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Methicillin –resistant Staphylococcus aureus (MRSA) Identification Among Health Care Workers in the Intensive Care of Nusa Tenggara Barat Regional General Hospital

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ABSTRACT

Healthcare workers (HCW's) are the primary providers of services in healthcare facilities. The high frequency of contact between healthcare workers and patients renders them susceptible to the transmission of MRSA. This research was conducted to detect the presence of the MRSA using Mec A gene and their sensitivity with antibiotics among HCWs in the intensive care of the Nusa Tenggara Barat Regional General Hospital. This is cross-sectional study with purposive random sampling nostril swabs and information about daily routines of changing outfit HCW's ICU, HCU, PICU rooms between January-March 2024. The examination included a microbiological examination and a PCR of the Mec A gene. Microbiological examination of 45 HCWs nostril swabs revealed the presence of 31 isolates of *S.aureus*, 25 of isolate were identified as Methicillin-Susceptible Staphylococcus aureus (MSSA), and 6 as MRSA. Four MRSA isolates from the HCW in ICU exhibited the highest resistant to Cefoxitin (75%), while two HCW's PICU isolates demonstrated 100% resistant to Amoxicillin. The six MRSA isolates were confirmed positive for the Mec A gene by PCR examination using the Mec A primer at 532 bp, and three of them is a HCW's who's didn't change clothes every day. The presence of the MRSA among HCWs in the intensive care unit of Nusa Tenggara Barat Regional General Hospital was 19%. Furthermore, it was found that all of *S.aureus* were still sensitif to vancomycin.

INTRODUCTION

MRSA can be transmitted from humans to animals through direct contact with contaminated animal skin or waste, farms with poor sanitation or places where animals often interact with humans. MRSA can begin when methicilin sensitive S.aureus (MSSA) bacteria get the mec A gene transfer from other organisms, the mec A gene contains penicillin binding protein 2a which can cause the bacterial wall to be weak in binding betalactam class antibiotics. S.aureus bacteria that have received the mec A gene transfer will be able to transfer genetic material to other bacteria The increasing prevalence of MRSA in a community can lead to increased transmission from communities such as hospitals to the surrounding environment and veterinary environment. As a bacterial pathogen, MRSA transmission can occur across regions, countries and even continents all related to the increasing human mobility.

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A study on the presence of MRSA in milked milk in the Tulungagung area, East Java, showed that contamination could be obtained from unhygienic hands during the milking process (Khairullah et al., 2023). As for human-to-human transmission spread through damaged skin surfaces such as open wounds and contaminated surfaces in the environment of health facilities, the MRSA population reported was found in surgical treatment rooms on scissors (83%), instrument tables (87%), bed sheets (67%) and infusion poles (75%) (Erlin et al., 2020). Health care workers can be a source of disease transmission MRSA because of their high intensity of encounters with patients directly or carriers, like the chain infection that occurs in health care settings from patients to health workers and vice versa.

MRSA frequently results in serious infectious illnesses in humans, such as foodborne illness, pyogenic endocarditis, suppurative pneumonia, otitis media, osteomyelitis, and pyogenic skin and soft tissue infections (Algammal et al., 2020). *Staphylococcus aureus* is a type of bacteria that can live on the skin and nose of humans that can develop into MRSA through genetic mutations that cause it to have resistant to antibiotics. The mobile genetic elemen *Staphylococcal chromosomal cassette mec* (SCCmec) carries the mecA gene, in MRSA the acquisition of SCCmec leads to the resistant to the β-lactam antibiotics (Liu, 2016). The mecA gene that has been acquired is responsible for coding penicillin-binding protein 2a (PBP2a), which effectively replaces the functions of natural PBPs. As it has a low affinity for β-lactam antibiotics, PBP2a becomes the primary factor leading to MRSA. Alongside PBP2a, other genetic elements that control the synthesis of cell walls, cell signaling pathways, and metabolism are necessary for the development of high-level resistant to β-lactam antibiotics in MRSA (Lade & Kim, 2023). This study aims to detect of the MRSA using Mec A gene among HCWs in the intensive care of the Nusa Tenggara Barat Regional General Hospital and their sensitivity to antibiotics. It is hoped that the results of the study can provide input for hospitals in preventing and controlling hospital acquired infections.

