



**UNIVERSITI PUTRA MALAYSIA**

**EFFECTS OF REPRODUCTIVE STAGES ON THE PREVALENCE OF  
THE NORMAL FLORA IN THE DOE**

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**EFFECTS OF REPRODUCTIVE STAGES ON THE PREVALENCE  
OF THE NORMAL FLORA IN THE DOE**

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## **CERTIFICATION**

It is hereby certified that we have read this project paper entitled “Effects of the Reproductive Stages on the Prevalence of the Normal Flora in the Doe”, by Norazmanita Edayu bt Ajaman and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999  
– Project

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*Specially dedicated to my beloved*

*parents and friends*



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**ABSTRACT**

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

**EFFECTS OF THE REPRODUCTIVE STAGES ON THE  
PREVALENCE OF THE VAGINAL NORMAL FLORA IN THE**

**DOE**

**By**

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**2016**

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**Co-supervisor:**

**Dr. Hasliza Abu Hassim**

**Assoc. Prof. Dr. Siti Khairani Bejo**

The genital tract of animals is known to harbour non-specific bacteria known as "normal flora" but under certain circumstances they can be opportunistic pathogen. Generally, variety of bacteria have been isolated but their role and mechanism are not well understood. This study was designed to determine the prevalence of normal bacteria in the vaginal of pregnant and non-pregnant doe with the aims to isolate and identify those bacteria and relate with the different stages of reproduction. For this purpose, a number of fourteen sterile vaginal swabs were collected from 2 groups of doe; non-

pregnant and pregnant doe (n = 7). The swabs were cultured and incubated for 24h and morphology of the colonies were recorded. Subculture was done to obtain the pure colonies. Gram staining was performed followed by biochemical test accordingly. Bacteria isolated from the vagina of the pregnant group showed higher percentage (55.6%) than non-pregnant group (44.4%). The most dominant bacteria belong to the *Stapylococci* (58.33%) followed by *Streptococci* and *Enterococcus* (8.33%) and other species were isolated at relatively lower rates in both groups. This study showed that hormonal changes during different stages of reproduction had effect on the vaginal bacterial population.

Keywords: *pregnant, non-pregnant, vagina, normal bacterial flora, sterile vaginal swabs, doe*

## **ABSTRAK**

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar sebagai memenuhi sebahagian daripada keperluan kursus VPD 4901-Projek.

### **KESAN PERINGKAT PEMBIAKAN KEATAS PREVALENS FLORA NORMAL VAGINA BAGI KAMBING BETINA**

Oleh

**Norazmanita Edayu Bt Ajaman**

2016

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Saluran peranakan haiwan merupakan kawasan pembiakan bakteria yang tidak spesifik yang dikenali sebagai flora normal tetapi dalam keadaan tertentu bakteria ini boleh menjadi patogen oportunistik. Secara umumnya, pelbagai bakteria telah dipencilkan tetapi peranan dan mekanisme mereka tidak difahami dengan baik. Kajian ini bertujuan untuk menentukan prevalens bakteria normal dalam vagina kambing betina yang hamil dan yang tidak hamil dengan tujuan untuk mengasingkan dan mengenal pasti bakteria yang berkaitan dengan pelbagai peringkat pembiakan. Untuk tujuan ini, empat belas swab vagina steril dikumpulkan dari 2 kumpulan kambing betina; kambing betina tidak hamil dan hamil ( $n = 7$ ). Sampel dikulturkan dan diinkubasi selama 24 jam dan morfologi

koloni telah direkodkan. Subkultur telah dilakukan untuk memperoleh koloni tunggal dan seragam. Gram pewarnaan dilakukan diikuti oleh ujian biokimia. Bakteria diasingkan daripada faraj kumpulan kambing hamil menunjukkan peratusan yang lebih tinggi (55.6%) daripada kumpulan yang tidak hamil (44.4%). Bakteria paling dominan tergolong dalam *Staphylococci* (58.33%) diikuti oleh *Streptococci* dan *Enterococcus* (8.33%) dan spesies lain telah diasingkan pada kadar yang lebih rendah dalam kedua-dua kumpulan. Kajian ini menunjukkan bahawa perubahan hormon semasa peringkat pembiakan mempunyai kesan ke atas populasi bakteria vagina

## 1.0 INTRODUCTION

Mucosal sites inhabited by commensal flora need to be capable of discriminating between commensals and the pathogenic organisms they encounter in order to generate protective immune responses (Gary, 2006). The lower female reproductive tract is one such site.

Hormones play a very important role in regulating host immunity in the genital tract, distinguishing this from other mucosal sites, notably oestrogen, progesterone and PGE2. Fluctuations in hormone levels during the reproductive cycle and pregnancy influencing immune surveillance and disease susceptibility (Gary *et al.*, 2006). Thus, the importance of studying such commensals flora is related to disease caused by them due to reduction of the immunity of the reproductive system (Al-Dahash and Fathall, 2000).

Considering these, the study was designed to assess the presence of normal bacteria in the vaginal environment of pregnant compared to non-pregnant doe with the aims to isolate and identify bacteria those are related with the different stages of reproduction and to determine the dominant and predominant groups capable of colonizing the vagina. Hence, the objectives are:

- i. To assess the presence and percentages of normal bacteria in the vaginal environment of pregnant compared to non-pregnant doe.
- ii. To isolate and identify the types of normal bacteria presence in the vagina of non-pregnant and pregnant doe.
- iii. To identify different types of isolated bacteria from the vagina of non-pregnant and pregnant does.

For this research, the following hypotheses were proposed:

- i. There will be higher number of the vaginal normal flora isolate from the non pregnant compared to the pregnant doe.
- ii. There will be more types of bacteria isolated from the vagina of non-pregnant than the pregnant doe

## **2.0 LITERATURE REVIEWS**

### **2.1 Vaginal physiology**

The vagina is a unique environment for bacterial colonization. It is subject to dramatic changes over the course of a lifetime, induced by developmental and hormonal changes (Phillip, 2002).

### **2.2 What is 'Normal flora'?**

Microbial flora has spatial and temporal complexity that differs by individual, body niche, age, geographic location, health status, diet and type of host (Savage, 1997). Even within the same individual, the composition of the microbial flora can vary according to changes in diet, stress, sexual behaviour, medication, hormonal changes and other host-related factors (Salminen *et al.*, 1995).

### **2.3 Types of bacteria found in the reproductive tract of female goats**

Several studies revealed there were several types of bacteria found in the reproductive tract of female goats. A study was conducted to determine the aerobic bacterial flora of the vagina, cervix, uterine body, right and left uterine horns and right and left oviducts which were taken from seventeen goat's genital systems in Al-Hilla Medhatia slaughterhouse. From their results, they concluded that Gram positive bacteria particularly *Staphylococcus aureus* were the most predominant micro-flora and *E.coli* from the Gram negative bacteria in normal cases. These bacteria may be present as opportunists which under conditions of stress may result in disease, the role played by these non-specific bacteria is not known but may result in disease under unfavourable conditions of stress (Saad *et al.*, 2014).

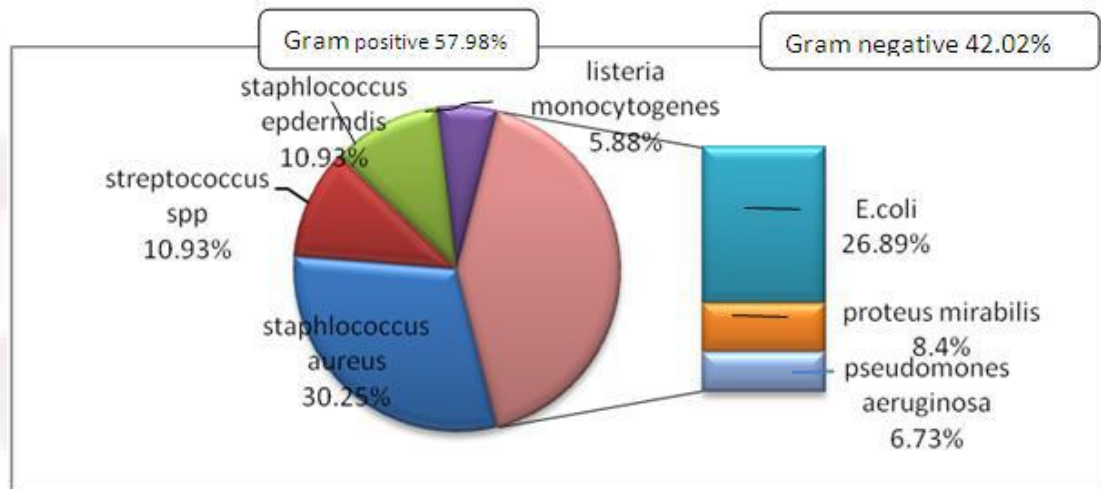


Figure 1: Percentage of different aerobic bacteria isolated from female genital system of goats at slaughter (Saad *et al*, 2014).

#### 2.4 Opportunistic bacteria

Opportunist bacteria may cause disease when the host are under favourable conditions of stress (Saad, 2014). They are usually harmless until the presence of predisposing factors such as trauma or another infection which may be pathogenic and cause disease (Martins *et al.*, 2010).

Genital infections in domestic ruminants are often caused by opportunistic secondary invaders, especially *Escherichia coli* species, that have frequently been isolated in majority from ewes (Manes *et al.*, 2010; Martins *et al.*, 2009; Sargison *et al.*, 2007), goats (Ababneh & Degefa, 2006) and cows (Sheldon *et al.*, 2008). Coliforms (of fecal origin) and other non-specific bacteria are also opportunistic pathogens in the reproductive tract. Under stressful conditions, these opportunist bacteria may cause genital infection that usually leads to reproductive failure in ruminants (Levinson & Jawetz, 1994; Shallali *et al.*, 2001). In addition, fluctuations in hormone levels during the reproductive cycle and pregnancy influencing immune surveillance and disease susceptibility.

