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Prevalence and antimicrobial sensitivity pattern of *Staphylococcus aureus* isolated from clinical and subclinical mastitis in small ruminant in Besut and Setiu, Terengganu, Malaysia

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ABSTRACT

Aims: The study was carried out to investigate *Staphylococcus aureus* in clinical and subclinical mastitis in small ruminant and to identify the antibiotic sensitivity profiles of the isolates.
Methodology and result: A total of 171 milk samples from lactating sheep and goats were collected from Besut and Setiu districts in Terengganu, Peninsular Malaysia. All animals were screened for mastitis using the California Mastitis Test (CMT). Phenotypic identification of *S. aureus* was determined using Gram-staining, Catalase test, Coagulase test, and Oxidase test. The genotypic identification was conducted using Polymerase Chain Reaction (PCR) to detect the *nuc* gene. The susceptibility of *S. aureus* to the antibiotic was tested by using the Kirby-Bauer method. In this study, subclinical and clinical mastitis were detected in 66/171 (39%) and 41/171 (24%) respectively. The cultures and PCR results showed that 18/39 (46%) samples (9 subclinical and 9 clinical mastitis) were positive for *S. aureus*. The antimicrobial susceptibility tests profiles shows 4/18 (22%) and 2/18 (11%) isolates were resistant to penicillin and tetracycline, respectively. However, all isolates were *tet*K and *tet*M negative. On the other hand, these isolates susceptible to amoxicillin, gentamicin, nitrofurantoin, oxacillin, cefoxitin, norfloxacin, chloramphenicol, amikacin, kanamycin, doxycycline and cefotaxime.
Conclusion, significance and impact of study: The presence of *S. aureus* from milk samples of both clinical and subclinical mastitis goats indicates, potential hazard on the livestock as well as public health settings. The occurrence of penicillin and tetracycline resistance should not be undermined. Milk from mastitis samples may play an important role as potential reservoir and transmission of this pathogen in posing disease regardless of antibiotics resistance background.

Keywords: Staphylococcus aureus, mastitis, small ruminant, milk, antibiotic susceptibility pattern.

INTRODUCTION

Mastitis is one of the major health problem affecting sheep and goats. The disease has caused decrease in milk production and quality; increased veterinary cost, treatment and culling rate (DeGraves and Fetrow, 1993). Gelasakis *et al.* (2016) reported that total milk production may experience a 15% decline due to the mastitis. In another study by Taylor and Field (2009) showed the milk production has declined almost 30%. Mastitis is generally classified as clinical or subclinical depending on the degree of inflammation in the mammary gland. Clinical mastitis is characterized by visible abnormalities in the milk or the mammary gland, while subclinical mastitis does not show a visible udder inflammation or milk changes. Although the milk appears normal, subclinically infected goats and sheep can be a source of infection to other animals in the herd.

The affected animals may experience anxiety, restlessness, pain, changes in feeding and behavioral pattern (Gougoulis *et al*., 2008; Gelasakis *et al*., 2015). There are several pathogens that can cause mastitis infection, but the most frequently reported is the *Staphylococcus* spp. and the coagulase negative staphylococci (CNS) (Turutoglu *et al.*, 2006; Contreras *et al*., 2007; Gelasakis *et al*., 2015). *Staphylococcus aureus* is a major mastitis-causing pathogen and frequently isolated from outbreak and sporadic cases (Contreras *et*

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al., 2007). It has been reported that *S. aureus* represent the main agent for mastitis and is responsible for sub-clinical cases in small ruminants (Merz *et al*., 2016). Severe *S. aureus* intra-mammary infection may turn to gangrenous and death (Contreras *et al*., 2003). Colonization by *S. aureus* usually occurs on teat opening or chapped teat skin. The infection can spread through by milkers hands, milking equipment or aerosol spray of milk from infected animals. Contamination of milks may cause staphylococcal food poisoning worldwide, as many traditional caprine and ovine dairy products are not subjected to pasteurization (Oliver *et al*., 2009). Furthermore, *S. aureus* has the ability to produce enterotoxin that can withstand high temperature and contaminated pasteurized milk (Gran *et al.*, 2003).

Antimicrobial treatment is an important tool to control mastitis. However, extensive use and frequent misuse of antibiotics may result in development of microbial resistance (Libera *et al*., 2010). Antimicrobial resistance of *S. aureus* to multiple drugs has been reported worldwide and become a concern to veterinarians and researcher. Study has reported that antibiotic resistance strains of *S. aureus* are increased mainly towards penicillin, methicillin, quinolone and vancomycin (Lowy, 2003). The organisms can transfer the resistance to a susceptible bacterium by conjugation known as R-plasmid mediated antibiotic resistance (Ahmadi *et al.*, 2010).

