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ORIGINAL ARTICLE **OPEN ACCESS**

Preliminary Study on Microbiota in Female Goat (*Capra Hircus*) at Different Reproductive Stages

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ABSTRACT
In recent years, the study of microorganisms has shifted from an in vitro emphasis to an ecological one, concentrating on how they interact intra- and inter-specifically in microbiomes. Microbiome refers to the variety of microorganisms that coexist with a species, yet changes in these natural populations may harm health and normal physiological circumstances. Animal behaviour depends on microbes and microbial products. Animals can taste and smell chemical compounds created by bacteria and utilize this sensory information to avoid harmful germs, indicate food sources or conspecifics, and send chemical messages. The healthy female goats were chosen to isolate the microbial strains present in female goats' urine and vaginal fluid profiles at different reproductive statuses. Bacterial isolates were obtained from the vaginal fluid of healthy female goats of different reproductive statuses. Bacteria isolated from the vagina were Serratia marcescent, E. coli, Klebsiella oxytoca, Proteus mirabilis, Providencia stuartil, Providencia rettgeri, and Citrobacter freudilli. No mycoplasmas were observed. In addition, no bacterial isolates were detected in the urine of goats. These findings stress that bacteria in the urine can escape detection by culture-dependent methods. Therefore, the current study suggests using methods to prove the presence of non-cultural bacteria; the urine samples and these microbes may be involve in pheromone compound production.
Keywords: *Microbiomes, E.coli, Female goats, Reproductive stages, Bacterial culture, Urine and Vaginal fluid.*

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INTRODUCTION

As the world's population increases, animal husbandry must produce more and better food animals. With a global population of 1 billion, dairy, goat, and beef production are important to worldwide agriculture and economics [1]. Goats are very useful for reducing poverty and improving food security and livelihoods in underdeveloped nations. Goats were among the first farm animals to be domesticated. As indicated by the archaeological evidence, they have been associated with man in a symbiotic relationship for up to 10,000 years [2]. Goats spread over the globe because of their tolerance to varied environments and dietary regimens. Goats are clever, autonomous, nimble, disease- and parasite-tolerant, and can take care of themselves better than other livestock. They may use poor forage and travel far to get nourishment. Their unusual eating habits make it simpler to pick diets. Farmers and pastoralists depend on goats for survival and money [3]. Goats offer their owners with a variety of goods and socioeconomic services and have played a major part in many people's social lives as presents, dowry, and in religious ceremonies and rites of passage.

Goats have become a model for biomedical research and surgical training. Clinical, applied, and basic research employ them [5]. Humans domesticated goats early on. They are cultivated globally for milk and meat. India contains 115 goat species out of 574 globally. Although human-designed treatments like as super ovulation and embryo transfer might increase reproductive efficiency, the livestock business relies on cow, goat, and pig abilities to establish and sustain a healthy pregnancy. The natural reproductive environment has been overlooked despite our efforts to study outside elements that impact fertility, such as diet.

Information flow in living beings is vital. Unidirectional or bidirectional information flow (non-living to living and between living organisms respectively). Particles and waves are used to communicate information. Living organisms have developed several communication mechanisms. Living species communicate chemically, but others do not (sound, vision). Bacteria, fungi, plants, and mammals commonly use chemical communication. Chemical information may have arisen as a byproduct before any receptors existed. Living beings release several chemical compounds. Chemical emissions arise due to the volatility of metabolic byproducts or because organisms must discard, waste materials or defend themselves against adversaries and abiotic stress. Since non-communicative compounds are emitted, they give several beginning places for unintentional communication.

In recent years, the study of microorganisms has shifted from an in vitro emphasis to an ecological one, concentrating on how they interact intra- and inter-specifically in microbiomes [7]. Microbiome refers to the variety of microorganisms that coexist with a species, yet changes in these natural populations may harm health and normal physiological circumstances [1]. With the rise of drug-resistant microorganisms, how these populations behave in their natural habitat may give medicinal solutions [8]. Many microorganisms in ewes and cows' vaginal microbiomes regulate pH levels to safeguard the reproductive environment, according to new study [9]. Animal biologists are acquainted with stomach symbionts and their effect on production, but less is known about reproductive tract microorganisms and their involvement in fertility [10]. Several findings imply future study may uncover a link between placental microbiomes and pregnancy loss and the potential to identify particular stages of pregnancy through microbial fingerprints. Both of these options would boost industrial output with less financial loss and cheaper, easier pregnancy screening [1].

