MICROBIOLOGY
INDONESIA
ISSN 1978-3477, eISSN 2087-8575
Vol.16, No.2, December 2022, p 15-23

Available online at
http://jurnal.permi.or.id/index.php/mionline
DOI: 10.5454/mi.16.2.15-23

Multidrug Resistance and Extensively Drug-Resistance in *Staphylococcus aureus, Staphylococcus epidermidis,* and *Staphylococcus haemolyticus*

CLIFF CLARENCE HALIMAN[1], DIMAS SETO PRASETYO[2], CONNY R TJAMPAKASARI[2], AND TJAHJANI MIRAWATI SUDIRO*[2]

[1] *Program of Clinical Microbiology Residency, Faculty of Medicine, Universitas Indonesia, Jakarta 10320, Indonesia;*
[2] *Department of Clinical Microbiology, Faculty of Medicine, Universitas Indonesia - dr. Cipto Mangunkusumo Hospital, Jakarta 10320, Indonesia*

Antimicrobial resistance in bacteria has become a leading global public health issue. *Staphylococcus sp.* has an efficient mechanism to deal with antimicrobial agents that make them hard to treat in hospital-acquired and community-acquired infections. This study was conducted due to limited data about multidrug resistance and extensively drug resistance in *Staphylococcus sp*. in Indonesia. This study was a descriptive retrospective study using a cross-sectional design to get the prevalence and antimicrobial susceptibility of *S. haemolyticus*, *S. aureus,* and *S. epidermidis.* The data were secondary data extracted from WHONET 2022 software. This study's data were from bacteria from samples sent to UKK LMK FKUI, Jakarta from 2017 to 2021 for routine diagnostic. In this study, we found that the prevalence of methicillin-resistant *S. aureus* was 24.9%, methicillin-resistant *S. epidermidis* was 65,5%, and methicillin-resistant *S. haemolyticus* was 86.8%. The prevalence of MDR *S. aureus* is less than *S. epidermidis* and *S. haemolyticus,* respectively. MDR *S. haemolyticus was* consistently above 85% each year, while *S. epidermidis* was above 50% and *S. aureus* was below 50%. XDR Staphylococcus was only found in *S. aureus* and *S. haemolyticus*, i.e. three and seven XDR isolates of *S. aureus and S. haemolyticus respectively* during 2017-2021. Although we could not find any pan-resistant isolates from all samples, we found methicillin-resistant *S. aureus* and *S. haemolyticus* isolates that were also resistant to vancomycin and linezolid. *S. haemolyticus* dan *S. epidermidis* were an important *coagulase-negative Staphylococcus* species that can't be neglected due to the high percentage of MDR and the discoveries of XDR in *S. haemolyticus* so that they have the potential to disseminate resistance plasmids to the more virulent bacteria. Therefore we need to control the use of antimicrobial agent to prevent this resistance.

Key words: Indonesia, Jakarta, MDR, *methicillin resistant*, *Staphylococcus aureus, Staphylococcus epidermidis*, *Staphylococcus haemolyticus,* XDR

Resistensi antimikroba adalah salah satu masalah kesehatan utama di dunia. *Staphylococcus sp*. memiliki mekanisme yang efisien dalam mengatasi antimikroba sehingga menyebabkan sulitnya pengobatan infeksi baik di *hospital acquired infection* maupun *community acquired infection.* Penelitian ini dilakukan karena keterbatasan data mengenai prevalensi *multidrug resistance (MDR) dan extensively drug resistance (XDR) Staphylococcus sp.,* di Indonesia. Penelitian ini menggunakan data sekunder yang diambil dari perangkat lunak WHONET 2022 dan merupakan penelitian deskriptif retrospektif dengan pendekatan potong lintang untuk mengetahui prevalensi dan pola kepekaan antimikroba dari *Staphylococcus haemolyticus, Staphylococcus aureus* dan *Staphylococcus epidermidis.* Sampel yang dianalisis merupakan sampel yang dikirim ke UKK LMK FKUI, Jakarta pada tahun 2017 sampai dengan 2021 untuk diagnosis rutin. Dari hasil penelitian ini ditemukan prevalensi *methicillin resistant S.aureus* adalah 24,9%, *methicillin resistant S.epidermidis* adalah 65,5% dan *methicillin resistant S.haemolyticus* adalah 86,8%. Prevalensi *S.aureus* yang merupakan MDR lebih sedikit daripada *S.epidermidis* dan *S.haemolyticus,* yaitu berturut-turut konsisten diatas 85% tiap tahun, konsisten di atas 50% dan konsisten di bawah 50%. Staphylococcus yang merupakan XDR, hanya ditemukan pada *S.aureus* dan *S.haemolyticus*, yaitu berturut turut pada *S.aureus* sebanyak tiga isolat dan pada *S.haemolyticus* sebanyak tujuh isolat selama tahun 2017-2021. Walaupun dari keseluruhan sampel, tidak ditemukan pan-resistensi, ditemukan *S.aureus* dan *S.haemolyticus* resisten metisilin yang juga resisten terhadap vankomisin dan linezolid. *S.haemolyticus* dan *S. epidermidis* merupakan *coagulase negative Staphylococcus* yang perlu diperhatikan, karena tingginya persentase MDR dan ditenukannya XDR pada *S.haemolyticus*, sehingga berpotensi dapat mendiseminasikan plasmid resistensi kepada organisme yang lebih virulen sehingga diperlukan adanya pengendalian penggunaan antimikroba untuk mencegah penyebaran resistensi tersebut.

