UNIVERSITI PUTRA MALAYSIA

THE ANTIMICROBIAL SUSCEPTIBILITY PROFILING OF *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* IN SMALL RUMINANTS IN *LADANG ANGKAT,* UNIVERSITI PUTRA MALAYSIA (UPM), SELANGOR, MALAYSIA

NASEEHA SAKINA BINTI DIAUUDIN

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CERTIFICATION

It is hereby certified that I have read this project paper entitled "The antimicrobial susceptibility profiling of *Staphylococcus aureus* and *Escherichia coli* in small ruminants in *Ladang Angkat*, Universiti Putra Malaysia (UPM), Selangor, Malaysia", by Naseeha Sakina binti Diauudin and in my opinion it is satisfactory in term of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999- Final Year Project.

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DEDICATIONS

This project paper is dedicated to Allah S.W.T., for His blessings and opportunity for me to embark my journey as a veterinary student,

To my beloved family,

My father, Diauudin bin Omar

My mother, Rohaya binti Muhammed

My siblings; Afifah Amila, Farah Nabila, Ikmal Nazreen,

My friends,

And to all my teachers who have committed themselves towards the noble cause of education. Thank you for the continuous support and care.

May this be an inspiration and motivation for your future ventures.

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LIST OF ABBREVIATIONS

%	: Percent
S. aureus	: *Staphylococcus aureus*
E. coli	: *Escherichia coli*
AMR	: Antimicrobial resistance
AST	: Antimicrobial sensitivity test

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek Ilmiah Tahun Akhir

PEMPROFILAN SUSEPTIBILITI ANTIMIKROB BAGI *STAPHYLOCOCCUS AUREUS* DAN *ESCHERICHIA COLI* DALAM KALANGAN RUMINAN KECIL DI LADANG ANGKAT, UNIVERSITI PUTRA MALAYSIA (UPM), SELANGOR, MALAYSIA

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Kerintangan antimikrob (AMR) telah menjadi satu masalah yang besar di seluruh dunia dengan impak kesihatan awam yang penting dalam populasi haiwan dan manusia. *Staphylococcus aureus* (*S. aureus*) adalah patogen

biasa dalam ternakan domestik dengan *Escherichia coli* (*E. coli*) sebagai flora normal dalam usus dan dianggap sebagai petunjuk yang berkesan dalam pengawasan AMR. Kajian ini bertujuan untuk menilai kerentanan *S. aureus* dan *E. coli* yang diasingkan daripada ruminan kecil di Ladang Angkat terhadap antimikrob berbeza. Sampel susu dan najis telah diambil daripada 36 kambing dari 3 Ladang Angkat berbeza dan sampel kemudian disediakan bagi pengasingan *S. aureus* dan *E. coli* masing-masing. Sebelas asingan *E. coli* telah diuji untuk ujian kepekaan antimikrob dan semua asingan menunjukkan rintangan terhadap antibiotik amoxicillin dan penisilin manakala 3 asingan *S. aureus* menunjukkan rintangan terhadap kelas antibiotik yang sama. Hasil kajian menunjukkan bahawa kedua – dua bakteria ini mempunyai kerentanan yang rendah terhadap antimikrob yang berbeza yang mana ini menunjukkan tahap kerintangan antimikrob yang tinggi dan penggunaan antibiotik beta-laktam yang lebih luas dalam kebanyakkan Ladang Angkat. Kajian ini memberikan maklumat terperinci mengenai profil kerintangan antimikrob untuk patogen biasa dan hubung kaitnya dengan amalan penggunaan antimikrob di Ladang Angkat.

Kata kunci: ***kerintangan antimikrob, kerentanan antimikrob, E. coli, S. aureus, ruminan kecil***

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Project.