## 2.5 Occurrence and identification of the opportunistic bacteria causing disease

A study had been carried out to determine the prevalence of vulvovaginitis in goats in Ibadan (Oluwatoyin *et al.*, 2011). Vaginal and vulvular swabs were collected from some of the does with or without clinical signs at different locations in Ibadan. The samples were immediately cultured for isolation and identification of the bacteria agents. The bacteria that were isolated and identified from the samples from affected does were coagulase positive and negative *Staphylococcus*, beta haemolytic *Streptococcus*, *Gemella* species, *E. coli*, *Proteus mirabilis* while from the unaffected does only coagulase positive *Staphylococcus* and faecal type *Streptococcus* with lower populations were isolated and identified.

It was observed that some of the does with or without the clinical signs of vulvovaginitis had *Staphylococci* and *Streptococci* species isolated from the vaginal and vulvular swabs, but the population of these bacteria was higher in does with clinical signs compared to does without clinical signs. This showed the normal vagina contains some bacterial flora and it also suggest that bacteria associated with the vulvovaginitis may be normal bacteria resident of the vagina and vulva and may become pathogenic when there is change in the vaginal and vulvular micro-environment.

## **2.6 Hormone levels during the reproductive cycle and pregnancy associated with immune level in goats**

Hormones play a very important role in regulating host immunity in the genital tract, distinguishing this from other mucosal sites, notably oestrogen, progesterone and PGE<sub>2</sub>.

Plasma levels of progesterone increase markedly in doe between days 90 and 125 gestation, then drop sharply before parturition. Levels of oestrogen increase from around day 120 and show a sharp rise 24 h prior to parturition. Levels of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) are also greatly elevated in the latter stages of pregnancy and around parturition.

Some of these hormones are known to directly influence immune function, notably oestrogen, progesterone and PGE<sub>2</sub>. Fluctuations of these hormone levels during the reproductive cycle and pregnancy influencing immune surveillance and disease susceptibility (Gary *et al.*, 2006).

Progesterone suppresses specific components of the immune system and natural killer cell activity. It is also reported that hormonal changes status such as oestrus cycle could be effect on vaginal bacterial population especially when progesterone levels were high (Gary *et al.*, 2006).

### 3.0 MATERIALS AND METHODS

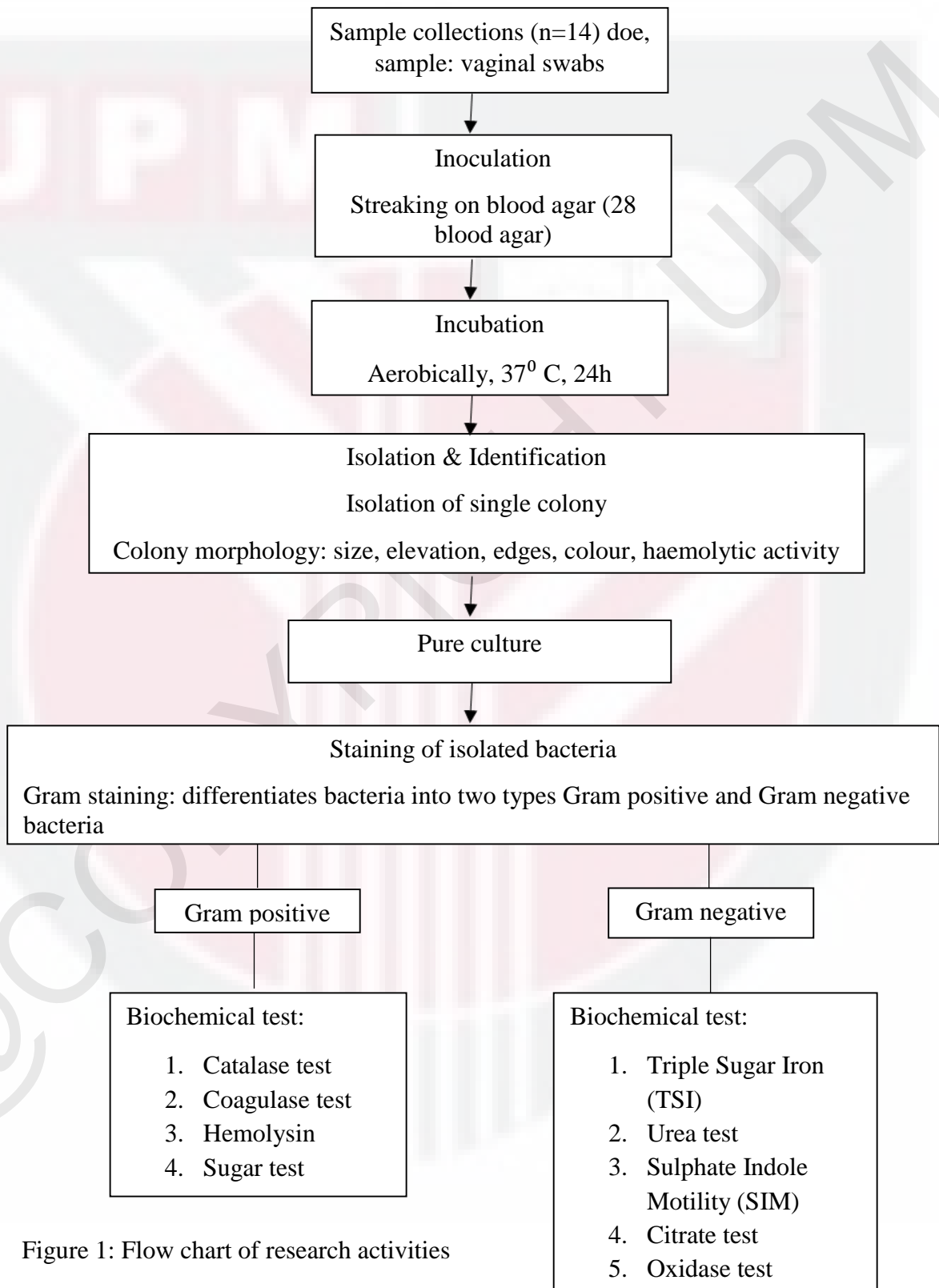


Figure 1: Flow chart of research activities

### **3.1 Selection of animals**

A total number of 7 pregnant doe and 7 non-pregnant doe were selected and examined. The doe were classified into pregnant and non-pregnant based on their records at the farm and confirmed by diagnosing the pregnancy status by ultrasound examination and physical changes appearance which includes increase in abdominal size, udder development and vulva swelling.

### **3.2 Sample collection**

The external genitalia of each animal were washed with chlorhexidine and water prior to sampling. Sterile swabs were inserted into the vagina. The swabs were rubbed gently on the mucosa to collect the largest amount of secretions, then taken out and kept in sterile glass test tubes. Each swab was kept in the ice box while transporting to the laboratory and cultured immediately on the blood agar.

### **3.3 Isolation and identification of bacteria:**

#### **3.3.1 Primary culture method**

Initial inoculum was streak on the blood agar. The swab was streak over the inoculation area to maximise transfer of organisms, taking care to avoid the edges of the plate. Dilution streaking were made by using sterile inoculating loop to isolate individual colonies on the blood agar and labelled. Then the plate was incubated at 37°C under aerobic conditions for 24 hours.

#### **3.3.2 Presumptive identification**

Many different colony types were noted in the initial cultures and each colony type seen was recorded and sub cultured on blood agar to provide a pure subculture. The

initial examination of colonies was made by naked eye and using microscope and recorded. The colonies seen were described in terms of their morphological characters such as size, elevation, outline, colour and their effect on the medium and these were recorded. Colonies were presumptively identified by these characters and their identity confirmed by further biochemical tests. Smears were made from colonies of interest, fixed and stained using Gram staining method and the morphology and the staining reactions were recorded.

### **3.3.3 Biochemical test:**

#### **3.3.3.1 Gram positive bacteria**

**Catalase test:** The bubbles resulting from production of oxygen gas clearly indicate a catalase positive result. The *Staphylococcus spp.* catalase positive. The *Streptococcus* and *Enterococcus spp.* are catalase negative.

**Coagulase test:** This test was used to differentiate *Staphylococcus aureus* which produces the enzyme coagulase, from *S. epidermidis* which do not produce coagulase. Clumping or clots formed within 10 seconds indicate coagulase positive and the organism is *S. aureus*. No clumping within 10 seconds indicate coagulase negative and the organism may be other *Staphylococci* or *S. epidermidis*.

#### **3.3.3.2 Gram negative bacteria**

**Oxidase test:** Positive result will shows deep blue-purple within 10 seconds. If no colour appears within 10 seconds the reaction is negative. *Escherichia coli* and other *Enterobacteriaceae* have oxidase negative.

#### **Triple Sugar Iron (TSI)**

**Urease test:** Testing for urease activity in differentiating *enterobacteria*. Urease positive organisms yield a bright pink or bright red to the agar (e.g. *Proteus* species). Yellow colour indicates negative reaction and no urease enzyme in the test organism (e.g. *E. coli*).

**Sulphide Indole Motility (SIM):** SIM is commonly used to differentiate members of *Enterobacteriaceae*. If hydrogen sulfide is produced, black colour will form in the medium. *Proteus mirabilis* is positive for H<sub>2</sub>S production. Indole reacts with added Kovac's reagent to form Rosindole dye which is red in color (indole +). *Escherichia coli* is indole positive. SIM tubes are inoculated with a single stab to the bottom of the tube. If an organism is motile than the growth will radiate from the stab mark and make the entire tube appear turbid. *Pseudomonas aeruginosa* and the strain of *Proteus mirabilis* that we work with are motile.

**Citrate test:** The alkaline pH turns the pH indicator (bromothymol blue) from green to blue. *Klebsiella pneumoniae* and *Proteus mirabilis* are examples of citrate positive organisms. *Escherichia coli* is citrate negative.

