

INTRODUCTION STUDY OF MDR STAPHYLOCOCCUS AUREUS IN PIG SLAUGHTER HOUSE IN KUPANG CITY

Maxs U.E. Sanam¹, Novalino H.G. Kallau^{1*}, Yeremia Y. Sitompul²

¹Department of Animal Diseases Sciences and Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Nusa Cendana, Kupang, East Nusa Tenggara, Indonesia

²Department of Veterinary Clinical, Reproduction, Pathology and Nutrition, Veterinary Medicine Faculty, Universitas Nusa Cendana, Kupang city, East Nusa Tenggara.

*Corresponding author: novalino.kallau@staf.undana.ac.id

ABSTRACT

Due to the unprudent use of antibiotics globally, it causes the incidence of antibiotic resistance with the emergence of bacteria that are resistant to antibiotics and has implications for the failure of the use of antibiotics in dealing with various cases of diseases caused by bacteria. The incidence of antibiotic resistance in the livestock sector occurs due to the use of antibiotics that are not based on recommendations and antibiotics are given in the long term. The presence of bacteria that carry resistance to one or several kinds of antibiotics in livestock is a threat to animal and human health. The city of Kupang as an area that has pig farms and a high pig population has a big challenge to the emergence of antibiotic resistance due to the use of antibiotics in maintaining livestock health. One of the bacteria that has a large number of distributions and is widely found in livestock and is an indicator of antibiotic resistance is *Staphylococcus aureus* (*S. aureus*).

This research has used an observational research method based on data collection methods and laboratory examinations, which are adjusted to the objectives that have been set. The approach that has been used in this research is a Cross Sectional Study, because it wants to get an overview of *S. aureus* bacteria that are resistant to antibiotics. The sample that has been needed is pig feces obtained by field observations. The data analysis technique that has been used is a descriptive analysis model, to explain the presence of antibiotic-resistant *S. aureus* bacteria in pig farms. The results showed that this isolation and identification process resulted in 28 (51.85%) samples that were positive for *S. aureus*. The highest prevalence of resistance was found in *S. aureus* which was resistant to the antibiotic Colistin sulfate (82.1%). The number of antibiotics tested from *S. aureus* isolates showed various variations from 0 to 3 types of antibiotics that were resistant in 1 isolate. The most common resistance pattern shown in *S. aureus* isolates was the CS (Colistin sulfate) pattern as many as 19 of the 28 samples. The conclusion of this study encourages the need to increase supervision related to the use of antibiotics and increase public awareness of the use of antibiotics and prevention of the increasing incidence of antibiotic resistance.

Keywords: Antibiotic resistance, Pig, *Staphylococcus aureus*

INTRODUCTION

The problem of antibiotic resistance is growing worldwide and can affect public health and animal health. The increase and spread of resistance events are increasingly widespread throughout the world and can reduce the level of public health and animal health. Resistance that has been formed due to the unwise use of antibiotics in animals and plants (Aziz, 2016). Uncontrolled application of antibiotics by breeders and farmers to animals and plants can further increase the incidence of resistance (Aziz, 2016). OIE has analyzed and provided estimates related to the possibility that resistant bacteria in 2050 will become the main cause of death if not treated early on (OIE, 2016). Control measures are a solution offered by OIE and WHO to suppress the spread of antibiotic-resistant bacteria. WHO and OIE have offered that intensive control efforts will be a solution in suppressing the spread of antibiotic-resistant bacteria.

Pig farming has been known to be one of the sites for the massive use of antibiotics as growth promoters, prophylactic and metaphylactic treatment (Barton, 2014) and a contributor to the emergence of antibiotic-resistant bacteria. Increasingly intensive livestock business encourages the use of antibiotics on a large scale. Not inferior to intensive farming, household or traditional scale farming is also suspected as a source

of the emergence of antibiotic-resistant bacteria. Resistant bacteria found on farms can later spread to humans and the environment around the farm area.

