IN VITRO ACTIVITIES OF 28 ANTIMICROBIAL AGENTS AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) FROM A CLINICAL SETTING IN MALAYSIA

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Abstract. Methicillin-resistant *Staphylococcus aureus* (MRSA), an established nosocomial and emerging community pathogen associated with many fatalities due to its hyper-virulence and multiple drug resistant properties, is on the continuous rise. To update the current status on the susceptibility of local MRSA isolates to various classes of antibiotics and to identify the most potent antibiotics, thirty-two clinical isolates comprised of hospital acquired (HA) and community acquired (CA) infections were investigated by disk diffusion test. Of the 32 MRSA isolates, 14 (43.75%) and 18 (56.25%) were community and hospital acquired MRSA, respectively. All isolates were multiple drug resistant to more than 3 classes of antibiotics despite the source or specimen from which it was isolated. The oxacillin MICs for all isolates ranged from 2 to ≥ 256 μg/ml. Twenty-five of 26 erythromycin-resistant MRSA isolates exhibited an inducible MLS_B resistance phenotype while one showed an MS phenotype. More than half the isolates (68.75%) were resistant to at least one of the six aminoglycosides tested, with netilmicin as the most susceptible. The most effective antistaphylococcal agents were linezolid, vancomycin, teicoplanin and quinupristin/dalfopristin exhibited 100% susceptibility. Since MRSA is under continuous pressure of acquiring multiple drug resistance, it is imperative to focus routine surveillance on HA and CA-MRSA strains to monitor and limit the spread of this organism.

INTRODUCTION

Despite the development and introduction of many new antimicrobials, methicillin-resistant *Staphylococcus aureus* (MRSA) infections remain an important cause of concern amongst the general public and physicians alike. This genetically adaptive organism is associated with a diverse range of infections, which include skin and soft tissue infections, endocarditis, bacteremia and toxin mediated diseases, such as scalded skin syndrome, toxic shock syndrome and gastroenteritis (Shopsin and Kreiswirth, 2001; Salgado *et al*, 2003). Although MRSA traditionally has been confined to nosocomial settings, the frequent isolation of MRSA from the community has become a new threat (Naimi *et al*, 2003; Tacconelli *et al*, 2003).

In Malaysia, the treatment of choice for serious MRSA infection is vancomycin (glycopeptides) (Norazah *et al*, 2002). However, antibiotics from many other groups (aminoglycosides, folate pathway inhibitors, glycopeptides, lincosamides, macrolides, oxazolidinones, phenicals, quinolones, tetracyclines, ansamycins) either individually or in combination have been used for the treatment of MRSA infections.

MRSA has acquired resistance to many structurally unrelated antibiotics; furthermore

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resistance develops rapidly whenever a new antibiotic is introduced (Lyon and Skurray, 1987; Lowy, 2003). Resistance to vancomycin, which is a drug of choice, documented in the United States in 2002 (CDC, 2002) is one such example. The phenomena of multiple drug resistance and emergence of vancomycin resistant MRSA strains have limited the therapeutic options for the treatment of MRSA infection. Although *S. aureus* isolates with reduced susceptibility or resistant to vancomycin has not been reported in Malaysia, its likely to happen is not too far. Assessing MRSA susceptibility to alternative antibiotics can prevent the abuse of glycopeptides and also identify other effective antibiotics for the treatment of *S. aureus* infection.

The aims of the present study were to analyze the antimicrobial susceptibility patterns of MRSA isolated from a clinical setting in Malaysia against 28 antimicrobials (old and new) and to identify the most effective antibiotics.

MATERIALS AND METHODS

Bacterial isolates

Clinical MRSA isolates were collected from the microbiology laboratory in a tertiary hospital from October 2006 to February 2007. Only one isolate per patient was included in the study. Criterion for a nosocomial infection was an infection which developed in a patient after 48 hours of hospitalization. Strains were considered as community acquired when isolated from patients that had not been hospitalized recently or within the first 48 hours of hospitalization. All isolates were identified in the hospital and were reconfirmed in our laboratory based on colony morphology, Gram staining, coagulase test and PCR assay for species specific *S. aureus* and the *mecA* gene (Murakami *et al,* 1991; Martineau *et al,* 1998) for methicillin-resistant *S. aureus*. All isolates were immediately stored at -80ºC for further study.