## **4.0 RESULT AND DISCUSSION**

### **4.1 Isolation and identification of bacteria**

#### **4.1.1 The morphology characteristics of colonies isolated**

Each type of the colonies seen from the pure culture were described in terms of their morphological characters such as size, elevation, outline, colour and their effect on the medium and were recorded in Table 1(a) and 1(b).

Table 1(a): The characteristics of colony morphology of each isolated bacteria from the vagina of the non-pregnant does.

<b>Animal ID</b>	<b>Colony morphology</b>							
	Form	Size	Structure	Texture	Colour	Elevation	Margin	Haemolytic activity
<b>Non-pregnant doe</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	1 mm	Shiny	Mucoid	Grey	Raised	Entire	-
	Circular	Pin point	Smooth	Moist	Grey	Raised	Entire	Alpha hemolytic activity
<b>2</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	Pin point	Shiny	Mucoid	White	Convex	Entire	-
	Circular	Pin point	Shiny	Mucoid	Grey	Raised	Entire	Alpha haemolytic activity
<b>4</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	Pin point	Shiny	Mucoid	White	Convex	Entire	-
	Circular	Pin point	Shiny	Mucoid	Grey	Raised	Entire	Alpha haemolytic activity
<b>6</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	1 mm	Shiny	Mucoid	Grey	Raised	Entire	-
	Circular	Pin point	Smooth	Moist	Grey	Raised	Entire	Alpha haemolytic activity
<b>7</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	<1 mm	Shiny	Mucoid	Yellow	Raised	Entire	-
	Circular	Pin point	Shiny	Mucoid	Grey	Raised	Entire	-
<b>H</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	1 mm	Smooth	Moist	Yellow	Raised	Entire	-
	Circular	1 mm	Shiny	Mucoid	White	Raised	Entire	-
<b>BR13</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
<b>BR20</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	<1 mm	Smooth	Moist	Yellow	Raised	Entire	-

Table 1(b): The characteristics of colony morphology of each isolated bacteria from the vagina of the pregnant does

Animal ID	Colony morphology							
	Pregnant doe	Form	Size	Structure	Texture	Colour	Elevation	Margin
<b>8</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	1.5 mm	Shiny	Mucoid	Yellow	Raised	Entire	-
	Circular	1.5 mm	Shiny	Mucoid	Grey	Raised	Entire	-
<b>9</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	1.5 mm	Shiny	Mucoid	Grey	Raised	Entire	-
<b>10</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	Pin point	Shiny	Mucoid	Grey	Raised	Entire	Alpha haemolytic activity
	Circular	1 mm	Shiny	Mucoid	Yellow	Raised	Entire	-
	Circular	Pin point	Shiny	Mucoid	Grey	Convex	Entire	-
<b>11</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	Pin point	Shiny	Mucoid	Grey	Convex	Entire	Alpha haemolytic activity
	Circular	1 mm	Shiny	Mucoid	Milky	Raised	Entire	-
	Circular	1 mm	Shiny	Mucoid	Yellow	Raised	Entire	Beta haemolytic activity
<b>12</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	1.5 mm	Shiny	Mucoid	Grey	Raised	Entire	-
<b>46</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	0.5 mm	Shiny	Mucoid	White	Raised	Entire	-
<b>1556</b>	Circular	Pin point	Smooth	Dry	White	Convex	Entire	-
	Circular	0.5 mm	Shiny	Mucoid	White	Raised	Entire	-

#### 4.1.2 Bacterial morphology and the Gram staining reactions

Bacterial morphology and the Gram staining reactions of each isolated colonies were described and tabulated in table 2(a) and table 2(b). *Staphylococcus intermedius* produced white coloured and pin-point size colony on blood agar as shown in Plate 1(a). All isolated of *Staphylococcus* were Gram positive and arranged in pair and catalase and coagulase positive. *S. aureus* were Gram positive, growth on blood agar with yellowish coloured and mucoid texture as shown in Plate 2(a). *Streptococcus spp.* produced pin-point in size, circular and convex colonies surround by Alpha-haemolytic activity on blood agar (Plate 3a). The isolate *Streptococcus spp.* was Gram positive and arrange in pairs and have coagulase negative. *Enterococcus* group produced pin-point in size, white to greyish coloured with mucoid texture colonies on blood agar (Plate 4a). *Enterococcus faecalis* produced whitish to milky coloured and mucoid texture colonies on blood agar (Plate 5a). Both were Gram positive cocci paired and have negative catalase test. *Proteus mirabilis*, Gram negative rods in paired arrangement. *Proteus mirabilis* produced whitish and mucoid colonies on blood agar (Plate 6a), have negative oxidase test, indole negative and positive motility (Plate 6b). *E.Coli* are Gram-negative, oxidase-negative and rod-shaped bacteria. *Pasteurella aeromonas* and *Pasteurella hemolytica* were Gram negative cocci, have oxidase test positive. *Klebsiella aeromonas* Gram negative coccobacilli bacteria and have indole and motility negative.

### 4.1.3 Biochemical test

Biochemical test result for bacterial identification for both Gram positive and Gram negative bacteria which were isolated from the vagina of the non-pregnant and pregnant doe were tabulated in Table 3 and Table 4.

Table 2(a): Gram staining characteristics of colonial isolated form the vagina swabs of non-pregnant does.

Animal ID	Staining characters		
	Gram staining reaction	Shape	Arrangement
<b>2</b>	Gram positive	Cocci	Single
	Gram negative	Rod	Single
	Gram negative	Cocci	Single
<b>4</b>	Gram positive	Cocci	Pairs
	Gram positive	Cocci	Pairs
	Gram positive	Cocci	Pairs
<b>6</b>	Gram positive	Cocci	Pairs
	Gram negative	Coccobacillus	Chains
	Gram positive	Cocci	Cluster
<b>7</b>	Gram negative	Coccobacillus	Single/pair
	Gram negative	Rod	Single/pair
	Gram positive	Cocci	Single/pair
<b>H</b>	Gram positive	Cocci	Pairs
	Gram positive	Cocci	Pairs
	Gram positive	Cocci	Pairs
<b>BR13</b>	Gram positive	Cocci	Pairs
<b>BR20</b>	Gram positive	Cocci	Pairs
	Gram positive	Cocci	Pairs

Table 2(b): Gram staining characteristics of colonial isolated form the vagina swabs of the pregnant doe.

Animal ID		Staining characters	
Pregnant doe	Gram staining reaction	Shape	Arrangement
<b>8</b>	Gram positive	Cocci	Pairs/ cluster
	Gram positive	Cocci	Cluster
	Gram negative	Cocci	Single/ pairs
<b>9</b>	Gram positive	Cocci	Pairs
	Gram negative	Rod	Single
<b>10</b>	Gram positive	Cocci	Pairs
	Gram positive	Cocci	Pairs
	Gram positive	Cocci	Pairs
	Gram negative	Rod	Single
<b>11</b>	Gram positive	Cocci	Single
	Gram positive	Cocci	Single
	Gram positive	Cocci	Single
	Gram positive	Cocci	Single
<b>12</b>	Gram positive	Cocci	Pairs
	Gram positive	Cocci	Cluster
<b>46</b>	Gram positive	Cocci	Pairs
	Gram positive	Cocci	Pairs
<b>1556</b>	Gram positive	Cocci	Pairs
	Gram positive	Cocci	Pairs

Table 3: Biochemical characteristics of Gram positive bacteria isolated from the vagina of the non-pregnant and pregnant doe

Gram Positive Bacteria	Biochemical test											
	Catalase test positive						Catalase test negative					
	Coagulase test	Hemolysins	VP	Maltose	Mannitol	ADH	Soluble hemolysin	6.5% NaCl	Bile esc	Lactose	Sorbitol	Trehalose
<i>Staphylococcus aureus</i>	+	+	+	+	+	+						
<i>Staphylococcus intermedius</i>	+	+	-	-	-	-						
<i>Streptococcus spp.</i>							+	+	-	+	+	+
<i>Enterococcus gp.</i>							-	+	+	-	-	-
<i>Enterococcus faecalis</i>							-	+	+	+	+	+

Table 4: Biochemical characteristics of Gram negative bacteria isolated from the vagina of the non-pregnant and pregnant doe

Gram Negative Bacteria	Biochemical test				
	Oxidase test	TSI	Urea	SIM	Citrate
<i>Escherichia coli</i>	-	Acid/Acid/Gas/no H <sub>2</sub> S	-	-/+/-	-
<i>Proteus mirabilis</i>	-	Alkali/Acid/No gas/No H <sub>2</sub> S	+	+/-/+	+
<i>Pasteurella aeromanas</i>	+	Acid/Acid/Gas/No H <sub>2</sub> S	-	-/+/+	-
<i>Mannheimia haemolytica</i>	+	Acid/Acid/Gas/No H <sub>2</sub> S	-	-/-/-	-
<i>Klebsiella aerogenes</i>	+	Acid/Acid/Gas/No H <sub>2</sub> S	+	-/-/-	-
<i>Acitenobacter</i>	-	Alkali/Alkali/No gas/No H <sub>2</sub> S	-	-/-/-	+

Identification was performed according to Jang *et. al.*, (2008)

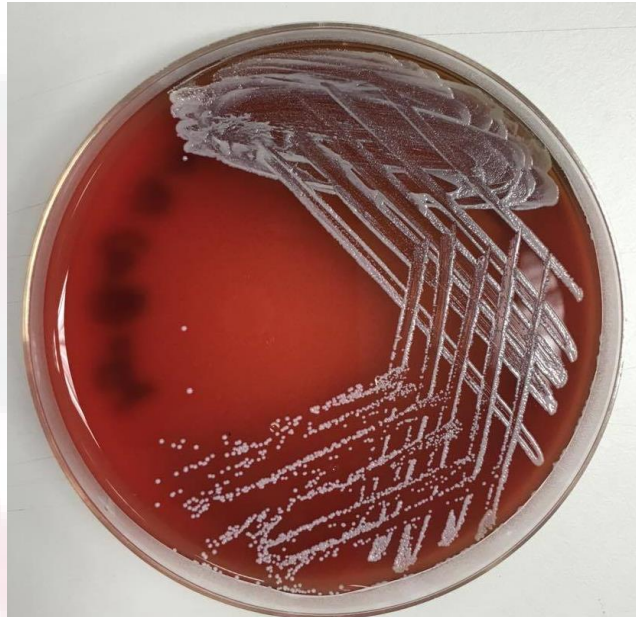


Plate 1(a): Circular, smooth, shiny, white colonies of *Staphylococcus intermedius*. (Blood agar, Aerobic culture, 24h)



Plate (1b): Positive result of Biochemical test for *Staphylococcus intermedius*.



