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Susceptibility of Staphylococci Isolated from a Burns Unit to Mupirocin and Other Antimicrobial Agents

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Key Words

Antibiotic susceptibility testing
Mupirocin resistance
Methicillin-resistant *Staphylococcus aureus*

Abstract

The activity of mupirocin (Bactroban) against coagulase-positive and coagulase-negative staphylococci isolated in a burns unit was tested to ascertain its effectiveness, and to detect any resistant isolates after continuous use of mupirocin for 2.5 years. A total of 395 staphylococci, consisting of 330 *Staphylococcus aureus* and 65 coagulase-negative staphylococci, were tested for resistance to mupirocin and other antimicrobial agents. The results showed that 94.5% of the isolates were fully susceptible to mupirocin (MIC $\leq$4 mg/l), and 5.3% expressed low-level resistance (MIC 8–128 mg/l). One *Staphylococcus haemolyticus* isolate expressed high-level resistance (MIC >1,024 mg/l). It transferred high-level mupirocin resistance to other staphylococci in conjugation experiments, which indicated a capacity to transmit mupirocin resistance between species. The results demonstrated that mupirocin was still highly effective against staphylococci in the burns unit. However, the demonstration that the resistant *S. haemolyticus* isolate could transfer high-level mupirocin resistance to other staphylococci was of concern. There is a compelling need to test staphylococci from clinical materials for mupirocin resistance. Early detection of resistance can prevent the establishment and spread of the mupirocin-resistant strains in the unit.

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Introduction

Mupirocin (Bactroban) is a naturally occurring antibiotic produced by *Pseudomonas fluorescens.* It inhibits bacterial protein synthesis by binding reversibly and specifically to bacterial isoleucyl transfer-RNA synthetase [1, 2]. Because of its excellent antistaphylococcal activity, mupirocin has been used to eradicate nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) in patients and healthcare workers [3–8], in the control of MRSA outbreaks [3, 9], in the treatment of infections of wounds, burns, ulcers and eczema [3, 7, 8, 10], as a prophylaxis before skin surgery [11], and for the reduction of staphylococcal load in atopic dermatitis [12, 13]. Mupirocin is very safe and does not appear to have any significant side effects. According to Moy et al. [14], no side effects were reported in patients after 4 years of treatment with mupirocin. It does not stain the skin or displace the host's normal flora [15]. In addition, it is structurally unrelated to any of the antibiotics in current use and there is no cross-resistance with them [15]. It was also found to be superior to topical antiseptics such as chlorhexidine, silver sulfadiazine, povidone iodine and azelaic acid because with these antiseptics, a significant number of MRSA usually persist after treatment [16]. These properties provide a compelling argument in favor of using mupirocin for treating burn wound infections, especially those caused by MRSA. Sadly, despite these qualities, plasmid-encoded high-level resistance to mupirocin has occurred in staphylococci isolated in some countries [17–20]. In a few instances the emergence of mupirocin resistance has been attributed to its prolonged use [6, 14].

MRSA is a persistent isolate in burns patients at the Ibn Sina Hospital, Kuwait. Subsequently mupirocin was introduced here in 1992 to treat burn wound infections and to eradicate nasal colonization in patients. The burns unit at the Ibn Sina Hospital, Kuwait, is a 70-bed special facility for treating burn patients for the whole of Kuwait. It has an intensive care unit with 12 beds. The bed occupancy in the burns unit is 70–100%. Both the nasal gel and skin ointments are administered to patients according to the guidelines provided by the manufacturer [15]. Although this treatment protocol has been successful in controlling MRSA in treated patients, MRSA continued to persist in the unit. It was therefore necessary to establish if staphylococci isolated in the unit has become resistant to mupirocin, which could, at least in part, explain the persistence of MRSA in the unit. An early detection of resistant strains would warrant prompt institution of appropriate infection control measures to prevent their spread.

