https://cejsr.academicjournal.io

Detection of Methicillin-Resistant Staphylococcus aureus (MRSA) Bacteria in Integrated Laboratory Handwashing Facilities

Kurniawan
Universitas Muhammadiyah Purwokerto, Purwokerto, Jawa Tengah, Indonesia
**Corresponding author e-mail: kurniawan@ump.ac.id*

Rena Rafinda Sufi
Universitas Muhammadiyah Purwokerto, Purwokerto, Jawa Tengah, Indonesia

Kurnia Ritma Dhanti
Universitas Muhammadiyah Purwokerto, Purwokerto, Jawa Tengah, Indonesia

Naufal Kurniawan
Pascasarjana UIN Sunan Kalijaga, Yogyakarta, Indonesia

Novericko Ginger Budiono
Institut Pertanian Bogor, Bogor, Jawa Barat, Indonesia

M. Yusuf
Sekolah Tinggi Agama Islam Darussalam Krempyang Nganjuk, Indonesia

ABSTRACT

Staphylococcus aureus is one of the normal flora bacterial species in humans which is susceptible to resistance to antibiotics. The example of which is Methicillin-Resistant Staphylococcus aureus (MRSA). It is resistant to the antibiotic β-lactam classes and its spread is quite widespread. It has spread not only in hospital, but also in the community environment. So, it is suspected that it has reached the campus environment which has several laboratories. One of the facilities in Universitas Muhammadiyah Purwokerto is an integrated laboratory which has a hand washing facility (sink). It is very potential to be a medium for transmission and spread of MRSA, yet the research related to this has never been done. Hence, the objective of this study is to reveal the presence or absence of MRSA and also the percentage of MRSA in the hand washing facility at the Integrated Laboratory of Universitas Muhammadiyah Purwokerto. It is an observational descriptive study, furthermore, the obtained data were analyzed by univariate analysis. The results indicated that there were 2 MRSA isolates scattered in the Microbiology and Biochemistry Laboratory, 2 isolates in the Botany and Genetics Laboratory, and 2 isolates in the Laboratory Equipment Washing Room. The percentage of the number of which was 27%. The conclusion indicated that Methicillin-Resistant Staphylococcus aureus (MRSA) was found in the three rooms of laboratory. Furthermore, the percentage of MRSA in the hand washing facilities in the Integrated Laboratory is 27%.

https://cejsr.academicjournal.io

KEYWORDS: *laboratory, MRSA bacteria, resistance, and S. aureus.*

Introduction

Bacteria *Staphylococcus aureus* is a common flora bacterium found in humans, particularly in the nose and skin, and is susceptible to antibiotic resistance (Nathwani *et al*., 2010; Brooks *et al*., 2012). Bacteria *Methicillin-Resistant Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that has a gene that makes it resistant to methicillin and several β-lactam antibiotics like flucloxacillin, cephalosporin, and carbapenem (Wildana, 2010).

MRSA bacteria have become a global health issue because they can cause difficult-to-cure infectious diseases with significant morbidity and treatment costs. Year after year, the spread of MRSA germs in Indonesia appears to be increasing. The incidence of MRSA bacterial infection in Indonesia was 2.5 % in 1986, and it continued to rise to 9.4 % in 1993, and 23.5% in 2006. (Asri, 2017).

A laboratory is a space that is equipped with scientifically-based equipment and materials. These facilities are typically used for scientific experiments, research, educating, learning practices testing, and/or the production of certain products (Pascalis, 2018). One source of bacterial transmission is the amount of treatments performed in the laboratory, which range from specimen handling to examination processes to tool washing. Laboratory staff or a laboratory environment that does not fulfill biosafety regulations can cause this spread. (Arifin, 2016).

A handwashing room or sink is one among the facilities of the integrated laboratory of Universitas Muhammadiyah Purwokerto (UMP). The sink should be free of microorganism contamination as a place to perform sanitation. MRSA research in handwashing facilities has never been done before at the UMP Laboratory. Based on this background, researchers believed it was necessary to do study on the detection of MRSA bacteria in handwashing facilities in the integrated laboratory of Universitas Muhammadiyah Purwokerto.

Materials and Methods

Sampling. Sampling was carried out by swabbing the surface of the sink faucet using a sterile cotton bud that had been moistened with peptone water. when swabbing a sterile cotton bud, rotate it slowly so that the surface of the cotton bud can be wiped evenly and the desired bacteria are obtained. After the swabbing procedure is complete, the cotton bud is inserted into the peptone water medium by cutting the part of the cotton bud that is being held using sterile scissors.