Kata kunci: Indonesia, Jakarta, MDR, *methicillin resistant*, *Staphylococcus aureus, Staphylococcus epidermidis*, *Staphylococcus haemolyticus,* XDR

Antimicrobial resistance in bacteria has become a leading global public health issue due to its ineffective treatment of Hospital Acquired or community-acquired infections. (Magiorakos *et al.* 2012; Patel *et al.* 2011)

*Corresponding author: Phone: +62-; E-mail: mrwtsoediro@gmail.com

Staphylococcus sp. has an efficient mechanism to deal with antimicrobial agents that make them hard to treat, especially in life-threatening diseases. (Almanaa *et al.* 2020; Kaur and Chate, 2015) Among all Staphylococcus species, *Staphylococcus aureus, Staphylococcus epidermidis,* and *Staphylococcus haemolyticus* are the most common species in nosocomial infection. (De Giusti *et al.* 1999; Dzen *et al.* 2005; Graham *et al.* 2000; Heilmann *et al.* 2019; Kim and Jang, 2017; Takeuchi *et al.* 2005; Suhartono *et al.* 2019) *Staphylococcus aureus* is the major pathogen species for humans. (Riedel *et al.* 2019, p.205) *Staphylococcus haemolyticus* inhabits human skin as commensal species, at first not considered a pathogen, now it is the second most common species after *Staphylococcus epidermidis* among the Coagulase-negative Staphylococcus group that can be isolated in nosocomial infection.(De Giusti *et al.* 1999; Dzen *et al.* 2005; Graham *et al.* 2000; Heilmann *et al.* 2019; Kim and Jang, 2017; Takeuchi *et al.* 2005) *Staphylococcus haemolyticus* has genomic flexibility that facilitates the survival mechanism to the antimicrobial agents and can be disseminated to other species in the Staphylococcus group. (Kim *et al.* 2012; Takeuchi *et al.* 2005).

In the previous study in Aceh, *Staphylococcus haemolyticus* infection prevalence in hospital is 32.2%, and among the isolate, 96.6% is *Methicillin-resistant Staphylococcus haemolyticus.* (Suhartono *et al.* 2019) While in a global study of multi drugs resistance to *Staphylococcus haemolyticus* that involved eight countries, the prevalence is 77.7%, where multi drugs resistance is defined as resistance to at least three antimicrobial agents. (Cavanagh *et al.* 2014; Czekaj *et al.* 2015).

Due to limited data about multidrug resistance and extensively drug resistance in *Staphylococcus sp*. especially in Indonesia. This study aims to determine the prevalence of multidrug resistance and extensively drug resistance in *S. haemolyticus*, *S. epidermidis,* and *S. aureus* from secondary data obtained from clinical isolates during routine diagnostic in UKK LMK FKUI, Jakarta.

MATERIALS AND METHODS

Staphylococcus sp. identification and antimicrobial susceptibility were obtained from secondary data extracted from WHONET 2022 software. This study was a descriptive retrospective study using a cross-sectional design to get the prevalence and antimicrobial susceptibility of *S. haemolyticus*, *S. aureus,* and *S. epidermidis.* This study's data were from bacteria from samples sent to UKK LMK FKUI, Jakarta from 2017 to 2021 for routine diagnostic. This study has been approved by The Ethics Committee of the Faculty of Medicine, Universitas Indonesia – Cipto Mangunkusumo Hospital. (No: KET-1272 /UN2.F1 /ETIK /PPM.00.02/ 2022). Clinical samples were inoculated to blood agar plates, and the plates were incubated for 24 hours in 37 ^{O}C before being identified by Gram staining. Blood samples were first inoculated into Bactec® blood culture system and positive samples were spread onto blood agar plates as above. Identification and drug susceptibility tests were generated using VITEK® 2 GP and VITEK® 2 AST-GP 67 (Biomerieux, France) and converted using BacLink Software (WHONET, Boston). Then the data of species and drug sensitivity were extracted and presented in frequency percentage using WHONET 2022 software (WHONET, Boston). GNU PSPP 1.6.2 (Free Software Foundation, Boston) and Microsoft Excel software was used to calculate the percentage that was not calculated in WHONET and make the graph.

The phenotype of Methicillin resistant Staphylococcus was detected by susceptibility to cefoxitin. (Magiorakos *et al.* 2012; Riedel *et al.* 2019,p.215) Multidrug resistance (MDR) was determined by antimicrobial susceptibility test results when the isolate has non-susceptibility to ≥1 antimicrobial agent in ≥3 antimicrobial categories phenotype or has Methicillin resistant phenotype (Magiorakos *et al.* 2012). While extensively drug resistance (XDR) was determined by antimicrobial susceptibility test results when the isolate has non-susceptibility to ≥1 antimicrobial agent in all but ≤ 2 antimicrobial categories phenotype (Magiorakos *et al.* 2012). A bacteria is determined as pandrug resistant (PDR) when the antimicrobial susceptibility test showed non-susceptibility to all antimicrobial categories (Magiorakos *et al.* 2012).

RESULTS

A total of 1099 samples were used to analyse the prevalence and sample characteristics of *S. aureus*, *S. haemolyticus,* and *S. epidermidis,* which can be seen in Table 1 and Table 2.

