International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.8, pp 326-331, 2016

The Resistance Patterns of Bacteria *Staphylococcus Aureus* against Various Antibiotics

Dahliatul Qosimah[1]*, and Supriyanto[2]

[1]Laboratory of Microbiology and Imunology, Brawijaya University, Indonesia
[2] Ministry of Agriculture, Indonesia

Abstract : Mastitis is a disease in cattle that is highly infectious, characterized by inflammation of the udder gland and can cause economic losses extremely high either decrease in the quality or quantity of milk. Mastitis incidence in Indonesia is largely a subclinical mastitis caused by Staphylococcus aureus bacteria. Currently the treatment of mastitis less effective because antibiotics are used less specific and not in accordance with causing bacteria. The aim of research to determine the resistance patterns of bacteria Staphylococcus aureus as a cause mastitis against various antibiotics. The sample used for the research were 22 isolates of Staphylococcus aureus originating from cows with subclinical mastitis that had been characterized from a dairy farm. Furthermore test of the sensitivity of bacteria to various antibiotics such as penicillin G, streptomycin, Amoxicillin, Tetracycline, Gentamycin, Chloramphenicol, Methicillin, Ciprofloxacin, Erythromycin and Trimethoprim+ Sulfamethoxazole using the Kirby-Bauer disk diffusion by measuring the diameter of inhibition zone. The results showed that Staphylococcus aureus isolates were resistant to antibiotics penicillin G, Amoxicillin, and Methicillin with the percentage of consecutive 14 isolates (63.6%), 18 isolates (81.8%), and 17 isolates (77.3%) while still as much as 100% sensitive to Streptomycin, Tetracycline, Gentamycin, Chloramphenicol, Ciprofloxacin, Erythromycin and Trimethoprim + Sulfamethoxazole. Conclusion that Staphylococcus aureus is still sensitive to non-beta-lactam group of antibiotics.
Keywords : Coccus Bacteria, mastitis, antibiotics, resistant.

1. Introduction

Mastitis is a disease that is highly infectious to cattle, especially dairy cattle and caused enormous economic losses to the farms industry [1] which is characterized by a decrease in the quality and quantity of milk due to inflammation in the mammary gland. Mastitis is also a zoonotic potential to cause a risk to human health through the food chain by consuming unsterilized milk [2]. In several cases of mastitis can also cause poisoning and premature death in cattle[3]. Approximately 65% indicated that the infection mastitis caused by S. aureus bacteria associated with biofilm formation[4].

Mastitis is classified into two groups based on the cause, namely: environmental mastitis and mastitis kontagius. Environmental mastitis, is caused by bacteria *Eschericia coli* (*E. coli*), *Streptococcus uberis*, *S. bovis, Klebsiella pneumonia (K. pneumonia), K. oxytoca, Enterobacter aerogenes* and also Coagulase Negative Staphylococcus [2,3] that infects and enter into nipple when cattle contact with contaminated or dirty environment [3] as well as some of contagious mastitis such as *Staphyloccocus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae* and Mycoplasma[2]. Bacteria *Staphylococcus aureus* (S. *aureus*) is a major pathogen of mastitis.

Mastitis can occur in two forms, namely clinical and subclinical manifestations. Bacteria S. uberis, Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa and Negative coagulase Staphylococcus is a clinical mastitis, while S. agalactiae and Enterococcus spp. cause subclinical mastitis. Subclinical mastitis is more harmful than clinical mastitis due to decreased quality and milk production without causing obvious clinical manifestations and often spread on a dairy farm[5]. S. aureus bacteria can cause mastitis both manifestations [2].

