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Staphylococcus aureus Resistance to Several Antibiotics Isolated from Broiler Chicken Meat at the Traditional Market in Banda Aceh-Indonesia

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Abstract

Staphylococcus aureus is a prevalent pathogen that frequently infects broiler chickens, with treatment typically involving the use of antibiotics. Antibiotic resistance refers to the ability of bacteria to withstand the effects of these drugs. This study aims to assess the prevalence of antibiotic-resistant *S. aureus* in broiler chicken meat sold at Traditional Market in Banda Aceh City. Employing a cross-sectional study, samples were collected through purposive sampling. A total of 100 breast meat samples from broiler chickens sourced from traditional markets in Banda Aceh City were analysed. The antibiotics tested in this study included ampicillin, streptomycin, ciprofloxacin, and erythromycin. All positive isolates of *S. aureus* underwent MRSA testing. Data were analysed descriptively. The findings reveal that out of the 100 samples, 10 tested positives for *Staphylococcus aureus*. Of these, four samples exhibited resistance to erythromycin (40%), two samples were resistant to ampicillin (20%), and one sample showed resistance to streptomycin (10%), while all ten samples were sensitive to ciprofloxacin (100%). Out of the 10 MRSA isolates analysed, the mecA gene was detected in one isolate. Consequently, it can be concluded that broiler chicken meat sold in traditional markets in Banda Aceh City demonstrates varying levels of resistance to several antibiotics.

Keywords: Antibiotics, broiler chicken, market, *Staphylococcus aureus*, resistance

Introduction

Traditional markets offer a diverse range of food essentials, including broiler chicken, sourced from various regions. Broiler chicken is a vital component of the Indonesian diet, fulfilling a significant portion of the population's demand for animal protein. One notable advantage of broiler chickens is their rapid growth rate, allowing them to be harvested as early as five weeks of age (Farrell, 2013). However, these birds are also vulnerable to diseases, particularly those caused by pathogenic bacteria. Common bacterial pathogens found in chicken meat include *Escherichia coli, Shigella flexneri, Pseudomonas spp., Salmonella spp., Clostridium perfringens, and Staphylococcus aureus* (Hyeon et al., 2015).

Staphylococcus aureus is typically present in the environment, including air, skin, and mucous membranes of both animals and humans. An elevated population of *Staphylococcus aureus* can lead to disease within animal hosts. The proliferation of *Staphylococcus aureus* is often facilitated by environments conducive to its growth and survival (Raji et al., 2016). Infections caused by *Staphylococcus aureus* are commonly treated with antibiotics.

Antibiotics are pharmacological agents designed to inhibit the growth of or kill microorganisms, particularly pathogenic bacteria. Rational and appropriate use of antibiotics is essential for effective treatment. However, inappropriate antibiotic usage can lead to the development of antibiotic resistance (Krupa et al., 2014). The emergence and persistence of antimicrobial resistant Staphylococcus aureus is widespread in farm animals is a global public health problem, affecting humans and animals (van der Mee-Marquet et al., 2014; Cuny et al., 2015; Becker et al., 2017). Antibiotic resistance arises from factors such as improper antibiotic use and the presence of mutations or acquired resistance genes (Darniati et al., 2024).

Resistance among *Staphylococcus aureus* isolates to various antibiotics has been widely documented. *S. aureus* is well-known for its characteristics of multidrug resistance and resistance to β-lactam-class antibiotics,sometimes known as methicillin-resistant Staphylococcus aureus (MRSA) (Green et al., 2012). MRSA and other antibiotic- resistant bacteria have led to nosocomial infections (Nimer, 2022). All β-lactam medications, such as cephalosporins and carbapenems, are ineffective against MRSA strains resistant to oxacillin and cefoxitin (Gajdács, 2019). Animal MRSA isolates were shown to have considerably higher levels of gentamicin, ciprofloxacin, and clindamycin resistance than human MRSA isolates (Fitranda et al., 2023).

MRSA strains mediate through the *mec*A gene (Rajabiani et al., 2014). A cellular genetic component called the staphylococcal cassette chromosome mec (SCCmec) contains this gene (Saber et al., 2017). Penicillin-binding protein 2a (PBP 2a), produced by this gene, has a lower affinity for β-lactam antibiotics than ordinary PBP (Fergestad et al., 2020). Thus, methicillin-resistant *Staphylococcus aureus* containing the mecA gene will resist β-lactam-class antibiotics (Ramandinianto et al., 2020). In recent years, severe epidemics have been brought on by antibiotic resistance. As a result, it is essential to conduct

resistance tests and culture experiments to track bacterial antibiotic resistance. Given this context, it is crucial to investigate the resistance patterns of *Staphylococcus aureus* in broiler chicken meat sold at Traditional Market in Banda Aceh City.