From all the data mentioned before, a total of 168 other than *S. aureus, S. epidermidis,* and *S. haemolyticus* were excluded from antimicrobial susceptibility

Table 1 Number of *Staphylococcus* sp. isolates in 2017-2021

Organism	2017 n (%)	2018 n (%)	2019 n (%)	2020 n (%)	2021 n (%)	Total
S. aureus	151(53.0)	74(32.5%)	69(27.0%)	42(29.2%)	53(28.5%)	389
S. epidermidis	51(17.9%)	55(24.1%)	70(27.3%)	37(25.7%)	42(22.6%)	255
S. haemolyticus	45(15.8%)	72(31.6%)	80(31.3%)	26(18.1%)	64(34.4%)	287
Other *Staphylococcus*	38(13.3%)[a]	27(11.8%)[a]	37(14.5%)[b]	39(27.1%)[c]	27(14.5%)[d]	168

[a] *S. capitis, S. cohnii, S. hominis, S. lentus, S. ludgunensis, S. pseudintermedius, S. saprophyticus, S. sciuri, S. warneri, S. xylosus.*
[b] *S. capitis, S. cohnii, S. hominis, S. lentus, S. ludgunensis, S. saprophyticus, S. sciuri, S. warneri*
[c] *S. capitis, S. cohnii, S. hominis, S. lentus, S. pseudintermedius, S. saprophyticus, S. sciuri, S. simulans, S. warneri, S. xylosus.*
[d] *S. capitis, S. cohnii, S. hominis, S. lentus, S. ludgunensis, S. pseudintermedius, S. saprophyticus, S. sciuri, S. warneri, S. xylosus*

Table 2 Characterization of *Staphylococcus* sp. isolates according to the specimen type of origin in 2017-2021

Specimen	Species			
	S. aureus (n (%))	*S. epidermidis* (n (%))	*S. haemolyticus* (n (%))	Other *Staphylococcus** (n (%))
Abdominal Fluid	1(0.3%)	1(0.4%)	2(0.7%)	2(1.2%)
Abscess and Pus	88(22.6%)	27(10.6%	11(3.8%)	11(6.5%)
Blood	34(8.7%)	71(27.8%)	20(7.0%)	55(32.7%)
Brain and CSF	-	4(1.6%)	-	2(1.2%)
Bronchoalveolar Lavage and Bronchial Washing	5(1.3%)	7(2.7%)	2(0.7%)	1(0.6%)
Cervix	-	1(0.4%)	3(1.0%)	-
Cornea and Eye	3(0.8%)	3(1.2%)	4(1.4%)	5(3.0%)
External urethra	1(0.3%)	3(1.2%)	8(2.8%)	-
Nose	47(12.1%)	12(4.7%)	4(1.4%)	9(5.4%)
Semen	-	2(0.8%)	19(6.6%)	7(4.2%)
Skin Swab	7(1.8%)	23(9.0%)	4(1.4%)	5(3.0%)
Sputum	57(14.7%)	43(16.9%)	36(12.5%)	3(1.8%)
Throat and Trachea	67(17.2%)	3(1.2%)	2(0.7%)	8(4.8%)
Tissue	30(7.7%)	17(6.7%)	9(3.1%)	8(4.8%)
Urine	16(4.1%)	19(7.5%)	145(50.5%)	38(22.6%)
Vagina	3(0.8%)	5(2.0%)	8(2.8%)	-
Wound and Ulcer	10(2.6%)	2(0.8%)	4(1.4%)	1(0.6%)
Others**	20(5.1%)	12(4.7%)	6(2.1%)	13(7.7%)
Total	389	255	287	168

* Other *Staphylococcus*: *S. capitis, S. cohnii, S. hominis, S. lentus, S. ludgunensis, S. pseudintermedius, S. saprophyticus, S. sciuri, S. simulans, S. warneri, S. xylosus.*

** Others: specimen less than 1% (Aspirate, bile, bone, central venous catheter, ear, joint fluid, kidney, leg swab, liver, male genital, mediastinum, mouth, no data, placenta, pleural fluid, rectal, sinus, prosthesis)

analysis to specify the antimicrobial susceptibility test only in three species. Therefore, 926 samples were used to analyse the antimicrobial susceptibility profile shown in Table 3, Table 4, and Table 5.

In total, *methicillin-resistant S. aureus (MRSA)* prevalence was 24.9%, *methicillin-resistant S. epidermidis (MRSE)* was 65.5% and *methicillin-resistant S. haemolyticus(MRSH)* was 86.8%.

From the antimicrobial susceptibility test, we found the prevalence of XDR in *S. aureus,* and *S. haemolyticus* were 0.77% and 2.43% respectively. While we could not found XDR in *S. epidermidis*. MDR and XDR phenotypes were found in *S. aureus, S. epidermidis,* and *S. haemolyticus* were shown in Table 6 and Fig 1 shows the percentage of methicillin-resistant phenotype. As shown in Fig 2, vancomycin-resistant were found in *S. aureus, S. epidermidis,* and *S. haemolyticus* in methicillin-resistant.

In this study, we found that among the methicillin-resistant phenotype, four isolates on *S. aureus* were vancomycin and linezolid resistant, and ten *S. haemolyticus* isolates were vancomycin and linezolid resistant. While in *S. epidermidis*, we did not find an isolate resistant to vancomycin and linezolid in the methicillin-resistant phenotype.

DISCUSSION

In this study, we found that *S. aureus, S. haemolyticus,* and *S. epidermidis*, respectively, were the most common species that can be isolated from clinical specimens during routine diagnostic tests. *S. aureus*, *S. epidermidis*, and *S. haemolyticus* are important hospital-acquired infection causative pathogens, especially in patients using a venous catheter and medical devices in the Intensive care unit.(Cerca *et al.* 2007; Daniel *et al.* 2014; Horan *et al.* 2008; Klingenberg *et al.* 2007).

In this study, *S. aureus* was commonly found in abscesses and pus, oropharynx and tracheal swab, and sputum. *S. epidermidis* was commonly found in blood, sputum, abscesses, and pus. At the same time, *S. haemolyticus* was commonly found in urine, sputum, and blood. *S. aureus* and Coagulase-negative Staphylococcus are bacteria that colonize human skin, nails, and nares. Hence, they can invade to form pus in the tissue if there is a disruption of human barriers, such as damage to the skin layer, hair follicle trauma, and using medical devices.(Do Carmo Ferreira *et al.* 2011; Lowy, 1998; Schuenck *et al.* 2008) While *S. haemolyticus* is associated with infection in the urinary tract. (Gunn and Davis, 1988; Hovelius *et al.* 1984; John and O'Dell, 1978; Lozano *et al.* 2015; Rupp *et al.*1992). From a study conducted in Aceh, *S. haemolyticus* was predominantly found in the Intensive care unit. (Suhartono *et al.* 2019) In another study conducted in Nepal, *S. aureus* was predominantly found from pus in the Intensive care unit, while *S. epidermidis* was predominantly found on catheter tips in the intensive care unit. (Shrestha *et al.*2018) In this study, we could not determine whether the Staphylococcus that we isolated were from the intensive care/ hospital ward or outpatient due to lack of data.

