, 1985). The role of the environment as a reservoir is still unclear, as it is not known whether the environmental isolates can be transmitted to patients and cause colonisation or infection or whether they have become secondarily contaminated with the true source of MRSA being elsewhere. There have, however, been some outbreaks reported which have been linked to an environmental source that seemed only to be controlled following extensive environmental cleaning (Layton *et al.*, 1993; Rampling *et al.*, 2001). Others have found that closure of a ward with extensive cleaning and refurbishment did not reduce the subsequent rate of MRSA in the ward upon re-opening (Barakate *et al.*, 1999). Embil *et al* reported showering equipment to be the source of an outbreak on a burns ward based on finding the same strain in the environment and patients (Embil *et al.*, 2001). Cessation of the outbreak occurred after showering procedures were changed, but several other control measures such as isolation and contact precautions were also used concurrently and not all patients who were colonised had been exposed to the showering equipment. Kumari *et al* documented colonisation of ventilation grilles in their ICU on one occasion and reported that cleaning the grilles and making other changes to the ventilation system terminated the outbreak (Kumari *et al.*, 1998). The data provided in these studies do not prove conclusively that the environmental contamination was the reservoir, but equally may have been a secondary phenomenon.

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The role of airborne transmission of MRSA is also difficult to assess. Although early authors found that some neonates acquired *S. aureus* via this route, it was of far less importance than hand-to-hand transmission (Mortimer *et al.*, 1962; Mortimer *et al.*, 1966; Wolinsky *et al.*, 1960). Airborne *S. aureus* has also been found via air sampling by some (Peacock *et al.*, 1980; Bauer *et al.*, 1990; Crossley *et al.*, 1979) but not others (Klimek *et al.*, 1976). Shiomori *et al* found increased airborne levels of MRSA following bed-making, some of which were in the respirable range (<4μm) (Shiomori *et al.*, 2002; Shiomori *et al.*, 2001). Cotterill *et al* suggested that airborne transmission of MRSA via a faulty ventilation system and window was responsible for an outbreak which was not terminated until they were fixed (Cotterill *et al.*, 1996). The difficulty in assessing the role of airborne transmission lies in the fact that there is always the possibility of other mechanisms of spread taking place, thus making it difficult to assess the relative importance of each (Solberg, 2000).

1.3.2 Colonisation and infection

Colonisation is defined as isolation of MRSA in the absence of symptoms and signs of infection. Various authors have used many different definitions of infection. More recently, many authors are using the Centers for Disease Control/National Nosocomial Infection Surveillance System definitions which combine clinical, laboratory and radiological criteria for determining the presence of infection (Garner *et al.*, 1988; Garner, 1996). Although designed primarily for surveillance, they are used by many to define infection in the context of a study to allow consistent comparisons between reports.

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Of patients who are MRSA carriers, between 30 and 64% have been reported to be infected at the time (Boyce *et al.*, 1981; Craven *et al.*, 1981; O'Toole *et al.*, 1970). However, cohort studies following up colonised patients have shown a lower rate of development of infection of 11.1% (Coello *et al.*, 1997), 11.8% (Longfield *et al.*, 1985) and 23.5% (Longfield *et al.*, 1985). Huang and Platt found that 29% of MRSA colonised patients became infected during an 18 month follow-up (Huang *et al.*, 2003). Coello *et al* found intensive care unit stay, presence of surgical wounds, pressure ulcers and intravenous catheterisation to be independent risk factors for development of MRSA infection in colonised patients during an outbreak (Coello *et al.*, 1997).

Nasal carriage of *S. aureus*, including MRSA, has been clearly documented to be a risk factor for subsequent infection in many scenarios. Williams *et al* described that nasal carriers of *S. aureus* were three times as likely to develop staphylococcal sepsis as non-carriers (Williams *et al.*, 1959). More recently, Von Eiff *et al* demonstrated identical *S. aureus* genotype in both nasal and blood isolates (of which 9.1% were methicillin resistant) in 82.2% of patients studied (Von Eiff *et al.*, 2001). Pujol *et al* showed that *S. aureus* carriers were more likely to develop bacteraemias than non-carriers, with MRSA carriers having a relative risk of 3.9 compared with methicillin-sensitive *Staphylococcus aureus* (MSSA) carriers (Pujol *et al.*, 1996). In a long-term care facility, Muder *et al* demonstrated that MRSA carriers were significantly more likely to develop clinical infection than either non-carriers or MSSA carriers, with colonising and infecting strains having the same subtype in most cases (Muder *et al.*, 1991). Other studies have shown *S.*

aureus nasal colonisation to be a significant risk factor for surgical site infection (Kluytmans *et al.*, 1997), including orthopaedic (Kalmeijer *et al.*, 2000) and cardiothoracic surgery patients (Kluytmans *et al.*, 1995; Weinstein, 1959). This increased risk for infection has also been shown in ICU patients (Corbella *et al.*, 1997; Mest *et al.*, 1994), in those with cirrhosis (Campillo *et al.*, 2001; Chang *et al.*, 1998; Dupeyron *et al.*, 2001), in those with HIV infection (Nguyen *et al.*, 1999), in liver transplant recipients (Bert *et al.*, 2000) and in chronic ambulatory peritoneal dialysis (CAPD) (Lye *et al.*, 1993) and hemodialysis patients (Ena *et al.*, 1994).

1.3.3 Risk factors

Analysis of risk factors associated with MRSA is hampered by various methodological issues and the heterogeneity between studies. The outcome assessed is sometimes colonisation (von Baum *et al.*, 2002), sometimes infection (Rello *et al.*, 1994; Shimada *et al.*, 1993) and sometimes both (Onorato *et al.*, 1999; Warshawsky *et al.*, 2000). Using infection as the outcome may result in misclassification, as unrecognised colonised patients may be inadvertently classified as controls (Paterson, 2002). In addition, studies have assessed risk factors for different types of infection, such as surgical site (Manian *et al.*, 2003), pneumonia (Lentino *et al.*, 1985) or blood stream infection (Pujol *et al.*, 1994; Rezende *et al.*, 2002). The settings have varied from different types of institution, including acute care facility (Muller *et al.*, 2003; Crowcroft *et al.*, 1996; Lucet *et al.*, 2003) and long-term care facility (O'Sullivan *et al.*, 2000; von Baum *et al.*, 2002; Washio *et al.*, 1997), different wards, including intensive care units (Westphal *et al.*, 1997; Ibelings *et al.*,

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1998; Merrer *et al.*, 2000; Rello *et al.*, 1994) and other wards. Some studies have taken place during an epidemic (Locksley *et al.*, 1982; Bitar *et al.*, 1987; Crowcroft *et al.*, 1996) and have examined different patient populations, including HIV infected patients (Onorato *et al.*, 1999), cirrhosis patients (Chang *et al.*, 1998) and surgical patients (Scriven *et al.*, 2003; Shimada *et al.*, 1993). Some have assessed risk factors for community acquired cases (Rezende *et al.*, 2002; Samad *et al.*, 2002; Warshawsky *et al.*, 2000) and some risk factors for hospital acquired cases (Asensio *et al.*, 1996; Bitar *et al.*, 1987). Studies also vary in their choice of predictor variables. The types of study include ecological, (Muller *et al.*, 2003), case-control (Asensio *et al.*, 1996; Crowcroft *et al.*, 1996; Graffunder *et al.*, 2002; Onorato *et al.*, 1999; Washio *et al.*, 1997) and cohort (Ho, 2003; Merrer *et al.*, 2000) and the choice of control often varies between patients with MSSA infections (Chang *et al.*, 1998; Graffunder *et al.*, 2002; Lentino *et al.*, 1985; Rello *et al.*, 1994) or patients without any *S. aureus* infection (Dziekan *et al.*, 2000; Onorato *et al.*, 1999; O'Sullivan *et al.*, 2000). In addition, many of the studies are univariate models and have not adjusted for confounding factors, such as length of stay or severity of illness (Scriven *et al.*, 2003; Westphal *et al.*, 1997; Hill *et al.*, 1998; Locksley *et al.*, 1982; Rello *et al.*, 1994). These methodological differences have meant that it is difficult to compare results of studies, some of which are conflicting.

Despite these methodological differences, there are several factors which have generally been consistently associated with a higher risk of the MRSA outcome assessed. These include increasing length of stay (Lucet *et al.*, 2003; Graffunder *et al.*, 2002; Ibelings *et*

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al., 1998; Lentino et al., 1985; Locksley et al., 1982; Chang et al., 1998; Scriven et al., 2003; Asensio et al., 1996; Crowcroft et al., 1996; Ho, 2003), age (Asensio et al., 1996; Lucet et al., 2003; Samad et al., 2002), presence of wounds (von Baum et al., 2002; Lucet et al., 2003), pressure areas (Crowcroft et al., 1996; O'Sullivan et al., 2000), urinary catheters (Onorato et al., 1999; Rezende et al., 2002; von Baum et al., 2002), intravenous catheters (Onorato et al., 1999; Pujol et al., 1994), nasogastric tubes (Thomas et al., 1989; Graffunder et al., 2002) or several concurrent medical devices (Ho, 2003), preceding hospitalisation (Lucet et al., 2003; Asensio et al., 1996; Samad et al., 2002; von Baum et al., 2002), antibiotic therapy (Ho, 2003; Dziekan et al., 2000; Shimada et al., 1993; Thomas et al., 1989; Washio et al., 1997; Rello et al., 1994; Lentino et al., 1985; Muller et al., 2003; Onorato et al., 1999; Rezende et al., 2002; Graffunder et al., 2002), intensive care unit stay (Asensio et al., 1996; Manian et al., 2003; Muller et al., 2003), colonisation pressure (usually defined as the ratio of MRSA carrier-days to total patient-days) (Merrer et al., 2000; Muller et al., 2003), severity of illness (Chang et al., 1998; Lentino et al., 1985; Ho, 2003) and previous surgery (Crowcroft et al., 1996; Graffunder et al., 2002; Bitar et al., 1987; Lucet et al., 2003). In summary, generally sicker patients with previous hospitalisation or intensive care unit stay, with longer stay and medical devices in place or having undergone procedures are at greatest risk.

1.3.4 Relationship between antibiotic usage and MRSA

Evidence exists showing that antibiotic usage is directly associated with rates of MRSA, including consistent associations between heavy antibiotic use and high MRSA prevalence

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and dose-effect relationships at the patient and hospital or unit level, accompanied by a plausible biological explanation (Monnet, 1998). At the geographical level, low antibiotic usage levels within some countries have been correlated with low rates of MRSA (Sørensen et al., 2000). At the ward level, those with higher rates of resistant organisms have higher antibiotic consumption (Fridkin et al., 1999) and at the patient level, associations have been made through many epidemiological studies. There is also some evidence that selective pressure exerted by certain antibiotics may play a major role in generation of endemic MRSA by selecting for small subpopulations of methicillin-resistant organisms amongst the sensitive S. aureus colonising individual patients (Schentag et al., 1998).

The relationship between prior antibiotic use and methicillin resistance has been investigated in many case-control and cohort studies, with conflicting results. Several studies using multivariate analysis, have shown exposure to antibiotics to be a risk factor (Graffunder et al., 2002; Ho, 2003; Mest et al., 1994; Onorato et al., 1999; Rezende et al., 2002; Washio et al., 1997), whereas others have not (Asensio et al., 1996; Hershow et al., 1992; Lucet et al., 2003). Methodological differences between studies are problematic and include inconsistent outcomes, choice of controls, selection of other variables and measures of antibiotic usage. Some of the studies with negative findings lack adequate subject numbers to reach statistical significance.

The origin of MRSA resides in the horizontal transfer of the *mecA* gene to *S. aureus*, which is thought to be a rare occurrence. Therefore the spread of MRSA across the globe has occurred primarily because of expansion of a few clones and not repeated *de novo* appearances of MRSA (Oliveira *et al.*, 2002). The relationship between exposure to antibiotics and MRSA lies in the creation of a milieu where MRSA may have a selection advantage over non-resistant *S. aureus* and spread is facilitated (Hiramatsu *et al.*, 2001).

1.4 Molecular epidemiology and typing

The ability to subtype bacterial isolates by various methods has given us new insights into the epidemiology of MRSA and other nosocomially acquired infections. Typing allows us to distinguish epidemiologically related or clonal strains from unrelated strains (Shopsin *et al.*, 2001). The major reasons for typing bacterial isolates include: identifying a source or quantifying the extent of an outbreak, differentiating sporadic or endemic strains from outbreak strains, as part of surveillance to identify acquisition of specific strains and distinguishing between recurrence or reinfection with a particular organism in an individual patient (Pitt, 1999). Numerous methods of MRSA subtyping have been developed and are usually divided into phenotypic and genotypic methods. Phenotypic methods detect characteristics expressed by the bacteria while genotypic ones involve direct analysis of chromosomal or extra-chromosomal genetic elements (Gemmell, 1999). The utility of the various tests is usually judged on criteria including typeability, reproducibility, discriminatory power and other factors such as cost, time to generate a result and technical skills required (Struelens, 1996). As all MRSA isolates across the

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world arise from only a few clones, typing techniques must be highly discriminatory to be useful (Oliveira *et al.*, 2002; Schmitz *et al.*, 1998). A summary of the advantages and disadvantages of some of the different typing methods is shown in Table 1-1 and some are discussed in further detail in the following paragraphs.

1.4.1 Antibiogram

An antibiogram refers to the antibiotic sensitivity pattern of an organism and may be used to compare isolates. It is usually routinely performed in the microbiology laboratory, but may be susceptible to variables such as antibiotic treatment and, because of the inherent instability of plasmids, may be unreliable over extended periods. It may not be reproducible, depending on laboratory conditions and is generally regarded as poorly discriminatory (Weller, 2000). Occasional isolates have a distinctive resistance pattern allowing it to be used to identify a particular strain (Archer *et al.*, 1983; Hoefnagels-Schuermans *et al.*, 1997; Kim *et al.*, 1998), but it is generally not regarded as useful for distinguishing subtypes of MRSA (Farrington *et al.*, 1990; Hoefnagels-Schuermans *et al.*, 1997; Struelens *et al.*, 1992). This is because many MRSA show similar resistance patterns (Mulligan, 1991). Conversely, identical genetic strains may have different antibiograms (Kostman *et al.*, 1995). Some have suggested that the antibiotic susceptibility pattern be used for initial screening of isolates to determine relatedness with other methods used for further discrimination (Tenover *et al.*, 1994). Others have found the antibiogram to be as discriminatory as PFGE (Montesinos *et al.*, 2002).

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1.4.2 Phage typing

Phage typing was developed in the 1940s for the typing of *S. aureus* isolates. It relies on the fact that some isolates contain temperate phages which lyse other bacteria of the same species, with different strains being identified by patterns of phage lysis (Weller, 2000). This is one of the few typing methods which has been standardised internationally but is time-consuming, technically demanding, lacks reproducibility and relies on propagating stocks of phages and therefore is only performed in a few reference laboratories. Another major disadvantage is that 15-20% of isolates may not be typable (Bannerman *et al.*, 1995). It is less discriminatory than PFGE, which has replaced it as the "typing method of choice" (Bannerman *et al.*, 1995).

1.4.3 Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis is now regarded as the "gold standard" for MRSA typing because of its reproducibility, typability, discriminatory power and excellent epidemiological correlations (Mickelsen, 1997; Struelens *et al.*, 1992). It involves digestion of DNA with a restriction endonuclease, most commonly *Sma*I, then separation of the fragments by size using pulsed-field electrophoresis. This unique type of electrophoresis uses alternating angles and frequency of electrical pulses to separate the large fragments. Gels are stained using ethidium bromide and photographed for interpretation (Bannerman *et al.*, 1995; Mickelsen, 1997). Tenover *et al* have proposed criteria to enable standardised interpretation of banding patterns (Tenover *et al.*, 1995). These criteria were intended for use in the outbreak situation and not for longer than one

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year, but in the absence of other criteria, they have been used more widely in other settings (Murchan *et al.*, 2003). Blanc *et al* have shown that MRSA strains produce PFGE banding patterns that are relatively stable over weeks to months (Blanc *et al.*, 2001). Computer software programs, such as GelCompar (Applied Maths, Kortrijk, Belgium), are available to enable assisted analysis of the gels and standardised comparison of banding patterns. This software is very expensive, however, and not readily available in all laboratories.

PFGE has been found to be more discriminatory than several PCR based methods, but a combination of PFGE with one other of these techniques allowed the best discrimination in one study (Schmitz *et al.*, 1998). Tenover *et al* compared PFGE with several other phenotypic and genotypic methods and found none to be superior. This group also concluded that a combination of typing methods may be the most discriminatory (Tenover *et al.*, 1994). Yoshida *et al* also found a combination of typing methods, including ribotyping and PFGE, to give the highest discriminatory power, although PFGE by itself was more discriminatory than either ribotyping or IS*431* typing (Yoshida *et al.*, 1997). The use of two or more molecular typing methods simultaneously is unlikely to be practical in the routine infection control laboratory, however. Several authors have found PFGE to be useful in the hospital setting to differentiate epidemic from endemic strains (Hartstein *et al.*, 1997; Macfarlane *et al.*, 1999; Meier *et al.*, 1996).

The major drawback of PFGE is the fact that it very time-consuming and takes several days to generate a result. Despite this, it is probably the most widely used typing

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technique for MRSA in the hospital setting. Many institutions have developed their own protocols and have considerable expertise in the technique. The HARMONY group in Europe has recently proposed a standardised protocol for the parameters, which now allows formation of an international database with the ability to compare and track the path of strains between different countries (Murchan *et al.*, 2003).

1.4.4 Ribotyping

Ribotyping uses ribosomal RNA as the probe for restriction fragment length polymorphisms using Southern hybridisation. Although all MRSA isolates are typable by this method and it is more discriminatory and reproducible than phenotypic methods, it is less discriminatory than PFGE (Prevost *et al.*, 1992; Yoshida *et al.*, 1997). In addition, it is time-consuming and technically demanding (Mulligan, 1991) and criteria for interpretation have not been standardised (Weller, 2000). An automated typing system based on the technique of ribotyping, the RiboPrinter® Microbial Characterization System (Qualicon, Wilmington, DE, USA) may overcome some of these problems. It is a fully automated system which can process up to 32-40 isolates in one day. It is linked to a software system that allows analysis of RiboPrint patterns and has a web-based database which allows comparison of RiboGroups between any institution. There are some published reports of its use in the subtyping of MRSA (Gales *et al.*, 2000; Landman *et al.*, 2003) but few direct comparisons. Fung *et al* found that the RiboPrinter® was unable to distinguish between 14 outbreak isolates of MRSA that were grouped into four clusters by antibiogram and PFGE (Fung *et al.*, 2001). Diekema *et al* found that the RiboPrinter® was

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less discriminatory than PFGE in all 26 PFGE types except one in their study (Diekema *et al.*, 2000). As it is based on the technique of ribotyping, it is not surprising that it is not as discriminatory as PFGE (Weber *et al.*, 1997). Its major drawback is its cost, which renders it prohibitive for many institutions to purchase.

1.4.5 Multilocus sequence typing

A multilocus sequence typing (MLST) system has been developed for *S. aureus* by a group in the United Kingdom (Enright *et al.*, 2000). This is a highly discriminatory method of characterising bacterial isolates based on the sequences of internal fragments of seven housekeeping genes. It allows each isolate to be given a sequence type defined by the allelic profile of each of these genes, which can be stored in a centralised web-based database. MLST typing correlates well with PFGE analysis. MLST is suitable for international comparisons and for long term evolutionary analysis of MRSA isolates (Oliveira *et al.*, 2002). However, its use at present is restricted to few reference laboratories.

In summary, many methods have been used to subtype MRSA over the years. Recently, the emphasis has been on genotypic ones, with PFGE being considered to be the "gold standard" as it is discriminatory and reproducible. Newer methods, such as MLST may be more useful for long-term epidemiological studies and for international comparisons, but are unlikely to replace PFGE in the routine infection control laboratory. Some institutions

have experience with other PCR based typing methods which may also be useful, but many

are not widely available.

Table 1-1 Summary of MRSA typing methods

METHOD	ADVANTAGES	DISADVANTAGES
1. PHENOTYPING		
a. Antibiogram	Easy to perform	Poor discriminatory ability
	Cheap	
	Readily available	
b. Phage typing	Standardised by International Subcommittee on Phage Typing	Time-consuming
		Technically demanding
		High proportion of isolates not typable
		Lack of reproducibility
c. Serotyping		Poor discriminatory ability
		Not used extensively for S. aureus
d. Protein electrophoresis		
Whole cell protein	Reproducible	Poor discrimination
	Lack of correlation with phage typing	Isolates need to be run in parallel
Immunoblotting	All isolates typable	Not discriminatory enough to correctly exclude unrelated organisms
Multilocus enzyme electrophoresis (MLEE)	All isolates typable	Comparison difficult – requires computer software
	Good reproducibility	Labour intensive
	Good discriminatory power	Not available in most laboratories

Zymotyping	All isolates typable	Not discriminatory enough
	Pattern is stable & reproducible	No correlation with antibiotic susceptibility pattern

2. GENOTYPING

a. Plasmid analysis	Easy to perform	Plasmids not present in every isolate – many organisms not typable
	Simple to interpret	
	Restriction endonuclease analysis or plasmid DNA (REAP) – more discriminatory than PFGE in some studies & less in others	Plasmid DNA may exist in more than 1 form, with different electrophoretic properties
		Lack of stability & reproducibility
		Plasmids easily gained & lost
		May be transfer of DNA between unrelated isolates
b. Restriction enzyme analysis of chromosomal DNA (REA)	All isolates typable	Large number of overlapping bands, making consistent analysis difficult
		Variable reproducibility and discriminatory power
c. Southern hybridisation		
Ribotyping	All MRSAs typable	Less discriminatory than PFGE
	More reproducible & discriminatory than phenotypic methods	Criteria for interpretation not standardised
		Time-consuming
		Technically complicated

Insertion sequences	Stable & reproducible results	Some strains not typable
		Discriminatory ability dependent on nature of isolates being tested
MecA:Tn554 probe typing		Most successful use of mecA:Tn554 hybridisation is with PFGE but this is very time-consuming
Binary typing	All MRSAs typable Results stable & reproducible	Fails to provide information on genetic relatedness of strains Technically subjective Time-consuming
d. Pulsed-field gel electrophoresis (PFGE)	All isolates typable Discriminatory ability high Superior discriminatory ability to phage typing, antibiogram, RAPD, ribotyping , zymotyping	Expensive Time-consuming Requires subjective interpretation & comparison of patterns
e. PCR typing		
Coagulase gene typing	Faster & less expensive than PFGE	Small number of isolates not typable Less discriminatory than PFGE
RAPD	All isolates typable Reproducible within same laboratory Relatively fast and simple	Discriminatory power variable, depends on number & sequence of primers Not as discriminatory as PFGE Poor interlaboratory reproducibility

Rep-PCR	Good reproducibility Good discriminatory power	Need to include an extra DNA purification step which increases procedure time Not as discriminatory as PFGE, but depends on group of MRSA tested
f. DNA sequence analysis		
Multilocus sequence typing (MLST)	Objective Genetic code is highly portable, easily stored and analysed in relational database	Labour intensive Time-consuming Expensive
Spa-typing	Fast Easy to use and interpret Compatible for building relational databases	Not as discriminatory as PFGE

From (Deplano *et al.*, 1997; Schmitz *et al.*, 1998; Shopsin *et al.*, 2001; Weber *et al.*, 1997; Weller, 2000)

1.5 Control of MRSA

1.5.1 Rationale for MRSA control

Although over the years there have been some who believe that MRSA control is not warranted, it is now generally accepted that we should attempt to actively control MRSA for the following reasons:

- MRSA infections occur in addition to, and do not merely replace, those caused by methicillin-sensitive *S. aureus* (MSSA) (Boyce *et al.*, 1983; Dominguez *et al.*, 1994; Haley *et al.*, 1982; Law *et al.*, 1988; Tam *et al.*, 1988), therefore controlling MRSA should have an impact on overall hospital infection rates. MRSA levels in an institution are believed to be an indicator of the overall performance of its infection control program (Herwaldt, 1999).

- Worldwide and in Australia, MRSA is a significant problem, with a large proportion of *S. aureus* isolates being methicillin resistant.

- MRSA colonisation and infection are preventable with potential enormous cost savings. Infection with MRSA has been shown to prolong hospital stay and increase attributable costs (Abramson *et al.*, 1999; Kim *et al.*, 2001; The Brooklyn Antibiotic Resistance Task Force, 2002) and control measures introduced to contain MRSA have been shown to be cost effective (Karchmer *et al.*, 2002; Papia *et al.*, 1999).

- MRSA is a virulent organism, capable of causing significant morbidity and mortality and there is some evidence that it may be more virulent than MSSA (Blot *et al.*, 2002; Romero-Vivas *et al.*, 1995).

- Because most (hospital acquired) MRSA are multi-resistant, there are a limited number of agents available for treatment of MRSA infections. These may include vancomycin, teicoplanin, rifampicin, fusidic acid, ciprofloxacin, linezolid and quinupristin/dalfopristin. Problems include necessity for parenteral administration for some, rapid development of resistance for others if used as monotherapy, expense and need for therapeutic monitoring.

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- Perhaps the most compelling reason for MRSA control is the global appearance of VISA and VRSA and VRE in the last few years. MRSA levels drive the empiric, therapeutic and prophylactic use of vancomycin (Garrouste-Orgeas *et al.*, 2001) and vancomycin use has been shown to be a risk factor for *S. aureus* with reduced susceptibility to vancomycin (Fridkin *et al.*, 2003) and for VRE (Bonten *et al.*, 2001). In addition, most VISA isolates are believed to have arisen from MRSA. Overall, this implies that failure to control MRSA will preclude control of VISA.

1.5.2 Limitations of current literature

Because of the nature of outbreaks and the difficulty in randomising many infection control interventions, much of the published material on MRSA control consists of retrospective observational studies and opinion, with a few prospective studies and fewer well designed interventional studies. One cannot assume that a fall in MRSA infections following an intervention results directly from that intervention, as rates of endemic MRSA tend to rise and fall with time (Goetz *et al..*, 1992). Interpretation of the literature is hampered by differing methods of reporting data and, in some studies, absence of denominators and lack of statistical analysis and power calculations. It is therefore often difficult to draw valid conclusions, let alone compare results.

Recent publication of guidelines for MRSA control (Muto *et al.*, 2003) have highlighted an issue that is rarely addressed in the literature: whether control of MRSA in one setting can be generalised to other settings. This question is most pertinent with regard to control of

epidemic and endemic MRSA, but also may apply to intensive care unit settings compared with other acute or non-acute wards and may apply to different patient groups with different characteristics, where the risk of MRSA transmission may not be the same. Many of the studies cited as a model for control of endemic MRSA have taken place in a ward, hospital or geographic area with little or no endemic MRSA. In this setting or in the setting of an outbreak, various measures are introduced simultaneously, often with apparently successful results. However, in the endemic context, measures must be efficacious, ongoing and sustainable. Without disputing that recommended measures are likely to be successful in controlling endemic MRSA, we currently lack an understanding of the *minimal* effective measures that are necessary and feasible in the endemic setting. The ability to respond to and the measures required to control MRSA in an outbreak or after introduction to an institute with no endemic MRSA may not necessarily be the same required to control MRSA in an institution where MRSA is highly endemic. Because of the likely contribution of multiple factors to the epidemiology and transmission of MRSA, there is unlikely to be a single solution for all institutions, but knowledge of the relative importance of the numerous recommendations would help to prioritise resources.

1.5.3 Prevention of transmission of MRSA

1.5.3.1 Surveillance

Surveillance is a means of identifying colonised or infected patients for whom specific control measures may be implemented. Surveillance may be passive, whereby laboratory results from clinical samples are monitored, or active, whereby patients are screened for

the presence of carriage in order to identify the complete reservoir, including patients who are asymptomatic but colonised. Passive laboratory-based screening for MRSA misses a significant proportion of the total number of colonised patients who can still remain a source for transmission (Farr et al., 2001). In three studies, up to 54.3% of colonised patients would not have been detected had active screening not been used (Coello et al., 1994; Girou et al., 1998; Lucet et al., 2003) and in another on a high-risk dermatology ward, 96% of MRSA carriers would not have been identified without a screening program (Girou et al., 1998). The extent of active screening has varied between studies from screening of all patients to screening of selected high risk-patients (with variable criteria) to screening of contacts of MRSA colonised patients. Only two studies have examined the ability of these selective strategies to detect all colonised patients (Girou et al., 2000; Lucet et al., 2003). Girou et al found that a selective screening strategy would have detected all colonised patients in a high-risk dermatology ward (Girou et al., 2000). In contrast, Lucet et al found that only universal screening would have detected MRSA carriage on admission to the ICU with an "acceptable sensitivity" (Lucet et al., 2003).

Active screening of patients has been recommended by several authorities as integral to control of MRSA by contact precautions (Ayliffe, 1998; Muto et al., 2003) and has been part of many successful active control programs (Cosseron-Zerbib et al., 1998; Mishal et al., 2001; Pittet et al., 2000). It has been shown to be cost effective in the situation where nosocomial transmission to as few as six patients is prevented (Papia et al., 1999). However, there are several studies that have shown control of MRSA without the use of

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active screening (Eveillard *et al.*, 2001) or when only screening contacts of MRSA cases (Jernigan *et al.*, 1995; Nicolle *et al.*, 1999; Valls *et al.*, 1994). The reasons why some control programs are successful without active screening are speculative. They may include a high proportion of patients being detected using clinical samples because of a low threshold for taking clinical samples or because of a high ratio of infected to colonised patients. Another reason may be that control of MRSA has been obtained through increased awareness and improved implementation of non-specific measures, such as hand hygiene, which are not reliant on knowledge of an individual's MRSA carriage status. Another reason may be the "Hawthorne effect", that is, because of awareness of the MRSA problem through initiation of multiple control measures and feedback, general improvement has taken place in many aspects of care.

The optimal anatomical site for culture to detect MRSA colonisation has been examined in several studies, although the method of reporting and the conclusions have differed to some extent. One study found that nasal cultures alone had a sensitivity of 93%, groin or perineum a sensitivity of 39% and axilla a sensitivity of 25% while addition of wound culture to nasal swabs gave a sensitivity of 100% (Sanford *et al.*, 1994). Another study found that only 59.8% of patients with clinical MRSA infection had nasal colonisation and 53% had rectal colonisation (Rimland *et al.*, 1986). This study also found that 41.5% of patients with a wound infection and 17.2% with other clinical infections did not have nasal or rectal MRSA colonisation. Other authors have found that 2% of patients with a negative nasal culture had a positive perianal culture and 16.7% of patients with a wound

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had a negative nose culture and a positive wound culture (Manian *et al.*, 2002). 26% of MRSA colonised dermatology patients were found to have positive nasal cultures in the absence of wound carriage (Girou *et al.*, 2000). Another study found that nasal or skin cultures detected 92% of MRSA positive patients whereas nasal or clinical cultures detected 37% (Lucet *et al.*, 2003). Coello *et al* found that nasal cultures alone had a sensitivity of 78.5% for detecting MRSA whereas nose, throat and perineum cultures had a sensitivity of 98.3% (Coello *et al.*, 1994). Cox *et al* demonstrated that only 82% of their patients had nasal colonisation, 9% had perineal colonisation alone and 9% had throat carriage alone (Cox *et al.*, 1995). In summary, nasal screening gives the highest yield for detection of MRSA carriage, but at least one other site such as wound, throat and perineum or groin should be screened in addition for maximal sensitivity.

If the purpose of active screening is to identify all the colonised patients making up the MRSA reservoir, then poor compliance with the recommended protocol may reduce the effectiveness of any control program. In one study, only 85% of patients who met the criteria for obtaining an admission screen had been swabbed by 72 hours (Papia *et al.*, 1999). Another issue for screening is the time required to obtain a result from MRSA cultures. Because of an intrinsic delay in generating results of screening cultures, active control measures may not be activated for MRSA colonised patients for several days. Some have recommended universal glove use in high-risk patients while these results are pending (Muto *et al.*, 2003), whilst others have used single room isolation of such patients until results were available during an outbreak (Shanson *et al.*, 1985). Physical labelling

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of patient records is inefficient and a mechanism for computerised tagging of electronic records has been developed by Bignardi and Askew (Bignardi *et al.*, 1998). Pittet *et al* reported a system that automatically alerts the infection control team when a previously colonised patient is readmitted. This significantly increased the proportion of MRSA colonised patients recognised at the time of admission from 13% to 40%, allowing earlier implementation of infection control measures (Pittet *et al.*, 1996).

1.5.3.2   *Isolation*

Recent guidelines issued by the British Society for Antimicrobial Chemotherapy, the Society for Healthcare Epidemiology of America (SHEA) and others have recommended active surveillance for MRSA in high risk patients and contact isolation precautions for those found to be colonised or infected (Ayliffe, 1998; Arnold *et al.*, 2002; Muto *et al.*, 2003). The current definition of contact isolation includes single room placement or cohorting, use of gloves when entering the room, use of gowns when entering the room if clothing will have substantial contact with the patient or environment, if the patient is incontinent or has wound drainage not contained by a dressing (Garner, 1996). The SHEA guidelines have recommend use of gloves and mask for entering a colonised patient's room and gown use for all patient and environmental contact (Muto *et al.*, 2003). Over the years, different levels of isolation and precautions have been used for MRSA control, including contact isolation, barrier precautions, patient cohorting and establishment of isolation wards. Evaluating the literature is hampered by changing isolation precautions over time and the various definitions used by different authors (Garner, 1996). Usually

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other infection control interventions have been introduced at the same time. Much of the literature supporting the use of active surveillance and contact precautions or cohorting concerns control of epidemics, often in wards, hospitals or geographical areas where the prevalence of MRSA is very low (Back *et al.*, 1996; Harbarth *et al.*, 2000; Jernigan *et al.*, 1996; Kotilainen *et al.*, 2001; Murray-Leisure *et al.*, 1990; Nicolle *et al.*, 1999; Saiman *et al.*, 2003; Selkon *et al.*, 1980; Shanson *et al.*, 1985; Valls *et al.*, 1994; Vriens *et al.*, 2002). It may be that the measures required to control an outbreak cannot be generalised to control of endemic MRSA, perhaps because of intrinsic differences between epidemic and endemic or sporadic strains (Dominguez *et al.*, 1994; Frénay *et al.*, 1994; Hoefnagels-Schuermans *et al.*, 1997; Van Belkum, 2000; Vriens *et al.*, 2002; Wagenvoort *et al.*, 2000), or because of the ability to implement and sustain the recommended precautions may be different in the two settings. Boyce calculated that in 46 published outbreak reports prior to 1991, definite or probable eradication of MRSA was achieved in all 11 hospitals with less than 20 cases compared with 71% of those hospitals with 20-39 cases and 10% of those with greater than 39 cases, suggesting that if measures are introduced at low levels of MRSA prevalence, the ability to control it is greater than if the prevalence is greater at the time of introduction (Boyce, 1991). Although eradication and control are not synonymous and it is usually accepted that eradication would be highly unlikely in the endemic setting, Boyce's data suggest that the starting point may have an impact on the success of the program.

There are numerous reports of control of MRSA outbreaks using active surveillance and contact precautions, although usually several other measures were also introduced at the same time (Murray-Leisure *et al.*, 1990; Nicolle *et al.*, 1999; Valls *et al.*, 1994). Haley *et al* used monthly screening with cohorting and other measures to eradicate MRSA from their neonatal ICU (Haley *et al.*, 1995). Harbarth *et al* reported a four year long outbreak that was eventually brought under control by the instigation of selective active screening of previously colonised patients on readmission, contacts of MRSA positive patients and admissions to the orthopaedic ward, accompanied by contact precautions and several other measures (Harbarth *et al.*, 2000). Jernigan *et al* calculated that during an outbreak in a neonatal ICU, the relative risk for transmission of MRSA for a patient not in contact isolation was 15.6 compared with one who was (Jernigan *et al.*, 1996). Vriens *et al* calculated that unidentified and therefore unisolated patients in their ICU were much more likely to transmit MRSA than recognised and isolated patients (Vriens *et al.*, 2002).

Despite repeated introduction of MRSA into their institution, Jernigan *et al* reported lack of significant transmission to other patients using active surveillance of roommates and "contact isolation" of MRSA cases, consisting of mask, gown and gloves, although the use of single room or cohorting was not explicitly stated (Jernigan *et al.*, 1995). However, after some time, there was a greater number of nosocomial MRSA cases, probably resulting from admission of increasing numbers of carriers, but suggesting that control measures were not fully effective. In addition, the failure to report any denominator means that other confounding factors, such as increasing numbers of admissions or changing

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length of stay, were not considered. Subsequently, authors from the same institution reported only 13 nosocomial MRSA bacteraemias in 1999 compared with 31-69 at four other institutions with similar bed size and patient illness severity (Calfee *et al.*, 2002). Hartstein *et al* have also reported reduction in the number of nosocomial cases, despite repeated introductions into their hospital, by using single room placement and glove use without active screening, however, only raw numbers were presented and not all outbreaks were prevented by this strategy (Hartstein *et al.*, 1995; Hartstein *et al.*, 1997). Farrington *et al* reported 10 years experience with a program of active screening of high-risk patients, single room isolation (although other precautions are not specified), staff screening, eradication therapy and ward closures if multiple cases were detected, keeping the numbers of MRSA low (Farrington *et al.*, 1998). With increasing numbers of MRSA-positive admissions, increasing nursing workload and increasing disruption caused by ward closures, the control measures were relaxed. Subsequent increases in MRSA numbers were attributed to reduced control measures, however, the increased numbers was originally one of the reasons for the initial relaxation. One year after numbers began to rise, only 25% of MRSA cases were in isolation because of lack of facilities, making it difficult to establish cause and effect. There are several other examples where category specific or contact isolation, with single room placement or cohorting of carriers, have produced falls in nosocomial MRSA cases (Cosseron-Zerbib *et al.*, 1998; Eveillard *et al.*, 2001; Nettleman *et al.*, 1991). Only two of these used active screening (Cosseron-Zerbib *et al.*, 1998; Nettleman *et al.*, 1991), two did not use gowns, gloves or masks and all instigated a program to increase hand washing at the same time.

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In contrast to the mostly retrospective observational studies, Ribner *et al* performed a prospective crossover study where the rates of MRSA transmission in the surgical and medical ICUs were not significantly different when strict and modified isolation were compared (Ribner *et al.*, 1986), although no indication of power estimations were given and whether single rooms were used was not disclosed (although one of the areas studied consisted only of single rooms). After implementation of the modified precautions throughout the hospital, there were significantly fewer MRSA infected but not colonised patients, perhaps because of better staff compliance with the modified precautions. Adeyemi-Doro *et al* reported a decrease in MRSA rates following a relaxation of isolation precautions from contact isolation, single room or cohort placement, patient and personnel screening and rigorous environmental cleaning to barrier precautions, education, emphasis on hand hygiene and isolation/cohorting only of patients with extensive skin lesions (Adeyemi-Doro *et al.*, 1997). The authors also commented that there was no other change likely to account for this fall. Thompson *et al* used selective screening of high risk patients with isolation appropriate to the site of colonisation in addition to education and emphasis on hand washing to produce a decrease in the prevalence and number of acquisitions of MRSA over a 12-month period (Thompson *et al.*, 1982). Mishal *et al* used active screening with gloves, handwashing and isolation of patients' personal belongings, but not single room placement of carriers, resulting in a significant decrease in MRSA (Mishal *et al.*, 2001). Blumberg and Klugman reduced MRSA bacteraemia using active surveillance, decolonisation and single room isolation in their ICU but also had reductions in another ward with no single rooms using active surveillance and decolonisation, with no cohorting or isolation (Blumberg *et al.*, 1994). In another study, even in the setting of a hospital-

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wide outbreak, single room isolation and cohorting did not reduce the numbers of MRSA cases (Linnemann Jr *et al.*, 1982). This epidemic resolved after the discontinuation of these precautions and remained at a low endemic level for the next 6-7 years, despite no specific infection control measures being used for MRSA control during this time. In another outbreak, active surveillance with contact precautions (strict handwashing but use of gloves, gowns and masks not reported), educational efforts and cohorting failed to control the outbreak, which was only terminated when the handwashing product was changed to hexachlorophene soap (Reboli *et al.*, 1989). Similarly, Zafar *et al* reported failure of aggressive infection control measures including cohorting, gowns, gloves, education and an emphasis on handwashing to control an outbreak in a neonatal unit (Zafar *et al.*, 1995). This was also controlled by changing the hand washing soap to another product. Cox *et al* reported an extensive outbreak of EMRSA-16 that continued to spread to almost all wards of three hospitals despite extensive screening of patients and staff and single room isolation of affected patients (Cox *et al.*, 1995). This outbreak was only terminated after institution of decolonisation of carriers and staff and creation of an isolation ward. Saiman *et al* also reported ongoing MRSA transmission during an outbreak in their neonatal ICU despite active surveillance and contact precautions including gowns, universal glove use, cohorting and mupirocin decolonisation (Saiman *et al.*, 2003). This outbreak was controlled only after the introduction of expanded swabbing sites for screening, environmental cultures, staff surveillance cultures with decolonisation of carriers and cohorting of nurses. Rampling *et al* also reported failure of active screening, single room isolation and barrier nursing, ward closures and education to

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control a 21-month outbreak of MRSA that was terminated only after extensive environmental cleaning (Rampling *et al.*, 2001).

