Prevalence and antibiotic Profiles of Mrsa, Thailand

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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS, COLLECTED AT THAMMASAT UNIVERSITY HOSPITAL, THAILAND, AUGUST 2012 - JULY 2015

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Abstract. We analyzed data of Staphylococcus aureus isolated from patients attending Thammasat University Hospital, Thailand from August 2012 to July 2015. In total, 232/502 (46%) S. aureus isolates were methicillin-resistant S. aureus (MRSA). There was a declining trend of proportion of MRSA infection, but the prevalence of MRSA in the last year of study remained high (38%). All 32 MRSA-infected outpatients had history of exposure to healthcare facilities during the previous two months and thus were not considered as having community-associated MRSA. In addition, all these strains were negative for pvl, suggesting that these strains were hospital-associated MRSA. All MRSA stains were susceptible to linezolid, teicoplanin and vancomycin, but resistance to erythromycin and clindamycin were nearly 100%. Fifty-two percent and 87% of MRSA strains were susceptible to tetracycline and trimethoprim-sulfamethoxazole, respectively. These results emphasize the necessity of long-term surveillance and monitoring of antimicrobial susceptibility pattern of MRSA.

Keywords: Staphylococcus aureus, antimicrobial susceptibility, MRSA, Thailand

INTRODUCTION

Resistant strains of Staphylococcus aureus especially methicillin-resistant S. aureus (MRSA) have become a worldwide threat to public health (Boucher and Corey, 2008). Infections with these strains are more difficult and expensive to treat, leading to significant morbidity and mortality and to an increase in healthcare burden. The pandemic of both hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) instigates a global concern. In Asia, a high prevalence of MRSA infection, where the proportion is greater than 70%, has been observed in several countries, including Japan, Korea, Taiwan, and Vietnam (Chen and Huang, 2014). Those countries also experience a high incidence of CA-MRSA infection (Chen and Huang, 2014).

In Thailand, data from two multicenter studies revealed MRSA prevalence of 53-57%, the majority being HA-MRSA.
with only 2.5% CA-MRSA (Song et al., 2011; Mendes et al., 2013). MRSA is usually resistant to all beta-lactams, but resistance to other antibiotic classes varies among strains (Chua et al., 2011). Predominant MRSA strains isolated from different geographic regions may manifest differences in antibiotic susceptibility patterns (antibiograms) (Chua et al., 2011). Given the diversity of MRSA strains and their evolving antibiogram, it is crucial to monitor the emergence of CA-MRSA and to conduct an antibiotic surveillance study. Currently, there is limited information of antibiotic susceptibility of MRSA isolated from Thammasat University Hospital. Thus, this study investigated the epidemiology of S. aureus and MRSA infections among patients visiting Thammasat University Hospital (a tertiary-care, academic hospital), Pathum Thani, Thailand and determined antibiograms of MRSA isolates. The data provide essential information for infection control monitoring and for establishing institutional guidelines for staphylococcal treatment.

MATERIALS AND METHODS

Study setting

Thammasat University Hospital, Pathum Thani is located approximately 40 km from Bangkok Metropolitan and provides tertiary medical care in all service sectors to an average of 1,000 outpatients per day, with 500 beds for inpatients.

Data collection

All S. aureus-positive clinical specimens sent to the Microbiology Laboratory, Thammasat University Hospital from August 2012 to July 2015 were included in the analysis. Conventional methods of strain identification included coagulase test, PR-glucose and PR-mannitol fermentation (Ishii et al., 2006). If all three tests were positive, the strain was identified as S. aureus.

Screening of MRSA strains

Cefoxitin disk diffusion test was used to screen all S. aureus isolates (CLSI, 2013). Isolates with a zone of growth inhibition ≥ 22 mm are defined as methicillin-sensitive S. aureus (MSSA), and those with a zone diameter < 22 mm as methicillin-resistant S. aureus (MRSA).

Criterion of CA-MRSA and HA-MRSA

Patients infected with CA-MRSA are defined by culture-confirmed MRSA infection when presenting at an outpatient clinic or within 48 hours of hospitalization without history of exposure to healthcare facilities during the previous two months. HA-MRSA-infected patients are defined as those whose history did not meet the definition of CA-MRSA.