No vertebrate has developed without symbiotic microorganisms in 600 million years. Each animal has deep relationships with microbial symbionts that presumably affect its biology. Many animal-microbe relationships seem to be mutualistic. Animal-associated bacteria activate host immune systems, exclude host pathogens, provide access to vitamins and minerals, and promote host tissue growth and function. Symbiotic bacteria may alter animal behaviour and may be especially important in chemical communication. Chemical signaling is the oldest and most common among animals. Animal chemical signals may be generated by signalers, collected from their environs, or derived via symbiotic microorganisms. Many animals scent mark using integument smell glands. Warm, wet, organic-rich, anaerobic smell glands look very favorable to fermentative symbiotic bacteria.

Animal behaviour depends on microbes and microbial products. Animals can taste and smell chemical compounds created by bacteria and utilize this sensory information to avoid harmful germs, indicate food sources or conspecifics, and send chemical messages. Germ-free mice spend less time with unfamiliar conspecifics than control mice. Raising mice in germ-free surroundings causes an increased physiological reaction to stress, which may be corrected by restoring normal microbial communities or Bifidobacterium infantis. Microbes in genial area may produce goat pheromones. The current research aims to identify microbial strains from urine and vaginal fluid profiles of female goats with varied reproductive statuses.

MATERIAL AND METHODS

Study area and experimental animals: The goat sample collection was carried out in the Sustainable Integrated Farming System (SIFS), Bharathidasan University, Tiruchirappalli – 620 024. The animals were maintained in the farm as free roaming in the daytime. Goats were locked inside the shed only at night (6 p.m. – 6 a.m.). They were allowed to roam freely in the farm at daytime (6 a.m. – 6 p.m.). A veterinarian affirmed the healthy state of each animal. The animals were fed with their natural feeds like grass, tree leaves, etc., no other prepared goat feeds were given. They were fed with water and feed *ad libitum*. As a part of normal herd management procedure, all the animals were monitored every day during the study period. Every examination was carried out under natural condition. Experimental animals of both male and female were maintained at separately during the investigation.

Scientific classifications of goat

Kingdom	:	Animalia
Phylum	:	Chordata
Class	:	Mammalia
Order	:	Artiodactyla
Family	:	Bovidae
Subfamily	:	Caprinae
Genus	:	Capra
Species	:	C. aegagrus
Subspecies	:	C. a. hircus
Trinomial name	:	Capra aegagrus hircus (Linnaeus, 1758)
Scientific name	:	Capra aegagrus hircus
Gestation period	:	150 days
Lifespan	:	15 – 18 years
Trophic level	:	Herbivorous

Sample collection: Healthy female goats were chosen for the isolation of bacterial strains. The urine and vaginal swab was obtained from four different reproductive stages (pre pubertal, pubertal, pregnant and after weaning). Sterile cotton swabs were used and smear was obtained from vaginal regions of the experimental goats. Then, the smear was inoculated into a culture tube containing 10 ml of sterile nutrient broth and incubated. After 24 hours, 1ml from each culture (10-4 – 10-7) was transferred aseptically into sterile nutrient agar plates. The plates were further subjected for incubation at 30°C ± 2°C for 24hrs for the isolation of bacterial colonies. The morphologically distinct colonies were picked, streaked and stored for further use. Totally there are 14 colonies were isolated and the following biochemical characterization was carried out.

Biochemical Characterization of Microbes: All 14 colonies were tested for biochemical analysis viz., Indole, Methyl red, Voges proskauer's, Citrate, Urease, TSI (Triple Sugar Iorn), Catalase & Gram staining for the preliminary confirmation of species type.