The pathogenesis of mastitis mediated by extracellular factors, toxins, invasive components such as adherence, biofilm formation and resistance to phagocytosis [6] be possessed by S. aureus bacteria. Some strains of bacteria can produce virulence factors such as hemolysins (K, L, Q or N), coagulase, leukocidin, Enterotoxins (AE), and toxic shock syndrome toxin 1 (TSST-1) 7. S. aureus bacteria that is transmitted during the milking process from infected udders gland to healthy udder subsequently colonize the teat orifice, teat canal and damaged skin epithelium[8], causing the gland inflmasi udder. Based on a survey conducted in Canadia showed that 19.8% and 48.5% of cases of mastitis in dairy cows occured in the phase of lactation[9].

Mastitis disease is still a major problem that until now there has been no settlement is mainly caused by the bacterium S. aureus. More than 60 years, antibiotics used in livestock to limit the spread of infectious diseases, improve the prognosis of the disease so that it can reduce the morbidity and mortality[10]. Despite mastitis has been treated with antibiotics but the is still frequently appears. Antibiotics commonly used single or in combination to improve the status of dairy cattle caused by mastitis through [11] healthy controls.

Handling of mastitis depends on many factors such as lactation period, the position of infected quarters, and the number of somatic cells of bacteria [4]. Successful treatment is determined by the accuracy of diagnosing microorganisms. Bacteria that infects cattle if that fails to respond to antibiotic will extend the curative to the livestock at risk of death or culling[12]. This causes farmers suffered economic losses high. Test sensitivity of S. aureus to antibiotics needs to be done to determine the appropriate type of antibiotic to kill the bacteria as cause mastitis so that to optimize the treatment and reduce the occurrence of bacteria resistance.

2. Experimental

2.1 Material

The materials used in this study were 22 isolates of Staphylococcus aureus (S. aureus) is obtained from Supriyanto, which comes from cows infected by subclinical mastitis based on CMT (California Mastitis Test) which showed positive one (+) to positive 4 (+ 4) and test Breed. Then Milk samples were grown in media Mannitol Salt Agar (Oxoid), subsequently staining gram, catalase test and coagulase test [5] for identification of S. aureus bacteria.

2.3 Antibiotic Sensitivity Test

Test sensitivity of Staphylococcus aureus to antibiotics using Disk diffusion method of Kirby-Bauer by planting a S. aureus bacterial suspension having a concentration in accordance with the standards of the Mac Farland 0.5 (bacterial concentration of about 108 CFU / ml) on MHA medium (Muller Hinton Agar) with dispersive method using sterile cotton bud and then put the paper in the test antibiotic disc on the media then media were incubated at 37ºC for 24 hours and observed and measured the formation of a clear inhibition zone diameter [11,13,14]. Diameters were measured matched to table the National Committee for Clinical Laboratory Standards (NCCLS) 1997[15.16] that shows antibiotics criteria: sensitive, Intermediate, and Resistant. Paper disk antibiotics (Oxoid) was used such as penicillin G, streptomycin, Amoxicillin, Tetracycline, Gentamycin, Chloramphenicol, Methicillin, Ciprofloxacin, Erythromycin and Trimethoprim + Sulfamethoxazole.

2.4 Analisis Statistika

Data is presented in descriptive form

3. Results And Discussion

The bacterial causes subclinical mastitis mostly has characteristics such as coccus, Gram-positive and catalase negative. Among all Gram-positive coccus bacteria, S. aureus bacteria commonly infects cattle, causing mastitis [5]. The opportunistic bacteria Staphylococcus sp, may persist and multiplication in the environment for

long periods and often cause disease in animals and humans such as mastitis, synovitis, arthritis, endometritis, furuncles, suppurative dermatitis, pyemia, and septicemia[6.17].

Pathogenesis of mastitis begins with S. aureus bacteria attach, adapt and multiply in the milk then enter to the top of the mammary glands and produce toxin. After that bacteria will stimulate macrophage activation and migration of neutrophils from blood to milk (causing an increase in somatic cells, mammary gland inflammation, and disorders of host immune cells)[6].