MATERIALS/METHOD

This research is a cross-sectional study with purposive random sampling. The sample used in this study was a 45 nostril swab that was taken from nurse with criteria nurse who works in the intensive care unit (HCU, ICU and PICU) and information about daily routines of changing outfit, the sample taken from January to March 2024. The sample is stored in a Nutrient Broth medium and incubated at a temperature of 37^0 C for 24 hours, on the second day the sample has been cultivated in a Manitol Salt Agar medium and reincubated to temperature 37^0 C for 24 hours. The subsequent growing colony is microbiologically identified and stated as *S.aureus* if the colony in the Manitol Salt Agar medium is yellow colony and coagulated positive. Disc diffusion method was used to ascertain the results of an antimicrobial susceptibi lity test using antibiotics disc Cefoxitin, Clindamycin, Vancomycin, Gentamycin, Ofloxacin, Ciprofloxacin, and Amoxicillin, and interpreted the results according to the CLSI (Clinical and Laboratory Standards Institute). Multi drug resistant *S.aureus* isolates were subsequently processed to DNA extraction using My Taq HS Red Mix Bio-25048 DNA isolation kit and amplification used Mec A primer referring to the research by Nam et.al.(Khairullah et al., 2023) : Mec A F 5’ AAA ATC GAT AAA GGT TGGC 3’ : Mec A R 3’ AGT TCT GCA GTA CCG GAT TTGC 5’ on 532 bp amplicon

RESULTS AND DISCUSSION

The microbiological culture examination of 45 nostril swabs from the health care workers HCU, ICU and PICU rooms found 31 (68.9%) *S.aureus* bacterial isolates and the remaining 14 (31%) were other bacteria with number from each room as shown in table 1.

Table 1. Number of positive samples for S.aureus from HCU, ICU and PICU

Bacteria	**HCU**	**ICU**	**PICU**	**Total**
S.aureus	8	13	10	31
Other bacteria	4	10	0	14
Total	12	23	10	45

S.aureus is commonly seen as a pathogenic bacterium because it can lead to various invasive infections. However, approximately 30% of people are asymptomatically colonized by S.aureus in their nostrils, either temporarily or continuously. Because of that it can also be considered a human commensal, despite the fact that carrying it raises the likelihood of infection (Laux et al., 2019, (Gehrke et al.,2023). Antibiotics susceptibility test from Cefoxitin, Clindamycin, Vancomycin, Gentamycin, Ofloxacin, Ciprofloxacin, and Amoxicillin was carried out on 31 isolates of S.aureus, from the results of the examination it was found that 25 isolates were in the Metichillin sensitive S.aureus (MSSA) category and 6 isolate were MRSA (Table 2).

Table 2. Precentage of Methicillin sensitive *S.aureus* (MSSA) and Methicillin resistant *S.aureus* (MRSA)

Bacteria	**HCU**	**ICU**	**PICU**	**Total**
MSSA	8	9	8	25
Precentage MSSA	100%	69%	80%	
MRSA	0	4	2	6
Precentage MRSA	0%	31%	20%	

S.aureus sensitivity to antibiotics tested in the HCU room reached 100%, whereas in the PICU and ICU respectively 80% and 69%. The highest resistant of S.aureus to the antibiotic tested was found in the ICU of 31% , while in HCU room no S.aureus resistant to tested antibiotics was found. High levels of antibiotic resistant in the ICU can be caused by the weakened immune systems of the patients being treated, so ICU patients require special care. In addition, the patient's companion, although limited, can also contribute to the transmission of bacteria from outside the treatment room.

Table 3. Antibiotic resistant test results on six *S.aureus* isolates

Isolate	**Antibiotic tested**							**Room**
Antibiotic class	**Betalactam**		**Linco samide**	**Glico peptide**	**Amino glicoside**	**Fluoroquinolon**		
	FOX	AX	DA	VA	CN	OF	CIP	
S31	R	R	S	S	R	R	R	ICU
S32	R	S	S	S	S	S	S	ICU
S33	R	S	R	S	S	S	S	ICU
S34	S	S	R	S	S	S	S	ICU
Resistant %	75%	25%	50%	0%	25%	25%	25%	ICU
S37	S	R	R	S	S	S	S	PICU
S41	R	R	S	S	S	R	R	PICU
Resistant %	50%	100%	50%	0%	0%	50%	50%	PICU