Gram staining: The staining reaction of bacteria is of greatest importance in their differentiation and identification. Gram staining reaction has the widest application in the broad classification of bacteria. Based on the reaction, all bacteria are grouped into two categories. Gram positive organisms are those that resist decolourisation and retain the crystal violet – iodine complex and appear purple or deep blue. Conversely, those cells that decolorize or give up the crystal violet – iodine complex more rapidly will accept the saffranin counter stain and appear red. These are the gram negative organisms.

Indole production test: Tryptophan is an essential amino acid that can undergo oxidation by the way of enzymatic activities of some bacteria. Conversion of tryptophan into metabolic products is mediated by the enzyme tryptophans. Tryptophan with the production of indole is not a characteristic feature of all microorganisms and therefore serves as a biochemical marker. The presence of indole is detectable by adding Kovac's reagent. This test is done to determine the ability of microorganisms to degrade amino acid tryptophan.

Methyl Red test: Hexose monosaccharide, glucose is major substrate to oxidize by all enteric organisms for energy production. The end products of this process will vary on specific enzymatic pathway process will vary on specific enzymatic pathway present in the bacteria. This test is done to determine the ability of microorganisms to oxidize glucose with the production of acid end products.

Voges-Proskauer test: Voges – Proskauer test determines the capability of organism produced non active or neutral and produces acetyl methyl carbinol, organic acids that results from glucose metabolism. This test is performed to differentiate further among enteric organisms such as E-coli and Klebsiella.

Citrate utilization test: In the absence of fermentable glucose or lactose some microorganisms are capable of using citrate as their carbon source for their energy. The ability depends on the presence of citrate in the cell. Citrate is the first major intermediate in the Krebs cycle and it is produced by the condensation of active acetyl with oxaloacetic acid. This test is performed to differentiate among enteric organisms such on the basis of their ability to ferment citrate as a sole carbon source.

Triple sugar ion test: The triple sugar ion (TSI) agar test is designed to differentiate among the different groups or genera of Entero bacteriaceae which are gram negative bacilli capable of fermenting glucose with the production of acid and distinguish Entero bacteriaceae from other gram negative intestinal bacilli, this differentiation is made on the basis of differences in carbohydrates fermentation patterns and hydrogen sulphide production by various groups of intestinal organisms. This test is performed to learn a rapid screening produced that will differentiate among the members of Entero bacteriaceae.

Urease test: Urease that is produced by some microorganisms is an enzyme that especially helps in the identification of *Proteus vulgaris*. Although other microorganisms may produce urease their action is found to be slower than that seen in *Proteus* species. This test serves to rapidly distinguish the members of these fermenting enteric organisms. This test is performed to determine the ability of microorganisms to degrade urea by means of the enzyme urease.

Catalase test: The enzyme catalase splits Hydrogen peroxide into water and oxygen. When small portion of the colony is introduced into H_2O_2, rapid evolution of bubbles indicates the positive result. To the 5ml of 48hrs old culture broth, 1ml of 3% H_2O_2 was added. Release of bubbles was observed and it was compared with the control.

Oxidase test: Oxidase Test is an important differential procedure which should be performed on all gram negative bacteria that are to be identified. In this test, oxidase discs were placed over the individual cultures on agar medium. Immediate colour change of oxidase discs from white to blue indicates the positive result and no colour change indicates the negative result.

Sample Collection in female goats: The urine samples were collected from both female of *Capra hircus* according to various reproductive stages. The samples were stored in ice condition, transferred to lab, and stored in -20 º c for further analysis.

RESULTS AND DISCUSSION

Biochemical characterisation of bacterial strains

Pregnant female goat: Two species were isolated from pregnant female vaginal swab. Both showed negative result in Indole test. Methyl red, Voges – proskaure, citrate utilization and Catalase assays showed positive results for one organism which was identified as *Serratia marcescens.* It is a gram positive bacterium. Further, it showed negative results in urease*.* Nature of the organism is alkaline. The other organism present in this sample showed positive results in Methyl red, Voges- proskauer, Citrate utilization and Catalase test. Urease test shows negative results. It is a gram negative bacterium. It was identified as *Klebsiella oxytoca.*

Normal adult female goat: In normal adult animal, the isolates gave negative results in indole and urease test. Methyl red, Voges- proskauer, Citrate utilization and Catalase assays showed positive results. It is a gram positive bacterium. Triple sugar iron test indicates that it is an alkaline organism. It was identified as *Serratia marcescens.*

Prepubertal female goat: In prepubertal animal *E.coli* strain was isolated from the vaginal swab. It was identified through the following test result, Indole, Voges- proskauer, Citrate. Methyl red, Urease, Catalase tests are negative. It is gram negative bacteria. Triple sugar test confirms its alkaline nature.

