Introduction

Mupirocin (Bactroban, Smith Kline Beecham, Welwyn Garden City, UK) was introduced into clinical practice in the UK in 1985, and has proved to be an extremely effective treatment of skin infections and one of the most successful topical antibiotics for the clearance of nasal Staphylococcus aureus isolates including those resistant to methicillin. It is currently registered for use in more than 90 countries worldwide. Unfortunately resistance was described shortly after its initial use. Many of the issues regarding its use are reviewed here, together with the mechanisms, genetics, surveillance and epidemiology of resistance, particularly in staphylococci. The various factors that increase resistance and how they might be controlled are also discussed.

Mechanism of activity

Mupirocin is bacteriostatic but appears to be bactericidal at a lower pH approximating that of many parts of the skin. Yanagisawa and co-workers have recently postulated that mupirocin binds to the isoleucyl-tRNA synthetase (IleS) target in the vicinity of an ATP-binding subsite and that mupirocin is a bifunctional inhibitor with characteristics of both isoleucine and ATP, i.e. an analogue of isoleucyladenylate. It was thought that the unique mode of action and the low incidence of purely low-level resistance from early studies would have made higher degrees of resistance a rather remote possibility.

Emergence of mupirocin resistance

Definitions of mupirocin resistance have varied but, as more strains have been encountered and the resistance mechanisms explored, it appears as though there are two resistant populations; those showing low-level resistance (MIC = 8–256 mg/L) of dubious clinical significance and those showing high-level resistance (MIC > 256 mg/L). In the USA no NCCLS guidelines exist for topical agents but in some papers, for example Fuchs et al., lower resistance breakpoints (4 mg/L) have been suggested. When considering the in-vivo relevance of mupirocin resistance breakpoints in predicting the outcome of therapy it is...
important to note that the concentrations of the agent encountered in the deeper layers of the skin may be lower than those in the nose. However, other factors are probably more relevant to therapeutic outcome, such as the presence of throat or rectal/faecal reservoirs of bacteria and irregular or ineffective applications. Mupirocin should be applied to the nares and massaged back into the nose for at least 1 min (or until the agent can be tasted) so that it can best penetrate the posterior nasopharynx. A further potential reason for apparent treatment failures is the reacquisition of the organism from other individuals on the same ward, usually via staff transiently carrying, or colonized with, the organism. Rarely, but more so in dermatology wards or burns units, airborne and environmental transmission may be relevant. In a recent mupirocin-resistant S. aureus outbreak, blood pressure cuffs used on patients with severe skin desquamation were thought to be implicated in the spread of the strain.

It was soon appreciated that low-level mupirocin resistance could be trained in vitro and low-level resistance was observed in an early in-vivo study. However, high-level resistance was described shortly after the agent was introduced into clinical practice.

### Mechanisms of mupirocin resistance

The level of mupirocin resistance is related to alterations in IleS. Farmer et al. showed that the antibiotic concentration that halved enzyme activity correlated well with the MIC: it was $3.3 \times 10^{-1} \text{mg/L}$ for sensitive isolates $1.3 \times 10^{-1} \text{mg/L}$, for low-level resistant (MuL) isolates and $7.5 \text{mg/L}$ in highly resistant (MuH) isolates.

Low-level resistance is probably due to mutations in a chromosomally encoded IleS, is stable and non-transferable. Recent work has shown that the substitution of a single amino acid in the synthetase of E. coli has significantly altered its mupirocin susceptibility. However, others have postulated that there may be other mechanisms for resistance, e.g. an altered tRNA synthetase protein complex that might reduce the ability of mupirocin to gain access to IleS, enzymatic destruction has yet to be described. High-level resistance has been shown to be due in vivo to the acquisition of an additional novel IleS.

### Clinical significance of resistance

The clinical significance of low-level mupirocin resistance is dubious. The initial reports of therapeutic failure may have had other explanations such as the complexities of multiple site S. aureus carriage, as was more recently shown by Gaspar et al. Others have shown that MuL strains (at least with an MIC of 32 mg/L) can be eradicated. The general consensus is that MuH strains of staphylococci cannot be eradicated with mupirocin.