There has been increasing interest of raising small ruminants in the east cost of Peninsular Malaysia. However, the study on mastitis and evaluation of the antibiotic sensitivity profile of *S. aureus* is still scarce. The prevalence and severity of mastitis is likely varied depending on the farming management, geographical area and control measures employed. Furthermore, antimicrobial resistance awareness among the smallholder farmers mainly at rural area is relatively low (Karimuribo *et al*., 2006). Moreover, most of the producers are selling unpasteurized goat milk due to the consumer demand. This will increase the risk of the transmission of antibiotic-resistance mastitis bacteria from animals to humans. Thus, this study was designed to address the information gap pertaining the detection of *S. aureus* mastitis in lactating small ruminants and antibiotic susceptibility pattern of the isolated *S. aureus* in the selected areas in Besut and Setiu, Terengganu, Malaysia.

MATERIALS AND METHODS

Sample isolation, growth maintenance and phenotypic identification

A total of 171 raw milk samples (145 goats and 26 sheep) were collected from different smallholder farms in Besut and Setiu, Terengganu, Peninsular Malaysia. Prior to the 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). Approximately, 6 mL of mid-stream milk was collected from both udders. The milk was collected by hand-stripping and stored in a sterile polystyrene tube before been transported to the Microbiology Laboratory, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus for further processing and microbiological analysis.

Milk samples were cultured onto Mannitol Salt Agar (MSA) (Merck, Germany) and incubated at 37 °C for 48 h as described by Quinn *et al.* (2004). The golden-yellow colonies on MSA indicated the presence of *S. aureus* due to mannitol fermentation (Figure 1). Colonies with desired phenotypic characters on MSA were classified as presumptive *S. aureus* and further subcultured onto Trypticase Soy Agar (TSA) (Oxoid, USA). Colonies which showed consistent results of positive reaction for Gram stain, tube coagulase and catalase test were phenotypically confirmed as *S. aureus*. Presumptive *S. aureus* colonies with yellowish colonies were further subcultured onto nutrient agar supplemented with 3.5% NaCl prior to further genotypic identification.

Genotypic identification of *S. aureus* isolates

Each presumptive *S. aureus* isolate was resuspended in PCR tube containing 100 µL Tris-EDTA buffers. DNA template was extracted using boiling method by heating the suspension containing *S. aureus* isolate at 99 °C for 10 min followed by centrifugation to separate supernatant containing DNA from boiled cells as described by Suhaili *et al.* (2018). DNA template was stored at –20 °C until further genotypic identification. PCR targeting *S. aureus* species-specific gene; *nuc* was carried out. The *nuc* primers were 5'-GCGATTGATGGTGATACGGTT-3' and 5'-AGCCAAGCCTTGACGAACTAAAGC-3'. The PCR parameters consisted of the following conditions: initial denaturation at 95 °C for 3 min followed by 30 cycles of 95 °C for 15 sec, 55 °C for 15 sec and 72 °C for 20 sec. The 279 bp PCR fragment was indicative of positive *nuc* gene PCR fragment.

Antibiotic susceptibility tests

The Kirby-Bauer disc diffusion method was used to determine the antibiotic susceptibility of *S. aureus* isolates. Methicillin resistance screening by using 1 µg of oxacillin and 30 µg of cefoxitin discs (Oxoid, USA) were used with reference strains *S. aureus* ATCC 700699 and ATCC 25923 as positive and negative controls, respectively. The susceptibility profiles of isolate towards other antibiotics are listed in Table 2 as suggested by Magiorakos *et al.* (2011). Guidelines from the Clinical and Laboratory Standard Institute (CLSI) and British Society for Antimicrobial Chemotherapy (BSAC) were followed to interpret the diameter of the inhibition zone (Andrews, 2001; Clinical and Laboratory Standards Institute, 2016). The presence of the tetracycline-resistance genes (*tet*K and *tet*M) was detected by PCR as described earlier. The *tet*K and *tet*M primers were 5'-GTAGTGACAATAAACCTCCTA-3' and 5'-GTAGCGACAATAGGTAATAGT-3', and 5'-CGGTAAAGTTCGTCACACAC-3' and 5'-

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GTGGACAAAGGTACAACGAG-3' respectively (Ng *et al*., 2001). The 360 and 406 bp PCR fragment were indicative of positive *tet*K and *tet*M respectively.

