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Original Article

The Emergence of Mupirocin Resistance among Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus* in Trinidad: a First Report

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SUMMARY: The objective of the study was to investigate the trend of mupirocin resistance among methicillin-resistant *Staphylococcus aureus* (MRSA) in Trinidad. No premarketing susceptibility surveillance was ever done following the introduction of mupirocin in 1986. A total of 188 MRSA strains recovered over a 2-year period from various body sites were tested for mupirocin resistance via the disc diffusion method. The major sources of MRSA were surgical site infections (74.0%) and bloodstream infections (8.0%). High-level and low-level mupirocin resistance were detected in 26.1 and 44.1% of MRSA stains, respectively. Resistances to other non-β-lactam antibiotics were also high. Ninety-eight percent of all MRSA were resistant to erythromycin. This was followed by resistance rates of 96.8, 95.2, 94.1, 93.6, and 93.1%, for gentamicin, ciprofloxacin, amikacin and tobramycin, co-trimoxazole, and tetracycline, respectively. No MRSA strains were found to be resistant to vancomycin, linezolid, and quinupristin-dalfopristin. The study showed that mupirocin resistance among Trinidadian MRSA strains was relatively high compared to that seen in other countries. Because of the increasing prevalence of MRSA at the San Fernando General Hospital (SFGH) and the apparently increasing resistance to mupirocin, frequent monitoring of MRSA susceptibility patterns and infection control initiatives may be helpful in reducing the incidence of MRSA with a concomitant decrease in mupirocin resistance. This report is the first after 20 years of continuous use of the drug at SFGH.

INTRODUCTION

Staphylococcus aureus has become the single most frequently isolated bacterial pathogen in hospitals (1,2), and the most common etiologic agent of nosocomial postoperative surgical wound infections (3-5). The impact of *S. aureus* infection on human health has dramatically increased as a result of the organism's remarkable ability to become resistant to antimicrobial agents (6-8). Soon after the introduction of methicillin, resistance of *S. aureus* to this drug emerged in Europe (9) and North America (10), and then worldwide (11-14). Resistance to methicillin implies resistance to all β-lactam antibiotics and is correlated with the development of increased resistance to other non-β-lactam antibiotic agents (15).

One of the few drugs that is still effective against methicillin-resistant *S. aureus* (MRSA) is mupirocin. Mupirocin was first introduced in the United Kingdom in 1985, and because of its success, has been widely used to treat various staphylococcal and streptococcal skin infections and exit-site infections in patients receiving peritoneal dialysis, and to eradicate nasal carriage of methicillin-susceptible *S. aureus* (MSSA) and MRSA (16-18). Two years after its introduction, resistance to mupirocin was reported (19). Since then, varying rates of mupirocin resistance have been reported. In 1993 in Ireland, a survey of 1,152 staphylococcal isolates from hospital and community sources found only 2% to be mupirocin resistant (20). In a 1997 European study, 3.9% of *S. aureus* strains were resistant to mupirocin (21). In the United States between 1990 and 1995, mupirocin resistance was found to be high (24%) among MRSA strains at a veterans' hospital where MRSA colonization was endemic and mupirocin was frequently used (22). Similarly, in one study (1994 - 1995) of a Brazilian hospital where usage of mupirocin was frequent, resistance to mupirocin was found to be >50%; in another nearby hospital where use was infrequent, mupirocin resistance was found to be 6% (23).

Mupirocin (pseudomonic acid A) is an analogue of isoleucine, which competitively binds to isoleucine-t-RNA synthetase, thereby inhibiting protein synthesis (21,24). The increased use of mupirocin in patients and healthcare workers has been accompanied by outbreaks of MRSA resistant to mupirocin (25,26). Although the frequency of resistance to the drug is low in some centers (21), the discovery of mupirocin-resistant strains may usher in a burst of resistance to the drug.

Mupirocin was introduced at the San Fernando General Hospital (SFGH) in 1986 to eradicate nasal carriage of MSSA and MRSA. This topical agent was considered safe, effective, and less costly, and lacked the untoward effects on normal body flora associated with oral antimicrobial agents. Several studies, however, have shown an association between increased mupirocin usage and the emergence of high-level mupirocin resistance (21,22,25). The purpose of this study was to investigate the prevalence of mupirocin resistance among clinical isolates of MRSA at SFGH.

MATERIALS AND METHODS

Collection of clinical isolates: A total of 188 clinical isolates of MRSA collected during 2005 and 2006 were included in the study. The isolates were associated with bloodstream

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infections, skin and soft tissue infections, urinary tract infections, nosocomial pneumonia and nasal colonization of health care providers. Repeated recovery of the same organism from the same patient was considered as one isolate.