Description :FOX : Cefoxitin, DA : Clindamycin, VA : Vancomycin, CN : Gentamycin, OF : Ofloxacin, CIP : Ciprofloxacin, AX : Amoxicillin,R :resistant, S : sensitive

Based on class antibiotic (Table 3.) also provide information that MRSA isolate from HCW's found is not only resistant to betalactam class antibiotics, but also occurs in lincosamide, Aminoglycosides, and Fluoroquinolone. The highest resistant to antibiotics in the HCWs ICU was found in Cefoxitin at 75%, followed by Clindamycin at 50%, This suggests that there is resistant to beta lactamase antibiotics which can lead to MRSA characteristics. The *S.aureus* resistant pattern to antibiotic in the HCW PICU room is the highest found in Amoxicillin at 100% meanwhile Vancomycine and Gentamycin appear to be sensitive. Study in Vietnam of *S.aureus* resistant in 55 HCWs in an intensive care unit, 24.5 HCWs were persistent nasal carriers of MRSA (Duong et al., 2022). Higher rates of healthcare-associated infections (HAIs) were linked to elevated levels of missed care and increased workload. Nurses' views on the frequency of central line-associated bloodstream infections (CLABSI) and ventilator-associated pneumonia (VAP) were positively correlated with the occurrence of these infections in the units they worked in.(Alanazi, 2023) Special importance is placed on ICU nurses in the prevention and control of Healthcare-associated infections (HAIs) due to their involvement in basic hygiene, leading quality improvement efforts, ensuring accurate microbiological sampling, and practicing antibiotic stewardship. The improvement in microbiological techniques and our enhanced understanding of the relationship between critically ill patients and their microbiota are causing us to reconsider our approach to defining HAIs and finding the most effective methods for diagnosing, treating, and preventing these infections in the ICU (Blot et al., 2022).

In this study, nurses' daily routines of changing outfit were observed. It was discovered that three nurses (50%) who worked in the ICU and did not change their clothes every day carried the Mec A gene (Table.4), with the electroforesis figure from three isolate marked by the formation of bands in the range of 532 bp in figure 1. This indicates that MRSA colonisation is related to the personal hygiene of HCWs. A study in the USA found that MRSA contaminated hands, clothes, cell phone, stethoscope and patient sign-out list before nurses entering the patient care room (Popovich et al., 2021).

Table 4. Antibiotic resistant and habit to changing outfit among nurse who's Mec A gene positif

Isolate	Antibiotic resistant	Rooms	Mec A gene (PCR)	Changing outfit
S31	FOX, CN, OF, CIP, AX	ICU	Positive	Every day
S32	FOX,	ICU	Positive	Not every day
S33	FOX, DA	ICU	Positive	Not every day
S34	DA	ICU	Positive	Not every day
S37	DA, AX	PICU	Positive	Every day
S41	FOX, OF, CIP, AX	PICU	Positive	Every day

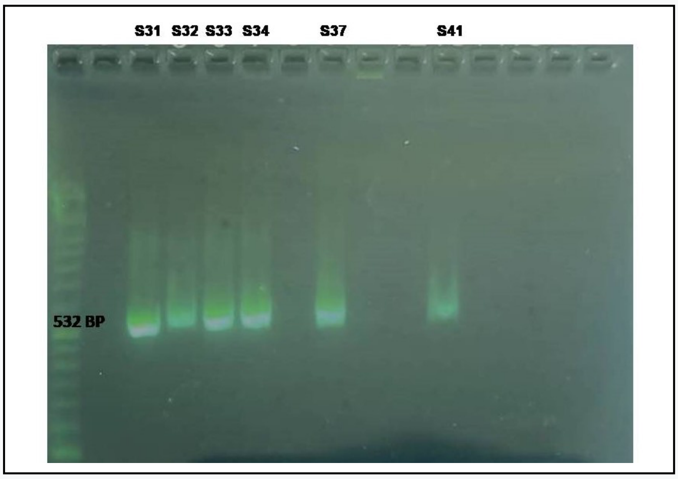

Figure 1. Electroforesis results of six *S.aureus* isolates with Mec A primers

CONCLUSIONS

The result in this study indicate that six out of 31 (9%) S. aureus isolates recovered from HCW's swabs were detected to have the mec A gene with three of them found in HCW's who did not change work clothes every day.

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