Data management and statistical analysis

Microsoft Excel spreadsheets were used for raw data management. Descriptive statistics such as percentage will be used to summarize the proportions of infected and non-infected sampled animals. The Cohen's Kappa statistics was performed by using Xlstat2016 to determine the correlation between phenotypic and genotypic identification techniques of *S. aureus* with statistically significant of $P < 0.05$.

Ethical approval

The study was performed with approval of by the Universiti Sultan Zainal Abidin (UniSZA) Animal and Plant Ethic Committee (UAPREC) Reff: UAPREC/17/005/UniSZA.0/3/374-3 (32).

RESULTS

Prevalence and bacterial characterization

Out of 171 milk samples, 107 (63%) were mastitis positive and 64 (37%) were normal. Out of the 107 mastitis positive samples, 41 (24%) were from clinical mastitis and 66 (39%) were from subclinical mastitis animals (Table 1). Bacteriological examinations revealed that 39/107 (36%) mastitis samples were positive for *S. aureus.* The 39 presumptive *S. aureus* were isolated from 16 (9%) clinical and 23 (13%) subclinical mastitis samples. Genotypic characterization by PCR showed that 18/39 (46%) presumptive *S. aureus* were positive for the presence of *nuc* gene (279 bp) (Figure 2), with distribution of 9 isolates from each clinical and subclinical mastitis milk respectively. A moderate agreement (Kappa = 0.52) ($P < 0.05$) was observed between the results obtained by phenotypic identification using conventional techniques and the results of the genotypic methods.

Antibiotic susceptibility profiles

The antimicrobial susceptibility profiles of the 18 *S. aureus* isolates to various antimicrobial drugs are summarized in Table 2. The results of antimicrobial susceptibility tests showed 4/18 (22%) isolates were resistant to penicillin and 2/18 (11%) isolates were resistant to tetracycline (Table 2). However, all isolates were *tet*K and *tet*M negative for PCR as depicted in Figure 3 (A) and 3(B). *Staphylococcus aureus* isolates tested in this study were fully sensitive (100%) to amoxicillin, gentamycin, nitrofurantoin, oxacillin, cefoxitin, norfloxacin, chloramphenicol, amikacin, kanamycin, doxycycline and cefotaxime.

DISCUSSION

In the present study, the prevalence of clinical and subclinical mastitis was reported at 24% and 39%, respectively. A scarce available information on caprine mastitis for developing countries like Malaysia are crucial. Therefore this current study, clinical mastitis detection was done by examining the gross signs of udder infection such as swelling, heat, hardness, redness or pain and abnormal milk in the form of clots, flakes, and watery milk. Clinical mastitis is considered severe than subclinical mastitis; however subclinical mastitis can be developed into clinical mastitis if untreated. Examination of subclinical mastitis by using CMT reagent offered a high level of sensitivity and provides quick and reliable SCC estimation in the subclinical cases as suggested by Tanwar *et al.* (2001). Thus, our study also observed subclinical mastitis cases by using the same method.

In the present study, 46% of the mastitis milk samples were positive for *S. aureus*. In addition, previous study by Faiq *et al.* (2017) reported the prevalence of *S. aureus* isolated from clinical and subclinical mastitis in small ruminant in Terengganu, Malaysia was 2% and 20% respectively. A recent study by Ariffin *et al.* (2019) showed that 16.3% of dairy goats with mastitis were positive for *S. aureus.* These bacteria have higher virulence capability compared to other commonly infectious agent such coagulase-negative staphylococci (CNS) and capable to develope subclinical mastitis into a clinical disease (Contreras *et al*., 2007). In the present study, a total of 39 isolates growth on MSA revealed *S. aureus* with all isolates appeared as gram-positive cocci in clusters. Identification for presumptive *S. aureus* based on growth on MSA has been reported elsewhere (Marwa *et al.*, 2013), and has been recommended by the American Public Health Association (APHA) for the counting of *Staphylococci* spp. in food and dairy products. In this study, *S. aureus* was confirmed

Table 1: Number of samples and the results of bacteriological examination and PCR assay.