The use of antibiotics is often used not only as medicine but also as a growth promoter that causes resistance antibiotic [5]. The results showed that S. aureus isolates were resistant more than 50% to β-lactam antibiotics group test like penicillin, Amoxicillin and Methicillin, respectively : 14 isolates (63.6%), 18 isolates (81.8%), and 17 isolates (77.3%) were shown in table 1. The high resistance of S. aureus bacteria to penicillin was equally with the study is conducted by[16] showed that 196 strains of S. aureus are resistant to penicillin 52.1%. Penicillin is a group of beta-lactam antibiotics. The occurrence of bacteria resistance to penicillin caused by bacterial beta-lactamase enzymes which have a structure similar to penicillin, there are four atoms in its molecular structure that works to break down or inactivate penicillin by hydrolyzing the beta-lactam ring of beta-lactam antibiotics [18]. The mechanism of antibiotic to inhibit bacterial cell wall synthesis. Approximately 50% of S. aureus strains produce beta-lactamase enzymes[12]. Additionally antibiotic bacteria resistance to antibiotics is influenced by their ability to form biofilms so that bacteria easily adhesi, invade and multiplication in cow mammary gland epithelial cells resulting in increased somatic cell bacteria and leukocytes. The increase in inflammatory cells will cause a decrease in milk quality[5].

Table 1. The Sensitivity test of *S. aureus* bacteria to various antibiotics

No	The sensitivity of *Staphylococcus aureus* to antibiotics																			
	1 *P (mm)*		2 *S (mm)*		3 *Amx (mm)*		4 *Te (mm)*		5 *CN (mm)*		6 *C (mm)*		7 *Met (mm)*		8 *Cip (mm)*		9 *E (mm)*		10 *SXT (mm)*	
1.	29	(S)	18	(I)	19	(R)	26	(S)	24	(S)	27	(S)	6	(R)	30	(S)	30	(S)	34	(S)
2.	23	(R)	19	(I)	1	(R)	25	(S)	25	(S)	26	(S)	6	(R)	34	(S)	35	(S)	36	(S)
3.	23	(R)	18	(I)	20	(S)	25	(S)	26	(S)	35	(S)	6	(R)	35	(S)	36	(S)	34	(S)
4.	24	(R)	19	(I)	17	(R)	23	(S)	24	(S)	33	(S)	7	(R)	34	(S)	33	(S)	32	(S)
5.	32	(S)	17	(I)	19	(R)	25	(S)	23	(S)	30	(S)	6	(R)	33	(S)	30	(S)	33	(S)
6.	23	(R)	19	(I)	18	(R)	24	(S)	24	(S)	> 40	(S)	6	(R)	> 40	(S)	> 40	(S)	> 40	(S)
7.	25	(R)	16	(I)	17	(R)	23	(S)	25	(S)	26	(S)	9	(I)	31	(S)	35	(S)	36	(S)
8.	33	(S)	19	(I)	20	(S)	25	(S)	26	(S)	31	(S)	9	(I)	33	(S)	33	(S)	32	(S)
9.	23	(S)	17	(I)	19	(R)	23	(S)	24	(S)	28	(S)	6	(R)	31	(S)	33	(S)	32	(S)
10.	24	(R)	18	(I)	19	(R)	22	(S)	26	(S)	29	(S)	8	(R)	32	(S)	31	(S)	33	(S)
11.	30	(S)	23	(S)	17	(R)	25	(S)	27	(S)	31	(S)	6	(R)	33	(S)	34	(S)	30	(S)
12.	23	(R)	16	(I)	18	(R)	27	(S)	25	(S)	31	(S)	6	(R)	34	(S)	35	(S)	31	(S)
13.	21	(R)	16	(I)	17	(R)	29	(S)	23	(S)	29	(S)	8	(R)	37	(S)	33	(S)	32	(S)
14.	25	(R)	18	(I)	16	(R)	24	(S)	24	(S)	32	(S)	9	(I)	36	(S)	32	(S)	35	(S)
15.	29	(S)	16	(I)	19	(R)	27	(S)	24	(S)	31	(S)	6	(R)	34	(S)	33	(S)	37	(S)
16.	20	(R)	19	(I)	20	(S)	29	(S)	26	(S)	34	(S)	7	(R)	32	(S)	36	(S)	34	(S)
17.	23	(R)	12	(R)	19	(R)	28	(S)	24	(S)	35	(S)	9	(I)	33	(S)	31	(S)	35	(S)
18.	25	(R)	18	(I)	18	(R)	26	(S)	27	(S)	29	(S)	9	(I)	30	(S)	34	(S)	34	(S)
19.	32	(S)	19	(I)	17	(R)	27	(S)	24	(S)	31	(S)	7	(R)	32	(S)	36	(S)	35	(S)
20.	29	(S)	16	(I)	19	(R)	27	(S)	25	(S)	32	(S)	6	(R)	33	(S)	38	(S)	36	(S)
21.	22	(R)	17	(I)	18	(R)	26	(S)	25	(S)	26	(S)	6	(R)	32	(S)	36	(S)	35	(S)
22.	21	(R)	18	(I)	20	(S)	29	(S)	25	(S)	29	(S)	7	(R)	31	(S)	35	(S)	37	(S)
	S : 8		S : 1		S : 4		S : 22		S : 22		S : 22		S : -		S : 22		S : 22		S : 22	
	I :-		I : 20		I : -		I : -		I : -		I : -		I : 5		I : -		I : -		I : -	
TOTAL	R : 14		R : 1		R : 18		R : -		R : -		R : -		R : 17		R : -		R : -		R : -	