Methods

The samples utilized in this study consisted of broiler chicken breast meat collected from traditional markets in Banda Aceh. Purposive sampling was employed, with criteria including the absence of gloves and aprons among vendors and the use of worn cutting boards. A total of 100 breast swab samples from 90 vendors were collected.

This research employed a cross-sectional design, with sample collection based on purposive sampling. The stages and procedures of the study included the identification of *Staphylococcus aureus* bacteria in the broiler chicken meat using Nutrient Broth (NB) as a bacterial culture medium and Mannitol Salt Agar (MSA) as a differential medium for *Staphylococcus aureus*. The sensitivity of *Staphylococcus aureus* to various antibiotics was assessed using the disk diffusion method according to the guidelines established by the Clinical and Laboratory Standards Institute (2012).

The collected swab samples were homogenized and incubated at 37°C for 24 hours. Following incubation, bacterial suspensions from NB were obtained using an inoculating loop and streaked onto MSA plates using the quadrant streaking technique. These plates were then incubated at 37°C for an additional 24 hours, resulting in the emergence of distinct colonies exhibiting cream or golden-yellow coloration. These bacterial colonies were subsequently inoculated onto slant NA media.

To differentiate between the genera Staphylococcus and Streptococcus, a catalase test was performed. A drop of hydrogen peroxide (H2O2) was placed on a glass slide, followed by the addition of one loopful of inoculum from the MSA. A positive catalase reaction, indicated by the production of gas bubbles (O2), confirms the presence of the Staphylococcus genus (Hayati et al., 2019).

Antibiotic resistance testing for *Staphylococcus aureus* isolates was conducted using the disk diffusion method. A colony of *Staphylococcus aureus* from the NA stock was selected with an inoculating loop and transferred to NB for incubation at 37°C for 24 hours. The turbidity of the bacterial suspension was adjusted to match the 0.5 McFarland standard; if it was less turbid, additional incubation was performed, and if it was too turbid, physiological saline (NaCl) was added to achieve the desired concentration. The adjusted suspension was then collected using a sterile swab and evenly spread onto MHA plates. Paper disks containing ampicillin, ciprofloxacin, streptomycin, and erythromycin were positioned on the surface of the MHA plates, which were subsequently labelled with the names of the respective antibiotics. The plates were incubated at 37°C for 24 hours, after which the diameters of the inhibition zones were measured. According to the Clinical and Laboratory Standards Institute (2012), the inhibition zones were categorized as sensitive, intermediate, or resistant.

Results and Discussion

From a sanitation perspective, the traditional markets were considered to have low sanitation standards. This can be observed from the slaughtering and cleaning of chickens were conducted in the same area, resulting in wastewater from the chicken cleaning process stagnating around the vendors. This condition naturally increases the chances of contamination spread by pathogenic bacteria such as *S. aureus*.
This study utilized swab samples from the breasts of chickens sourced from 90 broiler chicken vendors in traditional markets in Banda Aceh, with one breast swab sample taken from each vendor. Based on *S. aureus*. Detection tests, 10 samples (10%) tested positive. The results of the isolation can be seen in Figure 1.

Figure 1. Colonies of *Staphylococcus aureus* Growing on Mannitol Salt Agar (MSA)

The chicken breast swab was initially placed in NB medium. This medium is a general-purpose medium used to support the general growth of bacteria. It contains sources of carbon and nitrogen to meet the nutritional needs of bacteria (Hanson et al., 2011). The growth of *S. aureus* was then performed using MSA medium.

The growth of *S. aureus* is indicated by the presence of colonies on MSA. According to Abdalrahman, et al. (2015), MSA is a selective-differential medium used to identify the pathogenic bacteria *Staphylococcus aureus*, and only specific bacteria can thrive on it. On MSA, *S. aureus* displayed colonies with a yellowish-white color and a surrounding yellow zone due to its ability to ferment mannitol. The yellow zone indicates mannitol fermentation, whereby the acid produced causes the phenol red in the agar to turn yellow (Owuna, et al., 2015).

Contamination of chicken meat with *Staphylococcus aureus* can occur during the slaughtering process through direct contact with hands, water, and equipment. This

contaminant flora primarily originates from the animal's skin, the carcass being handled, and direct contact with dirty work areas during slaughtering (Wardana et al., 2021). The contamination can also come from the water used by vendors to wash their hands or cleaning the tools, especially if the water used is stagnant (Paerunan et al., 2018).