Mastitis status	No. of samples (%)	No. of samples positive for *S. aureus* isolation (%)	No. of samples positive for *nuc* gene (%)
Clinical mastitis	41 (24%) (34 goats, 7 sheep)	16 (9%) (12 goats, 4 sheep)	9 (5%) (7 goats, 2 sheep)
Subclinical mastitis	66 (39%) (58 goats, 8 sheep)	23 (13%) (19 goats, 4 sheep)	10 (5%) (9 goats, 1 sheep)
Normal milk	64 (37%) (53 goats, 11 sheep)	0	0

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Table 2: Percentage of antibiotic-resistant isolates of *S. aureus.*

Antibiotic	Number of isolates (%)		
	R	I	S
Penicillin	4 (22)	0 (0)	14 (78)
Amoxicillin	0	0 (0)	18 (100)
Tetracycline	2 (11)	0 (0)	16 (89)
Gentamycin	0 (0)	0 (0)	18 (100)
Nitrofurantoin	0 (0)	0 (0)	18 (100)
Oxacillin	0 (0)	0 (0)	18 (100)
Cefoxitin	0 (0)	0 (0)	18 (100)
Norfloxacin	0 (0)	0 (0)	18 (100)
Choramphenicol	0 (0)	0 (0)	18 (100)
Amikacin	0 (0)	0 (0)	18 (100)
Kanamycin	0 (0)	0 (0)	18 (100)
Doxycycline	0 (0)	0 (0)	18 (100)
Cefotaxime	0 (0)	0 (0)	18 (100)

R, resistant; I, intermediate; S, sensitive.

by PCR using species-specific primers (*nuc* gene), which is amplifying 279 bp of amplicon. Previously, the PCR assay has been used for quick identification of pathogenic bacterial genes in milk as well as used for standard identification and classification of *S. aureus* genotyping (Phuektes *et al.,* 2001., Brakstad *et al*., 1992). The identification of pathogenic bacteria could rapidly identified compared to the conventional bacteriological methods. Furthermore, the PCR techniques offer a prudent results which be able to distinguish between closely related bacteria such as other species of the Staphylococci (Riffon *et al.,* 2001). In another study by Abo-Shama (2014) stated that PCR amplification of *nuc* gene has been used as a practical method for the identification of *S. aureus* in milk samples, however, precautions must be taken to avoid false positive results due to the amplification of contaminating DNA. In this study, the comparison of the results obtained using the conventional phenotypic method and *nuc* PCR for *S. aureus* identification showed a moderate agreement (Kappa = 0.52) between methods. The coagulase test and growth on MSA are reliable for *S. aureus* identification (Kateete *et al*., 2010), particularly when genotypic methods are not available.

Antimicrobial susceptibility of 18 *S. aureus* isolates was evaluated using 13 antimicrobial agents. In this study, four (22%) *S. aureus* isolates were resistant to penicillin, and two (11%) isolates were resistant to tetracycline. These results were in agreement with the study of Chu *et al.* (2012) who reported that *Staphylococcus* isolated from mastitis of goats showed resistance to penicillin (50%) and tetracycline (100%). In another study the percentage of penicillin-resistant and tetracycline-resistant of *S. aureus* isolated from mastitis in cattle was equal to 27% and 7% respectively (Marimuthu *et al*., 2014). Moreover, Tel *et al.* (2012) found 27% of *S. aureus* isolated from ovine mastitis were resistant to penicillin. Franca *et al.* (2012) reported that *S. aureus* isolated from subclinical mastitis were resistant to different types of antibiotics including tetracycline. A recent study by Ariffin *et al.* (2019) reported that 6.45% of *S. aureus* isolated from dairy goats with clinical and subclinical mastitis were resistant to penicillin and tetracycline. The susceptibility tests in the current study disclose that clinical and subclinical mastitis caused by *S. aureus* isolates may be treated with amoxicillin, gentamicin, nitrofurantoin, oxacillin, cefoxitin, norfloxacin, chloramphenicol, amikacin, kanamycin, doxycycline and cefotaxime.

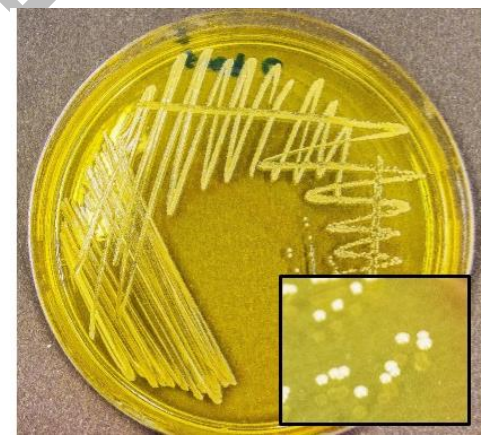

Figure 1: *Staphylococcus aureus* colony on Mannitol Salt Agar.

