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SENSITIVITY TEST OF *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS EPIDERMIDIS* IN WOMEN TAKING ROUTINE BEAUTY CARE OF CLINICS TO VARIOUS ANTIBIOTICS

Yusianti Silviani*, Ardy Prian Nirwana
Sekolah Tinggi Ilmu Kesehatan Nasional, Jl. Raya Solo - Baki, Bangorwo, Kwarasan, Kec. Grogol, Kabupaten Sukoharjo, Jawa Tengah 57552
*yusianti.silviani@gmail.com

ABSTRACT

Acne is a skin problem that is often experienced by teenagers. Acne treatment is mostly taken in beauty clinics. One of the causes of acne is bacteria, namely *Staphylococcus aureus* and *Staphylococcus epidermidis*. The use of antibiotics is often given by beauty clinics to treat acne problems. The irrational use of antibiotics and in a long term as well as within improper dose can cause resistance to bacteria. The purpose of this study is to determine the sensitivity of *Staphylococcus aureus* and *Staphylococcus epidermidis* to various antibiotics. The research was conducted in Bacteriology Laboratory of STIKES Nasional from January – December 2021.The study was conducted descriptively from January to December 2021. The population taken by quota sampling in this study was women having treatment at beauty clinics. The sensibility test was carried out by using the Kirby Bower method. *S. aureus* and *S. epidermidis* were found in 8 samples from 20 samples examined. Sensibility test showed 25% of *S. aureus* was sensitive to clindamycin 75% sensitive to Penicilin, 75% sensitive to Tetraciklin, 50% sensitive to Eritromicin, 75% sensitive to Cefoxitin, 100% sensitive to Ciproflosaxin and Gentamicin. Whilst 100% of *S. epidermidis* was resistant to clindamycin and erythromycin, 100% sensitive to Penicilin, Tetraciklin, Cefoxitin, Ciproflosaxin, Gentamicin. The conclusion of this study is that *S. epidermidis* has been resistant to erythromycin and clindamycin, whilst the highest resistance of *S. aureus* is to clindamycin.

Keywords: antibiotics; bacterial resistance; staphylococcus aureus; staphylococcus epidermidis

INTRODUCTION

Resistance has become a worldwide problem. The high cases of resistance are caused by as many as 50% of drugs being prescribed, distributed and sold inappropriately to patients. The irrational use of drugs, both types of drugs, doses, and duration of use triggers an increase in cases of resistance to microbes (Rukmini et al., 2019). Skin is the largest organ in humans, anatomically the skin consists of 4 tissues, namely epithelial tissue, connective tissue, muscle tissue and nervous tissue. The skin is the first defense against infection (Kalangi, 2014). Acne is one of the most common skin infections in adolescents aged 16-19 years to adults aged 30 years. Acne can cause permanent scar tissue (Wardhani & Sulistyani, 2015). Acne can cause chronic inflammation accompanied by comedones, nodules, papules, cysts and scars (Saragih et al., 2016)

The prevalence of acne sufferers in Indonesia ranges from 80-85% in adolescents with a peak incidence aged 15-18 years, 12% in women aged > 25 years and 3% at the age of 35-44 years

(Madelina & Sulistiyaningsih, 2018). The prevalence in men is 95-100% higher than in women 83-85% (Wardhani & Sulistyani, 2015). Bacteria that cause acne include Staphylococcus aureus, Staphylococcus epidermidis. S. aureus is a gram (+) coccus bacteria that can cause skin infections in the form of pimples or abscesses (Apriani et al., 2014). S. epidermidis is a normal flora of the skin, but if there is a change in skin condition, S. epidermidis can become a pathogen by causing skin infections (Pratami et al., 2013).Pengobatan terhadap jerawat yang disebabkan oleh bakteri dengan menggunakan antibiotik (Meilina dan Hasanah, 2018). Some antibiotics used in the treatment of acne are clindamycin, tetracycline, neomycin, and chloramphenicol but the use of these antibiotics causes irritation to the skin (Nizar et al., 2019). In addition to causing irritation, irrational use of antibiotics, both types of drugs, dosage, and duration of use triggers increased cases of resistance to microbes that can lead to resistance. As many as 50% of drugs are prescribed, distributed and sold inappropriately to patients, increasing cases of resistance (Rukmini et al., 2019)

