

Antimicrobial resistance in coagulase-negative staphylococci isolated from subclinical mastitis in Ettawa Crossbred goat (PE) in Yogyakarta, Indonesia

WIDODO SUWITO^{1,*}, WIDAGDO SRI NUGROHO², AGNESIA ENDANG TRI HASTUTI WAHYUNI³,
BAMBANG SUMIARTO^{2,**}

¹Assessment Institute for Agricultural Technology of Yogyakarta. Jl. Stadion Maguwoharjo No. 22, Wedomartani, Ngemplak, Sleman 55584, Yogyakarta, Indonesia. Tel./fax.: +62-274-447052, *email: widodosuwito6@gmail.com

²Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Jl. Fauna No. 2, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia. Tel./fax.: +62-274-560861, **email: b.sumiarto@gmail.com

³Department of Microbiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Jl. Fauna No. 2, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia

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Abstract. Suwito W, Nugroho WS, Wahyuni AETH, Sumiarto B. 2021. Antimicrobial resistance in coagulase-negative staphylococci isolated from subclinical mastitis in Ettawa Crossbred goat (PE) in Yogyakarta, Indonesia. *Biodiversitas* 22: 3418-3422. Subclinical mastitis (SCM) in Ettawa Crossbred Goat (PE) is most frequently caused by staphylococci with a significant reduction in milk yield. The aim of this study is to determine antimicrobial resistance patterns of coagulase-negative staphylococci (CoNS) from PE goat SCM. A total of 36 CoNS isolates originating from PE goat SCM were collected in semisolid tube use in this study. All CoNS isolates were further examined for antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. Antibiotic susceptibility of CoNS isolated samples according to Clinical Laboratory Standards Institute (CLSI). The CoNS isolates showed the highest resistance rate against sulfamethoxazole (65%), ampicillin (55.56%), penicillin (45%), cefoxitin (33.33%), erythromycin (25%), oxytetracycline (20%), tetracycline (15%), gentamicin and neomycin (11.11%), while oxacillin was sensitive. The highest of multiple antimicrobials resistance observed 15% in ampicillin, penicillin and tetracycline, then 5-10% in ampicillin, penicillin, erythromycin, tetracycline and oxytetracycline. The majority of CoNS in this study were resistant to sulfamethoxazole and then, followed by ampicillin, penicillin, cefoxitin, erythromycin, oxytetracycline, tetracycline, gentamicin and neomycin. In addition, most isolates were penicillin-resistant and multidrug-resistant (MDR).

Keywords: Antimicrobial, goat, staphylococci, subclinical mastitis

INTRODUCTION

Ettawa Crossbred Goat (PE) is the product of crossing Ettawah Goat of Jamanapari, India origin with local goat from Indonesia such as Kacang goat. Characteristically PE goat has a curved nose, horned, long ear drops up to 30 cm, long leg feathers with stripe white and black color. The body height of buck PE reaches 90-127 cm, while the body of doe PE is 76-92 cm. The bodyweight of buck PE is 68-91 kg. The average of milk production PE goat is 1340.00±76.38 mL/head/day (Suranindyah et al. 2018).

PE goat much more is found in Yogyakarta, especially in Sleman regency with a total 1.749 population. Sleman regency located at 100-2.500 m above sea level with a total area 57.428 ha, 107°15' 03" and 107°15' 03" coordinate, 107° 29' 30" East Longitude (BT), 7°34' 51" and 7°47' 30" South Latitude (LS) (Government of Sleman 2013). Sleman regency has a tropical climate with a rainfall average of 16.1 mm and a maximum speed of wind of 5.92 knots and a minimum of 1.3 knots. Consequently, Sleman regency has humidity relative 95.1% with the temperature at 21.5-33.8 °C.

Subclinical mastitis (SCM) is responsible for diseases in milk yield decrease in dairy goat herds (Koop et al.

2010). Subclinical mastitis characterized by an increased number of somatic cell counts (SCC) in milk without inflammation of the udder and when tested by California Mastitis Test (CMT) agglutination occurred (Carla et al. 2015). The CMT, qualitative measurement of the SCC in milk, is a screening test for SCM. The principle of the CMT test is indirect detection of total SCC in milk (Persson and Olofsson 2011).

Staphylococci bacteria have been reported as a common pathogen group associated with SCM in dairy goats (Padhy et al. 2015). The staphylococci bacteria group that causes more SCM in goats is coagulase-negative staphylococci (CoNS) (Mishra et al. 2018). The SCM in dairy goat, CoNS makes up 44.7% to 95.9% of the isolated pathogens from milk samples and *Staphylococcus aureus* (*S. aureus*) is usually considered to have the greater pathogenicity, accounts from 4.1% to 18.0% of SCM agents (Virdis et al. 2010).

