Antibiotics Resistant At Staphylococcus Aureus And Streptococcus Sp Isolated From Bovine Mastitis In Karang ploso, East Java, Indonesia

by Jola Rahmahani

Submission date: 25-Jul-2022 12:36PM (UTC+0800)
Submission ID: 1874838099
File name: Antibiotics_Resistant_At_Staphylococcus_Aureus_And.pdf (233.65K)
Word count: 2731
Character count: 15165

DOI Number: 10.5958/0973-9130.2019.00329.3

Antibiotics Resistant At *Staphylococcus Aureus* And *Streptococcus Sp* Isolated From Bovine Mastitis In Karangploso, East Java, Indonesia

Aswin Rafif Khairullah[1], Dadik Raharjo[2], Jola Rahmahani[3], Suwarno[3], Wiwiek Tyasningsih[4] , Nenny Harijani[2]

[1]Master student of Disease Science and Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Mulyorejo, Kampus C Unair, Surabaya, 60115, Indonesia; [2]Laboratory of Veterinary Public Health Department Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Mulyorejo, Kampus C Unair, Surabaya, 60115, Indonesia; [3]Laboratory of Virology and Immunology Department Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Mulyorejo, Kampus C Unair, Surabaya, 60115, Indonesia, [4]Laboratory of Bacteriology and Micology Department Microbiology, Faculty of Veterinary Medicine; Universitas Airlangga, Jl. Mulyorejo, Kampus C Unair, Surabaya, 60115, Indonesia

Abstract

Karangploso, District Malang is known as a milk producing area and is a suitable place for the development of dairy cattle business. Mastitis is one of the most common problems found in dairy farming in Indonesia that has a negative impact on the milk production economy. The study was conducted to examine the incidence of antibiotic resistance as an attempt to appropriate treatment for the treatment of mastitis in dairy cows. A total of 85 positive samples are *Staphylococcus aureus* and *Streptococcus sp* from farms in the KUD Karangploso region. Based on the sensitivity test against various antibiotics it is known that *Staphylococcus aureus* has been resistant to Penicillin (100%), Tetracycline (48.23%), Erythromycin (44.70%), Gentamicin (2.35%), and Ampicillin (1.18%). For *Streptococcus sp* has been resistant to antibiotic Penicillin (98.82%), Erythromycin (94.18%), Tetracycline (84.70%), Gentamicin (11.76%), Ampicillin (5.88%), and Cefalexin (3.53%).

Key words: *Subclinical mastitis, California Mastitis Test (CMT), Staphylococcus aureus and, Streptococcus sp, antibiotics resistant*

Introduction

Karangploso, District Malang is known as a milk producing area and is a suitable place for the development of dairy cattle business. This was marked by the operation of KUD Karangploso which was the second largest KUD after the SAE Pujon Cooperative with a total milk production of 5,000 - 6,000 liters per day.[1] Milk is one source of animal protein that is important for the growth and repair of cells and is able to increase the intelligence of the human brain.[2] To get high dairy products, it is important to pay attention to the maintenance management of dairy cows. One of the factors that greatly affects the success of dairy farming is the cleanliness factor. The environment of a cage that is not clean and milking that is not hygienic can disrupt livestock activities and also can cause germs, one of the diseases that can attack dairy cattle is mastitis.[3] Mastitis is still a major problem in dairy farming because it causes considerable losses due to reduced production, high quality of milk, high treatment and treatment costs.[4] Two pathogenic bacteria that were often found in subclinical mastitis cases in cow udders, namely *Streptococcus sp* ranged from 92% and *Staphylococcus aureus* ranged from 67%.[5] During this time mastitis treatment was done by using antibiotics, improper use of antibiotics caused the effects of antibiotic residues in milk, allergies, resistance and affected the processing

Corresponding author :
Dadik Raharjo,
email : dadik_tdc@yahoo.co.id

of milk products.[2] Research needs to be done to determine the presence of Staphylococcus aureus and Streptococcus sp bacteria as the cause of mastitis, and to check the level of antibiotic resistance as an effort for the right treatment for the treatment of mastitis in dairy cattle.