THE ANTIMICROBIAL SUSCEPTIBILITY PROFILING OF *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* IN SMALL RUMINANTS IN *LADANG ANGKAT,* UNIVERSITI PUTRA MALAYSIA (UPM), SELANGOR, MALAYSIA

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2017

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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 with *Escherichia coli* (*E. coli*) being a normal flora in gut and considered as effective indicators for AMR surveillance. This study

aimed to evaluate the susceptibility of *S. aureus* and *E. coli* isolated from small ruminants in *Ladang Angkat* against different antimicrobials. Milk and faecal samples were collected from 36 goats from 3 different *Ladang Angkat* and samples were prepared for the isolation of *S. aureus* and *E. coli* isolates respectively. Eleven *E. coli* isolates were tested for antimicrobial sensitivity test (AST) and all isolates showed resistance towards amoxicillin and penicillin antibiotics while 3 isolates of *S. aureus* showed resistance towards the same class of antibiotic. The results showed that both *S. aureus* and *E. coli* isolates have low susceptibility to different antimicrobials indicating high level of antimicrobial resistance and wider usage of β-lactam antibiotics in most of *Ladang Angkat.* This study provides detailed information about the antimicrobial resistance profile of the common pathogens in relation to the antimicrobial usage practice in *Ladang Angkat*.

Keywords: ***antimicrobial resistance, antimicrobial susceptibility, E. coli, S. aureus, small ruminants***

1.0 INTRODUCTION

1.1 Study background

The ability of bacteria to resist the inhibitory effect of antimicrobial drugs is termed as antimicrobial resistance (AMR). Antimicrobial resistance has become a major problem worldwide with a public health concern. One bacterium may develop multi-drug resistance and/or cross resistance (resistance to other antimicrobials in same or related class) and despite of their important roles in treating bacterial infections in human as well as animals (Wendlandt *et al*., 2015). *Staphylococcus aureus (S. aureus)* is a common pathogen in domestic livestock and has become resistant to many commonly used antibiotics with multiple antimicrobial susceptibility patterns have been observed in various animals (Chunping *et al*., 2009). Similarly, *Escherichia coli (E. coli)* is a normal flora in gut and considered as effective indicator for AMR surveillance in different populations and transfer of resistant bacteria between different environment (Alonso *et al*., 2016). Increased in antimicrobial resistance of these bacteria in veterinary medicine leads to difficulties in choosing the effective antibiotics to treat diseases and the usage of antimicrobial agents in agriculture will continue to increase due to lack of knowledge and little information available for reference on the choice of antimicrobial agents. Unfortunately, there is not many comprehensive studies done on antimicrobial susceptibility profiling of *S. aureus* and *E. Coli* among livestocks in Malaysia and only little

attention has been paid to the resistance in specific animal pathogens (Lanz *et al*., 2003). Therefore, this study helped in providing more information about the antimicrobial resistance profile of common pathogens causing diseases among small ruminants with zoonotic potential and eventually the data will be beneficial in improving the farm management strategies with prudent and responsible use of antimicrobials.

1.2 Justification

1. Lack of study on antimicrobial susceptibility profiling of *S. aureus* and *E. coli* among livestocks in Malaysia.
2. *S. aureus* is known to harbour multiple resistances to antimicrobial agents especially beta-lactam antimicrobials that can lead to complications in treating infections of both animals and human.
3. *E. coli* is an important zoonotic agent and a good indicator to determine the level of AMR in different populations and to evaluate the transfer of resistant bacteria between different ecosystems.

1.3 Objective

1. To evaluate the susceptibility of *S. aureus* and *E. coli* isolated from small ruminants in *Ladang Angkat*, Selangor against different antimicrobials.

1.4 Hypothesis

S. aureus and *E. coli* isolates have low susceptibility to different antimicrobials indicating high level of antimicrobial resistance.

2.0 LITERATURE REVIEW

2.1 Antimicrobial and Antimicrobial Resistance (AMR)

According to Frieden (2010), antimicrobial is a term generally used for drugs, chemicals, or other substances that either kill or suspend the growth of microbes. Examples are, antibiotic (which kill bacteria), antiviral agents (which kill viruses), antifungal agents (which kill fungi), and antiparisitic drugs (which kill parasites). Antimicrobial agent resistance has become an emerging worldwide problem in both human and veterinary medicine (R. Sayah *et al*., 2005) and it is generally accepted that increased in usage of antibiotic is the main risk factor for this problem (van Den Bogaard, & Stobberingh, 2000). Antibiotic usage in both animals and humans has been applied for therapy and prophylaxis of infectious diseases caused by bacteria. In addition, as reported by van Den Bogaard (2000), antibiotics for animals have also been incorporated into commercial livestock and poultry feed at sub therapeutic doses for growth promotion. All these exposures to antibiotics as growth promoters and therapeutics purposes have caused the emergence and spread of drug-resistance bacteria

among pathogenic and non-pathogenic bacteria strains including the normal intestinal flora of humans and animals (Adzitey *et al*., 2012).