PCR detection of S. aureus mecA and pvl

Total DNA was isolated from an inoculum of an overnight S. aureus culture using Genomic DNA Extraction Mini Kit (RBC Bioscience, New Taipei City, Taiwan). The primer pair used for mecA amplification was 5’-TC-CAGATTACAACCCCTCACCAGG-3’ and 5’-CCACTTCATATCTTGTAACG-3’, and for pvl 5’-ATCATTAGTAAATGTCTGGACATGATCC-3’ and 5’-GCATCAASTGGTATTGGATAGCAAAAGC-3’ (Integrated DNA Technologies, Singapore). PCR mixture consisted of 50-µl mixture of 10X PCR buffer, 50 mM MgCl₂, 10 mM dNTPs, 100 µM specific primer pair and 1.25 U Taq polymerase (RBC Bioscience). Thermocycling was conducted in a MyCycler™ Thermal Cycler (Bio-Rad, Hercules, CA) as follows: 94°C for 2 minutes; followed by 30 cycles of 94°C for 30 seconds, 51°C (for mecA) or 56°C (for pvl) for 30 seconds,
and 72°C for 1 minute; with a final heating at 72°C for 5 minutes. Amplicons (162 bp and 433 bp of mecA and pvl, respectively) were analyzed by 1% agarose gel electrophoresis, stained with GelStar™ Nucleic Acid Gel Stain (Lonza Rockland, Rockland, ME) and visualized under UV light. S. aureus N315 and KKU-MS14 strains, kindly provided by Dr Aroonlug Lulitanond, Faculty of Associated Medical Sciences, Khon Kaen University, Thailand, were used as mecA- and pvl-positive control, respectively.

**Antibiogram determination**

MRSA isolates were tested for antibiotic susceptibility using a standard disk diffusion method (CLSI, 2013), employing clindamycin (CD), erythromycin (E), fosfomycin (FOS), fusidic acid (FD), linezolid (LZD), teicoplanin (TEC), tetracycline (TE), trimethoprim-sulfamethoxazole (SXT), and vancomycin (VAN) (Liofilchem®, Arezzo, Italy).

**Statistical analysis**

Difference of proportion between MRSA and MSSA was tested using chi-square test (SPSS Statistics 22.0) (IBM, Armonk, NY). A p-value < 0.05 is considered significantly different.

**Ethical considerations**

The study was approved by the Human Research Ethics Committee of Thammasat University (approval no. MTU-EC-DS-6-015/57). All patients gave informed consent prior to the study.

**RESULTS**

**S. aureus isolates**

A total of 536 S. aureus isolates were obtained from clinical specimens of 502 patients during the 3-year study period. Only the first isolate from a patient with recurrent infections was used. Over the 3-year survey period, in the first (August 2012 - July 2013), second (August 2013 - July 2014) and third (August 2014 - July 2015) year there were 118 (57% MRSA), 150 (51% MRSA) and 234 (38% MRSA) S. aureus isolates, respectively. Although the incidence of S. aureus infection nearly doubled over the 3-year period, the proportion of MRSA significantly declined. All MRSA isolates harbored mecA encoding penicillin binding protein 2A (data not shown) (Ubukata et al, 1989). Distribution of S. aureus isolates according specimen types were as follows: sputum, 244 (67% MRSA); pus, 160 (18% MRSA); blood, 81 (37% MRSA); urine, 7 (86% MRSA); body fluid, 6 [4 synovial and 2 ascitic, 17% MRSA (from ascetic)]; and vaginal discharge, 4.

As regards the distribution of S. aureus among clinical wards, 109 (22%) isolates were from the Outpatient Department: Outpatient clinics, 63 samples (14% MRSA); Emergency room (ER), 42 (55% MRSA) and Hemodialysis center, 4. Among the Inpatient Departments, highest number of S. aureus samples was from Internal Medicine wards (239 isolates, 62% MRSA), followed by Surgery (78, 42% MRSA), Operation theaters (39, 23% MRSA), Pediatrics (34, 26% MRSA), and Obstetrics-Gynecology (3, 0% MRSA).

Review of outpatients’ illness history and based on the criterion for CA-MRSA, all 32 outpatients could be ruled out as putative CA-MRSA cases. All inpatients with culture-positive MRSA were detected after 48 hours of admission. No MRSA isolate in this study carried pvl, often associated with CA-MRSA (data not shown) (David and Daum, 2010).

**Antibiotic susceptibility of MRSA isolates**

According to antibiogram profiles, the 232 MRSA isolates can be classified
Table 1
Antibacterial susceptibility patterns of MRSA isolates collected at Thammasat University Hospital, Thailand during 2012-2015.