Antimicrobial susceptibility testing: Susceptibility to a panel of 14 antimicrobial agents was performed by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (27). Disks containing the following antimicrobials and concentrations (in parentheses) were used: gentamicin (10 μg), tobramycin (10 μg), vancomycin (30 μg), ciprofloxacin (5 μg), co-trimoxazole (trimethoprim-sulfamethoxazole) (1.25/23.75 μg), erythromycin (15 μg), tetracycline (30 μg), clindamycin (2 μg), chloramphenicol (30 μg), rifampin (5 μg), linezolid (30 μg), quinupristin-dalfopristin (15 μg), and mupirocin (5 μg and 200 μg). All isolates were tested via the disc diffusion method using a 5-μg mupirocin disc as a first step in determining resistance. Zone diameter breakpoints for susceptible and resistant isolates were set at ≥14 mm and ≤13 mm, respectively, as recommended by Finlay et al. (28). High-level resistance was confirmed using a 200-μg mupirocin disc. CLSI guidelines and breakpoints were used throughout the study. Reproducibility of mupirocin resistance was done using randomly selected isolates. Resistance to methicillin was identified using a 1-μg oxacillin disc, on Mueller-Hinton agar according to CLSI guidelines (27). Methicillin-resistant *Staphylococcus aureus* ATCC strain 25923 and methicillin-susceptible *Staphylococcus aureus* ATCC strain 49476, obtained from the Caribbean Epidemiology Center (CAREC), a branch of PAHO/WHO, were used as control organisms.

RESULTS

The distribution of MRSA isolates among the sites of infection is shown in Table 1. Of the 188 strains of MRSA, 139 (73.9%) were isolated from surgical site infections, 15 (8.0%) from blood, 11 (5.8%) from lower respiratory tract (LRT) specimens (see footnotes of Table 1), and 8 each (4.2%) from boils/abscesses and septic diabetic ulcers, respectively. The other sources of MRSA were from infected burn wounds (1.6%), swabs from peritoneal dialysis exit sites (1.6%) and urine (0.5%). The overall prevalence of high-level resistance to mupirocin was 26.1%, and a roughly equivalent percentage of strains (29.8%) were susceptible to mupirocin. Low-level resistance among MRSA was 44.1%. High-level resistance had heavy growth with no visible zone of inhibition around the disk; low-level resistant isolates produced hazy or no zone of inhibition. Susceptible strains had clear zones.

Table 1. Site of infection and frequency of resistance to mupirocin among 188 clinical isolates of MRSA at the San Fernando General Hospital

Site of infection	No. of mupirocin-sensitive and -resistant isolates			
	Total	Sensitivity	L-L resistance (5 μg)	H-L resistance (200 μg)
SSI	139 (74.0)	46 (30.1)	56 (40.3)	37 (26.6)
Blood	15 (8.0)	4 (26.7)	7 (46.7)	4
Pus/abscess	8 (4.2)	2 (25.0)	4 (50.0)	2 (25.0)
Septic ulcer	8 (4.2)	0	6 (75.0)	2 (25.0)
ETT[1)]	9 (4.8)	1 (11.1)	6 (66.7)	2 (22.2)
Burn	3 (1.6)	1 (33.3)	1 (33.3)	1 (33.3)
PD-exit site	3 (1.6)	0	2 (66.7)	1 (33.3)
Sputum[1)]	2 (1.1)	1 (50.0)	1 (50.0)	0
Urine	1 (0.5)	1 (100.0)	0	0
Total	188	56 (29.8)	83 (44.1)	49 (26.1)

[1)]: They are considered as LRT (lower respiratory tract) specimens.
MRSA, methicillin-resistant *Staphylococcus aureus*; L-L, low-level; H-L, high-level; SSI, surgical site infection; ETT, endotracheal tube; PD-exit site, peritoneal dialysis exit site.

Table 2. Antimicrobial susceptibility profile of 188 MRSA strains to non-β-lactam antimicrobial agents at the San Fernando General Hospital

Antimicrobial	No. (%) resistant to:
Erythromycin	184 (98.0)
Gentamicin	182 (96.8)
Ciprofloxacin	179 (95.2)
Amikacin	177 (94.1)
Tobramycin	177 (94.1)
Trimethoprim-sulfamethoxazole	176 (93.6)
Tetracycline	175 (93.1)
Clindamycin	130 (69.1)
Rifampin	6 (3.3)
Chloramphenicol	25 (13.3)
Quinupristin-dalfopristin	0
Linezolid	0
Vancomycin	0
Oxacillin	188 (100.0)

Table 2 shows the proportion of MRSA strains resistant to other antibiotics. The prevalence of resistance to erythromycin, gentamicin, ciprofloxacin, tobramycin and amikacin, co-trimoxazole, and tetracycline was 98.0, 96.8, 95.2, 94.1, 93.6, and 93.1%, respectively; while resistance rates for clindamycin was 69.1%. Each isolate was fully sensitive to quinupristin-dalfopristin, linezolid, and vancomycin, while resistant rates of 13.3 and 3.3% were observed for chloramphenicol and rifampin, respectively. Quinupristin-dalfopristin and linezolid are currently not listed in the hospital formulary.

