Research Article

Prevalence and Antibiotic Susceptibility Pattern of *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* in Dairy Goats with Clinical and Subclinical Mastitis

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Abstract | Mastitis is a common disease in lactating goats that widespread throughout the world. This study aims to determine the prevalence of *Staphylococcus aureus* (*S. aureus*), *Streptococcus agalactiae* (*S. agalactiae*) and *Escherichia coli* (*E. coli*) in clinical and subclinical mastitis in does, and to identify the antibiotic susceptibility of the isolated bacteria. A total of 145 milk samples were collected from different farms located around Besut and Setiu districts, Terengganu, Peninsular Malaysia. All does were screened for subclinical mastitis using the California Mastitis Test. Signs of clinical mastitis were recorded. The milk samples were inoculated on Mannitol salt agar, blood agar and Eosin-Methylene Blue agar. The isolated bacterial colonies were subjected to Gram's staining and biochemical tests (catalase, coagulase, oxidase, triple sugar iron and Christie, Atkins, and Munch-Peterson tests) for identification. The susceptibility of *S. aureus*, *S. agalactiae*, and *E. coli* to the antibiotic was tested using disc diffusion assay. The prevalence of clinical and subclinical mastitis was 23% (34/145) and 40% (58/145) respectively. The bacteriological examinations revealed that 15/92 (16.3%), 6/92 (6.5%) and 1/92 (1.1%) of the mastitis samples were positive for *S. aureus, S. agalactiae,* and *E. coli* respectively. Overall resistance levels were very low except for *S. aureus* towards penicillin (22%) and tetracycline (11%) and for *S. agalactiae* towards penicillin (33.3%). In conclusion, *S. aureus* was the most frequently isolated organisms from the caprine mastitis followed by *S. agalactiae* and *E. coli* in the study area.

Keywords | Mastitis, Goats, *S. aureus*, *S. agalactiae*, *E. coli*, Antibiotic susceptibility

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INTRODUCTION

Mastitis is an inflammation of the parenchyma of the mammary gland. This condition is a major endemic disease that can affect all lactating animals (Rinaldi et al., 2010). It is a particular concern for farmers and veterinarian in developing countries like Malaysia. Depending on the severity of the disease, mastitis causes the majority of economic losses to the dairy producers. Costs associated with mastitis include reduced milk production and quality, increased veterinary cost, increased risk of culling of infected animals and deaths (Seegers et al., 2003).

Mastitis can be classified as clinical or subclinical. Dairy goats with clinical mastitis usually show signs of inflammation such as redness, heat, pain and swollen udders (Sarker and Samad, 2011). Visible abnormalities in the milk like clots, flakes, abnormal colour and blood are also common (Dasohari and Kandula, 2017). Goats with subclinical mastitis do not show apparent signs and commonly detected by elevation of somatic cell count (SCC) or by California Mastitis Test (CMT). Mastitis is caused by various pathogens as either contagious or environmental. The most frequently isolated pathogens include *Staphylococcus* spp., *Streptococcus* spp., *E. coli,* and *Pasteurella* spp. (Contreras et al., 2007).

Antimicrobial therapy is used to treat mastitis in small ruminants along with antibiotic susceptibility testing to ensure optimum therapeutic action (Landfried et al., 2018). The emergence of multidrug-resistant bacteria has become a major threat to animal and human health (Parra et al., 2017). This problem may not only limit the option for effective treatment but also spreading of the resistance genes from contaminated milk to human normal flora (Hameed et al., 2007).

In major part of Malaysia, the disease is insufficiently investigated and published information regarding to its magnitude and causative agents among small ruminant is scant. Therefore the present study aimed to determine the prevalence and major bacterial causes of mastitis in goats. The antibiotic susceptibility pattern of the isolated bacteria were also investigated.

MATERIALS AND METHODS

Sample Collection

A total of 145 raw milk samples were collected from two districts (Besut and Setiu) of Terengganu, Peninsular Malaysia during January to May 2018. Prior to sampling, all lactating animals were screened and scored for mastitis using the California Mastitis Test (CMT) according to the method described by Lucia et al. (2017). Any signs of clinical mastitis such as swelling, heat, hardness, redness, or pain of the udder were recorded. Approximately, 6 ml of mid-stream milk was collected from both udders. The milk was aseptically collected by hand-stripping and stored in a sterile polystyrene tube. After collection, samples were placed in the icebox and transported to the Microbiology Laboratory, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus for further processing and microbiological analysis.