Antibiotic-resistant bacteria have been found in commensal and pathogenic bacteria. The use of antibiotics with many variations and often can lead to the occurrence of resistance in bacteria to one or more antibiotics (Coia, 2016). The nature of resistance to several antibiotics is often referred to as multi-antibiotic resistance. Resistance events in bacteria have been found in many livestock, environmental and human populations. *Staphylococcus aureus* as one of the most common types of bacteria found on the skin surface has shown an increasing number of cases related to MDR. The incidence of MDR *Staphylococcus aureus* that is often found is Methicillin Resistant *Staphylococcus aureus* (MRSA).

Staphylococcus aureus is a pathogenic bacterium that is often found in the respiratory tract, skin and digestive tract (Prescott et al., 2002). These bacteria can cause health problems for humans (Madigan, 2008). This is even more dangerous when these bacteria have become resistant to antibiotics. The incident that has developed today is that *Staphylococcus aureus* bacteria have become resistant to more than one type of antibiotic (multi-resistant) which is often called

Methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA can cause health problems in animals and humans. Disease disorders caused by MRSA have been found. MRSA consists of (Livestock Associated MRSA (LA-MRSA), Community Associated (CA-MRSA) and Hospital Associated MRSA (HA-MRSA).

In Indonesia, research has been carried out in the field of hygiene and sanitation related to contamination of antibiotic-resistant *Staphylococcus aureus* in pigs. This supports the research on antibiotic-resistant *S. aureus* in pig abattoirs in Kupang City to see its presence and distribution in order to monitor the incidence of antibiotic resistance.

MATERIALS AND METHODS

The research has been conducted using the cross sectional study method and has been taking place from August to November 2019. Sampling of pig feces has been carried out at the pig slaughterhouse

in Kupang City. Laboratory testing: isolation, identification and sensitivity tests have been carried out at the Laboratory of Microbiology and Veterinary Public Health FKH Undana.

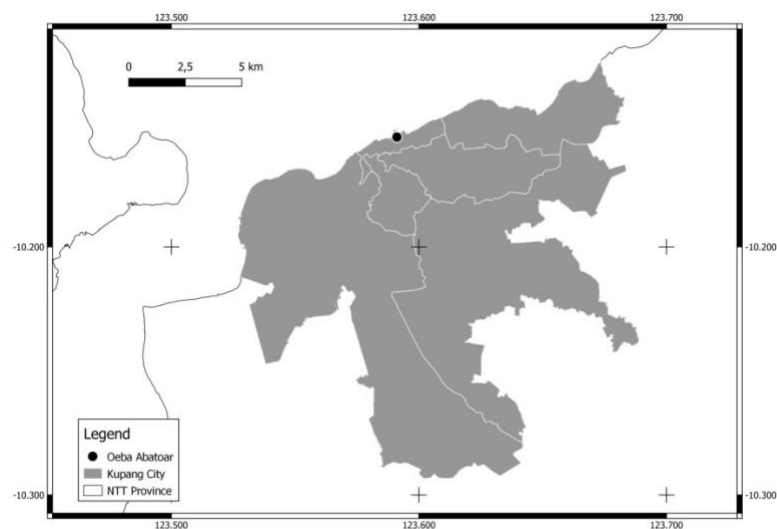


Figure 1. Map of sampling locations

Data collection technique

Sampling has been done by simple random sampling (simple random sampling). Data collection has been carried out through direct observation of pig oronasal swab samples obtained from pig abattoirs

in Kupang City. Samples were taken as many as 54 samples of pig feces from healthy pigs to be slaughtered at the Kupang City RPH. The swab sample was then isolated and identified for *S. aureus*. This identification was carried out to

determine the phenotypic character of *S. aureus* bacteria. The *S. aureus* isolates obtained were then confirmed by biochemical tests (catalase test, coagulase test and Gram stain) and microscopic examination. Testing the sensitivity of *S. aureus* to antibiotics using the Kirby-Bauer method (CLSI 2014).

Data analysis technique

After going through the data collection stage, the data that has been collected has been analyzed using a descriptive analysis model to relate the incidence of antibiotic-resistant *Staphylococcus aureus* contamination in pig abattoirs in Kupang City.