The combination of active surveillance and contact precautions for MRSA colonised and infected patients has been an effective control strategy in many settings, however, it has *not* been universally successful. In areas of zero or low prevalence, it seems to prevent MRSA transmission from colonised patients transferred from other institutions. The minimum level of isolation precautions required to control endemic MRSA cannot be determined because of disparate results from reported studies. Such a measure is not easily amenable to randomisation. In addition, single room isolation may have detrimental effects on patient care (Kirkland *et al.*, 1999; Lewis *et al.*, 1999; Peel *et al.*, 1997) and is not practical in many institutions because of lack of single rooms, need by other patients and inadequate staffing levels. Bartley *et al* reported an increase in the rate of MRSA infections during a hospital-wide outbreak of VRE as a consequence of increased burden on infection control resources (Bartley *et al.*, 2001). Success of isolation measures may occur because their use reinforces other infection control practices. There are two published studies which have shown that compliance with hand washing was significantly greater after caring for patients who were in isolation rooms compared with those who were not (Lai *et al.*, 1998; Kirkland *et al.*, 1999). If its major mode of transmission is from patient-to-patient on the contaminated hands of health-care workers, standard precautions should be adequate to control MRSA. Yet, experience with this strategy has been disappointing, perhaps because of lack of compliance with recommendations or

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because of other possible routes of transmission. Some authors have assessed how adherence to recommended contact precautions may have contributed to their failure in some instances. Pettinger *et al* reported only 41% compliance with isolation precautions by health-care workers, although the main reason for non-compliance was failure to wash hands (Pettinger *et al.*, 1991). Another study found that compliance with MRSA precautions (gowns, gloves and hand hygiene) was only 28%, although if the 35% hand hygiene compliance was excluded, compliance with gowns and gloves was 65% (Afif *et al.*, 2002). Kirkland *et al* reported 90% compliance with gowns and gloves for patients in contact precautions and noted that staff wearing gloves were twice as likely to wash their hands after patient care compared with those who were not (Kirkland *et al.*, 1999). Kim *et al* have demonstrated that glove use but not isolation precautions improved compliance with hand hygiene in health-care workers (Kim *et al.*, 2003). Introduction of stringent surveillance and isolation precautions to an institution unfamiliar with these measures requires major institutional change. Key factors to consider include surveillance and swab processing workforce issues, logistics such as availability of single rooms, psychological and other negative effects of isolation and compliance with recommended precautions.

1.5.3.3 *Gowns and gloves*

There is theoretical evidence that gowns and gloves may prevent contamination of health-care workers' hands and clothes. However, despite numerous situations where their use has been part of a successful multi-faceted MRSA control program (Jernigan *et al.*, 1995), there is no direct evidence they are indispensable and there are several reports of

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successful MRSA containment programs without them (Cosseron-Zerbib *et al.*, 1998; Nettleman *et al.*, 1991). Adequate hand hygiene should be sufficient to remove MRSA from hands, as has been demonstrated in several trials. Gloves have been shown to effectively prevent bacterial contamination of hands and may provide additional benefit in areas where hand hygiene compliance is poor, although this does not obviate the need for hand disinfection (Pittet *et al.*, 1999; Centers for Disease Control and Prevention, 2002). Boyce *et al* has demonstrated that environmental contamination occurred in the rooms of 73% and 69% of MRSA infected and colonised patients respectively (Boyce *et al.*, 1997). In this study, 65% of nurses' gowns and 58% of gloves had been contaminated after routine care of patients with MRSA in wounds or urine and 42% of nurses had contaminated their gloves after touching inanimate objects in the room without actually touching the patient. Theoretically, washing or disinfecting hands prior to all patient contact should be sufficient to prevent spread if hands have been contaminated by any mechanism. The ability of MRSA to be transmitted directly from clothing to patients is not known (Boyce *et al.*, 1997).

1.5.3.4   *Masks*

For masks to be effective in the prevention of transmission of MRSA, one would have to postulate that there is a relationship between the nasal acquisition of MRSA by health-care workers and the subsequent spread to a patient. Lacey *et al* found that wearing masks prevented nasal, throat and hand colonisation with MRSA in health-care workers (Lacey *et al.*, 2001). Although there are some reports of nasally colonised health-care workers being

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responsible for outbreaks of MRSA (Boyce *et al.*, 1993; Sherertz *et al.*, 1996) and *S. aureus* (Belani *et al.*, 1986) during upper respiratory tract infections, the relationship between nasal colonisation and spread to patients has not been quantitated and therefore the exact role of masks is unknown.

1.5.4 Decolonisation of carriers

Over the years, eradication of *S. aureus* colonisation has been attempted with various combinations of systemic and topical antibiotics, with intranasal mupirocin now accepted as the most efficacious (Boyce, 2001). It is more effective than placebo in eliminating nasal carriage in health-care workers (Reagan *et al.*, 1991), in hemodialysis and peritoneal dialysis patients (Boelaert *et al.*, 1989; Mupirocin Study Group, 1996) and in HIV infected patients (Martin *et al.*, 1999).

Decolonisation of MRSA nasal carriers is recommended in the endemic situation to reduce the pool of MRSA, although the evidence for efficacy mainly relies on non-randomised or uncontrolled studies (Talon *et al.*, 1995). In one uncontrolled study, nasal decolonisation and chlorhexidine baths with no other changes in infection control measures were reported to decrease the endemic rate of MRSA bacteraemia (Blumberg *et al.*, 1994). Conversely, when control measures did not include decolonisation, the rate of nosocomial transmission was maintained at low levels in one uncontrolled study (Jernigan *et al.*, 1995) and rates of infection and carriage reduced significantly in two others (Cosseron-Zerbib *et al.*, 1998; Eveillard *et al.*, 2001). In an uncontrolled trial of intra-nasal mupirocin in MRSA carriers

on a digestive diseases unit, Duyperon *et al* demonstrated good initial eradication, but over one quarter became recolonised after the first treatment and over two thirds after the second and third. There was also appearance of high-level mupirocin resistance in several cases after treatment (Dupeyron *et al.*, 2002). Similarly, Paterson *et al* had good eradication rates using intra-nasal mupirocin in liver transplant candidates, but had 37% recolonisation rate and no reduction in *S. aureus* infections compared with an historical control group although no mupirocin resistance was detected (Paterson *et al.*, 2003).

There has only been one published randomised placebo-controlled trial using intranasal mupirocin and chlorhexidine body washes for eradication of endemic MRSA colonisation at multiple body sites. This study found no significant difference in eradication of MRSA at any site, in the rate of MRSA infection or resource utilisation (Harbarth *et al.*, 1999). The authors suggested that "mupirocin should still be used with caution and may be targeted only at patients without chronic extranasal MRSA colonisation".

Widespread and prolonged use of mupirocin to eliminate MRSA colonisation in the long-term care setting has been associated with development of low- and high- level mupirocin resistance (Kauffman *et al.*, 1993). It is thought to arise particularly when applied to every patient, regardless of MRSA carriage status (Miller *et al.*, 1996), or when applied for prolonged periods, particularly to skin conditions (Kauffman *et al.*, 1993; Cookson *et al.*, 1990; Rahman *et al.*, 1987). However, resistance has also emerged in several hospitals where it was only used for the recommended five day course in colonised patients (Dos

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Santos *et al.*, 1996; Vasquez *et al.*, 2000) and not found despite blanket use in all patients for the duration of their ward stay (Mayall *et al.*, 1996).

Thus, recommendations for use of mupirocin in the endemic setting are based mainly on perceived contribution of mupirocin to the control of outbreaks and high eradication rates in particular settings. In the endemic setting, the only randomised placebo-controlled trial showed that eradication was no better in the mupirocin-treated group than in the control group. Several authors have shown control of endemic MRSA without the use of decolonisation. Combined with the risk of development of mupirocin resistance, it would seem reasonable to be cautious about widespread introduction of intranasal mupirocin to reduce endemic MRSA (Cosseron-Zerbib *et al.*, 1998).

In the only study to assess the independent use of antiseptic body washes for elimination of endemic *S. aureus* colonisation, little difference was found between intranasal and wound mupirocin alone and mupirocin accompanied by chlorhexidine body washes, but this was an inadequately powered, non-randomised study (Watanakunakorn *et al.*, 1995).

1.5.5 Prevention of infection in a colonised patient

1.5.5.1 Nasal decolonisation

Because of the strong association of *S. aureus* nasal colonisation with subsequent infection in various settings, it would seem reasonable to assume that eradication of nasal carriage

would prevent infections with *S. aureus*, but randomised-controlled trials do not unequivocally support this contention.

Use of intranasal mupirocin to prevent post-operative infection has been studied in several non-randomised trials, summarised in Table 1-2. Five trials using historical controls have demonstrated favourable responses to mupirocin for some outcomes (Cimochowski *et al.*, 2001; Gernaat-van der Sluis *et al.*, 1998; Kluytmans *et al.*, 1996; Wilcox *et al.*, 2003; Yano *et al.*, 2000). The two major randomised double-blind placebo-controlled trials, however, did not confirm these results, aside from a reduction in nosocomial *S. aureus* infections in *S. aureus* carriers in the study by Perl *et al* (Kalmeijer *et al.*, 2002; Perl *et al.*, 2002). Both of these trials had a lower rate of surgical site infection (SSI) in the placebo arm than anticipated, possibly reducing their power to detect a difference between the two groups. However, the other explanation for the lack of difference is that there really *was* no difference and that other factors may operate in the relationship between nasal carriage and SSI. Kalmeijer *et al* suggest that the intensive surveillance associated with the trial may be an explanation for the lower than expected rate of SSI in the control group and that this should be taken into account when determining sample size for these types of studies (Kalmeijer *et al.*, 2002). Another randomised trial in abdominal surgery failed to show a reduction in SSI, but did show a reduction in post-operative pneumonia (Suzuki *et al.*, 2003). This trial, however, had small numbers and other potential methodological flaws.

In summary, although the trials using historical controls suggest a benefit from use of pre-operative mupirocin to prevent SSI, randomised-controlled trials do not support these conclusions. Before recommending widespread use of mupirocin, further properly powered studies are required in different surgical disciplines and with larger numbers of *S. aureus* colonised patients.

In one study where all ICU patients received intranasal mupirocin, the treated group had significantly fewer *S. aureus* infections compared with historical controls (Talon *et al.*, 1995). However, in another study where *S. aureus* nasal carriers were treated with intranasal mupirocin and compared with an untreated historical control group, there was no reduction in the number of *S. aureus* infections, although the power of the study was not indicated (Brun-Buisson *et al.*, 1994). A randomised-controlled trial has recently been published where intranasal mupirocin was shown not to reduce non-surgical *S. aureus* infections, mortality or duration of hospitalisation compared with placebo (Wertheim *et al.*, 2004).

Failure to prevent surgical and non-surgical infections with intranasal mupirocin raises the question of the importance of non-nasal carriage sites. There have now been several studies published where eradication of gastro-intestinal MRSA carriage has reduced MRSA infection, although only one has been randomised. The randomised, placebo-controlled trial assessed selective decontamination of the digestive tract (SDD) with and without enteral and intranasal mupirocin (Nardi *et al.*, 2001). The mupirocin group had a

reduced rate of all pneumonia and pneumonia caused by *S. aureus* and of *S. aureus* isolation from tracheobronchial aspirates, but no reduction in the proportion of MRSA isolated. This study, however, excluded a large number of patients after randomisation and was not analysed on an intention-to-treat basis. In one of the historically controlled studies, enteral vancomycin was used to control an MRSA outbreak (Silvestri *et al.*, 2002) and in the other it was given to control MRSA in the endemic setting with a reduction in proportion of patients with MRSA in diagnostic samples from 31% to 2% (de la Cal *et al.*, 2004). Both of these studies did not document an increase in VRE or VISA, although this clearly remains a concern.

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Table 1-2 Use of mupirocin to prevent surgical site infection (SSI)

Study	Description of study	Subjects	Treatment arms	Surgical Site Infection
Kluytmans et al., 1996	Unblinded intervention trial with historical controls	Consecutive patients undergoing cardiothoracic surgery	Historical controls: no treatment Active group: intranasal mupirocin Concurrent controls: unintentionally no treatment	Significant reduction in overall SSI rate in active group Similar proportion of SSI caused by S. aureus (37.5 vs. 39.7%)
Cimochowski et al., 2001	Prospective cohort study	Patients undergoing cardiothoracic surgery	Group 1: no treatment Group 2: intranasal mupirocin	Significant reduction in overall SSI rate in active group Proportion of wound infections caused by S. aureus greater in active group (57.1 vs. 36.7%)
Yano et al., 2000	Unblinded intervention trial with historical controls	Patients undergoing upper gastrointestinal surgery	Historical controls: no treatment Active group: intranasal mupirocin	No significant difference in proportion of patients with SSI. Significant reduction in SSI caused by all S. aureus (0.71 vs 11.7%) & MRSA (0 vs 7%)

Gernaat-van der Sluis et al., 1998	Unblinded intervention trial with historical controls	Patients undergoing orthopaedic surgery	Historical controls: no treatment Active group: intranasal mupirocin	Significant reduction in all SSI (1.3 vs 2.7%). No significant reduction in SSI caused by S. aureus (0.6 vs 1.1%)
Wilcox et al., 2003	Unblinded intervention trial with historical controls	Orthopaedic surgery including insertion of metal prosthesis and/or fixation	Historical controls: no treatment Active group: intranasal mupirocin	Significant reduction in MRSA SSI (23/1000 operations vs 3.3/1000 operations)
Perl et al., 2002	Randomised, double-blind, placebo-controlled trial	Elective cardiothoracic, general, oncologic, gynecologic, neuro- surgery	Placebo group Active group: intranasal mupirocin	No significant reduction in: - rates of all nosocomial infections or those caused by S. aureus, - all SSI or those caused by S. aureus Significant reduction in all nosocomial infections caused by S. aureus in nasal carriers (4 vs 7.7%)

Kalmeijer et al., 2002	Randomised, double-blind, placebo-controlled trial	Elective orthopaedic surgery with implantation of prothetic material	Placebo group Active group: intranasal mupirocin	No significant reduction in overall SSI rate, S. aureus SSI or SSI caused by patient's endogenous S. aureus
Suzuki et al., 2003	Randomised trial	Abdominal digestive surgery	Control group: no placebo Active group: intranasal mupirocin	Significant reduction in pneumonia (0/202 vs 5/159) No significant reduction in SSI, but methodological flaws

Abbreviations: SSI – surgical site infection, *S. aureus* – *Staphylococcus aureus*, vs – versus

Table 1-3 summarises several trials that demonstrate successful use of mupirocin for prevention of *S. aureus* infections in dialysis patients (Boelaert *et al.*, 1989; Kluytmans *et al.*, 1996; Mupirocin Study Group, 1996). Such widespread use of mupirocin runs the risk of development of resistance and this has been reported by one group who used topical mupirocin for nasal and peri-peritoneal dialysis catheter *S. aureus* carriers (Pérez-Fontán *et al.*, 2002)

Table 1-3 Use of mupirocin in dialysis patients to prevent *S. aureus* infections

Study	Description of study	Subjects	Treatment arms	Surgical Site Infection
Kluytmans *et al.*, 1996	Unblinded intervention trial with historical controls	Patients on hemodialysis	Historical controls: no treatment Active group: intranasal mupirocin for 5 days then weekly for *S. aureus* nasal carriers	Significant decrease in bacteraemia rate (0.04 vs 0.25 per patient year of hemodialysis)
Boelaert *et al.*, 1989	Randomised, double-blind, placebo-controlled trial	Patients on hemodialysis with *S. aureus* nasal carriage	Placebo group Active group: intranasal mupirocin for 2 weeks then 3 times weekly	Significant decrease in *S. aureus* infections (1/104 patient-months vs 6/147 patient-months)
Mupirocin Study Group, 1996	Randomised, double-blind, placebo-controlled trial	Patients on continuous ambulatory peritoneal dialysis with *S. aureus* nasal carriage	Placebo group Active group: intranasal mupirocin for 5 days every 4 weeks	Significant decrease in *S. aureus* exit-site infections (1 in 99.3 vs 1 in 28.1 patient-months) No significant decrease in all exit-site infections, tunnel infections or peritonitis

In conclusion, there is reasonable evidence for the use of mupirocin decolonisation to prevent *S. aureus* infections in renal patients, although this requires long term, repeated

courses with a risk of development of resistance. There is weaker evidence for its use in the ICU setting and for prevention of infection in surgery and other settings. This is one area that is amenable to the performance of randomised-controlled trials and until these have been performed adequately, introduction of widespread use of mupirocin in these settings should proceed with caution.

1.5.6 Hand hygiene

Hand hygiene is considered to be the cornerstone in preventing transmission of nosocomial infections. Despite evidence dating back from the days of Semmelweis in the mid-1800s, compliance with hand washing has been notoriously low in most published reports, usually less than 50% (Brown *et al.*, 2003; Earl *et al.*, 2001; Harbarth *et al.*, 2002; Maury *et al.*, 2000; Pittet, 2000). Independent factors associated with poor compliance include being a physician or nursing assistant, working on a weekday, working in the ICU, undertaking procedures carrying a high risk for contamination and high patient care intensity (Pittet *et al.*, 1999). Other reasons include skin irritation, poorly accessible agents, higher priority for patient care, insufficient time, wearing of gloves, high workload, forgetfulness, lack of knowledge of guidelines, lack of role models and lack of institutional priority or safety climate (Boyce, 1999; Pittet, 2000). Voss and Widmer calculated that 100% compliance with hand washing would consume 16 hours of nursing time in their ICU or 17% of the total workforce and they concluded that it was unrealistic to expect full compliance with this measure (Voss *et al.*, 1997).

Many methods have been used to improve hand washing levels (Pittet, 2000), including education and monitoring compliance with feedback of results (Dubbert *et al.*, 1990; Rosenthal *et al.*, 2003) and empowerment of patients to ask health-care workers to wash their hands (McGuckin *et al.*, 1999). Many of these studies have only been associated with short-term improvements or have not studied compliance for extended durations. One study found minimal impact on long-term hand washing compliance despite an intensive program of feedback, education and increased sink automation (Larson *et al.*, 1997). In another study, Larson *et al* increased soap distribution (as a surrogate marker of hand hygiene) but failed to decrease MRSA rates significantly (Larson *et al.*, 2000).

One of the most important advances in hand hygiene has been the widespread introduction of waterless, alcohol-based hand disinfectants. These have been used for many years in Europe (Harbarth, 2002; Ojajarvi, 2003) and have more recently been recommended by authorities in the United States (Centers for Disease Control and Prevention, 2002) and United Kingdom (Teare *et al.*, 2001). These products contain a variable amount of alcohol and an emollient, with or without chlorhexidine and may be formulated as a rub or gel. Because they do not require water, they can be available at the bedside or in pocket-sized bottles carried by staff. They are quick to use and overcome the problem of lack of sinks or paucity of time to access one (Teare *et al.*, 2001). In addition, use of an alcohol-based hand disinfectant has been associated with less skin irritation than traditional soaps (Boyce *et al.*, 2000; Larson *et al.*, 2001; Mulberry *et al.*, 2001). They are recommended in all

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situations for hand hygiene except if hands are visibly soiled, where standard washing should be used.

Voss and Widmer found that full compliance with bedside use of an alcohol-based disinfectant would require only 2.7 hours per shift or <3% of the workforce, compared with 16 hours for traditional handwashing (Voss *et al.*, 1997). Several authors have shown that availability of these products in conjunction with promotional activities and education significantly improves compliance with hand hygiene (Bischoff *et al.*, 2000; Earl *et al.*, 2001; Hugonnet *et al.*, 2002; Maury *et al.*, 2000; Mody *et al.*, 2003; Pittet *et al.*, 2000). In a study in a paediatric hospital, although hand hygiene compliance improved significantly with the introduction of an alcohol hand rub, the final compliance level was only 30% (Harbarth *et al.*, 2002). Muto *et al* showed a failure to improve hand hygiene compliance with installation of alcohol rub dispensers and an educational campaign, although this study did not report whether it had an adequate sample size to detect a difference between the two groups (Muto *et al.*, 2000).

Alcohols are rapidly germicidal for Gram-positive and Gram-negative organisms, including MRSA and the most effective concentration is between 60 and 95%. Alcohol based products are at least as effective or more effective than plain or antimicrobial soaps for standard hand hygiene or preoperative hand disinfection (Centers for Disease Control and Prevention, 2002; Lucet *et al.*, 2002; Mulberry *et al.*, 2001; Parienti *et al.*, 2002; Zaragoza *et al.*, 1999). In a randomised-controlled trial, Girou *et al* showed that

handrubbing with an alcohol based product was significantly more effective in reducing bacterial hand contamination than handwashing with 4% chlorhexidine soap during routine patient care (Girou *et al.*, 2002). In a small, non-randomised study, Thakerar and Goodbourn found that an alcohol based hand rub was effective in removing MRSA from hands in 22 of 25 staff with MRSA hand contamination (Thakerar *et al.*, 2002). This effect on reducing hand contamination has also translated into reduced rates of MRSA transmission in several studies. Pittet *et al* showed that an increase in hand hygiene compliance by the use of an alcohol-chlorhexidine hand rub in conjunction with an educational campaign and strict infection control measures (including active surveillance and contact precautions) for MRSA colonised patients could produce a sustained reduction in MRSA transmission and other nosocomial infections over several years (Pittet *et al.*, 2000). Gopal Rao *et al* also showed a sustained reduction in MRSA acquisition after introduction of an alcohol hand gel with a promotional campaign (Gopal Rao *et al.*, 2002).

Although there is little contention that waterless alcohol-based disinfectants are now the hand hygiene product of choice, there still remains some controversy regarding choice of product. At equivalent concentrations, isopropanol is more effective than ethanol. Two groups have found that none of the gel based products that they tested met with European standards for antimicrobial efficacy, whereas all hand rubs did (Dharan *et al.*, 2003; Kramer *et al.*, 2002). In contrast, Kampf *et al* have found their gel based product met the European standards, perhaps because of a higher ethanol concentration (Kampf *et al.*, 2002). It is important to note, however, that these were *in vitro* studies using stringent but

arbitrary criteria for efficacy and it is not clear whether these findings are clinically relevant (McDonald, 2003). Although the gels did not meet the criteria, they still produced a several fold log reduction in bacterial counts and it is also not known what log reduction in bacterial count on staff hands is required to prevent cross-transmission in the clinical setting (Boyce *et al.*, 2002; Diekema, 2002). The success of waterless alcohol-based products may lie in their ability to improve compliance with hand hygiene, rather than only an increase in efficacy at reducing bacterial counts compared with hand washing. Although there have been no direct comparisons of the acceptability of gels versus rubs, some authors feel that staff prefer gels (McDonald, 2003; Boyce *et al.*, 2002; Diekema, 2002; Girard *et al.*, 2002; Hoffman *et al.*, 2002). One investigator used a gel based product to achieve a reduction in nosocomial MRSA acquisition (Gopal Rao *et al.*, 2002).

1.5.7 Antibiotic restriction

Although there is ample evidence that levels of MRSA are correlated with antibiotic usage, the evidence for success of antibiotic restriction in reducing MRSA is limited. Several studies have reported a decrease in MRSA temporally associated with antibiotic restriction (Frank *et al.*, 1997; Landman *et al.*, 1999; Smith, 1999; Stone *et al.*, 1998). However, some are confounded by factors such as failure to take into account year-to-year variability of MRSA rates. In addition, rather than reporting rates of methicillin resistance in isolates, some report clinical infection rates, which may be susceptible to the effect of concurrent infection control measures or confounders, such as length of stay (McGowan Jr, 1994; Phillips, 2001; Rice, 1999). It is interesting to note that five years after one of these

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reports, "the prevalence of MRSA had probably increased" at that institution (Farr et al., 2001).

1.5.8 Feedback

Feedback is not in itself a method of MRSA control, but is a tool which can be used to facilitate other control measures. Feedback involves the systematic delivery of MRSA rates to health-care providers as an indication of the effectiveness of infection control measures. Its use may encourage evaluation of infection control procedures, allows health-care providers to take responsibility for MRSA in patients under their care and allows early detection of outbreaks. Nettleman et al reported the results of a campaign where feedback of cases to the resident physician responsible for the patient was used as part of a campaign to control MRSA (Nettleman et al., 1991). Rates of MRSA were significantly reduced in the study period. However, the campaign included other aspects, including educational presentations, culturing of staff hands and encouragement of hand washing. Sheridan et al also used feedback in control of MRSA at a paediatric burns facility, but once again, this was part of a multi-faceted campaign (Sheridan et al., 1994). Curran et al have used annotated statistical process control charts as a means of concentrating infection control procedures in their hospital with sustained reductions in MRSA rates (Curran et al., 2002). These charts give an expected value of MRSA cases with control limits set at two and three standard deviations above or below the centre line, above which action can be taken to enhance infection control interventions.

1.6 Conclusion

MRSA has become a problem of increasing magnitude in most areas of the world over the last 40 years and is now established as one of the most significant nosocomial pathogens. There are many compelling reasons why it should be vigorously pursued. Rates of MRSA drive the use of vancomycin, which is associated with VRE and the more recent appearance of VISA. The few isolates of VRSA that have been found have contained the *van*A resistance gene from VRE. The implication is that unless we can control MRSA, we have little chance of controlling VISA, VRSA and possibly VRE.

Although there are many published studies on MRSA epidemiology and containment, there still remain many unanswered questions regarding its transmission dynamics and control. It is thought that colonised and infected patients constitute the major reservoir and that person-to-person spread on the contaminated hands of health-care workers is the predominant mode of transmission, yet the role of the environment, fomites and airborne spread is still not fully understood. This impacts on the recommended control measures. Although there are many reports of successful control of MRSA using active surveillance and contact precautions, the *minimum* effective measures required is not conclusively known. Analysis of the literature is hampered by the heterogeneity of studies, including different geographical settings, type of hospitals or ward, whether the study was conducted during an epidemic or in an endemic setting and often the methodological quality of studies has been poor. It is difficult to state whether these results can be generalised to all settings. One of the other major issues when assessing the effectiveness of infection

control interventions, is that in both the endemic and epidemic setting, usually multiple interventions are introduced at once, making the determination of which measures are most important usually impossible, although this has not prevented many authors from making such claims.

From this literature review, it can be seen that many, if not most, of the measures we currently use to contain MRSA do not have a methodologically sound evidence base. This leads to the conclusion that further studies are needed to improve the level of evidence on which we base our practice. Therefore, the major objective of this thesis was to rectify some of these deficiencies. Control of any pathogen requires local knowledge of its prevalence and transmission dynamics. As an initial step, the extent of the problem must be determined. Many studies have been performed in different countries and different settings which may not be generalisable to other locations because of different patient populations, hospital structures, control measures and antibiotic usage patterns. Once the extent of the problem is determined, an appropriate intervention may be introduced. Thus the initial step in this work was to generate local, quantitative data regarding MRSA levels.

Much of the literature regarding control of MRSA is based on control of outbreaks in areas where there is little or no background MRSA, using multiple simultaneous interventions. The *minimum* measures required for the containment of endemic MRSA have not been established. Although there is unlikely to be one solution that fits all situations, knowledge of the most important components would allow concentration on these areas,

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rather than implementing multiple measures that may be costly, labour intensive, impractical and unsustainable. The next aim of this work was therefore to implement an intervention to reduce MRSA that did not involve the many facets of other reported studies and did not require the levels of labour and monetary resources involved in some of these other studies, as described in Chapter 2.

Subtyping of MRSA may be performed using multiple different techniques, all of which have advantages and disadvantages. PFGE is widely accepted as the "gold standard", yet it is labour intensive, time-consuming, has a high turnaround time and requires a certain level of technical expertise for performance and analysis of banding patterns. Other methods have been compared, but none has been found to be as useful or accessible, despite its limitations. Clearly, a method requiring less labour input would be a major advantage. From a practical point of view, the RiboPrinter® would provide a solution to all of these issues because it is fully automated, has a turnaround time of one working day and provides a computer generated comparison of banding patterns. The study described in Chapter 3 of this thesis provides a comparison of PFGE and the RiboPrinter® with regard to discriminatory ability in order to determine whether RiboPrinting may be a viable alternative to PFGE for subtyping MRSA.

Many studies have been performed to determine risk factors for MRSA acquisition, although these have used different methodologies and have studied different populations. Chapters 5 and 6 of this thesis describe two cohort studies which were performed to

examine associations with MRSA acquisition in trauma patients, a group which was identified in this work as being at particular risk of becoming MRSA colonised.

The impetus for this thesis was that MRSA was believed to be a major problem in the intensive care unit and it was thought that a greater understanding of its epidemiology in this environment would lead to better control, preferably with relatively simple measures. Thus, the specific aims of this work were:

- To describe the extent of MRSA in the ICU by determining the prevalence of MRSA colonisation on admission and the incidence of MRSA acquisition in the ICU

- To determine whether demographic characteristics (such as previous ward, medical unit, gender, age and length of stay) were associated with MRSA colonisation on admission and whilst in the ICU

- To determine whether the RiboPrinter® was as useful as PFGE for subtyping of endemic MRSA isolates with regard to practical issues and discriminatory ability

- To determine whether MRSA acquisition in the ICU could be reduced by the introduction of a waterless, alcohol-based hand gel, a feedback campaign and an alert sign for colonised patients

- To determine whether certain patient characteristics were associated with MRSA acquisition in trauma patients, including mechanism of injury, procedures, surgery and antibiotics received

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2 CHAPTER 2. ACQUISITION OF MRSA IN THE ALFRED HOSPITAL INTENSIVE CARE UNIT

2.1 Introduction

Between 1991 and July 2001, at the Alfred Hospital in Melbourne, 60-75% of nosocomial and 50-100% of all intensive care unit (ICU) *S. aureus* blood culture isolates were resistant to methicillin. Systematic screening for MRSA had not been performed for approximately ten years and at that time, no denominator data had been collected to determine rates or proportions of patients who had acquired MRSA. Based on blood culture results, it was clear that there was a problem with MRSA, but its magnitude was not known. The major objective of this study was to document the extent of MRSA in the Alfred Hospital ICU. This was achieved by patient screening to determine the prevalence of MRSA on admission and as well as the incidence of colonisation in the ICU. The rationale for performing such a study was to generate local data on which a subsequent intervention could be based in order to decrease MRSA transmission.

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2.2 Aims

The aims of this study were to determine:

- The prevalence of MRSA colonisation on admission to the ICU

- Risk factors for MRSA colonisation on admission to the ICU

- The incidence of MRSA acquisition in the ICU

- Risk factors for acquisition of MRSA colonisation in the ICU

- The proportion of patients who were newly colonised with MRSA who developed MRSA infection

2.3 Methods

2.3.1 Setting

The Alfred Hospital is a 350 bed acute tertiary referral hospital. It is the major centre for trauma, burns, human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), hyperbaric medicine, cystic fibrosis and heart-lung transplantation in the state of Victoria. Approximately 700 multiply injured patients with an Injury Severity Score (ISS) of >15 are admitted annually. There are no paediatric or obstetric facilities. The 35 bed ICU is a combined medical and surgical unit with large numbers of trauma and cardiothoracic surgery patients. The patients are housed in one- or two- bed cubicles. They are cared for medically in three sections (trauma, cardiothoracic and general),

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although these are not physically separated. Standard infection control precautions (Garner, 1996) are used throughout, regardless of MRSA status. MRSA colonised patients are not specifically cared for in single rooms, although most of the cubicles are single. Gowns, gloves and masks are not routinely used unless indicated to fulfil standard precautions. Hand washing with antiseptic soap was the major method of hand hygiene used during this study, with Hibiclens® available for bedside hand disinfection.

This study was conducted from 31/7/00 to 20/5/01. During that period there was an average of 180 patients admitted to the ICU per month. Patients may have been admitted to the ICU more than once during the surveillance period and each admission was counted separately.

2.3.2 Study design and population

This study was a prospective cohort study. Patients who were admitted to the intensive care unit were screened for MRSA on admission and discharge using nose, throat, groin and axilla swabs. During the first 7½ months of the surveillance period, it was the responsibility of the nurse caring for the patient to take the screening swabs. For the remainder of the study, a nurse was employed to supervise the screening. She was responsible for taking the swabs herself or for encouraging the nurse directly caring for the patient to take swabs in her absence and for educating the nurses regarding the project and in the technique of swab taking. During the first period, methods to improve compliance with swabbing included oral presentations at the ICU nurses' ward meetings, brightly

coloured reminder signs in all patient cubicles, microbiology request slips filled out and signed by a medical officer in advance, assembly of four swabs and a signed request slip in specimen collection bags placed at convenient locations around the ward, informal discussion and meetings with unit managers and other nurses and reminder stickers placed on all patient care plans for admission and discharge swabs. These measures were introduced at various times and their effectiveness was unable to be assessed in this study.

2.3.3 Bacteriologic methods

Swabs were processed using selective mannitol-salt agar with 5mg/l methicillin (MS5 plates). Plates were read at 48 hours and colonies that turned the indicator dye from pink to yellow were subcultured on horse-blood agar plates and read at 24 hours. Colonies were then tested using a latex agglutination test (Pastorex Staph-Plus, BIO-RAD) and if the results were inconclusive, a coagulase test was performed. Susceptibility testing was performed using disk diffusion. Susceptibility testing was performed for penicillin, methicillin, oxacillin, erythromycin, tetracycline, rifampicin, fusidic acid, ciprofloxacin, trimethoprim and chloramphenicol.

2.3.4 Patient data

Patient data collected included age, gender, date of admission to hospital, dates of admission and discharge from the ICU and hospital wards in which the patient had resided prior to ICU admission. Follow up was for the duration of hospital stay. There was no active post-discharge surveillance, but if the patient was readmitted to the Alfred Hospital

with an MRSA infection related to the original admission reason, this was included in the analysis.

2.3.5 Definitions

2.3.5.1   *New colonisation and at risk patients*

Patients were said to be *at risk* of newly acquiring MRSA if both admission and discharge swabs were taken and MRSA was not isolated from the admission swab. If a patient had both admission and discharge screening performed and MRSA was not isolated from the admission swab but was isolated from the discharge swabs, the patient was said to have *acquired MRSA* or to be *newly colonised with MRSA*.

2.3.5.2   *Incidence of new MRSA colonisations*

This was calculated using the following formula:

(Number of new MRSA colonisations / Number of at risk patients) x 100

2.3.5.3   *Incidence of MRSA infection in colonised patients*

If a patient became newly colonised with MRSA during their ICU stay, their medical record was reviewed to determine whether MRSA infection was present using the Centers for Disease Control/National Nosocomial Infection Surveillance Systems (CDC/NNIS) criteria (Garner *et al.*, 1988) with MRSA isolated from a relevant specimen.

The incidence of MRSA infections in MRSA colonised patients was calculated using the following formula:

(Number of MRSA infections / Number of newly MRSA colonised patients) x 100

2.3.6 Statistical analysis

Proportions were compared using a Pearson χ^2 test. Risk factors for MRSA colonisation were analysed using univariate and multivariate logistic regression. Any conclusions of statistical significance were based on a p-value of <0.05. Analyses were performed using Stata software (Stata Corp, College Station, TX).

2.4   Results

2.4.1   Screening

There were 1662 admission to the ICU during the surveillance period. A total of 1328 (79.9%) patients had at least one set of swabs taken, that is, admission or discharge swabs or both. The mean age of the study patients was 57 years (range 12-97 years) and 887 (67%) were male. The mean length of stay (LOS) in ICU was 5.3 days (median 2, range <1-90 days) and the mean length of stay in the hospital prior to ICU admission was 3.9 days (median <1, range <1-224 days). Characteristics of the study patients are shown in Table 2-1.

Table 2-1 Characteristics of the study patients that had swabs taken

Patient characteristic	One swab only taken* (number = 732)	Both admission & discharge swabs taken (number = 596)	Total (number = 1328)
Hospital unit			
Trauma	161 (22.0%)	104 (17.5%)	265 (20.0%)
Cardiothoracic surgery	207 (28.3%)	229 (38.4%)	436 (32.8%)
Other	364 (49.7%)	263 (44.1%)	627 (47.2%)
Length of ICU stay			
≤1 day	259 (35.4%)	234 (39.3%)	493 (37.1%)
2–7 days	318 (43.4%)	245 (41.1%)	563 (42.4%)
>7 days	155 (21.2%)	117 (19.6%)	272 (20.5%)
Age group (years)			
<50	241 (32.9%)	180 (30.2%)	421 (31.7%)
50–70	256 (35.0%)	234 (39.3%)	490 (36.9%)
>70	235 (32.1%)	182 (30.5%)	417 (31.4%)
Gender			
Male	494 (67.5%)	393 (65.9%)	887 (66.8%)
Female	238 (32.5%)	203 (34.1%)	441 (33.2%)

*Only admission or discharge swab taken; used as comparison group for patients who had both swabs taken because accurate risk factor data were available for this group.

Table 2-2 shows the proportions of patients from whom admission, discharge or both swabs were taken, before and during the employment of the dedicated nurse. There was a significant difference in the numbers of patients from whom swabs were taken between the

two time periods, except for the proportion from whom only discharge swabs were taken (p-value =0.74).

Table 2-2 Swabs taken during the study period

Swabs taken	Prior to dedicated nurse (7½ months)	Dedicated nurse* (2 months)	Total	P-value
Admission Only	549 (40.6%)	40 (13.0%)	589 (35.4%)	<0.01
Admission & Discharge	358 (26.4%)	238 (77.3%)	596 (35.9%)	<0.001
Discharge Only	115 (8.5%)	28 (9.1%)	143 (8.6%)	0.74
None	332 (24.5%)	2 (0.6%)	334 (20.1%)	<0.01
Total Number of Patients Admitted to ICU	1354 (100%)	308 (100%)	1662 (100%)	

*Dedicated nurse refers to nurse employed to be responsible for swab taking.

2.4.2 MRSA isolation from swabs

Table 2-3 shows the proportion of swabs from which MRSA was isolated prior to and during employment of the dedicated nurse. Overall, MRSA was isolated from 6.8% of patients who had an admission swab taken with 11.4% of patients in the ICU acquiring MRSA. There was a significant difference in the proportion of swabs where MRSA was isolated between the periods where a dedicated nurse was and was not employed. Even after adjusting for length of stay in the ICU in a logistic regression, acquisition of MRSA

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patients had only nose, throat and groin, but not axilla swabs taken, 4/195 (2.1%) of positives would have been missed. If patients had only nose and throat swabs taken, 40/195 (20.5%) of positives would have been missed and if only nose swabs were taken, 60/195 (30.8%) would have been missed.

