http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.680.687

Research Article
High Proportion of Oxacillin-Susceptible *mecA*-Positive *Staphylococcus aureus* Isolates from Post-Viral Acute Rhinosinusitis Patients

[1,2]Imam Megantara, [3]Ronny Lesmana, [3]Nova Sylviana, [1]Adi I. Cahyadi and [1]Sunarjati Sudigdoadi

[1]Division of Microbiology, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[2]Department of Otolaryngology and Head and Neck Surgery, Santosa Hospital Bandung Central, Bandung, Indonesia
[3]Division of Physiology, Department Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

Abstract

Background and Objective: Detection of methicillin-resistant *S. aureus* have become a challenge in the presence of oxacillin-susceptible and *mecA*-positive *S. aureus* (OS-MRSA), concerning the misidentification events and therapeutic implications. This study aims to identify the OS-MRSA in clinical isolates of Post-viral acute rhinosinusitis, which, hopefully, can interfere with the therapeutic strategy. **Materials and Methods:** There were 60 patients diagnosed with Post-viral acute rhinosinusitis, recruited from an Ear, Nose and Throat (ENT) outpatient clinic. *Staphylococcus aureus* isolates were identified from the culture and were then tested for antibiotics susceptibility using a Kirby-Bauer disc diffusion test. The *mecA*, *mecC* and *blaZ* genes were determined using the Polymerase Chain Reaction (PCR) method. **Results:** *Staphylococcus aureus* was identified in 20 of the 60 samples from the patients (33.3%; 95% CI: 21.0-45.6). Of the 20 isolates, 19 isolates (95%) had a positive *mecA* gene, 19 (95%) had a positive *mecC* gene and 20 (100.0%) had a positive *blaZ* gene. The majority of the *mecA*-positive *S. aureus* showed an oxacillin-susceptible (85%) and 3 isolates (15.0%) were oxacillin-resistant toward the *S. aureus*. **Conclusion:** There was a high proportion of Oxacillin/cefoxitin-Susceptible *mecA*-positive *S. aureus* in the study population that indicate phenotypic susceptibility to antibiotics does not always indicate the absence of genes that carry resistant traits, thus allowing misidentification if the only phenotypic examination is carried out.

Key words: *Staphylococcus aureus*, oxacillin-susceptible, phenotype, genotype, *BlaZ* gene, *mecA* gene, rhinosinusitis

Citation: Megantara, I., R. Lesmana, N. Sylviana, A.I. Cahyadi and S. Sudigdoadi, 2021. High proportion of oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* isolates from post-viral acute rhinosinusitis patients. Pak. J. Biol. Sci., 24: 680-687.

Corresponding Author: Imam Megantara, Division of Microbiology, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran Bandung, Indonesia Tel: +62816612839

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Staphylococcus is a harmless bacteria and colonizes human skin and anterior nostrils, which acts as a typical flora. In some circumstances, this bacteria can cause various infections, ranging from skin and soft tissue infections to severe infections such as sepsis. Generally, *Staphylococcus* infections can be treated with antibiotics. However, due to the overuse of antibiotics over decades, a much more dangerous form of *Staphylococcus* has emerged, which has developed resistance toward methicillin and the β lactam-antibiotic, which is known as Methicillin-resistant *S. aureus* (MRSA). It is one of the most prominent pathogens known to cause community and Healthcare-associated infections[1,2].

Methicillin-resistant *S. aureus* (MRSA) is resistant to the entire class of β-lactam antibiotics, including penicillin, methicillin and cefazolin[3]. The resistance to β-lactam in *S. aureus* is known to be mediated by *the mecA* gene located in the mobile genetic element and also the Staphylococcal Cassette Chromosome *mecC* (SCCmec). These genes cause a mutation of the Penicillin Binding Protein (PBP2a), allowing the *S. aureus* to survive against the β lactam-antibiotic[4,5]. The *blaZ* gene in *S. aureus* is a gene known to be responsible for the resistance toward penicillin. The activated *blaZ* can encode the β-lactamase enzyme (penicillinase), which inactivates the antibiotic through hydrolysis of the peptide bond in the β-lactam ring[6].

