ANTIBIOTIC SUSCEPTIBILITY OF *Staphylococcus aureus* AND *Escherichia coli* ISOLATED FROM DAIRY GOATS IN SELECTED FARMS IN SELANGOR, MALAYSIA

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SUMMARY

Antimicrobial resistance (AMR) has become a major problem worldwide with significance public health impact in both animal and human populations. *Staphylococcus aureus* (*S. aureus*) is a common pathogen in domestic livestock and *Escherichia coli* (*E. coli*) is a normal flora in the gut and these organisms are considered as effective indicators for AMR surveillance. Increased in antimicrobial resistance of these bacteria in veterinary medicine leads to difficulties in choosing effective antibiotics to treat diseases they caused. This study aimed to evaluate the susceptibility of *S. aureus* and *E. coli* isolated from dairy goats in selected farms located in Selangor, Malaysia against different antibiotics. Milk and faecal samples were collected from 36 dairy goats and samples were prepared for the isolation of *S. aureus* and *E. coli* isolates respectively. The isolates were subjected to antibiotic susceptibility test. All 11 (100%) *E. coli* isolates showed resistance to amoxicillin and penicillin while 3 (27%) of the 11 *S. aureus* isolates, showed resistance towards the same class of antibiotic. Both *S. aureus* and *E. coli* isolates showed high susceptibility to four antibiotics, namely trimethoprim-sulfamethazole, neomycin, tetracycline and enrofloxacin. This study provided information regarding the antibiotic resistance of *S. aureus* and *E. coli* in relation to the antimicrobial usage practice in selected dairy goat farms located in Selangor, Malaysia.

Keywords: antibiotic resistance, antibiotic susceptibility, Staphylococcus aureus, Escherichia coli, dairy goats

INTRODUCTION

The ability of bacteria to resist the inhibitory effect of antimicrobial drugs / antibiotics is termed as antimicrobial / antibiotic resistance (AMR) has become a major problem worldwide with a public health concern. Increased in antimicrobial / antibiotic resistance of these bacteria in veterinary medicine leads to difficulties in choosing effective antibiotics to treat bacterial diseases. The usage of antimicrobial agents in particular antibiotics in agriculture continue to increase due to lack of knowledge and little information available for reference on the choice of antimicrobial agents / antibiotics. The exposures to antibiotics as growth promoters and prophylaxis in animals and therapeutics purposes in both animals and humans have caused the emergence and spread of drug-resistance among pathogenic and non-pathogenic bacteria strains including the normal intestinal flora of humans and animals (Adzitey *et al.*, 2012).

Staphylococcus aureus (*S. aureus*) is a common pathogen in domestic livestock and has become resistant to many commonly used antibiotics and various antibiotic susceptibility patterns have been observed in various animals (Lee, 2003). According to Marogna *et al.* (2012), *S. aureus* is one of the most commonly found pathogens in raw caprine and ovine milk and has become the main cause of clinical mastitis in small ruminants. Studies have shown an increase of staphylococci strains that exhibited resistance to methicillin/oxacillin termed as methicillin – resistant *S. aureus* (MRSA) which is found primarily in humans and later detected in animals (Lee *et al.*, 2004).

The most commonly reported livestock-associated MRSA (LA-MRSA) has shown to be able to colonise and caused serious infections in humans in close contact with animals primarily pigs, cattle and horses. Thus, there is a need to increase specific attention on the transfer of MRSA isolates between animals and humans (De Martino *et al.*, 2010). MRSA strains have also been reported in causing intramammary infection in goats (Aras *et al.*, 2012). This was shown in a study by Stastkova *et al.* (2009), which was the first reported MRSA detection from goats and/or goat's milk discovered only in aseptically collected goat milk. In addition, Alves *et al.*, (2009) reported that MRSA can cause subclinical mastitis in goats and could be shed in milk without any sign of infection. In subclinical infections, these microorganisms can then be transferred to milk without any alteration of the characteristics thus spreading the antibiotic resistance genes through the dairy food chain (Alves *et al.*, 2009).

Escherichia coli (*E. coli*) is a normal flora in the gut and considered as effective indicator for AMR surveillance in different populations and in the transfer of antibiotic resistant bacteria between different environment (Lambrecht *et al.*, 2017). According to a study done by Amadi *et al.* (2015), *E. coli* is a common inhabitant of the large and lower small intestines of mammals including goats and is excreted in faeces. Most *E. coli* strains are non-pathogenic, but the pathogenic strains may cause severe intestinal or extra intestinal diseases in humans and capable of causing zoonotic infections (Santos *et al.*, 2013). In a study by Adzitey *et al.* (2015), poor management of farm animals can cause faecal contamination of variety of sources including drinking water, thus, causing *E. coli* infection in both humans and animals by drinking contaminated water from such sources. The antibiotic resistant bacteria from

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animals can also infect human population via food products of animal origin (van Den Bogaard & Stobberingh, 2000). Any food vehicle in contact with ruminant faeces can be a potential exposure source of *E. coli* such as vegetables, meat products and milk and provide an opportunity of transfer of antibiotic resistance genes to other bacterial population (Day *et al.,* 2016).