Postpartum mother: Four species of microbes were isolated from this animal. The following tests results were confirmed this as *Providencia rettgeri* namely Indole, Voges- proskauer showed negative results. Methyl red, Citrate utilization, Urease, Triple sugar iron, Catalase showed positive results. It is a gram negative bacteria. *Providencia staurtil*is a microorganism which gives negative results in Indole, Voges-proskauer test. Methyl red, Citrate utilization, Urease, Triple sugar iron test showed positive test. It is gram negative bacteria. Another microorganism present in the sample is *Proteus mirabilis.* It is a gram negative bacteria. Methyl red, Citrate utilization, Urease, Catalase showed positive results. Indole and Voges-proskauer are negative. *Citrobacter freundili*is a gram negative bacteria. Indole, Voges- proskauer, results are negative. Methyl red, citrate utilization, urease, catalase gave positive results. Triple sugar iron test proved that it is alkaline in nature.

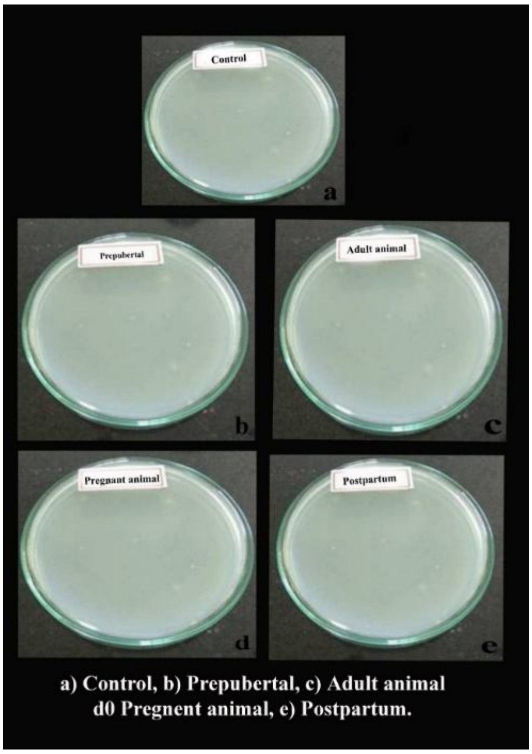
Symbiotic microbes can benefit their animal hosts by enhancing the diversity of communication signals available to them. Associations between animals and their symbiotic microbes can certainly be mutualistic. It is also becomingly increasingly clear that symbiotic bacteria can beneficially affect animal behavior, and that their contributions may be particularly prominent in chemical communication. Communication via chemical means appears to be the oldest and most widespread mode of signaling among animals. Many mammals communicate chemically by the secretions from urine, vaginal fluid and scent glands.

The high level of protein might be useful in indicating the estrus phase while high level of lipid denotes the onset of ovulation which corresponds to metestrus and diestrus phase. In addition, the consistent quantitative variation of the three fatty acids may additionally facilitate the communication of female in estrus to the male goat. However, the role of individual fatty acid in goat communication is yet to be studied. The above reports indicated the volatiles in the urine might be due to the presence of metabolic products of microbes. Hence, the present study was aimed to isolate the microbial strains present in the urine and vaginal fluid profiles of female goats at different reproductive status along with the volatile profiles.