### Genetic basis of mupirocin resistance

The original MuH strain carried the resistance element (subsequently named mupa) on an unusual plasmid that could be very effectively transferred by a conjugative process. Many subsequent isolates carried mupa on conventional closed circular plasmids which transferred or cured at varying rates and it was from one of these that the gene was cloned and sequenced.

MuH plasmids vary greatly in their molecular weight, ability to transfer and cure, and EcoRI restriction fragment length polymorphisms. Typing studies have shown that high-level (and indeed low-level) mupirocin resistance occurs in multiple clones of S. aureus. The stability of the resistance in vitro cannot be used to predict the behaviour in vivo. The original strain was easy to cure in vitro but persisted in one patient for more than 2 years despite no exposure to mupirocin or other agents that might have exerted selection pressure.

The mupa open reading frame A is a 30,272 bp region that encodes a 1024 amino acid polypeptide; the primary structure of this polypeptide shows significant homology with IleS. However, mupa only has 57% DNA identity with S. aureus IleS and may have arisen from another organism, although IleS database searches have failed to identify a likely candidate. It is likely to have arisen by mutations from native S. aureus as judged either from published sequence data or mupa probing of such trained isolates (N. W. Woodford, personal communication).

It is now known that mupirocin-susceptible strains can be trained to high-level resistance by sequential passage on media containing increasing concentrations of the agent, but may not survive in vivo. The Laboratory of Hospital Infection (LHI) has, thus far, seen few isolates with intermediary MICs (128–256 mg/L) but, as the resistance is stable and cumulative, we are concerned that a new form of high-level resistance may emerge and the LHI is prospectively screening all such isolates with the mupa probe. The use of mupirocin alone (without a systemic agent) in situations where penetration may not be optimal or possible (e.g. for multiple or large wounds, throat and rectal carriage) may encourage such mutations to occur, particularly if staphylococcal populations are large.

### Relationship between mupirocin resistance and other resistances

Although cross-resistance with other agents has not been described, and is unlikely given the unique mode of action of mupirocin, resistance may be selected for by other agents since co-transfer of other resistances with mupa has been described. These include triclosan, tetracycline,
and tetracycline, trimethoprim and cadmium. It is highly likely that the gene is transposable and we and others have seen mupA hybridize with a chromosomal region, although some of these isolates expressed low-level resistance. Copies of an insertion sequence (IS257) have been shown to flank mupA in direct repeats. In two plasmids, mupA and IS257 were seen to be duplicated, and in one of those plasmids a small tetracycline-resistant plasmid resembling pT181 was flanked by copies of IS257. These workers also showed that exposure of transconjugants to increasing concentrations of mupirocin resulted in plasmids initially with duplication of mupA and then unstable forms with three or four copies. They did not observe a relationship between mupirocin susceptibility and plasmid gene or even plasmid copy number, although such observations are limited by the amount of mupirocin that can be incorporated into test conditions. The promiscuity of the transposon is probably even greater. In one transfer experiment with the M uH and triclosan-resistant MRSA it was found that high-level resistance could co-transfer with a penicillinase plasmid (personal communication) and others have found that mupA probably integrated into a nucleic-acid binding resistance plasmid. M orton et al. found that IS257 recombination has probably enabled the mupA gene to replace the aminoglycoside, trimethoprim and quaternary ammonium resistance elements found on a conjugative plasmid.

Incidence of mupirocin resistance

There are several problems when trying to estimate the incidence of mupirocin resistance. An analysis of the first 136 responses to a recent UK infection control team (ICT) questionnaire illustrates some of these problems. Although 90.5% (133) of the laboratories tested susceptibility to mupirocin, only 19 centres tested all isolates; the rest concentrated their efforts in those areas where it was used clinically. Various methods were used to measure mupirocin resistance, but less than half of the laboratories used a method (breakpoint, MIC, 200 µg disc or E test) that could detect high level resistance. Detection of mupirocin resistance by disc diffusion methods can be insensitive, in that the zones of inhibition may be indistinct. In addition, in our experience some strains are heterogeneously resistant and large inocula are required to detect high-level resistance. Perhaps other genes are responsible for these effects, as for methicillin resistance.