RESULTS AND DISCUSSION

Prevalence of *Staphylococcus aureus*

The research has been carried out by taking nasal swab samples on pigs obtained at the Oeba Pig Slaughterhouse (RPH) Kupang City. The results of sampling have obtained as many as 54 samples. The samples that have been obtained are continued with the isolation and identification process to determine the presence of *Staphylococcus aureus* bacteria in pigs. This isolation and identification process resulted in 28 (51.85%) samples that were positive for *S. aureus*. The isolates of *S. aureus* that had been obtained

were then tested by resistance test using the Kirby Bauer method to obtain information about the condition of resistance that appeared in *S. aureus*. The incidence of resistance to *S. aureus* is shown in Table 1 below.

A high number of *S. aureus* has been possible because this bacterium is a commensal bacteria that is often found in the skin and oronasal areas, this is in line with Prescott *et al.* (2002). Hygiene and environmental sanitation conditions that are not considered properly can encourage an increase in the number of these bacteria in the environment.

Table 1 Prevalence of *S. aureus* in Pigs in Oeba Slaughterhouse Kupang City

Kind of Bacteria	Number of sample	%
<i>Staphylococcus aureus</i>	28	51,85%
Non <i>Staphylococcus aureus</i>	26	48,14%
Total	54	100%

Prevalence of resistant *Staphylococcus aureus*

A total of 28 isolates of *S. aureus* have been isolated and identified, followed by examination of the antibiotic resistance test. This

test was conducted to determine the sensitivity of *S. aureus* isolates to several antibiotics used. Several antibiotics such as Erythromycin, Methicillin, Amoxicillin and clavulanate origin, Tetracycline,

Colistin sulfate, Streptomycin, Trimethoprim-sulfamethoxazole, Ciprofloxacin, Cephalotin, and Cefotaxime have been used to determine the level of emerging resistance. Antibiotics that have been used are antibiotics that are often used in livestock and humans.

Figure 2 has shown the level of resistance that occurs in *S. aureus* in the Oeba pig abattoir. Resistance has been found to occur in several antibiotics namely Erythromycin, Methicillin, Colistin sulfate, Streptomycin and Cephalotin. The highest prevalence was found in *S. aureus* which was resistant to colistin

sulfate antibiotics (82.1%) and followed by Erythromycin, Meticillin, Streptomycin and Cephalotin at 10.7%. The important thing that has also been found in this study is that the potential for resistance at the intermediate level was also found in several antibiotics such as Streptomycin (75%), Erythromycin (25%), and Trimethoprim-sulfamethoxazole (3.6%). This needs to be watched out for, because it can develop into resistance in the future if the control of the use of antibiotics is not carried out.