From the result of *S. aureus* antimicrobial susceptibility that has been shown before, we found that the prevalence of methicillin-resistant *S. aureus (MRSA)* was below 40% each year. The highest prevalence was discovered in 2018 and decreased in 2019 and 2021. This prevalence is slightly lower compared to studies conducted in Afghanistan (Naimi *et al.* 2021) and Pakistan (Ullah *et al.* 2016) but similar to the study conducted in China (Wang *et al.* 2021). From the antimicrobial susceptibility profile, we found in this study that 90% of *S. aureus* is susceptible to nitrofurantoin, rifampicin, vancomycin, linezolid, and quinupristin/dalfopristin, tigecycline, and trimethoprim-sulfamethoxazole. While below 90% of *S. aureus* is susceptible to ciprofloxacin, clindamycin, erythromycin, gentamycin, levofloxacin, moxifloxacin, tetracycline, cefoxitin, oxacillin, and Penicillin G. Penicillin G was the least susceptible antimicrobial agent to *S. aureus.* Compared to other studies mentioned before, the resistance to penicillin G of *S. aureus* is similar to studies conducted in Afghanistan, Pakistan, and China, but slightly different from other antimicrobial agents such as in Afghanistan. They found that *S. aureus* is relatively resistant to erythromycin and ciprofloxacin, In China, they discovered that *S. aureus* is relatively resistant to erythromycin and clindamycin and in Pakistan, they found that *S. aureus* is relatively resistant to erythromycin.(Naimi *et al.* 2021; Ullah *et al.* 2016; Wang *et al.* 2021).

From the result of *S. epidermidis* antimicrobial susceptibility that has been shown before, we found that the prevalence of methicillin-resistant *S. epidermidis* was relatively high. This result is similar to the study conducted in Tianjin, China (Xu *et al.* 2020), which found a high prevalence of methicillin-resistant *S. epidermidis.* The result we found was slightly lower than their study result, but, in our discovery, *S.*

Table 3 Percentage of *S. aureus* antimicrobial sensitivity

Antibiotic	2017 (n=151)	2018 (n=74)	2019 (n=69)	2020 (n=42)	2021 (n=53)	Total (n=384)
Cefoxitin	90.7%	60.8%	65.2%	61.9%	73.6%	75.1%
Oxacillin	90.7%	60.8%	65.2%	61.9%	73.6%	75.1%
Penicillin G	9.9%	5.4%	21.7%	7.1%	20.8%	12.3%
Tetracycline	54.3%	73.0%	59.4%	81.0%	75.5%	64.5%
Erythromycin	84.1%	77.0%	72.5%	66.7%	71.7%	77.1%
Clindamycin	79.5%	79.7%	73.9%	66.7%	69.8%	75.8%
Ciprofloxacin	94.7%	78.4%	82.6%	78.6%	83%	86.1%
Moxifloxacin	94.7%	79.7%	81.2%	78.6%	83%	86.1%
Levofloxacin	94.7%	79.7%	82.6%	78.6%	83%	86.4%
Trimethoprim/Sulfamethoxazole	98.7%	90.5%	91.3%	83.3%	90.6%	93.1%
Gentamycin	93.4%	82.4%	82.6%	88.1%	90.6%	88.4%
Nitrofurantoin	100%	97.3%	95.7%	100%%	100%	98.7%
Vancomycin	87.4%	87.8%	92.8%	85.7%	100%	90%
Rifampicin	98%	90.5%	87%	95.2%	94.3%	93.8%
Tigecycline*	100%	97.3%	100%	97.6%	100%	99.2%
Quinupristin/Dalfopristin*	100%	98.6%	95.7%	100%	100%	99.0%
Linezolid	100%	97.3%	95.7%	100%	100%	98.7%

* Breakpoints according to EUCAST version 5.0

Table 4 Percentage of *S. epidermidis* antimicrobial sensitivity

Antibiotic	2017 (n=51)	2018 (n=55)	2019 (n=70)	2020 (n=37)	2021 (n=42)	Total (n=255)
Cefoxitin	23.5%	41.8%	52.9%	16.2%	23.8%	34.5%
Oxacillin	23.5%	41.8%	52.9%	16.2%	23.8%	34.5%
Penicillin G	2.0%	7.3%	37.1%	5.4%	2.4%	13.3%
Tetracycline	52.9%	76.4%	91.4%	78.4%	90.5%	78.4%
Erythromycin	15.7%	40.0%	54.3%	48.6%	26.2%	38.0%
Clindamycin	11.8%	43.6%	50%	43.2%	28.6%	36.5%
Ciprofloxacin	33.3%	69.1%	61.4%	45.9%	28.6%	49.8%
Moxifloxacin	35.3%	69.1%	61.4%	45.9%	28.6%	50.2%
Levofloxacin	33.3%	69.1%	61.4%	48.6%	28.6%	50.2%
Trimethoprim/Sulfamethoxazole	31.4%	65.5%	68.6%	40.5%	38.1%	51.4%
Gentamycin	39.2%	80.0%	67.1%	70.3%	47.6%	61.6%
Nitrofurantoin	100%	98.2%	98.6%	100%	100%	99.2%
Vancomycin	86.3%	83.6%	98.6%	97.3%	97.6%	92.5%
Rifampicin	86.3%	90.9%	77.1%	83.8%	54.8%	79.2%
Tigecycline*	98.0%	98.2%	98.6%	100%	97.6%	98.4%
Quinupristin/Dalfopristin*	100%	98.2%	100%	100%	100%	99.6%
Linezolid	100%	98.2%	98.6%	100%	100%	99.2%