Bacteriological Culture and Identification

Milk samples were plated on Mannitol salt agar (MSA), 5% goat blood agar and Eosin-Methylene Blue agar. The inoculated plates were incubated at 37°C for about 24 to 48 hours. Colonies suspected of being *S. aureus*, *S. agalactiae* and *E. coli* were initially identified by their colony morphology, Gram's staining and conventional biochemical tests (catalase, coagulase, oxidase, triple sugar iron (TSI) and Christie, Atkins, and Munch-Peterson (CAMP) tests) as per the method of Quinn et al. (2004).

Antibiotic Susceptibility Testing

The Kirby-Bauer disc diffusion method was used to determine the antibiotic susceptibility of isolates. A single colony from the fresh culture was picked with a sterile loop and suspended into Mueller Hinton Broth (MHB). The broth was then left at room temperature for 30 minutes. The densities of the bacterial suspensions were adjusted equal to the 0.5 McFarland standards. 10 µl from each isolate suspension was cultured on Mueller Hinton agar by sterile cotton swab. The susceptibility profiles of isolates towards different types of antibiotics are listed in Table 3 as suggested by Hinthong et al. (2017), Magiorakos et al. (2012) and Tomazi et al. (2018). The plates were incubated at 37 ºC for 18 to 24 hours. The breakpoints of Clinical Laboratory Standard Institute (CLSI) for the tested antibiotics were used to determine the susceptibility profiles. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were included as positive controls in each assay.

Statistical Analysis

The data generated from this experiment were organized in Microsoft Excel (2010) worksheet for further descriptive analyses. The prevalence (%) of *S. aureus*, *S. agalactiae* and *E. coli* mastitis was calculated by dividing the number of cases by the total number of the affected animals. One-way ANOVA was performed using GraphPad Prism for Windows, version 7 (GraphPad Software Incorporation, California, USA) to compare *S. aureus*, *S. agalactiae* and *E. coli* intramammary infection between the studied area and to compare between mastitis status.

RESULTS

Prevalence of *S. aureus*, *S. agalactiae* and *E. coli* in Dairy Goats with Clinical and Subclinical Mastitis

The findings of the CMT and clinical examination confirmed overall 63.4% prevalence of caprine mastitis in the study areas. Out of the 145 milk samples, 34 (23%) were clinical mastitis and 58 (40%) were subclinical mastitis respectively (Table 1). Our findings showed that the prevalence of mastitis varied with the categories of mastitis and the study area and the difference was significantly difference ($P<0.05$) (Figure 1). The bacteriological examinations revealed that 31/92 (33.7%), 6/92 (6.5%) and 1/92 (1.1%) of the mastitic samples were positive for *S. aureus*, *S. agalactiae* and *E. coli* (Table 2). There was no significant difference in finding bacteriologically positive samples between subclinical mastitis (n=58, 41.4%) and clinical mastitis (n=34, 41.2%) ($P>0.05$).

Table 1: Number and type of samples collected from goat mastitis.

Study area	Clinical mastitis	Subclinical mastitis	Total samples
Besut	3	19	22
Setiu	31	39	70
Total	34	58	92

Table 2: Prevalence of *S. aureus*, *S. agalactiae* and *E. coli* in goat mastitis in Besut and Setiu, Terengganu, Malaysia.

Besut, Terengganu									
Types of samples	**No. of samples**	***S. aureus***		***S. agalactiae***		***E. coli***		**Total isolates**	
		No	%	No	%	No	%	No	%
Clinical mastitis	3	0	0	1	33.0	0	0	1	33.3
Subclinical mastitis	19	8	44.4	0	0	0	0	8	44.4
Total	22	8	36.4	1	4.5	0	0	9	41.0
Setiu, Terengganu									
Types of samples	**No. of samples**	***S. aureus***		***S. agalactiae***		***E. coli***		**Total isolates**	
		No	%	No	%	No	%	No	%
Clinical mastitis	31	12	38.7	2	6.4	0	0	14	45.2
Subclinical mastitis	39	11	28.2	3	7.7	1	2.6	15	38.5
Total	70	23	32.8	5	7.1	1	1.4	29	41.4

Table 3: Overall antibiotic susceptibility pattern of *S. aureus*, *S. agalactiae* and *E.coli* isolated from clinical and subclinical mastitis in goats.