Plate 2(a): Circular, shiny, mucoid, yellow colonies of *Staphylococcus aureus*. (Blood agar, aerobic culture, 24h)



Plate 2(b): Positive result of Biochemical test for *Staphylococcus aureus*

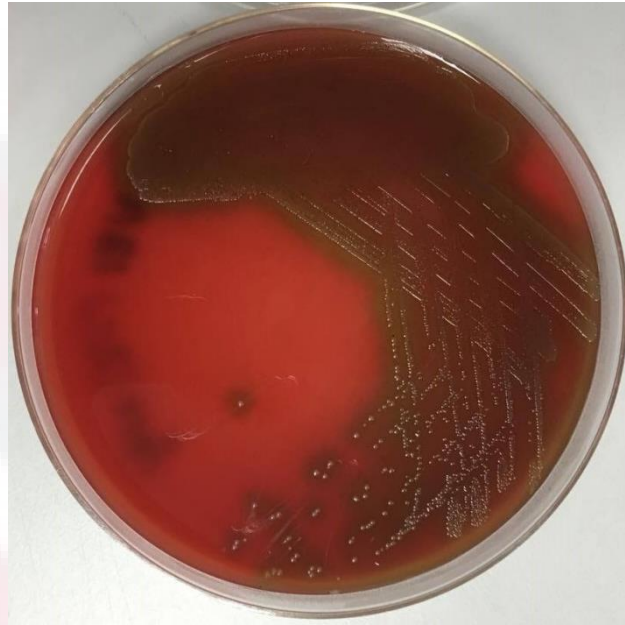


Plate 3(a): Circular, smooth, shiny colonies of *Streptococcus* spp. with partial haemolytic activity on blood agar.  
(Blood agar, Aerobic culture, 24h)



Plate 3(b): Positive result of Biochemical test for *Streptococcus* spp.

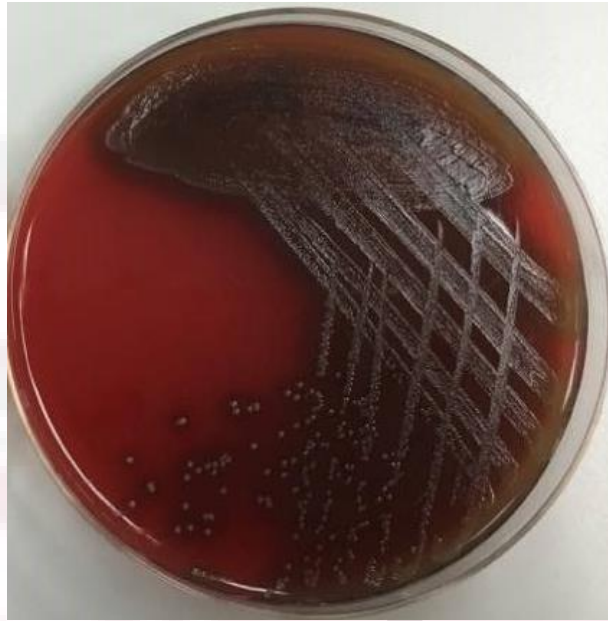


Plate 4(a): Circular, smooth, shiny, whitish colonies of *Enterococcus* group with partial haemolytic activity on the blood agar. (Blood agar, aerobic culture, 24h)



Plate 4(b): Positive result of Biochemical test for *Enterococcus* group



Plate 5(a): Circular, smooth, shiny, colourless colonies of *Enterococcus faecalis*. (Blood agar, aerobic culture, 24h)

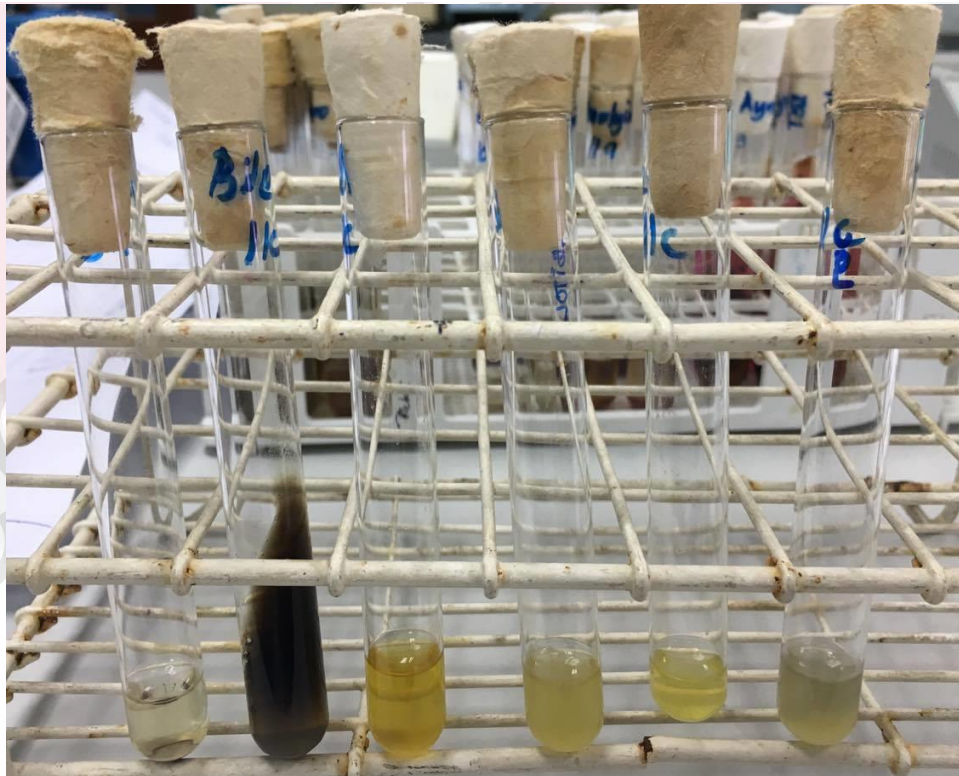


Plate 5(b): Positive result of Biochemical test for *Enterococcus faecalis*

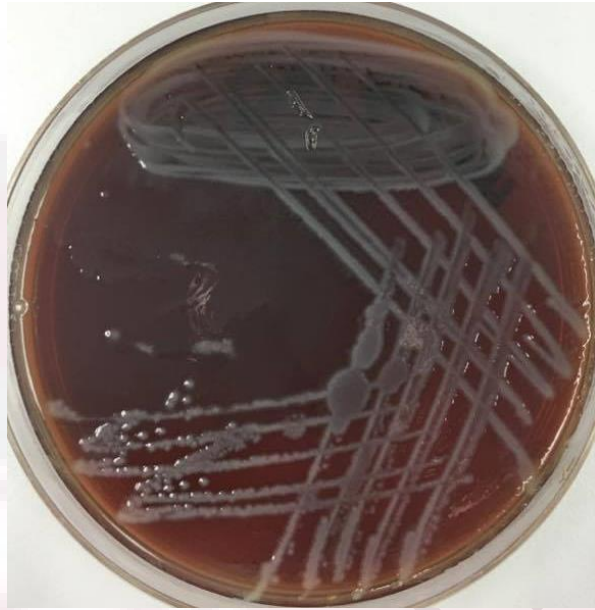


Plate 6(a): Circular, shiny, mucoid, small colonies of *Proteus mirabilis*.  
(Blood agar, aerobic culture, 24h)

