PROFILE OF ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI* IN MASTITIS FROM THE ENVIRONMENT OF DAIRY COW FARMING IN PANGALENGAN, WEST JAVA

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Abstract

Mastitis is a disease in cattle caused by E. coli bacteria. Pangalengan is a center for dairy farming that is still commonly found in cases of mastitis. The handling of mastitis cases is done by giving antibiotics. This study describes the detection of resistant antibiotics in E. coli that causes mastitis in milk and the livestock environment including water and cage floors. 192 subclinical mastitis milk samples, 9 water samples, and 9 cage floor swab samples were taken, identification of E. coli by planting on MacConkey agar and Levine-EMB agar, gram staining and biochemical testing of indole test. After that, antibiotic resistance testing is done using the Kirby Bauer method. The results showed that the highest resistance to Ampicillin (100%) in all samples, Sulphamethoxazole-trimethoprim (83%), and Oxytetracycline (66%) in the cage floor samples. This shows that the use of antibiotics is inappropriate and uncontrolled so that antibiotic resistance appears in E. coli in Pangalengan cattle farms, West Java.

Key words: Mastitis, Antimicrobial resistance, *E. coli*

INTRODUCTION

Mastitis is a disease in cattle that harms farmers because they cannot produce good quality milk. Manifestations of mastitis consist of clinical and subclinical. [4]. Mastitis occurs due to a bacterial infection that infects the udder of the cow. The main bacteria that infect the udder mastitis are *Staphylococcus aureus*, but there are also other bacteria such as *Streptococcus dysgalactiae*, or enterobacteria such as *Escherichia coli, Serratia* and *Klebsiella* [11]. Several studies indicate that *E. coli* is a pathogen that is often found in cases of mastitis after *S. aureus* [5].

Pangalengan is a center for dairy farming which some of the farms do not apply good farm management standards, so there are still many cases of clinical and subclinical mastitis. Prevention and treatment of mastitis can be done with a variety of farm management procedures, for example by maintaining the farm environment, staff hygiene, vaccination in cattle, antibiotic therapy, and good sanitation [6].

Treatment of mastitis is carried out with antibiotic therapy without following the direction of the dose given and the use of antibiotics is not appropriate by the breeder can cause antibiotic resistance, causing treatment failure [3]. Antibiotics used in the treatment of mastitis are beta-lactam antibiotics, fluoroquinolones [8], aminoglycosides, sulphonamide, chloramphenicol, and tetracycline [10].

This research will explain the detection of resistant antibiotics in *E. coli* as a cause of mastitis cases originating from milk, cage water, cow bedding, and feces. This research is expected to be able to find specific characteristics of resistant antibiotics so that effective prevention and treatment can be

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carried out especially in the area of Pangalengan Cattle Farming in West Java.

MATERIAL AND METHOD

Sampling

This study used milk, water, and cage floor samples taken from a dairy farm in Pangalengan, West Java. Milk samples of 192 subclinical mastitis samples that were previously carried out the CMT Test were put into sterile bottles. Water samples were 9 samples, and cage floor swabs were 9 samples.

Bacterial Isolation

The samples of milk, water, and cage floor swabs obtained were planted on MacConkey agar (Oxoid) and Levine-EMB Agar (Merck). Cage water samples were put into a sterile container and then diluted 10-5 by the scatter method and incubated for 24 hours at 37 ° C. Cow floor cage samples were carried out with a swab using a sterile cotton bud. *E. coli* which is pink on the McConkey agar medium and metallic green on the EMB agar is then selected and gram staining and indole test are performed.

Antibiotic susceptibility test

Selected *E. coli* were tested for antibiotic susceptibility by the Kirby-Bauer method, using the Mueller Hinton Agar (MHA) medium, then as much as 0.5 mc Farland, the bacteria in the swab used a sterile cotton bud to cover all areas of the MHA media, after 5 minutes it was placed Antibiotic discs Ampicillin (10 mcg), Chloramphenicol (30 mcg), Gentamicin (10 mcg), Oxytetracycline (30 mcg), Sulphamethoxazole-trimethoprim (25 mcg) and Ciprofloxacin (5mcg) (Oxoid).

RESULTS AND DISCUSSIONS

This study produced 172 (75%) *E. coli* isolates from 228 samples studied from the results of identification with morphological identification tests on MacConkey and Levine-EMB Agar media, as well as gram staining. Biochemical testing is done by an indole test. The morphology of *E. coli* on MacConkey media is characterized by the presence of lactose fermentation colonies, dry, pink, and surrounded by dark pink sediments [1]. *E. coli* in Levine-EMB agar is characterized by the presence of metallic green color in agar media [7]. Gram staining in *E. coli* is characterized by the morphology of the rod-shaped and red bacteria because it includes gram-negative bacteria that absorb the color of safranin. [1]. Indole testing on *E. coli* positivity was marked by the presence of a red ring layer after the addition of the Kovacs reagent [9].

