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Resistance of *Staphylococcus Aureus* Isolated from Chicken Meats to Some Antibiotics

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Abstract

The aim of this research was to study the resistance of Staphylococcus aureus isolated from chicken meats to some antibiotics. The present of S. aureus was detected by bacteriological analytic manual (BAM) method and the sensitivity test for S. aureus to some antibiotics was conducted using Kirby-Bauer method. This study used eight samples of chicken meat, and the antibiotics used were ampicillin, streptomycin, ciprofloxacin and erythromycin. The data were analyzed descriptively. The result of this study showed that all samples of chicken meat indicated the present of S. aureus. From sensitivity test, four samples of S. aureus were resistant to ampicillin (50%), six samples were resistant to erythromycin (75%), while all of eight samples were sensitive to ciprofloxacin (100%) and six samples were sensitive to streptomycin (75%). This study indicated that chicken meats used in this study were contaminated with S. aureus, and this bacterium showed a variety resistance level to some antibiotics.

Keyword: resistance, staphylococcus aureus, chicken meat, antibiotics

Introduction

Meat is a good media for diverse microflora growth, including the pathogen ones (Jay dkk., 2005). Chicken meat that is already contaminated with pathogen bacteria could be a source of foodborne disease. Some pathogen bacteria that are usually found in chicken meat are *Salmonella, Campylobacter, Listeria, dan Staphylococcus aureus* (Murtidjo, 2007).

Staphylococcus aureus is one of bacteria that caused foodborne disease, it can contaminate food and lead to food poisoning (BPOM, 2008). *S. aureus* was the cause of second biggest foodborne disease outbreak in France in 2000 (LeLoir, 2003). *S. aureus* infection in broiler chicken have a high impact in economic loss, due to high morbidity, stunted growth, and high treatment cost (Rasheed, 2011). Staphylococcus infection in chicken and other birds is called Staphylococcosis as the main source of disease in poultry, which causes arthritis, tenosynovitis, and gangrenous dermatitis (Tabbu, 2000).

Antibiotics are chemical substance that can inhibit or kill bacteria. The substance would meet the antibiotic criteria if they are the results of metabolism processes, have the ability to kill microorganism with low dosage, the chemical structure of synthesis product is similar to its original structure, and have antagonist effect on microorganism (Krisnaningsih, 2005). Some antibiotics that are usually used in poultry are group of aminoglycosides (gentamycin, neomycin, and streptomycin), beta lactam (penicillin, amoxicillin), and tetracycline (chlortetracycline, oxytetracycline, doxycycline, and ampicillin) (Lampang dkk., 2007). Antibiotics that are used to treat *S. aureus* infection including erythromycin, lincomycin, and spectinomycin (Syarif dkk., 2009). In poultry, antibiotics are added in feed to prevent infection and increase animals' growth (Khusnan dkk., 2008)

Resistance of antibiotics is the condition where the bacteria have developed the ability to defeat antibiotics, and the antibiotics no longer have the capability to inhibit bacteria's growth. Some bacterial strains might become resistant to more than one antibiotic (Clarks dkk., 2012).

Antibiotic resistance occurs when the use of antibiotic is irrational with inappropriate dosage, incorrect duration and time. In order to delay the resistance, the use of antibiotics must be seriously considered (Djide dan Sartini, 2008).

Materials and Methods

The method to detect *S. aureus* contamination was conducted according to Bacterial Analytical Manual (BAM), and the sensitivity of *S. aureus* to antibiotics was carried out using Kirby-Bauer method (diffusion disc method), where the data was analyzed based on Clinical and Laboratory Standard Institute (CLSI 2012). This study used eight samples of broiler chicken breast, *S. aureus* was then isolated and further tested for antibiotics sensitivity. The data was analyzed descriptively.

Results and Discussion

Staphylococcus aureus isolation

The samples used in this study was obtained from Ulee Kareng market in Banda Aceh, where each sample was attained from different seller. The result showed that all samples were contaminated with *S. aureus* (Table 1). The colony from each sample was then tested for sensitivity test.