Antimicrobial susceptibility testing

Susceptibilities to penicillins, cefotaxime, ampicillin, imipenem, cefoxitin, oxacillin, amikacin, gentamicin, kanamycin, netilmycin, streptomycin, neomycin, sulphamethoxazole/trimethoprim, trimethoprim, vancomycin, teicoplanin, clindamycin, erythromycin, linezolid, chloramphenicol, norfloxacin, ofloxacin, ciprofloxacin, quinupristin/dalfopristin, minocycline, tetracycline, fusidic acid, and rifampin were determined by the disk diffusion technique according to the guidelines of Clinical Laboratory Standards Institute (CLSI, 2005). MIC values for oxacillin were determined by E-test (AB Biodisk, Solna, Sweden) according to the manufacturer's recommendations included in the packaging inserts. ATCC 29247 and ATCC 700698 were used as quality controls. The breakpoints for resistance were those recommended by the CLSI. For fusidic acid, neomycin and streptomycin, which are not stated in the CLSI guidelines, the following were used; ≤ 18 mm - fusidic acid (Skov *et al,* 2001), ≤ 16 mm - neomycin, and ≤ 14 mm - streptomycin (Kim *et al*, 2004). Inducible clindamycin resistance was determined by the double disc diffusion test (D-test) by placing a 2-μg disk of clindamycin 15 mm away from the edge of a 15-μg disk of erythromycin on an agar plate. A truncated or blunted clindamycin zone of inhibition (D-shape) indicated inducible resistance. Constitutive resistance was recognized by a clindamycin zone diameter of ≤ 14 mm (Fiebelkorn *et al,* 2003). The resistant rate was calculated as the number of intermediate and resistant isolates divided by the total number of isolates. Multiple drug resistance was defined as resistance to β-lactams plus three or more of the following groups: aminoglycosides, glycopeptides, folate pathway inhibitors, tetracylines, quinolones, macrolides, phenicals, lincosamides, oxazolidinones, fusidic acid, ansamycins and streptogramins.

Table 1
Distribution of MRSA positive specimens from hospital and community sources.

Specimen	HA-MRSA (*n*=18, 56%)		CA-MRSA (*n*=14, 44%)	
	n	%	*n*	%
Pus/wound/abscess	10	55.5	8	57.1
Urine	2	11.1		
Tracheal aspirate	3	16.7		
Blood	3	16.7	6	42.9

RESULTS

A total of 32 MRSA isolates were studied, consisting of 14 (44%) community acquired MRSA specimens (CA) and 18 (56%) hospital acquired specimens, excluding consecutive samples from the same patient, were collected (Table 1). Concerning the origin of CA-MRSA isolates, 8 (57.1%) were from pus/wounds/abscesses and 6 (42.9%) were from blood specimens. For HA-MRSA, 10 (55.5%) were from pus/wound/abscess, 2 (11.1%) from the urine and 3 each (16.7%) from blood and tracheal aspirate specimens. All isolates showed gram-positive cocci in clusters and gave positive reactions on mannitol salt fermentation, catalase, tube coagulase, Sa442 and *mecA* PCR assay. The oxacillin MICs for all isolates ranged from 2 to ≥ 256 μg/ml, only 6.25% of the isolates were inhibited at an MIC below 256 μg/ml. Among the β-lactams tested, all isolates showed resistance to penicillins, ampicillin, oxacillin and cefoxitin, while with cefotaxime and imipenem, resistance rates ranged from 78% to 94% (Table 2). All isolates were multiple drug resistant, resistant to more than 3 classes of antibiotics (Table 3).

Of the 26 erythromycin-resistant MRSA isolates, 25 exhibited an inducible MLS_B resistance phenotype and one exhibited an MS phenotype. None of the isolates showed constitutive MLS_B resistance

Of the 32 isolates, 22 (68.7%) were resistant to at least one of the six aminoglycosides tested. Isolates were resistant to kanamycin in 98%, gentamicin in 95%, and amikacin in 90%. Most of the MRSA isolates were resistant to multiple aminoglycosides, there was resistance to amikacin in 78.1%, gentamicin in 78.1%, kanamycin in 75%, streptomycin in 81.2%, and neomycin in 87.5% (Table 2). Rates of resistance to netilmicin in less than 70%, the most effective aminoglycosides tested.

All isolates were susceptible to vancomycin, quinupristin/dalfopristin and linezolid (Table 2).

DISCUSSION

The widespread use of antibiotics has led to changes in the antibiotic susceptibility patterns of microorganisms, evidenced by increasing occurrences of antibiotic resistance among bacterial populations (Pfaller *et al,* 1999; Witte, 1999). Resistance rates vary from country to country depending on antibiotic policies, the strictness of infection control committees and under reporting. It is imperative to perform a local surveillance of frequently encountered dangerous pathogens, like MRSA, that are prone to acquire resistance rapidly. The antibiograms obtained update changes in susceptibility patterns.

