Prevalence and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* clones: A study at Taksin Hospital, Bangkok, Thailand

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ABSTRACT: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common pathogen in human diseases. Thirty-seven clinical clones from different patients were tested for a molecular study of the *mec* A gene and multilocus sequence types (ST). Total genomic extraction, followed by polymerase chain reaction (PCR) for DNA amplification with specific primers for *mec* A, and specific primers for various ST were used. Molecular typing for the study of genetic relationships among clones was performed by enterobacterial repetitive intergenic consensus (ERIC)-PCR. Antimicrobial susceptibility testing for all clones to 9 drugs was performed by the disk diffusion and vancomycin E-test. The presence of *mec* A was detected in all clones. The most common ST was MRSA-ST30, accounting for 81.1% of all MRSA tested, followed by MRSA-ST8/ST97/ST779 (8.1%), MRSA-ST239 (2.7%) and MRSA-nontypeable clones (8.1%). Molecular typing by ERIC-PCR demonstrated DNA fingerprints with corresponding results with sequence types (ST). Total genomic extraction, followed by a polymerase chain reaction (PCR) for DNA amplification and a danger to the global economy. The spread of MRSA in hospitals is a serious health threat to public health and hospital resources due to its resistance to several antimicrobial agents [1]. Its resistance is associated with the acquisition of a mobile genetic element called staphylococcal cassette chromosome *mec* that carries the *mec* A gene which encodes for the low-affinity penicillin-binding protein 2a [2]. Infections caused by multidrug-resistant (MDR) clones lead to prolonged hospital stays and increased mortality. The spread of MRSA in hospitals is a serious health threat and a danger to the global economy [3]. MRSA is highly prevalent in hospitals worldwide with high rates (≥50%) reported in Asia, Malta, North America, and South America [4]. A review of 15 studies showed that between 13% and 74% of worldwide *S. aureus* infections were MRSA [5]. In Thailand, data from two-multi-center studies revealed MRSA prevalence of 57%, with most cases being hospital-acquired MRSA [6]. At Thammasat University Hospital, Pathum Thani Province (adjacent to Bangkok) the prevalence of MRSA was reported to be 46% [7]. In a recent study, Chulalongkorn Memorial Hospital (a tertiary care university hospital in Bangkok) reported MRSA prevalence of 17% [8]. Two other provinces located outside Bangkok, Sa Kaeo Province (in eastern Thailand, near the Cambodian border) and Nakhon Phanom Province (in northeastern Thailand, near the border with Laos), reported MRSA prevalence of 10% [9]. Hospital-acquired MRSA from 12 Asian countries from Saudi Arabia to Philippines was identified by multilocus sequence typing [10]. Due to the high frequency of MRSA in Asia, data from the region suggests that at least 90% of hospital-acquired MRSA accounts for >60% of MRSA in the world and can be traced to a single clone (ST239 or multilocus sequence type 239) [10–12]. The ST239 sequence has also been found in 26 countries outside Asia [12]. The global dissemination of ST239 is consistent with high transmissibility. ST239 evolved from DNA recombination involving the import of DNA from ST30 into ST8. However, ST239 has been recently replaced by other clones in several countries [12, 13]. As for Thai MRSA, all four isolates tested in 2006 belonged to ST239 [11]. In 2008, 90% of MRSA, from northeast Thailand were linked to ST239 [12]. For other multilocus sequence types of MRSA, many studies have been conducted around the world. In 2019, a study from Northwestern China...
reported ST22 (2.02%), ST59 (11.8%) and ST239 (73.1%) [14]. In 2018, a study from East China reported ST59 (77.67%) [15]. Meanwhile in 2016, two Chinese hospitals in two different cities (Affiliated Hospital of Nantong University and Jiangsu Taizhou People’s Hospital) of Jiangsu Province reported ST5 (12.9%), ST7 (12.9%) and ST398 (16.1%) [16]. The aim of the present preliminary study was to determine the prevalence of sequence types and determine the current antibiogram profile of drug resistance in MRSA at Taksin Hospital.

