



Original Article

Staphylococcus Aureus Antibiotics Resistance's pattern in Osteomyelitis Cases at Hospital in Jambi City

Lipinwati^{1*}, Budi Justitia¹, Hanina¹

¹ Faculty of Medicine and Health Sciences, Universitas Jambi, Indonesia

E-mail Corresponding: lipinwati_fkik@unja.ac.id

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ABSTRACT

Background: Osteomyelitis is still a health problem in Indonesia, due to poor hygiene, late diagnosis, patients with open fractures who seek treatment late, treats osteomyelitis is long time and quite expensive. Osteomyelitis is an inflammation of the bone that is often caused by a bacterial infection. Osteomyelitis often occurs in open fractures, infections of the feet of diabetics, or surgical treatment of closed wounds. The bacteria that most often causes osteomyelitis is the genus Staphylococcus, which is found in 75% of cases of osteomyelitis, especially Staphylococcus aureus. We wanted to know how the prevalence of osteomyelitis with S. aureus infection was and the pattern of resistance of these bacteria to antibiotics.

Method: This research was a descriptive research with laboratory experiments. The research samples were patients with osteomyelitis who had signed informed consent form, samples were taken in the form of wound swabs/bone scrapings and blood using the total sampling method since August 2022 until December 2022. Wound swabs/bone scrapings were performed bacterial culture on Mannitol Salt Agar (MSA). MSA was incubated at 37°C for 18 - 24 hours. The cultures were done gram staining, catalase test and coagulase test. Cultures that grown in MSA with coccus positive gram, catalase test positive and coagulase test positive then tested resistance to antibiotics (Cefoxitin/FOX 30 mg, Ceftriaxone/CRO 30 mg, Gentamicin/CN 10 mg, Chloramphenicol/C 30 mg, and Ciprofloxacin/CIP 5 mg) using the disc diffusion method on Mueller Hinton Agar (MHA) with fresh culture bacteria 0.5 mc Farland. There are 18 samples swabs from wound, and 10 samples are identified as Staphylococcus sp.

Result: There are 9 samples that gram staining with coccus positive gram, Catalase positive test, and coagulase positive test. From 9 samples which done antibiotic sensitivity test, there are 2 samples, 3 samples, 1 sample, 3 samples, and 5 samples resistance to cefoxitin (Methicillin resistance Staphylococcus aureus/MRSA), Ceftriaxon, Chloramphenicol, Gentamisin and Ciprofloxacin respectively.

Conclusion: There are Fifty percents of osteomyelitis samples are staphylococcal infection with 22.22 percents infection of MRSA.

INTRODUCTION

Osteomyelitis is still a health problem in Indonesia, due to poor hygiene, late diagnosis, patients with open fractures who seek treatment late, osteomyelitis therapy takes quite a long time and costs are quite high. Osteomyelitis is an

inflammation of the bone which is often caused by a bacterial infection. Based on the onset of events, osteomyelitis is divided into two, namely acute osteomyelitis and chronic osteomyelitis [1,2]. In developing countries, the incidence of chronic osteomyelitis is higher than in developed

countries. Osteomyelitis often occurs with open fractures, diabetic foot infections, or surgical treatment of closed wounds and the significant infection within three month after open fractures [3].

The causes of Osteomyelitis consist of bacterial, fungal, or parasitic infections, and there are also unknown causes. The bacteria that most often causes osteomyelitis is the genus *Staphylococcus* found in cases of osteomyelitis as much as 75%, especially *Staphylococcus aureus* [4]. *Staphylococcus aureus* is most often found when isolating osteomyelitis samples related to bone implantation, and as many as 50% are caused by methicillin-resistant *S. aureus* (MRSA) [5].

The diagnosis is made by bone cultures, histology, and radiographs [6]. Finding the cause of the infection can be done by conducting a bacterial culture from a specimen at the site of the wound/bone scraping. If the causative bacteria are known, then treatment can be carried out properly so that healing can be faster. *Staphylococcus aureus* bacteria is a normal flora on the skin [7], and can act as an opportunistic pathogen if the bacteria move locations, for example when there is a wound on the skin. *Staphylococcus aureus* is a gram-positive cocci-shaped bacteria that can survive in environments with high salt concentrations such as mannitol salt agar.

Osteomyelitis is a severe infection of bone tissue, and bacterial culture examination can help confirm the diagnosis. In Jambi, examination of wound swab cultures in patients with osteomyelitis has rarely carried out, even though bacterial cultures can determine the type of bacteria that causes osteomyelitis and their resistance to antibiotics. Researchers want to see how the pattern of resistance of *S. aureus* to antibiotics?