The current study is limited by the small number of isolates, however, it is the total number of non-duplicate isolates obtained

Table 2
Numbers and percentages of MRSA isolates resistant to antimicrobial agents by CLSI diffusion method.

Antibiotic	Total isolates (*N*=32) *n*(%)	Hospital-acquired MRSA (*N*=18) *n*(%)	Community acquired MRSA (*N*=14) *n*(%)
β-lactams			
Penicillin	32 (100)	18 (100)	14 (100)
Cefotaxime	30 (93.7)	18 (100)	12 (85.7)
Ampicillin	26 (100)	12 (66.6)	14 (100)
Imipenem	25 (78.1)	15 (83.3)	10 (71.4)
Cefoxitin	32 (100)	18 (100)	14 (100)
Oxacillin	32 (100)	18 (100)	14 (100)
Aminoglycosides			
Amikacin	25 (78.1)	14 (77.7)	11 (78.5)
Gentamicin	25 (78.1)	15 (83.3)	10 (71.4)
Kanamycin	24 (75)	12 (66.6)	12 (85.7)
Netilmycin	22 (68.7)	12 (66.6)	10 (71.4)
Streptomycin	26 (81.2)	15 (83.3)	11 (78.5)
Neomycin	28 (87.5)	15 (83.3)	13 (92.8)
Folate pathway inhibitors			
Sulphamethoxazole / Trimethoprim	24 (75)	15 (83.3)	9 (64.2)
Trimethoprim	29 (90.6)	18 (100)	11 (78.5)
Glycopeptides			
Vancomycin	0 (0)	0 (0)	0 (0)
Teicoplanin	0 (0)	0 (0)	0 (0)
Lincosamides			
Clindamycin	0 (0)	0 (0)	0 (0)
Macrolides			
Erythromycin	26 (81.2)	14 (77.7)	12 (85.7)
Oxazolidinones			
Linezolid	0 (0)	0 (0)	0 (0)
Phenicals			
Chloramphenicol	7 (21.8)	4 (22.2)	3 (21.4)
Quinolones			
Norfloxacin	25 (78.1)	14 (77.7)	11 (78.5)
Ofloxacin	17 (53.1)	7 (38.8)	10 (71.4)
Ciprofloxacin	27 (84.3)	15 (83.3)	12 (85.7)
Streptogramins			
Quinupristin / Dalfopristin	0 (0)	0 (0)	0 (0)
Tetracylines			
Minocycline	21 (65.6)	13 (72.2)	8 (57.1)
Tetracycline	27 (84.3)	16 (88.8)	11 (78.5)
Fusidic acid	11 (34.3)	6 (33.3)	5 (35.7)
Ansamycins			
Rifampin	5 (15.6)	3 (16.6)	2 (14.2)

Table 3
Most common phenotypic resistance patterns of multi-resistant MRSA for 12 antimicrobial classes.

AM (CN)	FPI (SXT)	GLY (TEC)	LIN (DA)	MAC (ERY)	OXA (LZD)	PHE (C)	QUI (CIP)	STR (QD)	TET (TE)	FD (FD)	AN (RD)
R	R	S	S	R	S	R	R	S	R	S	S
R	R	S	S	R	S	S	R	S	R	S	S
R	R	S	S	R	S	S	R	S	R	R	S

AM=aminoglycosides, FPI=folate pathway inhibitors, GLY=glycopeptides, LIN=Lincosamides, MAC= macrolides, OXA=oxazolidinones, PHE=phenicals, QUI=quinolones, STR=streptogramins, TET=tetracyclines, FD=fusidic acid, AN=ansamycins, CN=gentamicin, SXT=sulphamethoxazole/trimethoprim, TEC=teicoplanin, DA=clindamycin, ERY=erythromycin, LED= linezolid, C=chloramphenicol, CIP=ciprofloxacin, QD=quinupristin/dalfopristin, RD=rifampin