* Breakpoints according to EUCAST version 5.0

epidermidis isolates were widely resistant to more than one antimicrobial agent. We found that the sensitivity of *S. epidermidis* to Penicillin G was below 10%, except in 2019,which is similar to several studies conducted in Tianjin, China(Xu *et al.* 2020), Shanghai, China(Du *et al.* 2013), and Scotland (Zalewska *et al.* 2021) where they have found more than 90% of *S. epidermidis* are resistant to Penicillin G. In 2019, Penicillin G sensitivity was slightly higher than usual, maybe this finding due to fewer methicillin-resistant phenotype that could be isolated from clinical specimens that year.

From the result of *S. haemolyticus* antimicrobial susceptibility that has been shown before, we found

Table 5 Percentage of *S. haemolyticus* antimicrobial sensitivity

Antibiotic	2017 (n=45)	2018 (n=72)	2019 (n=80)	2020 (n=26)	2021 (n=64)	Total (n=287)
Cefoxitin	17.8%	12.5%	12.5%	3.8%	15.6%	13,2%
Oxacillin	17.8%	12.5%	12.5%	3.8%	15.6%	13,2%
Penicillin G	6.7%	4.2%	1.3%	3.8%	6.3%	4,2%
Tetracycline	64.4%	61.1%	65%	53.8%	60.9%	62,0%
Erythromycin	20.0%	19.4%	40%	23.1%	21.9%	26,1%
Clindamycin	15.6%	13.9%	25%	19.2%	17.2%	18,5%
Ciprofloxacin	42.2%	33.3%	52.5%	42.3%	26.6%	39,4%
Moxifloxacin	44.4%	37.5%	50%	42.3%	29.7%	40,8%
Levofloxacin	44.4%	36.1%	52.5%	38.5%	28.1%	40,4%
Trimethoprim/Sulfamethoxazole	66.7%	61.1%	66.3%	50%	73.4%	65,2%
Gentamycin	62.2%	63.9%	73.8%	61.5%	51.6%	63,4%
Nitrofurantoin	97.8%	94.4%	100%	100%	98.4%	97,9%
Vancomycin	91.1%	83.3%	93.8%	76.9%	96,9%	89,9%
Rifampicin	75.6%	69.4%	76.3%	69.2%	71.9%	72,8%
Tigecycline*	88.9%	87.5%	86.3%	80.8%	84.4%	86,1%
Quinupristin/Dalfopristin*	93.3%	83.3%	93.8%	88.5%	98.4%	91,6%
Linezolid	93.3%	90.3%	97.5%	96.2%	98.4%	95,1%

* Breakpoints according to EUCAST version 5.0

Table 6 *Staphylococcus* sp MDR and XDR percentage from 2017-2021

	S. aureus		*S. epidermidis*		*S. haemolyticus*	
	MDR	XDR	MDR	XDR	MDR	XDR
2017	19.2%(29/151)	0%	84.3%(43/51)	0%	86.7%(39/45)	4.4%(2/45)
2018	44.6%(33/74)	1.4%(1/74)	60%(33/55)	0%	94.4%(68/72)	4.2%(3/72)
2019	44.9%(31/69)	2.9%(2/69)	50%(35/70)	0%	88.8%(71/80)	1.3%(1/80)
2020	40.5%(17/42)	0%	86.5%(32/37)	0%	100%(26/26)	0%
2021	41.5%(22/53)	0%	78.6%(33/42)	0%	90.6%(58/64)	1.6%(1/64)

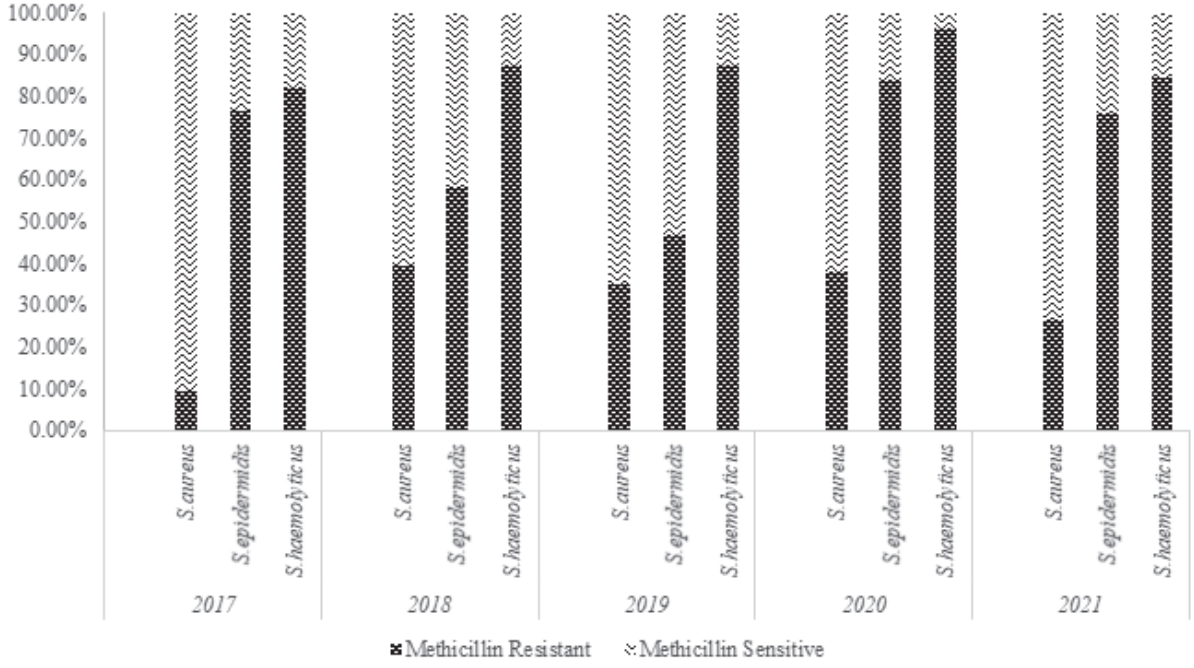

Fig 1 Percentage of Methicillin resistant *Staphylococcus* sp. in 2017 to 2021.