DISCUSSION

The prevalence of mupirocin resistance among MRSA during the study period was high (44.1 and 26.1%, for low-level and high-level, respectively) when compared to the overall resistance rates reported in the literature (1 - 13.8% for low-level and 2.4 - 14.0% for high-level resistance) (22,29, 30), but similar to that reported by Vasquez et al. (31), (low-level resistance, 58%; high-level resistance, 42%). The initial report of *S. aureus* resistance to mupirocin appeared shortly after the introduction of the drug into clinical practice (29,32); since then most reports of resistance to mupirocin have focused primarily on outbreaks (22,27,29,32-35). *S. aureus* outbreaks are usually due to MRSA, where mupirocin is preferentially used to eradicate nasal carriage and nosocomial infections due to such organisms. Most MRSA isolates at our institution are recovered from infected surgical sites and burn wounds (3,15). Topical use of mupirocin has increased because of efforts by the infection surveillance, control and prevention department to eradicate MRSA from the nasal passageways of health care workers, to prevent peritonitis and exit-site infections in patients having peritoneal dialysis, and to manage infected superficial wounds. None of the patients given mupirocin had any prior history of mupirocin usage. Infections, therefore, may have occurred via horizontal spread of resistant clones in the hospital. By this logic, analysis of the relationship of high mupirocin resistance prevalence

and strain clonality via pulsed-field gel electrophoresis would have been appropriate, had such a typing method been available in Trinidad.

Because of the increase in MRSA recovery from infected superficial sites and the anterior nares of healthcare workers, 294 prescriptions for mupirocin for inpatients and staff were filled by the hospital pharmacy in 2005. In 2006, 713 tubes of mupirocin (a 58.8% increase) were prescribed. The prevalence of MRSA at SFGH has been increasing over the years (9.8% in 1999 to 18.6% in 2004) (3,15); an increase in mupirocin resistance may be associated with this trend, due to the concomitant increase in mupirocin usage. In other words, once a patient is found to be colonized or infected with MRSA, it is very likely that mupirocin would be prescribed for long periods or even for their entire hospital stay. Mupirocin was first introduced in this country in 1986, but no structured premarketing susceptibility surveillance was ever done. Frequent and prolonged usage may usher in resistant strains, as was the case with endemic MRSA in a long-term care facility in the United States (36). Blanket treatment of healthcare workers or patients for MRSA carriage has also resulted in resistance to the drug (37). It is quite disheartening that despite these well documented reports, such practices are continuing, especially within our institution.

The emergence of mupirocin resistance among MRSA signals the potential loss of a major drug against these organisms. At our institution, the only agents for systemic infections to which most MRSA remain very sensitive are vancomycin, rifampin, and chloramphenicol. Judicious use of these drugs must be carefully employed. 'Blanket' use of mupirocin must be stopped, and eradication strategies must be carefully designed, as proposed by Cookson (22). The use of mupirocin in the prevention of peritonitis and exit-site colonization in peritoneal dialysis patients as well as surgical prophylaxis must be carefully monitored. If the prevalence of MRSA in our institution is contained, then the emergence of mupirocin resistance may also be contained. This connection between methicillin resistance and mupirocin resistance has been reported in the literature (24,30).

There are also increases in the frequent use of other drugs effective against MRSA in 2005 and 2006. For vancomycin, there was a 32.5% increase in 2006; and for rifampin, it was 45.7% (Hospital Pharmacy Records). To date, laboratory data has shown that no MRSA isolate has been found to be resistant to vancomycin, although 6 of 100 randomly selected MRSA strains were found to be resistant to rifampin. Other MRSA-sensitive agents such as linezolid, quinupristin-dalfopristin, daptomycin, and tigecycline are not yet available in the hospital formulary.

In this first report, the incidence of mupirocin resistance in a Trinidad hospital was determined. The resistance rate is relatively high when compared with that seen in other countries. Because no previous rates have been documented, no comparison with this study could be made. Therefore, it is open to speculation whether the resistance rate as now observed, will continue to increase unless steps are taken to contain this trend.

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