Cultivation of *Staphylococcus aureus* Bacteria. From the peptone water media, the dilution was carried out to 10-3 and then planted on MSA plate media and incubated at 37 °C for 24-48 hours. After the incubation period was completed, the growth of bacterial colonies was observed on the MSA plate medium and looked for yellow bacterial colonies with the media around the colonies also turning yellow. The yellow bacterial colonies were then separated and purified by growing several times on the TSA plate and after being completely pure, they were then grown on the inclined TSA medium as a culture stock.

Gram stain. A pure culture of bacteria suspected to be *S. aureus* was then taken a colony using an ose needle and then scratched on the center of the object-glass which had been dripped with 1-2 drops of sterile distilled water and stirred evenly. The bacterial smear was then carefully fixated over

a Bunsen flame. The object-glass was placed on the entire staining rack and the surface was flooded with crystal violet for 1 minute. Then rinsed with distilled water. The next step is that the object-glass is flooded with Lugol's iodine solution and left for 1 minute, then rinsed again using distilled water. The smear was dripped with acetone alcohol until the droplets became clear for a few moments and finally, the smear was dripped with safranin solution and allowed to stand for 1 minute. The smear was rinsed with distilled water and allowed to dry at room temperature. The dried Gram stain preparations were then covered with a cover glass and then observed using a microscope with 1000x magnification. If the observed bacterial cells are purple then the bacteria are included in the group of Gram-positive bacteria, but if the observed bacterial cells are red, they are included in the group of Gram-negative bacteria.

Coagulase Test. The coagulase test was carried out by taking a colony of bacteria suspected to be *S. aureus* bacteria using an ose needle to be placed on the surface of an object-glass and added with a sterile blood sample. Then the suspension of bacteria and blood was homogenized and observed for the formation of coagulants (clots). if it is formed, then the result of the coagulase test is said to be positive, but if it is marked by the absence of obstacles, then the test result is said to be negative.

Catalase Test. The catalase test was carried out by dripping 1-2 drops of hydrogen peroxide (H2O2) solution on a glass object and then mixed with bacterial colonies suspected of being *S. aureus* bacteria for further homogenization. After that, it was observed whether or not gas bubbles formed in the bacterial suspension. A positive catalase test result is indicated by the formation of gas bubbles, but if the test result is negative, no gas bubbles will form.

Test for Bacterial Resistance to Antibiotics. Positive cultures of *S. aureus* bacteria were grown on MSA media and then incubated for 6 hours or more, then made in the form of liquid cultures aged 18 hours with a density of 10-4 CFU/mL as test bacteria. *S. aureus* bacteria culture aged 18 hours was put into a 1 mL petri dish, then 15 mL of liquid MHA medium was poured and then moved slowly until the media and bacterial culture were evenly distributed in the petri dish and allowed to solidify. Five paper discs were placed on the MHA medium consisting of 3 paper discs containing penicillin (test material), 1 paper disc containing vancomycin (positive control), and 1 paper disc containing dimethyl sulfoxide (DMSO) (negative control). The distance between the paper discs is set to approximately 15 mm. The media was then incubated at 37°C for 24 hours. The zone of resistance is then measured using a caliper.

https://cejsr.academicjournal.io

Table 1. Morphological Characteristics of Bacterial Colonies

Sink Code	Growth	Colony Description
LM 01	None	-
LM 02	None	-
LM 03	Available	Round, yellowish white, shiny, convex, yellow zone present
LM 04	Available	Round, yellowish white, shiny, convex, yellow zone present
LM 05	Available	Round, white, flat surface, no yellow zone
LM 06	None	-
LM 07	None	-
LB 01	Available	Round, white, flat surface, no yellow zone
LB 02	Available	Round, colored yellowish white, shiny, convex, yellow zone present
LB 03	Available	Round, colored yellowish white, shiny, convex, yellow zone present
LB 04	None	-
LZ 01	Available	Round, yellowish white, shiny, convex, yellow zone present
LZ 02	None	-
LZ 03	Available	Round, yellowish white, shiny, convex, yellow zone present
LZ 04	Available	Round, yellowish white, shiny, convex, yellow zone present
LZ 05	Available	Round, yellowish white, shiny, convex, yellow zone present
LZ 06	None	-
RC 01	None	-
RC 02	Available	Round, yellowish white, shiny, convex, yellow zone present
RC 03	Available	Round, yellowish white, shiny, convex, yellow zone present
RC 04	Available	Round, yellowish white, shiny, convex, yellow zone present
RC 05	Available	Round, yellowish white, shiny, convex, yellow zone present