Description of code : P : Penicillin G 10 IU; S : Streptomycin 25µg; Amx : Amoxicillin 25µg; Te : Tetracycline 30µg; CN : Gentamycin 10µg; C : Chloramphenicol 30µg; Met : Methicillin 5µg; Cip : Ciprofloxacin 5µg; E : Erythromycin 15µg; SXT: Trimethoprim + Sulfamethoxazole 10µg

Bacteria isolates test there were no sensitive to the antibiotic methicillin because it has beta-lactamase enzymes such as penicillin. The emergence of strains of Methicillin resistant Staphylococcus aureus (MRSA) is a major problem in animal and human health [20]. This contrasts with research conducted by [21] which indicated that S. aureus bacteria isolated from cow's milk mastitis was still methicillin-sensitive so that it commonly used in the veterinary field [16].

All test bacteria as much as 100% were still sensitive to the antibiotic Tetracycline, Gentamycin, Chloramphenicol, Ciprofloxacin, and Erythromycin and Trimethoprim + Sulfamethoxazole. This was according to research conducted by [22] that S. aureus bacteria were derived from chicken is still sensitive to the antibiotic such as Tetracycline and Trimethoprim + Sulfamethoxazole. The high sensitivity of bacteria to amynoglikosida groups such as gentamycin and streptomycin because of both those antibiotics are still rare against mastitis cases in Indonesia. This study was contrary to that carried out by [12] in Uruguay which showed that the bacterium S. aureus had high resistance to the antibiotic trimethoprim / sulphamethoxazole (90%, 100%), and streptomycin (96%, 95%) because of the abundant use to both antibiotics and also the price is cheap so it is often used for curing cattle without considering the need to be done test to the sensitivity of bacteria to antibiotics. Mechanism of action of antibiotics Gentamycin by inhibiting bacterial DNA replication that would stop the bacterial cell replication and causing bacterial cell death [23].

The incidence of mastitis can only be reduced through a combination of sanitation programs, vaccination, improved nutrition and antibiotics [12]. Recurrent infections of subclinical mastitis caused by the bacteria's ability still persist in the udder host so it is difficult to reach by antibiotics. Antibiotics used during the mastitis is broad spectrum and bactericidal but mostly kills Gram-positive bacteria through intramamamry. Antibiotics are often used for mastitis, have the ability to penetrate the mammary gland include sulfonamides, penicillins, aminoglycosides, and early-generation cephalosporins [24].