MATERIALS AND METHODS

Bacterial isolates

Ethical approval for this study was obtained from the Human Research Committee of Siam University and the Bangkok Metropolitan Administration Human Research Ethics Committee with reference codes SIAMPY-IRB 2020/007 and E009h/63 NA, respectively. The sample size was calculated to be 35 MRSA isolates using the statistics formula \( n = Z^2 P(1-P)/d^2 \), where \( n \) = number of clones, \( Z = 1.96 \) at \( a = 0.05 \), \( P = 0.9 \). (The latter was due to 90% prevalence of MRSA-ST239 in Thailand as mentioned in a previous report [12].) Therefore, 37 MRSA clones were randomly collected from 37 different patients to prevent duplicates of the same antibiogram profile at a tertiary-care hospital (Taksin Hospital, Bangkok, Thailand) over six months (Jan–Jun 2020). MRSA was isolated from various specimens and identified according to standard bacteriological methods [1]. If the specimen was sputum, it was accepted for culturing if it contained more than 25 polymorphonuclear cells and less than 25 squamous epithelial cells per low-power field (10× magnification of microscope) [1]. The isolation and identification of MRSA was performed by standard techniques [1, 2]. First, \( S. aureus \) was confirmed by catalase and slide coagulase tests and if the result of a slide coagulase test was negative, a tube coagulase test was performed. Next, MRSA was confirmed using a disk diffusion test, according to CLSI. MRSA was defined if it was resistant to cefoxitin disk (30 \( \mu \)g). [2].

Antimicrobial susceptibility testing

We carried out testing following the standard disk diffusion method [2] and E-test (BioMerieux, USA), according to the manufacturer’s recommendation (vancomycin only). \( S. aureus \) ATCC 25923 was used as a control strain [2]. A clone was defined as multidrug-resistant (MDR) if it was resistant to \( \geq 3 \) classes of drugs [17].

A D-test was performed to study the presence of inducible macrolide, lincosamide-type B streptogramins resistance (iMLSBI) and constitutive MLSB resistance. Erythromycin (15 \( \mu \)g) and clindamycin (15 \( \mu \)g) disks were placed 15 mm apart from edge to edge on a lawn of MRSA suspension with a turbidity equaling the 0.5 McFarland standard, which was spread on Mueller Hinton agar as previously described [2].

Molecular study of \( S. aureus \) mecA and DNA sequence types

DNA was extracted from MRSA colonies grown on blood agar containing 5% sheep blood overnight at 35 °C by the Gentra Puregene Yeast/Bacteria kit (QIAGEN) and was used as a template for PCR reactions. PCR amplification was performed by using \( S. aureus \) mecA specific primers (mecA-F, TGTCGTAACCT GAACTAGC; mecA-R, TGCTATCCACCCCTAACACAG) and PCR conditions as previously described [18]. The 863-base pair (bp) amplicon was detected by 1% agarose gel-electrophoresis and ethidium bromide staining.

For identification of ST239 MRSA clones, PCR amplification was performed using primers ST8- and ST30-like sequences ([SA2003-F, CACCTTAAATATT GACCGAAAT; SA2003-R, TTGAAAAATGTATCTACG AA; 220 bp amplicon], and (SAO317-F, TGGCAGCT CGTGAACA; SAO317-R, AAATCGCTTGTGAACAC AAC ATT; 484 bp) amplicon) and PCR conditions as previously described [12]. The PCR product size(s) of each ST was (were) as follows: 484 bp for ST30; 220 bp for ST8/ST97/ST779; and 484 bp and 220 bp for ST-239.

In molecular typing for the study of genetic relationships among MRSA clones, enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) was performed using primers (ERIC1R, ATGTAAGCTC ATT; 484 bp) and PCR conditions as previously described [19].

Data analysis

Data were entered and analyzed with SPSS Statistics version 20 (SPSS Inc, Chicago, IL, USA) for descriptive analysis. Discrete variables were expressed as percentages, mean, standard deviation and proportions.

RESULTS

In this study, there were 64.9% (24/37) male patients (M:F = 24:13; sex ratio 1.8:1). The patient age ranged from 17–98, with a mean age and standard
deviation of 65±20.7 years. The sources of clinical specimens were sputum (24/37; 64.9%), pus (7/37; 18.9%), urine (3/37; 8.1%) (we found 10^4–10^5 CFU/ml), blood (2/37; 5.4%), and ascitic fluid (1/37; 2.7%) as shown in Table 1. MRSA clones were susceptible to fosfomycin (26/37; 70.3%), fusidic acid (37/37; 100%), gentamicin (34/37; 94.6%), tetracycline (32/37; 86.5%), trimethoprim-sulfamethoxazole (35/37; 94.6%) and vancomycin (37/37; 100%), but resistant to ciprofloxacin, clindamycin and erythromycin (susceptibility range 2.7–5.4%). Vancomycin’s minimal inhibitory concentration (MIC) range, MIC_{50} and MIC_{90} values was 0.25–1.0, 0.5 and 0.75 µg/ml, respectively.
All 37 randomly picked MRSA clones tested positive for mecA gene by PCR. Fig. 1 shows PCR products (519 base pairs) for the detection of mecA gene. For DNA sequence types, we found MRSA-ST30 (30/37; 81.1%) to be predominant (Fig. 2) in many clinical specimens such as sputum, pus, urine, blood and ascitic fluid. For MRSA-ST8/ST97/ST779, MRSA-ST239 and MRSA-nontypeable clones, we found these ST types less frequently [ST239 and MRSA-nontypeable clones, we found these DNA sequence types, we found MRSA-ST30 (30 mecA tative for clones were not ST-30, ST8 and (3 4 ScienceAsia due to long hospitalizations and prolonged mechanical infections. We did not find MRSA that was resistant to vancomycin in this study. However, vancomycin resistant S. aureus was reported recently [23]. On the other hand, a high number of vancomycin-resistant Enterococcus faecium isolates carrying the vanA gene was found in patients of hospitals in northeastern Thailand, which also led to hospital-associated infections [24].