Table 1. Isolation of *Staphylococcus aureus* in chicken meat

Seller	Sample	*Staphylococcus aureus contamination*
1	1	Positive
2	1	Positive
3	1	Positive
4	1	Positive
5	1	Positive
6	1	Positive
7	1	Positive
8	1	Positive

The present of *S. aureus* in meat could be identified by using Mannitol Salt Agar (MSA) as presented in Figure 1.

Mannitol Salt Agar is a selective and differential media which is used in the isolation of pathogenic *Staphylococcus.* The high content of sodium chloride makes this media selective for only *Staphylococcus* bacteria, while other bacteria could not survive in this media. Figure 1 showed the colony of *S. aureus* in MSA indicated by golden yellow color, and the media color was changed from pink to yellow as a result of mannitol fermentation by the bacteria. However, non-pathogenic bacteria such as *S. epidermidis* does not have the ability to ferment mannitol so it does not change the color of the media (Salamena, 2015).

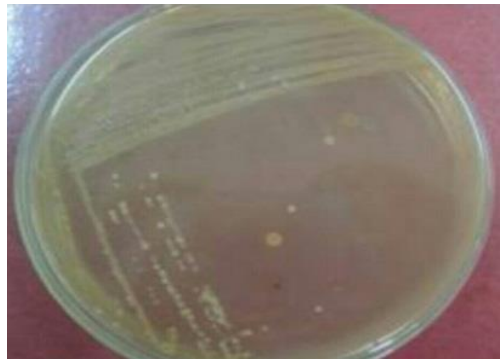

Figure 1**.** Colony of *Staphylococcus aureus* in *Mannitol salt Agar*

The colony in MSA media was further tested by Gram staining to check the nature and morphology of the bacteria. The result confirmed that the bacteria had the characteristic of *S. aureus*, it was Gram-positive and the morphology was round-shaped in cluster like grapes (Figure 2).

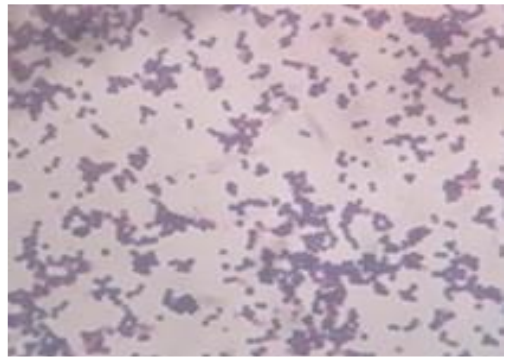

Figure 2. Gram staining of *Staphylococcus aureus*

Infection caused by *S. aureus* in chicken is mostly from the environment, because this disease is not transmitted from one chicken to others. Infection usually occurs when the skin or mucous has an open wound (Tabbu, 2000). The contamination among carcass are more common to occur, bad sanitation and bad hygiene during slaughtering and selling make the chicken is

more prone to *S. aureus* contamination. Contamination usually happens in traditional market where the bacteria is easily transmitted from the table, other materials, and environment. Since *S. aureus* can also live on human skin, the contamination could also spread from the hands of the seller and the buyer.

Sensitivity test of *Staphylococcus aureus* to antibiotics

The sensitivity test of *S. aureus* to antibiotics was conducted using disc diffusion method. The paper disc of antibiotic was placed on the surface of Mueller Hinton Agar which had been inoculated with *S. aureus*, and the inhibition zone was calculated (Figure 3). The result showed that each sample had different sensitivity to different antibiotic.