The principle behind the development of resistance is due to different types, concentrations and frequencies of exposure to antimicrobial agents experienced by the bacteria in the guts of humans and animals. Based on study done by Prescott *et al*. (2000), there are four general mechanisms of resistance which controlled by the action of specific genes and over time, selective pressure will select resistant bacteria that have specific fingerprints for resistance to the antimicrobial agents that have been used. Bacteria gain the resistance genes through mobile elements such as plasmids (Prescott *et al*., 2000) and mutations occurred in genes of binding sites or activation of portions of bacterial chromosomes. Once acquired, the resistance genes can be transferred between bacteria and a study by Lanz *et al*. (2003) has shown that *E. coli* can serve as reservoirs of these resistance genes. Being the normal intestinal flora of animals, *E. coli* not only act as reservoir of resistance genes for potentially pathogenic bacteria, but is able to be a good indicator for the resistance problems expected in pathogens (van Den Bogaard, & Stobberingh, 2000).

2.2 *Escherichia coli* in Small Ruminants

Escherichia coli is the most prevalent species of gram-negative bacteria (Bannerman *et al*., 2004) with a remarkably diverse species of

bacteria. Some strains of this bacteria live as harmless commensals in animal intestines (T. Farasat *et al.*, 2012). According to 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 disease in humans and also capable of causing zoonotic infections (Santos *et al*., 2013). The most important enterohaemorrhagic *E. coli* (EHEC) serotype associated with human disease is *E. coli* O157:H7 and the intestinal tracts of cattle, sheep and goats can serve as its reservoir (Amadi *et al*., 2015). These animals can easily contaminate the environment by shedding of the organism in their faeces that easily spread via soil, water and food (Battisti *et al*., 2006). The intestines of animals are considered as an ideal environment for the selection and transfer of antimicrobial resistance genes. Study by Lanz *et al*. (2003) has shown that *E. coli* can serve as reservoirs of antibiotic resistance genes. These genes have been efficiently transferred to other *E. coli* strains and also to other enteric pathogens of humans and animals (Oguttu *et al*, 2008).

2.3 *E. coli* and its Relation with Antimicrobial Resistance

In consequences to human health, there is a growing concern about the transfer of resistance bacteria from animals to human and *E. coli* have been responsible for a number of foodborne outbreaks (Centres for Disease

Control and Prevention; 2014) . In a study by Adzitey *et al*. (2015a), 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. Other than that, resistant bacteria from animals can also infect human population via food products of animals' 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 (Choffness *et al.,* 2012). For example, resistant bacteria from the intestinal flora of food animals can contaminate carcasses of slaughtered animals and reach the intestinal tract of humans via the food chain. These bacteria can then colonise humans and/or transfer their resistance genes to other bacteria of the endogenous flora of man. The higher the number of resistant bacteria in the intestinal flora, it is more likely for the resistance genes to be transferred and spread into the environment and from animals to foods of animal origin.

The problem of antimicrobial resistance need to be controlled and this can be achieved by reducing the amounts of antibiotics used in animals. As suggested by van Den Bogaard, & Stobberingh (2000), the requirement of antibiotics in veterinary therapy and bacterial infection prevention should be minimised by improving animal husbandry, disease eradication, optimum usage of existing vaccines and development of new vaccines.

2.4 *Staphylococcus aureus* in Small Ruminants

Staphylococcus aureus is among the most prevalent species of gram-positive bacteria in domestic livestock and is said to be the most important agent of mastitis in ruminants worldwide (Bannerman *et al*., 2004; Caruso *et al.,* 2016). 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 (Bergonier *et al*., 2003). In addition, study by Bochev and Russenova (2005) showed methicillin – resistant coagulase negative staphylococci (MRCNS) strains can be isolated from goats' milk with subclinical mastitis (SCM), thus confirming that staphylococci are the most common pathogens associated with SCM in dairy goats.