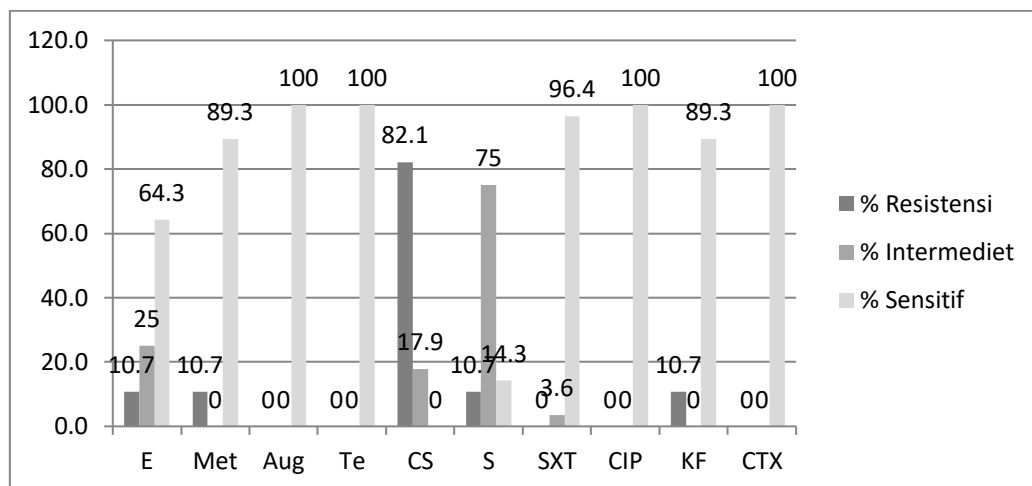


Figure 2 Percentage of prevalence of resistant, intermediate and sensitive *S. aureus* in Oeba pig abattoir in Kupang City, NTT; Eritromisin (E), Metisilin (Met), Amoksisilin + Asal klavulanat (Aug), Tetrasiklin (Te), Kolistin sulfat (CS), Streptomisin (S), Trimetoprim-sulfametoksasol (SXT), Siprofloksasin (Cip), Sefalotin (KF), Sefotaksim (CTX)

Attention also needs to be directed to colistin sulfate antibiotics, because colistin sulfate as a drug to treat resistance to MDR bacteria has shown a threat of failure to use due to the development of antibiotic-resistant bacteria. The spread of

resistance genes encoding resistance to colistin antibiotics are *mcr-1*, *mcr-2*, and *mcr-3* which are responsible for this (Bitrus et al. 2018).

The results of the *S. aureus* isolates in Figure 3 have shown variations in the emerging resistance

patterns. The total number of *S. aureus* isolates that showed resistance to 1 type of antibiotic (71.4%) was the highest followed by 2 or 3 antibiotics (10.7%) and 0 antibiotics (7.1%). The number of

antibiotics that have been tested from *S. aureus* isolates showed various variations from 0 to 3 types of antibiotics that were resistant in 1 isolate.

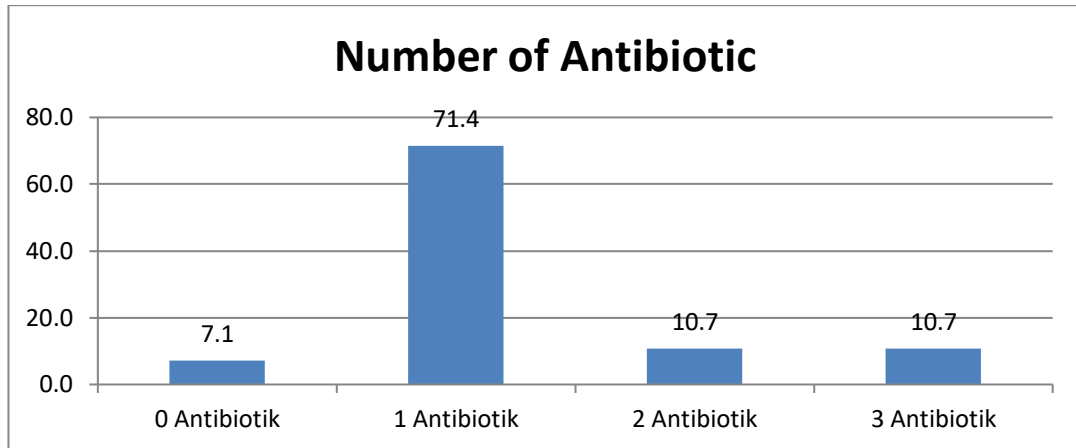


Figure 3. Resistance to *S. aureus* based on number of antibiotics

The pattern of resistance that most emerged and which has been shown in Figure 3 in *S. aureus* isolates was the CS (Colistin sulfate) pattern as many as 19 of the 28

samples and followed by the CS-S pattern as many as 2 samples. Other patterns such as E, E-CS, E-Met-KF, Met-S-KF, Met-CS-KF with 1 sample each (Figure 4).