Materials and Method

Research Samples

CMT examination and milk sampling were carried out in 3 villages in the Karangploso KUD area, Malang Regency namely Bocek Village (41 cows), Leban Village (23 cows), Donowarih Village (39 cows). The total number of CMT examinations carried out was 103 cows. Milk samples taken only in cattle detected mastitis, milk samples taken at each nipple (except nipples that do not work).

Milk Test with *California Mastitis Test* (CMT)

The test was carried out by taking 2 ml of milk placed on paddle then reacted with California Mastitis Test (CMT) reagent as much as 2 ml.

Milk Sampling

Squeeze the nipples that will be taken by milk samples. Immediately close the test tube after obtaining a sample volume that is cooled and labeled. Store samples in a cool box with a temperature of 4 - 5 ° C.

Isolation and identification of *Staphylococcus aureus*

For isolation and identification of Staphylococcus aureus dipped ose in the sample then streak in the form of zig zag lines on MSA isolation media. Media incubation at 37 ºC for 24 hours.

Catalase Test of *Staphylococcus aureus*

Colonies from MSA media were taken using ose then mixed with a drop of 3% on a glass object, then observed.

Coagulase Test of *Staphylococcus aureus*

The colony was taken from MSA media using ose, then put it into 4 ml Lurea Bertani Broth media and incubated for 24 hours at 37 ºC. After incubation, prepare 1 ml of rabbit plasma and then mixed until evenly using vortex then incubated for 24 hours.

Isolation and identification of *Streptococcus sp*

For isolation and identification of Streptococcus sp dipped ose in the sample then streak in the form of zig zag line on the isolation medium NA. Media incubation at 37 ºC for 24 hours.

Catalase Test of *Streptococcus sp*

Colonies from NA media were taken using ose then mixed with a drop of 3% on a glass object, then observed.

Christie, Atkins, Munch – Peterson Test (CAMP Test) of *Streptococcus sp*

CAMPtest was carried out on BloodAgar Media with *Staphylococcus aureus* as a marker of *Staphylococcus aureus* planted by making a line in the middle of BAP media using ose. Then plant Streptococcus sp. form a perpendicular line with *Staphylococcus aureus*. The culture was incubated for 24 hours at 37 ºC.

Making Suspension of *Staphylococcus aureus* and *Streptococcus sp*

To test the resistance of Staphylococcus aureus and Streptococcus sp bacteria, resistance testing was carried out by making a bacterial suspension with turbidity indicators equated with Mc Farland standard number 0.5 containing / ml of bacteria.

Resistance Test of *Staphylococcus aureus* and *Streptococcus sp*

After conforming to the Mc Farland turbidity standard number 0.5, inoculate 0.2 ml of sample suspension on MHA media with a cutton bud and then place the paper disk of Penicillin antibiotics, Gentamicin, Erythromycin, Ampicilin, Tetracycline, Cloxacilin, Cefalexin, and Kanamycin on top with a little pressure to attach perfectly to the MHA media. Then incubate 37 ºC for 24 hours. The test results show that there is a clear zone around the paperdisk as a barrier to bacterial growth.

Results and Discussion

Milk Test with California Mastitis Test (CMT)

The results of this study indicate that from 103 dairy cows that have been examined by California Mastitis Test (CMT) in 3 villages in KUD Karangploso area

(Bocek village, Leban village, Donowarih village), 22 dairy cows have shown positive results of subclinical mastitis (21.36%). And of the 22 dairy cows that were positive for subclinical mastitis, 85 milk samples were obtained.

California Mastitis Test (CMT) is one of the methods of diagnosis of subclinical mastitis which until now is considered simple and fast, namely the method by using a tool called paddle and using CMT reagents to determine the severity of subclinical mastitis experienced. This reaction is characterized by the presence or absence of changes in milk viscosity, then determined based on the California Mastitis Test (CMT) test that is (-) negative there is no precipitate in milk, (+1) there is a little sediment in milk, (+2) there are deposits it is clear but the gel has not yet formed, (+3) the mixture thickens and begins to form the gel, and (+4) the gel formed causes the surface to become convex.[6]

Isolation and identification of *Staphylococcus aureus* and *Streptococcus sp*

Based on table 1 the results of the *Staphylococcus aureus* identification test in the table, it showed 100% positive results in catalase and coagulase tests from 85 samples.