Goat and sheep milk may cause staphylococcal food poisoning in humans and this can happened as the bacteria gain access to milk either by direct excretion from udders or by secondary contamination (Scherrer *et al.,* 2004). There is higher risk of infection involved with small ruminants' milk as many traditional caprine and ovine milk products are not subjected to pasteurization and are often used for traditional, unpasteurized products such as raw milk cheeses (Merz *et al*., 2016).

In addition, human food poisoning can also be caused by strains of *S. aureus* through the production of an enterotoxin (C. Zhang *et al*., 2012).

Based on study done by Valle *et al*. (1990), staphylococcal enterotoxins (SE) are exoproteins which can cause symptoms of acute gastroenteritis when ingested by humans and several types of these enterotoxins have been identified serologically. Most enterotoxigenic strains are isolated from milk with very high percentages of coagulase-positive strains (CPS) which are *S. aureus* and *Staphylococcus hyicus* (*S. hyicus*) and lower percentages of coagulase-negative strains (CNS). This is proven by high percentage of enterotoxigenic CPS isolated from goat milk in study by De Buyser *et al.* (1987) and Valle *et al*. (1990). These studies may conclude that a high percentage of goats should be considered as carriers of enterotoxin-producing staphylococci.

2.5 *S. aureus* and its Relation with Antimicrobial Resistance

Recent studies have shown an increase of staphylococci strains that exhibited resistance to methicillin/oxacillin. Methicillin – resistant *S. aureus* (MRSA) is found primarily in humans and later detected in animals (Lee *et al*., 2004). MRSA strains are characterized by the presence of the mecA gene encoding low-affinity penicillin binding protein (PBP2), which mediate resistance to all classes of β-lactam antibiotics (Virdis *et al*., 2010). According to Vyletělová et al. (2011), MRSA are divided into three groups according to their epidemiological and genetic characteristics which are hospital acquired (HA-MRSA), community-associated (CA-MRSA), and livestock-associated (LA-MRSA). The most commonly reported LA-MRSA

is MRSA sequence-type 398 (ST398) genetically classified under Clonal Complex 398 (CC398) (Loncaric & Brunthaler, 2013). This strain has shown to be able to colonize and caused serious infections in humans in close contact with animals primarily pigs, cattle and horses (De Martino *et al.,* 2010) and thus, increase specific attention of the transfer of MRSA isolates between animals and humans. However, there is still a scarcity of information on infections with MRSA ST398 in goats (I. Loncaric, R. Brunthaler, 2013) and the absence of mecA gene and the low prevalence of antibiotic resistance suggest that SCM in goats does not play a significant role in the spreading of multiresistant staphylococci. In return, it did not represent a great public health concern (S. Virdis *et al*., 2010)

MRSA strains have also been reported in causing intramammary infection in goats (Aras *et al*., 2012). This is proven in a study by Stastkova *et al.* (2009), which first reported MRSA detection from goats and/or goat's milk where MRSA strains are discovered only in aseptically collected goat's milk but not found in any nasal or fecal swabs collected. In addition, Alves *et al*. (2009) reported that MRSA can cause subclinical mastitis in goats and this means the microorganism 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 resistance genes through the dairy food chain (Alves *et al*., 2009).

A study by Feingold *et al*. (2012) reported that handling or consumption of food of animal origin contaminated by MRSA could provide a potential vehicle for transmission to humans. This is further supported by a study which reported MRSA clones have been largely isolated from milk and dairy products (Karmal *et al*., 2013) and the isolates are considered potentially harmful for consumers. Kock et al., (2013) also reported human MRSA isolates showed a sequence type (ST398) which typically associated with livestock and it is highly suggested that the source of human infection could have been of animal origin. Transmission of MRSA between dairy cows and people working on farms has been reported by Antoci *et al.* (2013) and this provides evidence that people having close contact with MRSA ST398 shedding animals are at risk of being colonized by these strains. Method of MRSA spread has remained unclear until now but study by Stastkova *et al*. (2009) has confirmed the human to animal (or vice versa) transmission of MRSA. MRSA colonization is a risk for humans because family members who are living with MRSA carriers can be exposed to MRSA transmission and thus causing the high transmission of MRSA in community (Matsumoto *et al.,* 2001). Thus, the presence of MRSA in basic food production poses a risk of spreading the pathogens to other animal species, farm workers, foodstuffs and consequently to the general population