To substantiate the above speculation, an attempt was made in this current study to explore the microbial community in the urine and vaginal fluid of goat *Capra hircus* of four different reproductive phases. Bacterial vaginal culture, carried out in all the four different reproductive stages revealed the presence of different bacterial cultures. Microorganisms were isolated in goats with positive bacteriologic tests, respectively. Bacteria isolated from vagina were *Serratia masrcescens, E. coli, Klebsiella oxytoca, Proteus mirabilis, Providencia stuartil, Providencia rettgeri* and *Citrobacter freudilli*. No mycoplasmas were observed. In contrast to our finding, Al-Sariy, (2014) reported the presence of Staphylococcus aureus (30.25%) with the highest percentage which were positive for coagulase test, Escherichia coli (26.89%), Staphylococcus epidermidis and Streptococcus Spp. (10.93%), Proteus mirabilis. (8.4%), the pseudomonas aeruginosa (6.72%),and finally Listeria monocytogenes (5.88%). In fact, Groppetti *et al.,* (2012) reported the presence of Enterococcus faecalis, Streptococcus -haemolyticus, Pasteurella multocida, E. coli, Klebsiella pneumoniae, Proteus mirabilis, E. coli haemolyticus, *Arcanobacterium pyogenes*, *Streptococcus* spp., *Staphylococcus* spp. and Acinetobacter spp. in bitches.

The fermentation hypothesis for chemical recognition, introduced over 30 years ago, posits that, as bacteria ferment the protein and lipid-rich substrates in scent sources, they produce odorous metabolites that are co-opted by their mammalian hosts as components of chemical signals. If the fermentation hypothesis for group-specific social odors among scent marking mammals is correct, then minimally 1) mammalian scent sources should contain substantive populations of odour producing bacteria, and 2) the composition and/or structure of bacterial communities inhabiting the scent glands of members of the same social group should be more similar than those associated with members of different social groups. Historically,

technical limitations associated with cultivation-based surveys of symbiotic bacteria have impeded the ability to effectively test predictions of the fermentation hypothesis for chemical recognition and, therefore, evaluations of the hypothesis have typically either yielded ambiguous results or concluded that the bacterial diversity in scent sources is insufficient to underlie the observed diversity in chemical signals. Further, it lead to the speculation that, the odours in genital region of goat might vary according to the presence of microbes. To support the speculation in the current study variation in the microbial cultures were recorded in the present study. In addition, it is suspected that the vaginal fluid may contaminate with urine and the possibility for the presence on the same microbes in the urinary tract of the animal.
The urine samples were collected from female goats with various reproductive stages such as pre pubertal, adult, pregnant and lactating mother. At first, microbial culture test was used to confirm the animals are disease free, as goat urine does not contain any culturable microbe. According to culture results the urine samples are pathogen free. These findings stress that bacteria present in urine can escape detection by culture-dependent methods, and that the current view of bacterial diversity in urine thus may be incomplete. Investigations of healthy urine specimens have demonstrated the presence of non-cultural bacteria. Therefore, the current study suggests to carry out the methods to prove the presence of non-cultural bacteria. Even though certain pathogenic bacteria were present in the vaginal fluid of goad there was no pathogens in the urine. If the urinary tract contains any pathogens, it would have led to urinary infections that might have been reflected in the damage in kidney. Hence, in the current study kidney function test was conducted to prove that the animal's urinary tract is without infection. As expected, the levels of urinary biomarkers have no significant change according to various reproductive stages. This was a clear indication that the goats were healthy without any urinary infection. The main goal of our study is to identify volatile chemicals, which are co-expressed with reproductive stages and allow us to determine the reproductive status.



a) Control, b) Prepubertal, c) Adult animal d0 Pregnent animal, e) Postpartum.

Fig 1: Microbe isolation of female goat from urine in different reproductive stages.