Staphylococcus aureus produces catalase enzyme which is able to hydrolyze hydrogen peroxide into water and gas bubbles .[7] To prove that the isolate is *Staphylococcus aureus* then followed by coagulase test, coagulase is an extracellular protein produced by *Staphylococcus aureus* which can clot plasma.[8]

Based on table 2 the results of the identification test of *Streptococcus sp* in the table, it showed a negative result of 100% in the catalase test and a positive result of 0.05% in the CAMP test of 85 samples.

Based on the results of isolation on NA media there are cultures that are very diverse, but only a few colonies that have the characteristics of *Streptococcus sp* bacteria are round, small colonies with varying diameters (0.5-2 mm), smooth, transparent and convex.[9] Based on the results of a positive catalase test *Streptococcus sp* when no bubbles are formed on glass objects that have been dripped . Streptococcus sp does not produce the enzyme catalase to hydrolyze hydrogen peroxide into water and gas bubbles.[10] *Streptococcus sp*, specifically *Streptococcus agalactiae* increases hemolytic activity in Staphylococcal β-toxin to form arrow-like signs on CAMP reactions.[11]

Antibiotics Resistant Test of *Staphylococcus aureus*

From the results of Table 3 above, Staphylococcus aureus was resistant to Penicillin (100%), Tetracycline (48.23%), Erythromycin (44.70%). Sensitive to Cloxacilin (100%), Canamycin (100%), Cephalexin (80%).

Staphylococcus aureus bacterial resistance test showed very high resistance to Penicillin antibiotics. Mechanism of *Staphylococcus aureus* resistance to penicillin groups due to antibiotic inactivation by beta-lactamase, modification of target PBPs, damage to drug penetration into target PBPs, and the presence of an outflow pump producing beta-lactamase is the most common resistance mechanism.[12] *Staphylococcus aureus* bacterial resistance test showed high resistance to tetracycline antibiotics. The mechanism of tetracycline resistance when these broad-spectrum antibiotics inhibit bacterial protein synthesis by binding to the 16S part of the 30S ribosome subunit, thus preventing the aminoacyl-tRNA from attaching to the active site of the ribosome, this bond is naturally reversible, interfering with the attachment of tRNA that carries amino acids to the 30S ribosome from the 70S ribosome.[13] *Staphylococcus aureus* bacterial resistance test showed high resistance to Erythromycin antibiotics. The main mechanism of Erythromycin resistance is based on the RNA methylase enzyme which adds a methyl group to a specific adenine group in the 50S rRNA subunit. Macrolide antibiotics including erythromycin will not be bound to rRNA rRNA which is methylated.[14]

Antibiotics Resistant Test of *Streptococcus sp*

From the results of table 4 above shows *Streptococcus sp* has been resistant to Penicillin antibiotics (98.82%), Erythromycin (94.18%), Tetracycline (84.70%). Sensitive to Cloxacilin (98.82%), Ampicilin (89.41%), Gentamicin (84.70%).

Streptococcus sp bacterial resistance test showed sensitive results that were quite high against Penicillin antibiotics. The resistance of *Streptococcus sp* to penicillin can arise as a result of mutations that result in the production of different penicillin binders or due to

bacteria requiring new penicillin-acting protein genes.[15] *Streptococcus sp* bacterial resistance test showed high resistance to Erythromycin antibiotics. Erythromycin resistance in *Streptococcus sp* because there is a change in the erythromycin L4 or L12 protein coding gene in the 50S bacterial ribosome subunit, resulting in a decrease in erythromycin affinity for ribosomes.[14] *Streptococcus sp* bacterial resistance test showed high resistance to tetracycline antibiotics. The resistance of *Streptococcus sp* to tetracycline can arise because different cytoplasmic membranes are produced (changes) and prevent tetracycline binding to the 30S ribosomal subunit, so that protein synthesis can continue. Another mechanism for tetracycline resistance is eflux pump resistance, based on tetracycline transport out of cells rapidly, thus preventing tetracycline accumulation at toxic doses, so bacterial protein synthesis is not inhibited.[14]

Pratiwi, Sylvia T. Pharmaceutical microbiology. Erlangga. Jakarta. 2008.