Generally, bacterial resistance to antibiotic is often mediated through mechanism including active efflux, ribosomal protection, reduced permeability, ribosomal mutation and enzymatic inactivation (Markley and Wencewicz, 2018). Active efflux encoded by the *tet*K gene and ribosomal protection encoded by the *tet*M gene are the most common types of tetracycline resistance seen in *S. aureus* (Esposito *et al*., 2009; McCallum *et al*., 2010; Thaker *et al*., 2010). In this study, two *S. aureus* isolates were resistant to tetracycline but were negative to *tet*K and *tet*M genes. One possible explanation is that

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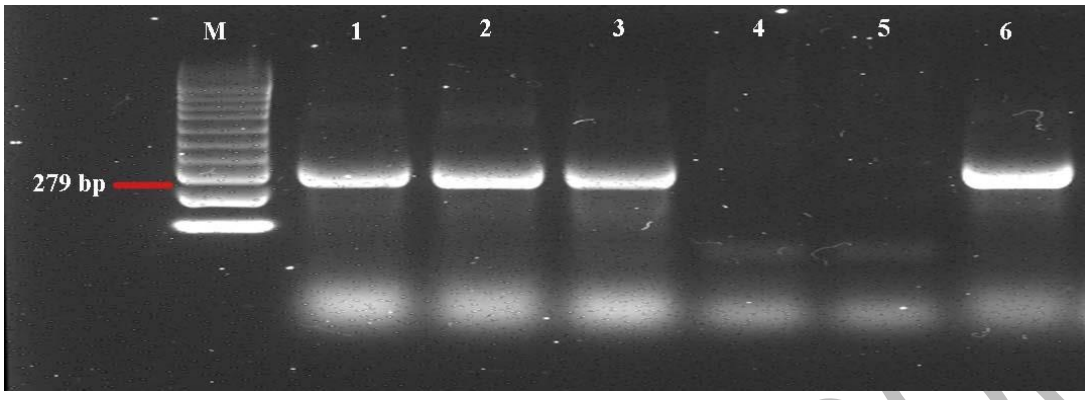

Figure 2: Agarose gel electrophoresis image of the *nuc* gene (279 bp). Lane 1-3: representative positive isolates; lane 4: representative negative isolate; lane 5: negative control (no template control); lane 6: positive control (ATCC 700699); lane M: 1000 bp ladder.

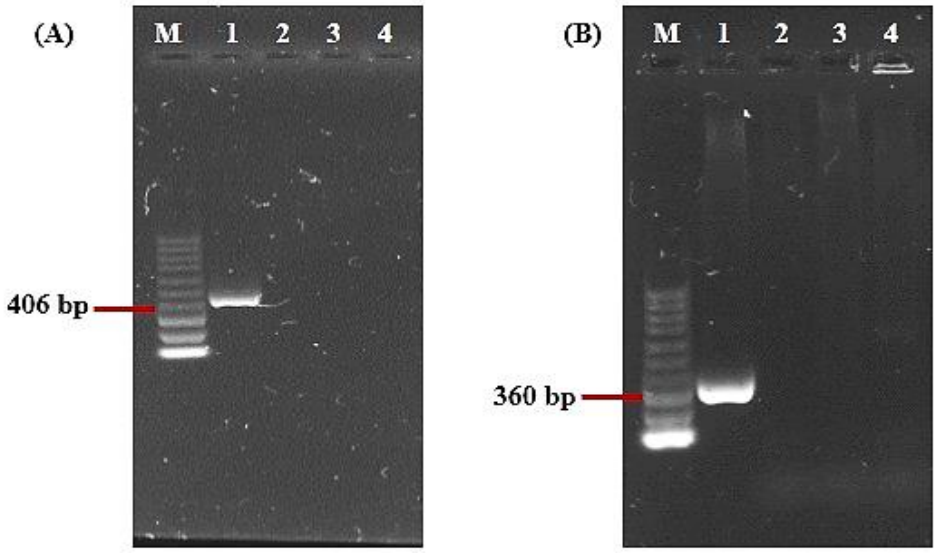

Figure 3: (A) Agarose gel electrophoresis image of the *tet*M gene (406 bp). Lane 1: positive control (ATCC 700699); lane 2: negative control (no template control); lane 3 - 4: *tet*M negative isolates; lane M: 1000 bp ladder. (B) Agarose gel electrophoresis image of the *tet*K gene (360 bp). Lane 1: positive control (ATCC 700699), lane 2: negative (no template control); lane 3 - 4 *tet*K negative isolates; lane M: 1000 bp ladder.

very low gene expression level result in apparent resistance to tetracycline or that tetracycline resistance in these isolates is mediated by a gene not included in our PCR assays. Tetracycline resistance through enzymatic inactivation by *tet*X gene has been recently found in a variety of environmental bacteria, human feces and hospital wastewater (Ming *et al*., 2017; Ohashi and Fujisawa, 2017; Wang *et al*., 2018).