A study in 1990 of one dermatological and four other English centres found only 0.3% of 7137 S. aureus isolates tested were resistant to mupirocin and only a sixth of these exhibited high-level resistance. There was no evidence of cross-colonization or transfer or re-admission of patients carrying these strains. In a Birmingham District General Hospital only one resistant isolate was found amongst 429 isolates tested over a 6 month period in 1991. However, over the same period, a dermatological hospital nearby had an incidence of 8.3% of 228 isolates and more than six times as much mupirocin usage. There were no data on whether prolonged courses of mupirocin had been given, which led to the suggestion that dosage regimens should be more than the twice-daily advocated previously, to reduce the likelihood of resistance emerging.

Bradley et al. reviewed mupirocin resistance recently. In the USA, a study in one centre showed 1% of 1309 nasal S. aureus isolates were mupirocin-resistant, although some outbreaks or clusters of cases were reported elsewhere in the USA. Reports of mupirocin resistance in other countries are appearing but the number of such isolates has been few, although occasional outbreaks are encountered. The agent was introduced clinically in New Zealand in 1986 and was made available without prescription in December 1991. There was no structured pre-marketing susceptibility surveillance. However, in 1991–92 3.7% mupirocin resistance (mainly high-level) was found amongst 4544 community-acquired S. aureus skin and wound infections in Auckland. Five of the 24 cases followed up had used mupirocin and two had not, for the remainder (17) there was uncertainty. Since this report, low-level (4.3%) and high-level (2.4%) mupirocin resistance have been described in MRSA referred to the New Zealand Health Communicable Disease Centre. Studies are planned to examine further the situation in the community. In Australia, 18% of 148 Western Australian MRSA were M uH compared with one isolate (3%) of 30 imported MRSA. It appears as though the high-level resistance is occurring in organisms from burns treated in the community, either frequently or for prolonged periods and the authors recommended less than 10 days' treatment and allowing at least 1 month between treatments. In Spain, high-level mupirocin resistance has been detected for the first time (after several years' use of the agent), in 12 MRSA isolates encountered over a 3 month period. It was not clear whether prolonged or repeated mupirocin resistance served as a marker of cross-colonization. However, the dynamics were complex as several different strains were present and a similar mupirocin resistance plasmid was probably present in all. A similar picture has been described in other countries that have a larger or longer experience of the problem and it is likely that mupirocin resistance will be seen in many other countries as suggested by recent reports of higher and low-level resistance in Canada, Brazil and Poland.

Further investigation of the mupA resistance elements is required to ascertain how much resistance is due to parallel evolution under the same selection pressures and how much to inter-strain transfer. It is probable that both will be seen. Although initial studies of the mupA gene showed relative conservation of the mupA flanking regions, more recent work has shown that these also can vary even within an epidemic MRSA strain. The epi-
demiological significance of these findings requires further study, but is unlikely to be simply explained by varying copy numbers of conventional IS257 elements.

In the UK, we have reported the increasing numbers of mupirocin-resistant S. aureus isolates, particularly MRSA, referred to our reference laboratory. These data are obviously biased and our ICT questionnaire revealed that most centres were seeing low numbers of mupirocin-resistant isolates amongst those tested over the previous 18 months (means of 15 for MRSA and three for MSSA strains). However, there was wide variation between centres (range for MRSA 0–174 and for MSSA 0–61) and as many had, as had not, seen an increase in MUr over the past 5 years. Although multiple mupirocin courses (35 hospital ICTs) and prolonged usage (26) were implicated, mupirocin resistance was most often seen in patients transferred from other hospitals (56). Clearly, although a hospital can audit its own mupirocin usage policies, increases in occurrence of resistance will be difficult to interpret if there is no system to monitor introduction and spread of MUr MRSA from other hospitals. These trends and the reasons for increases in mupirocin resistance must be watched closely as they ring warning bells for the future role of this agent in MRSA control as recommended in the MRSA guidelines drawn up by a UK working party and currently under review.41

The origin of mupirocin resistance

The origin of high-level mupirocin resistance is still a matter for conjecture. Interestingly, it has been described in stored isolates from before mupirocin was used clinically; three MUr isolates of S. aureus and Staphylococcus epidermidis from Nigeria in 196516 and, more recently, in a 1967 UK coagulase negative staphylococcus (CNS) isolate collection (author’s unpublished observations). Other workers have more recently observed MUr and MUL S. aureus without any apparent exposure to the agent.28,32

