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Effect of Antibiotics on Fosfolipase Production of Staphylococcus aureus Isolated from Preputium of Aceh Cattle

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Abstract

This study aimed to study the effect of antibiotic on phospholipase production of *Staphylococcus aureus* isolated from preputium of Aceh cattle. The parameters measured in this study were PzI of *Staphylococcus aureus* without any treatment, and PzI of *S. aureus* after being given antibiotics. The experiment was carried out by modifying the Samaranayake method on egg yolk agar media, incubated at 37 ° C for 48 hours, and the precipitation lines formed was measured. The antibiotics used were tetracycline, oxytetracycline and fosfomycin with concentrations of 10%, 20% and 30% (mg / mL, respectively). The results showed that phospholipase production of *S. aureus* isolated from preputium of Aceh cattle was suppressed, antibiotics were able to inhibit phospholipase production. However, the enzyme was still produced in positive category, with PzI = 0.287, the highest result was found in 30% tetracycline activity, which is equal to PzI = 0.341 and the lowest was in 10% fosfomycin which is equal to PzI = 0.332

Keywords: phospholipase, Staphylococcus aureus, antibiotics

Background

Pathogenesis and virulence of bacteria are determined by substances produced by the bacteria, one of which is extracellular enzyme known as exoprotein. Some pathogen bacteria are able to produce virulence factor either intracellular or extracellular. These factors play roles in bacterial growth and colonization in tissues and organs. These factors also explain why certain bacteria may cause infection in the entire organ of animals. These virulence factors will work alone or together synergistically which cause infection (Salasia *et al.*, 2005).

Polymorphonuclear leukocytes (PMN) are important factors in immune system that defend host from bacterial infection. Neutrophil as one of PMN uses biocidal mechanism in killing bacteria, including phagocytosis, reactive oxygen species, antimicrobial enzyme and peptide production, also formation of neutrophil extracellular traps (NETs) (Amulic *et al*., 2012).

Pathogenic bacteria have multi tools in protecting themselves from PMN phagocytosis. *Staphylococcus aureus*, as the main opportunistic bacteria, protect itself by hiding under fibrin pseudo capsule, destroy PMN by producing certain enzymes and toxins (proteinase, DNase, lipase/ phospholipase, *Leukocidin Panton-Valentine*), and decrease phagocytosis opsonin-dependent by protein A (Watkins *et al*., 2012).

Staphylococcus aureus (*S. aureus*) are capable in producing phospholipase, especially coagulase-positive (Warsa, 1994). Phospholipase is an enzyme that hydrolyzes phospholipids and other lipophilic substances. This enzyme helps the bacteria to survive and colonize in blood (Gordon dan Lowy, 2008), or in sebaceous glands, permeate through barrier of cutaneous and sub cutaneous tissue, and cause infection (Joklik *et al.*, 1992). Phospholipid will facilitate adhesion and penetration to host cells, the enzyme will breakdown cell membrane phospholipid, resulting in cell lysis and tissue damage (Lahkar *et al.*, 2017).

Infection caused by *S. aureus* is difficult to be treated, this bacterium is

resistant to beta-lactam antibiotics, and now there is an isolate of *S. aureus* that is resistant to methicillin, known as *Methicillin Resistant Staphylococcus aureus* (MRSA). Empirical studies showed that MRSA is not only resistant to methicillin, but also resistant to other antibiotics and called multi-resistance (Lowy, 2003). There is no effective therapy for MRSA infection, some antibiotics used as drugs of choice for MRSA infection have a delay bactericide effect, thus fail for therapy. Therefore, it is necessary to study the effect of antibiotics on virulence factor of *S. aureus* before treatment, and also to prevent more bacteria resistance on antibiotics (Yuwono, 2010).

Materials and Methods

This research was conducted in This experiment used *S. aureus* bacteria isolated from preputium of Aceh cattle. Procedure for bacteria isolation and identification was according to Bergey's Manual Determination (1994). The bacteria were cultured on nutrient broth and incubated at 37 ^{0}C for 24 hours. Then the bacteria were isolated to Mueller Hinton agar which was based with egg yolk, and incubated at 37 ^{0}C for 24 hours. The bacterial growth was observed and the precipitation line formed was measured.

Phospholipase Analysis

Phospholipase activity of *S. aureus* was detected according to Samaranayake (2005). A volume of 5 µL of *S. aureus* (10^8 Staphylococcus cell/ml) was inoculated in Mueller Hinton agar based with egg yolk, dried, and incubated at 37 ^{0}C for 24 hours. Phospholipase production was determined by precipitation line in the colony, and *S. aureus* ATCC was used as positive control. Phospholipase index (Pz) was ratio of colony diameter to total colony diameter plus precipitation area. Value indicator for Pz=1 means no phospholipase activity; Pz<1 indicated phospholipase production. The lower Pz value, the higher phospholipase production.

Data Analysis

The data was analyzed using analysis of variant (ANOVA).

Results and Discussion

This experiment was conducted to investigate the effect of antibiotics on phospholipase production of *S. aureus* isolated from preputium of Aceh cattle. Phospholipase is one of virulent factors that enables *S. aureus* to penetrate and colonize in tissues and organs of infected host. The results showed that *S. aureus* had the ability to produce phospholipase, as presented in Figure 1. The calculation of precipitation zone value was PzI = 0.287, which means that these bacteria was capable in producing phospholipase in category of strong positive. This is in accordance with phospholipase characteristics reported by Antonella (2013) which stated that if PzI < 0.5 was strong positive, PzI = 0.5 – 0.74 was moderate positive, PzI = 0.65 – 0.99 was weak, and PzI = 1 was negative.