One of the challenges in detecting and treating MRSA is the presence of an Oxacillin-Susceptible and *mecA*-positive *S. aureus* (OS-MRSA), which has been seen to be increasing recently[7-10]. In Microbiology laboratories, the diagnosis of the MRSA is confirmed by determining the Minimum Inhibitory Concentration (MIC) of oxacillin $\geq$4 μg mL^{-1}, or that of the cefoxitin MIC $\geq$8 μg mL^{-1}. It can also be detected by using *mecA* as a genetic marker from molecular-based assays[11]. Unlike Oxacillin-Resistance *S. aureus* (OR-MRSA) which phenotypically often exhibits a Multi-Drug Resistance (MDR), the OS-MRSA is less likely to be an MDR and shows a different MIC pattern from that of the OR-MRSA. This might lead to misidentified the Methicillin-Susceptible *S. aureus* (MSSA) in routine clinical laboratories, where the *mecA* detection is not performed[12]. However, even though the OS-MRSA is considered to be susceptible to β-lactam, the presence of the *mecA* and *blaZ* in the OS-MRSA tends to change its susceptibility pattern to the β-lactam, especially if the amount and duration of the antibiotic exposure increases, which in turn leads to the failure of the β-lactam treatment[7-10,13].

Data regarding the OS-MRSA in Indonesia is still very limited, therefore it must study the presence of this strain in clinical isolates. We considered taking samples from sinonasal mucosal secretions of Post-viral acute rhinosinusitis patients because the *S. aureus* colonization is very likely to occur in this area due to the horizontal translocation from the nostrils which precedes the bacterial infection. Various studies have shown that *S. aureus* is a bacteria that causes acute bacterial rhinosinusitis[14-18]. Moreover, the use of antibiotics in acute rhinosinusitis is relatively high, even though most studies have shown that antibiotics are not useful in mild, moderate and uncomplicated acute rhinosinusitis[19,20]. A survey of general practitioners and Ear, Nose and Throat (ENT) specialists in Asia has shown that antibiotics are among the top three drugs besides antihistamines and nasal decongestants, which are given in acute rhinosinusitis conditions. The use of antibiotics as the first line of treatment has been increasing in moderate (45.9%) and severe (60.3%) acute rhinosinusitis conditions[19]. This data indicates that antibiotics are overused in Asia. Studies in various European and American countries have also reported on antibiotics overuse, hereby increasing the potency of Multi-resistant bacteria[21,22].

Therefore, we conducted a study to assess the *S. aureus* colonization in the sinonasal mucosa, especially around the ostiomeatal complex of patients with clinically diagnosed post-viral acute rhinosinusitis, as well as the potency for the presence of OS-MRSA, by identifying the *mecA*, *mecC* and *blaZ* genes. We also conducted the Kirby-Bauer disk diffusion test to assess the susceptibility pattern to cefoxitin phenotypically. Hopefully, the information obtained from this study can increase the awareness of the existence of OS-MRSA and elucidate the importance of careful administration of antimicrobial therapies to reduce multi-resistance bacterial occurrences, especially that of the OR-MRSA.

MATERIALS AND METHODS

The study was carried out at the Division of Microbiology, Central Laboratory, Universitas Padjadjaran, Bandung, Indonesia from 1st August, 2018-1st December, 2019.

Study design and subjects: This study was performed on sixty patients, aged >18 years old, who were diagnosed with Post-viral acute rhinosinusitis in the ENT clinic. The diagnostic criteria for Post-viral acute rhinosinusitis included the sudden onset of two or more symptoms, which worsened after day 5-7 or was still present for more than 10 days. This included: (1) Nasal congestion/obstruction/blockage or anterior/posterior nasal drip, (2) Facial pain/pressure, reduction or loss of smell and (3) The presence of mucopurulent secretion and/or edema/thickening of mucosa in the meatus media region during endoscopic examination[23]. The patients in this study were not on antibiotics 10 days before the examination.

The exclusion criteria included: (1) Patients with a history of chronic or allergic rhinosinusitis, diabetes mellitus and asthma and or, (2) The presence of a dental infection at the upper premolar and molar. In this study, all of the patients who met the inclusion criteria agreed to participate and signed an informed consent form. The study was also reviewed and approved by The Health Research Ethics Committee, the Faculty of Medicine Universitas Padjadjaran, Bandung, Indonesia (No. 874/UN6.C.10/PN/2017).

Sampling: An ENT physical examination was performed, including a nasal endoscopic examination, which focused on the ostiomeatal complex. It was aimed at evaluating the presence of nasal secretions and edema/thickening of the nasal mucosa. Under direct endoscopic visualization, a sterile flocked swab was carefully inserted into the nasal cavity and placed in the ostiomeatal complex to take a sample from the secretion. The swab was carefully removed and inserted into a tube filled with STGG transport media, which had been labelled. The sample tubes were transported to the Microbiology laboratory by using an icebox within the first 12 hrs of the collection of the sample and stored in the refrigerator at a temperature of -80°C.

Culture and antibiotic susceptibility testing: The tubes containing the samples were gradually thawed and inoculated on a mannitol salt agar plate, sheep blood agar and chocolate agar media. These plates were incubated at 37°C and examined for bacterial growth across 24 and 48 hrs of the incubation period. The bacterial colony's growth was identified using a standard microbiology procedure and the susceptibility tests were performed on a Müller Hinton agar, in particular for the *S. aureus*, against 15 different antibiotics.