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Streak Plate

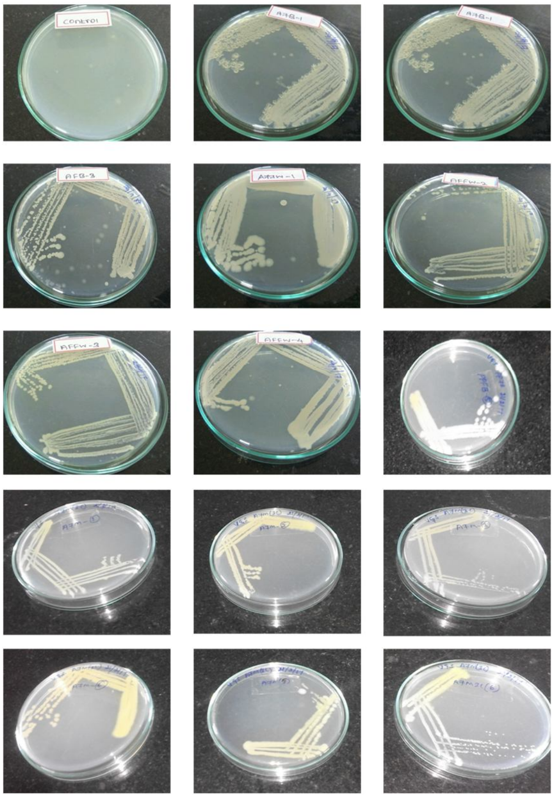

Fig 2: Microbe isolation of female goat from vaginal swab in different reproductive stages

Indole Test

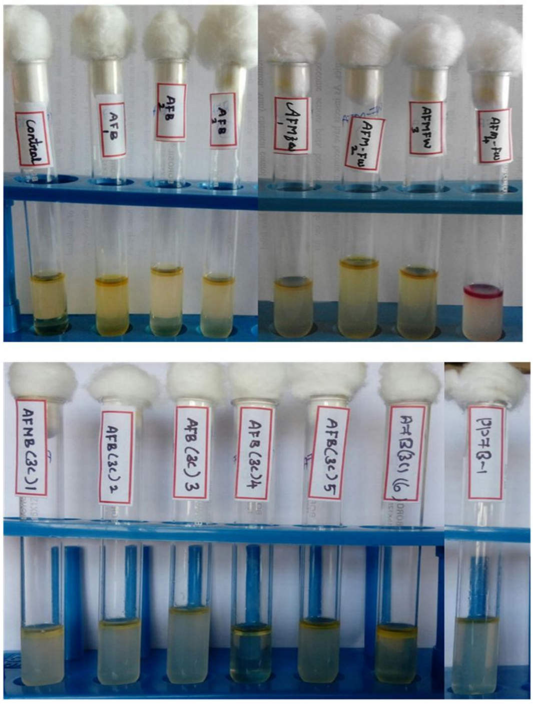

Fig 3: Indole test of female goat vaginal swab in different reproductive stages

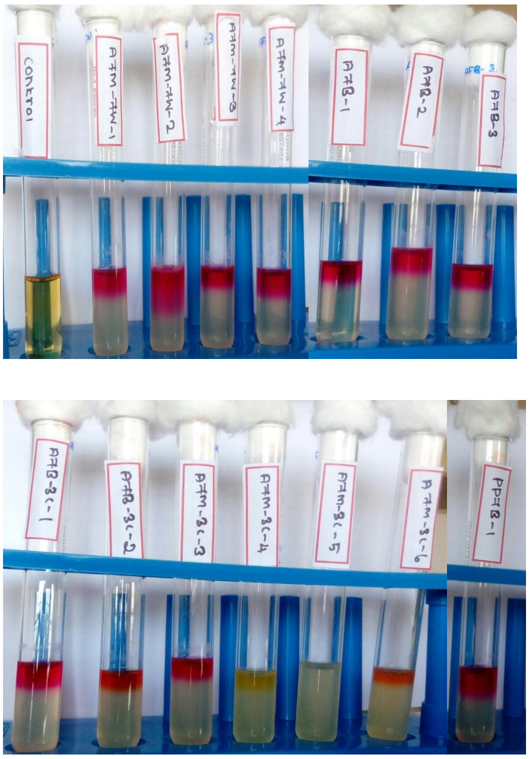

Fig 4: Methyl red test of female goat vaginal swab in different reproductive stages