				Number of isolates (%)		
Isolates	**Antimicrobials**	**Symbol**	**Disk Potency (µg)**	**Resistant**	**Intermediate**	**Sensitive**
S. aureus	Penicillin	P	10	2 (6.45)	0 (0)	29 (93.55)
	Gentamicin	CN	10	0 (0)	0 (0)	31 (100)
	Nitrofurantoin	F	300	0 (0)	0 (0)	31 (100)
	Oxacillin	OX	1	0 (0)	0 (0)	31 (100)
	Cefoxitin	FOX	30	0 (0)	0 (0)	31 (100)
	Norfloxacin	NOR	10	0 (0)	0 (0)	31 (100)
	Tetracycline	TE	30	2 (6.45)	0 (0)	29 (93.55)
	Amikacin	AK	30	0 (0)	0 (0)	31 (100)
	Kanamycin	K	30	0 (0)	0 (0)	31 (100)
	Doxycycline	DO	5	0 (0)	0 (0)	31 (100)
	Cefotaxime	CTX	30	0 (0)	0 (0)	31 (100)
	Amoxicillin	AMC	30	0 (0)	0 (0)	31 (100)
	Chloramphenicol	C	30	0 (0)	0 (0)	31 (100)
S. agalactiae	Vancomycin	VA	30	0 (0)	0 (0)	6 (100)
	Tetracycline	TE	30	0 (0)	0 (0)	6 (100)
	Clindamycin	DA	2	0 (0)	0 (0)	6 (100)
	Chloramphenicol	C	30	0 (0)	0 (0)	6 (100)
	Penicillin	P	10	2 (33.33)	0 (0)	4 (66.67)
E. coli	Chloramphenicol	C	30	0 (0)	0 (0)	1 (100)
	Gentamicin	CN	10	0 (0)	0 (0)	1 (100)
	Imipenem	IPM	10	0 (0)	0 (0)	1 (100)
	Norfloxacin	NOR	10	0 (0)	0 (0)	1 (100)
	Meropenem	MEM	10	0 (0)	0 (0)	1 (100)

Antibiotic Susceptibility Test

Antibiotic susceptibility pattern of the isolated *S. aureus*, *S. agalactiae* and *E. coli* is shown in Table 3. Different level of susceptibility and resistance against some antibiotics was observed. Of the 31 isolates of *S. aureus,* 87% (27/31) showed full susceptibility to amoxicillin, gentamicin, nitrofurantoin, oxacillin, cefoxitin, norfloxacin, chloramphenicol, amikacin, kanamycin, doxycycline and cefotaxime. However, 6.45% (2/31) were resistant to penicillin and 6.45% (2/31) were resistant to tetracycline respectively. All of the penicillin and tetracycline-resistant isolates were from clinical mastitis.Of a total of 6 isolates of *S. agalac-*

tiae, 66.7% (4/6) showed full susceptibility to vancomycin, tetracycline and clindamycin. However, 33.3% (2/6) of *S. agalactiae* isolates isolated from clinical mastitis were resistant to penicillin. All of the *E. coli* isolates revealed full susceptibility to chloramphenicol, gentamicin, imipenem, norfloxacin and meropenem.

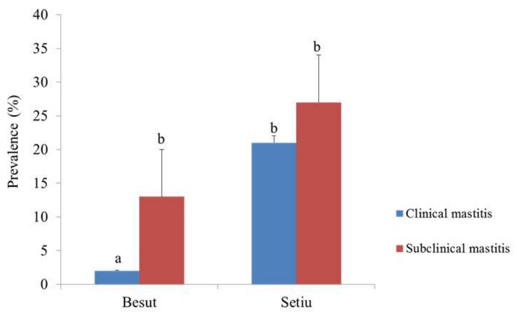

Figure 1: Prevalence of clinical and subclinical mastitis in the study area (P>0.05). Values (a & b) are significantly different within the study area according to category of mastitis

DISCUSSION

Mastitis is one of the important disease in goats that threaten public health and dairy industry worldwide. Diagnosis of clinical mastitis is straightforward, based on the appearance of the mammary gland and abnormality of the milk. In contrast, subclinical mastitis, which is a hidden form of intra-mammary infection is more problematic in term of diagnosis since the milk and udder appear normal. Diagnosis of subclinical mastitis routinely rely on direct measurement of the somatic cell count level or performing a CMT. In the present study, the prevalence of clinical and subclinical mastitis was 23% and 40% respectively. This result is in contrast with that of Abdalhamed et al. (2018) who recorded 7% and 11% prevalence rates of clinical and subclinical mastitis in goats based on the results of CMT. Previous study by Ebrahimi et al. (2007) reported mastitis was found in goats only with low prevalence (5.25%). However, higher mastitis prevalence (53.5%) in dairy goat was documented by Ali et al. (2010). The difference in the prevalence rates may be attributed to a difference in the study design, study area, geographical location, management system and time.