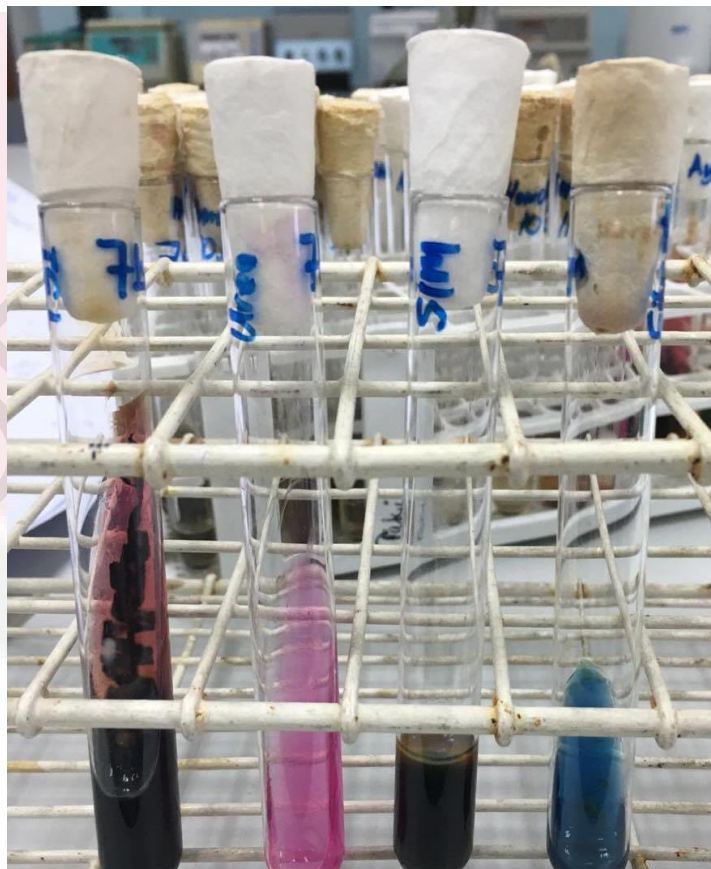


Plate 6(b): Positive result of biochemical test for *Proteus mirabilis*

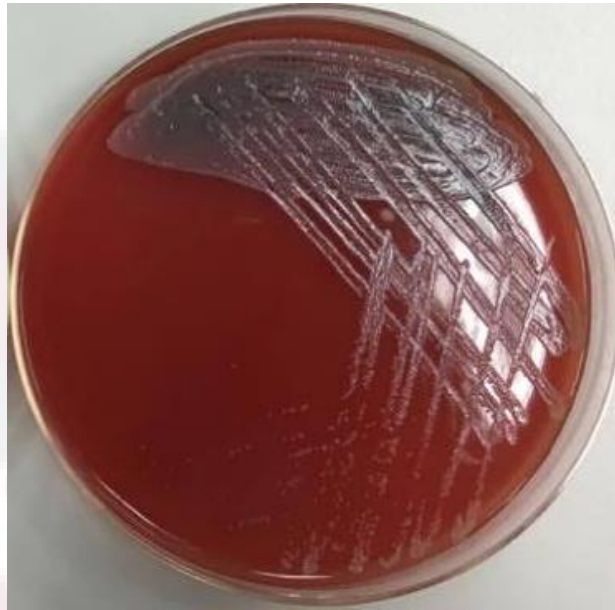


Plate 7(a): Circular, pin-point, smooth and shiny colonies of *Escherichia coli*. (Blood agar, aerobic culture, 24h)



Plate 7(b): Positive result of Biochemical test for *Escherichia coli*



Plate 8: Circular, shiny, mucoid colonies of *Pasteurella aeromonas*.  
(Blood agar, aerobic culture, 24h)

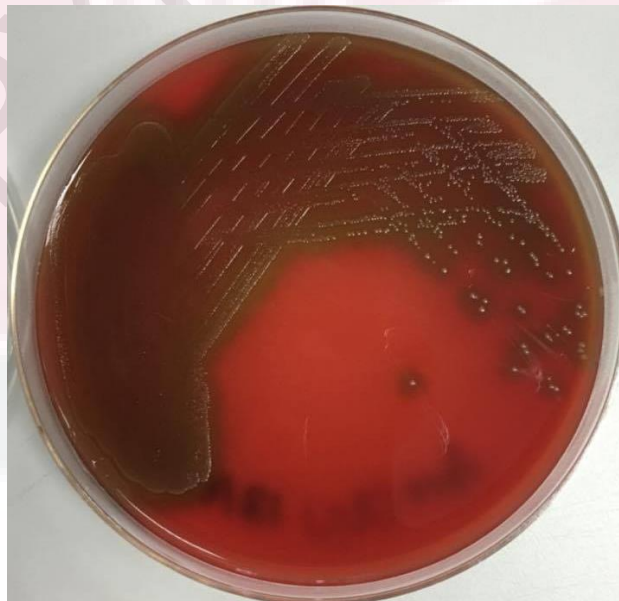


Plate 9: Circular, pin-point, shiny, smooth colonies of *Mannheimia haemolytica* with partial hemolytic activity on the blood agar. (Blood agar, aerobic culture, 24 h)



Plate 10: Circular, small, shiny, mucoid colonies of *Klebsiella aerogenes*.  
(Blood agar, aerobic culture, 24h)



Plate 11: Circular, small, shiny, mucoid colonies of *Acitenobacter*.  
(Blood agar, aerobic culture, 24h)

Table 5: Total number and percentages of bacteria isolated from vagina of non-pregnant and pregnant doe

Bacterial isolates		Frequency percentage of occurrence			
Family/ Group	Isolated	Non-pregnant doe		Pregnant doe	
		Total no. of isolated	% of isolated bacteria	Total no. of isolated	% of isolated bacteria
Gram +ve cocci	<i>Staphylococcus aureus</i>	2	12.5	6	30
Gram +ve cocci	<i>Staphylococcus intermedius</i>	6	37.5	7	35
Gram +ve cocci	<i>Streptococcus</i> spp.	2	12.5	1	5
Gram -ve rods	<i>Escherichia coli</i>	-	-	1	5
Gram -ve rods	<i>Proteus mirabilis</i>	1	6.25	1	5
Gram -ve rods	<i>Pasteurella aeromonas</i>	1	6.25	-	-
Gram -ve cocci	<i>Mannheimia haemolytica</i>	1	6.25	-	-
Gram -ve coccobacillus	<i>Klebsiella aerogenes</i>	1	6.25	1	5
Gram +ve cocci	<i>Enterococcus</i> gp.	2	12.5	1	5
Gram +ve cocci	<i>Enterococcus faecalis</i>	-	-	1	5
Gram -ve cocci	<i>Acitenobacter</i>	-	-	1	5
<b>Total</b>		16	44.44	20	55.56

From the total of 7 vaginal swab samples from non-pregnant doe, the dominant isolated bacteria were *Staphylococcus intermedius* (37.5%). Of the total of 7 vaginal swabs samples from pregnant doe, the dominant isolated bacteria were *Staphylococcus intermedius* (35%) and *Staphylococcus aureus* (30%) and other species were isolated at relatively lower rates. Total percentage of bacteria isolated from the vagina of non-pregnant doe is 44.44% compared to the percentage of bacteria isolated from the vagina

of pregnant doe is 55.56%. Bacteria isolated from the vagina of the pregnant doe showed higher percentage than non-pregnant doe and there were different types of bacteria isolated from the non-pregnant doe as compared to pregnant group (Table 5).

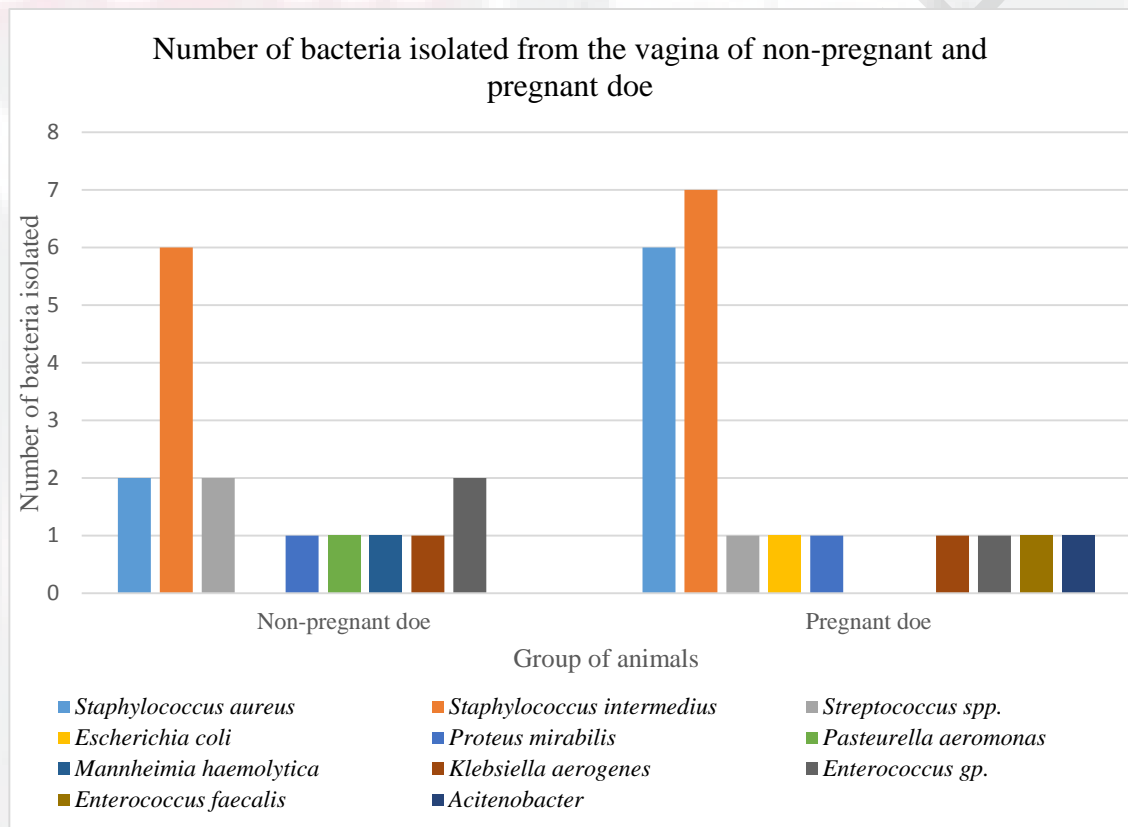


Figure 2: Total no. of bacteria isolated from the vagina of non-pregnant and pregnant doe