<table>
<thead>
<tr>
<th>Pattern</th>
<th>E (15 µg/ml)</th>
<th>TE (30 µg/ml)</th>
<th>FOS (200 µg/ml)</th>
<th>CD (1.25/23.75 µg/ml)</th>
<th>SXT (10 µg/ml)</th>
<th>FD (30 µg/ml)</th>
<th>VAN (30 µg/ml)</th>
<th>TEC (30 µg/ml)</th>
<th>LZD (30 µg/ml)</th>
<th>Number of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>NS</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>109 (47)</td>
</tr>
<tr>
<td>II</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>74 (32)</td>
</tr>
<tr>
<td>III</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>14 (6)</td>
</tr>
<tr>
<td>IV</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>11 (5)</td>
</tr>
<tr>
<td>V</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>11 (5)</td>
</tr>
<tr>
<td>VI</td>
<td>NS</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>3 (1)</td>
</tr>
<tr>
<td>VII</td>
<td>NS</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>3 (1)</td>
</tr>
<tr>
<td>VIII</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>1 (&lt; 1)</td>
</tr>
<tr>
<td>IX</td>
<td>NS</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>1 (&lt; 1)</td>
</tr>
<tr>
<td>X</td>
<td>NS</td>
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<td>NS</td>
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<td>S</td>
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<td>S</td>
<td>1 (&lt; 1)</td>
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<tr>
<td>XI</td>
<td>NS</td>
<td>S</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>1 (&lt; 1)</td>
</tr>
<tr>
<td>XII</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>1 (&lt; 1)</td>
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<tr>
<td>XIII</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>1 (&lt; 1)</td>
</tr>
<tr>
<td>XIV</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>1 (&lt; 1)</td>
</tr>
</tbody>
</table>

Number of susceptible isolates (%)
- E: 2 (1%) 120 (52%) 140 (60%) 3 (1%) 202 (87%) 229 (99%) 232 (100%) 232 (100%)

CD, clindamycin; E, erythromycin; FOS, fosfomycin; FD, fusidic acid; LZD, linezolid; STX, trimethoprim-sulfamethoxazole; TE, tetracyclin; TEC, teocplanin; VAN, vancomycin; NS, not susceptible; S, susceptible.

Table 2
Antibiotic susceptibility of MRSA isolates classified by period of collection, Thammasat University Hospital, Thailand.

<table>
<thead>
<tr>
<th>Period</th>
<th>Number of MRSA isolates</th>
<th>Number of susceptible MRSA isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>TE</td>
</tr>
<tr>
<td>1st year</td>
<td>67</td>
<td>1 (1)</td>
</tr>
<tr>
<td>2nd year</td>
<td>77</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3rd year</td>
<td>88</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

*All samples were susceptible to LZD, TEC and VAN.*
Table 3

Antibiotic susceptibility of MRSA isolated from different specimen types, collected at Thammasat University Hospital, Thailand during 2013-2015.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Total number of MRSA isolates</th>
<th>Number of MRSA susceptible isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SXT</td>
</tr>
<tr>
<td>Sputum</td>
<td>164</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Pus</td>
<td>31</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Blood</td>
<td>30</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Urine</td>
<td>6</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Body fluid</td>
<td>1</td>
<td>1 (100)</td>
</tr>
</tbody>
</table>

All samples were susceptible to LZD, TEC and VAN.

Antibiogram patterns I and II were predominant in MRSA isolates from Outpatient Clinics and ER and in all inpatient wards except the Pediatric wards, from which antibiogram patterns III, IV and V were obtained. It is noteworthy that all 32 putative non-CA-MRSA isolates from outpatients were susceptible to SXT whereas 170/200 (85%) isolates from the Inpatient Departments were susceptible to this antibiotic. Also all 9 MRSA isolates from the Pediatric wards were TE resistant while the overall TE susceptible rate was 52%.

There was no specific MRSA antibiogram pattern associated with a specimen type (Table 3). Three MRSA isolates from pus specimens were found resistant to FD, an antibiotic often used in topical form.

DISCUSSION

It is notable that the proportion of MRSA isolates detected at Thammasat University Hospital declined over 3-year study period (August 2013 - July 2015). Similar trends regarding MRSA infection were observed in the USA and Europe during the past decade (Johnson, 2011;
Nevertheless, despite the declining trend, MRSA constituted nearly 40% of S. aureus infections in the last year of the study.

The spread of CA-MRSA strains in many countries has created global concern (David and Daum, 2010). Thus, close monitoring of the emergence of CA-MRSA remains important. In this study, we did not find any instance of CA-MRSA infection among the inpatients. This suggests that HA-MRSA was most likely responsible for these community-onset infections.