To date, limited number of comprehensive studies on antibiotic susceptibility profiles of *S. aureus* and *E. coli* among livestocks in Malaysia is seen and little attention has been paid to AMR in specific animal pathogens. Hence, this study aimed to evaluate the susceptibility of *S. aureus* and *E. coli* isolated from dairy goats in selected farms in Selangor, Malaysia against different antibiotics.

MATERIALS AND METHODS

Animal Selection

A cross-sectional approach was used to collect samples and data over a 3-week period. A total of 36 female goats from three selected dairy goat farms located in Selangor, Malaysia were sampled (Farm A = 13 goats; Farm B = 16 goats; Farm C = 7). The does selected were lactating and were apparently healthy adults of more than one year old. Milk and faecal samples were collected from each animal. This project was approved by the Institutional Animal Care and Use Committee, Universiti Putra Malaysia (UPM/IACUC/AUP-FYP2016/FYP36).

Milk and Faecal Samples Collection

Milk samples were collected aseptically from lactating does. Dirty udders were cleaned thoroughly with mixture of water and chlorhexidine gluconate (Hibiscrub® 4%). Pre-dipping with germicidal teat dip was done for each teat before drying using clean paper towel. Few streams of milk were discarded and the end of each teat was cleaned with cotton ball soaked in 70% alcohol, starting from the farthest teats to the nearest teats. Milk collection started with nearest teat in sterile milk tubes placed at 45°C to prevent debris from entering the tube and to prevent the tube from touching the end of the teat. About 3 to 5 mL of milk was collected from each goat and milk samples were then stored in the ice box for transportation.

Faecal samples were collected manually from the rectum of individual animals using lubricated gloves and kept in a zip-lock bag.

In the laboratory, milk samples were prepared for the isolation of *S. aureus* and faecal samples for *E. coli*. A standard method for the isolation and identification of *S. aureus* and *E. coli* isolates as outlined in Diagnostic Manual of Veterinary Clinical Bacteriology and Mycology (2008) were performed.

Isolation and Identification of S. aureus from Milk Samples

A loopful of milk was taken from each milk sample using inoculating loop and cultured on the 7% blood agar. The agar plates were then incubated at 37°C for 24 to 48 hours. The suspected colonies that grew on the agar were then sub-cultured; Gram staining was performed, and Gram-positive cocci bacteria were examined. The identified colonies were then tested with catalase test and those that were tested positive were proceeded with the coagulase test. The colonies tested positive for coagulase test were then proceeded for identification by blood broth (haemolysin), Voges-Proskauer (VP), maltose, mannitol and Arginine Dihydrolase (ADH) tests. The bacterial isolates that were identified to be *S. aureus* based on the results of the biochemical tests were selected for antibiotic susceptibility test.

Isolation and Identification of E. coli from Faecal Samples

Faecal samples were inoculated on MacConkey agar using sterile swab. The agar plates were then incubated at 37°C for 24 to 48 hours. The MacConkey agar plates were examined for pink colonies that precipitated bile, lactose fermenters and had a dark red centre. Gram staining was then performed on the colonies smears and Gram-negative rods bacteria were examined. The identified colonies were then tested with oxidase test and those tested negative were then proceeded for identification by triple sugar iron (TSI) agar, sulphide indole motility (SIM) media, citrate and urease tests. The bacterial isolates that were identified to be *E. coli* based on the results of the biochemical tests were selected for antibiotic susceptibility test.