Identification of *mecA*, *mecC* and *blaZ* gene by PCR: Samples were inoculated into the TSB media and were incubated at 37°C. Afterwards, the bacterial DNA from the isolates were grown from the media and was extracted using the Wizard™ Genomic DNA Purification Kit (Promega, Madison, WI, USA). Bacterial plasmids were extracted using the Presto™ Mini Plasmid Kit (Geneaid, New Taipei City, Taiwan R.O.C).

The primers used to amplify the *mecA gene* (310 bp) were F:5'-GAAGATGGCTATCGTGTCACA-3' and R:5'-GGAACTTGTTGAGCAGAGGTT-3'. The *mecC* gene (138 bp) was F:5'-GGGTTCAGCCAGATTCATTTGT-3' and R: 5'-GTACTGTTGCTTCGTTCAATGG- 3' and the *blaZ* gene (373 bp) was F:5'-TTAAAGTCTTACCGAAAGCAG-3' and R: 5'-TAAGAGATTTGCCTATGCTT-3'.

The extracted chromosomal DNA was amplified. A 2 µL DNA template was mixed with a 10 µL master mix solution, consisting of 5 µL DreamTaq Green PCR and 0,5 µL of each primer (*mecA* or *mecC*), as well as 3 µL of nuclease-free water. The whole process consisted of five cycles, (1) initial denaturation conducted at 94°C for 3 min, (2) denaturation at 94°C for 30 sec, (3) annealing at 53°C for 30 sec, (4) extension at 72°C for 30 sec and (5) final extension at 72°C for 4 min. Denaturation, annealing and extensions were done 35 times.

As much as 4 µL amplicon was placed into a 1.5% agarose well, between positive and negative controls. The positive control was placed into the second well and the negative control was placed into the last well. Both of the *mecA* and *mecC* genes used the 100 bp ladder. Gel electrophoresis was performed at 90 Volts for 60 min. A distinct 310 bp band represented the *mecA* positive, while a distinct 138 bp band was associated with the *mecC* gene positive. A gene block synthesized by Integrated DNA Technologies (San Diego, CA USA) was used as a positive control.

The extracted plasmid DNA was then amplified. A 2 µL DNA template was mixed with 10 µL of the master mix solution, which consisted of 5 µL DreamTaq Green PCR, 0.5 µL of primer *blaZ* forward, 0.5 µL of primer *blaZ* reverse and 3 µL of nuclease-free water.

As much as 4 µL amplicon was put into the 1.5% agarose well between the positive and negative control. The positive control was put into the second well and the negative control was put into the last well. A 100 bp ladder was used. Gel electrophoresis was performed at 90 Volts for 60 min. A distinct 373 bp band depicted the *blaZ* positive. A gene block synthesized by Integrated DNA Technologies (San Diego, CA USA) was used as a positive control.

RESULTS

Profile of the bacteria in post-viral acute rhinosinusitis patients: Identification of the bacterial growth in the culture medium was conducted using standard microbiology procedures. In this study, the most common pathogens found were *S. aureus* (33.3%), *H. influenzae* (13.3%) and *S. pneumoniae* (8.3%). The profile of the bacteria on clinical isolates from the post-viral acute rhinosinusitis subjects are described in Table 1.

Detection of *mecA*, *mecC* and *blaZ* gene of *S. aureus*: In this study, twenty isolates (33.3%) of the *S. aureus* were identified from across 60 clinical isolates of the Post-viral acute rhinosinusitis subjects (Fig. 1a-c). The PCR test for the