Conclusion

Isolates of Staphylococcus aureus bacteria are resistant to beta lactam group of antibiotics such as penicillin, Amoxicillin and Methicillin but still sensitive to Streptomycin, Tetracycline, Gentamycin, Chloramphenicol, Ciprofloxacin, Erythromycin and Trimethoprim+ Sulfamethoxazole.

Acknowledgement

Researchers say thank to the DVM Supriyanto willing to provide samples of Staphylococcus aureus bacteria to be used in this study.

References

1. Gillespie, B.E and Oliver, S.P., Simultaneous Detection of Mastitis Pathogens, Staphylococcus aureus, Streptococcus uberis, and *Streptococcus agalactiae* by Multiplex Real-Time Polymerase Chain Reaction. J. Dairy Sci, 2005, 88, 3510–3518.
2. Cervinkova, D., Vlkova, H., Borodacova, I., Makovcova, J., Babak, V., Lorencova, A., Vrtkova, Marosevic, D and Jaglic, Z., Prevalence Of Mastitis Pathogens In Milk From Clinically Healthy Cows. *Veterinarni Medicina,* 2013, 58, 11: 567–575.
3. Kudi, A.C., Bray, M.B., and Niba, A.T., Mastitis Causing Pathogens within the Dairy Cattle Environment. International Journal of Biology, 2009, 1, 1, 3-13.
4. Moore, G. E., "Biofilm Production by Streptococcus uberis Associated with Intramammary Infections".University of Tennessee Honors Thesis Projects. http://trace.tennessee.edu/utk_chanhonoproj/1299, 2009.
5. Parada1, J.L., Gonçalves, D., Soccol, V.T., Lima, M and Soccol, C.R., Bovine Mastitis in the Metropolitan Area of Curitiba: Antibiotic Resistance and Antimicrobial Control of the Infection. Braz. Arch. Biol. Technol, 2011, 54, 4, 709-715.
6. Cucarella, C., Tormo, M.A.N., U´ Beda, C., Trotonda, M.P., Monzo, M., Crito`Fol Peris, Amorena, B., Lasa, N And Penade, J.R. Role Of Biofilm-Associated Protein Bap In The Pathogenesis Of Bovine *Staphylococcus Aureus.* Infection And Immunity, 2004, 72, 4, 2177–2185.