Molecular means were applied to distinguish between CA- and HA-MRSA. CA-MRSA strains usually carry staphylococcal chromosomal cassette mec (SCCmec) type IV and V as well as Panton-Valentine leukocidin (PVL) genes, whereas HA-MRSA strains carry SCCmec type I, II and III and seldom have pvl (David and Daum, 2010). No MRSA isolates in our study carried pvl, a finding consistent with previous reports (Song et al., 2011; Mendes et al., 2013). Overall data indicate that the prevalence of CA-MRSA infection remains very low in Thailand (Mekviwat-tanawong et al., 2006). The spread of various MRSA clones has already occurred between community and hospital and also between Asian nations (Song et al., 2011). Thai university students appear to have a prevalence of MRSA of around 1% (Kitti et al., 2011). Tertiary government hospitals in Thailand have also reported significant levels of MRSA nosocomial infections (Jariyasethpong et al., 2010). It was suggested that CA-MRSA found in an animal hospital might have come from humans and/or sick animals (Patchanee et al., 2014).

All MRSA strains tested were susceptible to LZD, TEC and VAN, indicating that these antibiotics are still effective. Interestingly, nearly all MRSA strains were resistant to CD and E; thus, both drugs are no longer recommended for managing MRSA infection in our institute. In particular, all MRSA specimens from the Pediatric Wards were TE resistant and 2/3 to SXT. The sporadic outbreak of MRSA strains isolated from four regions during 1996-1998 were also resistant to TE but less susceptible to SXT (81.5%), as compared to the tested strains in this study (Wongwanich et al., 2000). Resistance to at least 5 antimicrobial agents including cefazolin, erythromycin, gentamicin, ofloxacin and tetracycline was reported in a university hospital (Lulitanond et al., 2010). Multidrug resistance to MRSA was also found in a small animal hospital, Faculty of Veterinary Medicine, Chiang Mai University, Thailand (Patchanee et al., 2014). The strains were 100% susceptible to vancomycin but were 92% resistant to tetracycline, 69% to trimethoprim-sulfamethoxazole, and 62% to ceftriaxone. In comparison, the antibiogram of MRSA isolated from India during the same period (Abbas et al., 2015) showed all of its MRSA isolates were also sensitive to vancomycin and linezolid; however, resistance to E, TE and SXT was found to be less than in our study. This implies that good clinical practices in using antibiotics for MRSA infection treatment must be intensively monitored among physicians and medical personnel in order to reduce the spread of multidrug-resistant MRSA infections. In addition, HA-MRSA has a wider antimicrobial resistance pattern than CA-MRSA (Huang et al., 2006; Vysakh and Jeya, 2013; Abass et al., 2015).

The three FD-resistant MRSA isolates were from pus specimens of surgery patients. Frequent usage of this drug for topical applications in surgery patients may contribute to the emergence of FD-resistant MRSA strains. In New Zealand,
increased prevalence of FD-resistant MRSA was found in the youngest age group (< 5 years) with impetigo (Vogel et al, 2016). In addition, in Norway a growth in FD-resistance among S. aureus was reported in children with impetigo bullosa-like skin disease in the summer months (Tveten et al, 2002).

The most common specimen containing MRSA was sputum, consistent with previous report (Ray et al, 2012). Prolonged mechanical ventilation is known as one of the risks for nosocomial pneumonia (Lynch, 2001). In addition, more than 1/3 of all S. aureus bloodstream infections were MRSA, and they expressed various antibiogram patterns. However, there were no associations among antibiogram patterns and MRSA strains categorized according to clinical origins. Vancomycin is thus still the drug of choice for treating MRSA irrespective of site of infection. An active surveillance of vancomycin susceptibility is therefore encouraged. Moreover, meta-analysis data of vancomycin treatment indicated that high vancomycin trough levels are associated with risk of nephrotoxicity; however, the high vancomycin trough levels are not significantly different in mortality rate compared to the low vancomycin trough levels (Tongsai and Koomanachai, 2016). After vancomycin was introduced for MRSA infection treatment, MRSA with reduced susceptibility to vancomycin including VISA and hVISA were reported in 1997 (Hiramatsu et al, 1997a,b) and has increased globally. The isolates from Thammasat University Hospital will be further investigated for reduced susceptibility to vancomycin in order to prevent and control spread of the MRSA infection as recently described (Sirichoat et al, 2016).

In summary, this research reveals a recent situation of MRSA infection at Thammasat University Hospital. Although a declining trend in the proportion of MRSA among S. aureus infection was observed, the prevalence of MRSA infection in 2015 remained nearly 40%. CA-MRSA was not found in our institute, suggesting a low prevalence of this strain in the region. While linezolid, teicoplanin and vancomycin, still remained effective antibiotics for treatment of MRSA infection, vigilance of possible emerging resistance must be maintained. In addition, greater awareness of antibiogram profiles of MRSA strains prevailing in the various clinical wards should provide guidance in the appropriate choice of antimicrobial regimen in treating MRSA-infected patients.

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