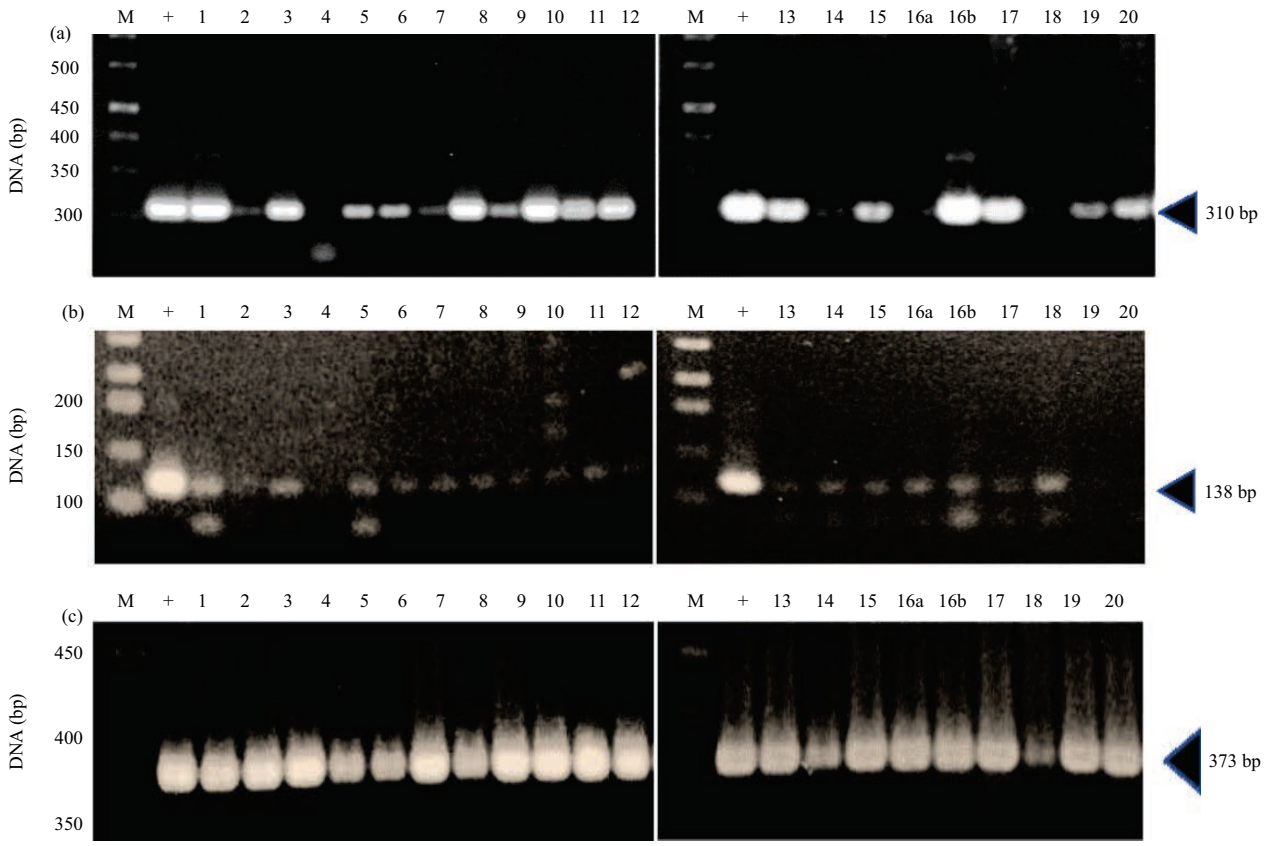

Fig. 1(a-c): Electrophoresis profile of the (a) *mecA,* (b) *mecC* and (c) *blaZ* gene of *S. aureus* in clinical specimens of Post-viral acute rhinosinusitis patients

Number of specimens is indicated on the top of the lane. Lane M: DNA size markers. Major bands are indicated by arrows. (a) A 310 bp bands of the positive gene in 19 samples of *S. aureus* isolates, (b) A 138 bands of the *mecC*-positive gene in 18 samples of *S. aureus* and (c) A 373 bands of *blaZ* gene in all of *S. aureus* isolates

Table 1: Frequency of bacteria in post-viral acute rhinosinusitis

Bacteria**	Frequency	Percentage
Staphylococcus aureus	20	33.3
Streptococcus pneumoniae	5	8.3
Haemophilus influenzae	8	13.3
Moraxella catarrhalis	0	0.00
Staphylococcus epidermidis	8	13.3
Streptococcus viridans	8	13.3
Others	13	21.6

**One subject may have more than one bacteria

20 isolates of the *S. aureus* showed that the number of *mecA* gene positives numbered 19 (95.0%) (Fig. 1a) and the *mecC* gene-positive sat at 18 (90.0%) (Fig. 1b), while all twenty isolates (100%) had a *blaZ* gene-positive (Fig. 1c).

Antibiotics susceptibility pattern: Bacterial culture examination showed twenty isolates (33.3%) of the *S. aureus* which were identified from across 60 clinical isolates of the post-viral acute rhinosinusitis subjects. The frequency of the resistance toward the antibiotics is shown in Fig. 2. The highest resistance was toward penicillin (n = 17, 85.0%), followed by amoxicillin (n = 14, 70.0%), co-amoxiclav (n = 9, 45.0%), azithromycin (n = 8, 40.0%), clindamycin (n = 8, 40.0%), ceftazidime (n = 3, 15.0%), tobramycin (n = 3, 15.0%), cefoxitin (n = 3, 15.0%), ciprofloxacin (n = 2, 10.0%), cefepime (n = 2, 10.0%), ceftriaxone (n = 2, 10.0%), meropenem (n = 2, 10.0%), cotrimoxazole (n = 2, 10.0%), streptomycin (n = 1, 5.0%) and imipenem (n = 0, 0.0%).