Table 2-4 Site of MRSA isolation

Site of swab	Number of swabs positive at this site	Percentage of all patients with MRSA positive swabs	Number of swabs positive only at this site	Percentage of all patients with MRSA positive swabs
Nose	125	64.1	21	10.8
Throat	113	58.0	24	12.3
Groin	105	53.9	32	16.4
Axilla	40	20.5	4	2.1
Total	195*	*	195*	*

*Numbers do not add to the total because patients may have had greater than one site positive

2.4.4 MRSA colonisation on admission to ICU

Overall, 156/1328 (11.8%) patients were found to be MRSA colonised at some time during their ICU stay. Of these, 80 (51.3%) were colonised on admission, 63 (40.4%) were newly colonised during their ICU stay and 13 (8.3%) had only discharge swabs taken, therefore, it was impossible to determine when MRSA was acquired in this last group. Patients may

have been on more than one ward, including the ICU, prior to their current ICU admission. As patients were not screened on wards other than the ICU, it was not possible to determine on which particular ward a patient may have become MRSA colonised.

Figure 2-1 shows the proportion of patients (with 95%CI) coming from various wards who were MRSA colonised on admission to ICU. 3.7% (95%CI 2.4-5.3%) of patients who had not been on another ward (that is, either admitted to the ICU from the emergency department or directly transferred from another hospital) were MRSA colonised on admission to ICU and only 1.2% (95%CI 0.1-4.6) of those on the cardiothoracic surgery ward were. In contrast, 36.1% (95%CI 25.9-47.4%) and 43.8% (95%CI 26.4-62.3%) who had previously stayed in the ICU or on the trauma/orthopaedics ward respectively were MRSA colonised at ICU admission.
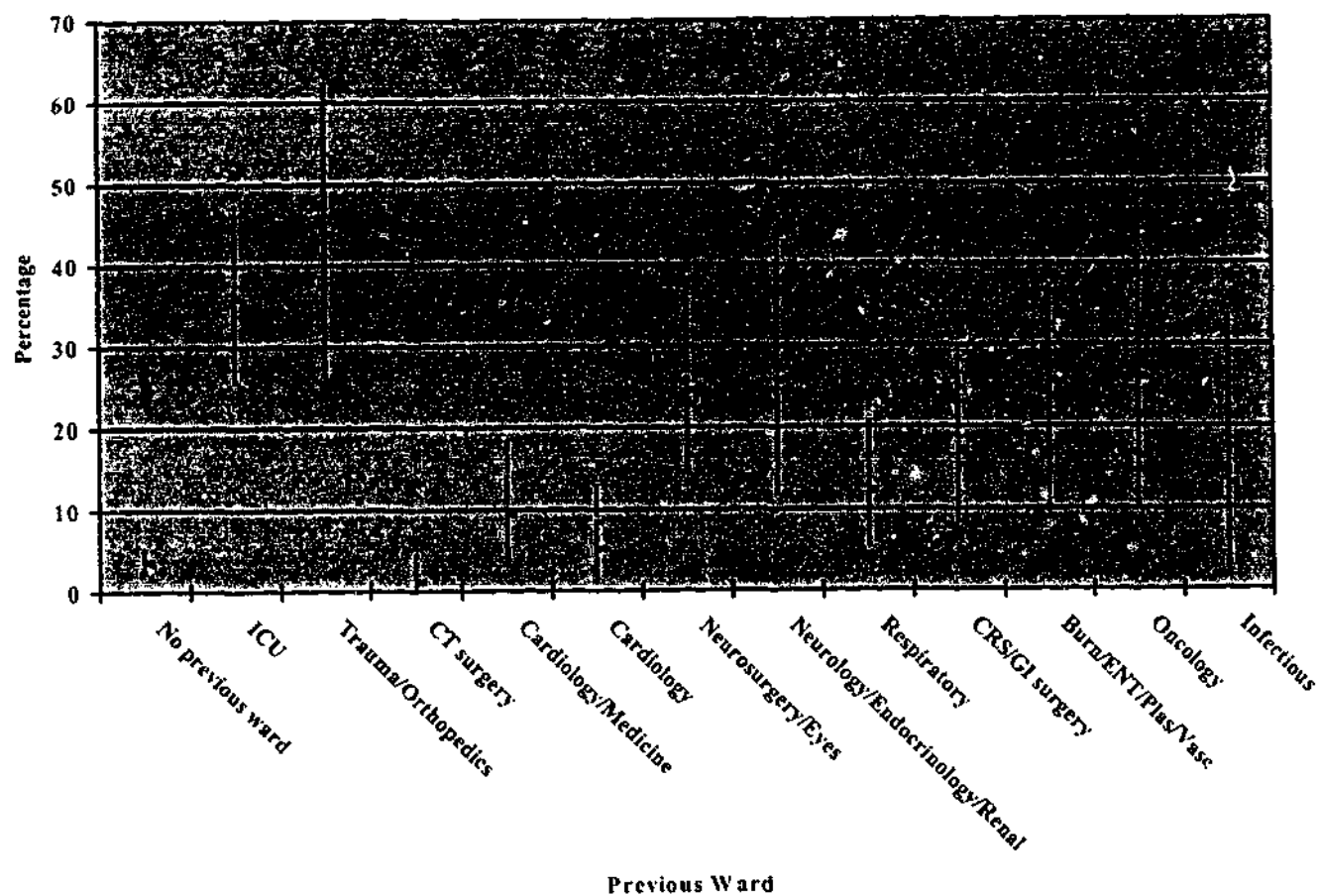
Horizontal line represents actual proportion and vertical line represents 95% confidence interval

Abbreviations: ICU-intensive care unit, CT-cardiothoracic, CRS-colorectal surgery,

GI-gastrointestinal, ENT-ear, nose and throat surgery, Plas-plastic surgery, Vasc-vascular surgery,

Infectious-infectious diseases

Figure 2-1 Proportion of patients on wards who were MRSA colonised on admission to ICU

The crude and adjusted odds ratios for MRSA colonisation at admission to the ICU are

listed in Table 2-5. On multivariate analysis with backward elimination of terms that had a

p-value >0.05, the variables that remained risk factors were having a previous admission to

the ICU (OR 3.3, 95%CI 1.7-6.6), the trauma/ orthopaedics ward (OR 2.9, 95%CI 1.2-7.2),

or the neurology/endocrinology/ rheumatology/renal ward (OR 2.6, 95%CI 1.0-6.9) and a

length of stay of more than three days prior to admission to the ICU (OR 8.6, 95%CI 4.4-

16.9). A previous admission to the cardiothoracic surgery ward (OR 0.1, 95%CI 0.02-0.4)

or to the cardiology/general medicine ward (OR 0.4, 95%CI 0.2-0.9) was protective

against having MRSA colonisation on admission to the ICU.

Table 2-5 Risk Factors for MRSA Colonisation on Admission to ICU

Risk factor	Not MRSA Colonised (Number)	MRSA Colonised (Number)	OR	95% CI	Adjusted OR†	95% CI
Wards prior to ICU*						
Hematol/Onc/Derm/Med	17	6	5.2	2.0-13.6	-	-
Infectious Diseases	20	3	2.1	0.6-7.3	-	-
Burns/Plas/Vasc/ENT/BES	30	8	4.0	1.8-9.0	-	-
CRS/CT Surg/Gastro/Urol	48	10	3.2	1.5-6.5	-	-
Respiratory/ACI	57	8	2.0	0.9-4.4	-	-
Cardiology/Med	67	7	1.5	0.7-3.4	0.4	0.2-0.9
Neurosurg/Gen Surg/Ophth	46	15	5.3	2.8-10.1	-	-
CCU/Cardiology	72	4	0.8	0.3-2.1	-	-
CT Surgery	152	2	0.2	0.04-0.7	0.1	0.02-0.4
Neurol/Endo/Rheum /Renal	23	8	5.2	2.3-12.1	2.6	1.0-6.9
Trauma/Orthopedics	18	14	12.8	6.1-26.9	2.9	1.2-7.2
ICU	53	29	11.28	6.6-19.2	3.3	1.7-6.6
No Previous Ward	465	15	0.3	0.2-0.6	-	-
Length of stay prior to ICU						
<1 day	645	17	1	-	1	-
1-3 days	274	8	1.1	0.5-2.6	1.3	0.6-3.1
>3 days	186	55	11.2	6.4-19.8	8.6	4.4-16.9
Gender						
Female	368	28	1	-	-	-
Male	737	52	0.9	0.6-1.5	-	-

in the ICU appeared more likely during the employment of the dedicated nurse (odds ratio [OR] = 1.9, 95% confidence interval [95% CI] = 1.1–3.5, p-value = 0.04).

Table 2-3 Proportion of patients at risk from whom MRSA was isolated

	Pre-dedicated nurse (7½ months)	Dedicated nurse (2 months)	Total	P-value
On Admission *	54/907 (6.0%)	26/278 (9.4%)	80/1185 (6.8%)	<0.05
	(CI 4.4-7.5%)	(CI 5.9-12.8%)	(CI 5.3-8.2%)	
At Discharge **	58/473 (12.3%)	57/266 (21.4%)	115/739 (15.6%)	<0.01
	(CI 9.3-15.2%)	(CI 16.5-26.4%)	(CI 13.0-18.2%)	
MRSA Acquired in ICU ***	30/335 (9.0%)	33/219 (15.1%)	63/554 (11.4%)	0.03
	(CI 5.9-12.0%)	(CI 10.3-19.8%)	(CI 8.7-14.0%)	
Total	907	278	1185	

*Proportion of total number of admission swabs where MRSA was isolated

** Proportion of total number of discharge swabs where MRSA was isolated

***Proportion of patients where both admission & discharge swabs were taken, MRSA was not isolated on admission swab and was isolated on discharge swab

CI = 95% confidence interval

2.4.3 Site of MRSA isolation

Table 2-4 shows the proportion of all MRSA positive swabs (admission and discharge) that were positive for MRSA at the four screening sites (nose, throat, groin and axilla). If

Age (years)						
<50	353	24	1	-	-	-
50-70	408	28	1.0	0.6-1.8	-	-
>70	344	28	1.2	0.7-2.1	-	-

Abbreviations: OR–odds ratio; CI–confidence interval; ACI–Allergy and Clinical Immunology; Hematol–hematology; onc–oncology; Derm–dermatology; Med–general medicine; Plas–plastic surgery; Vasc–vascular; ENT–otorhinolaryngology; BES–breast endocrine surgery; CRS–colorectal surgery; Gastro–gastroenterology; Urol–urology; Neurosurg–neurosurgery; Gen Surg–general surgery; ophth–ophthalmology; CCU–coronary care unit; CT-cardiothoracic; Neurol–neurology; Endo–endocrinology; Rheum–rheumatology

*Numbers exceed total number of patients with admission swabs as patients may have been on more than one ward prior to ICU admission. Odds ratios refer to excess risk of MRSA colonization on admission to ICU for patients previously on the specified ward compared with patients not on the specified ward prior to admission.

† Multivariate analysis considered all factors shown in table, with removal of terms according to the rule p-value >0.05 in a backwards elimination procedure.

2.4.5 MRSA acquisition in the ICU

The 554 patients who had a discharge swab taken and a negative admission swab spent a total of 2333 days in the ICU, giving a rate of new MRSA colonisations of 2.7 per 100 patient days. Table 2-6 shows the rates of new MRSA colonisations per 100 patient days for all of the medical units.

Table 2-6 Rate of new MRSA acquisitions in medical units per 100 patient days

Medical Unit	Rate per 100 patient days	95% confidence interval
Neurosurgery	2.5	1.0 – 6.7
Breast/Endocrine Surgery	2.6	0.4 – 18.2
Burns	1.3	0.2 – 9.4
Cardiology	2.5	0.6 – 10.1
Cardiothoracic Surgery	0.7	0.2 –1.7
Colorectal Surgery	10.5	3.4 – 32.6
Faciomaxillary Surgery	18.2	2.6 – 129.1
Gastroenterology	22.2	3.1 – 157.8
Infectious Diseases	3.9	0.5 – 27.3
Orthopaedics	4.4	0.6 – 31.6
Otolaryngology	10.0	2.5 – 40.0
Professorial General Medicine	1.9	0.6 – 5.9
Professorial Surgery	2.1	0.7 - 6.6
Renal	4.4	0.6 – 32.6
Respiratory	2.6	0.7 – 10.5
Trauma	6.0	4.2 – 8.6

There were no cases in other units not shown in table.

Figure 2-2 shows the proportion of patients under various medical units who acquired MRSA colonisation whilst in the ICU.

Medical Unit
Vertical line reprents 95% confidence interval with actual proportion marked
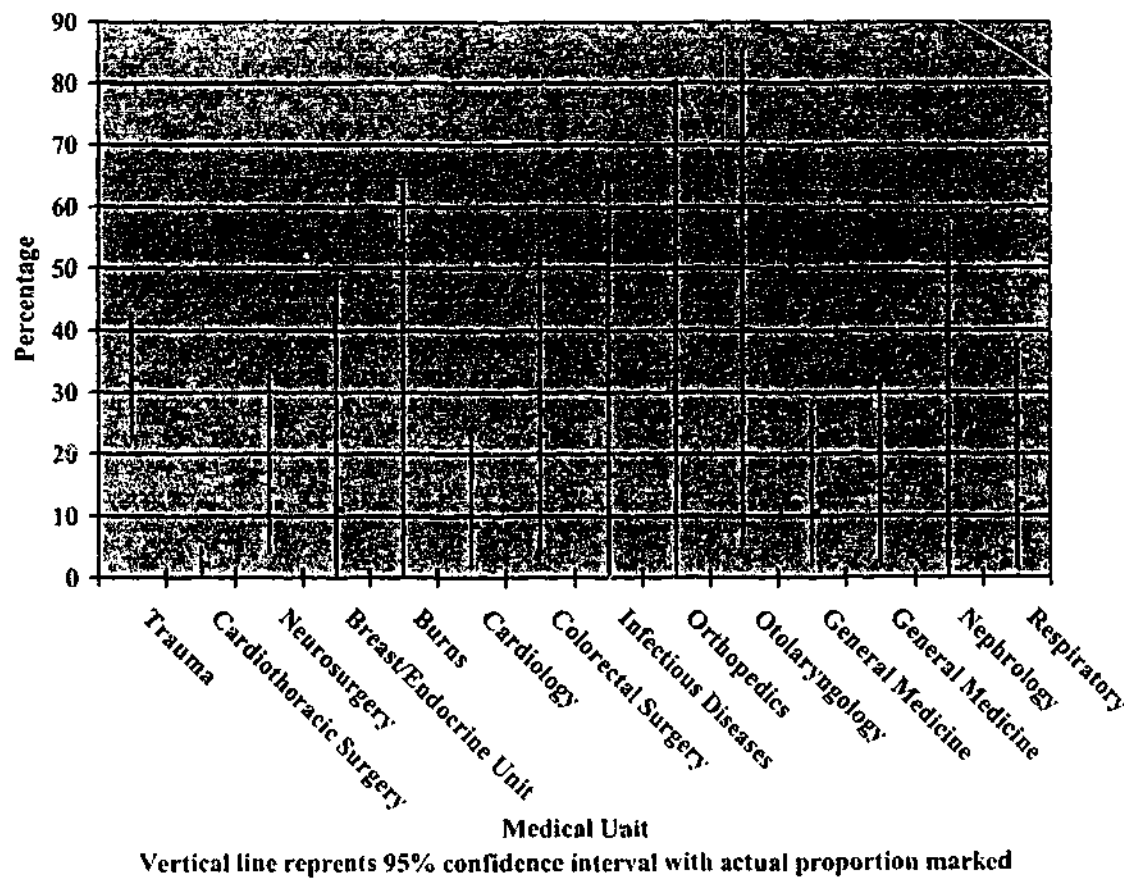
Figure 2-2 Proportion of patients in various units who acquired MRSA in ICU

The crude and adjusted odds ratios for new acquisition of MRSA in the ICU are listed in Table 2-7. On multivariate analysis, the variables remaining significant were being a trauma patient (OR 3.9, 95%CI 1.8-8.7), LOS of 2-7 days in the ICU (OR 11.1, 95%CI 1.4-86) and LOS of >7 days in the ICU (OR 109.8, 95%CI 14.5-833).

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2.4.6 Trauma patients

Of the 63 patients who acquired MRSA in the ICU, 31 (49.2%) were trauma patients, although trauma patients made up only 17.3% (96/554) of the total patients at risk. The odds ratio for MRSA acquisition in trauma patients compared with all other patients was 6.3 (p-value <0.001). The 96 at risk trauma patients had a mean length of stay in the ICU of 7.5 days compared with the remaining 458 at risk patients who had a mean ICU length of stay of 4.5 days (p-value <0.0001). However, even when adjusting for length of ICU stay by logistic regression, being admitted under the trauma unit was still a significant risk factor for MRSA acquisition.

Table 2-7 Risk factors for acquisition of MRSA in the ICU

Risk factor	MRSA not acquired (Number)	MRSA acquired (Number)	OR	95% CI	Adjusted OR†	95% CI
Hospital Unit						
Other units*	204	27	1	-	1	-
Trauma	65	31	3.6	2.0-6.5	3.9	1.8-8.7
Cardiothoracic Surgery	222	5	0.2	0.06-0.5	0.4	0.1-1.1
Length of ICU stay						
≤ 1 day	222	1	1	-	1	-
2-7 days	207	16	17	2.3-130	11.1	1.4-86
> 7days	62	46	165	22 -1218	109.8	14.5-833
Gender						
Female	178	15	1	-	1	-
Male	313	48	1.8	1.0-3.3	2.0	1.0 -4.1
Age (years)						
<50	137	32	1	-	1	-
50-70	202	18	0.4	0.2-0.7	0.9	0.4-2.0
>70	152	13	0.4	0.2-0.7	1.2	0.5-3.1

Abbreviations: OR – odds ratio; CI – confidence interval

* Other units include: allergy and clinical immunology, neurosurgery, breast/endocrine surgery, burns, cardiology, colorectal surgery, infectious diseases, orthopedics, otolaryngology, general medicine, general surgery, renal, respiratory, diabetes and endocrinology, gastroenterology, hematology, oncology, neurology, stroke, plastic surgery, rheumatology, hyperbaric medicine, urology, vascular surgery

† Multivariate analysis includes all factors shown in table.

2.4.7 Infection in colonised patients

Of the 63 patients who became colonised, 18 (29%, 95%CI 18–41%) developed an MRSA infection. Three patients developed MRSA blood stream infection and one had blood stream infection with a surgical site infection of a split skin graft of the hand. Seven other patients developed surgical site infections, including sternal, tracheostomy, laparotomy, lumbar, hand, scalp and radial artery incision site wound infections. Seven patients developed MRSA pneumonia.

2.5   Discussion

2.5.1 Incidence and prevalence of MRSA colonisation

In this study, 11.4% of patients admitted to the ICU acquired MRSA. The strongest risk factor was length of stay in the ICU, but certain units also had a higher risk, even after adjusting for length of stay. Some patients (6.8%) were already colonised with MRSA at admission to the ICU, with prior length of stay in the hospital being a significant risk factor.

Others have examined prevalence of colonisation on admission and incidence in the ICU, but because of differences in the type of ICU, differences in infection control precautions in place and the methodology used, it is difficult to compare results directly. The yield will depend on what proportion of patients are screened and the anatomical sites swabbed. Mest *et al* found a prevalence of nasal colonisation of 3.9% on admission to their surgical

ICU, but did not find prior length of stay in hospital to be a risk factor (Mest *et al.*, 1994). Merrer *et al* found that 6.5% of patients were nasal or cutaneous (axilla or perineum) MRSA carriers on admission when 98.2% of patients were screened, 15.4% were carriers when 48.9% were screened (defined as high risk) and 4.9% when 71.8% were screened (different definition of high risk) (Merrer *et al.*, 2000). Ho found that 12.1% of admissions to ten intensive care units were MRSA colonised and 11.1% became newly colonised in the ICU (Ho, 2003). Garroust-Orgeas *et al* found 5.1% of ICU admissions to be colonised with MRSA and 5.3% of study patients who were not colonised at admission to have acquired MRSA in the ICU (Garrouste-Orgeas *et al.*, 2001). Lucet *et al* found a prevalence of MRSA colonisation in the nose, skin and clinical sites of 6.9% in ICU admissions (Lucet *et al.*, 2003). In another study, 7.2% of patients were MRSA colonised in the nose, throat or perineum on admission to the ICU and 5.2% of patients who were in the ICU for greater than 48 hours became colonised (Hoefnagels-Schuermans *et al.*, 1997).

2.5.2 Risk factors

2.5.2.1   *Length of stay*

In the present study, length of stay in the ICU was strongly associated with acquisition of MRSA and length of hospital stay prior to ICU was also associated with colonisation on admission to the ICU. These findings have been mirrored by many other authors, although not universally. Mest *et al* found that length of stay prior to ICU was not a risk factor for nasal colonisation on admission, but like this study, found that previous stay in the ICU was associated (Mest *et al.*, 1994). Conversely, Lucet *et al* found that length of stay prior

to ICU admission was associated with MRSA carriage on admission (Lucet *et al.*, 2003). In the EPIC (European Prevalence of Infection in Intensive Care) study, length of stay in the ICU was the most important risk factor for MRSA infection, although this was a univariate analysis (Ibelings *et al.*, 1998). Ho also found that ICU length of stay was a significant factor in MRSA acquisition (Ho, 2003). Although length of stay is postulated as a risk factor for MRSA acquisition, it cannot be said with certainty that it is a causal factor. It may be that colonised patients stay longer in the ICU because of their MRSA. The only way to resolve this issue is by using frequent swabbing to determine when patients become colonised. If they become colonised late in the ICU stay, this suggests that length of stay may be causal. If they become colonised early in a long stay, the converse could be true.

2.5.2.2   *Trauma patients*

Even after an adjustment was made for length of stay, trauma patients had a greater risk of becoming colonised with MRSA prior to admission to the ICU and while in the ICU. It is known that neurosurgery and intracranial trauma patients are at increased risk of developing *S. aureus* pneumonia (Campbell *et al.*, 1999; Cazzadori *et al.*, 1997; Espersen *et al.*, 1981; Inglis *et al.*, 1993; Rello *et al.*, 1990) and that nosocomial infections are more common in trauma patients compared with other surgical patients in the ICU (Wallace *et al.*, 1999). Trauma has been found to be an independent risk factor for ICU-acquired infection (Vincent *et al.*, 1995), but few authors have examined trauma as a risk factor for MRSA acquisition. Grundmann *et al* found trauma and head trauma/neurosurgery not to be risk factors for MRSA acquisition in a multivariate analysis (Grundmann *et al.*, 2002).

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During an outbreak at their hospital, Boyce *et al* found that MRSA acquisition was significantly more common in burns patients than in trauma or head injury patients, but this analysis was not adjusted for potential confounders such as length of stay or severity of illness (Boyce *et al.*, 1981). In contrast, a history of major trauma was found to be an independent risk factor for acquisition of vancomycin-resistant enterococcus (VRE) during an outbreak (Byers *et al.*, 2001). With over half of the new MRSA acquisitions being among trauma patients, the finding concerning trauma patients represents a major problem for the Alfred Hospital.

2.5.2.3   *Cardiothoracic surgery patients*

From early 2000, cardiothoracic surgery patients at the Alfred Hospital received vancomycin and rifampicin as pre-operative prophylaxis because of a high rate of sternal wound infection with MRSA. In the subsequent two years, there were no sternal wound infections with MRSA and total vancomycin usage decreased (Spelman *et al.*, 2002). It may be that the overall burden of MRSA was decreased in the cardiothoracic surgery ward by reducing MRSA infections, which may explain why these patients did not have a lower risk of acquisition of MRSA in the ICU. The receipt of vancomycin and rifampicin itself is unlikely to be an explanation because it did not reduce the risk for patients in the ICU. Another explanation may be that this study did not have adequate power to detect a reduced risk for acquisition among cardiothoracic surgery patients in the ICU.

2.5.2.4   *Other variables*

Prior ICU stay has been reported in many studies as a risk factor for MRSA acquisition (Lucet *et al.*, 2003; Ho, 2003; Asensio *et al.*, 1996; Manian *et al.*, 2003; Muller *et al.*, 2003). The reasons for acquisition of multi-drug resistant organisms may be explained by many factors, including decreased host defences, use of invasive devices, high antibiotic usage, severity of illness and high patient acuity (Vincent, 2003).

Patients from the neurology/endocrinology/rheumatology/renal ward had a higher risk of being colonised with MRSA on admission to the ICU. The reasons for this remain unclear, and further analysis may be complicated by the wide variety of underlying illnesses and the admitting medical unit. It is also unclear why patients from the cardiology/general medicine ward had a reduced risk of MRSA colonisation on admission to the ICU.

2.5.3 Compliance with swabbing

The findings of this study may have been biased because only 80% of patients were screened at least once and only 36% were screened on both admission and discharge. There were, however, no substantial differences in age, gender or length of stay in the ICU between patients who had both admission and discharge swabs taken and those who had only one swab taken. There were some minor differences in the medical unit under which they were admitted, probably because of differences in staff compliance with swabbing protocol in the different areas of the ICU. Given the similarities between the two groups, it seems reasonable to infer that those patients swabbed on admission and discharge were

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representative of patients screened at least once regarding risk of infection in the context of an adjusted analysis of risk factors. The 80% response rate indicates that any effect of missing-data bias was unlikely to be great, although some impact on raw prevalence and incidence rates was possible.

Informal discussion with the nursing staff revealed several reasons for the poor compliance with swabbing. These included heavy workload and lack of time, perceived low priority of swabbing compared with other patient care tasks and the perception that swabbing did not change patient outcomes and therefore was of little value. Other reasons may have included lack of knowledge about the surveillance because of high staff turnover and use of agency/bank nurses. This surveillance was seen as a special project, not as routine patient care. Perhaps if MRSA screening were instituted as part of requisite routine care, it would not be perceived as an optional activity and the problem of non-compliance could be overcome. This would require sanctioning by the nursing, medical and management hierarchies.

A number of measures were introduced to try to improve compliance with swabbing. Despite their use, compliance with taking admission and discharge swabs remained low. It was therefore decided to employ a supervisory nurse to enable the study to accrue adequate numbers of patients. In the setting of active surveillance which is being used to identify MRSA carriers for the purposes of introduction of specific infection control procedures, poor compliance could play a major role in reducing the effectiveness of these measures if

the reservoir of MRSA colonised patients is not fully identified. There is little in the literature on this issue and it is not taken into account in recommendations for active surveillance as part of an MRSA containment programme (Muto *et al.*, 2003). In one study in ten intensive care units, patients were screened on admission and discharge for carriage of MRSA, VRE and ceftazidime-resistant Gram-negative bacilli using nose, throat and rectal swabs (Ho, 2003). This study reported that 74.4-100% (90.2% overall) of patients had both admission and discharge swabs taken. 98% of admissions overall and 85.4% of discharges were screened. Lucet *et al* reported that 5.2% of ICU admissions were not screened for MRSA during their study to estimate prevalence of MRSA on admission to the ICU (Lucet *et al.*, 2003). These studies show a much higher compliance rate with swab taking, although measures used to achieve these rates were not discussed.

In the present study, the employment of a nurse dedicated to the screening significantly improved compliance with swab taking, but still only 77.3% of patients had both admission and discharge swabs taken. The nurse worked for 1–2 hours per day (not including weekends) and took some swabs herself but also was responsible for educating and encouraging the primary nurses to take swabs. She also followed up some patients to ensure that swabs had been taken at the time of discharge from the ICU.

Patients screened during the time of employment of the dedicated nurse had a higher rate of detection of MRSA colonisation. This higher rate was not explained by any differences in age, length of stay in the ICU, gender or laboratory practices between the first and

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second time periods. It may have been the result of a genuine change in risk between the two time periods, although there is no indication why this would have occurred. Several nurses verbally reported improved swabbing technique during her employment as a result of education and observation, but it is impossible to quantify and determine if this could have been the predominant explanation for higher MRSA detection rates during this period.

2.6   Impact and consequences of study

The results of this surveillance were presented widely within the Alfred Hospital and at other institutions. Initially, they were presented to the ICU and Department of Trauma Surgery nursing and medical staff. They were also presented to a special infection control meeting at the Austin hospital. At the Alfred Hospital, the results were presented at the Bayside Health Infection Control Committee and were endorsed by the hospital executive. They were presented at the Alfred Hospital Trauma symposium which was telecast to other hospitals across Victoria. Presentations were made at the Monash Medical Centre Infectious Diseases Unit weekly meeting and the Melbourne Infectious Diseases Group (MIDG) fortnightly meeting. Results were also presented at the Australasian Society for Infectious Diseases (ASID) Annual Scientific Meeting in South Australia and the Australian Infection Control Association conference in Melbourne.

The data from this study were important at the Alfred Hospital because they quantified for the first time the extent of the MRSA problem in the ICU. The availability of this

information has given impetus to bring MRSA control once again to the foreground. The findings concerning trauma patients were of particular interest to the Department of Trauma Surgery as nosocomial infection is a major problem in these patients. Because of the large numbers of trauma patients admitted to the Alfred Hospital each year, intervening in this group could have a major impact on the levels of MRSA in the hospital as a whole. Interest in these results was widespread across Melbourne as they provided local data on incidence and prevalence in a large teaching hospital. The results may be generalisable to many of the other major hospitals with a similar patient make-up. This fact is important as many hospitals do not conduct active surveillance for MRSA and therefore this type of information is not available for all institutions. The findings from this study were used in the design of two cohort studies examining risk factors for MRSA acquisition in trauma patients, presented in Chapters 5 and 6. The subjects in this study also formed the control group for the intervention described in Chapter 4, the introduction of a new hand disinfectant. In addition, the MRSA isolates from this screening were subtyped by pulsed-field gel electrophoresis and the RiboPrinter®, as described in Chapter 3.

2.7 Conclusions

This study confirmed the initial belief that substantial numbers of patients were colonised on admission to the ICU and acquired MRSA in the ICU. It confirmed that length of stay is an important association with MRSA colonisation and found a novel association, that trauma patients were at particular risk. It has highlighted the importance of generating

local data as a prerequisite to implementing any interventions and for generating renewed

institutional interest in the perennial MRSA problem.

3 CHAPTER 3. COMPARISON OF SUBTYPING METHODS FOR MRSA

3.1 Introduction

The ability to subtype bacterial strains adds a greater dimension to the investigation of the epidemiology of MRSA. It allows delineation of epidemic from endemic disease, detailed investigation of transmission, determination of a point source for an outbreak and comparison of subtypes isolated from an individual patient. Numerous phenotypic and genotypic methods have been used to subtype MRSA, but all have inherent limitations. Pulsed-field gel electrophoresis (PFGE) is generally accepted as the "gold standard" although this term is given by convention rather than because it is the definitive typing method. Although it has good reproducibility, typability and discriminatory power, it is a time-consuming technique requiring technical expertise and has an inherent amount of subjectivity in the interpretation of gels, despite the publication of guidelines for this purpose (Tenover *et al.*, 1995; Tenover *et al.*, 1997). Thus it is difficult to obtain results in a timely manner for outbreak investigation. In addition, to subtype extensive numbers of isolates requires large amounts of time. Capital cost of equipment ranges from $12 000 to $20 000 and the cost of processing is from $50.00 per isolate, including labour.

In November 2001, a RiboPrinter® Microbial Characterisation System (Qualicon, Wilmington, Del.) became available in Melbourne. Because it is almost fully automated with a large, web-based database of RiboGroups, it allows rapid subtyping and gives an automatic, objective analysis of gels. Eight hours are required to generate a result, up to four runs may be commenced in a working day and each run can analyse eight strains, allowing up to 32 isolates to be typed per day. Its major drawback is its expense for initial set up and running costs ($250 000 purchase cost in 2001 and $125.00 per isolate including labour). It has been used widely, particularly in the food industry, but increasingly commonly to type other organisms. There have been few reports of its use for subtyping of MRSA isolates (Almer *et al.*, 2002; Landman *et al.*, 2003) and only two where its use has been validated against other typing methods (Diekema *et al.*, 2000; Fung *et al.*, 2001).

The aim of this chapter was to compare subtyping of the MRSA strains isolated in the ICU surveillance study described in Chapter 1 using the antibiogram, PFGE and the RiboPrinter® in order to determine the usefulness of each in terms of discriminatory power and practical aspects of their use.

3.2 Methods

3.2.1 Specimens

MRSA isolates were obtained from the surveillance study performed in the Alfred Hospital ICU as described in Chapter 1. Ten isolates were also included from five patients who

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were screened in the weeks leading up to the commencement of the surveillance project. Patients may have had more than one isolate from different anatomical sites (nose, throat, groin and axilla) on more than one occasion. Isolates were identified as described in Chapter 1, suspended in glycerol broth and stored at -70°C. All isolates underwent antibiotic susceptibility testing and 434 were available for analysis using PFGE. One hundred and thirty-six isolates were typed using the RiboPrinter®. Isolates were chosen to try to obtain a representative sample from all PFGE types in order to compare discriminatory ability. In addition, 76 isolates taken from clinically indicated sites in these patients were also typed using PFGE. Information was not available to determine whether these clinical isolates were significant or simply colonisers. Antibiotic sensitivity patterns from these isolates were not included in this analysis and none underwent typing using the RiboPrinter®. Altogether, 510 isolates were typed using PFGE.

3.2.2 Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the disk diffusion method. Antibiotics tested were penicillin 1.5 units, methicillin 5 micrograms (μg), oxacillin 1μg, erythromycin 5μg, tetracycline 10μg, rifampicin 1μg, fusidic acid 2.5μg, trimethoprim 1.25μg and chloramphenicol 30μg. Testing for reduced susceptibility to vancomycin using population analysis profile was not undertaken.

3.2.3 Pulsed-field gel electrophoresis

Five hundred and ten isolates were subtyped by PFGE using a modification of methods previously described (Bannerman *et al.*, 1995). Frozen isolates were cultured overnight on horse blood agar (HBA) at 37°C.

3.2.3.1 *DNA purification*

Enough isolate was added to PIV solution (10mM Tris-Chloride, 1M NaCl) to achieve an optical density of 0.45-0.50 at 590nm. The cells were pelleted twice by centrifugation at 2,000 x g for 15 min and then resuspended in equal volume of PIV solution and 2.4%w/v low melting agarose (Biotech Pharmacia). Approximately 100 microlitres (μl) were dispensed into each well of a plug mould. The set plugs were then added to 1ml EC lysis solution (6mM Tris-Cl, pH7.6, 1M NaCl, 100mM EDTA, pH 7.6, 0.2% deoxycholate and 0.5% sarkosyl with freshly added 1mg/ml lysozyme and 50μg/ml lysostaphin) and incubated for 2-4 hours at 37°C for cell lysis. The plugs were then added to 1ml ESP solution (0.5M EDTA, pH 9.0, 1% N-laurylsarcosine, 1mg/ml Proteinase K) and incubated in a waterbath at 50°C overnight.

3.2.3.2 *Restriction endonuclease digestion*

Plugs were washed once in TE1 buffer (10mM Tris-Chloride, 1mM EDTA, pH 8.0), containing 0.0175g/ml PMSF (phenylmethylsulphylfluoride) dissolved in 100% isopropanol and then a further five times in TE1 buffer alone. Plugs were then washed three times with Restriction Buffer A (Roche Diagnostics), before being suspended in

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Restriction Buffer A containing 30U of *Sma*I restriction enzyme (Roche Diagnostics) and incubated at 25°C overnight in a waterbath.

3.2.3.3   Electrophoresis

The plugs were set in Multipurpose Agarose Gel (1%) (Roche Diagnostics) and the gel was immersed in 2.5L of 0.5xTBE buffer (Tris Base, Boric Acid, 0.5M ECTA, pH8) in the Gene Navigator Control Unit (Amersham-Pharmacia). The gel was run at five seconds for eight hours, 20 seconds for eight hours and 80 seconds for ten hours at 170V and 120mA. The gel was then stained with ethidium bromide 10mg/ml and photographed.

3.2.3.4   Analysis of gel

Gels were analysed using the criteria published by Tenover *et al* (Tenover *et al.*, 1995). Isolates were considered to be *indistinguishable* if their restriction patterns were identical. According to these criteria, isolates were considered to be *probably related* if the restriction pattern varied by less than three bands, indicating a single genetic event, such as a point mutation. If the pattern differed by four to six bands, consistent with two independent genetic events, they were considered to be *possibly related* and if the pattern differed by more than six bands, consistent with three or more independent events, they were regarded as *unrelated*. In this study, isolates were compared with an isolate of the most common restriction pattern, which was labelled as Type 1, with probably and possibly related subtypes designated as Subtype 1a and 1b and so on respectively. Unrelated strains were designated Type 2, Type 3 and so on.

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3.2.4 RiboPrinter®

One hundred and thirty-six isolates were typed using the RiboPrinter® Microbial Characterization System. Isolates were chosen to give a representative sample of the different PFGE types. The Riboprinter® is an automated typing system using a technique based on ribotyping. It subtypes isolates into RiboGroups which it electronically analyses, stores and compares with other isolates in its web-based database.

Isolates were processed using a standard methodology (Bruce, 1996; Pfaller *et al.*, 2004) according to the manufacturer's instructions. Frozen isolates of MRSA were grown on blood agar plates and suspended in buffer in a micro-centrifuge tube. The suspension was then transferred to a sample carrier and heated (80°C for 30 minutes) to reduce viability of the organism and deactivate nucleases. After cooling, two lysis enzymes were added and the sample carrier was loaded into the instrument, where the remainder of the processing was fully automated. DNA was cleaved using the restriction enzyme *Eco*RI. The resulting restriction fragments were separated electrophoretically on agarose gel, transferred to a nylon membrane and hybridised with a labelled rRNA operon probe. The light intensity of the target DNA fragments produced by heating the membrane was detected by a customised charge-coupled device (CCD) camera and converted to digital information. A pattern was generated for each sample which was compared statistically to patterns obtained previously. A RiboGroup is a set of closely related patterns which are indistinguishable. A dendrogram was constructed using GelCompar software (Applied

Maths, Kortfijk, Belgium) using a band position tolerance of 1% and optimisation of 1.56%.

3.2.5 Criteria for comparison of typing methods

The following criteria were used to compare the usefulness of the typing methods:

- Time taken to generate results

- Amount of labour and time required to perform

- Level of expertise required for performance and analysis of results

- Equipment required

- Cost

- Discriminatory ability

3.3 Results

3.3.1 Specimens

One hundred and fifty-six patients had 339 positive swab sets (consisting of nose, throat, groin and axilla swabs) taken. 43/156 (27.6%) had one set taken, 64 (41%) had two, 34 (21.8%) had three, nine (5.8%) had four and six (3.8%) had five. Each patient had between one and five (average 1.4) positive swab sets. 109/156 (69.9%) patients had one

positive swab set, 32/156 (20.5%) had two, 11/156 (7.0%) had three, 2/156 (1.3%) had four and 2/156 (1.3%) had five.

There were 435 isolates of MRSA from 156 patients from any site swabbed at any time. Up to 13 isolates were obtained from an individual patient, with a mean number of 2.8 (median 2). Altogether 221 swab sets were positive, with an average of two of the four swabs positive per set. Ninety-one (41.2%) of the 221 swab sets had one of four swabs positive, 67 (30.3%) had two, 42 (19.0%) had three and 21 (9.5%) were all positive.

3.3.2 Antibiotic susceptibilities

Antibiotic susceptibility patterns are shown in Table 3-1 and Figure 3-1. Eight different antibiograms were found amongst the 435 specimens. All isolates were resistant to penicillin, methicillin and oxacillin and all were sensitive to chloramphenicol. Two hundred and ninety of the 435 (66.7%) fell into antibiogram I. Antibiograms VII and VIII were typical of non-multiresistant strains. The patient with antibiogram VII was admitted to hospital 16 days and to the ICU ten days prior to the positive swab, but had a negative screen one day after ICU admission, suggesting that the isolate was actually a hospital acquired non-multiresistant MRSA or that the initial swabs were all false negatives. One patient had three isolates from the same swab set with antibiogram VIII. This patient was admitted to hospital two days prior to ICU admission when the screen was performed and had no screening on other occasions. These isolates may have been a true community acquired strain.