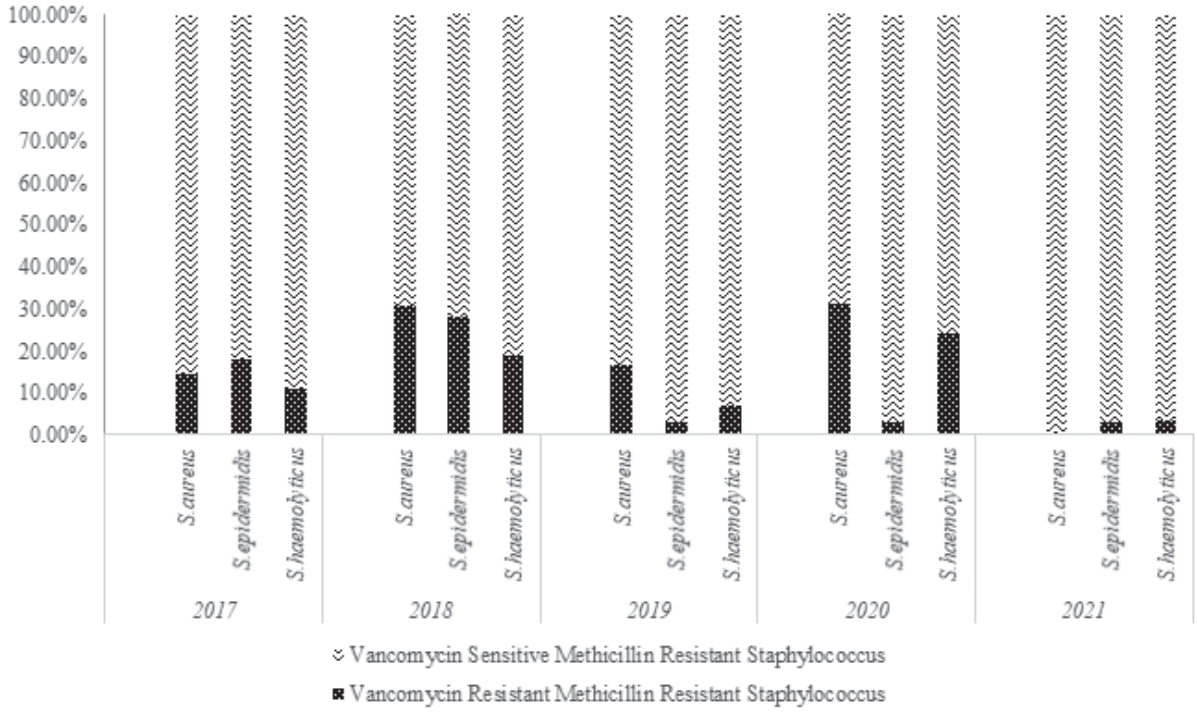

Fig 2 Percentage of vancomycin resistant *Staphylococcus* sp. among methicillin resistant Staphylococcus sp. in 2017 to 2021.

that the prevalence of methicillin-resistant *S. haemolyticus* was over 80% each year. This result is similar to a study conducted in Aceh (Suhartono *et al.* 2019) and Brazil (Barros *et al.* 2012), where 96.6 and 88% of *S. haemolyticus* from clinical specimens, respectively, were methicillin-resistant. Besides, we found that *S. haemolyticus* is widely resistant to antimicrobial agents such as erythromycin, clindamycin, ciprofloxacin, levofloxacin, moxifloxacin, oxacillin, and penicillin G. This result similar with a study conducted in Aceh, Indonesia (Suhartono *et al.* 2019) and review from several studies that conducted in Poland (Czekaj *et al.* 2015), they found that many *S. haemolyticus* are multidrug resistant.

Another important finding in this study is that we found vancomycin resistant in *S. haemolyticus* each year, and the highest prevalence was in 2020. This result differs from a study conducted in Brazil (Barros *et al.* 2012), where all *S. haemolyticus* isolates were susceptible to vancomycin. This finding needs further attention, since the drug of choice in methicillin-resistant Staphylococcal infections is vancomycin, and the drug of choice in vancomycin-resistant Staphylococcal infections is linezolid. (Choo and Chambers, 2016; Loomba *et al.* 2010) This study found isolates resistant to vancomycin and linezolid in the methicillin-resistant phenotype. Mainly we found them in *S. haemolyticus* and *S. aureus*, but we could not find them in *S. epidermidis*.

We found that *S. epidermidis* and *S. haemolyticus* were more resistant to antimicrobial agents than *S. aureus*. This was proven by the MDR percentage in *S. aureus* being lower than in *S. epidermidis* and *S. haemolyticus*, respectively. Interestingly, from our study, the prevalence of MDR *S. haemolyticus* was consistent above 85% each year, while *S. epidermidis was* above 50% and *S. aureus* was below 50% each year. Besides, we found that the XDR phenotype only can be found in *S. aureus* and *S. haemolyticus,* although the prevalence of XDR *S. haemolyticus* is higher. We discovered that only ten isolates have XDR phenotype where seven isolates of the XDR phenotype were *S. haemolyticus,* and three isolates were *S. aureus*. In this study, we could not find the PDR phenotype.