The *S. aureus* isolates obtained in this study were further tested using the catalase test. A positive catalase test for *S. aureus* is indicated by the presence of gas (O2) when homogenized with H2O2. The results of the catalase test showed in Figure 2.

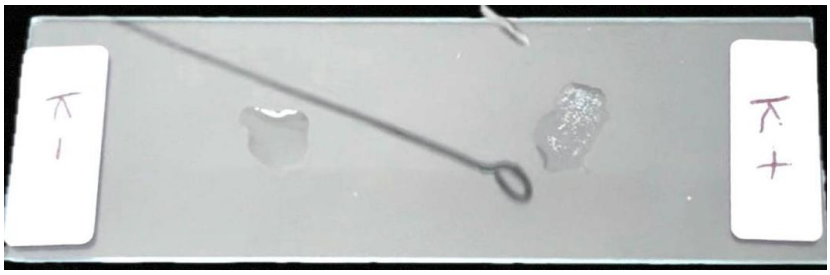

Figure 2. Catalase Test Results. Positive Catalase (k+) and Negative Catalase (k-)

The catalase test for the ten samples, grown on MSA in this study showed that all isolates exhibited a positive reaction. The catalase test for cocci bacteria is used to differentiate between *Staphylococcus* spp. and *Streptococcus* spp., where the *Staphylococcus* spp. group is catalase positive. Catalase is an enzyme that catalyses the breakdown of hydrogen peroxide into H2O and O2. Hydrogen peroxide is toxic to cells because it can inactivate enzymes within the cell. Hydrogen peroxide forms during aerobic metabolism, so microorganisms that grow in aerobic environments must break down this substance (Owuna et al., 2015).

Each colony of *S. aureus* from the ten chicken meat samples underwent antibiotic resistance testing using the disk diffusion method. After the *S. aureus* bacteria were placed in Petri dishes, antibiotic disks were separately placed using sterile forceps. The results of the antibiotic resistance measurements can be seen in Figure 3, and the antibiotic resistance measurement results are presented in Table 2.

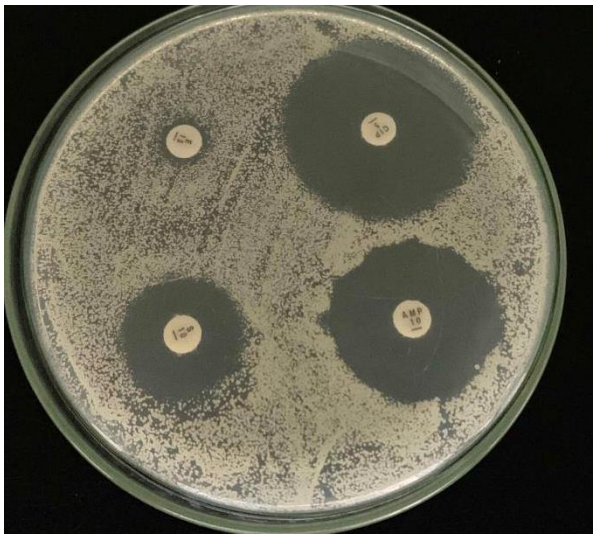

Figure 3. Measurement Results of Inhibition Zones on MHA. No Inhibition Zone Formed (a), Inhibition Zone Formed (b)

The diameter of the inhibition zone or the clear area around the antibiotic disk indicates the sensitivity of the bacteria to the antibiotics used. The inhibition zone formed around the disk is measured vertically and horizontally in millimeters using calipers (Magvirah et al., 2019). The results of the inhibition zone measurements are categorized into three groups: resistant, intermediate, and sensitive.

Table 1. Criteria for Assessing Inhibition Zones of Various Antibiotics Against *Staphylococcus aureus* (CLSI, 2012)

Antibiotic Group	Antibiotic	Disk Content (µg)	Inhibition Zone (mm) Standard Interpretation Diameter		
			S	I	R
β-lactam	Ampicillin	10	≥ 17	14-16	≤ 13
Aminoglycosides	Gentamicin (GN)	10	≥ 15	13-14	≤ 12
	Streptomycin (S)	10	≥ 15	12-14	≤ 11
Fluoroquinolones	Ciprofloxacin (CIP)	5	≥ 21	16-20	≤ 15
Macrolides	Erythromycin (E)	15	≥ 23	14-22	≤ 13
Sulfonamides	Sulfamethoxazole (SXT)	30	≥ 17	13-16	≤ 12
Tetracyclines	Tetracycline (TE)	30	≥ 19	15-18	≤ 14