However, our study revealed that from nineteen isolates of the *S. aureus* (95%), that the positive *mecA* and eighteen isolates (90.0%) of the positive *mecC* genes, that the majority of the strains showed a behaviour which was cefoxitin-susceptible, which was defined as the OS-MRSA. Three isolates (15.0%) were resistant to cefoxitin in the antimicrobial susceptibility test (AST), or OS-MRSA.

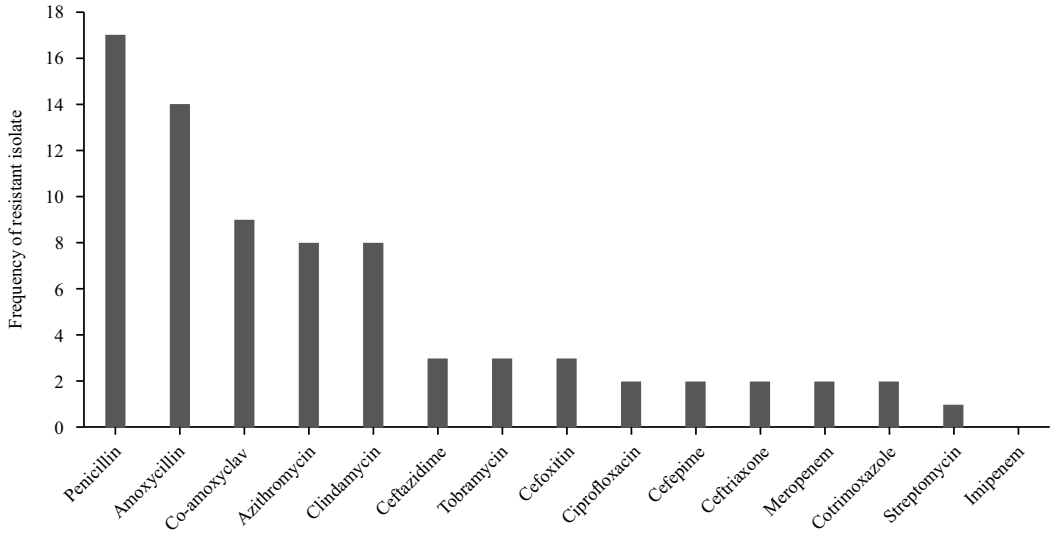

Fig. 2: Frequency of resistant isolates towards different antibiotics (n = 20)

DISCUSSION

Regarding the proportion of *S. aureus* in clinical isolates from the middle meatus of the post-viral acute rhinosinusitis patients in our study, the results suggested a similar prevalence of *S. aureus*. However, the proportion of the MRSA was much higher compared to another study which mentioned that 89 *S. aureus* (34.5%) were isolated from 258 enrolled patients, of which, 22 isolates (8.5%) were MRSA[24]. Another study found that 125 isolates (36.7%) were *S. aureus*, across 341 healthcare workers and 10 of those isolates (2.9%) were MRSA[25]. The different findings between our study and that of other studies may be due to the study population's difference. Joachim *et al.*[24] enrolled patients in hospitals, with any symptoms and/or signs of infection on admission. Varghese *et al.*[25] included relatively healthy participants (healthcare workers) who had been exposed to infections, whereas, subjects in our study were patients with a duration of symptoms for more than five days.

Moreover, our patients were recruited from an ENT Department in a referral hospital, where patients usually go when they have not been cured in a primary health care environment. It may also explain the high proportion of antibiotic use within one year before recruitment. A population-based survey in Indonesia showed a low prevalence of *S. aureus* (9.1%) and only two isolates had a positive *mecA* gene[26]. However, the study was conducted more than ten years ago and the prevalence might be higher today, even in the general population.

In this study, sixteen of the nineteen *mecA*-positive isolates were susceptible to cefoxitin, as determined via AST. It was therefore classified as Oxacillin/cefoxitin-Susceptible *mecA*-positive *S. aureus* (OS-MRSA). These isolates could be misclassified as Methicillin-Susceptible *Staphylococcus aureus* (MSSA) if the genetic detection of the *mecA* is not performed. These results indicated that both the *mecA* gene and the *mecC* gene do not always appear as a form of methicillin-resistance phenotype. The presence of the *mecA* is generally recognized as the most reliable method for detecting methicillin resistance. The *mecA*-positive staphylococcal strains are considered to be resistant to all β-lactam antibiotics[27,28]. *Staphylococcus aureus* which carries the *mecA* gene, but is phenotypically susceptible to oxacillin/cefoxitin has recently been reported worldwide[29,30]. Therefore, a combination of genotypic and phenotypic tests is necessary to avoid false positives or false negatives in identifying MRSA.