MATERIALS AND METHODS

The research sample was a wound swabs/bone scrapings of patients with osteomyelitis at Bratanata Hospital, Arafah Hospital and MattaHer Hospital. Osteomyelitis patients who met the inclusion: the patients with osteomyelitis up 18 years old, and exclusion criteria: the patient who didn't signed the informed consent. The samples were taken 2 swab samples of the wound/bone scraping using a sterile cotton swab and the method of collection was carried out in a sterile manner to prevent contamination by other types of bacteria. The sample was brought to the FKIK UNJA Biomedical Laboratory.

The wound swab obtained was immediately cultured on mannitol salt agar (MSA) petri dish which incubated at 37 °C for 18-24 hours. Bacterial colonies that grew were identified by bacteria using the Catalase test, Coagulase test, and gram staining [8]. Antibiotic sensitivity test based on the disc diffusion method by the Kirby-Bauer method was carried out by suspending the bacterial culture with OD 0.5 Mc-Farland equivalent to 1.5×10^8 CFU/ml and spreading it on a Mueller-Hinton agar (MHA) petri dish and leaving it for 0.5 hours [9]. Each bacteria is done in duplo. The bacterial culture on the MHA petri dish was given a disc of antibiotics cefoxitin, gentamicin, ceftriaxone, chloramphenicol and ciprofloxacin. The petri dishes were incubated aerobically at 37 °C for 24 h for bacterial growth. The zone of inhibition observed was measured and evaluated. The zone of inhibition was measured in mm, and average calculation of each bacteria was performed.

RESULT AND DISCUSSION

The number of Osteomyelitis Cases in Jambi City in August - December 2022 are 18 peoples (Table 1). Based on age, the distribution of the incidence of osteomyelitis is relatively even in all age groups [10].

From gender, Most of the samples were male (66.67%) with a history of trauma with located in the lower extremities (94.45%). it's in accordance with research conducted by Kremers, et al (2015) where the annual incidence was higher for men than for women [11]. All the samples is a chronic osteomyelitis. Indonesia is a developing country and the incidence of chronic

osteomyelitis is high [3,12]. Chronic osteomyelitis can also due to poor hygiene, late diagnosis, patients with open fractures who seek treatment late. There are three samples osteomyelitis with Diabetic foot (16.67%). Mostly Diabetic foot infection caused by gram gram positive bacteria such as staphylococcus aureus and osteomyelitis is common in diabetic foot ulcer [13].

Table 1. Patient Characteristics

Characteristics	Total	Percentage
Gender		
Male	12	66.7
Female	6	33.3
Age (year)		
17-25	2	11.1
26-35	5	27.78
36-45	4	22.22
46-55	1	5.55
56-65	3	16.67
>65	3	16.67
History of Trauma		
Yes	13	72.22
No	5	27.78
Osteomyelitis Location		
Upper Extremity	1	5.55
Lower Extremity	17	94.45
Growth ini MSA		
Growing	10	55.55
Not Growing	8	44.45
Diabetes Melitus		
Yes	3	16.67
No	15	83.3

Microbial identification test was carried out with MSA media, gram staining, coagulase test and catalase test. A total of 10 samples grew on MSA petri dish, and made the colour of phenol red turn yellow (figure 1A). eight samples did not grow on Mannitol Salt Agar media, Mannitol Salt Agar (MSA) is a selective and differential medium for the isolation and identification of *Staphylococcus aureus*. These 10 samples were *Staphylococcus* sp., then confirmed by the results of gram staining where the ten

samples showed gram positive coccus results (figure 1B). The catalase test for the ten samples were positive, while the coagulase test results showed nine positive samples and one negative sample (figure 2). Nine samples with positive coagulase and catalase test results were *Staphylococcus aureus*, while one sample was *Staphylococcus epidermidis*. The results of this test indicate that the prevalence of osteomyelitis caused by *S.aureus* in Jambi City is 50 percents, it's in

accordance with research conducted by Arciola, et al (2005) where the most common bacteria that cause osteomyelitis

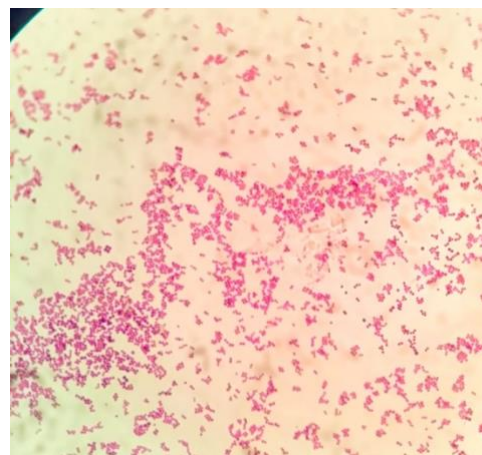
are *Staphylococcus* genus (75.5%) with the most species being *S.aureus* (44%) [4,11]..

Table 2. *Staphylococcus aureus* Sensitivity Test against various antibiotics*

Antibiotic	Concentration	Diameter Zone		
		S	I	R
Cefoxitin (FOX)	30 mg	22	-	21
Ceftriaxon (CRO)	30 mg	22	-	21
Chloramphenicol (C)	30 mg	18	13-17	12
Gentamicin (CN)	10 mg	15	14-13	12
Ciprofloxacin (CIP)	5 mg	21	16-20	15



(a.)