O'Leary, William. Practical Handbook of Microbiology. CRC Press Inc. Boca Raton. 1989.

Table 1 Results of *Staphylococcus aureus* identification test

Milk Sample	Identification Test			
	Catalase		Coagulase	
	Positive	Negative	Positive	Negative
85 sample	85 sample (100%)	0 sample (0%)	85 sample (100%)	0 sample (0%)

Table 2 Results of *Streptococcus* identification test

Milk Sample	Identification Test			
	Catalase		CAMP test	
	Positive	Negative	Positive	Negative
85 sample	0 sample (0%)	85 sample (100%)	5 sample (5,88%)	80 sample (94,18%)

Table 3 Results of *Staphylococcus aureus* resistance test according to the number of samples

Milk Sample	Criteria	Antibiotic Type							
		Tetracycline	Gentamicin	Penicilin	Eritromicin	Ampicilin	Cloxacilin	Cephalexin	Canamicin
85	S	34 (40%)	82 (96,47%)	- (0%)	17 (20%)	83 (97,65%)	85 (100%)	68 (80%)	85 (100%)
	I	10 (11,76%)	1 (1,18%)	- (0%)	30 (35,29%)	1 (1,18%)	- (0%)	17 (20%)	- (0%)
	R	41 (48,23%)	2 (2,35%)	85 (100%)	38 (44,70%)	1 (1,18%)	- (0%)	- (0%)	- (0%)

Notice : S = Sensitive I = Intermediate R = Resistant

Table 4 Results of *Streptococcus sp* resistance test according to the number of samples

Milk Sample	Criteria	Antibiotics Type							
		Tetracycline	Gentamicin	Penicilin	Eritromicin	Ampicilin	Cloxacilin	Cephalexin	Canamicin
85	S	8 (9,41%)	72 (84,70%)	1 (1,18%)	1 (1,18%)	76 (89,41%)	84 (98,82%)	40 (47,05%)	50 (58,82%)
	I	5 (5,88%)	3 (3,53%)	- (0%)	4 (4,70%)	4 (4,70%)	1 (1,18%)	42 (49,41)	35 (41,18)
	R	72 (84,70%)	10 (11,76%)	84 (98,82%)	80 (94,18%)	5 (5,88%)	- (0%)	3 (3,53%)	- (0%)

Notice : S = Sensitive I = Intermediate R = Resistant

Conclusion

Based on the test of bacterial resistance to various antibiotics from milk samples suspected mastitis in the KUD District Karangploso, Malang Regency it was found that *Staphylococcus aureus* was resistant to Penicillin (100%), Tetracycline (48.23%), Erythromycin (44.70%), Gentamicin (2.35%), and Ampicillin (1.18%). For *Streptococcus sp* was resistant to Penicillin antibiotics (98.82%), Erythromycin (94.18%), Tetracycline (84.70%), Gentamicin (11.76%), Ampicillin (5.88%), and Cephalexin (3.53%).

Competing Interests: The authors declare that they have no competing interests.

Source of Funding: The authors would like to thank the my parents, Drs. Gurendi Wiwoho, M.Pd. and Dr. Dra. Karmanis Karolan, M.Si

Ethical approval: The research does not need ethical approval. However, samples were collected as per standart collection methods without any harm and stress to the animals.