Note: R = Resistant; I = Intermediate; S = Sensitive

Table 2. Results of Resistance Measurements Based on Inhibition Zone Diameter (mm)

Sample	**Antibiotic**			
	Erythromycin	Streptomycin	Ampicillin	Ciprofloxacin
1	24,2 (S)	15,1 (S)	17,2 (S)	26,3 (S)
2	35,4 (S)	34,9 (S)	32,4 (S)	28,3 (S)
3	4,1 (R)	12 (I)	7,4 (R)	22,7 (S)
4	31,1 (S)	32,7 (S)	33,6 (S)	32,7 (S)
5	3,9 (R)	12,5 (I)	4,4 (R)	21,6 (S)

6	28,5 (S)	19,7 (S)	32,7 (S)	27 (S)
7	0,8 (R)	11 (R)	20,2 (S)	25,7 (S)
8	6,9 (R)	24,5 (S)	29,7 (S)	34 (S)
9	24 (S)	21,1 (S)	32,6 (S)	23,2 (S)
10	27,5 (S)	18,6 (S)	27,6 (S)	26,9 (S)
% (R)	40%	10%	20%	0%

Note: R = Resistant; I = Intermediate; S = Sensitive

The results of the resistance testing of *S. aureus* isolates, as shown in Table 2, indicated that the ten isolates exhibited varying sensitivities to the tested antibiotics, namely erythromycin, streptomycin, ampicillin, and ciprofloxacin. The highest resistance was observed for erythromycin (4/10 or 40%), followed by ampicillin (2/10 or 20%), streptomycin (1/10 or 10%), with intermediate resistance in 2/10 (20%) cases. In contrast, all isolates remained sensitive to ciprofloxacin (10/10 or 100%). This finding aligns with the research conducted by Rastina et al. (2020), which reported that Staphylococcus aureus showed the highest resistance to erythromycin and ampicillin, while showing greater sensitivity to streptomycin and ciprofloxacin. The study indicates a decrease in resistance levels compared to previous research, which is likely due to the increasing public awareness about the dangers of antibiotic use in livestock.

The research reveals that *S. aureus* isolates still exhibit high sensitivity to ciprofloxacin and streptomycin. This may be because these antibiotics are rarely used by farmers and are still under regulation, ensuring controlled usage. The high sensitivity to ciprofloxacin is attributed to its dual-target mechanism in bacterial cell killing, affecting both DNA gyrase and topoisomerase IV. Ciprofloxacin's efficacy is less affected by single mutations because if one enzyme undergoes mutation and becomes inaccessible, the drug can still target the other enzyme (Amri & Wulandari, 2022).

For streptomycin, one sample showed resistance while two samples exhibited intermediate resistance. Intermediate resistance refers to a condition where there is a shift from sensitivity to resistance, but not completely resistance (Artati et al., 2016). This could be due to the presence of *S. aureus* strains that are beginning to develop resistance around broiler chicken markets.

The relatively high resistance to ampicillin and erythromycin in the samples is suspected to be due to the frequent use of these antibiotics. The use of antibiotics as feed additives, especially for growth promotion, is one of the causes of antibiotic resistance in Indonesia. Observations in broiler farms indicate that all farms utilize commercial feed from feed mills, with the addition of antibiotics in feed being a primary driver of increased antibiotic resistance (Evarozani et al., 2023).

The differences in resistance levels among broiler chickens could be attributed to the varying origins of the broilers in the Al-Mahirah market, as each farmer has different husbandry practices. Some broiler farmers administer antibiotics to their livestock while others do not, leading to variability in antibiotic resistance levels.

Antibiotic resistance in animal-derived bacteria can have both direct and indirect impacts on humans. Direct effects may occur when humans come into contact with antibiotic-resistant bacteria from animals. Indirect effects can arise from exposure to antibiotic-resistant organisms that spread through the environment, such as water, air, and soil, due to residual antibiotic use in animals. Consequently, diseases caused by antibiotic-resistant bacteria can pose health risks to both humans and livestock, including increased healthcare costs, limited treatment options, prolonged treatment durations, and even death (Januari et al., 2019).

Conclusion

Based on the research, *S. aureus* isolated from the breast meat of broiler chickens sold in traditional markets in Banda Aceh showed varying resistance levels to several antibiotics: four samples were resistant to erythromycin (40%), two samples were resistant to ampicillin (20), one sample was resistant to streptomycin (10%), and all of ten samples were sensitive to ciprofloxacin (100%).

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