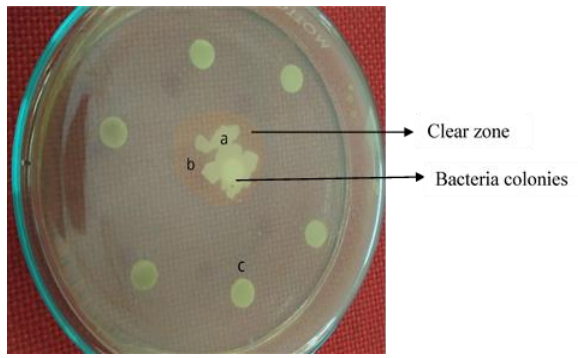

Figure 1. *S. aureus* colonies around blank disc. b. Clear zone formed by *S. aureus*. c. Clear zone do not exist around disc with antibiotic

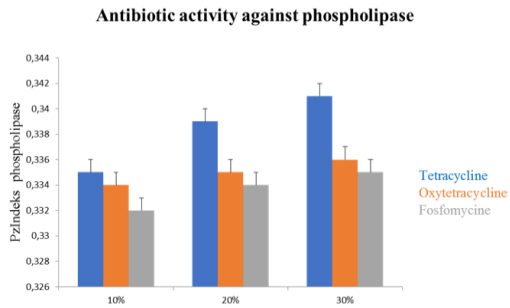

Figure 2. The graph of antibiotic activity against phopholipase of *S. aureus* isolated from preputium of Aceh cattle

Certain antibiotics have potential to suppress phospholipase production. In this experiment, antibiotics used were tetracycline, oxytetracycline, and fosfomycin, and these antibiotics had low

ability to suppress phospholipase production (PzI = 0.332 – 0.341), so the enzyme was still produced by *S. aureus* in significant level. The ability of these antibiotics to suppress phospholipase was listed in Table 1.

Table 1. Results of measurements of PzI phospholipase *S. aureus* isolates of Aceh cattle preputium using antibiotic treatment.

Antibiotic	Concentration (%)	PzI			
		1	2	3	$\bar{x}$
Tetracycline	10	0.336	0.335	0.335	0.335
	20	0.339	0.338	0.339	0.339
	30	0.341	0.340	0.342	0.341
Oxysitetracycline	10	0.334	0.334	0.333	0.334
	20	0.335	0.335	0.334	0.335
	30	0.336	0.337	0.335	0.336
Fosfomycin	10	0.333	0.332	0.332	0.332
	20	0.334	0.335	0.334	0.334
	30	0.335	0.336	0.335	0.335
S. aureus Aceh Cattle	-	0.288	0.287	0.285	0.287
S.aureus ATCC	-	0.278	0.285	0.277	0.276

The calculation of Pz index of *S. aureus* (Figure 2.) was increased in higher concentration of each antibiotic. The best phospholipase suppression was shown in 30% tetracycline with PzI value was 0.341, and the lowest phospholipase suppression was in 10% Fosfomycin with PzI value was 0.332. Statistical analysis showed that phospholipase production of *S. aureus* after antibiotics treatment was significantly different ($p<0.05$) for each antibiotic with different concentrations. Duncan test showed that tetracycline had different activity from oxytetracycline dan Fosfomycin, and different concentration also gave different result.

Treatment with 30% tetracycline had the highest ability to inhibit phospholipase production, however, the enzyme was still produced in strong positive category. Rahmaniar (2017) collected 20 samples isolated from dogs nose, 13 samples was positive with *S. aureus*, and 77% of those samples were sensitive to tetracycline. Putri (2017) tested the sensitivity of 10 samples of *S. aureus* isolated from mastitis milk to several antibiotics, and all of the samples were sensitive to tetracycline. Qolbaini (2014) reported that 48% of 105 samples isolated from mastitis milk was *S. aureus* positive, and all of the samples were sensitive to tetracycline. Tetracycline is a broad spectrum antibiotic used to treat Gram-positive and Gram-negative bacteria infection. Tetracycline is a bacteriostatic, working by inhibit protein synthesis of 30S subunit ribosome, change the genetic code of mRNA and resulting in new protein synthesis which is non-functional for bacteria (Choon *et al.*, 2017).

Staphylococcus aureus isolated from Aceh cattle was positive coagulase and capable in producing lipase/phospholipase which hydrolyzes phospholipids into fatty acids. As virulent factor, this enzyme works by avoiding PMN and suppress opsonin-dependent phagocytosis by protein A (Watkins *et al*., 2012). Tetracycline contains positive charge (cation) that have the ability to remove negative charge on bacteria surface which binds to substrate, and lower cell membrane hydrolysis that inhibit enzyme access into cell membrane (Movert *et al*., 2011).

Conclusion

From the experiment it can be concluded that *S. aureus* isolated from preputium of Aceh cattle had the ability to produced phospholipase in category of strong positive with PzI value was 2.287. Antibiotics had the ability to suppress phospholipase production, the highest phospholipase suppression was detected in 30% tetracycline with PzI value was 0.341, and the lowest was on 10% Fosfomycin with PzI value was 0.332.

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