Antibiotic Susceptibility Test

The standard Kirby-Bauer disk diffusion method was used to determine the antibiotic susceptibility of the isolates to six antibiotics. The procedure was done in accordance with the procedures described in the Clinical and Laboratory Standards Institute guidelines (CLSI, 2008) including suggested breakpoints to determine susceptibility and resistance of the isolates. The antimicrobial agents chosen were amoxicillin, trimethoprim/sulfamethoxazole, penicillin, neomycin, tetracycline, enrofloxacin, ampicillin, erythromycin and gentamicin. Different antimicrobial agents were chosen for *S. aureus* and *E. coli* isolates based on difference in susceptibility. *S. aureus* isolates were tested with ampicillin, erythromycin*,* penicillin, tetracycline, gentamicin and trimethoprim/sulfamethoxazole (Erskine *et al.,* 2002*)* while *E. coli* isolates were tested with amoxicillin, trimethoprim/sulfamethoxazole, penicillin, neomycin, tetracycline and enrofloxacin (Bogaard & Stobberingh, 2000). These antibiotics were selected based on their importance in bacterial infections and based on their ability to provide diversity for representation of different antibiotic classes (Sayah *et al.,* 2005). These antibiotics were also selected based on their importance in treating common bacterial infections in goats and based on the use of antibiotics in the investigated farms (from interview with the farm owners). The most commonly used antibiotics in these farms were amoxicillin which was classified in β-lactams group. The selected antibiotics in this study were listed under OIE's (World Organisation for Animal Health) criteria of Veterinary Critically Important Antimicrobial Agents

(VCIA). The standard suspension of each bacterial isolate was made using 0.5 McFarland standard solution. The suspension was spread on entire surface of Mueller-Hinton agar using sterile swabs. Six commercially prepared antibiotic discs were placed on each inoculated plate. The plates were incubated at 37°C for 24 hours. The diameters (in millimetres, mm) of the clear zones of growth inhibition around each antibiotic disc, including the 6 mm disc diameter, were measured by using a precision calliper and the results were interpreted as sensitive, intermediate, or resistant bacteria based on the breakpoints diameter according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2008).

RESULTS

From a total of 36 milk samples, 72% (26/36) were positive for *S. aureus* while from 36 faecal samples, 92% (33/36) were positive for *E. coli*. From Table 1, 100% (13/13) of *S. aureus* and 85% (11/13) of *E. coli* and were isolated from Farm A; 63% (10/16) of *S. aureus* and 94% (15/16) of *E. coli* were isolated in Farm B and 43% (3/7) of *S. aureus* and 100% (7/7) of *E. coli* were isolated in Farm C.

Table 1. *E. coli* and *S. aureus* isolated from faecal and milk samples respectively from dairy goats in 3 farms located in Selangor, Malaysia

Farm	Isolates (Samples)	
	E. coli (faeces) % (n)	*S. aureus* (milk) % (n)
A (13)*	85% (11)	100% (13)
B (16)*	94% (15)	63% (10)
C (7)*	100% (7)	43% (3)
Total	92% (33/36)	72% (26/36)

Number of samples; n=Number of positive samples

A total of 11 isolates of each *S. aureus* and *E. coli* representing each farm were selected for the antibiotic susceptibility test (AST). As shown in Table 2, 27% (3/11) of *S. aureus* isolates were resistant to both ampicillin and penicillin. However, 9% (1/11) and 18% (2/11) of *S. aureus* isolates were found to be resistant to erythromycin and tetracycline respectively. As for *E. coli*, 100% of the isolates were found to be susceptible to all antibiotics tested except to amoxicillin and penicillin. Four isolates of *S. aureus* in Farm A were found to be resistant towards ampicillin, penicillin and tetracycline while one isolate of *S. aureus* in Farm B were resistant towards erythromycin and tetracycline. However, in Farm C, all *S. aureus* isolates were susceptible to all antibiotics.

DISCUSSION

The results of this study showed a total of 33 out of 36 (92%) *E. coli* were isolated from faecal samples from 3 different farms. The occurrence of *E. coli* in the goats ranged from 85 to 100%. This indicated that healthy goats harbour *E. coli* in their gastrointestinal tracts and the

Table 2. Susceptible and resistant *E. coli* and *S. aureus* isolates towards six types of antibiotics

Farm (No. of isolates)	*S. aureus* Susceptible isolates (%)			Total resistant isolates (%)
	A (4)	B (4)	C (3)	
Antibiotics	n (%)	n (%)	n (%)	
Ampicillin	1(25)	4(100)	3(100)	3(27)
Trimethoprim-sulfamethazole	4(100)	4(100)	3(100)	0
Penicillin	1(25)	4(100)	3(100)	3(27)
Erythromycin	4(100)	3(75)	3(100)	1(9)
Tetracycline	3(75%)	3(75)	3(100)	2(18)
Gentamicin	4(100)	4(100)	3(100)	0

Farm (No. of isolates)	*E. coli* Susceptible isolates (%)			Total resistant isolates (%)
	A (4)	B (4)	C (3)	
Antibiotics	n (%)	n (%)	n (%)	
Ampicillin	0(100)	0	0	11(100)
Trimethoprim-sulfamethazole	4(100)	4(100)	3(100)	0
Penicillin	0	0	0	11(100)
Erythromycin	4(100)	4(100)	3(100)	0
Tetracycline	4(100)	4(100)	3(100)	0
Gentamicin	4(100)	4(100)	3(100)	0

occurrence is widespread among goats in the investigated farms. The high occurrence showed that *E. coli* is a common inhabitant of the intestines of goats and is excreted in faeces (Amadi *et al*., 2015).