Materials and Methods

Bacterial Strains

A total of 395 staphylococci consisting of 230 MRSA, 100 methicillin-sensitive *S. aureus* (MSSA) and 65 coagulase-negative staphylococci (CNS) isolated from clinical materials and submitted to the microbiology laboratory from patients between April 1994 and May 1995 were studied. The isolates were from patients in the burns unit and were studied whether or not the patients from whom they were isolated had mupirocin therapy. Strains were initially identified as *S. aureus* or coagulase-negative staphylococci by the tube coagulase test using rabbit plasma (Difco Laboratories, Detroit, Mich., USA). The coagulase-negative staphylococci were speciated according to biochemical reactions using the API-20, Staph identification scheme (bioMérieux SA, Marcy-l'Étoile, France). They consisted of *S. epidermidis* (n = 42), *S. haemolyticus* (n = 9), *S. saprophyticus* (n = 6), *S. simulans* (n = 4), *S. hominis* (n = 2), *S. scuiri* (n = 1) and *S. capitis* (n = 1).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed by disk diffusion method on Mueller-Hinton agar accord-

Table 1. Sources of the staphylococcal isolates (%)

Specimens	MRSA	MSSA	CNS	Total (%)
Blood	23 (10)	18 (18)	36 (55.3)	77 (19.5)
Swabs[1]	174 (75.6)	58 (58)	14 (21.5)	246 (62.2)
Fluids	5 (2.1)	6 (6)	4 (6.1)	15 (3.8)
ETS	4 (1.7)	–	–	4 (1.01)
Sputum	2 (0.8)	1 (1)	1 (1.5)	4 (1.01)
Urine	–	–	1 (1.5)	1 (0.2)
Catheter tips	4 (1.7)	4 (4)	5 (7.6)	13 (3.3)
Axillae	5 (2.1)	3 (0)	–	8 (2.02)
Nasal swab	8 (3.4)	7 (7)	4 (6.1)	19 (4.8)
Groin	3 (1.3)	3 (3)	–	6 (1.5)
HVS	2 (0.8)	–	–	2 (0.5)
Total	230	100	65	395

[1] The swabs consisted of wound swabs, high vaginal swabs and ear swabs.

ing to the NCCLS guidelines [21, 22]. For susceptibility to mupirocin, disks containing 5 and 200 μg mupirocin (UniPath, Basingstoke, England) were used. For susceptibility to other antibacterial agents, commercial disks (UniPath) with the following concentrations of antibacterial agents were used: methicillin (5 μg), penicillin (10 μg), gentamicin (10 μg), kanamycin (30 μg), neomycin (30 μg), streptomycin (30 μg), erythromycin (15 μg), clindamycin (2 μg), chloramphenicol (30 μg), tetracycline (10 μg), minocycline (30 μg), trimethoprim (2.5 μg), fusidic acid (10 μg), rifampicin (5 μg), ciprofloxacin (5 μg), teicoplanin (30 μg), and vancomycin (30 μg). For sensitivity to heavy metals and nucleic acid-binding compounds 6-mm disks were impregnated with antimicrobial agents: cadmium acetate (50 μg), propamidine isethionate (50 μg), and ethidium bromide (60 μg). Zone sizes for each antibiotic were measured and compared to those produced by *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228. Minimum inhibitory concentration (MIC) to mupirocin was determined by the agar dilution method using a replicator instrument (Sigma Chemical Co.) with an inoculum of 10^5 cfu/ml in Mueller-Hinton agar containing doubling dilutions of the antibiotic with final concentrations of 2–1,024 μg/ml. The plates were incubated at 35 °C for 18 h and the MIC was recorded as the lowest concentration of antibiotic which completely inhibited bacterial growth.

Genetic Manipulations

Attempts were made to transfer mupirocin resistance from *S. haemolyticus* isolate CN216 to laboratory recipients and clinical isolates of *S. aureus* and coagulase-negative staphylococci in conjugation experiments as described previously [23]. Donor and transconjugants were screened for plasmid carried by the cetyltrimethylammonium bromide method as described previously [23].