Table 2 shows that 96.7% (29/30) of MRSA-ST30 clones were multiple drug resistant (MDR). The most common pattern was resistance to three drugs, including ciprofloxacin, clindamycin and erythromycin (19 clones), followed by resistance to four drugs including ciprofloxacin, clindamycin, erythromycin and fosfomycin (9 clones). For MRSA-ST8/ST97/ST779, all three clones were MDR with three patterns (ciprofloxacin, clindamycin, erythromycin, fosfomycin; ciprofloxacin, clindamycin, erythromycin and gentamicin; and ciprofloxacin, clindamycin, erythromycin, gentamicin and tetracycline). There was one MRSA-ST239 clone, which was the most resistant MRSA in this study (resistance to six drugs: ciprofloxacin, clindamycin, erythromycin, gentamicin, trimethoprim-sulfamethoxazole and tetracycline). For MRSA-nontypeable, two clones were MDR with two patterns (ciprofloxacin, clindamycin, erythromycin, trimethoprim-sulfamethoxazole, tetracycline and clindamycin, erythromycin, tetracycline).

DISCUSSION

Most patients in this study were elderly, which is similar to a report from Shanghai, China between 2013–2018 in which patients’ median age was 59 and age range was 7 months–97 years. In the same study, 67.5% of patients were male [20] which is close to our result of 64.9%. Most patients in this study were in-patients and a few were out-patients. This investigation confirmed results of several other studies that MRSA is mostly isolated from clinical specimens of sputum, followed by pus, blood or urine [8, 21, 22]. The prevalence of MRSA was found mostly in sputum due to long hospitalizations and prolonged mechanical ventilation known for increased risk of nosocomial pneumonia. For the mecA gene study, there was an additional mecC gene, which also conferred methicillin resistance in S. aureus, but it was rare and less important than the mecA gene [1, 2, 23]. Vancomycin is still considered the drug of choice for treatment of MRSA infection. For the D-test, inducible macrolide, lincosamide-type B streptogramins resistance (iMLSB) and constitutive MLSB was observed (2/37; 5.4% and 34/37; 91.9%, respectively). One clone was susceptible to both erythromycin and clindamycin (1/37; 2.7%). Two iMLSB clones were found in MRSA-ST30 and MRSA-ST nontypeable clones.

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MRSA is a nosocomial bacterial pathogen and can also cause community-acquired infections [29]. Macrolide, lincosamide-type B streptogramin (MLSB) i.e. erythromycin and clindamycin are often used to treat skin and soft tissue infection caused by S. au- reus. The inducible macrolide, lincosamide-type B streptogramin resistance (iMLSB) (i.e., resistance to clindamycin) occurs when there is erythromycin that acts as an inducer of clindamycin resistance [30]. Antimicrobial susceptibility testing by the standard disk diffusion method may not be able to detect inducible clindamycin resistance. Therefore, treatment failure will occur if the D-test is not routinely performed in a clinical bacteriology laboratory. The iMLSB report from India in 2015 [31], Iran in 2020 [32] and Nepal in 2019 [30] was 11.8%, 21.7% and 43.8%, respectively, which is higher than our result.

In an antimicrobial susceptibility test, the most common MDR pattern was resistance to ciprofloxacin, clindamycin and erythromycin (19 isolates) found in MRSA-ST30 clones. In a recent report in Poland, 92.9% of MRSA clones were MDR and the most common pattern was resistance to ciprofloxacin, clindamycin, erythromycin and levofloxacin [33]. The clonal transmission of MDR-MRSA is usually spread by direct contact with an infected wound or from contaminated hands, usually those of healthcare providers. Also, people who carry MDR-MRSA but have no signs of infection (people who are colonized by this bacterium) may spread the bacteria to other people.
There was a limitation of a small sample size and funding in this study. It would be useful to further investigate larger sample sizes and conduct a multicenter study. In conclusion, DNA sequence types and antibiogram profiles of drug resistance are useful and important epidemiological markers. These preliminary data should support ongoing studies by increasing the number of clinical MRSA isolates to improve the quality of epidemiological studies and surveillance of drug resistance.

Acknowledgements: This work was supported by the Siam University Council research grant number 002/03/2563. The authors thank Thanakorn Watcharasupat for molecular epidemiology testing.

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