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One patient had eight isolates from two swab sets taken on two consecutive days with three different antibiograms (types I, II and III). Fourteen patients had between two and nine isolates with two different antibiograms (types I and II, I and III or I and IV). Two patients had two antibiograms in the same swab set and one had two in two sets taken on the same day. Others had sets taken up to five months apart. Of the ten patients who had more than one positive set, eight had organisms with a different antibiogram isolated from the same anatomical site on different occasions.

Table 3-1 Proportion of isolates with antibiotic susceptibility patterns

ABG	NO.	%	PEN	METH	OX	ERYTH	TET	RIF	FA	CIP	TRIM	CHLOR
I	290	66.7	R	R	R	R	R	S	S	R	R	S
II	65	14.9	R	R	R	R	S	S	S	R	R	S
III	64	14.7	R	R	R	R	R	S	S	S	R	S
IV	9	2.1	R	R	R	R	R	R	S	R	R	S
V	1	0.2	R	R	R	R	R	R	S	S	S	S
VI	2	0.5	R	R	R	R	S	R	R	S	S	S
VII	1	0.2	R	R	R	S	S	S	S	S	S	S
VIII	3	0.7	R	R	R	S	S	S	S	R	S	S
TOTAL	435	100.00										

Abbreviations: ABG- antibiogram, NO.-number, %-proportion, PEN-penicillin, METH-methicillin, OX-oxacillin, ERYTH-erythromycin, TET-tetracycline, RIF-rifampicin, FA-fusidic acid, CIP-ciprofloxacin, TRIM-trimethoprim, CHLOR-chloramphenicol.
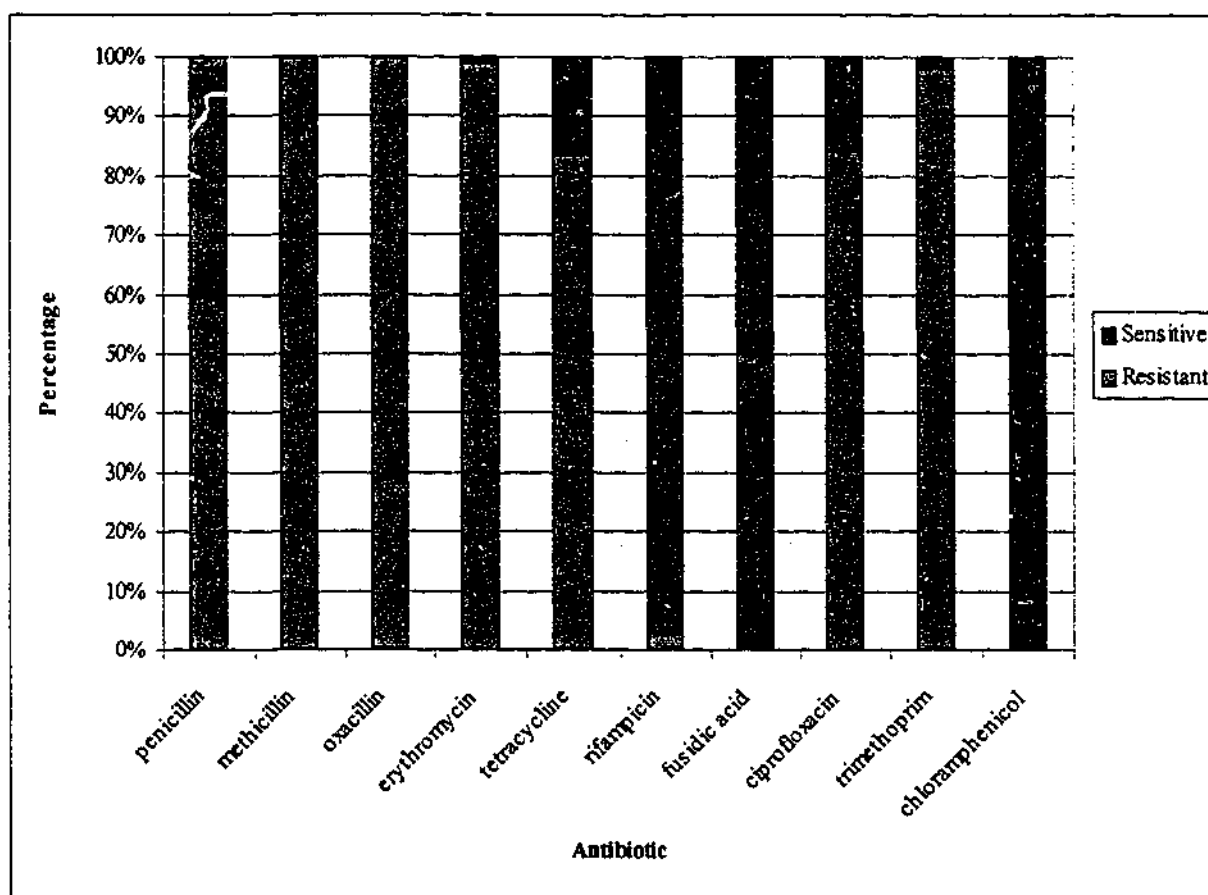
Figure 3-1 Proportion of isolates resistant to antibiotics tested

3.3.3 Pulsed-field gel electrophoresis

3.3.3.1   PFGE types and subtypes

Five hundred and ten isolates were typed using PFGE, including 76 from clinical sites.

The most prevalent pattern was designated as Type 1, with the parental type (to which all

others were compared) being designated as Subtype 1. The isolates were separated into 21

Types altogether, using the criteria of being greater than or equal to seven bands different

from Subtype 1 (see Figure 3-2). There were 17 subtypes (1a-1q) that were probably or

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closely related to Subtype 1. The screening isolates were divided into 16 Types, with 15 subtypes (1a-1o) being probably or possibly related to Subtype 1. Subtypes 1d, 1g, 1k and 1p were probably related to Subtype 1 (1-2 bands difference), with the other subtypes in this strain being possibly related to Subtype 1 (4-6 bands difference). In this discussion, Subtype 1 refers to the parental strain whereas Type 1 refers to all isolates that were probably or possibly related to Subtype 1 (1a-1q) and includes Subtype 1.

MW – molecular weight marker, * - most prevalent type

Figure 3-2 PFGE patterns and corresponding Types and Subtypes

Figure 3-3 shows the number of isolates in each PFGE type. 93/510 (18.2%) were Subtype 1, with 275/510 (53.9%) being closely or possibly related to this subtype (making a total of 368/510 [72.1%] Type 1). The next most frequent types were Subtype 1a (88/510, 17.3%), Type 2 (51/510, 10.0%), Subtype 1b (44/510, 8.6%), Subtype 1g (42/510, 8.2%), Subtype 1d (27/510, 5.3%), Type 9 (26/510, 5.1%) and Subtype 1e (19/510, 3.7%). Seventy-six

isolates were probably related to Subtype 1 (Subtypes 1d, 1g, 1k, 1p), with 199 being possibly related (Subtypes 1a, 1b, 1c, 1e, 1f, 1h, 1i, 1j, 1l, 1m, 1n, 1o, 1q).

Of the two non-multiresistant strains, the one that was isolated 16 days after admission to hospital was assigned a PFGE subtype of 1l, with a difference of five bands from the most prevalent type (Subtype 1). This means that it was possibly related to Subtype 1 and strengthens the probability, but does not prove, that this was a hospital acquired non-multiresistant MRSA. The other non-multiresistant isolate fell into two PFGE groups, Type 14 and 15. Although they differed from the parental type (Subtype 1) by more than six bands, they only differed from each other by one band, indicating a close relationship.
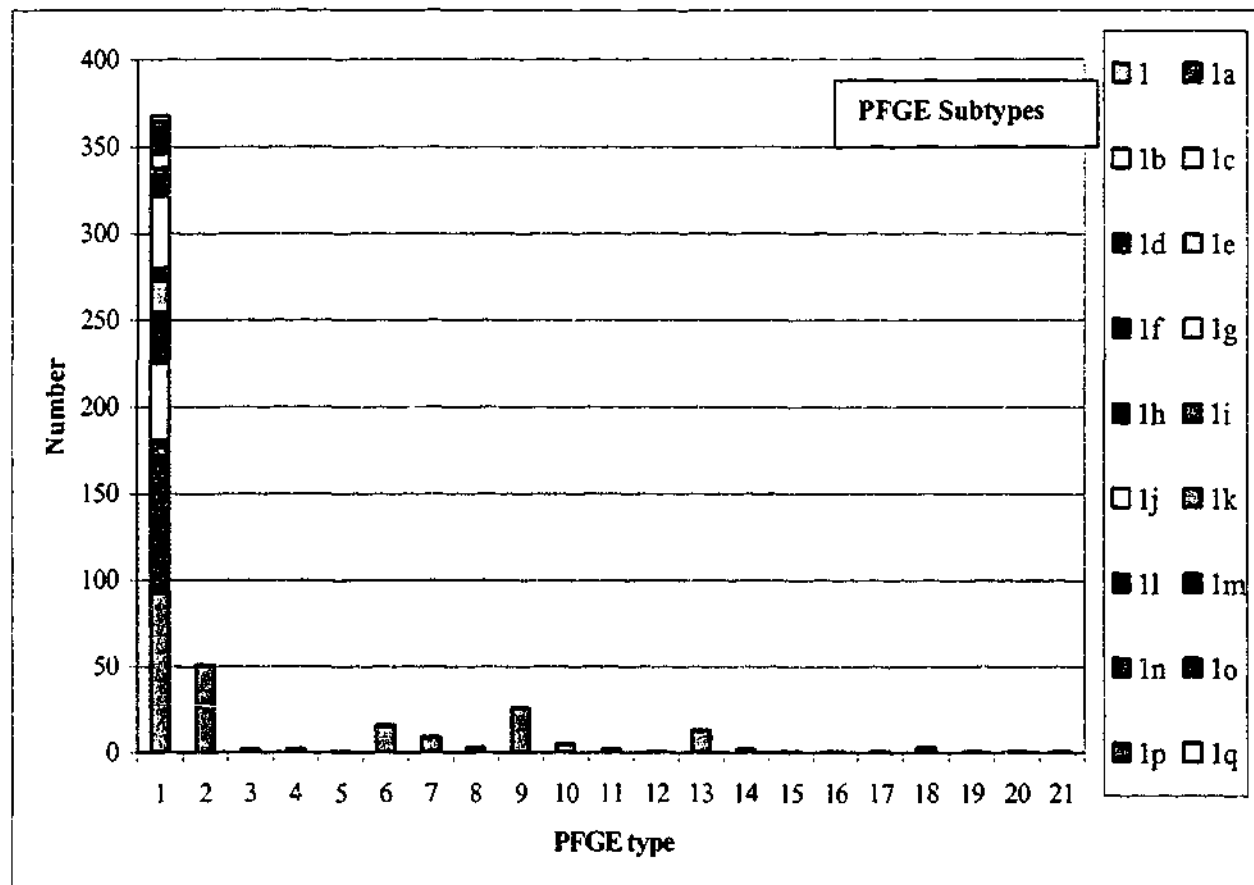
Figure 3-3 Number of isolates in each PFGE type/subtype

3.3.3.2 Comparison of PFGE and antibiogram

A comparison of PFGE types/subtypes and antibiogram is shown in Figures 3-4 and 3-5. PFGE was much more discriminatory than antibiotic sensitivity testing, with eight antibiograms falling into 16 PFGE types (or 31 groups if subtypes of Type 1 were included) (Figure 3-4). Conversely, there were several PFGE types/subtypes that were further discriminated into two (1,1b,1e,1k,10) or three antibiograms (1a,1d,8) (Figure 3-5).
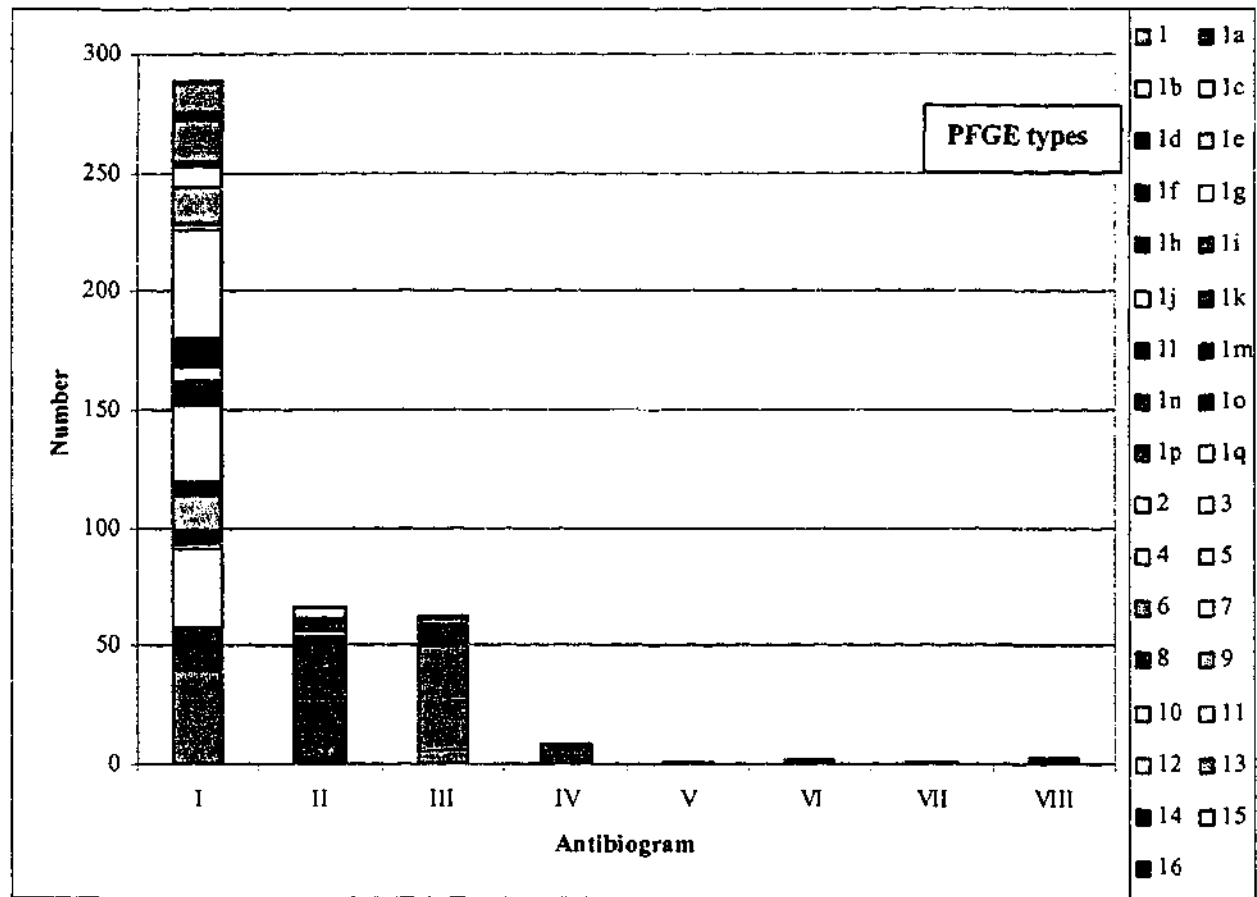
Figure 3-4 Comparison of antibiogram and PFGE types/subtypes
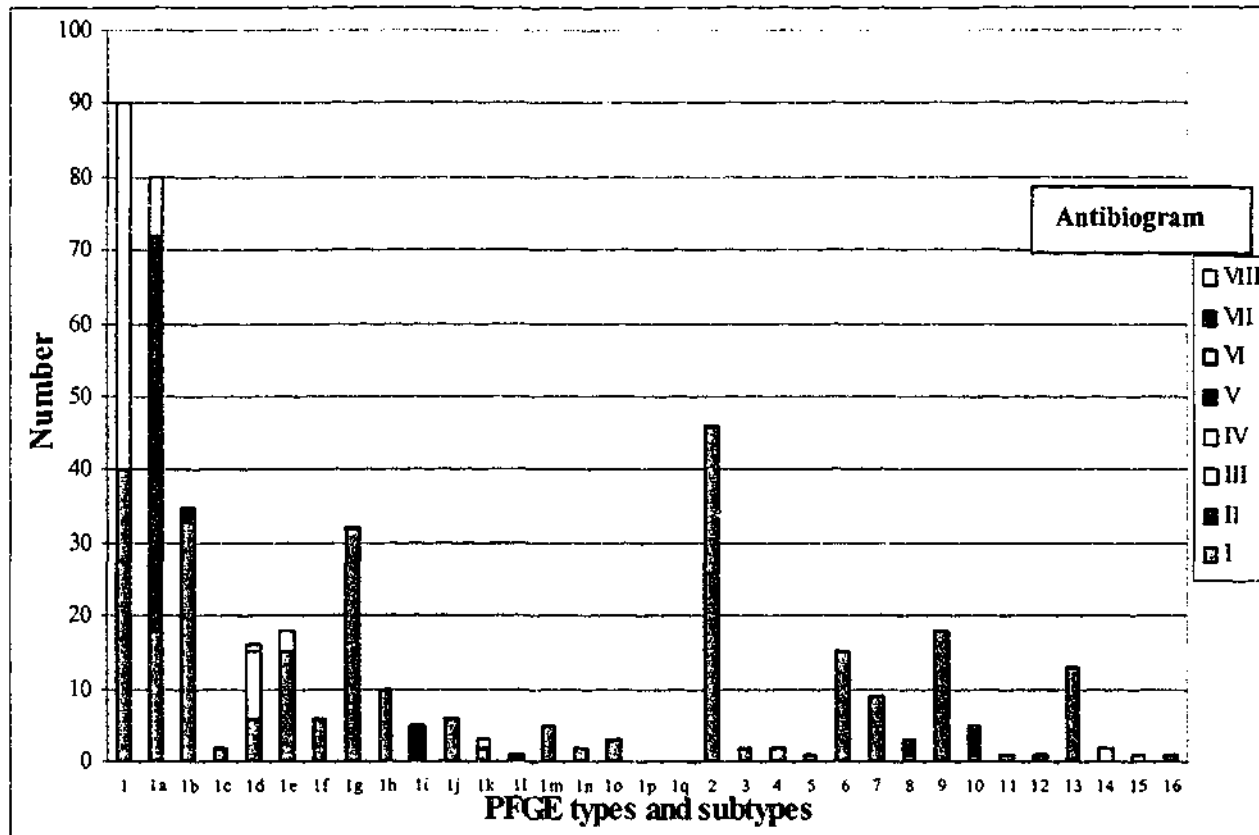
*Subtypes 1p and 1q belonged to clinical isolates for which antibiogram data was not included in this analysis

Figure 3-5 Comparison of PFGE types/subtypes and antibiogram

3.3.3.3 *Clinical and screening isolates*

Fifty patients had both screening and clinical isolates typed by PFGE. If all subtypes of type 1 were considered as Type 1, 32/50 (64%) of clinical and screening isolates were identical, 13/50 (26%) were different types and 5/50 (10%) had more than one screening type, one of which was the same type as the clinical isolate, whilst the other(s) were different. Of the 18 who displayed more than one type, one had three subtypes and 17 had two types.

One hundred and nineteen patients had more than one specimen positive for MRSA (including clinical and screening samples). The maximum number of occasions (includes swab sets and clinical samples) where MRSA was isolated was eight. The maximum number of different PFGE types per swab set was two (if all type 1 subtypes were considered as Type 1). 99/119 (83.2%) patients had a maximum of one PFGE type per swab set and 20/119 (16.8%) had a maximum of two. 82/119 (68.9%) patients had a maximum number of one PFGE type altogether, 36/119 (30.3%) had a maximum number of two and 1/119 (0.8%) had a maximum number of three.

3.3.4 RiboPrinter®

3.3.4.1   RiboGroups

One hundred and thirty-six isolates were typed using the RiboPrinter®, which were grouped into 18 RiboGroups. However, the single isolate in RiboGroup R17 resembled the other six from that patient which were fell into RiboGroup R1, but also contained some extra bands which may represent partial lysis. As the organism was not available for re-typing, the results could not be confirmed (see last isolate in Figure 3-11). This means that there were probably only 17 RiboGroups. An example of one gel containing eight samples is shown in Figure 3-6.
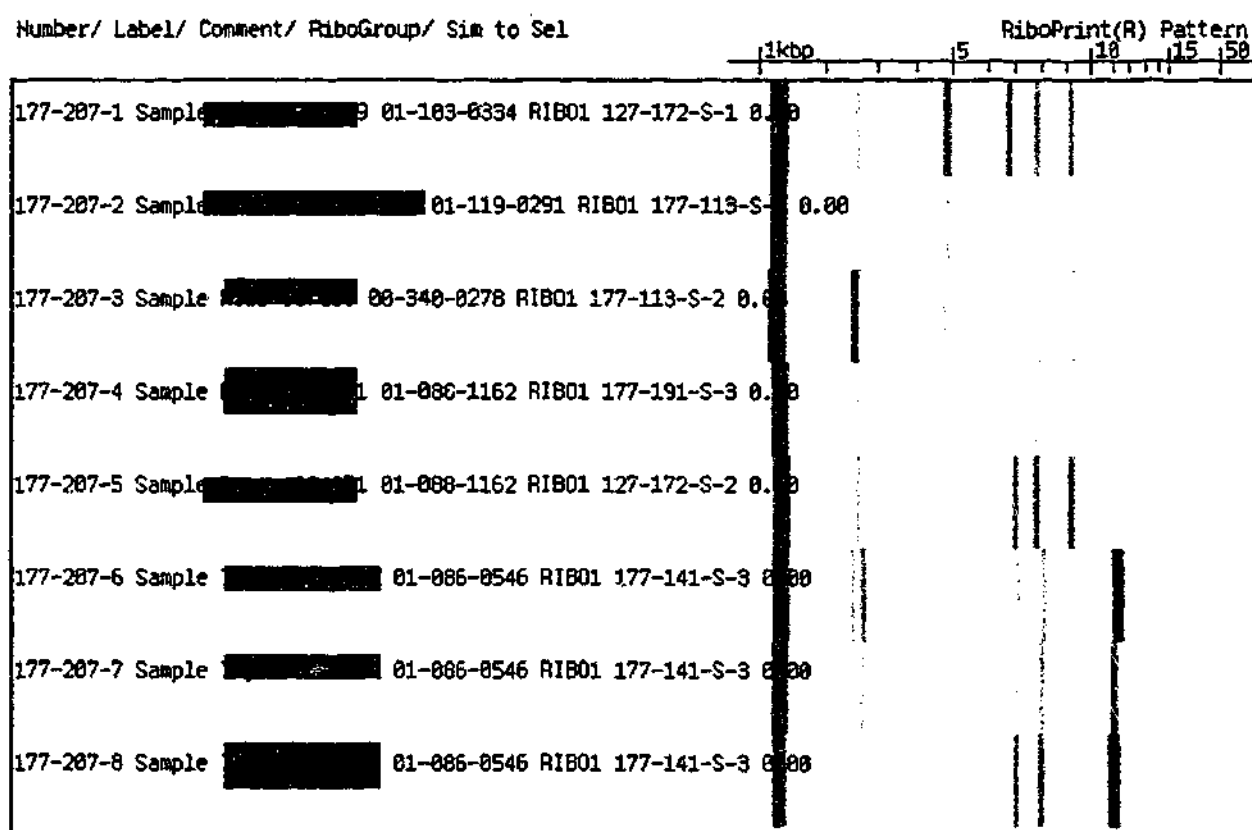
Figure 3-6 Example of RiboPrinter® gel

3.3.4.2 *Comparison between RiboPrinter® and antibiotic susceptibility testing*

RiboPrinting appeared more discriminatory than antibiotic susceptibility testing, with the 97 isolates with antibiogram I falling into 12 RiboGroups, the 20 with antibiogram II falling into four groups and the 12 tested with antibiogram III falling into three groups. Conversely, isolates in RiboGroups R1 to R5 displayed two different antibiograms respectively (Table 3-2). No isolates with antibiogram IV were typed using the RiboPrinter®.

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Table 3-2 Correlation between RiboGroups and antibiograms

		Antibiogram (Number)							
RiboGroup	Number	ABG I	ABG II	ABG III	ABG IV	ABG V	ABG VI	ABG VII	ABG VIII
R1	30	29	1	-	-	-	-	-	-
R2	6	5	-	1	-	-	-	-	-
R3	16	1	15	-	-	-	-	-	-
R4	21	12	-	9	-	-	-	-	-
R5	5	4	1	-	-	-	-	-	-
R6	40	40	-	-	-	-	-	-	-
R7	2	-	-	2	-	-	-	-	-
R8	2	-	-	-	-	-	2	-	-
R9	1	1	-	-	-	-	-	-	-
R10	1	1	-	-	-	-	-	-	-
R11	1	1	-	-	-	-	-	-	-
R12	1	-	-	-	-	1	-	-	-
R13	3	-	3	-	-	-	-	-	-
R14	1	1	-	-	-	-	-	-	-
R15	1	-	-	-	-	-	-	1	-
R16	3	-	-	-	-	-	-	-	3
R17	1	1	-	-	-	-	-	-	-
R18	1	1	-	-	-	-	-	-	-
Total	136	97	20	12	0	1	2	1	3

3.3.4.3   Comparison of RiboPrinting with PFGE

PFGE appeared to have similar discriminatory power to RiboPrinting, with the 17 (or 18 if R17 is included) RiboGroups falling into 16 PFGE types (Type 1-16), if all subtypes of Type 1 were included in one group. A comparison of PFGE types (including subtypes of Type 1) and RiboGroups is shown in Figures 3-7 and 3-8. Both figures show that some PFGE types were divided into multiple RiboGroups and vice versa. Figure 3-9 shows a comparison of RiboGroups according to PFGE type where all subtypes of Type 1 were considered together. PFGE Type 1 contained 11 RiboGroups, Type 2 contained four, Types 7, 10 and 13 contained three each and Types 6,8 and 9 contained two each. Eight PFGE types were represented by only one RiboGroup, three of which had only one isolate tested and five of which contained only two isolates. Figure 3-10 shows a comparison of PFGE Types according to RiboGroup. RiboGroup 6 was differentiated into nine, R1 into seven, R5 into four, R3 into three and R16 into two PFGE types respectively. The remaining 12 (or 13 if R17 is included) contained only one PFGE Type. Seven (or eight) of these contained one isolate only.
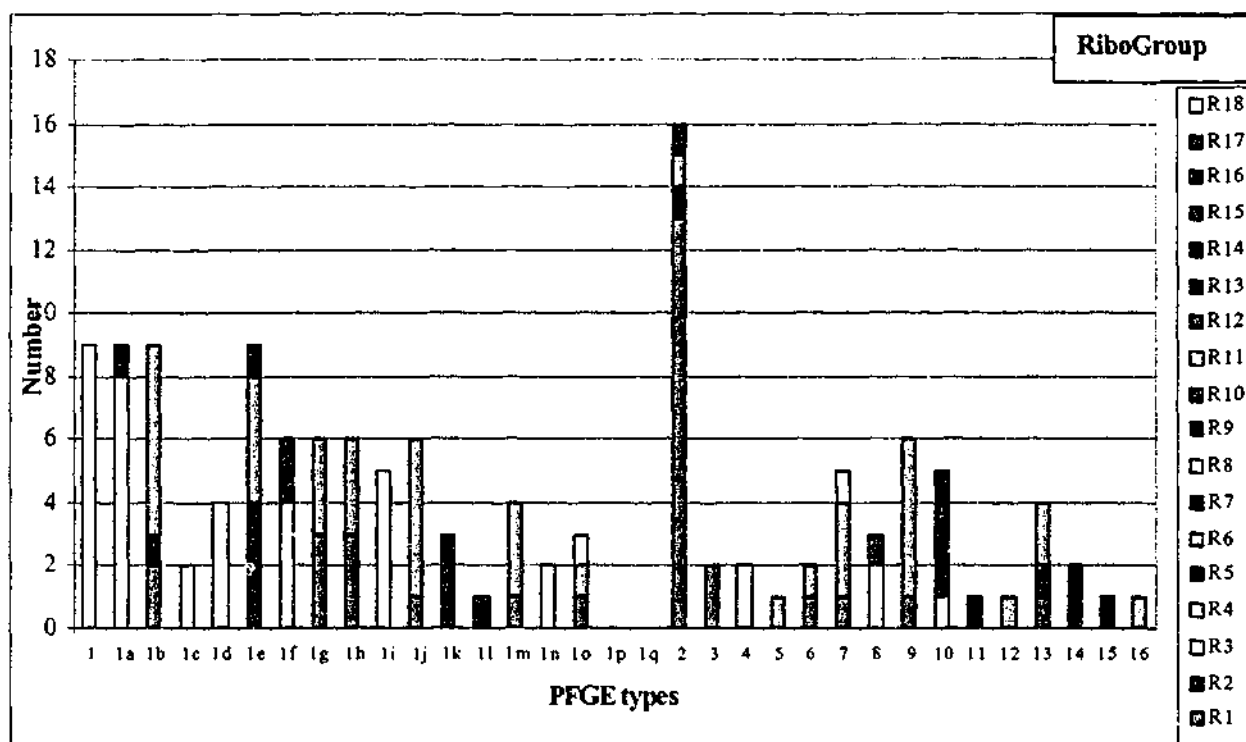
No isolates belonging to PFGE Types 1p and 1q were typed using the RiboPrinter®

Figure 3-7 Comparison of RiboGroups according to PFGE Type and Subtype
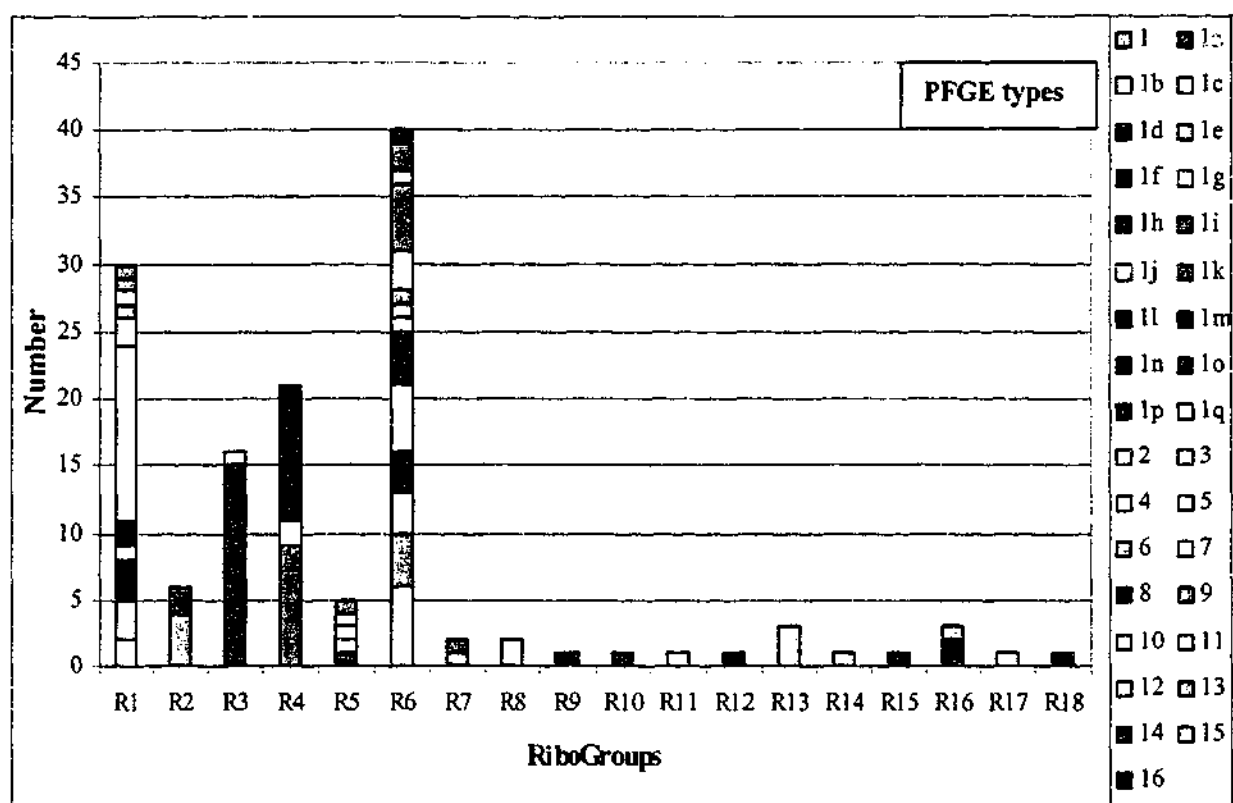
Figure 3-8 Comparison of PFGE Types and Subtypes according to RiboGroup
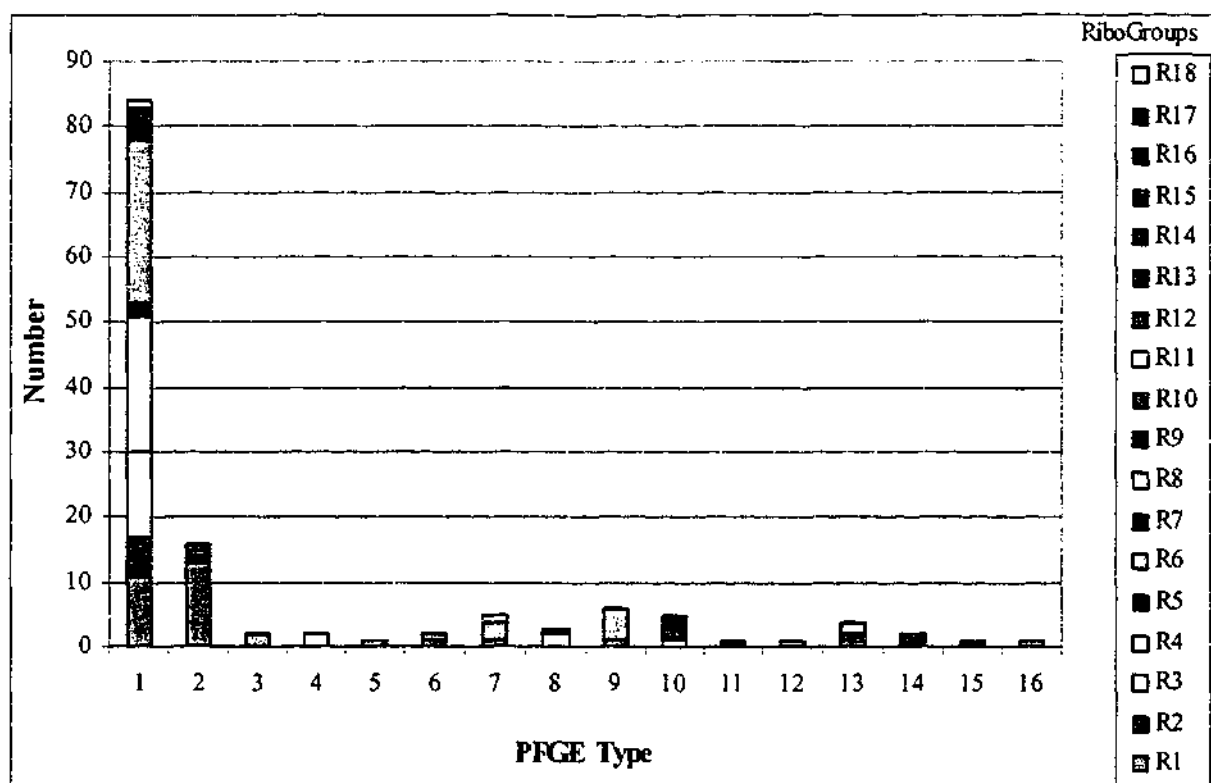
Figure 3-9 Comparison of RiboGroups according to PFGE Type with all Subtypes of Type 1 being included as Type 1
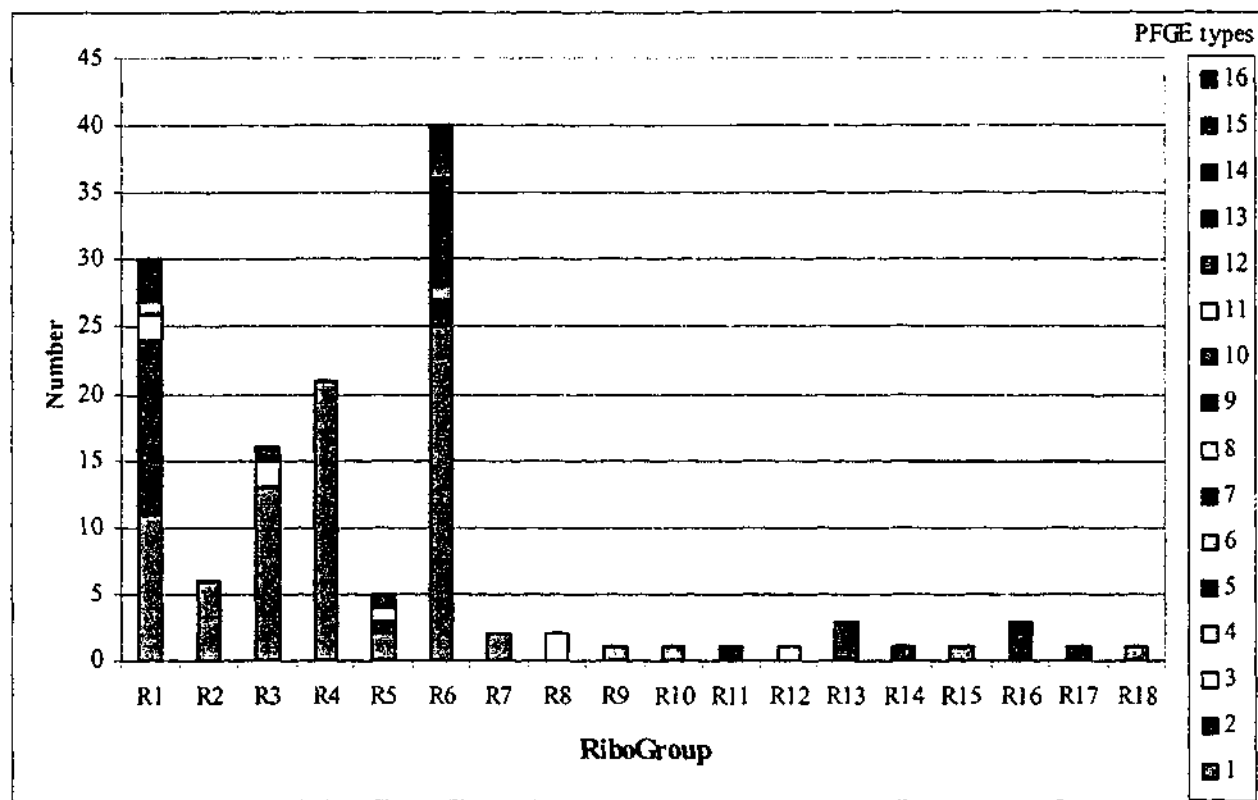
Figure 3-10 Comparison of PFGE Types according to RiboGroup with all Subtypes of Type 1 being included as Type 1

Figure 3-11 shows a dendrogram constructed using GelCompar software to determine relationships between the isolates according to RiboPrinting. It shows several which were very similar, even though designated as a different RiboGroup by the RiboPrinter®. Visual examination of the banding patterns (not shown) shows some of these RiboGroups were very similar. For example, the bands of RiboGroups R5 and R6 were identical in position, but differed slightly in intensity, probably accounting for their designation into different RiboGroups. Examination of the dendrogram also shows these isolates to be similar, although they are represented by several different PFGE groups. Several isolates

had a clearly distinctive banding pattern on RiboPrinting, including Patients K, LL and

MM. These have been designated different RiboGroups and different PFGE groups.