Results

The 395 staphylococci studied were isolated from different clinical materials which are summarized in table 1. The majority, 246 (62.2%), of the isolates were from wound or related swabs. However, the majority of the CNS isolates were from blood cultures, an indication of the growing importance of these organisms in bloodstream infections. Although mupirocin has not been used specifically to eradicate colonization by CNS, the CNS isolates were tested for resistance to mupirocin because CNS has been found to express high-level resistance to mupirocin which

Table 2. Susceptibility of staphylococci to mupirocin

Isolates	n	Sensitive, %	Resistant, %	
		MIC ≤4 mg/l	MIC ≥16 mg/l	MIC >512 mg/l
MRSA	230	219 (95.2)	11 (4.8)	0.0
MSSA	100	98 (98.0)	2 (2.0)	0.0
CNS	65	56 (86.2)	8 (12.3)	1 (1.5)[a]
Total	395	373 (94.5)	21 (5.3)	1 (0.2)

[a] This isolate had an MIC >1,024 mg/l.

are borne on conjugative plasmids [18, 25]. All of the 395 isolates were tested for susceptibility to mupirocin and other antimicrobial agents. Their susceptibilities to mupirocin are as summarized in table 2. The results showed that 94% of the isolates were fully susceptible (MIC ≤4 mg/l).

Twenty-one (5.3%) of the 395 isolates expressed low levels of resistance (MIC 8–128 mg/l). One CNS isolate, identified as *S. haemolyticus* (CN216), expressed high-level resistance (MIC >1,024 mg/l). They were all susceptible to teicoplanin and vancomycin but were resistant to the other antimicrobial agents as shown in table 3. Resistance to gentamicin, streptomycin, tetracycline, trimethoprim, erythromycin, clindamycin, ciprofloxacin and fusidic acid was more common in MRSA than in MSSA isolates. Also the MRSA were more resistant to the non antibiotic agents, cadmium, propamidine isethionate and ethidium bromide. Forty-six percent of the CNS isolates were resistant to methicillin. These were also more resistant to other antimicrobial agents than the MRSA and MSSA isolates.

In clinical laboratories, susceptibility to mupirocin by the disk diffusion method is usually performed with two disks, one containing 5 μg and the other containing 200 μg of mupirocin. The 5-μg disk is used to detect low-level resistance while the 200-μg disk is used to detect high-level resistance. Therefore, for each isolate, two mupirocin disks are needed. Both disks could be used together on the same test plate or the 5-μg disk is used first to detect the presence of low-level resistance followed by the use of the 200-μg disk to confirm high-level resistance. This is technically

Table 3. Resistance of the isolates to other antimicrobials

Antimicrobial agents	% resistance		
	MRSA	MSSA	CNS
Methicillin	100.0	0.0	46.5
Penicillin G	100.0	86.0	80.0
Gentamicin	99.0	4.0	61.5
Tetracycline	94.8	46.0	44.6
Streptomycin	88.3	0.0	24.6
Trimethoprim	86.1	7.0	56.9
Erythromycin	66.5	7.0	63.1
Clindamycin	23.5	1.0	35.4
Ciprofloxacin	53.0	1.0	26.1
Chloramphenicol	25.2	2.0	32.1
Fusidic acid	21.7	0.0	24.6
Rifampin	0.9	0.0	12.3
Cadmium	96.5	47.0	47.7
Propamidine isethionate	11.6	7.4	17.2
Ethidium bromide	11.6	7.4	22.5

Table 4. Comparison of MIC values with zone diameters using different mupirocin disk concentrations

MIC, mg/l	Zone 5 μg	Diameters 200 μg
≤4	25–30	36–42
8	18–20	32–34
16	14–16	28–30
32	8–10	25–26
64	6	22–24
128	6	14–16
>512	6	6

laborious, time-consuming and costly. In this study, we compared zone sizes obtained by the two mupirocin disk concentrations with their corresponding MIC values to assess if the 200-μg disk could be used alone to accurately detect both low- and high-level mupirocin resistance. This would save time and reduce cost. The results are as shown in table 4. With the 5-μg disk, growth to the edge of the disk (zone size 6 mm) was obtained with MICs greater than 32 mg/l, but this did not distinguish between low- and high-level resistance. On the other hand, growth of the organism to the edge of the 200-μg disk corresponded with MIC >512 mg/l, and different zone sizes corresponded with different MIC values.