This study also identified that all *S. aureus* isolates (100%) had the presence of *blaZ*. This gene is responsible for the resistance to penicillin, which was related to seventeen isolates of the *S. aureus,* which is resistant to penicillin (85%). It was also found that 14 isolates were resistant to Amoxicillin (70%). The activated *blaZ* can encode the β-lactamase enzyme (penicillinase), which inactivates the antibiotic through hydrolysis of the peptide bond in the β-lactam ring[6]. In this study, not all *blaZ*-positive *S. aureus* isolates exhibited the penicillin-resistant phenotypically, which corresponded well to previous findings[31]. The additional compound of beta-lactamase inhibitors (clavulanic Acid) to the Amoxicillin showed a lower incidence of resistance (45%). Clavulanic Acid is a semisynthetic beta-lactamase inhibitor that is isolated from Streptomyces. Clavulanic Acid contains a beta-lactam ring and binds strongly to beta-lactamase, at or near its active site, thereby preventing its enzymatic activity[32].

This study also showed that eight isolates of the *S. aureus* (40%) were resistant to clindamycin and azithromycin. The results were in line with a few reports, revealing the high incidence in clindamycin and azithromycin resistance, either in MRSA or in Methicillin-Susceptible *S. aureus* (MSSA)[33,34]. Some of the oral antibiotic preparations above are often used as empirical antibiotic therapies in acute bacterial rhinosinusitis[23]. Despite the high resistance against many antibiotics, we found a high susceptibility of the *S. aureus* against Imipenem (100%), Ciprofloxacin (90%) and Cotrimoxazole (90%). Most guidelines recommend Amoxicillin as a first-line of therapy, because of its safety, effectiveness, low cost and narrow microbiologic spectrum. In terms of high resistance to Amoxicillin, we suggest using Ciprofloxacin and Cotrimoxazole as an alternative antibiotic when it is needed, as an antibiotic treatment[18,23,35]. Even so, along with most guidelines, we suggest using antibiotics wisely. Even for Acute Bacterial Rhinosinusitis (ABRS), antibiotic treatment might not be necessary unless symptoms have persisted for at least seven days[18,35].

Detection and treatment of Methicillin-Resistant *S. aureus* (MRSA) become a challenge in the presence of Oxacillin-Susceptible-*mecA*-positive *S. aureus* (OS-MRSA) concerning misidentification and therapeutic implication. Clinically, the sensitivity of *S. aureus* to cefoxitin/oxacillin can be interpreted as susceptible to β-lactam, but the presence of the *mecA* and *blaZ* genes causes susceptibility of OS-MRSA to β-lactam easily change, especially if they use of these antibiotics increased, leading to failure of β-lactam treatment. Our study shows that *S. aureus* is the most common bacteria found in patients with acute post-viral rhinosinusitis and that there is a high proportion of *blaZ* and *mecA*-sensitive cefoxitin positive *S. aureus* in the study participants. Therefore, this study implies the importance of examining the *mecA* and *blaZ* genes to avoid misinterpretation of MRSA as MSSA. We recommend that *mecA* and *blaZ* genes are included in the standard routine laboratory tests in a clinical setting, especially during MRSA identifying process.

CONCLUSION

The presence of *S. aureus mecA*, *mecC* and *blaZ* gene was found to be highly distributed. However, phenotypically, most *S. aureus* was susceptible to oxacillin/cefoxitin. It is likely to make up a high proportion of the cefoxitin-susceptible *mecA*-positive *S. aureus* in the study participants. Other than that, all *S. aureus* isolates had a *blaZ* gene-positive with the majority of isolates exhibited the penicillin-resistant phenotypically, however, a small portion of the *blaZ*-positive *S. aureus* was still sensitive.

SIGNIFICANCE STATEMENT

This study discovers the examination of *blaZ* and *mecA* genes is required for identifying MRSA that can be beneficial for rational use of antibiotics in acute rhinosinusitis patients. This study will help the researcher to uncover the critical areas of antibiotic resistance that many researchers were not able to explore. Thus, a new theory on these combinations of oxacillin/cefoxitin Minimum Inhibitory Concentration (MIC) examination and detection of *blaZ* and *mecA* as genetic markers in the diagnosis of MRSA in acute rhinosinusitis, may be arrived at.

ACKNOWLEDGMENTS

We would like to thank the Head of Infectious Disease Research Centre, Faculty of Medicine, Universitas Padjadjaran, Indonesia and other members who contribute to this research while collecting and analyzing data for research. This research was funded by Ministry Research, Technology and Higher Education to RL (2019) and the grant number is PDUPT No: 3854/Unc.6/2019.