3.0 MATERIALS AND METHOD

3.1 Experimental Design

A cross – sectional approach was used to collect samples and data over a 3-week period. Three farms were chosen randomly among the *Ladang Angkat* small ruminants farms (foster farms) of University Veterinary Hospital (UVH), UPM. All selected farms were managed semi-intensively.

3.2 Animal selection

A total of 36 goats from 3 farms were sampled. The goats selected were lactating, apparently healthy adults and aged more than one year old. Milk and faecal samples were collected from each animal.

3.3 Samples collection

Milk samples were collected aseptically from lactating does. Dirty udders were cleaned thoroughly with mixture of water and chlorhexidine gluconate (Hibiscrub® 4%) and dried using clean towel. 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 and the first 1 to 2 streams of milk were removed from each teat before collecting the milk. The milk was collected in sterile milk tubes and the tube was hold at 45° to prevent debris from entering the tube and to prevent the tube from touching the end of the teat (Bewley *et al*., 2010). About 3 to 5 ml

of milk was collected for each sampling 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.

Both milk and faecal samples were prepared for the isolation of *S. aureus* and *E. coli* isolates respectively.

3.4 Isolation and identification of *S. aureus* from milk samples.

Standard methods for the isolation, identification, and biochemical confirmation of *S. aureus* isolates as outlined in Diagnostic Manual of Veterinary Clinical Bacteriology and Mycology (Jang *et al*., 2008) were performed. A loop of milk was collected from the milk sample by 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 colonies that grew on the agar were then sub-cultured and Gram staining was performed on the colonies smears 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 biochemical confirmation by blood broth (hemolysin), Voges-Proskauer (VP), maltose, mannitol and Arginine Dihydrolase (ADH) tests. Only the bacterial isolates

that were confirmed to be *S. aureus* based on the results of the biochemical tests were selected for antimicrobial agent susceptibility testing.

3.5 Isolation and identification of *E. coli* from faecal samples.

Standard methods for the isolation, identification, and biochemical confirmation of *E. coli* isolates as outlined in Diagnostic Manual of Veterinary Clinical Bacteriology and Mycology (Jang *et al*., 2008) were performed. Faecal samples were inoculated on MacConkey's agar by using sterile swab. The agar plates were then incubated at 37°C for 24 to 48 hours. The MacConkey agar plate was 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 proceed for biochemical confirmation by triple sugar iron (TSI) agar, sulfide indole motility (SIM) media, citrate test and urease test. Only the bacterial isolates that were confirmed to be *E. coli* based on the results of the biochemical tests were selected for antimicrobial agent susceptibility testing.

3.6 Antimicrobial agent susceptibility testing

Once a single *S. aureus* and *E. coli* isolate was isolated and identified from each sample collected, the standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial agent sensitivity profiles of the isolates for 9 antimicrobial agents. The procedure was done in accordance with the standards described in the National Committee for Clinical Laboratory Standards (NCCLS) M31-A document (National Committee on Clinical Laboratory Standards, Wayne, PA), including suggested breakpoints to determine susceptibility and resistance. 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 antimicrobial agents were selected based on their importance in bacterial infections and on the basis of their ability to provide diversity for representation of different antimicrobial agent classes (Sayah *et al.,* 2005). Standard suspension 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 antimicrobial agent discs were placed on each inoculated plate. Total of 11 plates of *S. aureus* and *E. coli* isolates were prepared for antimicrobial agent susceptibility test respectively. The plates were incubated at 37°C for 24 hours. The diameters (in millimeters) of the clear zones of growth inhibition around the antimicrobial agent discs, including the 6-mm disc diameter, were measured by using precision callipers and the results were interpreted as sensitive, intermediate, or resistance based on the breakpoints diameter according to Clinical and Laboratory Standards Institute guidelines (CLSI., 2006).