The prevalence of *E. coli* in milk samples was found as many as 136 (70.8%) of 192 milk samples taken. This indicates the presence of *E. coli* contamination in milked milk glands. *E. coli* is normally present in the digestive tract of warm-blooded animals [6]. The cause of *E. coli* entry into the mammary gland is caused by poor farm management, lack of hygienic enclosure and unhygienic sanitation which has the potential to spread *E. coli* such as sleeping mats, milking devices, breeder hands, flies and contracting from other cows [2]. The udder infection is caused because Gram-negative bacteria enter the udder channel and multiply in the mammary glands. Although the mammary gland is not a natural habitat for coliform bacteria, many strains are able to survive and multiply in the mammary glands [6]. Public farms managed by cattle ranchers do not usually meet livestock standards, so this is the cause of cases of mastitis in Indonesia is still high, in contrast to farms managed by private companies that have met the standards of dairy farming management.

The results of antibiotic resistance tests on milk samples (Figure 1) show a high level of resistance to ampicillin antibiotics that is equal to 100% while other antibiotics still show high sensitivity such as the antibiotics Sulphamethoxazole-trimethoprim (SxT), Chloramphenicol (C), Oxytetracycline (OT), Gentamicin (Cn). Ciprofloxacin in milk samples showed an intermediate percentage of 70%. Other levels of resistance can also be found in cage water samples (Figure 2), ampicillin antibiotics showed a percentage of 100% followed by Sulphamethoxazole-trimethoprim at 66% and Oxytetracycline 13%. In the cage floor sample (Figure 3), the highest resistance level was also found in ampicillin antibiotics by 100%, followed by the antibiotic

Sulphamethoxazole-trimethoprim by 83% and Oxytetracycline 66%.

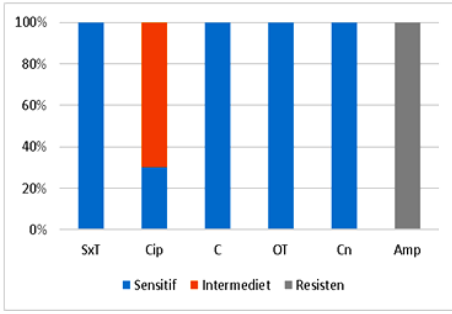

Fig 1. Percentage prevalence of *E. coli* Resistant, Intermediate and Sensitive to the antibiotics of Sulphamethoxazole-trimethoprim (SxT), Ciprofloxacin (CIP), Chloramphenicol (C), Oxytetracycline (OT), Gentamicin (Cn) and Ampicillin (Amp) in Cow Milk Samples

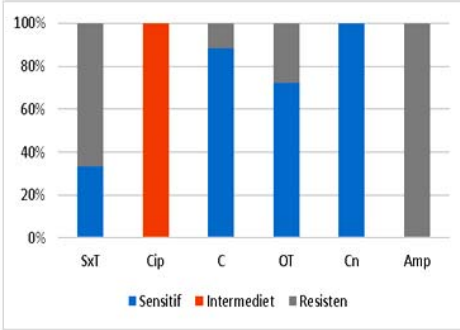

Fig 2. Percentage prevalence of *E. coli* Resistant, Intermediate and Sensitive to the antibiotics of Sulphamethoxazole-trimethoprim (SxT), Ciprofloxacin (CIP), Chloramphenicol (C), Oxytetracycline (OT), Gentamicin (Cn) and Ampicillin (Amp) in Water Cage Samples

The similarity in the antibiotic model does not differ greatly, indicating that there is an association of *E. coli* bacteria in a water environment sample and the cage floor with *E. coli* causing subclinical mastitis, this is also supported by research [5] which states that the similarity of patterns resistance to milk and water indicates the potential for contamination of milk containing *E. coli* from water or other environmental sources.