Sitohang et al., (2019) S. aureus was resistant to clindamycin 14.3% and tetracycline 0%. In line with this study, Madelina & Sulistiyaningsih (2018) stated that resistance to clindamycin was greater than tetracycline. Thus, S. aureus was resistant to clindamycin compared to tetracycline. In the study of Yunita Hapsari et al., (2019), it was stated that S. epidermidis was sensitive to the antibiotic erythromycin (11%), intermediate (3%), and resistant (74%), resistant to azithromycin (38.1%), erythromycin (33.3%).), and clindamycin (28.6%). The aims of this study were to 1) determine the presence of S. aureus and S. epidermidis in routine care women in beauty clinics. 2) Knowing the description of the sensitivity of S. aureus and S. epidermidis to various antibiotics.

METHOD

The design of this research is descriptive observational with a cross sectional approach. This research was conducted at the Bacteriology laboratory of the National Health Sciences College in January – December 2021. The tools used were object glass, petri dishes, ohse, sterile cotton swabs, tube racks, test tubes, dropper pipettes, tweezers, drupple plate, spirit burner, painting racks, microscopes, calipers, incubators, electric stoves, scales, ovens, autoclaves. The materials used in this study were disc antibiotics clindamycin, tetracycline, erythromycin, penicillin, cefoxitin, ciproflosaxin, gentamicin. Blood Agar Plate (BAP) media, Muller Hinton Agar, Gram stain, sterile distilled water, H2O2, Mc Farland's solution number 0.5, immersion oil and Mannitol Salt Agar (MSA), plasma citrate. The population of this study were women who did routine maintenance at a beauty clinic. The sampling technique used is Quota sampling with the minimum criteria of doing maintenance once a month and having done maintenance for at least 3 months.

RESULTS

The research was conducted at the Bacteriology Laboratory of the National College of Health Sciences. A total of 20 respondents who met the criteria were subjected to facial swabs and identification of bacteria. The results of the identification of bacteria obtained results as shown in Table 1

Table 1.
Bacterial Identification Results in Face Swab Samples

Sampel	Cat gram	Test katalase	NA miring	MSA	Test koagulase	Spesies bakteri
A	Coccus, bergerombol , gram (+)	+	Kuning	+	+	*S.aureus*
			Putih susu	-	-	*S. epidermidis*
B	Coccus, bergerombol , gram (+)	+	Kuning	+	+	*S.aureus*
			Putih susu	-	-	*S. epidermidis*
C	Coccus, bergerombol , gram (+)	+	Kuning	+	+	*S.aureus*
			Putih susu	-	-	*S. epidermidis*
D	Coccus, bergerombol , gram (+)	+	Kuning	+	+	*S.aureus*
			Putih susu	-	-	*S. epidermidis*
E	Coccus, bergerombol, gram (+)	+	kuning	-	-	*S. saprophyticus*
	Batang,tersebar, gram (-)	-	Identifikasi tidak dilakukan			-
F	Coccus, bergerombol , gram (+)	+	kuning	-	-	*S. saprophyticus*
G	Batang, tersebar, gram (-)	-	Identifikasi tidak dilakukan			-
H	Coccus, berderet, gram (+)	-	Identifikasi tidak dilakukan			-
	Batang, tersebar, gram (-)	-	Identifikasi tidak dilakukan			-
I	Coccus, berderet, gram (+)	-	Identifikasi tidak dilakukan			-
	Batang, tersebar, gram (-)	-	Identifikasi tidak dilakukan			-
J	Coccus, berderet, gram (+)	-	Identifikasi tidak dilakukan			-
	Batang, tersebar, gram (-)	-	Identifikasi tidak dilakukan			-
K	Batang, tersebar, gram (-)	-	Identifikasi tidak dilakukan			-
L	Coccus, berderet, gram (+)	+	Identifikasi tidak dilakukan			-
M	Batang, tersebar, gram (-)	-	Identifikasi tidak dilakukan			-
N	Coccus, bergerombol, gram (+)	+	kuning	-	-	*S. saprophyticus*
O	Batang,tersebar, gram (-)	-	Identifikasi tidak dilakukan			-
P	Coccus, bergerombol , gram (+)	+	Kuning	+	+	*S.aureus*
			Putih susu	-	-	*S. epidermidis*
Q	Coccus, bergerombol , gram (+)	+	Kuning	+	+	*S.aureus*
			Putih susu	-	-	*S. epidermidis*
R	Batang, tersebar, gram (-)	-	Identifikasi tidak dilakukan			-
S	Coccus, bergerombol , gram (+)	+	Kuning	+	+	*S.aureus*
			Putih susu	-	-	*S. epidermidis*
T	Coccus, bergerombol , gram (+)	+	Kuning	+	+	*S.aureus*
			Putih susu	-	-	*S. epidermidis*