In the present study, the highest percent bacteria revealed were *S. aureus* (34%) followed by *S. agalactiae* (6.5%) and *E. coli* (1.1%). The lower isolation rate of *S. aureus* is contrary to the previous studies reported from Pakistan (Najeeb et al. 2013). The rate of *S. aureus* was stated as 61.6%, mainly isolated from subclinical mastitis cases in dairy goat. The prevalence of *S. aureus* mastitis in goat that has been reported by the other studies in Malaysia (20%) (Faiq et al., 2017) and China (15%) (Zhao et al., 2015) were considerably lower than the prevalence reported in this study. According to Rahman et al. (2016), different climatic conditions, herd management and diversity of breeds may explain the differences in the prevalence of *S. aureus* mastitis between studied areas. *S. aureus* can spread during manual contact of mammary gland by the herdsman, contaminated bedding material from infected does and lambs sucking does other than their dam (Rainard et al., 2018).

Caprine mastitis caused by *E. coli* can range from being a subclinical infection of the mammary gland to a severe systemic infection. In the current study, *E. coli* was detected in 1/58 (1.7%) subclinical cases. This result is in agreement with that reported by White and Hinckley (1999). Jeph (2012) reported that *E. coli* (19%) was the main aetiological agent responsible for clinical mastitis in goats. In another study by Pirzada et al. (2016) showed that the prevalence of *E. coli* mastitis was 5.26%. Recently, in Bangladesh, *E. coli* were isolated from 27.6% of clinical mastitis cases in doe (Rudra and Dutta, 2018). *E. coli* mastitis is mainly attributed to environmental factors such as low levels of hygiene, contaminated litter, milking and post-milking areas pollution may increase the likelihood of invading and colonization of the pathogens. Thus, low prevalence of *E. coli* mastitis seen in this study might be due to improvements in herd environmental management in the study areas.

Mastitis caused by *S. agalactiae* is often reported in dairy goats. In this study, 6/92 (6.5%) mastitis samples were positive for *S. agalactiae*. The low prevalence of *S. agalactiae* was comparable with the findings of previous studies (Pirzada et al., 2016; Wakwoya et al., 2006). According to Hawari et al. (2014), the prevalence of *S. agalactiae* mastitis can be increased as high as 30%. *S. agalactiae* mastitis is a highly contagious bacterial infection that can be easily transmitted from one animal to other lactating animals (Barkema et al., 2009).

Mastitis pathogens have been showed to change over time, therefore bacteriological monitoring need to be regularly executed at the herd level. Microbial culture is considered most reliable and economical method to identify the disease-causing pathogens. The gold standard for identifying intramammary infections is to perform a bacteriological milk culture, with identification of the pathogen and sensitivity testing. The purpose of antimicrobial susceptibility test is to determine which specific antibiotics a particular bacteria is sensitive or resistant to, thus helping in the choice of drug to be used for treatment.

The antibiotic susceptibility pattern of *S. aureus* reported by Shamila-Syuhada et al. (2016) is in agreement with the current study where 22% isolates showed resistant to penicillin. Our results are also in line with the work by Seyoum et al. (2018) who reported that *S. aureus* from mastitis milk has low tetracycline resistance rates (14.2%). Multiple drug resistance (MDR) among bacteria is a worldwide problem. The emergence of MDR is due to indiscriminate and prolonged use of antimicrobial agents (Wegener, 2012). Moreover, drinking mastitis infected milk may increase risk of transmitting antibiotic resistance genes to gut microbiota of human beings (Najeeb et al., 2013). *S. aureus* is remarkable in its ability to acquire resistance to any antibiotic (Chambers and DeLeo, 2009). In the present study, 33.3% of *S. agalactiae* were classified as penicillin-resistant. Our findings are similar to the results of the previous study with 57.1% antimicrobial resistance against *S. agalactiae* for penicillin (Najeeb et al., 2013). In this study, chloramphenicol, gentamicin, imipenem, norfloxacin and meropenem were found to be the most effective antimicrobials against *E. coli* isolates, confirming the data of Rudra and Dutta (2018). Vasquez-Garcia et al. (2017) reported that gentamicin was a less effective antimicrobial (50%) for *E. coli* isolates. In another study by Aydin et al. (2009) reported 100% resistance of *E. coli* isolates to norfloxacin which is in contrast with findings of the present study.

CONCLUSIONS

In conclusion, *S. aureus* was the most prevalent organisms in caprine mastitis followed by *S. agalactiae* and *E. coli* in the study area.The presence of *S. aureus*, *S. agalactiae* and *E. coli* from milk samples of both clinical and subclinical mastitis goats poses a threat to public health and dairy industry. Routine inspection with CMT is imperative in the diagnosis of mastitis, and bacterial isolation and antibiotic susceptibility testing are essential to control the disease and achieve effective treatment.

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CONFLICT OF INTEREST

None of the authors have any potential conflict of interest to declare.

AUTHORS CONTRIBUTION

All authors contributed equally.

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