7. Leitner, G., Krifucks, O., Glickman, A., Younis, A and Saran, A., Staphylococcus aureus strains isolated from bovine mastitis: virulence, antibody production and protection from challenge in a mouse model. FEMS Immunology and Medical Microbiology, 2003, 35, 99-106.
8. Petersson-Wolfe, C.C., Mullarky, I.K and Jones, G. M., *Staphylococcus aureus* Mastitis: Cause, Detection, and Control. Virginia Tech Invent The Future, 2010.
9. Gill, J.J., Pacan, J.C., Carson, M.E., Leslie, K.E., Griffiths, M.W and P. M. Sabour., Efficacy and Pharmacokinetics of Bacteriophage Therapy in Treatment of Subclinical Staphylococcus aureus Mastitis in Lactating Dairy Cattle American Society for Microbiology. Antimicrobial Agents and Chemotherapy, 2006,50, 9, 2912–2918.
10. Omran, L and Askar, E., Antibiotic Sensitivity Patterns of the Most Common Bacteria Isolated from Al-Mouwasat University Hospitalin 2015, Syria. International Journal of Pharmtecht Research, 2016, 9, 1, 113-119.
11. Pillar , C. M., Stoneburner , A., Shinabarger , D.L., Abbeloos , E and Goby, L., In vitro susceptibility of bovine mastitis pathogens to a combination of penicillin and framycetin: Development of interpretive criteria for testing by broth microdilution and disk diffusion. J. Dairy Sci. 2014, 97, 6594–6607.
12. Alekish, M.O., Al-Qudah, K.M and Al-Saleh, A., Prevalence Of Antimicrobial Resistance Among Bacterial Pathogens Isolated From Bovine Mastitis In Northern Jordan. *Revue Méd. Vét.*, 2013, 164, 6, 319-326.
13. Prabu J. and Johnson., Hydrothermal Synthesis of ZnO Nano-Honeycomb Structures and their Activity against Pathogenic Bacteria. International Journal of PharmTech Research, 2015,.8, .6, 65-71
14. Kamali, M., Khosroyar, S and Mohammadi, A., Antibacterial activity of various extracts from Dracocephalum kotschyi against food pathogenic microorganisms. International Journal of PharmTech Research, 2015, 8, 9, 158-163.
15. Dzen, S.M., Santoso, S., Roekistiningsih, dan Santosaningsih, D., Perbedaan Pola Resistensi *Staphylococcus Koagulase Negatif* Isolat Darah Terhadap Antibiotika Di Rsu Dr Saiful Anwar Malang Tahun 2000-2001 Dengan 2004-2005. Jurnal Kedokteran Brawijaya, 2005, XXI, 3.
16. Pitkala¨, A., Haveri, M., Pyorala, S., Myllys, V and Honkanen-Bu, T., Bovine Mastitis in Finland 2001—Prevalence, Distribution of Bacteria, and Antimicrobial Resistance. J. Dairy Sci, 2004, 87, 2433–2441.
17. Nadjir, L. K., Ayimba1, E., Alfa, T., Kokou, A. Dakey, Medhat, M. Abozid, Midzodzi P, and Kpotsra, A., Ameyapoh, Y., Nour-Eddine, C, and Drider, D., Salmonella typhimurium and Staphylococcus epidermidis biofilms resistance to Chlorinated water on plastic surface. International Journal of PharmTech Research, 2014, 6, 6, 1846-1843.
18. Sah, S.J and Hemalatha, S., Extended spectrum Beta lactamase (ESBL) Mechanism of antibiotic resistance and Epidemiology. International Journal of PharmTech Research, 2015, 7, 2, 303-309.
19. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. 2011. CLSI document M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute.
20. Karsono, Patilaya, P, Azisah, N and Nerdy. Comparison of Antimicrobial Activity of Red Betel (Piper Crocatum Ruiz & Pav) Leaves Nanoparticle and Powder Ethanolic Extract against Methicillin Resistant Staphylococcus Aureus. International Journal of PharmTech Research, 2015, 8, 4, 696-701
21. Cucarella, C., Solano, C., Valle, J., Amorena, B., Lasa, N and Penadés, J.R., Bap, a *Staphylococcus aureus* Surface Protein Involved in Biofilm Formation. J. Bacteriol., 2001, 18, 9, 2888-2896.
22. Amri, I.A., Murwani, S., Qosimah, D and Pradikta, R.A., Multiple Resistance of Pathogenic Bacteria in Poultry from Malang District, Indonesia. International Journal of ChemTech Research, 2016, 9, 06, 214-224.
23. Onanuga, A. Oyi, A. R., Olayinka, B.O. And Onaolapo, J. A., Prevalence Of Community-Associated Multi-Resistant *Staphylococcus Aureus* Among Healthy Women In Abuja, Nigeria. African Journal Of Biotechnology, 4, 9, 942-945.
24. Bogni, C., Odierno, L., Raspanti, C., Giraudo, J., Larriestra, A., Reinoso, E., Lasagno, M., Ferrari, M., Ducrós, E., Frigerio, C., Bettera, S., Pellegrino, M., Frola, I., Dieser, S and Vissio, C. War against mastitis: Current concepts on controlling bovine mastitis pathogens. Science against microbial pathogens: communicating current research and technological, 2011.

*****