Antibiotic is frequently used in the treatment of mastitis (Radostits *et al*., 2016). It can lead to high risk of overuse antibiotic in treatment especially in the rural area with low surveillance from the veterinarian. The resistance of staphylococcus isolates may related to the production of beta-lactamases which can hydrolyse and destroy beta-lactam antibiotics including penicillin or the prevention of access to target by expression of penicillin-binding protein 2a (PBP 2a) (Abed and Hamim, 2015).

The occurence of bacteria causing mastitis that are resistant to antibiotic has serious consequences to the infected animals. The resistant strain can make the mastitis treatment become more challenging, particularly when the bacteria have multidrug resistance phenotypes. Antimicrobial resistance in *S. aureus* is a major concern in dairy industry (Sasidharan *et al*., 2011). Thus, antimicrobial resistance surveillance in common mastitis pathogens is essential not only to optimize the selection of antibacterial agents by veterinarians towards more successful therapy but also helps to protect the public health.

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CONCLUSION

In conclusion, the presence of *S. aureus* from milk samples of both clinical and subclinical mastitis goats indicates, there is a potential hazard on the livestock as well as public health. Furthermore, there were no multidrug resistance isolates observed among isolates tested. However, the presence of low penicillin and tetracycline resistance rates should not be undermined. Our study suggests that milk from mastitis samples may play an important role as potential reservoir and transmission of these pathogens in posing disease regardless of antibiotics resistance background.

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REFERENCES

Abed, M. K. and Hamim, S. S. (2015). Molecular detection of *Staphylococci* isolated from mastitis in sheep and cows in Thi-Qar province. *International Journal of Research in Applied, Natural and Social Sciences* **3, 33-44.**

Abo-Shama, U. H. (2014). Prevalence and antimicrobial susceptibility of *Staphylococcus aureus* isolated from cattle, buffalo, sheep and goat`s raw milk in Sohag Governorate, Egypt. *Assiut Veterinary Medical Journal* **60, 63-72.**

Ahmadi, M., Rohani, S. M. R. and Ayremlou, N. (2010). Detection of *Staphylococcus aureus* in milk by PCR. *Comparative Clinical Pathology* **19, 91-94.**

Andrews, J. M. (2001). BSAC standardized disc susceptibility testing method. *Journal of Antimicrobial Chemotherapy* **48, 43-57.**

Ariffin, S. M. Z., Hasmadi, N., Syawari, N. M., Sukiman, M. Z., Faiq, T. A. M., Chai, M. H. and Ghazali, M. F. (2019). Prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* in dairy goats with clinical and subclinical mastitis. *Journal of Animal Health and Production* **7, 32-37.**

Brakstad, O. G., Aasbakk, K. and Maeland, J. A. (1992). Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. *Journal of Clinical Microbiology* **30, 1654-1660.**

Clinical and Laboratory Standards Institute (2016). *Performance Standards for Antimicrobial Susceptibility Testing. CLSI supplement M100S. Clinical and Laboratory Standards Institute, Wayne, PA.*

Chu, C., Yu, C., Lee, Y. and Su, Y. (2012). Genetically divergent methicillin-resistant *Staphylococcus aureus* and sec-dependent mastitis of dairy goats in Taiwan. *BMC Veterinary Research* **8, 39.**

Contreras, A., Luengo, C., Sanchez, A. and Corrales, J. C. (2003). The role of intramammary pathogens in dairy goats. *Livestock Production Science* **79, 273-283.**

Contreras, A., Sierra, D., Sanchez, A., Corrales, J. C., Marco, J. C., Paape, M. J. and Gonzalo, C. (2007). Mastitis in small ruminants. *Small Ruminant Research* **68, 145-153.**

DeGraves, F. J. and Fetrow, J. (1993). Economics of mastitis and mastitis control. *The Veterinary Clinics of North America: Food Animal Practice* **9, 421-434.**

Esposito, S., Leone, S., Petta, E., Noviello, S. and Ianniello, F. (2009). Treatment options for skin and soft tissue infections caused by meticillin-resistant *Staphylococcus aureus*: Oral vs.parenteral; home vs. hospital. *The International Journal of Antimicrobial Agents* **34, 30-35.**