For *S. aureus*, 26 out of 36 (72%) isolates were isolated from the milk samples. The high occurrence of *S. aureus* in goat milk in Farm A could possibly due to subclinical mastitis (SCM). This assumption was made based on a study by Bochev and Russenova (2005) who reported that *S. aureus* is the most common pathogens associated with SCM in dairy goats. Our study however was done on the apparently healthy does without any screening on the subclinical mastitis. In addition, identification of affected animals with SCM can be challenging, as in contrast to cattle, because high somatic cell counts and positive results in the California Mastitis Test (CMT) are not necessarily reliable indicators of intramammary infections among small ruminants (Merz *et al*., 2016).

In this study, AST revealed high susceptibility of 11 *E. coli* isolates to four out of six antibiotics tested. All of the *E. coli* isolates (100%) were found susceptible to all of the antibiotics tested except for amoxicillin and penicillin. This is similar to the findings of Amadi *et al*. (2015) which tested *E. coli* isolates from healthy goats with a result of 99 to 100% isolates susceptible to enrofloxacin and gentamicin. Their result also revealed low resistance rate (ranging from 1% to 19%) of 12% to streptomycin and 2% each to trimethoprim-sulfamethoxazole and tetracycline. Although gentamicin and streptomycin were not used for *E. coli* isolates in this

study, these antibiotics were classified under the same class of aminoglycosides with neomycin, which was used in this study. Thus, based on similar low resistance rate (increased susceptibility) to the antibiotics in the same class as shown by the *E. coli* isolates, it can be safely assumed that antibiotics from the same class may cause cross resistance among antibiotics in the same class. In contrast, the *E. coli* isolates in this present study showed high resistance rate of 100% towards amoxicillin and penicillin whereas *E. coli* isolates from the study of Amadi *et al.* (2015) showed low resistance rate of 7% to ampicillin. Although ampicillin was not used in this study, it was classified in class of β-lactams together with amoxicillin and penicillin which were used in this study. Even though being in the same class of antibiotics, ampicillin did not show the same result with amoxicillin and penicillin. This could be because amoxicillin was the most used antibiotics in the investigated farms. It has been established that antibiotic resistance patterns vary from one region to another and the differences are due to a number of factors such as in time and samples examined, sampling methodology employed and the extent to which antibiotics are used in various regions (Adzitey, 2015).

As for *S. aureus*, 27% isolates were resistant to both ampicillin and penicillin, 9% to erythromycin while 18% were resistant to tetracycline (Table 2). According to Virdis *et al.* (2010), resistance against β-lactam or aminoglycosides is the most common trait observed for *S. aureus*. This can be observed in this study which showed resistance rate of 27% of the isolates towards ampicillin and penicillin of β-lactam group which was low when compared to Zhang *et al.* (2012) who reported the high resistance rate of 90% to penicillin but found 100% susceptibility of *S. aureus* towards trimethoprim - sulfamethoxazole. Contrarily, resistance towards aminoglycosides was not seen in this study based on the 100% susceptibility towards gentamicin. A study by Zhang *et al.* (2012) reported high resistance rate to erythromycin (85.6%) which was in contrast with this study which showed only 9% of the *S. aureus* isolates was resistant to erythromycin. According to Virdis *et al.* (2010), the susceptibility of *S. aureus* was high for oxytetracycline (84%) and this can be compared with low resistance rate (increased susceptibility) in this study with only 18% isolates were resistant to tetracycline which was classified under the same group as oxytetracycline.

This study was limited in the number of animals, farms and region and only provided baseline information on the antibiotic susceptibility of *E. coli* and *S. aureus*. Susceptibility patterns for various bacteria could be similar between studies, but few studies had compared trends in susceptibility patterns over a period of several years. The comparisons of susceptibility patterns from this study to previous studies must be made cautiously because of differences in selection of animals, regional differences in pathogen populations and the extent to which antibiotics are used in various regions.

CONCLUSION

S. aureus is a common pathogen of goats based on the high number of isolates from the milk and *E. coli* as commensals in the intestine. The *S. aureus* isolates were found resistant to four of six antibiotics ranging from 9% to 27% while 100% *E. coli* isolates were found resistant to two of six antibiotics. Both isolates showed high susceptibility to antibiotics and low antibiotic resistance except to ampicillin, amoxycillin and penicillin.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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