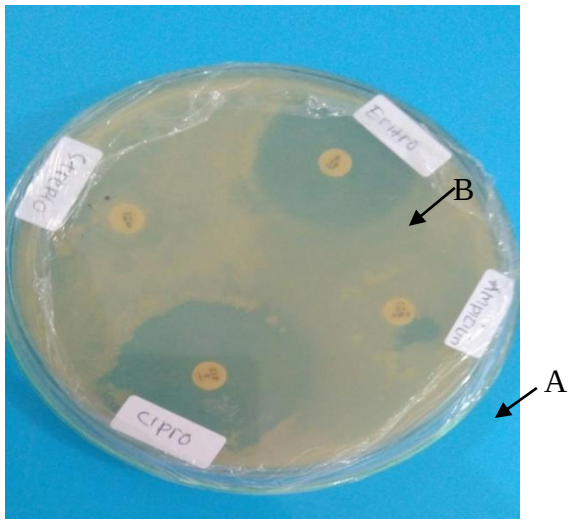

Figure 3. The result of sensitivity test on MHA medium. A. Inhibition zone formed. B. No inhibition zone.

Based on Kirby-Bauer method, there are three category of sensitivity test: a. susceptible, if microorganism give response to antibiotic and other antimicrobial substance and the growth is inhibited; b. intermediate, if the microorganism have moderate sensitivity to antibiotics which could be related to low dosage, infection specific, and high toxic effect; c. resistant, if the microorganism does not respond to antibiotics. The sensitivity test result is listed in Table 3.

Table 3. Result of sensitivity test based on inhibition zone (mm).

Sample	Antibiotic			
	Ampicillin	Erithromycin	Streptomycin	Ciprofloxacin
1	41 (S)	28(S)	14 (I)	41 (S)
2	0 (R)	10 (R)	32 (S)	36 (S)
3	32 (S)	8 (R)	29 (S)	32 (S)
4	9 (R)	0 (R)	27 (S)	30 (S)
5	29 (S)	17 (I)	28 (S)	32 (S)
6	8 (R)	0 (R)	23 (S)	36 (S)
7	36 (S)	11 (R)	29 (S)	29 (S)
8	0 (R)	12 (R)	13 (I)	21 (S)

From Table 3, it was shown that all samples had different sensitivity level on different antibiotics. Four of the samples were susceptible to ampicillin, and the other samples were resistant to ampicillin. For erythromycin, one sample is susceptible, one sample showed moderate level, and the other six were resistant. Six of the samples were susceptible to streptomycin, while the other two were moderate. All of the samples exhibited susceptible towards ciprofloxacin. The difference level of sensitivity showed by *S. aureus* probably because the samples were obtained from different seller and originated from different chicken farm (Effendi, 2008).

According to CLSI (2012), microorganism is categorized as resistant to antibiotic if the inhibition zone is ≤13 mm for ampicillin, ≤11 mm for streptomycin, ≤15 mm for ciprofloxacin, and ≤13 for erythromycin. 50% of the samples showed resistance to ampicillin, while 75% was resistance to erythromycin. The bacteria were not resistant to both streptomycin and ciprofloxacin. This result was in accordance with a study conducted by Salamena (2015) where *S. aureus* was more resistant to ampicillin and erythromycin, but more susceptible to streptomycin and ciprofloxacin.

According to Jacob (2005), *S. aureus* was susceptible to ciprofloxacin because this antibiotic has two targets in the bacterial cell e.g. DNA gyrase and topoisomerase IV. If one enzyme was mutated and unapproachable, there was another enzyme to attack. Ciprofloxacin was not affected by

single mutation and still have the capability to kill bacteria. However, the use of this antibiotic must be rational to avoid resistance in the future.

In chicken farm, sometimes the use of antibiotic is not rational, all flocks are given antibiotic despite the condition where only several chickens have symptoms. This could lead to antibiotic resistant, especially to erythromycin and tetracycline which are used widely in poultry (Salamena, 2015). Antibiotic could also have negative impact due to its residue in livestock products, and it also affect microflora in the intestine (Murdiati, 1997). Anadon et al. (1994) reported that the concentration of doxycycline would decrease to lower level of tolerance after five days of administration.

Conclusion

From this study on sensitivity test of *S. aureus* isolated from chicken breast obtained from Ulee Kareeng market in Banda Aceh, 50% of samples showed resistant to ampicillin, 75% was resistant to erythromycin, while 100% was susceptible to ciprofloxacin and 75% was susceptible to streptomycin.

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