In conclusion, *S. haemolyticus* and *S. epidermidis* were important *coagulase-negative Staphylococcus* species that can't be neglected, although in earlier times, they were not considered a pathogen species, due to their high prevalence in clinical isolate. Besides, *S. haemolyticus* are resistant to many antimicrobial agents in a high percentage. Thus, we should worry about their potential ability to disseminate the plasmid to virulent species. (Kim and Jang 2017) Moreover, with the finding of *S. haemolyticus* resistant to vancomycin and linezolid, controlling antimicrobial

agent usage to prevent this resistance is imperative.

ACKNOWLEDGMENTS

This study has been presented in the International Seminar of the Indonesian Society for Microbiology (12th ISISM) 2022. We Thank dr. R. Fera Ibrahim, M.Sc., Ph.D., Sp.MK(K)., head of UKK LMK FKUI, for giving access to isolates data.

REFERENCES

Almanaa TN, Alyahya SA, Khaled JM, Shehu MR, Alharbi NS, Kadaikunnan S, Alobaidi AS, Khalid Alzahrani A. 2020. The extreme drug resistance (XDR) *Staphylococcus aureus* strains among patients: A retrospective study. Saudi J Biol Sci. 27:1985–1992. Doi: 10.1016/j.sjbs.2020.04.003.

Barros EM, Ceotto H, Bastos MCF, Dos Santos KRN, Giambiagi-deMarval M. 2012. *Staphylococcus haemolyticus* is an important hospital pathogen and carrier of methicillin resistance genes. J Clin Microbiol. 50:166–168. Doi: 10.1128/JCM.05563-11.

Cavanagh JP, Hjerde E, Holden MTG, Kahlke T, Klingenberg C, Flægstad T, Parkhill J, Bentley SD, Ericson Sollid JU. 2014. Whole-genome sequencing reveals clonal expansion of multiresistant *Staphylococcus haemolyticus* in European hospitals. J Antimicrob Chemother. 69:2920–2927. Doi: 10.1093/jac/dku271.

Cerca N, Oliveira R, Azeredo J. 2007. Susceptibility of *Staphylococcus epidermidis* planktonic cells and biofilms to the lytic action of staphylococcus bacteriophage K. Lett Appl Microbiol. 45, 313–317. Doi: 10.1111/j.1472-765X.2007.02190.x.

Choo EJ, Chambers HF, 2016. Treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. Infect Chemother. 48:267–273.Doi: 10.3947/ic.2016.48.4.267.

Clinical and Laboratory Standard Institute. 2021. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute.

Czekaj T, Ciszewski M, Szewczyk EM. 2015. *Staphylococcus haemolyticus* – An emerging threat in the twilight of the antibiotics age. Microbiol (United Kingdom). 161:2061–2068. Doi: 10.1099/mic.0.000178.

Daniel B, Saleem M, Naseer G, Fida A. 2014. Significance of *Staphylococcus haemolyticus* in hospital acquired infections. J Pioneer Med Sci. 4:119–125.

De Giusti M, Pacifico L, Tufi D, Panero A, Boccia A, Chiesa C, 1999. Phenotypic detection of nosocomial mecA-positive coagulase-negative staphylococci from neonates. J Antimicrob Chemother. 44:351–358. Doi: 10.1093/jac/44.3.351.

Do Carmo Ferreira N, Schuenck RP, Dos Santos KRN, De Freire Bastos M. do C, Giambiagi-deMarval M. 2011. Diversity of plasmids and transmission of high-level mupirocin mupA resistance gene in *Staphylococcus haemolyticus*. FEMS Immunol Med Microbiol. 61:147–152. Doi: 10.1111/j.1574-695X.2010.00756.x.

Du X, Zhu Y, Song Y, Li T, Luo T, Sun G, Yang C, Cao C, Lu Y, Li M. 2013. Molecular analysis of *Staphylococcus epidermidis* strains isolated from community and hospital environments in China. PLoS One 8. Doi: 10.1371/journal.pone.0062742.

Dzen SM, Santoso S, Roekistiningsih, Santosaningsih D. 2005. Perbedaan pola resistensi Staphylococcus koagulase negatif isolat darah terhadap antibiotika di RSU dr Saiful Anwar Malang tahun 2000-2001 dengan 2004-2005. J Kedokt Brawijaua. 21:127–32.

Graham JC, Murphy OM, Stewart D, Kearns AM, Galloway A, Freeman R. 2000. Comparison of PCR detection of mecA with methicillin and oxacillin disc susceptibility testing in coagulase-negative staphylococci. J Antimicrob Chemother. 45:111–113. Doi: 10.1093/jac/45.1.111.

Gunn BA, Davis CE., 1988. *Staphylococcus haemolyticus* urinary tract infection in a male patient. J Clin Microbiol.26:1055–1057.

Heilmann C, Ziebuhr W, Becker K. 2019. Are coagulase-negative staphylococci virulent? Clin Microbiol Infect. 25:1071–1080. Doi: 10.1016/j.cmi.2018.11.012.

Horan TC, Andrus M, Dudeck MA. 2008. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 36:309–332. Doi: 10.1016/j.ajic.2008.03.002.

Hovelius B, Colleen S, Mårdh PA., 1984.Urinary tract infections in men caused by *Staphylococcus saprophyticus*. Scand J Infect Dis. 16:37–41. Doi: 10.3109/00365548409068407.

John JFPK, O'Dell NM. 1978. Species identification of coagulase-negative staphylococci from urinary tract isolates. J Clin Microbiol. 8:435–437. Doi: 10.1128/jcm.8.4.435-437.1978.