In the original MUr MSSA outbreak9 and in a subsequent MUr MRSA outbreak,42 CNS with high-level mupirocin resistance were encountered and might be the reservoir for such resistance in mupirocin-treated patients. However, in the MUr MRSA outbreak, the mupA gene was on a different EcoRI fragment from that of the outbreak MUr MRSA.42 Others have described the transfer of mupA between CNS and S. aureus.43 More recently, Udo & Jacob44 described the emergence of MUr in a Staphylococcus haemolyticus isolate from a burns unit where the agent had been used for 2 years. Interestingly, the resistance could be transferred to a restriction-deficient S. aureus and then to a restriction-positive S. aureus, but not directly into the latter. This may provide clues as to why mupirocin resistance does not necessarily emerge in S. aureus when there is a CNS reservoir of resistance available to it.

MUr CNS have also been seen in settings where there was no exposure to the agent: Connolly and co-workers43 found MUr CNS from patients on peritoneal dialysis and these often differed in species, antibiogram and plasmid profile even from the same patient. Thus a possible natural reservoir for MUr may be CNS which have acquired the gene from a hitherto undescribed source. Perhaps such an ecological niche could be in varicose ulcers colonized by Pseudomonas fluorescens or another pseudomonads producing mupirocin-like substances. The presence of IS257 surrounding mupA perhaps provides a clue, since these sequences are not only found in several staphylococcal species, but have also been described in enterococci.21 However, mupA probing of enterococci has thus far failed (N. Woodford, personal communication).

We need to learn more about the control mechanisms for expression of high-level mupirocin resistance. Searches for the mupA reservoir will have to be extended to MUL organisms now that chromosomal mupA has been described in MUL S. aureus strains.12,23 In the LHI we have also detected a mupirocin- and methicillin-susceptible S. aureus that has a plasmid that hybridizes with the mupA probe (N. Woodford, personal communication). The recent description of a chromosomal mecA-like gene in methicillin-susceptible Staphylococcus sciuri15 raises the possibility that other resistance genes, such as mupA, may be present in the chromosome of mupirocin-susceptible organisms performing an IleS function. Investigations of mupirocin-resistant outbreaks should also address the possibility of mupA being in ‘innocuous’ MUL strains during the course of outbreaks of high-level resistance.18

MUr and MUL isolates can occur on the same patient, but curing of the MUr strain has, until now, resulted in a fully sensitive strain. Thus it appears that, as in vitro experiments, low-level resistance is not a pre-requisite for mupA transfer. It is not yet known if MUL isolates increase the likelihood of mupA transfer, but it can be predicted that, as the numbers of MUL strains increase, isolates with both mupA encoded high-level resistance and chromosomal low-level resistance will be encountered.

Treatment of mupirocin-resistant staphylococci

Elimination of MUr (and indeed even susceptible and MUL) staphylococcal isolates requires a strategic approach. Wounds and other colonized/infected sites must be treated. Systemic treatment must be considered for eradication of nasal or skin S. aureus carriers if the organism is also in the throat or faeces. Many MUr isolates have emerged while patients are on mupirocin therapy and it is unlikely that the agent can eliminate these from the skin or, probably, the nose. Alternative agents have been suggested but not tested in well controlled clinical trials.

A number of agents have been shown to have in-vitro activity against MUr and MUL S. aureus. These include