In Yogyakarta, SCM in PE goats is rarely diagnosed by veterinarians. It is caused that SCM in PE goat no clinical symptom and CMT test is rarely done by veterinarians. Administration antibiotic in PE goat given during dry period for reducing the incidence of SCM. The administration of antibiotics at the end of a lactation period

will complement the above procedures and will contribute to improved mammary health for the forthcoming lactation period (Petridis and Fthenakis 2014).

Currently, in Yogyakarta, antibiotics are sold freely without the control of veterinarians. It causes the PE goat farmers to easily obtain the antibiotics and freely use them. Administration of antibiotics without paying attention to the right dose may lead to antibiotic resistance. Concomitant with the increased number of antibiotics sold freely in Yogyakarta, especially to PE goat farmers, the aim of this research is to determine antimicrobial resistance patterns of CoNS from PE goat SCM.

MATERIALS AND METHODS

Animal ethical commission

Letter of approval from the Animal Ethical Commission is not required in this study because of no invasive procedure in the PE goat.

Collection of samples

A total of 150 PE goat lactation from 25 PE goat farms located in individual and communal houses in Yogyakarta, Indonesia is using in this study. Samples of PE goat milk collected when the milking process in the morning. The PE goat milk samples were aseptically collected from the udder healthy PE goat that PE goat farmer property in Yogyakarta, Indonesia. Briefly, the teats were wiped with swabs soaked in 70% ethanol, and thereafter, few streams of milk were discarded. The CMT test PE goat has done in the farm location and then, 10-15 mL of milk was collected into a sterile tube, labeled, and immediately brought to the Veterinary Public Health, Faculty of Veterinary Medicine, UGM, Yogyakarta for bacteriological examination.

Determination of SCM in PE goat

The CMT is using in the determination of SCM in PE goat. Briefly, the CMT assay was done by mixing 3-4 ml of PE goat milk with equal volume CMT reagent and then circular motion. The PE goat was called SCM if CMT assay showed a score of +2 or +3 and occur the gel formation (Persson and Olofsson 2011).

Bacteriological examination of milk samples

Bacteriological examination was performed according to Bacteriological Analytical Manual (BAM 2011). Briefly, 0.01 mL of milk with CMT positive +2 and +3 were streaked onto a half of a Mannitol Salt Agar plate (MSA CM:005, Oxoid Ltd., Basingstoke, United Kingdom) and overnight incubation at 37°C. The colony was confirmed by morphology, Gram staining, catalase, and tube coagulase test. CNS isolates were stored at Brain-Heart semi-solid medium (BHI CM:1135, Oxoid Ltd., Basingstoke, United Kingdom) with 15% (wt/vol) glycerol at refrigerator until further identification.

Antibiotic susceptibility

An antibiotic susceptibility test was performed using the disc diffusion method (CLSI 2018). Pure cultures of isolates CNS from PE goat SCM were standardized to 0.5 CFUs/mL McFarland standard. Mueller Hinton Agar plate (MHA; CM:0037, Oxoid Ltd., Basingstoke, United Kingdom) were inoculated with standardized inoculums of the test organism. Ten antibiotics (Thermo Fisher Scientific, Oxoid Ltd) were used for the susceptibility test in this study. The antibiotic discs were used ampicillin (10 µg), cefoxitin (30 µg), erythromycin (15 µg), gentamicin (10 µg), neomycin (30 µg), oxytetracycline (30 µg), oxacillin (5 µg), penicillin (10 IU), sulfamethoxazole (300 µg) and tetracycline (30 µg). The discs were placed on the Mueller Hinton Agar plate (MHA; CM:0337, Oxoid Ltd., Basingstoke, United Kingdom) surface. Zone of inhibition (mm) produced by each disc antibiotic after overnight incubation at 37°C was measured and then compared with CLSI breakpoints (CLSI 2018).

Data analysis

Data obtained from the CMT test, bacteriological examination of milk samples and antibiotic susceptibility were analyzed by using SPSS 25.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Determination of SCM

Determination of SCM in PE goat from in Yogyakarta based on CMT is presented in Table 1. The PE goat milk samples with CMT scores of 0 and +1 were considered as negative, while the samples with scores +2 and +3 were taken as positive. A total of 91 PE goat's milk were done a test by CMT and it showed that 54 samples were identified as SCM (59.34%) and non-SCM in 37 samples (40.7%).