Faiq, T. A. M., Ghazali, M. F., Chai, M. H. and Ariffin, S. M. Z. (2017). Isolation of *Staphylococcus aureus*: Clinical and subclinical mastitis infections in sheep and dairy goats. *In:* Proceedings of the 29th Veterinary Association Malaysia Congress. Haron, A. W., Saleha, A. Z., Faez, F. J. A. and Hua, K.K. (eds.).Veterinary Association Malaysia, Putrajaya. Malaysia. **pp. 60.**

Franca, C. A., Peixoto, R. M., Cavalcante, M. B., Melo, N. F., Oliveira, C. J. B., Veschi, J. A. and Veschi, J. L. A. (2012). Antimicrobial resistance of *Staphylococcus* spp. from small ruminant mastitis in Brazil. *Brazilian Veterinary Research* **32, 747-753.**

Gelasakis, A. I., Mavrogianni, V. S., Petridis, I. G., Vasileiou, N. G. C. and Fthenakis, G. C. (2015). Mastitis in sheep - The last 10 years and the future of research. *Veterinary Microbiology* **181, 136-146.**

Gelasakis, A. I., Angelidis, A. S., Giannakou, R., Filioussis, G., Kalamaki, M. S. and Arsenos, G. (2016). Bacterial subclinical mastitis and its effect on milk yield in low-input dairy goat herds. *Journal of Dairy Science* **99, 3698-3708.**

Gougoulis, D. A., Kyriazakis, I., Papaioannou, N., Papadopoulos, E., Taitzoglou, I. A. and Fthenakis, G. C. (2008). Subclinical mastitis changes the patterns of maternal-offspring behaviour in dairy sheep. *Veterinary Journal* **176, 378-384.**

Gran, H. M., Wetlesen, A., Mutukumira, A. N., Rukure, G. and Narvhus, J. A. (2003). Occurrence of pathogenic bacteria in raw milk, cultured pasteurised milk and naturally soured milk produced at small-scale dairies in Zimbabwe. *Food Control* **14, 539-544.**

Karimuribo, E. D., Fitzpatrick, J. L., Bell, C. E., Swai, E. S., Kambarage, D. M., Ogden, N. H. and French, N. P. (2006). Clinical and subclinical mastitis in smallholder dairy farms in Tanzania: Risk, intervention and knowledge transfer. *Preventive Veterinary Medicine* **74, 84-98.**

Kateete, D. P., Kimani, C. N., Katabazi, F. A., Okeng, A., Okee, M. S., Nanteza, A. and Najjuka, F. C. (2010). Identification of *Staphylococcus aureus*: DNase and mannitol salt agar improve the efficiency

of the tube coagulase test. *Annals of Clinical Microbiology and Antimicrobials* **9, 1-7.**

Libera, D. A., Blagitz, M. G., Souza, F. N., Batista, C. F., Azedo, M. R., Benites, N. R., Melville, P. A. and Gomes, V. (2010). Antimicrobial susceptibility of coagulase-negative *Staphylococci* isolated from meat-producing ewes with mastitis. *Brazilian Journal of Veterinary and Animal Science* **62, 1499-1502.**

Lowy, F. (2003). Antimicrobial resistance: The example of *Staphylococcus aureus*. *Journal of Clinical Investigation* **111, 1265-1273.**

Lucia, M., Rahayu, S., Haerah, D. and Wahyuni, D. (2017). Detection of *Staphylococcus aureus* and *Streptococcus agalactiae*: Subclinical mastitis causes in dairy cow and dairy buffalo (*Bubalus bubalis*). *American Journal of Biomedical Research* **5, 8-13.**

McCallum, N., Berger-Bächi, B. and Senn, M. M. (2010). Regulation of antibiotic resistance in *Staphylococcus aureus*. *International Journal of Medical Microbiology* **300, 118-129.**

Magiorakos, A., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G. and Hindler, J. F. (2011). Bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Microbiology*, **18, 268-281.**

Marimuthu, M., Abdullah, F. F. J., Mohammed, K., Poshpum, S. S., Adamu, L., Osman, A. Y. and Tijjani, A. (2014). Prevalence and antimicrobial resistance assessment of subclinical mastitis in milk samples from selected dairy farms. *American Journal of Animal and Veterinary Sciences* **9, 65-70.**

Markley, J. L. and Wencewicz, T. A. (2018). Tetracycline-inactivating enzymes. *Frontiers in Microbiology* **9, 1058**.