from a tertiary hospital over a 5 months period. The prevalence rates for CA and HA-MRSA in the hospital were similar, which shows that both strains are equally potent in causing infections. The prevalence of MRSA has increased worldwide, as is evidenced by many surveillance studies (Diekema *et al*, 2001; Jones *et al,* 2003). The highest rates of MRSA prevalence have been noted in developed countries, especially in Western Pacific regions, both in community-acquired and hospital infections (Diekema *et al,* 2001). In Malaysia the prevalence of MRSA increased from the range of 10-25% in 1985-1986 to more than 40% in 1996 according to surveys conducted in several hospitals (Lim *et al,* 1988; Rohani *et al,* 2000). The current study identified a large number (43.7%) of CA-MRSA from infectious samples isolated within 48 hours of admission. CA-MRSA identification was further supported by genetic analysis through staphylococcal cassette chromosome *mec* (SCC*mec)* typing. A positive signal for SCC*mec* type V in CA-MRSA, and SCC*mec* type III in HA-MRSA isolates, reconfirms the MRSA isolate source as either HA or CA. Although there was no CA-MRSA reported from the hospital setting in Malaysia, a previous study by us on nasal carriage of MRSA in healthy individuals in the community identified 3 CA-MRSA strains (Neela *et al*, 2007). Although this study is the first report of the isolation of CA-MRSA from hospital setting in Malaysia, CA-MRSA is increasingly being reported in the neighboring country of Singapore (Hsu *et al,* 2006; Chua and Lee, 2006). Our findings are in accordance with Gonzalez *et al* (2006) who reported 24 (65%) of 37 MRSA isolates from blood cultures were CA-MRSA. For most of the MRSA isolates (93.7%), the oxacillin MIC was ≥ 256 μg/ml. The high level of oxacillin resistance attained through acquisition of the *mecA* gene was detected by PCR on all isolates tested, and is in accordance with the report by Kim *et al* (2004).

No matter what the origin of the isolates, all were multidrug resistant (Table 3). This finding is in agreement with Randrianirina *et al* (2007), who also found no difference in the rates of resistance in strains isolated from nosocomial infections and community acquired infections. Although 20 different multiple drug resistant patterns were identified, only 3 patterns (Table 3) were found to dominate.

About 81.2% of MRSA strains were resistant to erythromycin, but all were susceptible to clindamycin. An inducible MLS_B phe-

notype was detected in 96.1% of MRSA strains, while 3.9% showed an MS phenotype. A recent survey in South Africa found that 100% of MRSA strains were D-test positive (Shittu and Lin, 2006) which is in accordance with our observations. However, in Korea 86% of MRSA strains exhibited constitutive resistance (Kim *et al*, 2004). The constitutive type MLS_B phenotype is a common feature among MRSA isolates in Turkey (Aktas *et al*, 2007) and Belgium (Denis *et al,* 2004). These observations indicate the incidence of constitutive and inducible MLS_B resistance in staphylococcal isolates varies by geographic region. Out of the 26 erythromycin resistant strains tested in the current study only one isolate exhibited an MS phenotype. It is important to distinguish inducible MLS_B strains from those that contain the *msrA* (MS phenotype) gene to encode an efflux pump that affects only macrolides, not clindamycin. The D-test plays an important role in identification of clindamycin resistant strains, which is not possible by routine antimicrobial susceptibility testing. Our study found, more than 90% of erythromycin resistant strains are also clindamycin resistant, which emphasizes the point that the D-test can reduce treatment failures due to clindamycin.

High numbers of isolates were resistant to aminoglycosides, which is in agreement with Kim *et al* (2004) who found more than 90% of MRSA isolates are resistant to aminoglycosides. Although most MRSA isolates are resistant to aminoglycosides, when given in combination with beta-lactam antibiotics or vancomycin, they are effective in treating serious staphylococcal infections due to their synergistic effect (Lowy, 1998).

The rates of MRSA resistance to rifampin, fusidic acid and chloramphenicol were much lower (14 to 36%) than those of other antibiotics (Table 2). Although vancomycin is the mainstay of therapy for systemic MRSA infections, not all infections are death causing, hence oral antibiotics can provide an alternative mode of therapy, particularly when prolonged therapy is needed. Rifampin in combination with fusidic acid, chloramphenicol or clindamycin are widely available oral agents in Malaysia, which demonstrate better tissue penetration than glycopeptides.

Linezolid showed excellent activity, equal to that of vancomycin. The complete susceptibility of *S. aureus* to quinupristin/dalfopristin observed in this study agrees with the data from a previous study in Malaysia (Norazah *et al,* 2005) indicating that quinupristin/dalfopristin is an excellent and effective agent for the treatment of *S. aureus* infections in Malaysia. Based on the results of the current study, the most effective therapeutic options for MRSA infections identified are vancomycin, teicoplanin, linezolid and quinupristin/dalfopristin.

In summary, the rates of multiple drug resistance in MRSA isolates obtained from a tertiary hospital, both in community and in hospital acquired infections, are very high. First line anti-staphylococcal antibiotics are less effective against MRSA. Antibiotic susceptibility testing is crucial to monitor the changing patterns of resistance. The most effective antibiotics in this time are vancomycin, teicoplanin, linezolid and quinupristin/dalfopristin. We are left with few choices of antibiotics, so let us preserve the effective ones by judicial use.

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