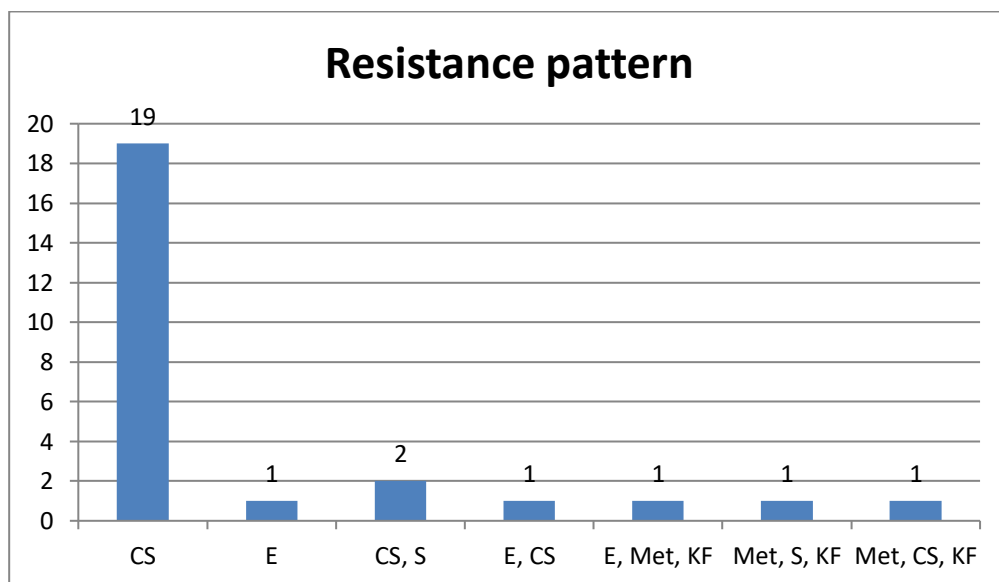


Figure 4 *S. aureus* Resistance Pattern; Eritromisin (E), Metisilin (Met), Amoksisilin + klavulanat acid (Aug), Tetrasiklin (Te), Kolistin sulfat (CS), Streptomisin (S), Trimetoprim-sulfametoksasol (SXT), Siprofloksasin (Cip), Sefalotin (KF), Sefotaksim (CTX)

CONCLUSION

The results of our study showed that this isolation and identification process resulted in 28 (51.85%) samples that were positive for *S. aureus*. The highest prevalence of resistance was found in *S. aureus* which was resistant to the antibiotic Colistin sulfate (82.1%). The number of antibiotics tested from *S. aureus* isolates showed various variations from 0 to 3 types of antibiotics that

were resistant in 1 isolate. The most common resistance pattern shown in *S. aureus* isolates was the CS (Colistin sulfate) pattern as many as 19 of the 28 samples. The conclusion of this study encourages the need to increase supervision related to the use of antibiotics and increase public awareness of the use of antibiotics and prevention of the increasing incidence of antibiotic resistance.

REFERENCES

- Aziz F, Lestari FB, Nuraidah S, Purwati E, Salasia SIO. 2016. Deteksi Gen Penyandi Sifat Resistensi Metisilin, Penisilin dan Tetrasiklin pada Isolat *Staphylococcus aureus* Asal Susu Mastitis Subklinis Sapi Perah. *Jurnal Sain Veteriner*. 34(1):60-69
- Barton MD. 2014. Impact of antibiotic use in the swine industry. 19:9-15.doi:10.1016/j.mib.2014.05.017.
- Coia JE. 2016. MRSA - seeing the bigger picture. 93(4):364-365.doi:10.1016/j.jhin.2016.05.017.
- Davies J, Davies D. 2010. Origins and evolution of antibiotic resistance. *Microbiology and molecular biology reviews* : *MMBR*. 74(3):417-433.doi:10.1128/MMBR.00016-10.
- Dierikx CM. 2013. Beta-lactamases in Enterobacteriaceae in broilers [Thesis]. Netherlands: Wageningen UR.
- Erfianto GI. 2014. Escherichia coli Yang Resistan Terhadap Antibiotik Yang Diisolasi Dari Sapi Potong Yang Diimpor Melalui Pelabuhan Tanjung Priok Jakarta [Tesis]. Bogor: Institut Pertanian Bogor.
- FAO, OIE, WHO. 2010a. The FAO-OIE-WHO Collaboration Sharing responsibilities and coordinating global activities to address health risks at the animal-human-ecosystems interfaces. [internet]. [diunduh Date Accessed]; Volume(Issue):Pages. Tersedia ada: URL.doi:DOI].