Marwa, I. A. E. and Mahmoud M. B. (2013). Association between agralleles and toxin gene profiles of *Staphylococcus aureus* isolates from human and animal sources in Egypt. *International Journal of Advanced Research* **1, 133-144.**

Merz, A., Stephan, R. and Johler, S. (2016). *Staphylococcus aureus* isolates from goat and sheep milk seem to be closely related and differ from isolates detected from bovine milk. *Frontiers in Microbiology* **7, 1-7.**

Ming, D. S., Chen, Q. Q. and Chen, X. T. (2017). Analysis of resistance genes in pan-resistant Myroides odoratimimus clinical strain PR63039 using whole genome sequencing. *Microbial Pathogenesis* **112, 164–170.**

Ng, L. K., Martin, I., Alfa, M. and Mulvey, M. (2001). Multiplex PCR for the detection of tetracycline resistant genes. *Molecular and Cellular Probes* **15, 209-215.**

Ohashi, Y. and Fujisawa, T. (2017). Detection of antibiotic resistance genes in the feces of young adult Japanese. *Bioscience of Microbiota, Food and Health* **36, 151-154.**

Oliver, S. P., Boor, K. J., Murphy, S. C. and Murinda, S. E. (2009). Food safety hazards associated with consumption of raw milk. *Foodborne Pathogens and Disease* **6, 793-806.**

Phuektes, P., Mansell, P. D. and Browning, G. F. (2001). Multiplex polymerase chain reaction Assay for simultaneous detection of *Staphylococcus aureus* and *Streptococcal* causes of bovine mastitis. *Journal of Dairy Science* **84, 1140-1148.**

Quinn, P., Bryan, M., Carter, M. and Carter, G. R. (2004). Clinical Veterinary Microbiology Mosby Publishing, London. **pp. 43-55, 327-344.**

Radostits, O. M., Gay, C. C. and Hinchcliff, K. W. C. (2016). Veterinary medicine: A textbook of the disease of cattle, horses, sheep, pigs and goats. Elsevier, New York. **pp. 1045-1046.**

Riffon, R., Sayasith, K., Khalil, H., Dubreuil, P., Drolet, M. and Lagace, J. (2001). Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. *Journal of Clinical Microbiology* **39, 2584-2589.**

Sasidharan, S., Prema, B. and Yoga, L. L. (2011). Antimicrobial drug resistance of *Staphylococcus aureus* in dairy products. *Asian Pacific Journal of Tropical Biomedicine* **1, 130-132.**

Suhaili, Z., Rafee, P., Mat Aziz, N., Yeo, C. C., Nordin, S. A., Abdul Rahim, A. R. and Mohd Desa, M. N. (2018). Characterization of resistance to selected antibiotics and Panton-Valentine leukocidin-positive *Staphylococcus aureus* in a healthy student population at a Malaysian University. *Germs* **8, 21-30.**

Tanwar, R. K., Vyas, S. K., Fakhruddin and Singh, A.P. (2001). Comparative efficacy of various diagnostic tests in diagnosis of subclinical mastitis in Rathi cows. *In:* Proceedings of Round Table Conference of the Indian Association for the Advancement of Veterinary Research (IAAVR) on Mastitis. Verma, R. and Mamtani, A. P. (eds.). IAAVR, Ludhiana, India. **pp. 161-163.**

Taylor, R. E. and Field, T. G. (2009). Scientific Farm Animal Production: An Introduction to Animal Science. Gilfillan, A., Fox, D. and Dimmick, L. (eds.). Pearson, New York. United State. **pp. 445.**

Tel, O., Aslantas, O., Keskin, O., Yilmaz, E. and Demir, C. (2012). Investigation of the antibiotic resistance and biofilm formation of *Staphylococcus aureus* strains isolated from gangrenous mastitis of ewes. *Acta Veterinaria Hungarica* **60, 189-197.**

Thaker, M., Spanogiannopoulos, P. and Wright, G. D. (2010). The tetracycline resistome. *Cellular and Molecular Life Sciences* **67, 419-431.**

Turutoglu, H., Ercelik, S. and Ozturk, D. (2006). Antibiotic resistance of *Staphylococcus aureus* and Coagulase-Negative *Staphylococci* isolated from bovine mastitis. *Bulletin Veterinary Institute in Pulawy* **50, 41-45.**

Wang, Q., Wang, P. and Yang, Q. (2018). Occurrence and diversity of antibiotic resistance in untreated hospital wastewater. *Science of the Total Environment* **621, 990-999.**