References

1. Indonesian Milk Cooperative Association. GKSI Member Cooperative Data. East Java. Malang. 2011.
2. Sudarwanto, M. Milkcheker, a tool for detecting subclinical mastitis. 1999. Med Vet (4): 1.
3. Wahyuni, A.E.T.H., Wibawan, I.W.T., and Wibowo, M.H. Characterization of hemagglutinin *streptococcus agalactiae* and *staphylococcus aureus* causes subclinical mastitis in dairy cattle. Veteteriner Sain Journal, Microbiology Section of FKH-UGM, Yogyakarta. 2005. Vol. 23 No. 2.
4. Wibawan, I.W.T., Pasaribu, F.H., Huminto, H., and Estuningsih, S. Characteristics of *Streptococcus Agalactiae* Biovar as a Guide to Cross Infection Between Cows and Humans. Competitive Grant Report IV Stage-1. Faculty of Veterinary Medicine, Bogor Agricultural University. 1997.
5. Sarwono, B. and Arianto, H.B. Fattening Cattle Quickly. Self-helpers. Jakarta. 2002.
6. Setiawan, T and Tanius, A. Etawa Breeding Goat Breeding. Issue 1. Penebar Swadaya, Jakarta. 2003.
7. Toelle, N.N., and Viktor, L. Identification and Characteristics of *Staphylococcus sp*. and *Streptococcus sp*. From Ovarian Infection in Commercial Laying Chicks. 2014.
8. Dewi, K.A. Isolation, Identification and Sensitivity Test of *Staphylococcus aureus* against Amoxicillin from Ettawa (PE) Goat Milk Samples of Mastitis Patients in Girimulyo Region, Kulonprogo, Yogyakarta. Veterinary Sain Journal. 2014. 31: 2. 140-141.
9. Poeloengan, M.. Effect of Lemongrass Essential Oil (Andropogon citratus) on Bacteria Isolated from Subclinical Mastitis Cows, Research Journal, Center for Veterinary Research, Bogor. 2009.
10. Lay, B.W. Analysis of Microbes in the Laboratory. Rajawali Press. Jakarta. 1994.
11. Lang, S. and Palmer, M. Characterization of

Streptococcus agalactiae CAMP factor as a pore-forming toxin. J Biol Chem. 2003. 278(40):38167–38173.

12. Katzung, B. G. Basic Pharmacology and Clinics. XIII Edition. Book 3. Translation of Basic and Clinical Pharmacology Eight Edition Subtitles by Pharmacology Section Faculty of Medicine, Airlangga University. Jakarta: Salemba Medik. 2004.
13. Yuwono, T. Molecular Biology. Laboratory of Microbiology, Faculty of Agriculture, Gadjah Mada University. 209 - 215. Jakarta. Erlangga. 2009.

Antibiotics Resistant At Staphylococcus Aureus And Streptococcus Sp Isolated From Bovine Mastitis In Karang ploso, East Java, Indonesia

ORIGINALITY REPORT

3%	3%	2%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.frontiersin.org Internet Source	1%
2	www.lia-edu.ca Internet Source	1%
3	B J Tuasikal, T Handayani, D Priyoatmojo, A C Trinugraha, T Tjiptosumirat. "Characterizations of Streptococcus agalactiae from subclinical mastitis cases in dairy cow", IOP Conference Series: Materials Science and Engineering, 2021 Publication	<1%
4	D. Sergelidis, T. Papadopoulos, D. Komodromos, E. Sergelidou, T. Lazou, M. Papagianni, A. Zdragas, A. Papa. " Isolation of methicillin-resistant from small ruminants and their meat at slaughter and retail level in Greece ", Letters in Applied Microbiology, 2015 Publication	<1%

5	Thirumananseri Kumarevel, Hiroshi Mizuno, Penmetcha K. R. Kumar. "Structural basis of HutP-mediated anti-termination and roles of the Mg2+ ion and L-histidine ligand", Nature, 2005 Publication	<1 %
6	etd.repository.ugm.ac.id Internet Source	<1 %

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		

Antibiotics Resistant At Staphylococcus Aureus And Streptococcus Sp Isolated From Bovine Mastitis In Karang ploso, East Java, Indonesia

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6