Based on Table 1, it is known that S. aureus and S. epidermidis were found in samples A, B, C, D, P, Q, S, T while in samples E, F, N found S. saprophyticus, gram (-) stem and gram (+)

coccus in a row. In this study, the sensibility test of the Kirby Bower method was used with 7 antibiotics. The range of antibiotic readings is shown in Table 2.

Table 2.
Standard Interpretation of Inhibitory Zone Diameter Results (CLSI, 2021)

Disk antibiotik	Zona Interpretasi Hasil		
	S	I	R
Clindamycin 2μg	$\geq$ 21	15-20	$\leq$ 14
Penicilin 10 μg	$\geq$ 29		$\leq$ 28
Tetraciklin 30μg	$\geq$ 19	15-18	$\leq$ 14
Eritromicin 15μg	$\geq$ 23	14-22	$\leq$ 13
Cefoxitin 30μ	$\geq$ 25		$\leq$ 21
Ciproflosaxin 5μg	$\geq$ 21	16-20	$\leq$ 15
Gentamicin 10μg	$\geq$ 15	13-14	$\leq$ 12

Description : S: Sensitive, I: Intermediate, R: Resistant

S. aureus that was found was then tested for sensibility with 2 repetitions using 7 antibiotics namely Clindamycin, Tetraciclin, erythromycin, penicillin, cefoxitin, ciproflosaxin, gentamicin. S. aureus sensibility results are shown in Table 3.

Table 3.
Average Inhibitory Zones of Various Antibiotics against S. aureus

Kode sampel	Hasil (dalam mm)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
A	6	12.5	27.3	24.8	27	41	31.3
B	34	34.7	28.4	30.6	28	29.7	27.2
C	6	26.1	27.4	6	29.5	33	29.9
D	6	6	13.2	11.3	12.7	32.1	22
P	6	13.1	29.1	24.5	27	42.7	31.3
Q	35.3	34	28.4	29.3	29	30.4	29.8
S	6	26.1	26.9	6	31.8	33.9	30.2
T	6	6	13	10.3	10.5	30.7	20

Description: 1: Clindamycin 2μg, 2: Penicillin 10 g, 3: Tetracycline 30μg, 4: Erythromycin 15μg, 5: Cefoxitin 30μg, 6: Ciproflosaxin 5μg, 7: Gentamicin 10μg