REFERENCES

1. Chambers, H.F., 2005. Community-associated MRSA-resistance and virulence converge. N. Engl. J. Med., 352: 1485-1487.
2. Klevens, R.M., M.A. Morrison, J. Nadle, S. Petit and K. Gershman *et al*., 2007. Invasive methicillin resistant *Staphylococcus aureus* infections in the United States. J. Am. Med. Assoc., 298: 1763-1771.
3. Stapleton, P.D. and P.W. Taylor, 2002. Methicillin resistance in *Staphylococcus aureus*: Mechanisms and modulation. Sci. Prog., 85: 57-72.
4. Hiramatsu, K., L. Cui, M. Kuroda and T. Ito, 2001. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol., 9: 486-493.
5. Llarrull, L.I., J.F. Fisher and S. Mobashery, 2009. Molecular basis and phenotype of methicillin resistance in Staphylococcus aureus and insights into new β-lactams that meet the challenge. Antimicrob. Agents Chemother., 53: 4051-4063.
6. Takayama, Y., T. Tanaka, K. Oikawa, N. Fukano, M. Goto and T. Takahashi, 2018. Prevalence of blaZ gene and performance of phenotypic tests to detect penicillinase in *Staphylococcus aureus* isolates from Japan. Ann. Lab. Med., 38: 155-159.
7. Saeed, K., N. Ahmad, M. Dryden, N. Cortes and P. Marsh *et al*., 2014. Oxacillin-susceptible methicillin-resistant *Staphylococcus aureus* (OS-MRSA), a hidden resistant mechanism among clinically significant isolates in the Wessex region/UK. Infection, 42: 843-847.