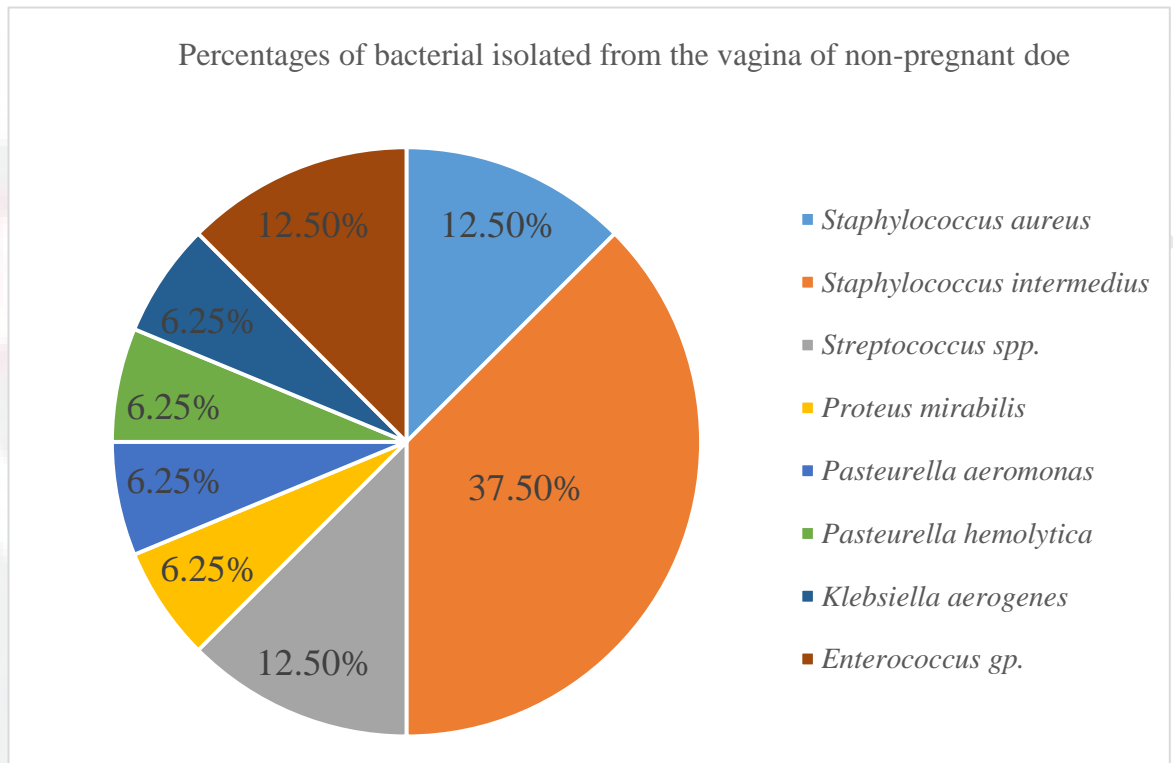


Figure 3: Percentages of bacterial isolated from the vagina of non-pregnant doe

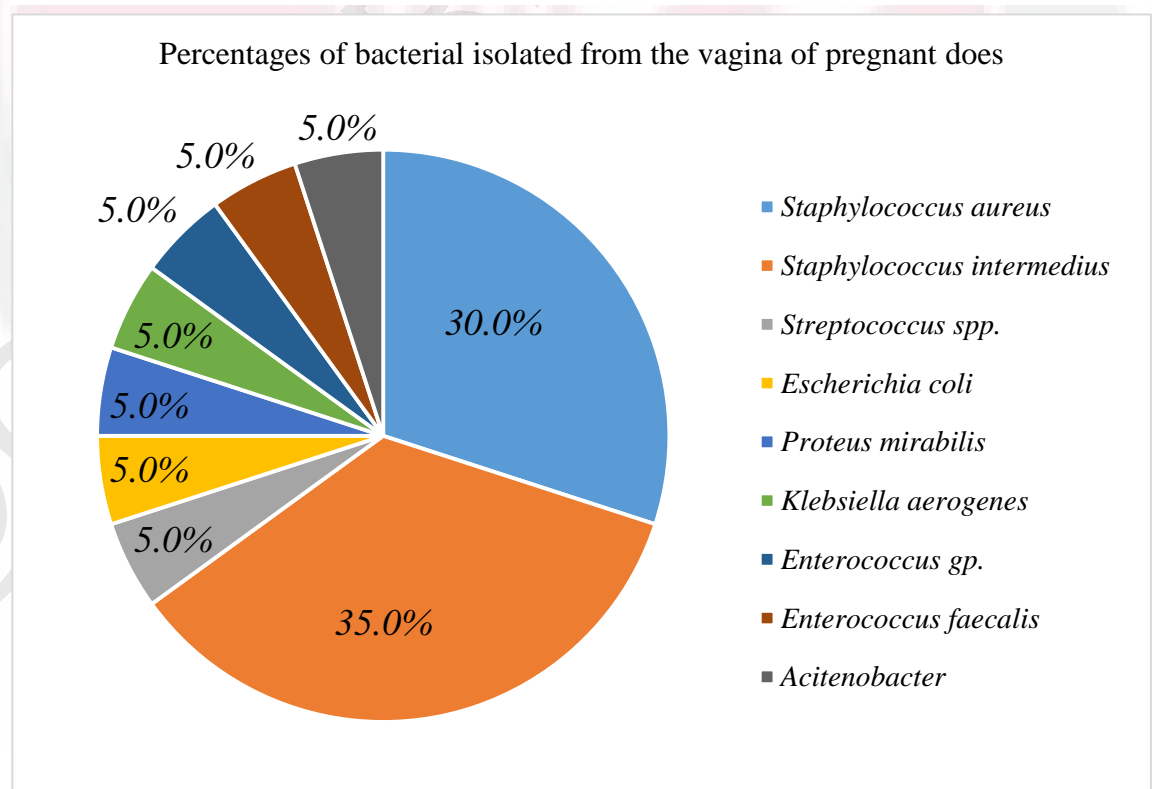


Figure 4: Percentages of bacterial isolated from the vagina of pregnant does

Figure 3 showed the percentages of bacteria isolated from the vagina of non-pregnant doe. The most dominant isolated bacteria is *Staphylococcus intermedius* (37.5%) and the predominant species are *Staphylococcus aureus*, *Streptococcus* spp. and *Enterococcus* group with the same percentages 12.50%. Other species of bacteria were isolated at lower rates.

Figure 4 is showed the percentages of bacteria isolated from the vagina of pregnant doe. The most dominant species is *Staphylococcus intermedius* (35.00%) and the predominant bacteria isolated is *Staphylococcus aureus* (30.00%). Other species of bacteria are only isolated at lower rates.

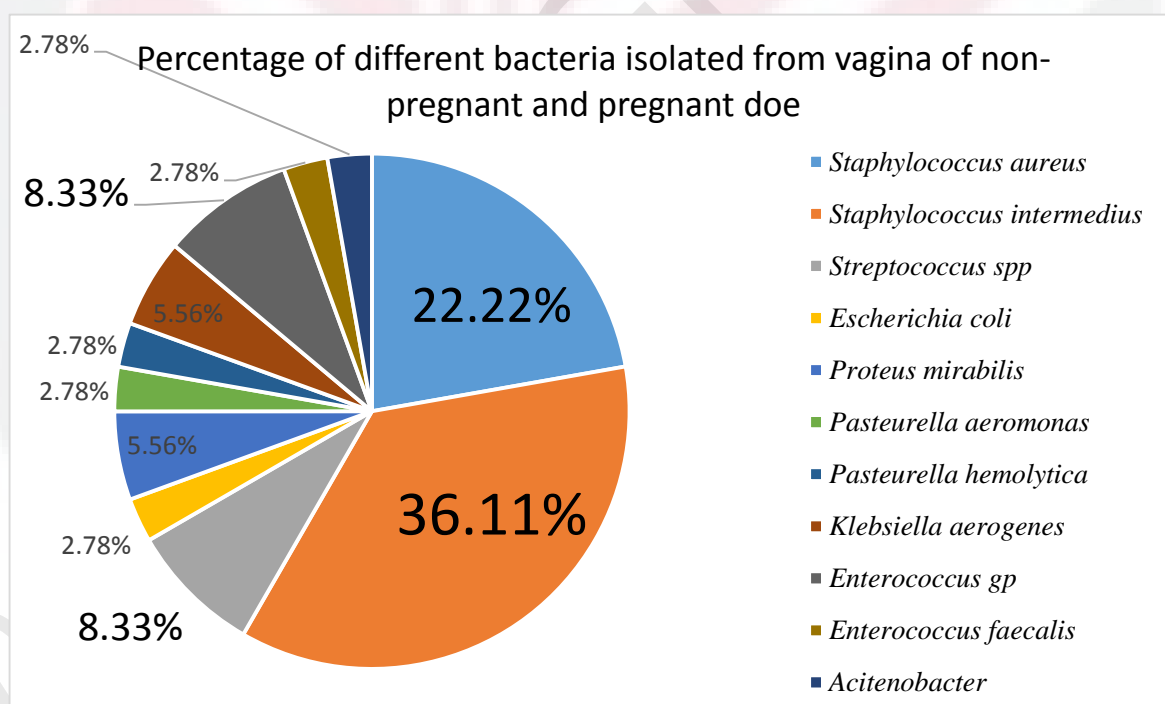


Figure 4: Percentage of different bacteria isolated from the vagina of the non-pregnant and pregnant doe

The distribution of the bacterial isolates appear from the total 14 samples from the vagina in both of pregnant and non-pregnant does is shown in Figure 4. The Gram-

positive cocci were most dominant among the isolates; *Staphylococcus intermedius* (36.11%) and predominant bacterium was *Staphylococcus aureus* (22.22%), followed by *Streptococcus* spp. and *Enterococcus* group (8.33%). Other species were isolated at relatively lower rates.

## 5.0 DISCUSSION

Bacteria that had been isolated from the vagina of the does are classified as normal flora which were include *Staphylococcus intermedius*, *Staphylococcus aureus*, *Streptococcus* species, *E. Coli*, *Proteus mirabilis*, *Pasteurella aeromonas*, *Mannheimia haemolytica*, *Klebsiella aerogenes*, *Enterococcus* group and *Acitenobacter*. The most dominant bacteria belong to the *Stapylococci* (58.33%) followed by *Streptococci* and *Enterococcus* (8.33%). A study conducted by Saad, 2014 showed that Gram positive bacteria (57.98%) is higher as compared to Gram negative bacteria (42.02%). Among the Gram positive bacteria, the most dominant bacteria isolated was *Staphylococcus aureus* while for Gram negative bacteria, *E.Coli* was the most dominant isolated bacteria present. In my study, we only isolated 5.0% of *E.Coli*. This may suggest that least contamination with fecal during sampling.