Table 4.
Average Inhibitory Zones of Various Antibiotics against S. epidermidis

Kode sampel	Hasil (dalam mm)						
	1	2	3	4	5	6	7
A	6	33.9	20.7	6	32.6	31.3	31.8
B	6	29.5	27.9	6	31.7	35	28.9
C	6	28.3	28	6	32.8	33.5	30.7
D	6	28.4	27.2	6	33.8	34.9	30.4
P	6	32.3	19	6	31.3	31.3	31.4
Q	6	32.5	30.7	6	32.5	34.9	31.1
S	6	30.2	29.6	6	32.2	33.8	32
T	6	28.7	27.1	6	32.3	33.8	30.5

Description: 1: Clindamycin 2μg, 2: Penicillin 10 g, 3: Tetracycline 30μg, 4: Erythromycin 15μg, 5: Cefoxitin 30μg, 6: Ciproflosaxin 5μg, 7: Gentamicin 10μg

Based on the results in Table 3 and the standard interpretation in Table 2 it was found that S. aureus was 25% sensitive to Clindamycin, 75% sensitive to Penicillin, 75% sensitive to Tetracycline, 50% sensitive to Erythromycin, 75% sensitive to Cefoxitin, 100% sensitive to Ciproflosaxin. and Gentamicin. S epidermidis found was then tested for sensibility with 2 repetitions using 7 antibiotics namely Clindamycin, Tetracilin, erythromycin, penicillin, cefoxitin, ciproflosaxin, gentamicin. S. epidermidis sensibility results are shown in Table 4.

DISCUSSION

Based on the results in Table 4 and the standard interpretation in Table 2, it was found that S. epidermidis was 100% resistant to Clindamycin and Erythromycin, 100% sensitive to Penicillin, Tetracycline, Cefoxitin, Ciproflosaxin, and Gentamicin. S. aureus and S. epidermidis were isolated from proband skin swabs that were routinely treated at a beauty clinic. Of the 20 probands who were willing to conduct the study, 8 samples were found to have S. aureus and S. epidermidis. Facial skin swabs were carried out using 0.9% NaCl which was then fertilized using Blood Peptone Broth. Furthermore, from the results of the fertilization, gram staining was carried out and gram (+) coccus bacteria were found in clusters. Staphylococcus sp is a gram-positive bacterium that has peptidoglycan, polysaccharides (teichoic acid), and a small amount of lipid in its cell wall (Jawetz et al., 2014)

The results of observations of Staphylococcus sp colonies on Blood Agar Plate (BAP) media found golden yellow colonies, namely S. aureus (Guo et al., 2020) and smooth, round, prominent, shiny colonies, soft consistency, white porcelain. These bacteria produce the enzyme catalase which breaks down H202 into H20 and O2 (Faradiba, 2014. On MSA media S. aureus showed golden yellow colonies and the color of the media changed from pink to yellow. S. aureus was able to ferment mannitol so that it was able to change the color of the media from red to yellow, the same as the color of its colonies (Phasor et al., 2015), while S. epidermidis showed negative results because S. epidermidis bacteria could not ferment mannitol and were negative coagulase.

The coagulase test is used to differentiate S. aureus from other Staphylococcus species (Sanu et al., 2015). Coagulase is an enzyme-like protein that can coagulate plasma citrate. Bacteria that have coagulase can potentially become pathogens (Hendawan, 2018). According to Faradiba (2017) Staphylotrombin is formed from the binding of extracellular protein (coagulase) with host prothrombin. S. aureus test results showed positive results while S. epidermidis showed negative results.

Based on Table 3, it was found that S. aureus was 25% sensitive to Clindamycin, 75% sensitive to Penicillin, 75% sensitive to Tetracycline, 50% sensitive to Erythromycin, 75% sensitive to Cefoxitin, 100% sensitive to Ciproflosaxin and Gentamicin. Based on observations, it was found that the cream used came from different clinics so that the composition contained in it was different, in sample A and sample C from the same beauty clinic, while samples B, D, P, Q, S, T came from different beauty clinics. . If the cream contains antibacterial agents, it is possible for Staphylococcus sp bacteria to develop resistance. The cause of the occurrence of resistance in microorganisms to antibiotics due to frequent use of antibiotics, with excessive doses, and used for a long time. This condition triggers mutations so that bacterial genes become resistant, besides that resistance genes are also obtained from plasmid exchange (gene transfer) (Pratiwi, 2017).