Table 1. Determination of SCM in PE goat from Yogyakarta, Indonesia based on CMT

Goat milk samples	CMT assay	
	(-)	(≥++)
PE goat milk (n: 91)	37	54
Total (%)	40.7	59.3

Note: (-): Negative SCM; (≥ ++): Positive SCM

Table 2. Isolation staphylococci from PE goat milk SCM

Sample	Isolation bacteria		
	Staphylococci	Staphylococci	Other
	Coa+	Coa-	bacteria
PE goat milk (n: 54)	13	36	5
Total (%)	24.1	66.7	9.2

Table 3. Antibiotic susceptibility of CNS from PE goat SCM in Yogyakarta, Indonesia

Antibiotics	Number isolate (n: 36)			MDR(%)
	S (%)	R (%)	I (%)	
Ampicillin (10µg)* ^Δ	44.44	55.56	0	*15; ^Δ 5-10
Cefoxitin (30µg)	87	12	1	none
Erythromycin (15µg)	73	25	2	^Δ 5-10
Gentamicin (10µg)	88.89	11.11	0	none
Neomycin (30µg)	87.89	11.11	1	none
Oxacillin (5µg)	100	0	0	none
Oxytetracycline (30µg)	79	20	1	^Δ 5-10
Penicillin G (10 IU)* ^Δ	48	45	7	*15; ^Δ 5-10
Sulfamethoxazole (300µg)	32	65	3	none
Tetracycline (30µg)* ^Δ	84	15	1	*15; ^Δ 5-10

Note: S: Susceptible, R: Resistant, I: Intermediate, MDR: Multidrug-resistant. *: multiple resistance penicillin G, ampicillin, and tetracycline. ^Δ: multiple resistance penicillin G, ampicillin, tetracycline, erythromycin, and oxytetracycline

Bacteriological examination

Subclinical mastitis in PE goat milk was caused by staphylococci Coa + in 13 samples (24.1%), Coa – in 36 samples (66.7%) and the other 5 samples (9.2%) were not associated with staphylococci is presented in Table 2.

Antibiotic susceptibility

Ampicillin, penicillin G, and sulfamethoxazole are antibiotics that presented a high level of resistance 55.56%, 45%, and 65%, respectively, and they are presented in Table 3. Multiple resistance occurred in ampicillin, penicillin G and tetracycline 15%, while in penicillin G and ampicillin are 5-10%.

Discussion

Mastitis in PE goats is an important disease, and it is responsible for serious economic loss to PE goat farmers in Yogyakarta mainly encountered in PE goats in subclinical form. In goat with SCM cannot be diagnosed by general physical examination such as palpation (Lakshmi and Jayavardhanan 2016). It is making goat farmers in Yogyakarta rarely knowing with SCM in PE goat. Overall, the occurrence of SCM in PE goats in Yogyakarta was assessed as 59.34%. This result is similar to that reported by Akter et al (2020). Some researchers reported that the prevalence of SCM in goats is 37.19%, 38%, 45.82%, and 50% (Islam et al. 2011; Pirezada et al. 2016; Zhao et al. 2015; Mahlangu et al. 2016). Many risk factors that affected the prevalence of SCM in goats such as milk production, age of goat, litter size, parity, body condition score (BCS), and weaning age (Margatho et al. 2019; Koop et al. 2010; Suwito et al. 2014). Prevalence SCM in Barbari and Jamunapari goats were found as 24.21% and 15.12%, respectively (Mishra et al. 2018). Prevalence SCM among the breeds of goat may be attributed to the difference in genetic resistance, hygiene, milking practices, management systems, and technical knowledge of the investigators along with methods used for the diagnosis of the disease (Islam et al. 2011).

Isolation of the bacteria associated with SCM in PE goat helps in designing effective preventive and control strategies. In our study, CoNS were found to be the most prevalent bacteria in causing SCM in the PE goats in

Yogyakarta. This result is in agreement with other studies that CoNS is the majority causal agent SCM in dairy goats (Bourabah et al. 2013; Salaberry et al. 2015; Dore et al. 2016). Commonly, CoNS was detecting in SCM goat milk and CoNS is frequently causes persistent SCM infection for several months, even though during the dry period (Gabli et al. 2019). In DIY, PE goat was still traditionally milked by hand and risk for staphylococci contaminant. The CoNS are opportunists and adhere to metal devices to produce a protective biofilm (Limoli et al. 2015). A biofilm is bunch of bacteria that attached to surface with carbohydrate covered which produced by this bacteria. The ability to produce biofilm causes CoNS to persist in milking equipment as well as in the milker's hands, which serves as a major of source staphylococcal spread (Lee et al. 2014).