VCA	Patient	Isolate*	RiboGroup	PFGE	Antibiogram
	A	1N	R4	i	3
	A	1G	R4	i	3
	B	1G	R4	i	1
	A	2T	R4	i	3
	A	2A	R4	i	3
	C	1N	R4	i	3
	D	1T	R4	ic	1
	D	2T	R4	ic	1
	E	1G	R4	id	1
	E	2G	R4	i	1
	F	1G	R4	i	3
	C	2N	R4	id	3
	G	1G	R4	id	3
	H	1T	R4	id	1
	A	2G	R4	i	3
	C	1T	R2	ie	1
	C	3N	R2	ie	1
	C	1A	R2	ie	1
	C	2A	R2	ik	1
	C	2T	R2	ie	3
	C	3G	R2	ik	1
	I	1N	R4	if	1
	I	1G	R4	if	1
	J	2N	R4	if	1
	J	2T	R4	if	1
	J	1N	R9	if	1
	J	1A	R10	if	1
	K	1T	R4	in	1
	K	1G	R4	in	1
	L	1N	R7	ie	3
	L	2N	R7	ik	3
	REPEAT				
	REPEAT				
	M	1A	R12	8	5
	N	1N	R15	11	7
	O	1A	R6	ie	1
	O	2N	R6	ie	1
	O	1T	R6	ie	1
	O	1N	R6	ic	1
	G	2N	R6	ig	1
	P	1T	R6	1b	1
	C	3T	R6	12	1
	Q	1T	R6	5	1
	B	1N	R6	13	1
	Q	1G	R6	ib	1
	B	2N	R5	ib	1
	B	2G	R6	ib	1
	B	2T	R5	13	1
	R	1N	R6	ij	1
	R	4N	R6	ij	1
	R	3N	R6	ij	1
	R	1G	R6	ij	1
	S	2N	R1	2	1
	T	1N	R6	9	1
	U	1N	R1	3	c
	U	2N	R1	2	1
	V	1T	R6	9	1
	W	1A	R6	7	1
	U	1T	R1	2	1
	T	1A	R6	9	1
	U	3T	R1	2	c
	S	1G	R1	2	1
	O	1G	R6	2	c
	Q	2T	R6	ig	1
	L	3G	R6	1b	1
	L	2G	R6	1b	1
	U	3N	R1	2	1
	S	2T	R1	2	1
	U	2T	R1	2	1

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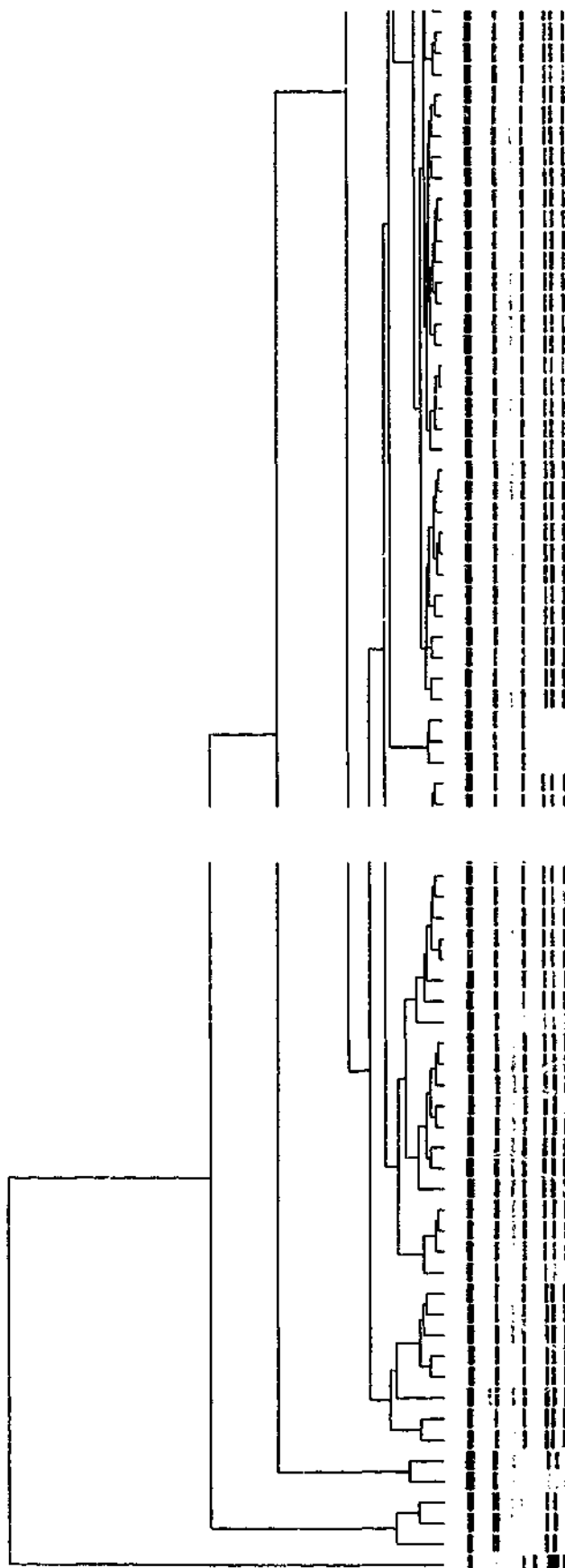
	REPEAT				
	X	1G	R6	6	1
	X	1T	R6	1m	1
	Y	1G	R6	1g	1
	Z	3G	R6	13	1
	R	4G	R6	1j	1
	W	2N	R6	7	1
	W	2A	R1	7	1
	H	1G	R6	9	1
	Y	1T	R6	1m	1
	Y	1N	R1	1g	1
	T	1T	R6	9	1
	AA	1A	R1	2	1
	BB	3N	R1	1b	2
	W	1T	R6	7	1
	T	1G	R6	16	1
	P	2G	R6	1b	1
	P	1N	R6	1b	1
	Y	2T	R6	1m	1
	B	1T	R6	1b	1
	L	1G	R6	1h	1
	S	1N	R1	2	1
	G	1A	R1	1b	1
	A	2N	R1	2	1
	G	1N	R1	1g	1
	G	2A	R1	1g	1
	O	2G	R1	1m	1
	AA	1N	R1	2	1
	F	1A	R1	6	1
	AA	1T	R1	3	1
	R	2N	R1	1j	1
	CC	1T	R1	2	1
	V	1A	R1	9	1
	A	1A	R5	1a	2
	DD	1N	R5	11	1
	A	1T	R5	2	1
	REPEAT				
	REPEAT				
	BB	3T	R3	1a	2
	BB	3G	R3	1a	2
	S	1A	R3	1a	2
	BB	1A	R3	8	1
	BB	2T	R3	1a	2
	EE	1T	R3	11	2
	BB	3A	R3	1a	2
	P	1G	R3	8	2
	FF	2G	R3	1i	2
	FF	1G	R3	1i	2
	EE	1N	R3	1i	2
	EE	1G	R3	1a	2
	FF	1N	R3	1i	2
	EE	1A	R3	1a	2
	GG	1G	R3	10	2
	S	2G	R3	1a	2
	HH	1A	R13	10	2
	HH	1G	R13	10	2
	HH	2G	R13	10	2
	GG	1A	R14	10	1
	II	1G	R1	1o	1
	JJ	1G	R1	1b	1
	KK	1N	R18	1o	1
	Z	1A	R1	13	1
	KK	1G	R6	1o	1
	CC	2A	R11	7	1
	JJ	1N	R1	1h	1
	JJ	1T	R1	1h	1
	LL	1N	R8	4	6
	LL	1T	R8	4	6
	MM	1G	R16	15	8
	MM	1N	R16	14	8
	MM	1A	R16	14	8
	U	3G	R17	2	1

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* Isolate represents each swab set with the site from which MRSA was isolated, for example, 1N indicates the nose swab from one swab set. The numbers are not in chronological order but represent different swab sets taken on different occasions.

Figure 3-11 Comparison of PFGE, RiboGroups and antibiogram using dendrogram (Previous pages)

3.4 Discussion

3.4.1 Antibiotic susceptibility pattern

The antibiotic susceptibility pattern was available for all the screening isolates in this study. Antimicrobial resistance rates have been published for MRSA isolates obtained from hospitals around Australia, however, these were clinical isolates that may have been obtained from any site in any patient (Nimmo *et al.*, 2003). Data are also available from the SENTRY Antimicrobial Surveillance Program which monitors antimicrobial resistance patterns from any patient in selected hospitals across the globe, including several in Australia (Diekema *et al.*, 2001). A comparison is shown in Table 3-3, although data may not be directly comparable because of the differences in source patients (ICU in this study versus any in the others and inclusion of other countries in the SENTRY study) and the nature of the samples (screening in this study versus clinical in the others).

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Table 3-3 Comparison of antimicrobial resistances rates in this study and published studies (proportion of MRSA isolates resistant to various antibiotics)

	Antibiotic						
Study	Rifampicin	Fusidic acid	Cipro-floxacin	Tetra-cycline	Erythro-mycin	Trimetho-prim	Chloram-phenicol
Current	2.8%	0.5%	84.4%	83.7%	99.1%	98.4%	0%
Australia 1999 *	7.7%	4.9%	75.9%	80.1%	88.9%	82.4%	NR
SENTRY Western Pacific **	10.5%	NR	88.1%	82.0%	94.7%	NR	9.6%

NR – not reported * (Nimmo *et al.*, 2003) ** (Diekema *et al.*, 2001)

This work suggested that PFGE and RiboPrinting were more discriminatory than the antibiotic sensitivity pattern for subtyping of the MRSA isolates collected in the Alfred Hospital ICU. There are no published comparisons of antibiotic susceptibility patterns and RiboPrinter® results for MRSA. PFGE is generally considered to be more discriminatory than antibiotic susceptibility testing, but there have been occasional strains reported where the antibiogram was sufficiently discriminatory for differentiation from others (Archer *et al.*, 1983; Hoefnagels-Schuermans *et al.*, 1997; Kim *et al.*, 1998).

3.4.2 Pulsed-field gel electrophoresis

Banding patterns were analysed and compared using the criteria published by Tenover *et al* (Tenover *et al.*, 1995). These authors state that their guidelines were intended to be used for relatively small sets of isolates (typically less than or equal to 30) in an outbreak situation and not for large populations of organisms collected over extended periods of greater than one year. Although this analysis is of a large collection of isolates not taken during an outbreak, the study period spanned less than one year. In addition, there are no other published criteria for comparison of large numbers of isolates where computer-assisted analysis cannot be used. Several authors have used the criteria published by Tenover *et al* for visual interpretation of large numbers of isolates (Diekema *et al.*, 2000; Landman *et al.*, 2003; Murchan *et al.*, 2003). One of the inherent problems of any electrophoresis-based method of band separation is the assumption that any bands in the identical position represent the same region of chromosomal DNA in different isolates, even though they may represent different chromosomal areas with coincidental identical *Sma*I restriction sites. This is less of a problem with smaller numbers involved in an outbreak but becomes more significant in the setting of larger numbers of isolates (personal communication, Richard V. Goering).

The "Tenover criteria" are ideal for identification of isolates that are identical or completely different, which is why they are most useful in an outbreak. A major problem arises when trying to determine the level of relatedness between isolates with similar banding patterns. In addition, strict use of the criteria allows a comparison only with the

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outbreak or parental type, but does not permit comparison between the other types. In other words, isolates with 1-6 band difference (that is, subtypes 1a, 1b, 1c etc.) are classified as related to the parental type (subtype 1), but no assumptions can be made about the relationship between these subtypes, which may differ to a greater extent from each other than each differs from the parental subtype (Goering *et al.*, 1997). This is also an issue when comparing unrelated types (greater than six bands difference from the parental type), as these types may still be related to each other but by definition, are not related to the parental subtype. For example, PFGE Types 14 and 15 varied from each other only by one band but were classed as unrelated using the Tenover criteria as they each varied from the parental type (Subtype 1) by more than six bands. The relationship between subtypes and types may be more easily determined using computer software, which is able to generate a dendrogram, although even this process is subject to a certain amount of subjectivity in selecting settings of band tolerance and optimisation. However, one group of authors has suggested that computerised analysis of gels may be more useful for identifying identical isolates than reliably differentiating unrelated ones (Van Belkum *et al.*, 1998). The gels in the current study were compared by eye rather than using computer software because the photographic images were unable to be resolved to the quality required by the available software, which has been an issue raised in other studies (Murchan *et al.*, 2003; Van Belkum *et al.*, 1998). Certainly, the ability to use computer software for this study would have made analysis of this large number of isolates easier with less subjectivity. Ideally, the gels should have been re-run using photographic equipment which was able to resolve the images to the quality required for use of computer-assisted analysis, but this was beyond the scope of this study as the equipment

was unavailable and the time required to repeat the PFGE analysis on over 500 isolates would have been prohibitive.

The interpretation of the relatedness of MRSA isolates is also complicated by the fact that MRSA is very clonal and isolates may have similar or identical PFGE patterns with no epidemiological link, which has prompted some to change to very conservative evaluation (even regarding a single band difference as significant), particularly in detecting outbreak strains among endemic disease (Goering, 2004). Conversely, because bacteria are likely to undergo genetic events over time, some have suggested that using the criteria of a single genetic change, that is, 2-3 bands difference, to represent the same strain may not be valid in patients from whom MRSA is repeatedly isolated over several months (Hartstein et al., 1995). Blanc et al studied the constancy of PFGE restriction patterns in long-term carriers of MRSA by comparing with another typing method to determine clonality (Blanc et al., 2001). These authors found that when comparing the first with subsequent isolates from the same patient, those with 1-6 fragment differences on PFGE were clonally related whereas those with 14-24 fragment differences were not, however they did not comment on strains with 7-14 bands difference. Because of all of these variables, it may be necessary to perform more than one typing method to establish more fully the relationship between isolates and above all, relating the molecular typing back to the clinical situation and epidemiologic data is paramount (Goering, 2004). In this study, detailed epidemiologic data, such as bed numbers, were not available to assist interpretation, but the fact that all isolates were taken from patients in a single ICU over several months is a strong epidemiological link.

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The MRSA isolates were subtyped into 21 different types (differing by more than six bands), although over 80% fell into two types (Type 1 and Type 2). Most MRSA isolates in Victoria fall into two major groups, Aus EMRSA-2 and Aus EMRSA-3 (Turnidge, 2003). These are the same using multiple typing systems (urease, phage, coagulase, ST and SCCmec) but differ only by PFGE type and mercury susceptibility. This study was designed only to compare isolates with each other and not to group into standardised well-characterised strains, such as Aus EMRSA –2 and –3. The fact that 21 PFGE groups were found in this study may be accounted for by several reasons. Protocols and running conditions may have varied between our molecular laboratory and that used to characterise these other strains. Because of the close geographical and chronological relationship of these isolates, a very conservative interpretation of the Tenover criteria was used, particularly for small shifts of bands (personal communication, Richard V. Goering). Once again, because the Tenover criteria can be used only compare with the parental type and not between types, it may be that the different types were actually closely related to each other, but still not related to the parental type. Further analysis of the isolates using a third typing method was beyond the scope of this study and computer-assisted analysis was not possible for reasons outlined above.

This study showed that patients could be colonised by multiple types of MRSA. It also showed that clinical isolates were of the same PFGE type in 74% of patients. Data were not available to analyse whether clinical isolates were significant or colonisers and only the first clinical isolate from a patient was available for testing. Patients could also carry different PFGE types at the same time in different sites or different types at different times.

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An analysis of temporal relationship between these isolates was not made. The significance of these findings with further evaluation is found in the Discussion section of Chapter 4.

3.4.3 RiboPrinter®

Use of the RiboPrinter® provided time-efficient, standardised and automated typing of the MRSA isolates. Only a proportion of the isolates was typed in this study, with the choice based on taking a representative sample of PFGE types. The RiboPrinter® assigns a RiboGroup to each isolate after comparing it to other banding patterns that it has encountered before and stored in its database. A RiboGroup is created by combining individual pattern data and making an average composite pattern. A similarity coefficient is calculated depending on location and intensity of the bands. Isolates are assigned a different RiboGroup if their similarity coefficients vary by more than 0.93. As with all methods of molecular analysis, use of the RiboPrinter® requires visual interpretation and comparison with epidemiological data to paint the full picture. In this study, the majority of isolates were assigned to RiboGroups R1 and R6, however, visual inspection of the banding and the dendrogram suggests that these isolates were in fact very similar and may have been separated into different RiboGroups because of a slight difference in intensity of the bands. Although a standardised amount of specimen is supposed to be analysed, it is possible that there were small variations in the amount of isolate picked off the subculture plate, which may account for some differences in banding density. When a cut-off of 85% similarity was used when examining the dendrogram, the isolates fell into three main

clusters (if the last isolate, Patient U, isolate 3G is ignored because of the probable partial lysis described in section 3.3.4.1). Two clusters consisted of only one patient each (MM and LL). All of the other patients had similar banding patterns on inspection and fell into the third cluster. However, this cut-off value of 85% for RiboPrinting is an arbitrary one, based on visual inspection of the dendrogram with no validation. McDougal *et al* used an 80% cut-off value for comparison of PFGE types in a large study of MRSA, but this was validated using epidemiological and other typing data (McDougal *et al.*, 2003).

3.4.4 Comparison of PFGE and RiboPrinting

In this study, comparison of PFGE types and RiboGroups showed that PFGE and RiboPrinting were about equally discriminatory. Individual RiboGroups were divided into up to nine PFGE types (if all subtypes of Type 1 were considered as one group) and individual PFGE groups were separated into up to 11 RiboGroups, rendering it difficult to say that one was more discriminatory than the other. However, because of the fact that interpretation of the PFGE bands gave only a relative comparison between types, comparison with the RiboPrinter® may not be valid. In addition, if the exact relationship between all the PFGE types could be determined using computer-assisted software, there may be even fewer PFGE types delineated. The only ways to resolve this would be to determine the exact relationship between all the PFGE types using a dendrogram and/or type all isolates using a third system and compare results with epidemiological information.

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There have only been two direct comparisons of RiboPrinting and PFGE for MRSA published. In one, which was conducted during an MRSA outbreak, the RiboPrinter® was unable to discriminate between 14 isolates that had been differentiated into several clusters/types by PFGE and randomly amplified polymorphic DNA (RAPD) PCR analysis (Fung *et al.*, 2001). Diekema et *al* found PFGE to be more discriminatory than RiboPrinting, with four to ten PFGE types within some RiboGroups, although a difference of four or more bands was defined as a different type in the PFGE analysis (Diekema *et al.*, 2000). Hollis *et al* found that 22 isolates of *S. aureus* in five RiboGroups could be further differentiated into two or three PFGE groups, although a three band difference was used to differentiate between PFGE types and these results may not be able to be extrapolated to MRSA, because of its high clonality (Hollis *et al.*, 1999).

Struelens *et al* have classified subtyping methods into two broad categories, *comparative* and *library* typing methods (Struelens *et al.*, 1998). Comparative methods are used to differentiate strains that are closely related from those that are unrelated and are best suited for outbreak investigation where only local relevance is important. These methods must be highly discriminatory and include PFGE, RAPD and rep-PCR typing. Library typing methods, such as multilocus sequence typing (MLST), are used to map the longer-term evolution of an organism which occurs in prospective surveillance. These methods must be highly reproducible and stable with standardised nomenclature and the ability to analyse and store patterns using computer software. These methods have broad epidemiological relevance and are used for the longer term monitoring of geographic spread and shifts in prevalence of epidemic and endemic clones (Pfaller *et al.*, 2004). The RiboPrinter® is an

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example of an emerging library system. Establishment of the HARMONY protocol may allow PFGE to become a library typing method (Murchan *et al.*, 2003), but using individualised protocols and interpretation using the Tenover criteria, as in this study, allows it only to be used as a comparative method. This delineation of typing methods into these two categories further explains why PFGE as it was performed in this study cannot be validly compared with the RiboPrinter®.

3.5 Conclusions

Although antibiotic susceptibility testing is performed routinely in the microbiology laboratory, does not require high levels of expertise, is not labour intensive, costly or time-consuming, its discriminatory ability is not as good as PFGE in most situations. It may be useful particularly for discriminating non-multiresistant community acquired strains from multi-resistant hospital acquired strains or for occasional outbreak strains with a distinctive susceptibility pattern.

The most important question examined in this study was whether the RiboPrinter® was as useful as PFGE. In terms of labour and time requirements and turnaround time to generate results and analyse bands, the RiboPrinter® was far superior to PFGE. The RiboPrinter® requires about 30 minutes of hands on time, is highly reproducible, takes one working day to generate results and presents them in a convenient format that can be relatively easily interpreted by eye or by using computer-assisted analysis. PFGE takes four to five days to generate a result, is labour intensive and requires high levels of technical expertise to

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analyse by eye or alternatively requires computer-assisted analysis. The cost of equipment and costs for analysing each isolate are far greater for the RiboPrinter® than for PFGE, although cost effectiveness studies have not been formally performed. In terms of expertise, the RiboPrinter® probably requires a lower level of experience to become proficient, largely because most steps are automated. In addition, results are analysed automatically. Despite this, however, a certain level of expertise is still required to interpret results.

In this study, PFGE was able to distinguish a similar number of strains to the RiboPrinter® and thus seemed to be about equally discriminatory. The RiboPrinter® was clearly able to distinguish strains that were distinctive (for example, R16 and R8) and this was corroborated by a different PFGE type and, in some cases, a different antibiogram. However, there were several groups of isolates with similar appearance which were further distinguished by PFGE typing and vice versa. Discriminatory power is particularly important for MRSA, which is very clonal, and it is important to be able to detect small differences between isolates, especially for detection of outbreaks.

This study has highlighted the limitations and difficulties associated with some typing methods. Visual interpretation of banding patterns may be difficult, especially for large numbers of isolates. The Tenover criteria are valuable, but still have an element of subjectivity and are most useful for differentiating multiple strains from a parental or outbreak strain. A limitation of this study was that because only visual interpretation using

the Tenover criteria was used, the PFGE types were assigned using a comparison to the parental type, but assignment into absolute types taking into consideration relationships between the groups was not possible. On the other hand, the RiboPrinter® compares isolates with each other. This makes comparison between the two typing methods difficult. Determination of the relatedness of isolates using computer-assisted analysis of PFGE banding patterns would allow legitimate comparison of PFGE and RiboPrinting and should be used in further studies to compare the relative discriminatory power of the two typing methods before any firm conclusions can be drawn.

Ultimately, there currently exists no perfect typing system for MRSA. Different methods (comparative or library) may be suited to different purposes, such as outbreak investigation, assessing effectiveness of intervention programs or long term analysis of *S. aureus* lineages (McDougal *et al.*, 2003; Struelens *et al.*, 1998) and may be complimentary. The choice of typing method(s) chosen should depend upon the purpose of the typing and the facilities and expertise available. It is also imperative that any results should be taken in the context of the clinical and epidemiological setting and not considered in isolation. The RiboPrinter® provides a convenient, serviceable typing system, but at present its use is limited by availability and cost. Because of its reliability and the expertise available, PFGE will probably continue to be widely used. Further larger comparative studies are needed, which include assessment of cost effectiveness.

4 CHAPTER 4. INTRODUCTION OF A NEW HAND HYGIENE AGENT TO REDUCE MRSA IN THE INTENSIVE CARE UNIT

4.1 Introduction

It is believed that the major mode of MRSA transmission is via the contaminated hands of health-care workers and it is known that MRSA can be removed from hands using adequate hand washing or disinfection. There are multiple reasons for poor compliance with optimal hand washing recommendations (see Chapter 1 for references). These include insufficient time to comply fully because of recommended duration of washing, inaccessible products, pressure of time, absence of good role models and skin irritation. Use of waterless alcohol based hand hygiene products may provide the solution to some of these problems. Programs to improve hand hygiene using these products have shown improved rates of compliance with hand hygiene, reduced transmission of MRSA and decreased nosocomial infections. Often these programs have also involved several other simultaneous interventions, including monitoring and feedback of compliance levels with hand hygiene, active surveillance for MRSA, single room placement and contact precautions for MRSA colonised or infected patients with placement of alert signs. One such program also included a rotation of posters aimed to improve hand hygiene which were designed by individual wards and completed by graphic artists (Pittet et al., 2000).

Others have shown that introduction of these new hand hygiene products with a brief educational campaign have not improved compliance (Muto *et al.*, 2000).

Extensive programs which include all or even some of these components are resource intensive, requiring dedicated funding and increased personnel for compliance monitoring, screening, laboratory processing, poster production and rotation. The level of resources required for this kind of project is significant and may not be sustainable in the long term, depending on the funding source. It is impossible to determine which is the most important component of the program because of the multi-faceted approach. If the belief that contaminated hands are the major mode of transmission is correct, optimal hand hygiene alone should reduce transmission of MRSA. This has not been tested, however, although the National Institute of Allergy and Infectious Diseases (NIAID) in the United States is commencing a randomised-controlled trial comparing improved hand hygiene and Standard Precautions with active surveillance and Contact Precautions for MRSA and VRE colonised patients (personal communication, W. Charles Huskins, Bacteriology and Mycology Study Group protocol chair). This trial is randomising intensive care units (who have not previously used active surveillance and contact precautions) to either active surveillance with contact precautions or standard precautions and a hand hygiene promotion campaign, with the primary outcome measure being the incidence density of new colonisations with MRSA and VRE.

The aim of this project was to reduce MRSA acquisition in the Alfred Hospital ICU using a three-pronged approach. This involved the introduction of a new hand disinfection agent, SteriGel+®, placement of alert signs to identify MRSA colonised patients and hospital-wide dissemination of information regarding the project using the Public Relations department with feedback of results. This involved a multi-disciplinary team which was overseen by the Infection Control and Hospital Epidemiology Unit.

The previous screening study (Chapters 2 and 3) found that multiple swabs taken from the same site in the same patient were not consistently positive and that some patients had initial positive swabs with subsequent negative ones. Because of the intensive screening for MRSA that was being undertaken in this study to determine the outcome, results from all the swabs were used in an analysis of the screening, including sensitivity and consistency of results. This is not an issue that has been examined well in the literature and yet it impacts on recommendations for frequency of screening, recommended sites for screening and whether a negative swab truly represents clearance of colonisation. Because the screening program included twice weekly swabbing, timing of MRSA acquisition was also determined.

4.2 Objectives

4.2.1 Primary

- To determine whether introduction of a new hand hygiene product in a multi-faceted intervention project would reduce MRSA acquisition in the Alfred Hospital ICU

4.2.2 Secondary

- To determine the sensitivity of MRSA detection by swabbing various combinations of anatomical sites (nose, throat, groin, axilla)

- To determine the consistency between results from the four anatomical sites swabbed on multiple occasions and compare with results obtained from clinical samples

- To determine the acceptability of the newly introduced hand hygiene product

- To assess usage of hand hygiene product using distribution data

4.3 Methods

4.3.1 Study design and setting

This was a prospective cohort study of patients admitted to the Alfred Hospital intensive care unit between 16th December 2002 and 30th September 2003 inclusive. The primary outcome was the proportion of patients who newly acquired MRSA during their ICU stay.

These results were compared with those identified in the MRSA screening project described in Chapter 2. The study involved three main components:

- Introduction of a waterless, alcohol based hand disinfectant gel, SteriGel+®

- Placement of an "**Antibiotic Resistant Organism**" sign in the cubicle of patients colonised with MRSA

- Hospital-wide publicity and feed-back about the project

This study was endorsed by the Alfred Hospital Infection Control Committee and the hospital executive as a quality improvement initiative. The ICU has been described in Chapter 2. Because of a structural problem which was unrelated to this current project, immunocompromised patients, including transplant patients, had been admitted to a geographically separate intensive care ward (2C) from April 2002, eight months prior to the commencement of this project. In the present study, if a patient was transferred directly between the main ICU and ward 2C, the admission was considered as one ICU admission. If a patient spent any period of time outside these areas and was then readmitted, this was classed as a separate admission. During this project, another study was commenced involving antibiotic prescribing habits before and after commencement of regular ICU ward rounds by an infectious diseases physician, although this was not expected to impact on MRSA acquisition.

4.3.2 Description of interventions

4.3.2.1   Description of SteriGel+®

SteriGel+® (Les Enterprises Solumed Inc, Quebec City, Canada) is a waterless, alcohol-based gel containing 70% ethylalcohol, 0.5% chlorhexidine, an emollient and a fragrance. It is available in 500ml pump packs and 150ml pocket sized containers (see Figure 4-1). Approval was given by the Therapeutic Goods Administration (TGA) to use this product on a trial basis and the project was approved by the Alfred Hospital Ethics Committee. This product was chosen on the basis that it contained alcohol and chlorhexidine and was the most popular product trialed amongst nursing and medical staff. At the time of this study, there were a limited number of alcohol based hand hygiene products available commercially in Victoria.

Four to five pump packs of SteriGel+® were placed at convenient sites in each patient cubicle and each permanent ICU staff member was given their own pocket-sized container. Distribution of these pocket containers was monitored by the Infection Control and Hospital Epidemiology Unit, with new bottles only being distributed upon return of the old empty one. An information leaflet was distributed to all staff with each bottle (see Appendix 1). Staff were instructed to use SteriGel+® as a substitute for hand washing in all situations except where the hands were visibly soiled, in which case hand washing with detergent-based products and water was recommended. Products available already in the ICU (Bioprep®, Microshield® and Hexol®) were left in place to allow staff the choice of

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hand hygiene product. All staff, including nurses, doctors, physiotherapists, radiographers and support staff were encouraged to use SteriGel+®.
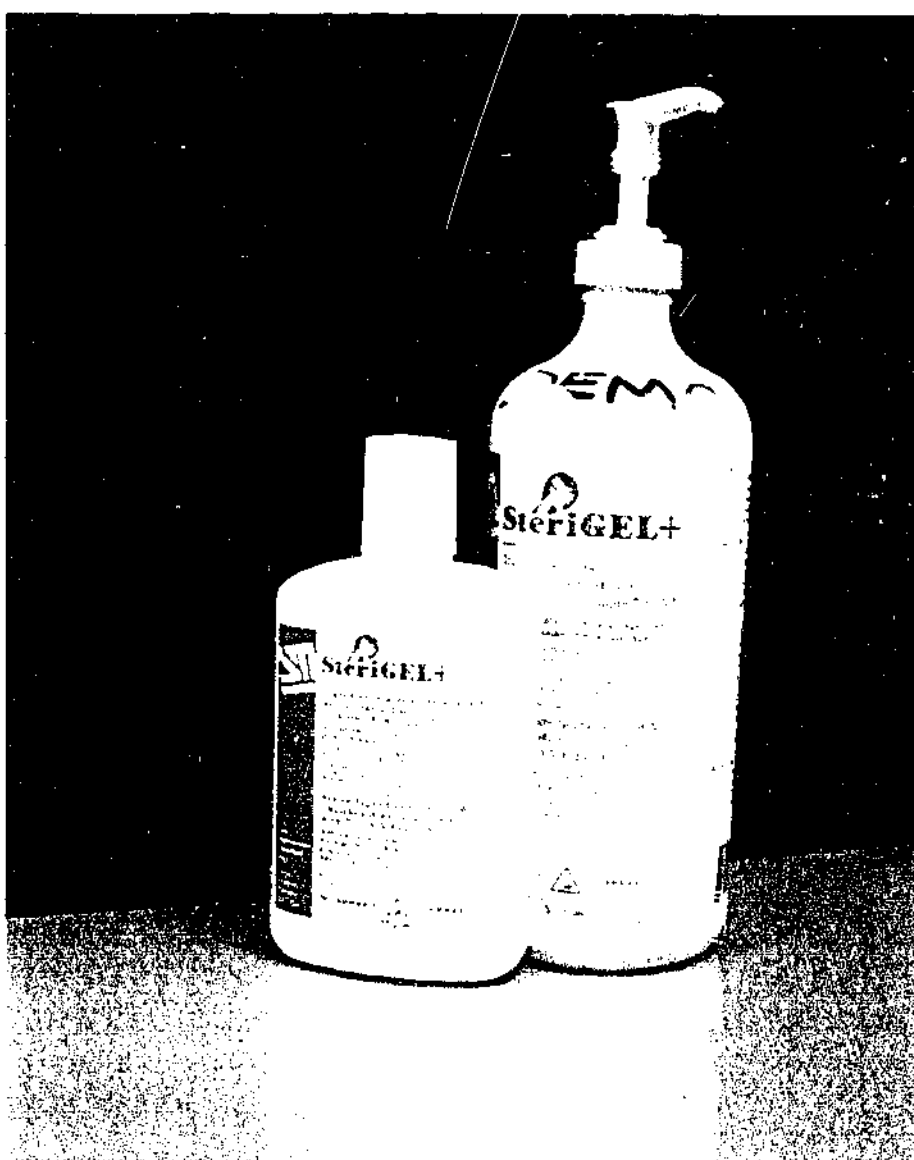
Figure 4-1 Pocket sized and pump pack of SteriGel+®

Adverse reactions to SteriGel+® were reported to ICU nurse managers or to Infection Control and Hospital Epidemiology Unit staff. The affected staff member was then referred to the Allergy and Immunology Clinic for further assessment. Seven months after commencement of the project, user acceptability was determined using a modified version of the assessment tool distributed by Les Enterprises Solumed (Appendix 2).

Because of lack of suitable resources, compliance with hand hygiene could not be directly observed and assessed. Volume of product distributed per 1000 patient days was used as a surrogate marker of compliance. This has been used by other authors, but usually in conjunction with formal observation (Pittet *et al.*, 2000) and is recommended in the Guideline for Hand Hygiene in Health-Care Settings as a performance indicator (Centers for Disease Control and Prevention, 2002). This method of assessment may give an indication of the quantity and pattern of usage, but is unable to quantitate whether hands are being disinfected at all appropriate opportunities.

At the beginning of the project, staff were given a plain language statement concerning the project (Appendix 1) and several in-service lectures were given, including to support staff. Aside from informal discussion about the hand gel, there was no ongoing formal education campaign conducted for promotion of the product.

4.3.2.2 Placement of alert sign

Once MRSA had been isolated from a screening swab, the microbiology laboratory was required to notify a member of the Infection Control team. A red sign, with the words "Antibiotic Resistant Organism" (see Appendix 3) was placed in the patient's cubicle to alert treating staff and others. Infection Control team members were available to discuss the issue with patients or their family members if required. The numbers of signs placed was counted and the reasons for non-placement of a sign were also recorded.

4.3.2.3 Feedback and dissemination of information regarding project

Feedback was disseminated to several groups and consisted of several components:

1. Feedback of compliance with screening (described in Section 4.3.3)

2. Feedback of results of numbers of new clinical MRSA isolates using statistical process control charts. These are commonly used in industry and increasingly in the healthcare sector, particularly in infection control (Benneyan, 1998; Benneyan, 1998). They are used as a mechanism to monitor and improve performance and in one institution, as a tool for feeding back MRSA rates with a subsequent 50% reduction (Curran *et al.*, 2002). The control charts were not used as an intervention in this study, but were used as a method of disseminating raw numbers of MRSA isolates in relatively simple terms. Control charts were not used in this study as an outcome measure, but as a timely feedback tool. Clinical and not screening isolates were used in these control charts and no attempt was made to determine whether the isolates were significant for the purposes of the feedback.

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The number of MRSA isolates per month were charted with three control lines, the centre line, the upper control limit and the lower control limit, which corresponded to the mean (or expected value) and three standard deviations above and below this line respectively. In general, if rates occur within these control lines, they are said to be "in statistical control". If they go above (or below) the control limits, the rate is deemed to be "out of control" and a cause is sought. Other criteria may also be used to determine if a rate is out of control, although still within the upper and lower control limits; these are designated as "supplementary criteria". Rule 1 states that eight consecutive values occurring on the same side of the centre line constitutes a "within limits" lack of control. The type of chart used was a simplified Stewhart C chart, which assumes constancy of the monthly ward census (Benneyan, 1998; Benneyan, 1998).

3. The results of compliance and new MRSA clinical isolates were given to ICU staff in their communications book and on the ICU infection control notice board. Results were also conveyed to nurse managers, key medical staff and administration via personalised memorandums. The public relations department was used to disseminate information regarding the hand gel and the project throughout the hospital. Other media used included "The Alfred Matters", a newsletter which is published regularly and distributed around the hospital, where results were presented and interviews with Infection Control team members were published (see Figure 4-2). The project was also reported in the Herald-Sun newspaper (the most widely read daily newspaper in the state of Victoria). For the purposes of this project, there was no formal assessment of the effects of the feedback campaign.

Bringing Infection Control into the Public Arena

Alfred Matters:

Infection control program coordinator Glenys Harrington (right) with Gabby Burdeau, Nat Ballan and Carlos Scheinkestel from Intensive Care.
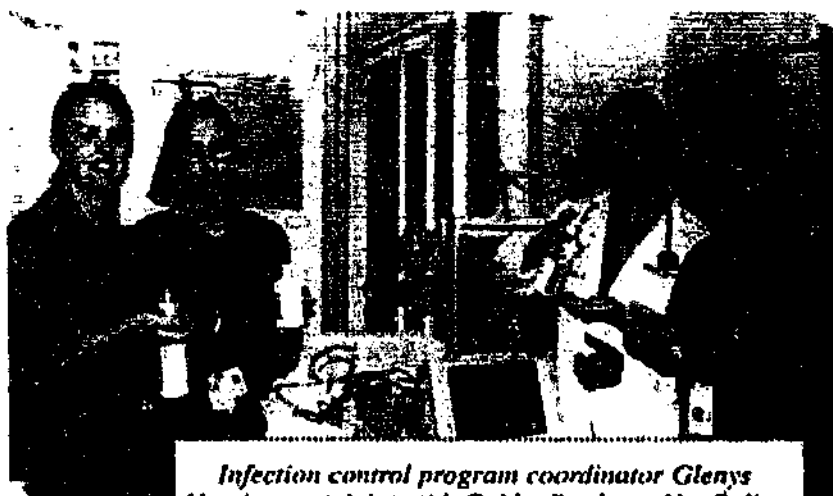
Initiatives to monitor and control the spread of antibiotic resistant organisms will be trialed at The Alfred from this month.

Figure 4-2 Use of hospital media for feedback concerning hand hygiene

4.3.3 Screening and compliance

ICU patients were screened on admission and discharge and every Monday and Thursday using nose, throat, groin and axilla swabs. To measure compliance with the screening protocol in this study, the proportion of patients who were swabbed was calculated by the microbiology scientist receiving and processing the swabs each day. The percentage compliance was calculated by the following formula:

(Number of swabs sets received/number of eligible patients) x 100

The number of eligible patients was determined using a daily computer census of the admissions, discharges and in-patients on Mondays and Thursdays. This information was calculated every fortnight for the general, surgical and immunocompromised sections of the ICU. It was communicated to the ICU nursing and medical staff, as well as the hospital executive. When compliance levels were considered to be suboptimal, a letter was sent to the hospital management for action.

4.3.4 Clinical samples

The date of the first of any MRSA positive clinical samples for a patient was also recorded. Results of clinical samples were available from 1997. A clinical sample was defined as any specimen that was sent at the request of the treating doctor and not as part of the current screening. Whether the clinical sample was a significant result was not determined for this study, therefore some of these isolates may have been colonisers. The number of days between positive clinical and screening samples was determined. For patients who had positive clinical samples but no positive screening samples, the relationship of the date

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of the first clinical sample to the ICU admission dates was determined. Because subtyping of all isolates was not available for this study and because of the potentially long periods of time between the first clinical sample and the screening sample without knowledge of samples taken in between, there was uncertainty regarding the exact relationship between the clinical samples and the screening samples. As a result of this uncertainty and because the definitions had been determined prior to the study and therefore prior to knowledge of any discordance between clinical and screening samples, clinical samples were not used in any of the calculations regarding acquisition or sensitivity of swabs.

4.3.5 Microbiological processing of swabs

This has been described in Chapter 2.

4.3.6 Statistical analysis

Proportions were calculated with 95% confidence intervals and were compared using a χ^2 test. Agreement between different swabbing sites was calculated using a Kappa statistic. Stata software was used for all calculations. A p-value of <0.05 was considered to be statistically significant

4.4   Results

4.4.1   Patient population

A total of 3730 swab sets (nose, throat, groin and axilla) were taken over the period from 16th December 2002 to 30th September 2003 inclusive. Swabs were taken from 1181 patients who had 1306 admissions to either the main ICU, 2C or both during that time period; for analysis, these were considered to be separate admissions. The mean length of ICU stay was 6.1 days (median 2, range <1-160 days). The mean age was 56.6 years (range 15-94 years) and 907 (65.5%) were male. Characteristics of the current study patients compared with the previous screening study patients are shown in Table 4-1.