Plasmids are extrachromosomal DNA that replicates in the host cell and then is transferred to other bacterial cells. The transfer of genetic information between bacteria can occur through the process of transduction, namely the transfer of genetic information between viral DNA and bacterial DNA. The process of transferring viral DNA to bacterial DNA is carried out by transposons with a mechanism known as transposition. Transposons leave the plasmid and join the new host DNA or into the plasmid after conjugation. The genetic information contained in the transposon is still alive even though the plasmid used for transfer is dead (Pratiwi, 2017). S. epidermidis resistance occurs because S. epidermidis forms a biofilm (Cabrera-Contreras et al., 2013).

There are various mechanisms of bacterial resistance to antibiotics. This clindamycin resistance occurs because bacteria have RNA genes in plasmids that can cause ribosome methylation, thereby reducing the strength between the drug and the ribosome and resulting in decreased antibiotic activity. The mechanism of action of the antibiotic clindamycin binds to the 50S ribosome and suppresses protein synthesis of the main pathogenic bacteria causing suppurative infections, which can produce -lactamases, so that they can easily become resistant to -lactam antibiotics. Clindamycin is used topically in gel preparations, solutions, especially for the treatment of acne. The addition of benzoyl peroxide to clindamicin gel can inhibit antibiotic resistance to clindamycin (Putri et al., 2021).

Penicillin resistance occurs due to the activity of the -Lactamase enzyme in destroying the beta lactam ring on antibiotics, antibiotics are unable to penetrate the outer membrane of Gram negative bacteria to reach Penicillin Binding Protein (PBP), drug efflux across the outer membrane of Gram negative bacteria, and low affinity between antibiotics. and target PBPs (Pratiwi, 2017), while bacterial resistance to erythromycin occurs due to a decrease in bacterial cell walls, changes in drug receptors on ribosomes, and drug hydrolysis by esterases produced by certain bacteria (Khasanah et al., 2019). S aureus was found to be resistant to cefoxitin by producing Amp C Beta Lactamase which lyses the beta lactam ring (Dharmawan & Layanto, 2019).

According to research by Agustina et al., (2019) S aureus is resistant to ampicillin sulbactam, 67% sensitive to ciprofloxacin, 50% sensitive to cotrimoxazole, 83% sensitive to levofloxacin, 83% sensitive to gentamicin and 50 sensitive to chloramphenicol, this happens because the presence of the ErmC resistance gene and has a beta-lactamase enzyme. According to Saba et al.(2017) as many as 17% of S aureus isolated from hospitals in Ghana found MRSA, namely resistance to oxacilin, ampicillin, ciprofloxacin, etracyline, streptomycin, erythromycin and sulfamethoxazole 17, 13, 9, 28,89, 13 and 11% (Saba et al., 2017). Research by Deyno, et all (2017) in Ethiopian Hospital found that S. aureus was resistant to penicillin 75%, amoxicillin 77%, ampicillin 76%, carhatolin 34%, cephalotin 30%, cefriaxone 34%, cefoxitine 27%, Cipro 19% , tetraciline 62%, erythromycin 41%, chloramphenicol 37%, gentamicin 26% (Deyno et al., 2017).

CONCLUSION

Based on the research conducted, it was found that 40% of the samples contained S. aureus and 40% of the samples contained S. epidermidis. 25% of S. aureus 25% sensitive to clindamycin, 75% sensitive to penicillin, 75% sensitive to tetracycline, 50% sensitive to erythromycin, 75% sensitive to cefoxitin, 100% sensitive to ciproflosaxin and gentamicin. S. epidermidis 100% resistant to clindamycin and erythromycin, 100% sensitive to Penicillin, Tetracycline Cefoxitin, Ciproflosaxin, Gentamicin.

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