(b.)

Figure 1. (a.) The samples that grew on MSA, the MSA media turned yellow. (b.) Gram stain with a positive gram coccus result



(a.)



(b.)

Figure 2. (a.) Positive Catalase test. (b.) Positive Coagulase test.

The antibiotic's sensitivity test is done with disc diffusion method by Kirby-Bauer in Mueller Hinton Agar petri dish.

Staphylococcus aureus samples tested with disc antibiotics for coccus positive gram are Cefoxitin, Ceftriaxone, Chloramphenicol,

Gentamicin, Ciprofloxacin with CLSI 2020 (Table 2). The average diameter zone inhibition were shown in table 3, and the

percentage of antibiotics sensitivity test were shown in table 4.

Table 3. Antibiotic's Sensitivity Test with many Antibiotics with Disc Diffusion Method

Antibiotics	Sample 1	Sample 2	Sample 6	Sample 7	Sample 8	Sample 9	Sample 13	Sample 17	Sample 18
Cefoxitin (FOX)	31.89 (S)	33.26 (S)	25.53 (S)	- (R)	29.24 (S)	18.85 (R)	27.43 (S)	30.71 (S)	30.16 (S)
Ceftriaxon (CRO)	31.78 (S)	32.83 (S)	21.56 (R)	- (R)	24.07 (S)	- (R)	22.30 (S)	32.93 (S)	31.82 (S)
Chloramphenicol (C)	28.55 (S)	29.11 (S)	12.76 (R)	27.53 (S)	29.74 (S)	30.86 (S)	26.55 (S)	28.26 (S)	23.73 (S)
Gentamisin (CN)	25.75 (S)	25.45 (S)	12.99 (R)	- (R)	29.21 (S)	- (R)	24.54 (S)	24.29 (S)	22.19 (S)
Ciprofloxacin (CIP)	29.89 (S)	29.46 (S)	9.11 (R)	11.94 (R)	12.79 (R)	- (R)	27.35 (S)	0 (R)	27.12 (S)



Figure 3. Antibiotic's Sensivity Test with Sensitive results (duplo).

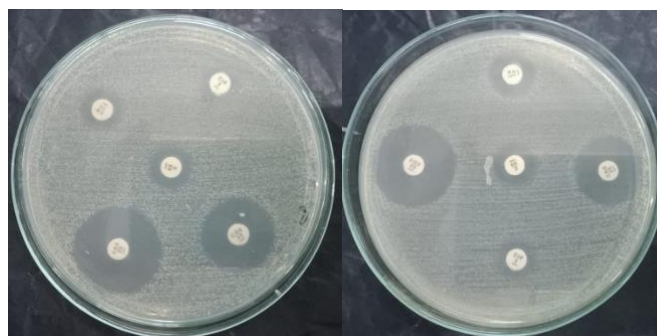


Figure 4. Antibiotic's Sensivity Test with Resistance results (duplo).

There are 4 samples (44.44%) sensitive to all antibiotics (figure 3). Our research found that there are 2 samples are resistant to cefoxitin, ceftriaxone, gentamicin and ciprofloxacin antibiotics (figure 4) but sensitive with chloramphenicol. This sampel which resistant to

cefoxitin due to MRSA infection (22.22%). *Staphylococcus aureus* is most often found when isolating osteomyelitis samples related to bone implantation, and as many as 50% are caused by methicillin-resistant *S. aureus* (MRSA) [5]. MRSA is frequently multidrug resistant [14].

Table 4. Sample Sensitivity Test Results Against Various Antibiotics

Antibiotic	Concentration	Total	
		S (%)	R(%)
Cefoxitin (FOX)	30 mg	7 (77.78)	2 (22.22)
Ceftriaxon (CRO)	30 mg	6 (66.67)	3 (33.33)
Chloramphenicol (C)	30 mg	8 (88.89)	1 (11,11)
Gentamicin (CN)	10 mg	6 (66.67)	3 (33.33)
Ciprofloxacin (CIP)	5 mg	4 (44.44)	5 (55.56)

MRSA samples were sensitive to chloramphenicol antibiotic. Chloramphenicol is a broad-spectrum bacteriostatic antibiotic drug that stops bacterial growth by inhibiting protein-chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosome. Fayyaz et al presents that MRSA clinical isolates have shown susceptibility to chloramphenicol

with minimum inhibitory concentrations (MICs) of $\leq 8 \mu\text{g/mL}$ [15].

CONCLUSION

Osteomyelitis infection caused by *Staphylococcus aureus* (SA) is 50 %, and there are 22.22% bacteria that are resistant to cefoxitin antibiotic (Methicillin Resistant *Staphylococcus aureus*/MRSA).

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