14
azelaic acid, nitrofurazone, silver sulphadiazine and a mixture of a mupirocin-like substance (most closely resembling pseudomonic acid C) and holothin, a pyrrothione antibiotic has even greater activity than mupirocin against a S. aureus isolate and an M. RSA. The topical agent, magainin MSI-78, appears to show in-vitro activity against M. H S. aureus in clinical trials. When the first UK epidemic M. RSA (EMRSA) acquired M. H, there was so much concern that a 'search and destroy' strategy was adopted. Throat carriage resulted in relapse of carriage and combined systemic (ciprofloxacin and rifampicin) and topical (bacitracin and fusidic acid) therapy was required. It should be noted when interpreting the impact of these regimens that five patients died of unrelated causes whilst still colonized with M. H EMRSA and so decreased the M. H reservoir. Similar observations have been made in a vancomycin-resistant enterococcal outbreak. Others have used topical colistin, bacitracin and fusidic acid to eradicate a M. H EMRSA although there are concerns about the use of a valuable systemic agent in this way. Now that high-level mupirocin resistance is evident in so many EMRSA strains, the use of topical agents with potential systemic use might be considered less advisable, although this is still an area of some controversy. The emergence of mupirocin resistance and the potential loss of a valuable agent in M. RSA control has resulted in a considerable debate about the indications for its use. Use outside the product licence have been reviewed by Hudson and include nasal peri-operative treatment in various types of surgery, prolonged use to eradicate nasal S. aureus carriage from patients on haemodialysis, continuous peritoneal dialysis, in intensive care units, or in familial outbreaks of staphylococcal infection and the eradication of methicillin-resistant S. epidermidis from staff and patients on a cardio-thoracic unit. Mupirocin preparations are not sterile and, because of the polyethylene glycol base, should be applied with caution to granulation tissue. However, Boyce et al recently have shown that the agent was non-toxic to cultured human keratinocytes and fibroblasts, and clinicians will no doubt continue to use mupirocin to treat infected ulcers and wounds, particularly where other regimens have failed or are not available.

Some investigators are addressing the cost-effectiveness of these treatments and resistance must be monitored closely, especially where the agent is used repeatedly or for prolonged periods. History is repeating itself with the emergence of mupirocin resistance after prolonged use of mupirocin in a long-term care facility with endemic M. RSA in the USA. Although there is a place for prophylactic use of the agent at the start of an MSSA or M. RSA outbreak in critical care areas whilst the results of screening are awaited, some have advocated 'blanket' use for outbreak control and long-term prophylaxis of endemic M. RSA in intensive care and surgical wards. Unfortunately, after 18 months of such a practice, high level mupirocin resistance emerged. Others who have also recently treated all patients with mupirocin for their entire hospital stay, have similarly experienced dramatic increases in resistance. Fortunately, the incidence of resistance returned to low levels after this practice was stopped. It is a matter for some concern that such practices continue, despite these well documented consequences. Mupirocin resistance may take some time to emerge when used in these ways. Geographical variation in the timing or frequency of the emergence of antibiotic resistance, despite similar patterns of drug usage, is well described and perhaps reflects different resistance gene reservoirs or the existence of certain species or strains to facilitate resistance gene transfer, as illustrated by the experiments of Udo et al.

Prevention of mupirocin resistance

The emergence of mupirocin resistance and the potential loss of one of the major weapons in M. RSA control emphasizes the importance of using the agent judiciously. M. RSA eradication strategies should be designed carefully with reliable laboratory screening for resistance and periodic antibiotic prescribing and infection control audits. In this way, if resistance does emerge, it is more likely to be detected rapidly and action taken early and effectively to minimize spread. The same strategies will be very important for any other agent that is used for control of M. RSA or M. H M. RSA. Any agent should not be used as a substitute for poor infection control and antibiotic prescribing practices, but as part of an overall policy developed, audited and reviewed by the local relevant health care workers.

Prolonged or widespread 'blanket' use of mupirocin in hospital or closed community outbreaks must be stopped. Policies for repeated therapies should be agreed locally, audited and reviewed. Prophylactic or prolonged therapy in other areas such as surgical prophylaxis or prevention of infection in dialysis patients must be assessed in well designed case-controlled studies, their cost-effective-
ness established and staphylococcal resistance monitored closely. Marjolein and co-workers, for instance, addressed the issue of mupirocin resistance in their recent cost-effectiveness study and advocated prophylactic perioperative mupirocin use for documented S. aureus carriers rather than for all patients having cardiac surgery. We must not continue to rely on man’s ingenuity to discover new agents or further develop old ones, and then use them in the same haphazard manner.

Acknowledgement
I gratefully acknowledge colleagues who have given permission to cite unpublished work and observations.

References
The emergence of mupirocin resistance


Received 20 February 1997; returned 29 April 1997; revised 17 July 1997; accepted 7 October 1997