Table 4-1 Characteristics of study population

Patient characteristic	Current study (number=1306)	Previous study (number=1328)
Hospital unit		
Trauma	293 (22.4%)	265 (20.0%)
Cardiothoracic surgery	337 (25.8%)	436 (32.8%)
Other	676 (51.8%)	627 (47.2%)
Length of ICU stay		
≤1 day	525 (40.2%)	493 (37.1%)
2-7 days	475 (36.4%)	563 (42.4%)
>7 days	306 (23.4%)	272 (20.5%)
Age group		
<50 years	431 (33.0%)	421 (31.7%)
50-70 years	507 (38.8%)	490 (36.9%)
>70 years	368 (28.2%)	417 (31.4%)
Gender		
Male	907 (69.5%)	887 (66.8%)
Female	399 (30.5%)	441 (33.2%)

4.4.2 Compliance with screening

Compliance with screening was determined fortnightly for admission, discharge, Monday and Thursday swabs. The total figures for compliance overall are shown in Table 4-2. These calculated figures (4082 swabs taken) do not equate to the total number of swabs actually processed (3730). Possible reasons include an error in calculation of the figures at

the time. Because the overall number of swabs received could not be determined until the screening was finished and the compliance was determined prospectively, it was impossible to reconcile the numbers retrospectively. In addition, the number of swabs taken on each of the four occasions is not equal (for example 1042 admission swabs received in the compliance figures compared with 1088 admission swabs actually processed) because of some reclassification of labels depending on the actual date a swab was taken correlating with admission and discharge dates. For example, a swab labelled "Thursday" was counted as a Thursday swab for the compliance figures, but if this fell on the discharge day, it was relabelled as "Discharge" swab for the purposes of the subsequent analysis.

Table 4-2 Compliance with swabbing

Timing of swab	Number (percentage) of eligible patients who had swabs taken
Admission	1042/1241 (84.0%)
Discharge	809/1298 (62.3%)
Monday	1069/1218 (87.8%)
Thursday	1162/1351 (86.0%)
Total	**4082/5108 (79.9%)**

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4.4.3 Acquisition of MRSA

1088 patients had an admission swab taken, of which, 75 (6.9%, 95%CI 5.5-8.6) were MRSA positive. 889 patients had a discharge swab taken, of which 132 (14.9%, 95%CI 12.5-17.2) were positive. 675 patients had both admission and discharge swabs taken where the admission swab was negative. Of these, 58 (8.6%, 95%CI 6.6-11.0) had a positive discharge swab, that is 8.6% of patients acquired MRSA using the definition used in Chapter 2 (admission and discharge swabs only considered). If the two ICU locations were considered separately, 49/534 (9.2%, 95%CI 6.9-12.0) of patients in the main ICU and 8/137 (5.8%, 95%CI 2.5-11.2) in the immunocompromised unit (Ward 2C) acquired MRSA. Acquisition of MRSA (in both areas combined) using only admission and discharge swabs compared with acquisition in the first screening period (prior to the dedicated nurse and during her employment) is shown in Table 4-3.

Table 4-3 Comparison of proportion of patients acquiring MRSA (using admission & discharge swabs only)

	1st study period	2nd study period
Pre-dedicated nurse	30/335 (9.0%)	NA
Dedicated nurse	33/219 (15.1%)	NA
Total	63/554 (11.4%)	58/675 (8.6%)

NA – not applicable as dedicated nurse not employed in second study period

319/1306 (24.4%) of patients had only one swab set taken and 987 (75.6%) had two or more taken (range 1-28). Of these 987 patients, the first swab was negative for MRSA in 904. Of these 904, 116 (12.8%, 95%CI 10.7-15) had a positive second swab, that is 12.8% of patients acquired MRSA if any two swabs were considered.

Table 4-4 shows the proportion of patients in various medical units that acquired MRSA (using the same definition as the first screening study). Of note is the marked reduction in the proportion of trauma patients acquiring MRSA (32.3% versus 12.7%).

Table 4-4 Proportion of patients in different medical units acquiring MRSA in two studies (using only admission and discharge swabs)

Unit	Number (percentage) acquiring MRSA in 1st study	Number (percentage) acquiring MRSA in 2nd study
Other	27/231 (11.7%)	33/316 (10.4%)
Trauma	31/96 (32.3%)	22/173 (12.7%)
Cardiothoracic surgery	5/227 (2.2%)	3/186 (1.6%)
Total	**63/554 (11.4%)**	**58/675 (8.6%)**

4.4.4 Timing of MRSA acquisition

Of the 116 patients who acquired MRSA (using any two sets of swabs), the mean time to the first positive swab set from the negative swab set was 8.3 days (median 6, range <1-42 days). In order to determine whether the timing of MRSA acquisition after ICU admission could be reasonably approximated to these figures, the number of days to the first swab set being taken was determined. The average number of days between admission and the first swab set being taken was 0.6 (median <1, range <1-131 days [some of these patients were admitted prior to the commencement of the study]). 868/904 (96.0%) had their first swab set taken within two days of admission. Of the 116 patients who acquired MRSA, the mean number of days between admission and the first swab set taken was 2.2 days (median <1, range <1-131 days) and 89.7% of these patients had their first swab set taken within two days of admission. These figures allow a rough approximation of the number of days between negative and positive swab sets to the average length of stay in ICU prior to MRSA acquisition. The exact number of days to acquisition is impossible to determine as patients were swabbed only twice per week and some of these swab sets were missed. For the 116 patients who acquired MRSA, the average number of days between the last negative and first positive swab sets was 3.3 (median 3, range <1-7 days), with 89.7% having four or less days between the negative and positive swab sets. In other words, for most patients who acquired MRSA, the timing can be determined with an accuracy of up to three to four days.

4.4.5 Analysis of anatomical sites positive for MRSA

4.4.5.1 Anatomical sites positive for MRSA

There were 686 swab sets where MRSA was isolated from at least one site and 224 patients who had MRSA isolated from at least one site. Table 4-5 shows the proportion of the total number of positive swab sets that was positive at each anatomical site and the proportion of the total number of MRSA colonised patients that was positive at each site.

Table 4-5 Anatomical sites positive for MRSA

Anatomical site	Number of swabs sets positive at this site	Percentage of all positive swab sets	Number of patients positive at any time	Percentage of all positive patients
Nose	444	64.7%	155	69.2%
Throat	465	67.8%	160	71.4%
Groin	391	57.0%	151	67.4%
Axilla	205	29.9%	97	43.3%
Total number	686*	*	224*	*

* Numbers do not add up to total as more than one site positive in many swab sets

4.4.5.2 Frequency of combinations of positive sites

Table 4-6 shows the frequency of combinations of sites that were positive in any individual swab set. Nose and throat and nose, throat and groin being positive were almost equally

the most common combinations (16.8% and 16.5% respectively), with all four sites being

positive the next most common (13.1%).

Table 4-6 Frequency of combinations of anatomical sites positive in each swab set

Anatomical site positive for MRSA	Number of all positive swab sets	Proportion of all positive swab sets
Nose only	49	7.2%
Throat only	66	9.6%
Groin only	84	12.2%
Axilla only	20	2.9%
Nose & throat only	115	16.8%
Nose & groin only	25	3.6%
Nose & axilla only	5	0.7%
Throat & groin only	29	4.2%
Throat & axilla only	7	1.0%
Groin & axilla only	24	3.5%
Nose, throat & groin only	113	16.5%
Nose, throat & axilla only	33	4.8%
Throat, groin & axilla only	12	1.8%
Nose, groin & axilla only	14	2.1%
Nose, throat, groin & axilla only	90	13.1%
Total	686	100.0%

4.4.5.3 Sensitivity of combinations of sites for detecting MRSA colonised patient

The sensitivity of swabbing different combinations of anatomical sites in each patient was calculated using being positive at any site at any time as the denominator (224). These results are shown in Table 4-7. This indicates, for example, that if the nose only was swabbed in an individual patient, the sensitivity of detecting this patient to be MRSA colonised at some stage was 69.2%.

Table 4-7 Sensitivity of combinations of swabbing sites for detecting MRSA carrier

Anatomical site swabbed	Number of MRSA carriers detected	Sensitivity
Nose only	155	69.2%
Throat only	160	71.4%
Groin only	151	67.4%
Axilla only	97	43.3%
Nose or throat	183	81.7%
Nose or groin	198	88.4%
Nose or axilla	175	78.1%
Throat or groin	203	90.6%
Throat or axilla	180	80.4%
Groin or axilla	167	74.6%
Nose, throat or groin	216	96.4%
Nose, throat or axilla	198	88.4%
Nose, groin or axilla	206	92.0%
Throat, groin or axilla	211	94.2%
Nose, throat, groin or axilla	224	100%

4.4.5.4   Sensitivity of individual site for detection of positive swab set

Table 4-8 shows the sensitivity of an individual anatomical site or combinations of sites to detect a swab set that was positive at any site. Swab sets where one or more sites were missing were excluded, giving a total of 675 as a denominator. This indicates, for

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example, that the sensitivity of swabbing the nose for detecting MRSA colonisation at any

of the four sites in that swab set was 64.7%.

Table 4-8 Sensitivity of single swab for detecting swab set at any site

Anatomical site swabbed	Number positive	Proportion positive
Nose only	437	64.7%
Throat only	464	68.7%
Groin only	384	56.9%
Axilla	200	29.6%
Nose or throat	550	81.5%
Nose or groin	583	86.4%
Nose or axilla	498	73.8%
Throat or groin	604	89.5%
Throat or axilla	523	77.5%
Groin or axilla	423	62.7%
Nose, throat or groin	655	97.0%
Nose, throat or axilla	593	87.9%
Nose, groin or axilla	609	90.2%
Throat, groin or axilla	628	93.0%
Nose, throat, groin or axilla	675	100%

4.4.5.5   Agreement between swabbing sites

The Kappa statistic was calculated to determine the level of agreement between anatomical sites swabbed. These results are shown in Table 4-9. The agreement represents the proportion of swabs whose results were concordant, that is positive-positive or negative-negative at the two sites. The expected agreement represents the amount of agreement that would be expected by chance and the Kappa statistic, as a percentage, shows the percentage of the way between random agreement and perfect agreement. For example, by chance, we would expect 78.6% agreement between results for nose and throat swabs. In this study, the results were in agreement 94.5% of the time or 74% of the way between expected and perfect agreement. This is interpreted as substantial agreement between the two sites.

Table 4-9 Amount of agreement between anatomical sites swabbed

Anatomical sites swabbed	Agreement	Expected agreement	Kappa statistic	P-value	Interpretation
Nose & throat	94.5%	78.6%	0.74	<0.001	Substantial
Nose & groin	90.6%	80.11%	0.53	<0.001	Moderate
Nose & axilla	90.2%	83.9%	0.39	<0.001	Fair
Throat & groin	90.1%	79.7%	0.52	<0.001	Moderate
Throat & axilla	89.7%	83.4%	0.38	<0.001	Fair
Groin & axilla	91.5%	85.2%	0.43	<0.001	Moderate

4.4.5.6   Consistency between results

Two hundred and fifty-three patients had two or three swab sets taken on the same day, some inadvertently and some because of admission and discharge on the same day. 215/253 (85.0%) were taken from patients who never had any MRSA positive swabs during that admission, including the six patients who had three sets taken on the same day, that is, all two or three sets taken on the same day were negative for MRSA. 38/253 (15.0%) patients had two swab sets taken on the same day and were positive at some stage in their stay. The results of these swabs were concordant in 16/38 (42.1%), of which eight were two negative sets on the same day and eight were two positive sets on the same day. 22/38 (57.9%) had discordant results, that is, of two swab sets taken on one day, one was positive for MRSA and one was negative. In other words, of 253 patients who had more than one swab set taken on the same day, 22 (8.7%) had discordant results (one set positive and one set negative).

One hundred and sixty-three patients had between two and 20 swab sets taken where at least the first was positive for MRSA. These patients had up to seven different combinations of anatomical sites positive for MRSA in consecutive swab sets (see Table 4-10). For example, 13 patients had five positive swab sets taken with two to five different combinations of swabbing sites that were positive per patient. The various possible combinations of positive swabbing sites are shown in Table 4-6. 25/163 (15.3%) patients had the same combination of swabbing sites positive in all swab sets. One patient had four consecutive swab sets positive at the same site, three had three positive at the same site and 21 had two. 22/163 (13.5%) had a least one positive swab set followed by at least one

negative swab set. 12/163 (7.4%) were initially positive, becoming negative and then positive again in subsequent swab sets. One of these 12 changed status (positive to negative or vice versa) nine times over the course of the admission.

Table 4-10 Number of swab sets taken per patient (after and including 1st positive set) and number of combinations of positive sites in patients

Number of swab sets taken after and including 1st positive set	Number of patients	Number (range) of different combinations of anatomical sites positive per patient
2	54	1–2
3	37	1-3
4	22	1-4
5	13	2–5
6	15	2–6
7	7	3–6
8	2	3–4
9	2	2–4
10	3	3–6
11	1	6
12	2	4
14	1	7
15	1	3
16	1	6
20	1	5
26	1	7
Total	163	

4.4.6 Clinical samples

One hundred and seventy of 1306 patients (13.0%) had a positive clinical sample prior to or during the study period. One thousand one hundred and ninety-eight patients had a negative first or only swab set taken. Sixteen of these patients (1.3%) had a positive clinical sample prior to or on the same day as their first (negative) swab set was taken. The mean number of days between the positive sample and the negative first swab set was 266 (range 0-1604 [4.4 years], median 69 days). One hundred and fifty-four of 224 patients with any positive screening sample (68.8%) and 16 of 1082 patients who never had a positive MRSA screening sample (1.5%) had a positive clinical sample. Sixty-four of 154 (41.6%) had the positive clinical sample before the first positive screening sample and 90 (58.4%) had the positive clinical sample taken on the same day as or after the screening sample. The mean number of days between a positive clinical sample and a subsequent positive screening sample was 168 (median 19.5, range 1-1680 days [4.6 years]).

Seventy-six of 116 (65.5%) of patients who acquired MRSA (using any two swabs) had a positive clinical sample and 20/76 (26.3%) had a positive clinical sample prior to the positive screening sample. For these patients, the mean time between positive clinical and screening samples was 29.6 days (range 1-956 days). Forty-eight of 76 (63.2%) had the positive clinical isolate on the same day as the first positive screening sample. The remainder (8/76, 10.5%) had the positive clinical sample after the screening sample.

Of the 16 patients with a positive clinical but no positive screening sample, six (37.5%) were positive prior to or on the admission date, two (12.5%) became positive during the admission and eight (50%) became positive after the discharge date. If the number of new MRSA acquisitions was calculated using any negative first swab and a subsequent positive clinical swab, two extra acquisitions would have been found, altering the overall number of new MRSA acquisitions (using any two swabs) from 116 to 118.

4.4.7 Usage of hand gel

4.4.7.1 *Compliance*

Compliance with hand hygiene was not formally assessed by observation. As a surrogate measure, the volumes of Bioprep®, Microshield®, Hexol® and SteriGel+® that were distributed per 1000 patient days were calculated. The average amount per month of product was also calculated for the three products that were available in both periods. These results are shown in Table 4-11. The average amount of Bioprep®, Microshield® and Hexol® that was distributed per 1000 patient days was roughly similar in the periods before and during the study but an additional 42.7 litres of SteriGel+® per 1000 patient days was distributed. The total average amount of hand hygiene product distributed before and after the introduction of SteriGel+® was 78.9 and 120.6 litres per 1000 patient days respectively.

Table 4-11 Distribution of hand hygiene products in the ICU (litres per 1000 patient days)

Month	Bioprep	Microshield	Hexol	Sterigel
Jun-02	14.3	11.6	8.9	-
Jul-02	11.7	28.7	27.4	-
Aug-02	49.3	11.0	19.2	-
Sep-02	25.1	32.8	19.5	-
Oct-02	35.5	1.3	18.4	-
Nov-02	23.7	45.3	53.2	-
Dec-02	35.2	11.1	5.2	-
Dec 1-Dec 15	70.3	22.1	10.4	-
Average	35.4	21.9	21.6	-
Dec 16-Dec 31	70.5	23.5	22.2	216.4
Jan-03	23.8	19.9	21.9	1.0
Feb-03	25.5	33.3	19.1	44.0
Mar-03	44.5	25.3	21.6	17.9
Apr-03	35.2	29.4	20.2	21.7
May-03	34.2	25.9	11.4	23.4
Jun-03	34.5	21.7	9.6	25.8
Jul-03	10.9	25.5	18.8	20.0
Aug-03	10.4	16.2	14.5	35.5
Sep-03*	31.9	21.8	17.7	0.4
Average	33.8	25.5	18.6	42.7

* Patient day data was incomplete for September 2003 and was calculated from available data

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4.4.7.2   Acceptability

Two hundred and fifty-five user acceptability questionnaires were distributed, with only a 29.4% (75/255) response rate (Appendix 2). These results are shown in Appendix 4. These results show that the majority of respondents were satisfied with SteriGel+®. In summary, 89% reported that they liked SteriGel+® with 88% reporting that if SteriGel+® were available all the time in the ICU, they would use it.

4.4.7.3   Adverse reactions

The exact number of staff that used SteriGel+® was not available, but of all those who did, only one made an official complaint of a potential adverse skin reaction to the product. This staff member was medically assessed and it was felt that the skin condition was not related to SteriGel+®.

4.4.8 Other aspects of the project

4.4.8.1   Alert signs

"Antibiotic Resistant Organism" alert signs were placed in the cubicle of 72/224 patients who were found to be MRSA positive. Table 4-12 shows the reasons why alert signs were not placed in the cubicle of all patients.

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Table 4-12 Reason for failure to place "Antibiotic Resistant Organism" signs in patient cubicles

Reason	Number	Proportion
Patient gone to ward	71	46.7%
Patient discharged from hospital	1	0.7%
Missed	7	4.6%
Deceased	9	5.9%
Project finished by the time result known	8	5.3%
Unknown	56	36.8%
Total	**152**	**100%**

4.4.8.2   Feedback

Feedback regarding new clinical MRSA isolates was given on a regular basis to ward staff in the form of Stewhart control charts. The final chart is shown in Figure 4-3. This chart has 24 months of previous MRSA data and shows a reduction of eight consecutive points on the same side of the centre line.
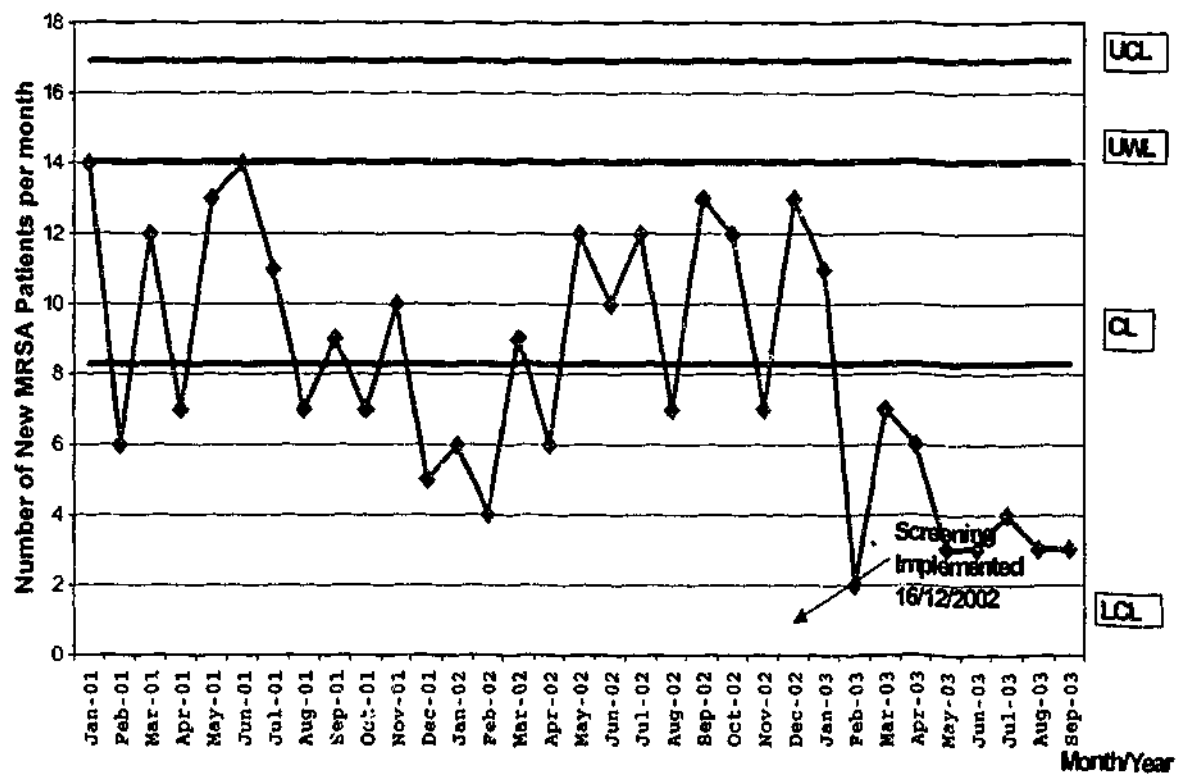
Abbreviations: UCL-upper control limit, UWL- upper warning line, CL-centre line, LCL-lower control limit

Figure 4-3 Control chart of new MRSA clinical isolates per month during study period

4.5 Discussion

4.5.1 Acquisition of MRSA and effectiveness of hand gel

4.5.1.1 Effect of the intervention

In this study, 8.6% of patients admitted to the ICU acquired MRSA using the definition of acquisition used in the first screening study (admission swab negative, discharge swab positive). The overall acquisition rate of MRSA in the first study was 11.4%, which indicates a reduction in the second. Because of the possible heterogeneity of the two studies, it was felt that the most appropriate type of analysis was descriptive, without formal application of a test to determine statistical significance, which may have been misleading and an oversimplification of the multiple factors likely to be operating. The studies were not necessarily comparable for the following reasons:

1. The second study started 19 months after the completion of the first. To avoid the problems inherent in effectively comparing the results of the intervention with historical controls, the ideal situation would have been to re-establish the proportion of patients acquiring MRSA in the time leading up to the intervention. In order to do this with reasonably narrow confidence intervals, it was calculated that approximately 3-4 months of re-screening patients would be needed (assuming good compliance with the screening protocol). This was unable to be performed because of lack of resources (laboratory staff and consumables) and because of a desire by the hospital executive to begin using the new hand hygiene product as soon as possible, once it had been sourced and the ethics approval had been given.

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2. During the first study, the compliance with the screening regimen varied widely according to the presence or absence of the dedicated nurse. Because of the differences in the methods used to calculate the compliance with screening in the two study periods, they may not be directly comparable. Lack of resources prevented a dedicated nurse being employed for the second study. Because the project had the approval of the hospital executive who received feedback on compliance levels with swabbing, it was felt that compliance would be better than in the first study prior to when the dedicated nurse was employed. This highlights another problem with using historical controls to test the effectiveness of an intervention.

3. Between the first and second studies, the second intensive care unit was opened for care of immunocompromised patients, although some other (non-immunocompromised) patients spent time in both the main and the immunocompromised unit (ward 2C). The proportion of patients that acquired MRSA was lower in the immunocompromised unit than the main ICU (5.8% versus 9.2%). This may not have been a real difference or may have resulted because from the difference in the numbers and types of patients in the two units. Colonisation pressure (number of MRSA-carrier patient-days/total number of patient-days) has been shown to be an independent risk factor for MRSA acquisition (Merrer et al., 2000). These figures are not available for the two ICUs in this study, but it is possible because of the different patient mix, the colonisation pressure may have varied in each of the units.

4. There were small differences in the characteristics of the study populations in the two studies, including medical unit, age, length of stay and gender (see Table 4-1), although the impact of this could not be determined.

5. Comparison of the two studies was not planned *a priori* before initiation of the first study. This was unavoidable because the first study was an exploratory study and the second was designed in response to the results of the first. Had MRSA acquisition rates been re-established prior to the second study, this issue could have been avoided.

Interestingly, the major reduction in the proportion of patients acquiring MRSA occurred in the trauma patients (32.3% versus 12.7%). A test of statistical significance was not reported because of the possible heterogeneity of the studies, but the size of the reduction suggests a real result. One could speculate that because of the knowledge of the high rate of MRSA acquisition during the first study, the staff caring for trauma patients were more particular with use of the new hand gel or other procedures. Another explanation is that the finding in the first study of trauma patients being at particular risk of acquiring MRSA was spurious. However, with a 95% confidence interval for the proportion of 23.1-42.6%, even the lower limit of 23.1% was higher than the upper limit of the 95% confidence interval of the proportion who acquired MRSA in the second study (8.0-17.8%), suggesting (but not proving) that a spurious result was unlikely. Another explanation is that there may have been differences between the trauma patients in the two time periods, for example, in the number or types of injuries, although this was unable to be confirmed.

It is also possible that reduction in the overall burden of MRSA in the whole unit had the greatest impact on trauma patients, because of the large numbers making up this group.

It is impossible to quantify the individual effects of the three parts of the intervention project (the hand gel, the "Antibiotic Resistant Organism" card and the feedback of results and dissemination of information regarding the project). It is likely that the resistant organism sign had the least effect because many of the patients did not have a sign displayed and because of the time taken to generate results, there was usually a delay in mounting the sign. Feedback of compliance results did not seem to have much effect on impact with the screening unless accompanied by letters from the hospital general manager. It is likely that the hand gel had the major impact on MRSA acquisition, with feedback probably serving to reinforce its use. It is also possible that there was a Hawthorne effect and that general practices, such as aseptic technique for procedures, improved because of the publicity surrounding the project. This is a potential issue when interpreting any intervention project.

Stewhart control charts were used as a method for timely feedback of results to the ICU staff. According to "Rule 1" (Benneyan, 1998; Benneyan, 1998), eight consecutive values on the same side of the centre line suggest a "within limits lack of control". In this study, it suggests a real sustained reduction in new MRSA clinical isolates. However, the numbers per month were small and, as previously stated, these charts were only used as a method of feedback of results and were not intended to be used as an outcome measure

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prior to the commencement of the study. Therefore, a detailed discussion of the advantages and disadvantages of control charts will not be undertaken here. Control charts were also constructed for several other wards that accepted many patients from the ICU, such as the trauma/orthopaedic ward. Reduction in the number of new MRSA isolates (one per patient) per month in these wards mirrored that seen in the ICU (charts not shown). Although there may have been many other factors operating, this consistent reduction in these other wards suggests that a reduction in MRSA in the ICU had flow-on effects to other areas. If this is a real phenomenon, this is important and warrants further examination.

4.5.1.2 *Compliance with hand hygiene*

Although compliance with use of the hand gel was not quantified, it was generally well accepted throughout the unit, with informal feedback indicating that it was well liked and tolerated, with no adverse effects. This was corroborated by the increased overall usage of hand hygiene products, essentially attributable to the introduction of SteriGel+®. The response rate to the user acceptability questionnaire (29.4%) was too low to draw any reliable conclusions, although the results were consistent with verbal feedback. During informal observation by infection control staff, it was clear that the nursing staff were using the gel frequently. There was strong opposition to withdrawal of the product at the end of the project. Although the data from the user acceptability survey were not sufficient to confirm that the product was found to be acceptable, it was unlikely that a significant problem or dislike of it would not have been noticed or reported.

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Compliance with the hand hygiene product is likely to be a significant factor in reduction of MRSA and other nosocomial infections. In this study, it may have been important because the product chosen (a gel containing ethylalcohol) may not produce as great a reduction in MRSA hand contamination as a rub containing isopropanol (Dharan *et al.*, 2003; Kramer *et al.*, 2002). However, it is not known exactly what log reduction *in vitro* correlates with a reduction of colonisation or infection (Boyce *et al.*, 2002; Diekema, 2002). The level of compliance may depend on the product chosen and several authors have described a preference for gel based products (McDonald, 2003; Boyce *et al.*, 2002; Diekema, 2002; Girard *et al.*, 2002; Hoffman *et al.*, 2002). The experience during this study would be consistent with the belief that acceptance of the product is an important factor in ensuring its use by staff. It is possible that improving compliance may be equally as important as the content of the product when trying to reduce transmission of nosocomially acquired organisms (Sickbert-Bennett *et al.*, 2004).

4.5.2 Screening results

4.5.2.1 Anatomical sites

This study showed that the various combinations of MRSA positive anatomical sites varied in individual patients from swab set to swab set, with patients frequently losing and acquiring colonisation at a particular site. Only 15.3% of patients with more than one swab set taken with at least one positive set had consistent colonisation of the same anatomical sites. Others went from positive to negative and back to positive again. Regardless of the reasons, this raises the question of what is the "gold standard" or

appropriate denominator for calculating sensitivity of swabbing sites for detecting MRSA. It also raises further questions about the definition of MRSA acquisition used in this and other studies.

4.5.2.2 Molecular epidemiology of isolates

A parallel study was performed for the purposes of an honours thesis where 377 of the screening and clinical isolates from 32 patients in this study were subtyped using PFGE and interpreted using the "Tenover criteria" (Tenover *et al.*, 1995; unpublished data, Lim, Marshall and Spelman, 2003). This study found that 16/32 carried multiple subtypes of MRSA and 4/32 patients carried different several types. Two of these four carried three different types at different times. Similar results were found in the analysis of PFGE types of isolates taken during the ICU screening project (Chapter 3), where it was found that patients could carry multiple types of MRSA at the same time or at different times. These data suggest that patients may lose and acquire different strains of MRSA with time, as well as losing or acquiring carriage at different sites. Although long term MRSA carriage has been demonstrated by several authors (Blok *et al.*, 2001; Scanvic *et al.*, 2001) and two studies have examined the cumulative yield of repeat swabbing (three times in one study and comparing repeat swabbing at one hour versus one day for *S. aureus*), no other studies have intensively screened patients over days to weeks as in the present study. Several authors have demonstrated that in the long term (months to years), patients may carry different subtypes of MRSA (Herwaldt *et al.*, 2002; Maslow *et al.*, 1995; Sanford *et al.*, 1994) and two have shown carriage of different subtypes within a two and three month period respectively (Herwaldt *et al.*, 2002; Maslow *et al.*, 1995).

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4.5.2.3   Definition of MRSA acquisition

If any two sets of swabs, with the first negative and the second positive, was used to define MRSA acquisition, 12.8% of patients acquired MRSA compared with 8.6% using only admission and discharge swabs in the definition.   Inclusion of more patients in the definition increased the denominator, but should not have increased the proportion that acquired MRSA.   This may have resulted from the fact that patients not infrequently lost and acquired MRSA at various screening sites and therefore, there may have been many false negative swabs. Swabbing the patient on multiple occasions is likely to have reduced the number of patients who may have acquired MRSA but not been detected because of a false negative discharge swab, as in the first study.   In other words, repeated swabbing would have increased the proportion of MRSA colonised patients detected, making it less likely that a colonised patient would have been missed through only taking admission and discharge swabs. The other explanation for possible false negatives may be because of technical reasons, such as poor swabbing technique or laboratory factors. This discrepancy highlights a problem which has been demonstrated in other areas of this study, that of definitions of MRSA acquisition. If clinical samples were also used in the definition, that is a negative first screening sample and a subsequent positive screening or clinical sample, a further two patients would have been classified as MRSA acquisitions. It was impossible to determine whether a negative swab was a true negative or a false negative in the absence of a true gold standard.

4.5.2.4 Clinical isolates

Use of results of clinical samples also added another dimension of complexity to the analysis. Sixteen patients had positive clinical samples taken from day 0 (that is, the same day as the negative screening set) to 1604 days before the study, yet had a negative first swab in this study. It was impossible to determine whether clinical samples were of the same type as the screening isolates because subtyping was not performed. Because some patients had their first clinical isolate up to several years before this study, it is possible that they had cleared that MRSA subtype and had subsequently re-acquired another and therefore were a true acquisition in this study. Only records of the first clinical isolate were available, so it was not known whether patients had MRSA isolated on more than one occasion prior to this study. Because of these uncertainties and because the definitions of acquisition were decided upon prior to the study, previous positive clinical samples were not taken into account in determining whether a patient did or did not acquire MRSA during this study or used in calculating sensitivity of swabbing sites.

4.5.2.5 Screening sites

This study found that the proportion of patients colonised in the nose, throat and groin to be similar (69.2%, 71.4% and 67.4% respectively) whereas the axilla was colonised in 43.3% of patients. It is difficult to directly compare frequency of isolation of MRSA from various anatomical sites with that found in the literature because of differences in the sites swabbed, the frequency of screening, the denominator used (for example, all patients or only MRSA infected patients), the patient group screened (for example, in ICU or another

unit) and whether there was an outbreak occurring or not. In this study, subjects were screened on multiple occasions, which increased the detection rate for each site. This is demonstrated by the fact that the proportion of total number of patients who were positive at a particular site at some stage was greater than those found in the total number of swab sets, which may loosely represent what would occur if patients were only swabbed once. In other words, the yield for detection of MRSA at an individual site was increased by repeated swabbing at that site, particularly in the groin and axilla. In general, the nose is believed to be most commonly colonised, with all of the other sites less commonly colonised. For example, one study during a hospital wide outbreak found the nose, throat, groin and axilla to be colonised in 88.4%, 41.2%, 22.1% and 11.2% of patients respectively (including repeated swabbing) (Coello *et al.*, 1994). Few studies have examined colonisation sites ICU patients. One study showed 84% of patients to be colonised in the nose, but did not give adequate figures for other sites (Girou *et al.*, 1998).

The relationship between the nose and throat makes intuitive sense because of the close anatomical connection between the two. This has not been borne out in other studies, but may be particularly strong in this study because of it being performed in an ICU where the majority of patients were intubated, many with nasogastric tubes present and undergoing nasopharyngeal suction, which may have impaired the normal anatomy of the area and facilitated spread between the two sites. Concordance of results between nose and throat swabs was substantial, probably for the same reason. This study found that the groin was almost as frequently colonised as the nose and throat. Several other studies have screened the perineum or groin and found a much lower rate of colonisation than the nose and or

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throat (Coello *et al.*, 1994; Gnanalingham *et al.*, 2003; Manian *et al.*, 2002). Again these discrepancies may be a result of the differences between the patient populations and the screening regimens in different studies.

Analysis of the sensitivity of the various combination of swabbing sites for detection of MRSA in an individual patient (allowing for multiple swabbing occasions) or in a single swab set (which is often all that is taken in other studies) has again highlighted the relative unimportance of the axi'la as a swabbing site, although if it were not swabbed, 3% of colonised patients would have been missed. Taking a nose swab only would have missed approximately 30% of colonised patients in this study. Others have similarly found the nose, throat and perineal swabs to give the highest sensitivity (Coello *et al.*, 1994). In the current study, taking of either a nose or throat swab was essential for maximal sensitivity of MRSA detection, although addition of a grcin swab to either or both of these incremented the result substantially.

The agreement between results of nose and throat swabs (Kappa 0.74) and the knowledge that either nose or throat swabs were necessary for maximal sensitivity of MRSA detection in an individual swab set or in a patient highlights the strong relationship between the two sites. This study also demonstrates that the groin may be an under-appreciated site of MRSA colonisation. Importance of MRSA colonised sites other than the nose may explain why not all patients with clinical infections are nasal carriers of MRSA. In two studies, only 82.2% and 84% of patients with *S. aureus* bacteraemia had an identical strain

colonising the nose (Pujol *et al.*, 1996; Von Eiff *et al.*, 2001). Amato *et al* have shown that for patients with continuous ambulatory peritoneal dialysis (CAPD) peritonitis, the most frequently colonised site with a strain of *S. aureus* that was identical to that causing the peritonitis was the catheter exit site (Amato *et al.*, 2001). Paterson *et al* found that 43.8% of liver transplant carriers with *S. aureus* infections never had previous nasal colonisation (Paterson *et al.*, 2003). The current study found that the groin was a relatively important site of MRSA colonisation, with a sensitivity for only swabbing the groin of 67.4%, which was nearly as high as only swabbing the nose (69.2%) for detecting an MRSA carrier. Rimland and Roberson have demonstrated that 59.8% of patients known to be MRSA positive in clinical samples were rectal carriers of MRSA compared with 53.0% who were nasal carriers (Rimland *et al.*, 1986). In a liver transplant unit, Squier *et al* found 93.2% of patients to be nasal and 57.3% to be rectal carriers of *S. aureus* (approximately half of which were MRSA). Patients who were both rectal and nasal carriers of *S. aureus* were significantly more likely to develop infection than nasal carriers alone (Squier *et al.*, 2002). The relationship between rectal and groin or perineal carriage of MRSA is not known and one could postulate that groin or perineal carriage is a surrogate marker for rectal carriage.

Importance of colonised sites other than the nose may also explain why pre-operative intra-nasal mupirocin has not been conclusively shown to reduce surgical site infections. However, the population in the current study was different from that in the mupirocin trials and it may not be reasonable to compare MRSA colonisation in unwell ICU patients with relatively healthy patients carrying (mainly) methicillin-sensitive *S. aureus* pre-operatively. Although one study has shown that intra-nasal mupirocin can eradicate hand carriage of *S. aureus* in healthy health-care workers, this may not be able to be

extrapolated to sick MRSA carriers. Harbarth *et al* have shown that intranasal mupirocin and antiseptic body washes do not eradicate multi-site MRSA carriage in ICU patients (Harbarth *et al.*, 1999). Another group has shown that only low concentrations of mupirocin reach the pharynx after intranasal application of mupirocin (Watanabe *et al.*, 2001), which may explain, for example, why intranasal mupirocin would not prevent a clinical infection in a throat carrier. Corroboration that non-nasal sites may be important in the pathogenesis of MRSA infection is also given by several studies that have demonstrated reduction in MRSA infections after enteral administration of anti-MRSA agents (mupirocin and vancomycin) (de la Cal *et al.*, 2004; Nardi *et al.*, 2001; Silvestri *et al.*, 2002).

4.6   Conclusions

This study has demonstrated success of a multifaceted program (primarily based on introduction of a new hand hygiene agent) for reduction of MRSA acquisition. This conclusion is strengthened by the results of Stewhart control charts in the ICU, with a possible flow on effect to other wards. It is also supported by the literature and is biologically plausible. Thus, although it was felt to be inappropriate to assess the results merely in terms of statistical significance testing because of methodological limitations, the available facts are all consistent with a reduction in new MRSA acquisitions as a result of the intervention.

The intervention only consisted of three facets, of which use of an alert sign was likely to be the least important. This means that use of a novel alcohol and chlorhexidine based gel with feedback of new MRSA clinical isolates was likely to be responsible for any reductions. This is significant because most other interventional programs shown to reduce MRSA transmission have included multiple components, including active surveillance and use of contact precautions and single room isolation (or cohorting) of MRSA colonised patients. These results suggest that MRSA rates can be reduced without resource intensive infection control interventions. Aside from the extra microbiologist to process MRSA screening swabs, the intervention was implemented with no extra infection control resources. This study also suggests that compliance with a hand hygiene product may be dependent on acceptability of the product to staff and that intensive programs are not required to ensure its use. It is not known whether this seemingly good compliance is sustainable in the long term.

Analysis of the screening results from this study has demonstrated that results from individual swabs can vary and may therefore be unreliable for use in definitions of MRSA acquisition rates and sensitivity. However, in the absence of a "gold standard", it is necessary to continue to use this method for MRSA detection. It is important to appreciate the variability and unreliability and therefore not to make unqualified statements when interpreting these results. Repeated swabbing over time is likely to give a more accurate picture of whether a patient is a carrier of MRSA and use of subtyping may assist in determining if recrudescence or re-acquisition of MRSA has occurred. This study also highlights the importance of using local data, as extrapolation from other studies

performed in different settings and which may have used different methods for detection may not be generalisable to other situations.