4.0 RESULTS

4.1 Selective isolates of *E.coli* and *S. aureus*

Table 1 : Number of *E. coli* and *S. aureus* isolates obtained from faecal and milk samples respectively from dairy goats from 3 *Ladang Angkat* farms

	ISOLATES (SAMPLES)	
FARM	***E.coli* (faecal)**	***S.aureus* (milk)**
A	85% (11/13)	100% (13/13)
B	94% (15/16)	63% (10/16)
C	100% (7/7)	43% (3/7)
TOTAL	**92% (33/36)**	**72% (26/36)**

Table 1 shows the number of bacterial isolates, isolated from faecal and milk samples from 3 different farms. *E. coli* and *S. aureus* were selectively collected from faecal and milk samples respectively. From all 3 farms, a total of 92% samples were positive for *E. coli* and 72% samples were positive for *S. aureus*. For Farm A, 85% *E. coli* and 100% *S. aureus* were isolated, while from Farm B, a total of 94% *E. coli* and 63% *S. aureus* were cultured. For Farm C, both *E. coli* and *S. aureus* were 100% and 43% isolated respectively.

4.2 Antimicrobial Susceptibility Test (AST)

A total of 11 isolates of each *E.coli* and *S. aureus* were selected for the antimicrobial susceptibility test (AST).

As shown in Table 2, all (100%) of the 11 *E. coli* isolates were found to be susceptible to all of the antimicrobials tested except for amoxicillin and penicillin. As for *S. aureus*, 3 out of 11 (27%) isolates were resistant to both ampicillin and penicillin. One out of 11 (9%) of the *S. aureus* isolates was resistant to erythromycin while 2 out of 11 (18%) isolates were resistant to tetracycline.

For each Farm A and B, 4 *E. coli* isolates were resistant to 2 antimicrobials which were amoxicillin and penicillin while in Farm C, 3 *E. coli* isolates were resistant towards the same antimicrobials. As for *S. aureus*, Farm A had 4 isolates that were resistant towards 3 antimicrobials which are ampicillin, penicillin and tetracycline while Farm B had 1 isolates that were resistant towards erythromycin and tetracycline. However in Farm C, all 3 isolates were susceptible to all antimicrobials tested.

Table 2: Antimicrobial susceptibility test (AST) result for *E. coli* and *S. aureus* isolated from dairy goats in Farms A, B and C

FARM	A		B		C		
	SUSCEPTIBLE ISOLATES – *S. aureus*						
ANTIBIOTICS	n	%	n	%	n	%	TOTAL ISOLATES RESISTANT (%)
Ampicillin	1/4	25%	4/4	100%	3/3	100%	3/11 (27%)
Trimethoprim - sulfamethazole	4/4	100%	4/4	100%	3/3	100%	0/11 (0%)
Penicillin	1/4	25%	4/4	100%	3/3	100%	3/11 (27%)
Erythromycin	4/4	100%	3/4	75%	3/3	100%	1/11 (9%)
Tetracycline	3/4	75%	3/4	75%	3/3	100%	2/11 (18%)
Gentamicin	4/4	100%	4/4	100%	3/3	100%	0/11 (0%)
TOTAL ISOLATES - RESISTANT AM	**4-3**		**1-2**		**0**		-

FARM	A		B		C		
	SUSCEPTIBLE ISOLATES – *E. coli*						
ANTIBIOTICS	n	%	n	%	n	%	TOTAL ISOLATES RESISTANT (%)
Amoxycillin	0/4	0	0/4	0	0/3	0%	11/11 (100%)
Trimethoprim - sulfamethazole	4/4	100%	4/4	100%	3/3	100%	0/11 (0%)
Penicillin	0/4	0	0/4	0	0/3	0%	11/11 (100%)
Neomycin	4/4	100%	4/4	100%	3/3	100%	0/11 (0%)
Tetracycline	4/4	100%	4/4	100%	3/3	100%	0/11 (0%)
Enrofloxacin	4/4	100%	4/4	100%	3/3	100%	0/11 (0%)
TOTAL ISOLATES - RESISTANT AM	**4-2**		**4-2**		**3-2**		-