Transfer of Mupirocin Resistance

Genetic studies conducted earlier [24] demonstrated that the *S. haemolyticus* isolate CN216 carried five plasmids whose sizes were approximately 40, 24, 3.5, 1.8 and 1.6 kb. In experiments designed to study the genetic nature of the mupirocin resistance determinant of CN216, it was demonstrated that the high-level mupirocin resistance was carried out on a conjugative plasmid which was transferable to clinical isolates of *S. epidermidis, S. haemolyticus* and *S. saprophyticus.*

Discussion

Plasmid-mediated resistance to high-level mupirocin has been reported in *S. aureus* [17–20] and CNS [18, 25] with resistance reported after prolonged use of mupirocin in a few institutions [6, 14]. The results presented here have demonstrated that 94% of the staphylococci isolated in a burns unit were fully susceptible to mupirocin after 2 years of continuous use of mupirocin for treatment of staphylococcal infections and the eradication of colonization. The low level of resistance to mupirocin that was observed is in agreement with those of other published reports [5, 6, 8]. However, it was in contrast to the reports of Kauffman et al. [6] and Moy et al. [14], where resistance to mupirocin developed after prolonged use. It is important to note that while Kauffman et al. [6] and Moy et al. [14] used mupirocin for more than 10 days at a time on a patient which led to the development of resistance, mupirocin is usually administered for 5 days per patient in our unit. This practice, which is in accordance with the recommendations of the manufacturer of mupirocin [15], may have helped to keep the level of resistance low. Our finding was reassuring because it indicated that mupirocin was still an effective agent against MRSA isolated in the burns unit.

Characteristically, the MRSA isolates were more resistant to other antimicrobial agents than the MSSA. Also, methicillin resistance was high among the CNS but this was comparable to the incidence of methicillin resistance reported for CNS in another Kuwait hospital [22]. Nevertheless, mupirocin was equally effective against MRSA, MSSA and the CNS irrespective of their resistance to other agents. This is consistent with the absence of cross-resistance between mupirocin and other antibiotics [2, 15]. Although the patient from whom the resistant CNS was isolated had

been treated with mupirocin previously, it is not clear whether the *S. haemolyticus* isolate developed the resistance during therapy or whether the patient acquired an already resistant strain. No staphylococcal isolates from the burns unit were tested for resistance to mupirocin prior to mupirocin use. Nevertheless, high-level mupirocin resistance has been reported in some staphylococci isolated prior to the production and clinical use of mupirocin [18, 25]. Notwithstanding the original source of the mupirocin resistance determinant in the *S. haemolyticus,* its detection was timely because it helped to raise the awareness of staff to the presence of a potential problem. Since then, all the MRSA from the unit are tested for resistance to mupirocin. The finding that the high-level resistance to mupirocin in the *S. haemolyticus* was carried on a conjugative plasmid which was transferable to other staphylococci is of concern because it implies that the mupirocin resistance genes can be readily transferred to other staphylococci in vivo.

The results comparing MIC values of mupirocin to the zone sizes obtained by the 5-μg and 200-μg disks suggested that the 5-μg disk, used alone, would give exaggerated levels of resistance. On the other hand, using the 200-μg disk alone would miss low-level resistance. However, since the clinical significance of low-level resistance is presently not clear, it would be better to use the 200 μg to detect high-level resistance if one were to choose between the two disks, since only the high-level resistance is clinically significant at this stage [1, 2]. It appears that strains expressing low-level mupirocin resistance can be eradicated with mupirocin treatment [27]. In addition, low-level resistance can be detected with the 200-μg disk if the zone sizes are carefully measured (table 4).

In conclusion, the results of this study which demonstrates that mupirocin was highly effective against the majority of staphylococcal isolates in the burns unit is reassuring. However, it also drew attention to the real threat of high-level mupirocin resistance developing factor. This can occur by strains developing resistance during therapy or by acquiring resistant genes from other resistant staphylococci which might be introduced into the unit [26]. There is a strong need for constant vigilance through surveillance so as to detect any of these events early. Early detection of resistance and the institution of infection control measures will prevent the establishment and spread of high-level mupirocin-resistant strains in the unit. Finally, the results also emphasize the need for judicial use of mupirocin according to the recommendations of the manufacturer if the development of resistance during therapy is to be avoided.

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