5 CHAPTER 5. RISK FACTORS FOR MRSA ACQUISITION IN TRAUMA PATIENTS IN THE INTENSIVE CARE UNIT I

5.1 Introduction

In the surveillance study described in Chapter 2, trauma patients were identified as being particularly at risk of being MRSA colonised on admission to the ICU and of acquiring MRSA during their stay. During the 9½ month study period, 96 trauma patients were able to be evaluated for possible MRSA acquisition in the ICU. Approximately one third (31/96, 95%CI 23-43%) of these patients became colonised with MRSA in the ICU, of whom one third (10/31) developed clinical infections. Approximately 500-600 trauma patients are admitted to the Alfred Hospital ICU annually. If the results from the screening study are extrapolated, this could mean that 160-200 trauma patients per year may become MRSA colonised with up to 70 developing MRSA infection. Clearly this represents a major problem for this group of patients. As half (31/63) of all the MRSA acquisitions were in trauma patients, this also represents a major problem for the ICU. If MRSA colonisation and infection could be reduced in trauma patients, this may have a significant impact on the overall rates of MRSA in this ICU. By gaining an understanding of the risk factors for MRSA acquisition within this group of patients, we are likely to be able to improve and direct specific preventive measures.

There are no published studies examining risk factors for MRSA acquisition in trauma patients. The aim of this study was to examine factors that may be associated with trauma patients becoming MRSA colonised. This was a cohort study of the 96 trauma patients identified in the previous screening study. Because of the fixed number of subjects and lack of relevant literature in this area to guide its design, the aim was to use it as an hypothesis generating study. The study presented in Chapter 6 was a larger prospective cohort study designed using the results of this study to examine similar variables using more appropriate statistical analysis.

5.2 Aim

To determine whether certain patient factors were associated with an increased or decreased risk of acquiring MRSA in trauma patients in the intensive care unit.

5.3 Methods

5.3.1 Setting

The setting for this study was the Alfred Hospital ICU, which has been described in Chapter 2. This study was approved by the Alfred Hospital Ethics Committee.

5.3.2 Study design and population

This was a cohort study using prospectively and retrospectively collected data. Study subjects were identified during the previous screening project (see Chapter 2) and consisted of the 96 trauma patients who had both admission and discharge swabs taken with no MRSA isolated from the admission swab. MRSA acquisition was defined as having a negative admission and positive discharge screen for MRSA. This study compared the 31 patients in this group who acquired MRSA with the 65 who did not.

5.3.3 Data collection and definitions

Patient data was routinely collected by the Intensive Care Department Database, the Department of Trauma Surgery Database and Health Information Services coding system. Additional information was collected retrospectively from the patients' medical records.

Data collected included age, gender, length of ICU stay, mechanism of trauma, body sites injured, extent of trauma using the Injury Severity Score (ISS) (Stevenson *et al.*, 2001), surgical and other procedures performed, use of medical devices and antibiotics received. Mechanism of trauma was divided into two groups: motor vehicle accident (including car/truck/motor cycle accident, pedestrian and cyclist) and other (including falls, assault, crush injuries and collision other than with a motor vehicle). Several measures of injury severity were collected (APACHE II, Glasgow Coma Scale [GCS], Injury Severity Score [ISS], New Injury Severity Score [NISS], Trauma Injury Severity Score [TRISS] and the Revised Trauma Score [RTS] (Senkowski *et al.*, 1999)). The Injury Severity Score was

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chosen as the measure of severity of trauma as it is widely used as a scoring system in trauma research (Hurr *et al.*, 1999) and accurately calculated values were available for all patients in this study. It has been shown to be predictive of nosocomial infections in trauma patients in some studies (Hurr *et al.*, 1999; Croce *et al.*, 2001) but not as predictive as the NISS in others (Jamulitrat *et al.*, 2002). Scores below 16 are considered to be minor trauma and those 16 and above are considered to be moderate to severe trauma.

Body site injured and surgery were categorised in a simplified anatomical way which would allow practical identification of specific patients who may be at particular risk. Head injury included only those with intracerebral injury/ haemorrhage, but not those with a minor head injury, defined as a brief loss of consciousness and GCS of 13-15 with normal computerised tomography (CT) scan of the brain and no neurological sequelae. Surgical procedures were included if they occurred on the day of the trauma, en route to the ICU or during the ICU stay. Other procedures and medical devices analysed included presence of a tracheostomy (surgical or percutaneous), central venous catheter, arterial line, Swan-Ganz catheter, intracranial pressure monitor, naso- or oro- gastric tube with or without enteral feeds, intercostal catheter, bronchoscopy and duration of mechanical ventilation. Antibiotic administration was analysed as a dichotomous variable (received or not received) and did not include those given in the emergency department or as pre-operative prophylaxis in the operating theatre, as patients were sometimes transferred from other institutions and this information was often not available. Enteral and parenteral but not intrathecal or topical antibiotics were included.

5.3.4 Statistical analysis and power calculation

Univariate odds ratios (OR) with 95% confidence intervals were calculated for all variables using logistic regression. Odds ratios were adjusted for length of stay in a multivariate logistic regression. A p-value of <0.05 was considered to be statistically significant. Data were analysed using Stata software (StataCorp, College Station, TX).

With 96 subjects and for a risk factor with 33% prevalence in the 65 non-colonised patients, this study had 88% power to detect an odds ratio of four (risk factor having 67% prevalence in colonised patients) but only 33% power to detect and odds ratio of two (risk factor having 30% prevalence in colonised patients).

5.4   Results

5.4.1 Patient characteristics

Of the 96 patients in the study, 31 acquired MRSA and 65 did not. The average age was 39 years (range 15-82 years) and the mean ICU length of stay was 7.5 days (range <1-37 days). The mean ISS was 27 (range 9-50). The mechanism of trauma is shown in Table 5-1. There was no significant difference between the proportion of patients in each group with an ISS <16 or 16-75 (p-value = 0.4).

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Table 5-1 Mechanism of trauma

Mechanism of injury	Number	Proportion of total	Number with ISS 16-75	Proportion of each group with ISS 16-75
Car/truck accident	52	54.2%	45	86.5%
Motor cycle accident	13	13.5%	12	92.3%
Pedestrian/cyclist	12	12.5%	11	91.7%
Other (includes assault, falls, machinery injury, other collision)	19	19.8%	14	73.7%

5.4.2 Associations with MRSA acquisition

Information was collected on all patients except antibiotic data for one patient whose medical record was unavailable. Crude odds ratios for all variables and odds ratios adjusted for length of stay are shown in Table 5-2. Length of stay was a strong univariate predictor of MRSA acquisition (OR 13.7). The factors which remained significant after adjustment for length of ICU stay were laparotomy (OR 6.3), motor vehicle accident compared with other mechanisms of trauma (OR 10.4), receipt of ticarcillin/clavulanic acid (OR 4.5) and receipt of a glycopeptide (OR 5.9). Further division of mechanism of trauma into four variables (car/truck, pedestrian/cyclist, motorcycle, other) did not significantly alter results (not shown). Other variables, such as head injury (OR 3.0), craniotomy (OR 4.0), tracheostomy (OR 8.8), central venous catheter (OR 3.3), intracranial pressure monitor (OR 3.8), gastric tube with feeds (OR 15.1), intercostal catheter (OR 2.9) and bronchoscopy (OR 6.2) and receipt of other antibiotics, such as a third generation

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cephalosporin (OR 4.4), carbapenem (OR 19.6), aminoglycoside (OR 7.0), erythromycin (OR 12.1) and fluconazole (OR 6.0) were significant predictors on univariate analysis, but not after adjustment for length of ICU stay. Abdominal injury was not a significant predictor in the univariate (OR 2.1) or adjusted analysis (OR 1.6) and laparotomy still remained significant after adjustment of length of stay and abdominal injury (OR 9.1, 95%CI 1.4-57.3). Adjusting for other factors, such as ISS or mechanism of injury or both, did not significantly alter the odds ratios after adjustment for length of stay. Adjustment for severity of trauma using other scoring systems (New Injury Severity Score or Glasgow Coma Scale) gave similar odds ratios as using the ISS.

5.4.3 Laparotomy

Histories were available for 12/13 patients in this study who underwent laparotomy. All patients underwent their surgery on the same day as their ICU admission, except one who had it on the following day. Laparotomies were performed by five different surgical teams in six different theatres and often in conjunction with other procedures, such as orthopaedic or neuro- surgery, which were not associated with increased risk. Findings at laparotomy were sometimes multiple and included splenic rupture or laceration (four patients), lacerated or contused liver (five patients), free intraperitoneal air with no cause found (one patient), mesenteric tears (five patients), retroperitoneal haematoma (two patients), non-viable areas of bowel from a crush injury (one patient), adrenal haematoma (one patient) and bleeding gall bladder bed (one patient). Three patients underwent splenectomy, one repair of a splenic laceration, two packing of liver lacerations, four

oversewing of liver lacerations, one resection of small and large bowel, one formation of a sigmoid colostomy, two oversewing of serosal tears and one ligation of the Falciform ligament and hepatic vessels. Five patients had post-operative wound ooze, two developed post-operative ileus and one a subphrenic abscess. Four underwent re-operation, for reasons including removal of packs and revision of colostomy.

Table 5-2 Risk factors for MRSA acquisition in trauma patients in the ICU

Variable	MRSA not acquired (number)	MRSA acquired (number)	Crude odds ratio	95%CI	Odds ratio adjusted for LOS	95%CI
General						
Age						
< 25 years	20	10	1.0	~	1.0	~
25-45 years	25	11	0.9	0.3-2.5	1.2	0.3-4.2
> 45 years	20	10	1.0	0.3-2.9	1.6	0.4-6.0
Gender						
Female	22	7	1.0	~	1.0	~
Male	43	24	1.8	0.7-4.7	1.5	0.5-4.9
ICU length of stay						
< 7days	52	7	1.0	~	1.0	~
7 days or greater	13	24	13.7	4.9-38.7	~	~
Mechanism of trauma						
Other*	18	1	1.0	~	1.0	~
Motor vehicle accident**	47	30	11.5	1.5-90.6	10.4	1.2-93.7
Injury						
Head Injury	38	25	3.0	1.1-8.2	1.8	0.6-5.8
Face Injury	19	11	1.3	0.5-3.3	1.0	0.3-3.0
Spinal Cord Injury	3	2	1.4	0.2-9.0	0.8	0.1-6.9
Abdominal Injury	15	12	2.1	0.8-5.3	1.6	0.5-4.7
Thoracic Injury	41	21	1.2	0.5-3.0	0.8	0.3-2.4
Pelvic Injury	14	11	2.0	0.8-5.1	1.8	0.6-5.5
Orthopaedic Injury	56	28	1.5	0.4-6.0	0.7	0.1-3.6
Soft Tissue Injury	54	24	0.7	0.2-2.0	0.5	0.1-1.7
Surgery						
Craniotomy	7	10	4.0	1.3-11.7	2.5	0.7-9.1
Facial bone surgery	5	5	2.3	0.6-8.7	2.2	0.4-11.0
Thoracotomy	3	1	0.7	0.1-6.9	0.4	<0.01-5.6
Laparotomy	5	8	4.2	1.2-14.1	6.3	1.4-28.9

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Orthopaedic surgery	32	18	1.4	0.6-3.4	1.0	0.4-2.8
Soft tissue surgery	17	10	1.4	0.5-3.4	1.2	0.4-3.6

Other Procedures

Tracheostomy	7	16	8.8	3.1-25.4	1.7	0.4-6.8
Central venous catheter	40	26	3.3	1.1-9.6	1.1	0.3-4.1
Arterial line	52	28	2.3	0.6-8.9	1.0	0.2-4.7
Swan-Ganz catheter	1	3	6.9	0.7-68.8	1.7	0.2-18.4
ICP monitor	13	15	3.8	1.5-9.5	1.5	0.5-4.5
Gastric tube						
No gastric tube	27	2	1.0	~	1.0	~
Gastric tube, no feeds	13	1	1.0	0.1-12.5	0.7	0.1-9.5
Gastric tube with feeds	25	28	15.1	3.3-70.1	5.2	0.9-28.3
Intercostal catheter	17	15	2.9	1.2-7.1	2.4	0.8-6.9
Bronchoscopy	6	12	6.2	2.1-18.8	2.3	0.6-8.2
Ventilation						
None	23	2	1.0	~	1.0	~
1-5 days	32	9	3.2	0.6-16.4	2.5	0.5-13.6
> 5 days	10	20	23.0	4.5-117.6	3.3	0.4-30.8

Injury Severity Score

< 16	11	3	1.0	~	1.0	~
16-75	54	28	1.9	0.5-7.4	0.8	0.2-4.1

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Antibiotics ***						
Penicillin/Amoxycillin	6	6	2.3	0.7-7.9	1.6	0.4-7.0
Flucloxacillin	5	6	2.8	0.8-10.1	1.3	0.3-5.8
Ticarcillin/clavulanic acid	7	18	11.3	3.9-32.6	4.5	1.3-15.0
1st gen. cephalosporin	38	16	0.7	0.3-1.7	0.4	0.1-1.2
3rd gen. cephalosporin	8	12	4.4	1.6-12.5	2.3	0.7-7.8
Ceftazidime	2	2	2.1	0.3-15.9	0.5	0.1-4.0
Imipenem/Meropenem	2	12	19.6	4.0-95.3	5.5	1.0-30.3
Aminoglycoside	21	24	7.0	2.6-18.9	2.4	0.7-8.1
Glycopeptide	6	19	15.3	5.1-46.4	5.9	1.7-21.0
Erythromycin	1	5	12.1	1.3-108.8	3.2	0.3-30.4
Ciprofloxacin	3	5	3.9	0.9-17.6	0.9	0.2-4.5
Metronidazole	16	12	1.9	0.8-4.7	0.9	0.3-2.8
Fluconazole	2	5	6.0	1.1-32.7	1.5	0.2-8.8

* Includes falls, assault, crush injury ** Includes car, truck, motorcycle, pedestrian, cyclist

*** Other antibiotics not shown were not received by any subjects

Abbreviation: LOS-length of stay

5.5   Discussion

5.5.1 Major findings

5.5.1.1   Length of stay

Length of stay in the ICU was found to be a strong predictor of MRSA acquisition in this study, confirming findings from the Alfred Hospital MRSA screening (see Chapter 2) and other studies (Graffunder et al., 2002; Ibelings et al., 1998; Lucet et al., 2003). This may be because longer length of stay increases exposure time to MRSA. What is not certain is whether the risk of acquisition is constant for a given period of time at different times during the stay. Length of stay may be an indicator of severity of trauma or may be an

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indicator of pre-existing illness or co-morbidities. In this study, additional adjustment for severity of illness using the Injury Severity Score or other illness severity scores did not significantly change the odds ratios or confidence intervals for MRSA acquisition after adjustment for length of stay. It is also possible that the association between length of stay and MRSA acquisition exists because MRSA acquisition prolongs length of stay in the ICU because of predisposition to MRSA infection.

5.5.1.2   Receipt of antibiotics

Other authors have found receipt of antibiotics to be an independent risk factor in multivariate analysis for MRSA colonisation or infection, although these studies have used different methodologies and were performed on different populations, so are not directly comparable with the current study (Graffunder *et al.*, 2002; Mest *et al.*, 1994; Onorato *et al.*, 1999; Rezende *et al.*, 2002). It is not surprising that glycopeptide administration was associated with MRSA acquisition in this study, as these drugs are used to treat MRSA. Many of the patients who received a glycopeptide may have received it after acquisition of MRSA, therefore, only association and not causation can be concluded from the information available. In this study, receipt of ticarcillin/clavulanic acid was significant risk factor and the odds ratio for imipenem/meropenem receipt nearly reached statistical significance (OR 5.5, 95%CI 1.0-30.3); with larger subject numbers, this may become significant. Use of broad spectrum agents, including anti-pseudomonal penicillins and imipenem, has been shown to be an independent risk factor for MRSA colonisation or infection in HIV patients (Onorato *et al.*, 1999). Receipt of ticarcillin/clavulanic acid and carbapenems has been shown to be a risk factor for acquisition of vancomycin-resistant

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enterococcus (VRE) (Padiglione *et al.*, 2003) and this may be explained by its lack of activity against this organism and consequent selection for it. A similar explanation for the association of these antibiotics may also apply to MRSA acquisition, although other antibiotics without MRSA activity, such as cephalosporins, were not found to be associations in this study.

5.5.1.3 Mechanism of trauma

Mechanism of injury was the other independent association found in this study. A road traffic accident victim (car/truck, pedestrian, cyclist, motor cyclist) was at greater risk of acquiring MRSA than a patient who had suffered other mechanisms of injury. It is known that patterns of injury differ between different mechanisms of trauma (Cameron *et al.*, 1995), which may confer certain characteristics on patients making them more susceptible to MRSA acquisition. For example, certain mechanisms of trauma may result in more skin defects, such as open versus closed fractures, or require more surgical procedures, providing a portal of entry for organisms. Other factors may include the total number of individual injuries or the total number of invasive procedures performed per patient. This is only speculation, however, as there are no similar reported findings.

5.5.1.4 Laparotomy

Laparotomy was another unexpected association with MRSA acquisition in this study. There are no similar reports in the literature of it being a risk factor for MRSA colonisation or infection. At the time of the study, there was no outbreak of MRSA infections related to

a point source. There were several different findings at laparotomy and they were performed in different theatres by different surgical teams. Abdominal injury was not a significant risk factor and laparotomy may have been a marker for another factor which was not examined or was unable to be detected because of limited subject numbers. There were no outstanding features of patients who underwent laparotomy who acquired MRSA compared with those who did not and numbers were too small to make any meaningful statistical comparisons.

Abdominal injury was not a significant risk factor and even when adjusted for abdominal injury in addition to ICU length of stay, laparotomy remained significant. Croce *et al* found that need for any of emergency craniotomy, laparotomy or femoral fixation was an independent predictor of post-traumatic pneumonia, although the individual effect of laparotomy was not assessed (Croce *et al.*, 2001). In addition, the consequences of laparotomy on respiratory function and its role in development of pneumonia are more biologically plausible than its possible role in acquisition of skin or mucosal MRSA. A laparotomy wound could be a portal of entry for MRSA, but the relationship to nasal or mucosal MRSA colonisation is not clear. Most surgical site infections are believed to arise from endogenous MRSA colonisation of nasal mucosa, rather than the other way round. Results of abdominal wound swabs were not correlated with screening swabs for the purposes of this study.

5.5.2 Limitations of study

This study had several limitations. Firstly, it had a restricted sample size with inadequate power to detect factors with odds ratios below 3-4, hence, it was impossible to rule out small to moderate sized associations for risk factors that were found not to hold statistically significant associations in this study. Secondly, because timing of acquisition was not taken into account, predictor variables may have taken place after acquisition and therefore do no necessarily demonstrate causality. This was likely to have been the case for glycopeptide administration. A less plausible explanation is that glycopeptide use was a risk factor for MRSA acquisition. Similarly, length of stay in the ICU had a strong association, but it cannot be determined whether this was because of a longer exposure time or whether MRSA acquisition caused a prolonged length of stay. These issues are addressed in the study described in Chapter 6 where timing of MRSA acquisition is more accurately determined by more frequent screening of patients.

A third limitation of this study was potential selection bias. In the screening study from which these patients were drawn, less than half of the trauma patients had both admission and discharge swabs taken. 80% of all patients admitted during that time had at least one swab set taken. Patients who had one swab set taken were used as a control group for comparison with those who had both admission and discharge swabs taken, as data were not collected on patients who had no swabs taken. If trauma patients who had only one swab taken were compared with those who had both admission and discharge swabs taken, the two groups were very similar with respect to length of ICU stay, age and gender (see

Appendix 5). Although other differences between the patients who were swabbed on admission and discharge and those who were not cannot be excluded, these data indicate that there was unlikely to be any systematic bias in swabbing of patients which may have resulted in alteration of results.

5.6 *Conclusion*

This study found that length of stay was a strong predictor of MRSA acquisition and after adjustment for this, mechanism of trauma, laparotomy and receipt of ticarcillin/clavulanic acid or glycopeptides remained as predictors. Because of methodological limitations of this study, its results were used as the basis for estimating sample size for the larger study examining risk factors for MRSA acquisition in trauma patients described in Chapter 6. Nevertheless, this study is important as there is little literature dealing with MRSA and trauma patients. Given that high prevalence of MRSA is a major problem for many intensive care units and that trauma patients are predisposed to many types of nosocomial infections and particularly those caused by *S. aureus*, it seems reasonable to presume that MRSA would be a significant issue for severely injured patients in other institutions. Admission of high numbers of patients to specialist trauma referral centres gives us the unique opportunity to perform large studies in areas that have been identified as problematic, such as MRSA colonisation and infection. It is unlikely that many of the predisposing factors, except for receipt of antibiotics, can be altered, but if particular patients that are at higher risk of MRSA acquisition can be identified, preventive interventions may be directed at this group.

6 CHAPTER 6. DETERMINATION OF RISK FACTORS FOR ACQUISITION OF MRSA IN TRAUMA PATIENTS IN THE INTENSIVE CARE UNIT II

6.1 Introduction

In Chapter 5, a description was given of the cohort study conducted to determine risk factors for MRSA acquisition in trauma patients in the ICU. This was a study examining subjects identified during the screening project described in Chapter 2. Prior to this first study, because of the paucity of literature in the area of MRSA and trauma patients, calculation of the subject numbers required to conduct an adequately powered study was difficult to perform. The aim of the study reported in this chapter was also to examine risk factors for MRSA acquisition in trauma patients in the ICU. In this study, patients were more frequently swabbed, allowing for time of acquisition of MRSA to be taken into account in the analysis. This study also defined acquisition of trauma slightly differently because more frequent swabbing was performed.

6.2   Aims

The aims of this study were to determine whether certain patient factors were associated with an increased or decreased risk of acquiring MRSA in trauma patients in the ICU and whether the results found in the first cohort study could be confirmed.

6.3   Methods

6.3.1   Setting

The setting for this study was the Alfred Hospital ICU, which has been described in Chapter 2. This study was approved by the Alfred Hospital Ethics Committee.

6.3.2   Study design and population

This was a prospective cohort study of trauma patients who were patients in the Alfred Hospital ICU between 16th December 2002 and 30th September 2003. Subjects for this study were trauma patients who were screened for MRSA as part of the hand hygiene project (see Chapter 4). In this study, patients were screened on admission, discharge and every Monday and Thursday, using nose, throat, groin and axilla swabs. The subjects who acquired MRSA were compared with those who did not whilst in the ICU.

6.3.3 Data collection and definitions

Patient data were routinely collected by the Intensive Care Department Database, the Department of Trauma Surgery Database and Health Information Services coding system. Other information regarding antibiotic administration was collected by the Infection Control and Hospital Epidemiology Unit. Any discrepancies in the data were corrected by examination of the patient medical record. Data from all sources were collated using an Access database..

MRSA acquisition was defined as a patient who had any two sets of swabs taken, with the first being negative and the second being positive for MRSA. Other definitions and data collected were identical to that described in Chapter 5. In the present study, however, dates of procedures, surgery and antibiotic receipt were recorded and taken into account in the analysis. Days between the first (negative) swab set and either the first positive swab set (the outcome or failure event) or the last negative swab set (for those who did not acquire MRSA) were used to calculate the patient days at risk. Procedures that occurred after MRSA acquisition or after the last negative swab set were excluded. Duration of ventilation was not included, as information was not available regarding whether ventilation took place before the first swab or after the last swab set was taken.

6.3.4 Sample size estimation

For the power calculation, a conservative MRSA acquisition rate in trauma patients of 20% was used (based on the actual 30% acquisition rate found in the screening study described in Chapter 2). With 325 subjects, for a univariate analysis, it was calculated that there would be at least 80% power to detect risk ratios of two for any risk factor with a prevalence between 25% and 50% and to detect risk ratios of three for any risk factors with a prevalence between 5% and 80%. This number was inflated by 10% for a multivariate analysis, giving a desired number study subjects of approximately 360.

6.3.5 Statistical analysis

For patients who acquired MRSA, the duration of exposure was calculated from the day of the first negative swab set to the day of the first positive swab set. For patients who did not acquire MRSA, the duration of exposure was calculated from the first negative swab set to the last negative swab set. Days on each antibiotic were accounted for in the analysis. The association of the predictor variables with acquisition of MRSA was assessed by Cox proportional hazards regression models for univariate and multivariate analysis. A forward stepwise selection was performed using a p-value <0.05 for inclusion and >0.1 for exclusion.

In order to compare results with those in the cohort study described in Chapter 5, a similar logistic regression was performed using the same definition (any two swab sets) and the definition used in Chapter 5 (admission and discharge swabs only). A p-value of <0.05 or

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95% confidence interval that did not cross one were considered to be statistically significant. Data were analysed using Stata software (StataCorp, College Station, TX).

6.4 Results

6.4.1 Patient characteristics

During the study period, there were 293 trauma patients admitted to the ICU. Six had a first swab set taken which was positive for MRSA. There were 239 patient admissions where more than one MRSA swab set was taken, with the first set being negative. The other patients either had no swabs or only one swab set taken. In other words, there were 239 subjects in this study, of whom forty-one (17.2%) acquired MRSA and 198 (82.8%) did not. The mean age was 43.6 years (range 15-85 years). There were 70 females (29.3%) and 169 males (70.7%). Forty-one (17.2%) had an ISS of ≤15 or less, 83 (34.7%) an ISS of 16-25, 79 (33.1%) an ISS of 26-40 and 36 (15.0%) an ISS of >40. One hundred and sixty-six (69.5%) were road traffic accident victims and 73 (30.5%) suffered other forms of trauma.

One hundred and seventy-three patients had both admission and discharge swabs taken where the first was negative. Using the definition of MRSA acquisition from the first cohort study (described in Chapter 5) of a negative admission and a positive discharge swab set would have given 22 (12.7%) MRSA acquisitions.

The overall rate of MRSA acquisition was 2.6 per 100 patient days (95%CI 1.9-3.5). The Kaplan-Meier survival curve for the probability of remaining MRSA free over time is shown in Figure 6-1. Rates of MRSA acquisition per 100 patient days at risk for individual variables are shown in Table 6-1. As an example, the Kaplan-Meier survival curve for the probability of remaining MRSA free with and without a tracheostomy are shown in Figure 6-2. This graph shows that the probability of remaining MRSA free drops rapidly in patients without a tracheostomy.
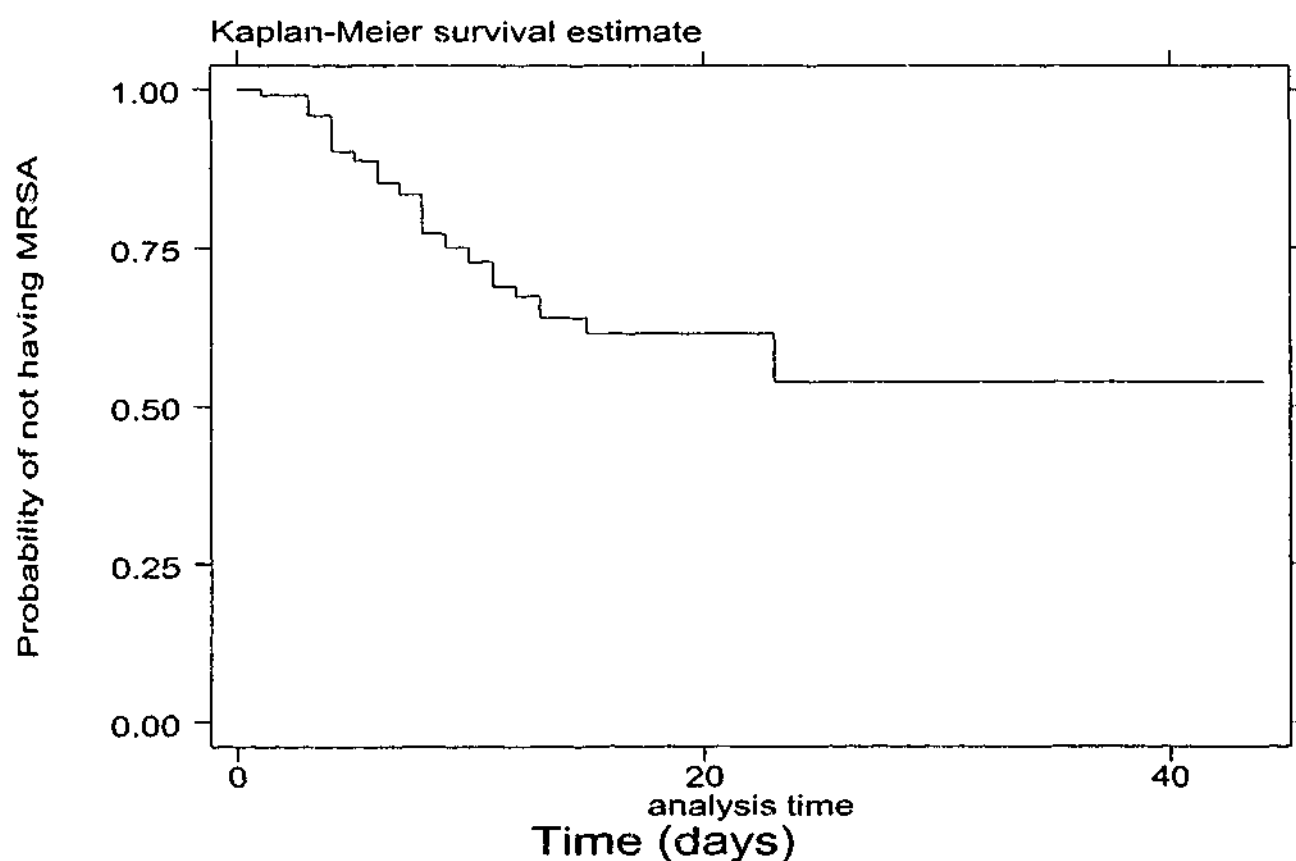
Figure 6-1 Probability of remaining MRSA free over time

Kaplan-Meier survival estimates, by Trache
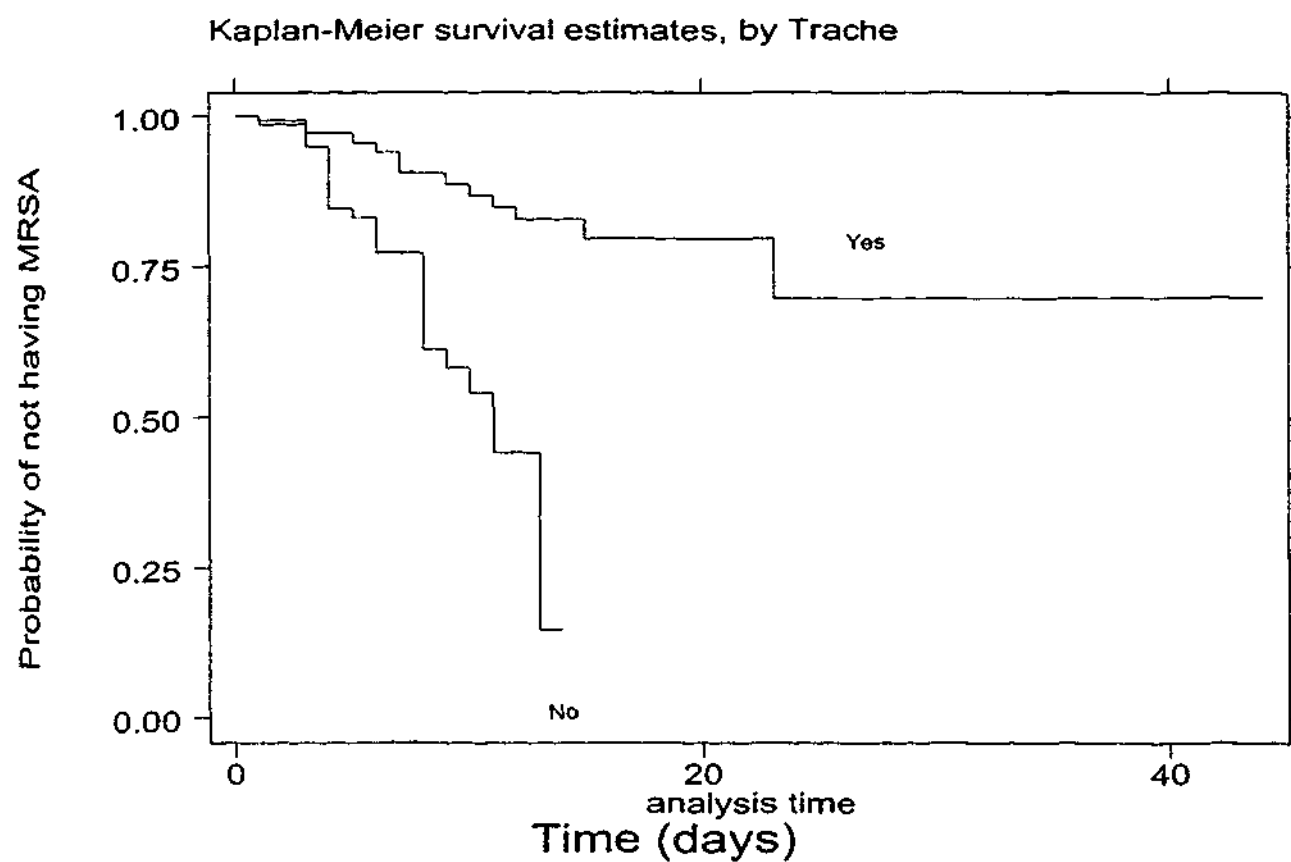
Abbreviation: Trache – tracheostomy (Yes = present, No = absent)

Figure 6-2 Probability of remaining MRSA free with and without tracheostomy

Table 6-1 Numbers and rates of MRSA acquisition for predictor variables

Variable	MRSA not acquired	%	MRSA acquired	%	Rate per 100 days
Overall rate	198	82.8	41	17.2	2.6
General					
Age					
<25 years	54	91.5	5	8.5	1.7
25-45 years	68	84.0	13	16.0	2.4
>45 years	76	76.8	23	23.2	3.1
Gender					
Female	56	80.0	14	20.0	2.7
Male	142	84.0	27	16.0	2.6
ICU length of stay					
<7 days	121	95.3	6	4.7	2.1
7 days or greater	77	68.8	35	31.2	2.7
Mechanism of trauma					
Other	61	83.6	12	16.4	2.7
Motor vehicle accident	137	82.5	29	17.5	2.6
Injury					
Head Injury	113	85.6	19	14.4	2.0
No	85	79.4	22	20.6	3.5
Face Injury	59	84.3	11	15.7	2.0
No	139	82.3	30	17.7	3.0
Spinal Cord Injury	6	100.0	0	0	0
No	192	82.4	41	17.6	2.6
Abdominal Injury	50	76.9	15	23.1	3.1
No	148	85.1	26	14.9	2.4
Thoracic Injury	107	78.7	29	21.3	2.8
No	91	88.4	12	11.6	2.3
Pelvic Injury	27	67.5	13	32.5	4.4
No	171	85.9	28	14.1	2.2
Orthopaedic Injury	133	80.1	33	19.9	2.9
No	65	89.1	8	10.9	1.9

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Soft Tissue Injury	148	80.0	37	20.0	2.9
No	50	92.6	4	7.4	1.3

Surgery

Craniotomy	31	79.5	8	20.5	2.4
No	167	83.5	33	16.5	2.7
Facial bone	6	60.0	4	40.0	5.0
No	192	83.8	37	16.2	2.5
Thoracotomy	8	80.0	2	20.0	1.9
No	190	83.0	39	17.0	2.7
Laparotomy	27	73.0	10	27.0	3.1
No	171	84.7	31	15.3	2.5
Orthopaedic surgery	72	78.3	20	21.7	2.8
No	126	85.7	21	14.3	2.4
Soft tissue surgery	66	76.7	20	23.3	3.2
No	132	86.3	21	13.7	2.2

Other procedures

Tracheostomy	60	83.3	12	16.7	1.3
No	138	82.6	29	17.4	4.4
CVC	148	78.7	40	21.3	2.7
No	50	98.0	1	2.0	1.0
Arterial line	191	82.3	41	17.7	2.6
No	7	100.0	0	0	0
ICP monitor	74	84.1	14	15.9	2.0
No	124	82.1	27	17.9	3.1
EVD	54	83.1	11	16.9	1.7
No	144	82.8	30	17.2	3.2
Gastric tube/ enteral feeds					
No gastric tube	31	88.6	4	11.4	8.1
Tube, no feeds	32	97.0	1	3.0	2.0
Tube with feeds	135	79.0	36	21.0	2.4
ICC	75	77.3	22	22.7	2.9
No	123	86.6	19	13.4	2.3
Bronchoscopy	48	78.7	13	21.3	2.0
No	150	84.3	28	15.7	3.1

ISS					
<16	38	92.7	3	7.3	2.3
16-75	160	80.8	38	19.2	2.6

Abbreviations: CVC-central venous catheter, ICP-intracranial pressure, EVD-external ventricular drain, ICC-intercostal catheter, ISS-injury severity score

6.4.2 Associations with MRSA acquisition

The results of univariate and multivariate analysis for the acquisition of MRSA are shown in Table 6-2. In the univariate analysis, factors which were significant were head injury (HR 0.5, 95%CI 0.3-1.0), tracheostomy (HR 0.2, 95%CI 0.1-0.4), external ventricular drain (HR 0.5, 95%CI 0.2-0.9), gastric tube and enteral feeds (HR 0.07, 95%CI 0.02-0.2), receipt of penicillin or amoxycillin (HR 3.5, 95%CI 1.6-7.6) and receipt of a macrolide (erythromycin or roxithromycin) (HR 0.2, 95%CI 0.1-0.7).

In the multivariate analysis, the variables which remained significant were presence of a central venous catheter (HR 10.0, 95%CI 1.2-87.0), gastric tube without enteral feeds (HR 0.03, 95%CI <0.01-0.3) and with enteral feeds (HR 0.05, 95%CI 0.01-0.2), tracheostomy (HR 0.2, 95%CI 0.1-0.4) and receipt of penicillin or amoxycillin (HR 4.6, 95%CI 2.0-10.5).

Table 6-2 Risk factors for acquisition of MRSA (univariate and multivariate analysis)

Variable	MRSA not acquired (Number)	MRSA acquired (Number)	Hazard ratio	95% CI	Adjusted hazard ratio	95% CI
General						
Age						
<25 years	54	5	~	~		
25-45 years	68	13	1.3	0.5-3.7		
>45 years	76	23	1.8	0.7-4.7		
Gender						
Female	56	14	~	~		
Male	142	27	1.0	0.5-1.9		
ICU length of stay						
<7 days	121	6	~	~		
7 days or greater	77	35	0.7	0.3-1.8		
Mechanism of trauma						
Other	61	12	~	~		
Motor vehicle accident	137	29	0.9	0.4-1.8		
Injury						
Head Injury	113	19	0.5	0.3-1.0		
Face Injury	59	11	0.7	0.3-1.3		
Spinal Cord	6	0	~	~		
Abdominal Injury	50	15	1.2	0.7-2.3		
Thoracic Injury	107	29	1.2	0.6-2.3		
Pelvic Injury	27	13	1.9	1.0-3.6		
Orthopaedic Injury	133	33	1.6	0.7-3.4		
Soft Tissue Injury	148	37	2.2	0.8-6.3		
Surgery						
Craniotomy	31	8	0.8	0.4-1.7		
Facial bone	6	4	2.0	0.7-5.7		
Thoracotomy	8	2	0.9	0.2-3.8		
Laparotomy	27	10	1.3	0.6-2.7		
Orthopaedic	72	20	1.2	0.6-2.2		

Soft tissue surgery	66	20	1.4	0.8-2.6		
Other procedures						
Tracheostomy	60	12	0.2	0.1-0.4	0.2	0.1-0.4
CVC	148	40	1.7	0.2-13.0	10.0	1.2-87.0
Arterial line	191	41	~	~		
ICP monitor	74	14	0.6	0.3-1.1		
EVD	54	11	0.5	0.2-0.9		
Gastric tube/enteral feeds						
No gastric tube	31	4	~	~		
Tube, no feeds	32	1	0.19	0.02-1.7	0.03	<0.01-0.3
Tube with feeds	135	36	0.07	0.02-0.2	0.05	0.01-0.2
ICC	75	22	1.3	0.7-2.3		
Bronchoscopy	48	13	0.5	0.3-1.0		
ISS						
≤15	38	3	~	~		
16-75	160	38	0.9	0.3-3.0		
Antibiotics*						
Penicillin/amoxycillin	22	12	3.5	1.6-7.6	4.6	2.0-10.5
Flucloxacillin	25	7	1.0	0.3-3.3		
Ticarcillin/clavulanate	58	15	0.6	0.3-1.4		
1st generation cephalosporin	99	30	0.6	0.2-1.8		
3rd generation cephalosporin	33	14	2.1	0.9-4.8		
Ceftazidime/cefepime	10	4	0.7	0.2-3.1		
Meropenem	44	21	0.8	0.3-1.8		
Aminoglycoside	75	30	0.9	0.4-1.9		
Glycopeptide	83	36	1.2	0.6-2.4		
Macrolide	50	17	0.2	0.1-0.7		
Metronidazole	46	16	1.0	0.4-2.5		

* Actual number of patients who received individual antibiotic shown, but for analysis, days on antibiotic used in calculations

There were insufficient subjects in one or other group (MRSA acquisition or not) on ciprofloxacin, fusidic acid, rifampicin, cotrimoxazole and clindamycin or with an arterial line or spinal cord injury to generate hazard ratios

6.4.3 Logistic regression

Logistic regression was performed on the current dataset using the same method as that used in the cohort study described in Chapter 5, where the dichotomous variable of MRSA acquisition or not was the outcome. Crude odds ratios and odds ratios adjusted for length of stay are shown in Tables 6-3. This table shows results when MRSA acquisition was define using any two swab sets. The only variable that remained significant when adjusted for length of ICU stay was presence of a tracheostomy, with an adjusted odds ratio of 0.3 (95%CI 0.1-0.7). Performing a similar logistic regression using only admission and discharge swabs in the definition of MRSA acquisition (as used in Chapter 5) gave roughly similar odds ratios (results not shown). Similarly, the only variable that remained significant when adjusted for length of ICU stay was presence of a tracheostomy, with an adjusted odds ratio of 0.2 (95%CI <0.1-0.6).