The bacteria isolated showed higher percentage in pregnant does as compared to the non-pregnant does. Changes in the environment of the vagina may lead to alterations on the microflora, e.g. due to pH modifications during estrus or pregnancy. Previous study has stated that hormonal changes during oestrus cycle had effect on vaginal bacterial population especially when progesterone levels were high (Gary, 2006).

The species of bacteria recognized as pathogens are present in the genitalia of the doe. The mere presence of these bacteria in the genitalia of the doe does not necessarily mean that an active infection exists. In fact, they may be protective. In present study, those bacteria may be present as saprophytes or opportunists which under conditions of stress may result in disease, the role played by these non-specific bacteria is not known but may result in disease under unfavourable conditions of stress (Bukar

et al., 2007). As mentioned by Levinson & Jawetz, 1994 in their studies, bacteria colonizing the vagina and uterus are likely to cause reproductive failure in ewes and other domestic ruminants. Vaginal bacteria get access into the uterus during the peripartum period leading to metritis and endometritis and cause subsequent reduction in the reproductive capacities of these animals. *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella* spp were the most common genital bacterial isolates observed in ewes associated with genital infections (Sokkar *et al.* 1980).

In this study, doe showed no abnormalities in the reproductive tract. Given that the tracts were grossly normal, the bacteriological findings should reflect the “normal” flora of the reproductive tract of the doe. Keeping of the ewes under non stressful condition is warranted to avoid clinical infection of reproductive tract with opportunistic bacteria.

## 6.0 CONCLUSION

From our results, Gram positive bacteria particularly *Staphylococcus* spp. is the most dominant microflora that has been isolated from the vagina of the doe and the predominant bacteria are *Streptococcus* spp. and *Enterococcus* group in normal cases. There were higher number of the vaginal normal flora isolated from the non pregnant compared to the pregnant doe. Hormonal changes during different stages of reproduction may had an effect on the vaginal bacterial population. All bacteria species that had been isolated are normal flora and these bacteria are capable to become opportunist which in under conditions of stress may cause disease.

## 7.0 RECOMMENDATIONS

In the future, our findings can be more comprehensive if the complete hormonal profiles of the doe throughout the oestrous cycle can be monitored. The hormones such as estrogen and progesterone can be measured using Radioimmunosay (RIA). In this case, the association between the prevalence of the normal flora and the reproductive hormones can be determined.

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## 8.0 APPENDICES

Cultural colony characteristics and Gram staining reaction of the bacteria from vagina of non-pregnant doe

Animal ID: 2

Colony	2(a)	2(b)	2(c)
Colony morphology	Pin-point and whitish in colour. Has round entire edge and smooth texture with convex elevation.	About 1mm in size and greyish in colour. Has round entire edge with mucoid texture and raised elevation	Pin-point and greyish in colour. Has entire edge and smooth texture with raised elevation. Has partial haemolytic activity.
Gram staining	Gram positive cocci, single	Gram negative rod, single	Gram negative cocci single
Biochemical test for Gram positive bacteria:			
Catalase test	Positive		
Slide coagulase test	Positive		
Hemolysin	Positive		
Acetoin production (VP)	Negative		
Maltose	Negative		
Mannitol	Negative		
ADH	Negative		
Biochemical test for Gram negative bacteria:			
Oxidase test		Positive	Positive
TSI: Slant Butt Gas H <sub>2</sub> S <sub>2</sub>		Alkaline Acid No gas Negative	Acid Acid No gas Negative
Urea		Negative	Negative
SIM H <sub>2</sub> S <sub>2</sub> Indole Motility		Negative Negative Positive	Negative Negative Negative
Citrate		Negative	Negative
Identified organism	<i>Staphylococcus Intermedius</i>	<i>Pasteurella aeromonas</i>	<i>Pasteurella haemolytica</i>

Animal ID: 4

Colony	4(a)	4(b)	4(c)	
Colony morphology	Pin-point and whitish in colour. Has circular entire edge and rough texture with convex elevation.	Pin-point and whitish in colour. Has entire edge and mucoid texture with convex elevation.	Pin-point and greyish in colour. Has entire edge and mucoid texture with convex elevation and have partial haemolytic activity.	
Gram staining	Gram positive, cocci in pairs	Gram positive, cocci in pairs	Gram positive cocci in pairs	
Biochemical test for Gram positive bacteria:				
Catalase test	Positive	Positive	Negative	
Slide coagulase test	Positive	Positive	Soluble hemolyin	Positive
Hemolysin	Positive	Positive	6.5% NaCl	Positive
Acetoin production (VP)	Negative	Negative	Bile esculin	Negative
Maltose	Negative	Negative	Lactose	Positive
Mannitol	Negative	Negative	Sorbitol	Positive
ADH	Negative	Negative	Trehalose	Positive
Biochemical test for Gram negative bacteria:				
Oxidase test				
TSI				
Urea				
SIM				
H <sub>2</sub> S <sub>2</sub>				
Indole				
Motility				
Citrate				
Identified organism	<i>Staphylococcus Intermedius</i>	<i>Staphylococcus Intermedius</i>	<i>Streptococcus spp.</i>	

Animal ID: 6

Colony	6(a)	6(b)	6(c)	
Colony morphology	Pin-point and whitish in colour. Has round entire edge and rough texture with convex elevation.	About 1mm in size and greyish in colour. Has round entire edge with mucoid texture and raised elevation	Pin-point and greyish in colour. Has entire edge and smooth texture with raised elevation. Has partial haemolytic activity.	
Gram staining	Gram positive cocci in pair	Gram negative coccobacillus in chain	Gram positive cocci in cluster	
Biochemical test for Gram positive bacteria:				
Catalase test	Positive		Negative	
Slide coagulase test	Positive		Soluble hemolysin	Negative
Hemolysin	Positive		6.5% NaCl	Positive
Acetoin production (VP)	Negative		Bile esculin	Positive
Maltose	Negative		Lactose	Negative
Mannitol	Negative		Sorbitol	Negative
ADH	Negative		Trehalose	Positive
Biochemical test for Gram negative bacteria:				
Oxidase test		Negative		
TSI Slant		Alkaline		
Butt		Acid		
Gas		No gas		
H <sub>2</sub> S <sub>2</sub>		Negative		
Urea		Negative		
SIM		Negative		
H <sub>2</sub> S <sub>2</sub>		Negative		
Indole		Negative		
Motility		Negative		
Citrate		Negative		
Identified organism	<i>Staphylococcus Intermedius</i>	<i>Klebsiella aerogenes</i>	<i>Enterococcus gp.</i>	

Animal ID: 7

Colony	7a	7b	7c	
Colony morphology	Pin point, whitish, has entire edge with convex raised and mucoid texture. Non haemolytic reaction.	1 mm in size, yellowish, has entire edge with raised elevation and mucoid texture. Non haemolytic reaction.	Pin point, yellowish, has entire edge and raised elevation and mucoid texture. Non haemolytic reaction.	
Gram staining	Gram negative coccobacillus in pair/single	Gram negative rod in pair/single	Gram positive cocci in single/pair	
<b>Biochemical test for Gram positive bacteria:</b>				
Catalase test			Negative	
Slide coagulase test			Soluble hemolysin	Negative
Hemolysin			6.5% NaCl	Positive
Acetoin production (VP)			Bile esculin	Positive
Maltose			Lactose	Negative
Mannitol			Sorbitol	Negative
ADH			Trehalose	Positive
<b>Biochemical test for Gram negative bacteria:</b>				
Oxidase test	Negative	Negative		
TSI Slant Butt Gas H <sub>2</sub> S <sub>2</sub>	Alkali Acid No gas Positive	Alkali Acid No gas Positive		
Urea	Positive	Positive		
SIM H <sub>2</sub> S <sub>2</sub> Indole Motility	Positive Negative Positive	Positive Negative Positive		
Citrate	Positive	Positive		
Identified organism	<i>Proteus mirabilis</i>	<i>Proteus mirabilis</i>	<i>Enterococcus gp.</i>	

Animal ID: H

Colony	H (a)	H (b)	H (c)	
Colony morphology	Pin point in size, whitish colour, rough texture and has convex elevation.	About <1 mm in size, yellowish, has smooth texture and raised elevation.	About 1 mm in size, whitish, has mucoid texture and raised elevation.	
Gram staining	Gram positive cocci in pairs.	Gram positive cocci in pairs.	Gram positive cocci in single.	
Biochemical test for Gram positive bacteria:				
Catalase test	Positive	Positive	Negative	
Slide coagulase test	Positive	Positive	Soluble hemolysin	
Hemolysin	Positive	Positive	6.5% NaCl	
Acetoin production (VP)	Negative	Positive	Bile esculin	
Maltose	Negative	Positive	Lactose	
Mannitol	Negative	Positive	Sorbitol	
ADH	Negative	Positive	Trehalose	
Biochemical test for Gram negative bacteria:				
Oxidase test				
TSI Slant Butt Gas H <sub>2</sub> S <sub>2</sub>				
Urea				
SIM H <sub>2</sub> S <sub>2</sub> Indole Motility				
Citrate				
Identified organism	<i>Staphylococcus intermedius</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus spp.</i>	

Animal ID: BR13

Colony	BR13 (a)	
Colony morphology	Pin point in size, whitish colour, has rough texture, entire edge and has convex elevation.	
Gram staining	Gram positive cocci in pairs.	
Biochemical test for Gram positive bacteria:		
Catalase test	Positive	
Slide coagulase test	Positive	
Hemolysin	Positive	
Acetoin production (VP)	Negative	
Maltose	Negative	
Mannitol	Negative	
ADH	Negative	
Biochemical test for Gram negative bacteria:		
Oxidase test		
TSI Slant Butt Gas H <sub>2</sub> S <sub>2</sub>		
Urea		
SIM H <sub>2</sub> S <sub>2</sub> Indole Motility		
Citrate		
Identified organism		<i>Staphylococcus intermedius</i>