- FAO, OIE, WHO. 2010b. WHO, FAO, and OIE unite in the fight against Antimicrobial Resistance.
- FDA. 2012. Antimicrobial Resistance.
- Gebreyes WA, Wittum T, Habing G, Alali W, Usui M, Suzuki S. 2017. Spread of Antibiotic Resistance in Food Animal Production Systems. In: Dodd CER, Adsworth TG, Stein RA, editors. Foodborne Diseases. 3 ed. United Kingdom: Academic Press (Elsevier). p. 105-130.
- Guilfoile PG. 2007. Deadly Disease And Epidemics: Antibiotic-resistant bacteria. New York: Infobase Publishing.
- Heuer H, Schmitt H, Smalla K. 2011. Antibiotic resistance gene spread due to manure application on agricultural fields. *Current opinion in microbiology*. 14(3):236-243.doi:10.1016/j.mib.2011.04.009.
- Jiang HX, Lu DH, Chen ZL, Wang XM, Chen JR, Liu YH, Liao XP, Liu JH, Zeng ZL. 2011. High prevalence and widespread distribution of multi-resistant *Escherichia coli* isolates in pigs and poultry in China. *Veterinary journal*. 187(1):99-103.doi:10.1016/j.tvjl.2009.10.017.
- Krisnaningsih MMF, Asmara W, Wibowo MH. 2005. Uji Sensitivitas Isolat *Escherichia coli* Patogen Pada Ayam Terhadap Beberapa Jenis Antibiotik. *J Sain Vet*. 1:13-18
- Madigan MT, Martinko JM, Dunlap PV, Clark DP. 2008. Biology of Microorganisms 12th edition. San Francisco: Pearson.
- Molton JS, Tambyah PA, Ang BS, Ling ML, Fisher DA. 2013. The global spread of healthcare-associated multidrug-resistant bacteria: a perspective from Asia. *Clin Infect Dis*. 56(9):1310-1318.doi:10.1093/cid/cit020.
- OIE. 2016. The OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials. Di dalam: OIE,editor. [Internet]. OIE.hlm.Tersedia pada: http://www.oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/PortailAMR/EN_OIE-AMRstrategy.pdf.
- Parisi A, Caruso M, Normanno G, Latorre L, Miccolupo A, Fracalvieri R, Intini F, Manginelli T, Santagada G. 2019. MRSA in swine, farmers and abattoir workers in Southern Italy. 82:287-293.doi:10.1016/j.fm.2019.03.003.
- Park JH, Kim YJ, Binn K, Seo KH. 2018. Spread of multidrug-resistant *Escherichia coli* harboring integron via swine farm waste water treatment plant. *Ecotoxicology and*

- environmental safety*. 149:36-42.doi:10.1016/j.ecoenv.2017.10.071.
- Prescott LM, Harley JP, Klein DA. 2002. Microbiology. 5th Ed. Boston: McGraw-Hill.
- Salasia, S. I. O., Khusnan, Z., Lammler, C., and Zschock, M. (2004) Comparative studies on pheno-and genotypic properties of *Staphylococcus aureus* isolated from bovine subclinical mastitis in central Java in Indonesia and Hesse in Germany. *J Vet. Sci.* 5 (2): 103–109.
- Susanto E. 2014. *Escherichia coli* Yang Resisten Terhadap Antibiotik Yang Diisolasi Dari Ayam Broiler Dan Ayam Lokal Di Kabupaten Bogor. Bogor: Institut Pertanian Bogor.
- Szmolka A, Nagy B. 2013. Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. *Frontiers in microbiology* [internet]. [diunduh Date Accessed];Volume(Issue):Pages. Tersedia ada: URL. doi:DOI].
- Todar, K. 2002, *Todar's Online Textbook of Bacteriology : Streptococcus pyogenes*, Departement of Bacteriology Universitas of Wisconsin, Madison.
- Wulf M, Voss A. 2008. MRSA in livestock animals-an epidemic waiting to happen? 14(6):519-521.doi:10.1111/j.1469-0691.2008.01970.x.
- Yenny, Herwana E. 2007. Resistensi dari bakteri enterik : aspek global terhadap antimikroba. *Universa Medicina*. 26 No. 1:46-56