Table 6-3 Risk factors for MRSA acquisition (crude and adjusted odds ratios)

Variable	MRSA not acquired	MRSA acquired	Crude odds ratio	95% CI	Odds ratio adjusted for LOS	95% CI
General						
Age						
<25 years	54	5	1.0	~	1.0	~
25-45 years	68	13	2.1	0.7-6.1	1.6	0.5-5.1
>45 years	76	23	3.3	1.2-9.1	2.3	0.8-6.8
Gender						
Female	56	14	1.0	~	1.0	~
Male	142	27	0.8	0.4-1.6	1.0	0.5-2.2
ICU length of stay						
<7 days	121	6	1.0	~	~	~
7 days or greater	77	35	9.2	3.7-22.8		
Mechanism of trauma						
Other	61	12	1.0	~	1.0	~
Motor vehicle accident	137	29	1.1	0.5-2.2	1.0	0.4-2.1
Injury						
Head Injury	113	19	0.7	0.3-1.3	0.6	0.3-1.2
Face Injury	59	11	0.9	0.4-1.8	0.7	0.3-1.5
Spinal Cord	6	0	~	~	~	~
Abdominal Injury	50	15	1.7	0.8-3.5	1.5	0.7-3.2
Thoracic Injury	107	29	2.1	0.99-4.3	1.5	0.7-3.3
Pelvic Injury	27	13	2.9	1.4-6.4	2.2	1.0-5.1
Orthopaedic Injury	133	33	2.0	0.9-4.6	1.7	0.7-4.1
Soft Tissue Injury	148	37	3.1	1.1-9.2	2.9	0.9-8.7
Surgery						
Craniotomy	31	8	1.3	0.6-3.1	0.9	0.4-2.3
Facial bone surgery	6	4	3.5	0.9-12.9	2.6	0.6-10.7
Thoracotomy	8	2	1.2	0.3-6.0	1.2	0.2-6.4
Laparotomy	27	10	2.0	0.9-4.6	1.7	0.7-4.0

Orthopaedic surgery	72	20	1.7	0.9-3.3	1.3	0.7-2.8
Soft tissue surgery	66	20	1.9	1.0-3.8	1.8	0.9-3.8
Other procedures						
Tracheostomy	60	12	1.0	0.5-2.0	0.3	0.1-0.7
CVC	148	40	13.5	1.8-100.9	4.3	0.5-35.6
Arterial line	191	41	~	~	~	~
ICP monitor	74	14	0.9	0.4-1.8	0.6	0.3-1.2
EVD	54	11	1.0	0.5-2.1	0.5	0.2-1.0
Gastric tube/ enteral feeds						
No gastric tube	31	4	1.0	~	~	~
Tube, no feeds	32	1	0.2	0.3-2.3	0.3	0.03-2.8
Tube with feeds	135	36	2.1	0.7-6.2	0.3	0.05-1.5
ICC	75	22	1.9	1.0-3.7	1.4	0.7-2.8
Bronchoscopy	48	13	1.5	0.7-3.0	0.6	0.3-1.3
ISS						
<16	38	3	1.0	~	1.0	~
16-75	160	38	3.0	0.9-10.3	1.7	0.5-6.2
Antibiotics						
Penicillin/amoxycillin	22	12	2.3	0.7-7.8	3.4	0.8-14.1
Flucloxacillin	25	7	0.9	0.2-4.1	0.5	0.1-2.4
Ticarcillin/clavulanate	58	15	3.1	0.7-13.3	2.6	0.5-13.3
1st generation cephalosporin	99	30	1.3	0.7-2.6	1.3	0.6-2.7
3rd generation cephalosporin	33	14	0.5	0.1-3.8	0.3	0.04-2.7
Ceftazidime/cefepime	10	4	~	~	~	~
Meropenem	44	21	1.1	0.2-5.2	0.8	0.2-4.0
Aminoglycoside	75	30	2.6	1.0-6.5	2.4	0.9-6.7
Glycopeptide	83	36	1.2	0.4-3.9	1.1	0.3-3.6
Macrolide	50	17	3.8	0.8-17.8	2.2	0.4-10.8
Ciprofloxacin	16	4	~	~	~	~
Metronidazole	46	16	1.4	0.6-3.1	1.1	0.5-2.7

6.5 Discussion

6.5.1 Study findings

This study found that having a tracheostomy or a gastric (naso- or oro- gastric) tube without and with enteral feeds was protective against acquisition of MRSA. It also found that having a central venous catheter or receipt of penicillin or amoxycillin were risk factors for MRSA acquisition. There are no published studies examining risk factors for MRSA acquisition in trauma patients. There are numerous others detailing the association of various factors with MRSA infection or other outcomes in different populations, but none are directly comparable with these results. These other studies were case-control or prospective studies using logistic regression (Graffunder *et al.*, 2002; Ho, 2003; Thomas *et al.*, 1989; Pujol *et al.*, 1994; Ibelings *et al.*, 1998). As with the first cohort study described in Chapter 5, assessing the outcome (which varied from study to study) without taking into account the timing of MRSA acquisition often has the inherent problem of not being able to determine whether the predictor variable of interest took place before or after the outcome. Even in studies that have used the obvious outcome of clinical infection, patients are likely to have become colonised prior to development of infection, the timing of which is usually unknown. Therefore, in this study, frequent MRSA screening took place and a Cox proportional hazards regression model was performed, as it took into account timing of MRSA acquisition and in particular, accounted for number of days on each antibiotic. This method of analysis has been used in another study examining risk factors for acquisition of VRE (Padiglione *et al.*, 2003).

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6.5.1.1 Inference of causality

Criteria used for inferring causality in epidemiological studies include strength of association, consistency with other studies, biological plausibility, temporal relationship, specificity, reversibility with intervention, biological gradient and analogy (Farr, 2004). For this study and the first cohort study, the associations according to the magnitude of the risk ratios were strong, with the positive odds ratios and hazard ratios being well above two.

Because of the type of analysis performed in the second study (Cox regression), the temporal relationship of the associations is consistent, that is, the predictor variables occurred before the outcome. The temporal relationship of the associations in the first study could not be proven for some of the variables because of the type of analysis performed. As discussed above, this is also an issue for other published studies, which generally have not examined the issue of timing of associations in such detail.

Specificity refers to whether the association is the only cause of the outcome. In a population such as this, there are multiple factors which could also have caused the outcome, many of which cannot be or were not measured. These other factors may be confounders and may be an explanation for some of the unexpected findings. In theory, if withdrawal/addition, reversal or treatment of one of these factors could take place with reduction in the outcome, this would support the association as being causal. It would be impossible to reverse many of the significant factors found in this study and because of the

likely multifactorial nature of the reasons for MRSA acquisition, it is unlikely that reversal of just one factor could reduce the outcome enough to infer causality convincingly.

Analogy refers to whether other similar factors also can cause the problem. This may refer to other types of intravascular lines being associated with MRSA acquisition, which has been found in the literature and to the association of other antibiotics with MRSA acquisition. Biological gradient refers to a dose response. This may be relevant to duration of certain procedures or dose of antibiotics. For example, this study did not examine duration of the presence of tracheostomy but it did examine duration of antibiotics. Biological coherence refers to evidence from animal studies, which is probably not relevant to this study.

Although most of these criteria should be fulfilled to infer causality, the ones that are probably most relevant to this study are consistency, temporal relationship and biological plausibility. Consistency and biological plausibility are discussed further in the following paragraphs.

6.5.1.2   *Presence of tracheostomy*

In this study, presence of a tracheostomy was protective against acquiring MRSA. Other studies have found tracheostomy not to be associated with MRSA acquisition (Pujol *et al.*, 1994; Shimada *et al.*, 1993). Presence of a tracheotomy may be protective because aspiration is prevented and this may be a route of entry for MRSA. However, the fact that

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nasal and throat swabs were taken in this study and were by definition negative in patients who did not acquire MRSA, argues against aspiration of nasopharyngeal secretions being a primary cause of MRSA acquisition. In other words, prevention of MRSA deposition in the lungs may be prevented by having a tracheostomy, but it is difficult to postulate how prevention of aspiration could prevent initial acquisition in the nasopharynx, unless the nasopharynx is not the primary site of acquisition. In addition, most patients who did not have tracheostomies were ventilated using a closed circuit system, which should also prevent aspiration and contamination or were weaned from ventilation and therefore unlikely to be aspirating. Presence of a tracheostomy may have been a reason for nursing and other staff to disinfect hands more frequently, which may explain why tracheostomy was protective. Another reason may be that there would be less physical trauma to the nasopharyngeal region with a tracheostomy as opposed to nasal or oral intubation, although most of the patients who had tracheostomies had already been intubated for some time. In addition, insertion of a tracheostomy is an invasive procedure leaving an open wound, which may predispose to MRSA acquisition. Thus, the protective association is not fully understood and it is possible that having a tracheostomy was measuring another confounding factor which was not examined in this study or that the result occurred by chance. Tracheostomies were usually inserted because of difficulty in weaning patients from the ventilator. It may not be unreasonable to suggest early rather than later tracheostomy for prevention of MRSA colonisation, but the decision is usually multifactorial. Prevention of MRSA infection by early insertion is not proven and thus is unlikely to play a significant role in the ultimate decision of when a tracheostomy is performed.

6.5.1.3   Gastric tube and enteral feeds

This study found enteral tubes without or with enteral feeding to be protective against acquiring MRSA. Others have found enteral feeding tubes to be a risk factor for MRSA (Graffunder *et al.*, 2002; Thomas *et al.*, 1989). This seems biologically plausible, as presence of a gastric tube may serve as a portal of entry for MRSA. One explanation for why a gastric tube was protective is that nursing and other staff were more likely to disinfect hands when caring for these patients, although it is difficult to postulate why this would not occur with other invasive devices, such as an intercostal catheter or external ventricular drain, for example. Similarly to presence of a tracheostomy, other reasons may be that having a gastric tube represented another factor which was not examined and therefore not adjusted for in this analysis or that the result occurred by chance.

6.5.1.4   Central venous catheterisation

Presence of a central venous catheter was found to be a risk factor for MRSA acquisition with a hazard ratio of 10, although the 95% confidence interval was very wide. This is consistent with other studies (Onorato *et al.*, 1999; Pujol *et al.*, 1994), but these studies were not directly comparable because of different methodology and populations studied. A central venous catheter could serve as a portal of entry for MRSA and constitutes an ongoing open wound whilst *in situ* and thus is biologically plausible as a risk factor. However, catheter entry site swabs were not examined and it is not known whether this was the primary site of colonisation with spread to the nasopharynx and other skin sites or vice versa. There is a large literature on this topic and it is recommended that intravenous

catheters be removed as soon as no longer needed (Mermel *et al.*, 2001). Results from this study would support these guidelines.

6.5.1.5 Receipt of penicillin or amoxycillin

This study found that receipt of penicillin or amoxycillin was a risk factor for MRSA acquisition. Numerous others have shown association of antibiotics with MRSA, although methodological differences are prominent and results are sometimes contradictory. None have used a Cox proportional hazards regression model and many have not examined individual antibiotics, but rather receipt of any antibiotic. Use of broad-spectrum antibiotics makes more biological sense as a risk factor for MRSA acquisition and this has been found in HIV patients and in a geriatric hospital (Onorato *et al.*, 1999; Washio *et al.*, 1997). One study has found that use of "penicillins" was an independent risk factor for MRSA acquisition, but it is not clear which penicillins were included and what other antibiotics were used (Ho, 2003). Conversely, others have not found penicillins (not defined) or beta-lactams (not defined) to be significant in a multivariate analysis (Muller *et al.*, 2003; Pujol *et al.*, 1994). It makes biological sense that broad-spectrum antibiotics are more likely than narrow spectrum agents to facilitate the spread of MRSA, but on close examination and comparison of reported studies, it has not been proven that receipt of a particular antibiotic in an individual patient is associated with MRSA colonisation. Further larger studies using appropriate statistical analysis are needed to conclusively show the exact relationship between various classes of antibiotics and MRSA acquisition.

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6.5.2 Comparison with first cohort study

The findings from the second cohort study do not confirm the findings of the first (described in Chapter 5), which found that length of stay, laparotomy, being a road traffic accident victim and receipt of a glycopeptide or ticarcillin/clavulanic acid were associated with MRSA acquisition. These two studies differed in the following ways:

- Number of subjects – the second study had 2½ times the number of subjects as the first (239 versus 96). This gave a greater power of the second study to detect differences between the two groups

- The definition of MRSA acquisition differed – in the first study, only admission and discharge swabs were used whereas in the second any two swab sets were used. There should not have been any difference between the proportion of patients acquiring MRSA in the two groups. However, because all patients did not have admission and discharge swabs taken but may have had two other sets of swabs taken, there were greater numbers in the denominator using the second definition compared with the first. The proportion of patients who acquired MRSA was greater using the second definition than the first (17.2% versus 12.7%). This may have related to false negative discharge swabs causing misclassification of cases and controls. However, in the absence of a gold standard for MRSA detection, this will remain a perennial problem in any study using skin or mucosal swabs for MRSA detection.

- Timing of MRSA acquisition – because of the frequent swabbing which took place in the second study, the timing of MRSA acquisition in relationship to the time of

occurrence of the various predictor variables studied could be taken into account by using a Cox proportional hazards regression analysis.

Despite these differences, it should be possible to compare the two studies. Because the timing of MRSA acquisition in relation to occurrence of risk factors could not be accounted for in the first study, it is not surprising that receipt of a glycopeptide was not found to be associated with MRSA acquisition in the second. The same may apply to receipt of ticarcillin/clavulanic acid. Mechanism of accident is not a time dependent factor and because most of the laparotomies took place on the day of ICU admission, it is reasonable to assume that acquisition of MRSA would have occurred after the laparotomy. Thus, performance of a time-dependent analysis should not have been necessary for these variables. Interestingly, when a logistic regression similar to that performed in the first cohort study was performed on the data from the second study, none of the variables found to be significant in the first study were found to be so in the second. In the second study, use of logistic regression found only tracheostomy to be significant.

One of the reasons for the discrepancies between the studies may be because of a true difference between the study populations. With less than 100% compliance with the swabbing protocol, there may have been some selection bias that differed in the two studies. This was felt to be unlikely in the first study where data regarding patients who were only swabbed once (as a surrogate for patients who were not swabbed at all) were available to compare whether the groups were similar. In the second study, compliance

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with the swabbing protocol was greater than 80%, suggesting that the effect of selection bias would not have been great. This, however, is speculation, and it must be acknowledged that selection bias could have been an issue.

Another reason for the discrepancies between the two studies may have been because of falsely negative results. In Chapter 3, it was shown that results of swabs could change between positive and negative multiple times. The fact that the proportion of patients acquiring MRSA differed when the two definitions of MRSA acquisition were used suggests that false negative results may have had an impact. The impact of false negative swabs could have been to misclassify cases and controls. This means that if a first swab was falsely negative, the patient could have been classed as an MRSA acquisition if the second were positive. Likewise, if a discharge swab was falsely negative, the patient could have been falsely classed as not acquiring MRSA. The occurrence of false negatives may have been less likely to have an impact in the second study because patients were swabbed multiple times, increasing the chance of detecting MRSA colonisation. The impact of this could have been to bias the results in either direction, depending on the number of cases and controls that might have been misclassified.

Another possible reason for the discrepancy may be that some (or all) of the odds ratios may have been significant purely because of chance. By definition, there will be a one in twenty possibility that any result will be significant by chance. Because of the multiple variables (over 40) examined, this may have been the case.

6.5.3 Power of study

The number of patients calculated in the sample size estimation was 360. These calculations were based on previous numbers of trauma patients admitted to the ICU. During the study period, however, only 293 trauma patients were admitted, thus making it impossible to recruit enough subjects. With less than 100% swabbing compliance and excluding patients who had MRSA isolated from their first swab set, only 239 patients were eligible to be entered in the study. In addition, an MRSA acquisition rate of 20% was used in the sample size calculations. The actual acquisition rate was 17.2%, thus further reducing the power.

6.6 Conclusions

The aim of performing risk factor analysis for trauma patients acquiring MRSA was to determine whether it was possible to identify a group that were at high risk and whether this group could be targeted for particular interventions, such as infection control measures or prophylactic antibiotics to eradicate MRSA. This study took place during the introduction of a new alcohol and chlorhexidine hand disinfectant (SteriGel+®). The study had been initiated because trauma patients were found to be at particular risk of MRSA acquisition, with about one third acquiring MRSA during their ICU stay. During the time of this cohort study, 17.2% of trauma patients acquired MRSA, probably (but not definitely) reduced as a result of the hand hygiene intervention. From the two cohort studies conducted, it was not possible to identify confidently particular trauma patients at risk of MRSA acquisition, as the findings from the two studies were disparate and because

some of the findings in the second study were unexpected and not easily explained in terms of biological plausibility or based on previous literature. Because of the striking reduction in MRSA acquisition in trauma patients whilst SteriGel+® was in use, the next course of action may be to determine whether even further improvements in hand hygiene may be the best way to proceed. Performance of further risk factor studies for MRSA acquisition in trauma patients may suffer from the same issues as the two already performed and may again give disparate results. In light of these findings, further efforts should be directed towards enhanced hand hygiene.

7 CHAPTER 7. CONCLUSIONS AND FUTURE DIRECTIONS

Disease caused by methicillin-resistant *Staphylococcus aureus* is a serious and increasing problem in hospitals and is now emerging as a significant community acquired pathogen. Levels of MRSA drive the empirical, prophylactic and therapeutic use of vancomycin, which in turn, promotes and facilitates the emergence and spread of other resistant organisms, such as vancomycin-resistant enterococcus (VRE) and *Staphylococcus aureus* with reduced susceptibility to vancomycin (VISA and VRSA). Despite the fact that MRSA first appeared over 40 years ago, there is still controversy regarding the best methods for its control and our knowledge regarding its transmission dynamics remains incomplete. Detailed examination of the literature has demonstrated that many of the studies concerning MRSA control are methodologically unsound. A critique of current MRSA containment guidelines has also shown that many of the recommendations are based on studies that may not be generalisable to other epidemiological settings. This thesis aimed to expand our knowledge of MRSA epidemiology as the basis for improving its control.

7.1 Acquisition of MRSA in the ICU

This was a cohort study designed to determine the prevalence of MRSA colonisation on admission to the ICU and the incidence of new acquisitions in the ICU. It found that 6.8% of patients were colonised with MRSA on admission. Length of stay of greater than three days in hospital before ICU admission and prior stay in the ICU, the trauma/orthopaedics ward and another mixed ward were significant risk factors. Admission to the cardiology/general medicine and the cardiothoracic surgery wards were protective. This study also found that 11.4% of patients in the ICU became newly colonised with MRSA, for which length of ICU stay greater than two days and being a trauma patient were significant risk factors. It was also demonstrated that compliance with the screening program was poor and that improvements ensued after employment of a supervisory nurse, during which time the detection of patients who acquired MRSA also increased.

The importance of this study was three-fold. Firstly, it generated local data which were presented widely across Melbourne and interstate. These data were used to show that the Alfred Hospital had a significant problem with MRSA and justify why interventions were necessary, at a time when complacency about MRSA was increasing. Because of differences between ICUs within Australia and overseas, it is important to use data that is specific to local hospitals. Secondly, this study produced some novel findings concerning trauma patients and their increased risk of acquiring MRSA. This was important for the Alfred Hospital, as it is the major trauma centre in Victoria, but also had global implications as similar findings have not previously been published. Thirdly, results from

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this study allowed formulation of further questions, some of which were examined in the subsequent studies presented in this thesis.

7.2 Comparison of subtyping methods for MRSA

MRSA isolates from the ICU screening study were subtyped using three methods, antibiotic susceptibility testing, pulsed-field gel electrophoresis and RiboPrinting, in order to compare the utility and discriminatory power of each. This study showed that antibiotic susceptibility testing was not sufficiently discriminatory to differentiate between the PFGE types. A comparison between the RiboPrinter® and PFGE showed that both had similar discriminatory power. From a practical point of view, the RiboPrinter® was easier and faster to use, being fully automated, but set-up and running costs may limit its availability.

This study also highlighted some important factors regarding typing and analysis of electrophoretic gels. Because of technical issues, it was not possible to use computer-assisted analysis to compare the pulsed-field gels. A comparison was made by eye using the "Tenover criteria", which are most useful when determining whether a strain of MRSA is identical to an outbreak strain. As designation of types is dependent on the relationship of the banding pattern to the parental or outbreak strain, absolute types cannot be assigned to strains and comparisons between other subtypes and types cannot be made. This impacted on the comparison of PFGE types in this study with the other typing methods. The ability to use computer-assisted analysis to generate a dendrogram would have

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allowed delineation of the exact degree of similarity between PFGE types. Ultimately, these results confirmed the oft repeated recommendations to use more than one typing method and always to correlate any results with clinical and epidemiological data.

7.3 *Improving hand hygiene in the ICU*

In the study presented in this chapter, a new waterless, alcohol-based hand hygiene gel (SteriGel+®) was introduced into the ICU in order to improve compliance with hand hygiene and reduce acquisition of MRSA. This intervention also involved the placement of an "Antibiotic Resistant Organism" sign in the cubicle of any MRSA colonised patient. Feedback regarding compliance with swabbing and new clinical MRSA isolates was given to staff and the public via several media. The major outcome assessed was the proportion of patients who newly acquired MRSA. This was reduced when compared with the proportion in the initial ICU screening study and supported by other data, including statistical control charts. The proportion of trauma patients who acquired MRSA, in particular, was reduced between the two time periods.

Although compliance with hand hygiene was not formally assessed by observation, the overall amount of all hand hygiene products increased between the two periods, largely as a result of the introduction of SteriGel+®. Informal feedback revealed that this product was very popular and well used by staff. Use of the "Antibiotic Resistant Organism" sign was likely to have been the least useful aspect of the project.

Two major hypotheses have arisen from this study:

1. That good hand hygiene may be adequate to reduce MRSA transmission.

2. That compliance with a hand hygiene product may not require labour and cost intensive support campaigns and may be dependent on user acceptability of the product.

The first hypothesis is currently being tested in the randomised-controlled trial being conducted by the NIAID comparing active surveillance and contact precautions with adequate hand hygiene and standard precautions. The second could be tested by comparing compliance with a product in two groups given different levels of educational and other support either in a randomised-controlled trial or a cross-over trial.

Analysis of the screening swabs demonstrated that patients lose and reacquire MRSA colonisation at various anatomical sites and may be colonised by more than one PFGE type at the same or different times. These findings impact on definitions of MRSA acquisition and clearance and also on the reported sensitivity of swabbing. This study also found that the nose and throat were intimately related in terms of MRSA carriage. The groin was found to be frequently colonised, with a sensitivity for MRSA detection similar to that of the nose and throat. It is not known whether groin colonisation represents enteral MRSA carriage, but this could be examined in a study involving swabbing of both sites. The findings of this section again highlight the importance of having local data, as results from different settings may not be generalisable to others.

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Three hypotheses have arisen from this part of the study with review of the literature:

1. That non-nasal colonisation may more important than generally believed and may explain why not all clinical infections are associated with nasal colonisation

2. That non-nasal colonisation may explain why intra-nasal mupirocin has not been successful in preventing *S. aureus* infections

3. That eradication of enteral MRSA carriage may reduce MRSA infections and reduce the overall burden of MRSA, thus reducing the colonisation pressure with a concomitant decrease in MRSA transmission

The first has been examined in a limited number of studies, particularly with regard to gastrointestinal/faecal carriage, but could be examined further in a detailed screening study with larger numbers which correlates infecting with screening isolates from nasal and non-nasal sites using subtyping. If a correlation is found, further studies examining alternative methods for elimination of non-nasal carriage could be carried out with subsequent testing for prevention of infection. There are now a limited number of studies examining use of enteral agents (mupirocin and vancomycin) for eradication of MRSA and prevention of subsequent infection. This could be examined further in a randomised-controlled trial using existing or novel agents to eradicate enteral MRSA carriage.

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7.4 Risk factors for MRSA acquisition by trauma patients

These cohort studies were performed based on the findings from the ICU screening that trauma patients were particularly at risk of MRSA colonisation. The first study was performed using data available from the 96 trauma patients identified in the first screening study. The findings confirmed that length of stay was an important risk factor and also that laparotomy, mechanism of trauma and usage of certain antibiotics (ticarcillin/clavulanic acid and glycopeptides) were also significant. This study had the major limitation of being small and therefore underpowered for detecting some rarer associations. Also, because the timing of MRSA acquisition could not be determined accurately, it was impossible to say whether some of the associations had taken place after MRSA acquisition and were therefore not causal.

The second cohort study was designed to have greater power for detection of associations and because of the frequent screening of patients, the timing of MRSA acquisition was able to be determined more accurately with performance of more appropriate statistical analysis. Because fewer than expected trauma patients were admitted to the ICU during the study period, significantly less subjects were accrued than anticipated, giving the study reduced power to detect differences between the two groups. The MRSA acquisition rate of 17.2% was markedly reduced when compared with the 32.3% found in the initial screening study. This is likely to be attributable to the introduction of the new hand disinfectant, although other factors, which may include a Hawthorne effect, may have played a role.

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The second cohort study showed presence of a tracheostomy or naso- or oro- gastric tube with or without enteral feeding to be protective against MRSA acquisition, whereas having a central venous catheter or receipt of penicillin or amoxycillin were associated with increased risk. On face value, presence of a tracheostomy or gastric tube being protective against becoming MRSA colonised seems difficult to explain, but there may be a number of factors which were not examined that may account for the relationship. In the literature, antibiotic use has been generally been associated with MRSA acquisition. Although receipt of broad spectrum agents would seem more likely to select for MRSA acquisition and spread, this was not the finding of this study. Presence of a central venous catheter as a risk factor for MRSA acquisition can be more easily understood and reinforces recommendations for early removal of unnecessary intravenous lines.

To apply particular infection control or other preventive measures selectively to high risk groups requires either rapid laboratory detection of MRSA or a reliable, sensitive and specific discriminant rule to predict at risk patients. Despite two detailed cohort studies attempting to elucidate risk factors to identify particular subsets of trauma patients at risk of MRSA acquisition, definite conclusions could not be drawn. In order to examine this further, even larger studies would be required with subsequent validation of any discriminant rules on large numbers of patients. Experience gained with the studies in this thesis questions whether this would be the most fruitful approach to take. On the other hand, experience with enhanced hand hygiene has suggested that this type of generic measure may be the best solution. The hypothesis that has arisen from this study is that

enhanced hand hygiene has the greatest impact on MRSA levels in trauma patients. This could be tested by looking at rates of MRSA acquisition in different subgroups as part of other studies promoting hand hygiene and whether rates of MRSA can be brought back to the baseline level in trauma patients using only hand hygiene promotion. If rates in trauma patients continue to remain significantly elevated despite good reductions in other patient groups and good compliance, further targeted strategies may still be necessary.

7.5   Future directions

From the literature, it is clear that we still do not have a complete understanding of how best to control MRSA transmission and how to prevent infections in colonised patients. Work performed as part of this thesis has attempted to expand this knowledge and generate further hypotheses. Evidence from published studies and this thesis now supports hand hygiene as a major factor in reduction in MRSA transmission. What remains to be elucidated is the level of other infection control measures required to support this.

Work in this thesis has also supported findings in the literature that non-nasal sites of MRSA colonisation may play a role in the development of infection. In particular, there is evidence that gastrointestinal carriage of MRSA may be important and several studies have shown promising results by eradicating MRSA from this site. In the published studies, reduction in MRSA carriage and infection occurred in individual patients with a concomitant reduction in the overall burden of MRSA in the unit. Although there may be

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concerns about giving widespread oral vancomycin, there are other novel agents which could be tested in randomised-controlled trials.

It is imperative that MRSA levels be quantified locally because the transmission dynamics, patient population, infection control procedures and hospital layout, culture and management differ between institutions. There is not one solution to fit the problem of MRSA control. In circumstances where randomised-controlled trials are not practicable, it is still important to perform well designed studies to answer these questions. This involves use of adequate subject numbers to give statistically significant results, good surveillance before and after introduction of an intervention, using standardised criteria for determining outcomes, standardised reporting of rates with analysis of individual interventions and appropriate statistical tests to analyse the data.

Experience has shown that the health-care profession, including medical, nursing and administrative personnel, has failed to adequately control MRSA and to prevent the development of vancomycin resistance in *S. aureus* and the appearance of community acquired MRSA. A new approach is mandatory. This is already underway, with recent successes in improving hand hygiene but should be continued in studies looking at novel strategies to halt transmission and prevent infection in colonised patients. It is up to us to tackle the problem with all available resources, before the ever-changing *S. aureus* eludes us again.

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8 APPENDICES

APPENDIX 1

Plain language statement given to intensive care unit staff on commencement of the hand hygiene project.

This Plain Language Statement is 4 pages long. Please make sure you have all the pages.

PROJECT TITLE

Improving Hand Hygiene in the Intensive Care Setting to Reduce Hospital Acquired Infections and Methicillin Resistant Staphylococcus aureus (MRSA) Acquisition.

INFORMATION SHEET

You are invited to take part in this research project. This plain language statement contains detailed information about the research project. Its purpose is to explain to you as openly and clearly as possible what the project involves. Please feel free to take a copy of this statement. A number of questions have been answered below, but should you require additional information please contact Infection Control on extension 3139 or through the hospital switch board (dial 9) and either Glenys Harrington, Infection Control Program Coordinator or another Infection Control Practitioner will take your inquiry.

What is the purpose of the project?

The purpose of the project is to try to decrease infections and acquisition rates of MRSA ("golden staph") in patients in ICU by introducing a number of Infection Control strategies to improve hand hygiene and awareness about hospital acquired infections and antibiotic resistant organisms including the introduction of a new hand hygiene product.

What is the new hand hygiene product?

The product is a waterless alcohol (70%), chlorhexidine (0.5%) gel with emollients (softening agents), which can be rubbed directly onto visibly clean hands.

What are the benefits of using the new hand hygiene product?

There may be benefits for both healthcare workers and patients.

Health-care workers

Health-care workers may be required to disinfect their hands from a few times per hour to as many as 40-50 times per hour. Such frequent hand disinfection can result in skin irritation and reduces compliance with performing hand disinfection.

Waterless alcohol based gel hand disinfectants containing emollients (softening agents) have been developed to:

Reduce exposure of health-care workers to more irritant disinfectants

Reduce the time it takes to disinfect hands by washing with a disinfectant and water

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Improve accessibility to disinfectant products (the gels come in free standing pump packs and pocket size containers)

Patients

The benefits for the patients will come from improved hand disinfecting compliance which has been shown to decrease infections and also decrease the spread of resistant organisms from one patient to another.

Do I still need to wash my hands with the currently available hand hygiene product in the Unit?

Yes.

If your hands are visibly soiled you will still need to wash your hands with a disinfectant solution and water followed by drying with paper towel.

Who will be involved in the project?

The project involves all staff working in ICU (permanent, part time, bank and agency) and staff who visit ICU to see patients or assist ICU nursing and medical staff in the care of the patients.

How will I find out about the results of the project?

Part of the project is a proactive promotional campaign, which will involve regular feedback of progress and results to staff in the intensive care unit and hospital wide via presentations, newsletters and the Infection Control and Hospital Epidemiology web page.

What time frame will the project run over?

The project will run over an 8-month period from approximately April to November 2002 and be overseen by a multidisciplinary project team established by the Infection Control and Hospital Epidemiology Unit.

What are the possible risks involved in the project?

You may develop irritant or contact dermatitis or a hypersensitivity to the waterless alcohol based hand gel.

Allergic contact dermatitis or hypersensitivity to alcohol is rare and very uncommon with chlorhexidine. The concentration of alcohol in the waterless alcohol based hand gel is 70% and the concentration of chlorhexidine in the waterless alcohol based hand gel is very low (0.5%).

These concentrations are the same as what is used in skin prep products used prior to patient procedures such as operations or insertion of drips.

How will I know of I have an allergic reaction to the waterless alcohol based hand gel?

Signs and symptoms of irritant or contact dermatitis include the following:

Erythema, dryness, scaling, cracking or itchy skin on hands.

What will I do if I think I have an allergy?

Contact the Alfred Staff Clinic on extension 2434 and a referral and assessment in the Asthma and Allergy Clinic will be arranged.

or

You can contact the Asthma and Allergy Nurse Educator directly on extension 2886

or

You can contact Infection Control on extension 3139 or through the hospital switch board (dial 9) and either Glenys Harrington, Infection Control Program Coordinator or another Infection Control Practitioner will arrange for your assessment in the Asthma and Allergy Clinic.

What are the alternatives to participating in the use of the waterless alcohol based hand gel?

If you decide not to use the waterless alcohol based hand gel other hand disinfectants will still be widely available for use in the Intensive Care Unit.

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These include the following:

A disinfectant hand product

Name: Bioprep

Composition: 4% chlorhexidine gluconate

Dispenser type: wall mounted dispenser that contains a 750 ml disposable cartridge (bladder)

Chlorhexidine hand lotion

Name: Chlorhexidine hand lotion

Composition: Chlorhexidine gluconate 10g/L and Ethanol 70% v/v in and emollient perfumed lotion.

Dispenser type: 500ml pump pack.

How will my confidentiality be protected if I participate in the project?

No information that will identify health-care workers is being collected as part of this project. To determine if the product is being used distribution data (ie how much of the product is supplied to the Unit or issued to staff) will be used as a proxy for usage rather than direct observation of healthcare workers.

Is participation is voluntary?

Participation in this project is voluntary. If you do not wish to take part you are not obliged to. If you decide to take part and later change your mind you are free to withdraw from participation in the project.

Has an ethics committee approved the project?

The Human Research Ethics Committee of the Alfred Hospital, Bayside Health, has approved the ethical aspects of this research project. Ms Rowan Frew, Ethics Manager can be contacted on 92733848 if you have any concerns in this area.

APPENDIX 2

Questionnaire to determine user acceptability of SteriGel+®

1. Do you like Sterigel?

- ❏ YES

- ❏ NO

- ❏ UNSURE

1. Which size Sterigel do you use?

- ❏ POCKET SIZE ONLY (150ML)

- ❏ PUMP PACK AT PATIENT'S BEDSIDE ONLY (500ML)

- ❏ BOTH (Pocket size & Pump Pack)

- ❏ NEITHER

2. Does Sterigel evaporate fast enough?

- ❏ YES

- ❏ NO

3. Do you like the fragrance once Sterigel has evaporated?

- ❏ YES

- ❏ NO

FOR OPTIONS 5-7, MORE THAN ONE ANSWER MAY BE APPLICABLE

4. How would you describe the sensation on your hands after using Sterigel?

       ❑ SOFT

       ❑ SLIPPERY

       ❑ TACKY

       ❑ NO SENSATION

       ❑ OTHER

       (describe...)

5. Did you experience any of the following side effects on your hands after using Sterigel?

       ❑ REDNESS

       ❑ DRYNESS

       ❑ RASH

       ❑ CHAPPED HANDS

       ❑ NO SIDE EFFECTS

❑ OTHER (describe...)

6. **If you did experience side effects, did you notify anyone about it?**

      ❏ NO

      ❏ YES, NURSE MANAGER

      ❏ YES, INFECTION CONTROL

❏ YES, OTHER:

      Who did you notify?...

7. **If Sterigel was available all the time in the Unit would you use it?**

      ❏ YES

      ❏ NO

      ❏ NOT SURE

APPENDIX 3

"Antibiotic Resistant Organism" sign placed in patient cubicle if found to be MRSA colonised .

Antibiotic Resistant Organism

The Alfred Hospital Infection Control & Hospital Epidemiology Unit

APPENDIX 4

Results of SteriGel+® user acceptability questionnaire.

SteriGel+® USER ACCEPTABILITY QUESTIONNAIRE

Q1 Do you like Sterigel?   **Number**

Yes   88

No   9

Unsure   0

No Response   2

Total   **99**

Q2 Which size Sterigel did you use?

Pocket size only (150 ml)   0

Pump pack at patients bedside only (500 ml)   87

Pump and Pocket Sterigel   8

Neither   2

No Response   2

Total   **99**

Q3 Does Sterigel evaporate fast enough?

Yes   88

No   8

No Response   3

Total   **99**

Q4 Do you like the fragrance once Sterigel has evaporated?

Yes   80

No   12

No Response   5

Q5 How would you describe the sensation on your hands after using Sterigel?

	Yes	No	No Response
Soft	45	50	4
Slippery	13	83	3
Tacky	22	74	2
No Sensation	18	78	3
Other Comments	15		

Q6. Did you experience any of the following side effects on your hands?

	Yes	No	No Response
Redness	7	90	2
Dryness	24	73	2
Rash	3	94	2
Chapped hands	12	85	2
No side effects	64	33	2

Q7 If you did experience side effects, did you notify anyone about it?

No   29

Yes, Nurse Manager   1

Yes, Infection Control   2

Yes, Other.   1

Yes, No indication of who was notified   1

Q8. If Sterigel was available all the time in the unit would you use it?

Yes   87

No   7

Unsure   2

No Response   3

APPENDIX 5

Comparison of characteristics of trauma patients who had one swab set taken and those who had admission and discharge swabs taken during screening study described in Chapter 1.

Comparison of length of stay in ICU

ICU length of stay	1 swab set received	Admission & discharge swabs received	Total
1 day	37 (23.0%)	19 (18.3%)	56 (21.1%)
2-7 days	64 (39.8%)	52 (50.0%)	116 (43.8%)
>1 week	60 (37.3%)	33 (31.7%)	93 (35.1%)
Total	161 (100.0%)	104* (100.0%)	265 (100.0%)

Comparison of age

Age group	1 swab set received	Admission & discharge swabs received	Total
<50	110 (68.3%)	76 (73.1%)	186 (70.2%)
50-70	32 (19.9%)	20 (19.2%)	52 (19.6 %)
>70	19 (11.8%)	8 (7.6%)	27 (10.2%)
Total	161 (100%)	104* (100%)	265 (100%)

Comparison of gender

Gender	1 swab set received	Admission & discharge swabs received	Total
Male	115 (71.4%)	74 (71.2%	189 (71.3%)
Female	46 (28.6%)	30 (28.9%)	76 (28.7%)
Total	161 (100%)	104* (100%)	265 (100%)

*104 trauma patients had both swabs taken because this includes those who had a positive swab on admission

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