Animal ID: BR20

Colony	BR20 (a)	BR20 (b)
Colony morphology	Pin point in size, whitish colour, has smooth texture, entire edge and has convex elevation.	About <1 mmm in size, yellowish colour, has smooth texture, entire edge and has raised elevation.
Gram staining	Gram positive cocci pairs	Gram positive cocci pairs
Biochemical test for Gram positive bacteria:		
Catalase test	Positive	Positive
Slide coagulase test	Positive	Positive
Hemolysin	Positive	Positive
Acetoin production (VP)	Negative	Positive
Maltose	Negative	Positive
Mannitol	Negative	Positive
ADH	Negative	Positive
Biochemical test for Gram negative bacteria:		
Oxidase test		
TSI Slant Butt Gas H <sub>2</sub> S <sub>2</sub>		
Urea		
SIM H <sub>2</sub> S <sub>2</sub> Indole Motility		
Citrate		
Identified organism	<i>Staphylococcus intermedius</i>	<i>Staphylococcus aureus</i>

Cultural colony characteristics and Gram staining reaction of the bacteria from vagina of pregnant doe

Animal ID: 8

Colony	8(a)	8(b)	8(c)		
Colony morphology	Pin-point and whitish in colour. Has round entire edge and smooth texture with convex elevation.	About 1.5mm in size and yellowish in colour. Has entire edge with mucoid texture and raised elevation.	About 1.5mm in size and cloudy/ greyish in colour. Has entire edge with mucoid texture and raised elevation.		
Gram staining	Gram positive cocci in pair/ cluster	Gram positive cocci in cluster	Gram negative cocci in pair/ single		
Biochemical test for Gram positive bacteria:					
Catalase test	Positive	Positive			
Slide coagulase test	Positive	Positive			
Hemolysin	Positive	Positive			
Acetoin production	Negative	Positive			
Maltose	Negative	Positive			
Mannitol	Negative	Positive			
ADH	Negative	Positive			
Biochemical test for Gram negative bacteria:					
Oxidase test			Negative		
TSI Slant Butt Gas H <sub>2</sub> S <sub>2</sub>			Alkaline Alkaline No gas Negative		
Urea			Negative		
SIM H <sub>2</sub> S <sub>2</sub> Indole Motility			Negative Negative Positive		
Citrate			Negative		
Identified organism			<i>Staphylococcus Intermedius</i>	<i>Staphylococcus aureus</i>	<i>Acitenobacter</i>

Animal ID: 9

Colony	9(a)	9(b)	
Colony morphology	Pin-point and whitish in colour. Has circular entire edge and smooth texture with convex elevation.	About 1.5mm and greyish in colour. Has circular entire edge and mucoid texture with raised elevation.	
Gram staining	Gram positive cocci in pairs	Gram negative rod single	
Biochemical test for Gram positive bacteria:			
Catalase test	Positive		
Slide coagulase test	Positive		
Hemolysin	Positive		
Acetoin production (VP)	Negative		
Maltose	Negative		
Mannitol	Negative		
ADH	Negative		
Biochemical test for Gram negative bacteria:			
Oxidase test		Positive	
TSI Slant Butt Gas H <sub>2</sub> S <sub>2</sub>		Acid Acid Gas Negative	
Urea		Negative	
SIM H <sub>2</sub> S <sub>2</sub> Indole Motility		Negative Positive Negative	
Citrate		Negative	
Identified organism		<i>Staphylococcus intermedius</i>	<i>Klebsiella aerogenes</i>

Animal ID: 10

Colony	10a	10b		10c	10d
Colony morphology	Pin point, whitish, entire edge, convex raised, rough texture and non-hemolytic	Pin point, greyish colour, entire edge, raised elevation, mucoid texture, partial haemolytic activity		About 1 mm in size, yellowish cloudy in colour, mucoid texture, raised elevation and non-hemolytic activity.	Pin point, greyish colour, convex elevation, mucoid texture, and non-hemolytic reaction.
Gram staining	Gram positive cocci in pair	Gram positive cocci in pair		Gram positive cocci in pair	Gram negative rod single
Biochemical test for Gram positive bacteria:					
Catalase test	Positive	Negative		Positive	
Slide coagulase test	Positive	Soluble hemolysin	Negative	Positive	
Hemolysin	Positive	6.5% NaCl	Positive	Positive	
Acetoin production (VP)	Negative Negative	Bile esculin	Positive	Positive	
Maltose	Negative	Lactose	Negative	Positive	
Mannitol	Negative	Sorbitol	Negative	Positive	
ADH	Negative	Trehalose	Negative	Positive	
Biochemical test for Gram negative bacteria:					
Oxidase test					Negative
TSI Slant					Alkali Acid No gas Negative
Butt Gas H <sub>2</sub> S <sub>2</sub>					
Urea					Negative
SIM H <sub>2</sub> S <sub>2</sub> Indole Motility					Negative Positive Negative
Citrate					Negative
Identified organism	<i>Staphylococcus Intermedius</i>	<i>Enterococcus gp.</i>		<i>Staphylococcus aureus</i>	<i>E. Coli</i>

Animal ID: 11

Colony	11a	11b		11c		11d
Colony morphology	Pin point, whitish, rough texture, convex elevation, and non-hemolytic activity	Pin point, greyish colour, entire edge, convex elevation, mucoid texture, and has partial hemolytic activity.		< 1 mm size, milky colour, entire edge, mucoid texture, raised elevation, and non-hemolytic activity.		About 1 mm in size, yellowish in colour, mucoid texture, raise elevation, entire edge and has complete haemolytic activity.
Gram staining	Gram positive cocci pair	Gram positive cocci pair		Gram positive cocci pair		Gram positive cocci single/ pair
Biochemical test for Gram positive bacteria:						
Catalase test	Positive	Negative		Negative		Positive
Slide coagulase test	Positive	Soluble hemolysin	Negative	Soluble hemolysin	Negative	Positive
Hemolysin	Positive	6.5% NaCl	Positive	6.5% NaCl	Positive	Positive
Acetoin production (VP)	Negative	Bile esculin	Negative	Bile esculin	Positive	Positive
Maltose	Negative	Lactose	Positive	Lactose	Positive	Positive
Mannitol	Negative	Sorbitol	Positive	Sorbitol	Positive	Positive
ADH	Negative	Trehalose	Positive	Trehalose	Positive	Positive
Biochemical test for Gram negative bacteria:						
Oxidase test						
TSI Slant Butt Gas H <sub>2</sub> S <sub>2</sub>						
Urea						
SIM H <sub>2</sub> S <sub>2</sub> Indole Motility						
Citrate						
Identified organism	<i>Staphylococcus Intermedius</i>	<i>Streptococcus spp.</i>		<i>E. faecalis</i>		<i>Staphylococcus aureus</i>

Animal ID: 12

Colony	12(a)	12(b)
Colony morphology	Pin-point and whitish in colour. Has circular entire edge and smooth texture with convex elevation.	About 1.5mm and greyish in colour. Has circular entire edge and mucoid texture with raised elevation.
Gram staining	Gram positive cocci in pairs	Gram positive cocci in cluster
Biochemical test for Gram positive bacteria:		
Catalase test	Positive	Positive
Slide coagulase test	Positive	Positive
Hemolysin	Positive	Positive
Acetoin production (VP)	Negative	Positive
Maltose	Negative	Positive
Mannitol	Negative	Positive
ADH	Negative	Positive
Biochemical test for Gram negative bacteria:		
Oxidase test		
TSI Slant Butt Gas H <sub>2</sub> S <sub>2</sub>		
Urea		
SIM H <sub>2</sub> S <sub>2</sub> Indole Motility		
Citrate		
Identified organism	<i>Staphylococcus intermedius</i>	<i>Staphylococcus aureus</i>

Animal ID: 46

Colony	46 (a)	46 (b)
Colony morphology	Pin point in size, whitish colour, has convex elevation, entire edge and smooth texture.	About 0.5 mm in size, whitish colour, has raised elevation, entire edge and mucoid texture.
Gram staining	Gram positive cocci in pairs	Gram positive cocci in pairs
Biochemical test for Gram positive bacteria:		
Catalase test	Positive	Positive
Slide coagulase test	Positive	Positive
Hemolysin	Positive	Positive
Acetoin production (VP)	Negative	Positive
Maltose	Negative	Positive
Mannitol	Negative	Positive
ADH	Negative	Positive
Biochemical test for Gram negative bacteria:		
Oxidase test		
TSI Slant Butt Gas H <sub>2</sub> S <sub>2</sub>		
Urea		
SIM H <sub>2</sub> S <sub>2</sub> Indole Motility		
Citrate		
Identified organism	<i>Staphylococcus intermedius</i>	<i>Staphylococcus aureus</i>

Animal ID: 1556

Colony	1556 (a)	1556 (b)
Colony morphology	Pin point in size, whitish colour, has smooth texture, entire edge and convex elevation.	About 0.5 mm in size, whitish colour, has mucoid texture, entire edge and raised elevation.
Gram staining	Gram positive cocci in pairs.	Gram positive cocci in pairs.
<b>Biochemical test for Gram positive bacteria:</b>		
Catalase test	Positive	Positive
Slide coagulase test	Positive	Positive
Hemolysin	Positive	Positive
Acetoin production (VP)	Negative	Positive
Maltose	Negative	Positive
Mannitol	Negative	Positive
ADH	Negative	Positive
<b>Biochemical test for Gram negative bacteria:</b>		
Oxidase test		
TSI Slant Butt Gas H <sub>2</sub> S <sub>2</sub>		
Urea		
SIM H <sub>2</sub> S <sub>2</sub> Indole Motility		
Citrate		
Identified organism	<i>Staphylococcus intermedius</i>	<i>Staphylococcus aureus</i>