Sulfamethoxazole is rarely used in treatment SCM in PE goats in Yogyakarta than ampicillin and penicillin G, but it is high quite of resistance. It was possible that caused by a genetic mutation in the plasmid. Sulfamethoxazole is often carried along with tetracycline resistance in plasmids (Munita and Arias 2016). An interesting in isolate observation is that high isolate resistant and multi-drug resistance (MDR) toward tetracycline and trimethoprim-sulfamethoxazole as reported in other studies, although these molecules are only occasionally used for treating mastitis (Saed and Ibrahim 2020). Staphylococci resistance to sulfamethoxazole is mediated by following five mechanisms such as permeability barrier or efflux pumps, naturally insensitive enzymes target, regulation changes in the enzymes target, change of mutational or recombinational in the enzymes target and acquired resistance by drug-resistant enzymes target (Munita and Arias 2016).

In our studies, resistance to penicillin and ampicillin are higher than the other countries such as Italy that it reached up to 12% (Viridis et al. 2010). Other researches were reported that resistance *S. aureus* toward penicillin in clinical mastitis (CM) and SCM at goat in Malaysia is 22% (Ariffin et al. 2019). The difference of resistance to antibiotics among the others countries is possibly be affected by some factors such as management in the farm, especially intensity application antibiotic. Carelessly and limitation PE goat farmers in using antibiotic is potential to

resistance. Penicillin and ampicillin are antibiotics that destroy the wall cell of Gram-positive bacteria (Munita and Arias 2016). The chemical structure of penicillin has a ring beta-lactam, whereas ampicillin is a derivative of penicillin. Resistance to penicillin and ampicillin in CoNS from PE goat SCM in Yogyakarta may be caused by produce beta-lactamase enzyme and destroy the ring beta-lactam. Consequently, it was required to substitute antibiotics such as oxacillin or cefoxitin.

The MDR isolate is a condition of bacterial isolate that is resistant to antibiotics at least ≥ 2 antibiotic groups (CLSI 2018). The MDR was discovered in CoNS from PE goat SCM in Yogyakarta about 5-15%. It was discovered in ampicillin, penicillin, and tetracycline (15%) and ampicillin, penicillin, erythromycin, tetracycline, and oxytetracycline (5-10%). The occurrence of MDR in PE goat in Yogyakarta is lower than in other countries which reached up to 44.4-73.86% (Ceniti et al. 2017). The majority of SCM at the cow in Uganda was caused by *S. aureus* and 71% showed MDR (Kasozi et al. 2014). Occurrence differences of MDR in CoNS isolate were caused by some factors such as antibiotic utilization in the PE goat farms. Penicillin, ampicillin, tetracycline, and oxytetracycline are widely used antibiotic in PE goat farms in Yogyakarta because it is easily and freely available for sale. Consequently, antimicrobial resistance (AMR) in ampicillin, penicillin G, tetracycline, and oxytetracycline much more occur than with other antibiotics. The AMR was caused by mutations protein on active site receptor that recognizes antibiotic and formation of plasmids that encode gene resistance to several antibiotics (Munita and Arias 2016). Plasmids are extrachromosomal deoxyribose nucleic acid (DNA), while episome is a plasmid that bounded at extrachromosomal DNA. R-plasmid is responsible for antibiotic resistance. R-plasmid consists of 2 units, namely Resistance Transfer Factor (RTF) segment and determinant-r (unit-r). The RTF segment is responsible for moving of R-plasmid, whereas unit-r is carried with properties antibiotic resistance (Munita and Arias 2016).

Occurrence of MDR in CoNS toward penicillin or ampicillin caused by staphylococci produces β -lactamase and destroys the structure of ring β -lactam at penicillin. Staphylococci with MDR are known as methicillin-resistant *S. aureus* (MRSA) and generally, the bacteria were resistant to β -lactam antibiotics. The MRSA was formed of substitution in gen that Penicillin Binding Protein (PBP2) encoding to PBP2a, so that active side receptors for β -lactam antibiotic cannot be recognized (Genovese et al. 2021). Antibiotic resistance occurred to change in the active side of amino acids into the other amino acid which occurs at several points, for example at position 106 serine change to amino acid glycine, serine 70 change to alanine and serine 42 change to asparagine (Munita and Arias 2016).

The majority of CoNS in this study were resistant to sulfamethoxazole and then, followed by ampicillin, penicillin, cefoxitin, erythromycin, oxytetracycline, tetracycline, gentamicin and neomycin. In addition, most isolates were penicillin-resistant and MDR.

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