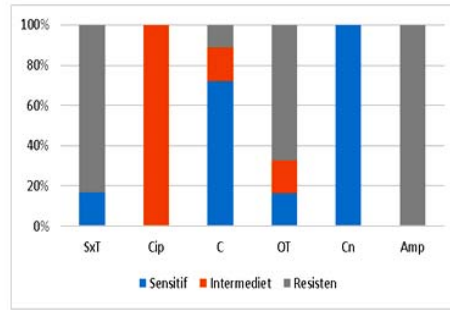

Fig 3. Percentage prevalence of *E. coli* Resistant, Intermediate and Sensitive to the antibiotics of Sulphamethoxazole-trimethoprim (SxT), Ciprofloxacin (CIP), Chloramphenicol (C), Oxytetracycline (OT), Gentamicin (Cn) and Ampicillin (Amp) in Cage Floor Samples

Based on the three samples tested, special attention is needed to ampicillin antibiotics that are not effective in killing *E. coli* in both milk samples and cattle breeding environments in Pangalengan, West Java such as cage water and cage floor, also with high antibiotic resistance to Sulphamethoxazole-Trimethropim and Oxytetracycline. Ciprofloxacin antibiotics that are at the intermediate level of milk, water and cage floor samples can also be potentially resistant, if the use is not appropriate or not controlled.

CONCLUSIONS

This study shows that *E. coli* causes mastitis in dairy farms in Pangalengan, West Java, which has high resistance especially to ampicillin 100% antibiotics found in milk samples and environmental samples such as water and cage floor. Likewise with high resistance to sulphamethoxazole-trimethoprim antibiotics and oxytetracycline in water samples and cage floors. Therefore, more appropriate antibiotic treatment is needed so that the treatment of mastitis can be cured, as well as more stringent use of antibiotics to prevent antibiotic resistance in the future.

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REFERENCES

[1] Baban, Soza. (2017). Prevalence and Antimicrobial Susceptibility Pattern of Extended Spectrum Beta-Lactamase-Producing Escherichia coli Isolated from Urinary Tract Infection among Infants and Young Children in Erbil City.

[2] Derakhshani, H., Fehr, K. B., Sepehri, S., Francoz, D., De Buck, J., Barkema, H. W., … Khafipour, E. (2018). Invited review: Microbiota of the bovine udder: Contributing factors and potential implications for udder health and mastitis susceptibility. Journal of Dairy Science, 101(12), 10605–10625. https://doi.org/10.3168/jds.2018-14860

[3] Fazel, F., Jamshidi, A., & Khoramian, B. (2019). Phenotypic and genotypic study on antimicrobial resistance patterns of *E. coli* isolates from bovine mastitis. Microbial Pathogenesis, 132(May), 355–361. https://doi.org/10.1016/j.micpath.2019.05.018

[4] Gonçalves, J. L., Kamphuis, C., Martins, C. M. M. R., Barreiro, J. R., Tomazi, T., Gameiro, A. H., … dos Santos, M. V. (2018). Bovine subclinical mastitis reduces milk yield and economic return. Livestock Science, 210(June 2017), 25–32. https://doi.org/10.1016/j.livsci.2018.01.016

[5] Hinthong, W., Pumipuntu, N., Santajit, S., Kulpeanprasit, S., Buranasinsup, S., Sookrung, N., … Indrawattana, N. (2017). Detection and drug resistance profile of Escherichia coli from subclinical mastitis cows and water supply in dairy farms in Saraburi Province, Thailand. PeerJ, 2017(6), e3431. https://doi.org/10.7717/peerj.3431

[6] Hogan, J., & Smith, K. L. (2003). Coliform mastitis. Veterinary research, 34(5), 507-519.

[7] Leininger, Dagny & Roberson, Jerry & Elvinger, François. (2001). Use of Eosin Methylene Blue Agar to Differentiate Escherichia Coli from Other Gram-Negative Mastitis Pathogens. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc. 13. 273-5.

[8] Liu, Y., Liu, G., Liu, W., Liu, Y., Ali, T., Chen, W., … Han, B. (2014). Phylogenetic group, virulence factors and antimicrobial resistance of Escherichia coli associated with bovine mastitis. Research in Microbiology, 165(4), 273–277. https://doi.org/10.1016/j.resmic.2014.03.007

[9] MacWilliams, M. P. (2012). Indole test protocol. American Society for Microbiology, Washington, DC.

[10] Saini, V., McClure, J. T., Léger, D., Keefe, G. P., Scholl, D. T., Morck, D. W., & Barkema, H. W. (2012). Antimicrobial resistance profiles of common mastitis pathogens on Canadian dairy farms. Journal of Dairy Science, 95(8), 4319–4332. https://doi.org/10.3168/jds.2012-5373

[11] Zadoks, R. N., Middleton, J. R., McDougall, S., Katholm, J., & Schukken, Y. H. (2011). Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. Journal of Mammary Gland Biology and Neoplasia, 16(4), 357–372. https://doi.org/10.1007/s10911-011-9236-y