8. Andrade-Figueiredo, M. and T.C. Leal-Balbino, 2016. Clonal diversity and epidemiological characteristics of *Staphylococcus aureus*: High prevalence of oxacillin-susceptible mecA-positive *Staphylococcus aureus* (OS-MRSA) associated with clinical isolates in Brazil. BMC Microbiol., Vol. 16. 10.1186/s12866-016-0733-4.
9. Song, Y., L. Cui, Y. Lv, Y. Li and F. Xue, 2017. Characterisation of clinical isolates of oxacillin-susceptible mecA-positive *Staphylococcus aureus* in China from 2009 to 2014. J. Glob. Antimicrob. Resist., 11: 1-3.
10. Conceição, T., C. Coelho, H. de Lencastre and M. Aires-de-Sousa, 2015. Frequent occurrence of oxacillin-susceptible mecA-positive *Staphylococcus aureus* (OS-MRSA) strains in two African countries. J. Antimicrob. Chemother., 70: 3200-3204.
11. Clinical and Laboratory Standard Institute, 2019. Performance Standards for Antimicrobial Susceptibility Testing: 20th Informational Supplement. Clinical and Laboratory Standard Institute, Wayne, PA., USA.
12. Pu, W.X., Y. Su, J.X. Li, C.H. Li, Z.Q. Yang, H.P. Deng and C.X. Ni, 2014. High incidence of oxacillin-susceptible mecA-positive *Staphylococcus aureus* (OS-MRSA) associated with bovine mastitis in China. PLoS ONE, Vol. 9. 10.1371/journal.pone.0088134.
13. Duarte, F.C., T. Danelli, E.R. Tavares, A.E.B. Morguette and G. Kerbauy *et al*., 2019. Fatal sepsis caused by mecA-positive oxacillin-susceptible *Staphylococcus aureus*: First report in a tertiary hospital of southern Brazil. J. Infect. Chemother., 25: 293-297.
14. Thanaviratananich, S., S. Chusakul, G. Moungthong, T. Luxameechanporn and S. Tantilipikorn *et al*., 2015. A surveillance bacteriological study of acute bacterial rhinosinusitis in Thailand and the clinical responses to the culture-directed antibiotics. J. Med. Assoc. Thailand Chotmaihet Thangphaet, 98: S204-S216.
15. Kirtsreesakul, V., J. Tuntaraworasin and B. Thamjarungwong, 2008. Microbiology and antimicrobial susceptibility patterns of commensal flora in the middle nasal meatus. Ann. Otol. Rhinol. Laryngol., 117: 914-918.
16. Meltzer, E.O. and D.L. Hamilos, 2011. Rhinosinusitis diagnosis and management for the clinician: A synopsis of recent consensus guidelines. Mayo Clinic Proc., 86: 427-443.
17. Brook, I., 2011. Microbiology of sinusitis. Proc. Am. Thoracic Soc., 8: 90-100.
18. Aring, A.M. and M.M. Chan, 2011. Acute rhinosinusitis in adults. Am. Family Physician, 83: 1057-1063.
19. Wang, D.Y., R.S. Wardani, K. Singh, S. Thanaviratananich and G. Vicente *et al*., 2011. A survey on the management of acute rhinosinusitis among asian physicians. Rhinol. J., 49: 264-271.
20. Young, J., A. De Sutter, D. Merenstein, G.A. van Essen and L. Kaiser *et al*., 2008. Antibiotics for adults with clinically diagnosed acute rhinosinusitis: A meta-analysis of individual patient data. The Lancet, 371: 908-914.
21. Goossens, H., M. Ferech, R.V. Stichele and M. Elseviers, 2005. Outpatient antibiotic use in Europe and association with resistance: A cross-national database study. Lancet, 365: 579-587.
22. Gill, J.M., P. Fleischut, S. Haas, B. Pellini, A. Crawford and D.B. Nash, 2006. Use of antibiotics for adult upper respiratory infections in outpatient settings: A national ambulatory network study. Family Med., 38: 349-354.
23. Fokkens, W.J., V.J. Lund, J. Mullol, C. Bachert and I. Alobid *et al*., 2012. EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. Rhinology, 50: 1-12.
24. Joachim, A., S.J. Moyo, L. Nkinda, M. Majigo and E. Mmbaga *et al*., 2017. Prevalence of methicillin-resistant *Staphylococcus aureus* carriage on admission among patients attending regional hospitals in Dar es Salaam, Tanzania. BMC Res. Notes, Vol. 10. 10.1186/s13104-017-2668-8.
25. Varghese, S., D. Ramakrishnan, J. Rajahamsan, S.K. Balakrishnan and I.R. Mercybai *et al*., 2018. *Staphylococcus aureus* carrier state among surgery and orthopedics health care personnel of a tertiary care center. Clin. Epidemiol. Global Health, 6: 105-108.
26. Lestari, E.S., J.A. Severin, P.M.G. Filius, K. Kuntaman and D.O. Duerink *et al*., 2008. Antimicrobial resistance among commensal isolates of *Escherichia coli* and *Staphylococcus aureus* in the Indonesian population inside and outside hospitals. Eur. J. Clin. Microbiol. Infect. Dis., 27: 45-51.
27. Jonas, D., M. Speck, F.D. Daschner and H. Grundmann, 2002. Rapid PCR-based identification of methicillin-resistant *Staphylococcus aureus* from screening swabs. J. Clin. Microbiol., 40: 1821-1823.
28. Peacock, S.J. and G.K. Paterson, 2015. Mechanisms of methicillin resistance in *Staphylococcus aureus*. Annu. Rev. Biochem., 84: 577-601.
29. Jannati, E., M. Arzanlou, S. Habibzadeh, S. Mohammadi and P. Ahadi *et al*., 2013. Nasal colonization of mecA-positive, oxacillin-susceptible, methicillin-resistant *Staphylococcus aureus* isolates among nursing staff in an Iranian Teaching Hospital. Am. J. Infect. Control, 41: 1122-1124.
30. Hososaka, Y., H. Hanaki, H. Endo, Y. Suzuki and Z. Nagasawa *et al*., 2007. Characterization of oxacillin-susceptible mecA-positive *Staphylococcus aureus*: A new type of MRSA. J. Infect. Chemother., 13: 79-86.

31. Matono, T., M. Nagashima, K. Mezaki, A. Motohashi and S. Kutsuna *et al*., 2018. Molecular epidemiology of β-lactamase production in penicillin-susceptible *Staphylococcus aureus* under high-susceptibility conditions. J. Infect. Chemother., 24: 153-155.
32. Doi, Y. and H.F. Chambers, 2014. Penicillins and β-Lactamase Inhibitors. In: Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, Bennett, J.E., R. Dolin and M.J. Blaser (Eds.)., Elsevier, New York, pp: 263-277.
33. Lall, M. and A.K. Sahni, 2014. Prevalence of inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. Med. J. Armed Forces India, 70: 43-47.
34. Carrel, M., M. Goto, M.L. Schweizer, M.Z. David, D. Livorsi and E.N. Perencevich, 2017. Diffusion of clindamycin-resistant and erythromycin-resistant methicillin-susceptible *Staphylococcus aureus* (MSSA), potential ST398, in United States Veterans Health Administration Hospitals, 2003-2014. Antimicrob. Resist. Infect. Control, Vol. 6. 10.1186/s13756-017-0212-1.
35. Kaplan, A., 2014. Canadian guidelines for acute bacterial rhinosinusitis: Clinical summary. Can. Family Physician, 60: 227-234.