Table 2. Observations of Gram's Staining

Isolate Code	The Results of Gram's Staining	Cell Shape	Isolate Code	The Results of Gram's Staining	Cell Shape
LM 03	Purple (Gram Positive)	Cocci	LZ 04	Purple (Gram Positive)	Basil
LM 04	Purple (Gram Positive)	Cocci	LZ 05	Purple (Gram Positive)	Basil
LB 02	Purple (Gram Positive)	Cocci	RC 02	Purple (Gram Positive)	Cocci
LB 03	Purple (Gram Positive)	Cocci	RC 03	Purple (Gram Positive)	Cocci
LZ 01	Purple (Gram Positive)	Cocci	RC 04	Purple (Gram Positive)	Basil
LZ 03	Purple (Gram Positive)	Basil	RC 05	Purple (Gram Positive)	Basil

Results

Table 3. Biochemical Test Results of Bacteria Suspected of S. aureus

Isolate Code	Biochemical Test			Isolate Code	Biochemical Test		
	Coagulase	Catalase	Mannitol Fermentation		Coagulase	Catalase	Mannitol Fermentation
LM 03	+	+	A	LZ 01	-	+	A
LM 04	+	+	A	RC 02	+	+	A
LB 02	+	+	A	RC 03	+	+	A
LB 03	+	+	A				

Table 4. Results of Antibiotic Resistance Test of Suspected S. aureus

Isolate Code	Inhibition zone diameter (mm)					Average of P (mm)	Standard (mm)
	C (+)	P_1	P_2	P_3	C (-)		
LM 03	09,7	11,2	11,4	11,2	0	11,6	Resistant: ≤ 28
LM 04	17,4	13,1	13,5	13,1	0	13,2	
LB 02	15,4	11,8	11,6	11,3	0	11,5	
LB 03	16,4	11,3	11,3	11,1	0	11,2	Sensitive: ≥ 29
RC 02	0	0	0	0	0	0	
RC 03	0	0	0	0	0	0	

Table 5. Distribution of MRSA in Handwashing Facilities in the Integrated Laboratory

Laboratory Rooms	Sensitivity Level of *S. aureus*	
	Resistant	Sensitive
LM	2	0
LB	2	0
LZ	0	0
RC	2	0

Discussion

Morphological Characteristics of Bacterial Colonies on *Mannitol Salt Agar* (MSA) Media. Based on Table 1, there were 12 isolates suspected of being bacteria *S. aureus*, two isolates suspected of not being *S. aureus*, and eight isolates that did not grow on MSA media. The presence of *S. aureus* bacteria was indicated by the formation of yellow bacterial colonies and a color change in the zone around the colonies from red to yellow. MSA medium is a selective medium for isolating Staphylococcus coagulase-positive (*S. aureus*) pathogenic bacteria and other halophilic bacteria, but inhibits the growth of bacteria that are not included in the group of coagulase-positive Staphylococcus bacteria, namely the non-pathogenic group of coagulase-negative Staphylococcus bacteria (Dewi, 2013).

Morphological Characteristics of Isolated Bacterial Cells. The results of cell morphology observations using Gram staining revealed that there were seven isolates in the form of cocci that belonged to the Gram-positive bacteria group (Table 2). It can be seen in the purple, cocci-shaped bacterial cells with single, paired, or chain-shaped cell conformations. *S. aureus* is a Gram-positive bacterium with a cocci-shaped form and a purple Gram stain color. The bacteria retain the first color, crystal violet, which results in the purple color. The thickness of the peptidoglycan content of Gram-positive bacteria is thicker than that of Gram-negative bacteria, which influences the difference in

Gram's characteristics. (Dewi, 2013).

Biochemical Test Results. Catalase and coagulase tests were used to perform bacterial biochemical tests. The results of the catalase test showed that seven isolates were identified as *S. aureus* (Table 3). The catalase test is a biochemical test that is used to distinguish *Staphylococcus* sp. from *Streptococcus* sp. *Staphylococcus* sp. can produce catalase enzymes, which can catalyze the degradation of toxic hydrogen peroxide (H_2O_2) into a water molecule (H_2O) and oxygen (O_2). Based on the results of the characteristics of the coagulase test, six of the seven isolates were identified as *S. aureus* (Table 4). The coagulase test is a biochemical test that determines whether bacteria have the ability to create coagulase enzymes that are attached to cell walls. This enzyme will react to form a complex that can cleave fibrinogen and cause fibrin clotting. The ability to produce this coagulase enzyme is one of the characteristics that distinguishes *S. aureus* from other *Staphylococcus* sp. (Dewi, 2013).