Kaur DC, Chate SS. 2015. Study of antibiotic resistance pattern in methicillin resistant *staphylococcus aureus* with special reference to newer antibiotic. J Glob Infect Dis. 7:78–84. Doi: 10.4103/0974-777X.157245.

Kim HJ, Jang S. 2017. Draft genome sequence of multidrug-resistant *Staphylococcus haemolyticus* IPK_TSA25 harbouring a *Staphylococcus aureus* plasmid, pS0385-1. J Glob Antimicrob Resist. 11:8–9. Doi: 10.1016/j.jgar.2017.08.010.

Kim JS, Kim HS, Park JY, Koo HS, Choi CS, Song W, Cho HC, Lee KM. 2012. Contamination of X-ray cassettes

with methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus haemolyticus* in a radiology department. Ann Lab Med. 32:206–209. Doi: 10.3343/alm.2012.32.3.206.

Klingenberg C, Rønnestad A, Anderson AS, Abrahamsen TG, Zorman J, Villaruz A, Flægstad T, Otto M, Sollid EJ. 2007. Persistent strains of coagulase-negative staphylococci in a neonatal intensive care unit: Virulence factors and invasiveness. Clin Microbiol Infect. 13:1100–1111. Doi: 10.1111/j.1469-0691.2007.01818.x.

Loomba P, Taneja J, Mishra B. 2010. Methicillin and vancomycin resistant *S. aureus* in hospitalized patients. J Glob Infect Dis. 2:275. Doi: 10.4103/0974-777x.68535.

Lowy FD. 1998. *Staphylococcus aureus* infections. N Engl J Med. 339(8):520-32. Doi: 10.1056/NEJM199808203390806.

Lozano V, Fernandez G, Spencer PL, Taylor SL, Hatch R. 2015. *Staphylococcus epidermidis* in urine is not always benign: a case report of pyelonephritis in a child. J Am Board Fam Med. 28:151–153. Doi: 10.3122/jabfm.2015.01.140118.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 18:268–281. Doi: 10.1111/j.1469-0691.2011.03570.x.

Naimi HM, André C, Bes M, Tristan A, Gustave CA, Vandenesch F, Nazari QA, Laurent F, Dupieux C. 2021. Antibiotic resistance profile and molecular characterization of *Staphylococcus aureus* strains isolated in hospitals in Kabul, Afghanistan. Eur J Clin Microbiol Infect Dis. 40:1029–1038. Doi: 10.1007/s10096-020-04130-0.

Patel H, Shah A, Mistry M, Chanda S. 2011. In vitro antimicrobial susceptibility study in clinical isolates of Streptococci and Enterococci. African J Microbiol Res. 5:1374–1378. Doi: 10.5897/ajmr10.161.

Riedel S, Morse S, Mietzner T, Miller S. 2019. The Staphylococci. In Jawetz, Melnick & Adelberg's medical microbiology, 28th ed. McGraw Hill, New York, p.205-15.

Rupp ME, Soper DE, Archer GL. 1992.Colonization of the female genital tract with *Staphylococcus saprophyticus*. J Clin Microbiol.30:2975–2979.

Schuenck RP, Pereira EM, Iorio NLP, Dos Santos KRN. 2008. Multiplex PCR assay to identify methicillin-resistant *Staphylococcus haemolyticus*. FEMS Immunol Med Microbiol. 52:431–435. Doi: 10.1111/j.1574-695X.2008.00387.x.

Shrestha PD, Rai S, Gaihre S. 2018. Prevalence of hospital acquired infection and its preventive practice among health workers in a tertiary care hospital. J Nepal Health Res Counc. 16:452-456.

Suhartono S, Hayati Z, Mahmuda M. 2019. Distribution of *Staphylococcus haemolyticus* as the most dominant species among staphylococcal infections at the Zainoel Abidin Hospital in Aceh, Indonesia. Biodiversitas. 20:2076–2080. Doi: 10.13057/biodiv/d200739.

Takeuchi F, Watanabe S, Baba T, Yuzawa H, Ito T, Morimoto Y, Kuroda M, Cui L, Takahashi M, Ankai A, Baba SI, Fukui S, Lee JC, Hiramatsu K. 2005. Whole-genome sequencing of *Staphylococcus haemolyticus* uncovers the extreme plasticity of its genome and the evolution of human-colonizing staphylococcal species. J Bacteriol. 187:7292–7308. Doi: 10.1128/JB.187.21.7292-7308.2005.

Ullah A, Qasim M, Rahman H, Khan J, Haroon M, Muhammad Niaz, Khan A, Muhammad, Noor. 2016. High frequency of methicillin-resistant *Staphylococcus aureus* in Peshawar Region of Pakistan. Springerplus. 5:4–9. Doi: 10.1186/s40064-016-2277-3.

Wang X, Lin D, Huang Z, Zhang J, Xie W, Liu P, Jing H, Wang J. 2021. Clonality, virulence genes, and antibiotic resistance of *Staphylococcus aureus* isolated from blood in Shandong, China. BMC Microbiol. 21:1–11. Doi: 10.1186/s12866-021-02344-6.

Xu Z, Cave R, Chen L, Yangkyi T, Liu Y, Li K, Meng G, Niu K, Zhang W, Tang N, Shen J, Mkrtchyan HV. 2020. Antibiotic resistance and molecular characteristics of methicillin-resistant *Staphylococcus epidermidis* recovered from hospital personnel in China. J Glob Antimicrob Resist. 22:195–201. Doi: 10.1016/j.jgar.2020.02.013.

Zalewska A, Wilson J, Kennedy S, Lockhart M, Macleod M, Malcolm W. 2021. Epidemiological Analysis of Antimicrobial Resistance in *Staphylococcus epidermidis* in Scotland, 2014-2018. Microb Drug Resist. 27:485–491. Doi: 10.1089/mdr.2019.0502.