5.0 DISCUSSION

The results of this study showed a total of 33 out of 36 (92%) *E. coli* isolated from faecal samples from 3 different farms. Table 1 shows tabulation of the isolates ranging from 85% to 100% of *E. coli* isolates according to farms. In Farm A, there were 11 out of 13 (85%) *E. coli* isolates. Farm B showed a total of 15 out 16 (94%) isolates while Farm C with 7 out of 7 (100%) isolates. This indicated that healthy goats harbour *E. coli* in their gastrointestinal tract and the occurrence is widespread among goats in the investigated farms. The high isolates number proven 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. As shown in Table 1, 13 out of 13 (100%) samples from Farm A showed presence of *S. aureus*. From the high number of isolates, it can be safely assumed that the goats from this farm may be having subclinical mastitis (SCM). This assumption was made based on a study by Bochev and Russenova (2005) which confirmed that *S. aureus* are the most common pathogens associated with SCM in dairy goats. This study however did not target on goats with SCM and that was why sampling was done on the apparently healthy goats without any test to detect SCM pre-sampling.

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). Aside from Farm A, there were 10 out of 16 (63%) isolates from Farm B and 3 out of 7 (43%) isolates from Farm C.

A total of 11 isolates of each *E.coli* and *S. aureus* were then selected for the antimicrobial susceptibility test (AST). Two separate groups of antimicrobial agents with 6 different agents in each group were utilized for AST; one group for gram-positive cocci (*S. aureus*) and the other for gram negative rods (*E. coli*). Different antimicrobial agents were chosen based on difference in susceptibility and to provide diversity for representation of different antimicrobial agent classes. *S. aureus* isolates were tested with ampicillin, erythromycin, penicillin, tetracycline, gentamicin and trimethoprim/sulfamethoxazole (Erskine *et al*., 2002). *E. coli* isolates were tested with amoxicillin, trimethoprim/sulfamethoxazole, penicillin, neomycin, tetracycline and enrofloxacin (Bogaard & Stobberingh, 2000). These antibiotics were also selected based on their importance in causing bacterial infections 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) and were registered with the National Pharmaceutical Control Bureau (NPCB) of the Ministry of Health, Malaysia (Health Action International Asia Pacific (HAIAP), 2013).

In this study, the result obtained from AST revealed high susceptibility of the 11 *E. coli* isolates to four out of six antibiotics tested (Table 2). Table 2 showed all (100%) of the 11 *E. coli* isolates were found to be susceptible to all of the antimicrobials tested except for amoxicillin and penicillin. This is similar to the findings of Amadi *et al.* (2015) who tested *E. coli* isolates from healthy goats in Grenada with 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) of the antibiotics in the same class towards 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 the other and the differences are due to variations 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*, 3 out of 11 (27%) isolates were resistant to both ampicillin and penicillin. One out of 11 (9%) of the *S. aureus* isolates was resistant to erythromycin while 2 out of 11 (18%) isolates 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. This is also similar to the finding by Zhang *et al.* (2012) which reported the high resistance rate of 90% observed with penicillin and also 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. Study by Zhang *et al.* (2012) reported high resistance rate observed with erythromycin (85.6%) which was in contrast with this study which showed only one out of 11 (9%) of the *S. aureus* isolates was resistant to erythromycin. According to Virdis (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 2 out of 11 (18%) isolates were resistant to tetracycline which was classified under the same group as oxytetracycline.

In conclusion, this study is limited in scope of region and only provides baseline information about the antibiotic susceptibility of *E. coli* and *S. aureus.* Susceptibility patterns for various bacteria could be similar between studies, but few studies have 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.

6.0 CONCLUSION

From this study, it can be concluded that *S. aureus* and *E. coli* are common pathogens of small ruminants based on the high number of isolates from the milk and faecal samples respectively. The isolates of these bacteria have low susceptibility to different antimicrobials indicating high level of antimicrobial resistance.

7.0 RECOMMENDATION

Recommendation that can be suggested for future study is to further conduct minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as a more quantitative measure of susceptibility after conducting AST using standard Kirby-Bauer disk diffusion method. Another suggestion is to conduct California mastitis test (CMT) before milk collection to check for subclinical mastitis.

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