Antibiotic Resistance Test Results. The results of the resistance test showed that there were six bacterial isolates (27 %) were found to be resistant to penicillin antibiotics (Table 4.). The standard value of the penicillin inhibition zone refers to the Clinical and Laboratory Standards Institute (CLSI), with a resistance value of 28 mm and a sensitive value of 29 mm (Hudzicki, 2016). Resistance to the penicillin group is due to a modification of the *Penicillin Binding Protein* (PBP) which has undergone a change in affinity. This change in affinity causes a change in the nature of PBP which should be able to bind to penicillin to change, so that it is unable to bind, and there is an outflow pump for β-lactamase production (Azis, 2017). The resistance test of six *S. aureus* isolates to vancomycin antibiotics (positive control) revealed that three of the six isolates were vancomycin resistant (Table 4). The diameter of the bacterial inhibitory zone is less than the usual value of 12 mm, indicating this. Vancomycin is a glycopeptide antibiotic with a mechanism of action involving the alteration of the D-alanine-D-alanine binding site on peptidoglycan, which causes *S. aureus* cell wall thickening. (Afifurahman, 2014).

Percentage of *Methicillin-Resistant Staphylococcus aureus* (MRSA) in the Integrated Laboratory. Based on the results of the study, MRSA bacteria were most commonly found in handwashing facilities in the Microbiology and Biochemistry Laboratory, Botany and Genetics Laboratory, and Laundry Room for Laboratory Equipment (Table 5). This can be caused by the room's sink being used more frequently than in other rooms. The more the number of users, the higher the percentage of bacterial contamination Longadi (2015). In addition, the differences in the characteristics of MRSA in the handwashing facilities in each of these rooms were caused by variety of factors,, including room cleanliness, room conditions, facilities in the room, and differences in the characteristics of laboratory users.

Acknowledgement(s)

We would like to thank the Institute for Research and Community Service, Universitas Muhammadiyah Purwokerto, which has funded this research to completion.

References

1. Afifurahman K, Samadin H, Aziz, S. 2014. Pola kepekaan bakteri *Staphylococcus aureus* terhadap antibiotik Vancomycin di RSUP Dr. Mohammad Husain Palembang. Majalah Kedokteran Sriwijaya 46(4).
2. Arifin A., Hayati Z, Jamil, K.F., 2016. Isolasi dan identifikasi bakteri di Lingkungan

Laboratorium Mikrobiologi Klinik RSUDZA Banda Aceh. Jurnal Ilmiah Mahasiswa Kedokteran Komunitas. 1(4):1-8.

3. Asri, R.C, Rasyid R, dan Edison. 2017. Identifikasi MRSA pada diafragma stetoskop di Ruang Rawat Inap dan HCU Bagian Penyakit Dalam. Jurnal Kesehatan Andalas. 6(2): .....
4. Azis M.A., 2017. *Pengaruh penambahan putih telur terhadap aktivitas penisilin dalam menghambat bakteri Staphylococcus aureus yang resisten penisilin*. (Skripsi). Universitas Islam Sultan Agung, Semarang.
5. Brooks G.F, Carroll K.C, Butel J.S, Morse S.A., and Mietzner, T.A. 2013. *Jawetz, Melnick, & Adelberg. Mikrobiologi Kedokteran* Edisi 26. EGC. Jakarta.
6. Dewi K.A. 2013. Uji sensitivitas *Staphylococcus aureus* terhadap amoxilin dari sampel susu kambing peranakan etawa (PE) penderita mastitis di Wilayah Grimulyo, Kulonprogo Yogyakarta. Sains Veteriner. 31(2):140-141.
7. Hudzicki J. 2016. *Kirby-Bauer Disk Diffusion Susceptibility Test Protocol*. American Society for Microbiology.
8. Longadi Y.M., Waworuntu O dan Soeliongan S. 2015. Isolasi dan identifikasi bakteri aerob yang berpotensi menjadi sumber penularan infeksi nosokomial di Irina A RSUP Prof. Dr. R. Kandou Manado. Universitas Sam Ratulangi, Manado.
9. Nathwani D, Davey P.G, and Marwick C.A. 2010. MRSA: treating people with infection. Clinical evidence 10(922): 1–18.
10. Wildana, Senang N dan Rusli B. 2010. Resistensi terhadap methicillin (*Methicillin Resistant*) *Staphylococcus aureus* di Instalasi Rawat Inap. Indonesian Journal of Clinical Pathology And Medical Laboratory 17(1): 5-8.