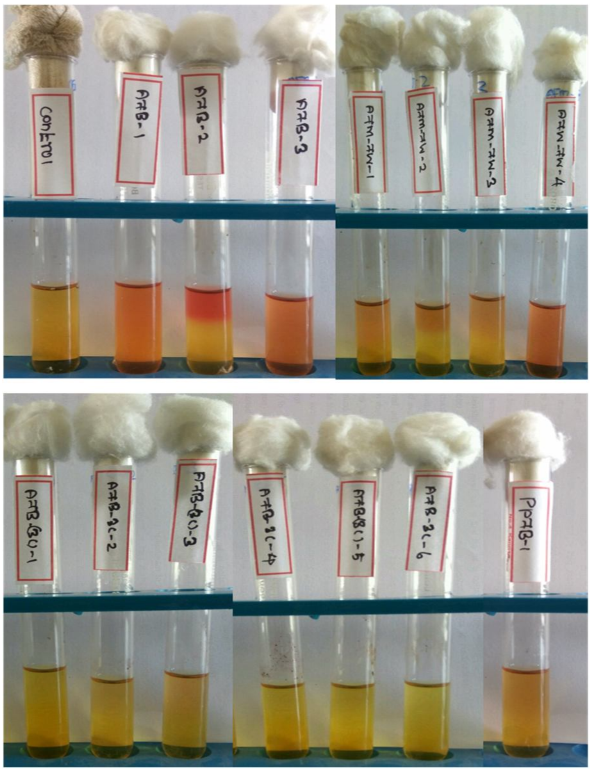

Fig 5: Voges - Proskauer's test of female goat vaginal swab in different reproductive stages

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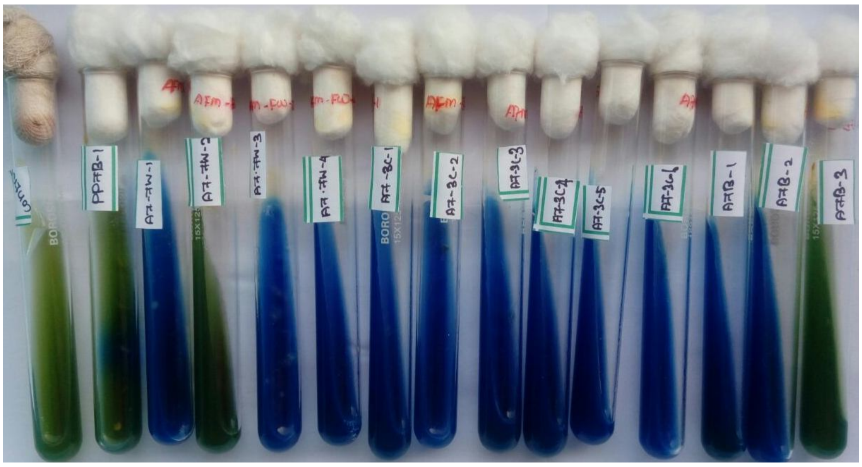

Fig 6: Citrate agar test of female goat vaginal swab in different reproductive stages

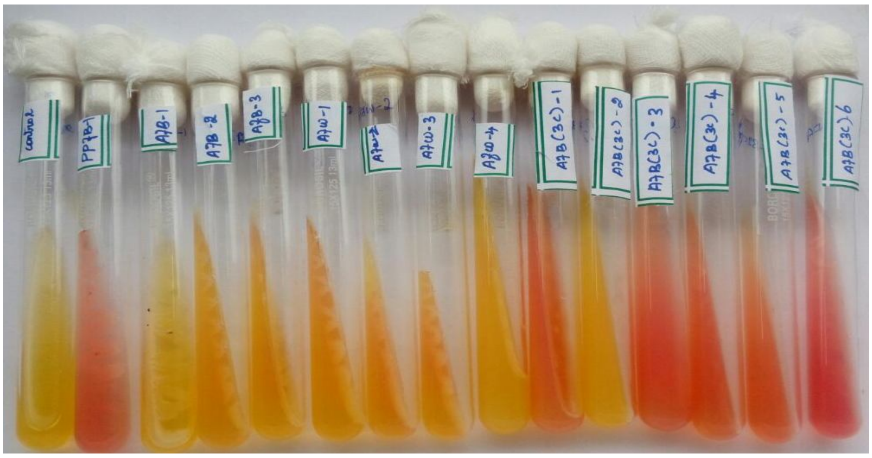

Fig 7: Urease test of female goat vaginal swab in different reproductive stages

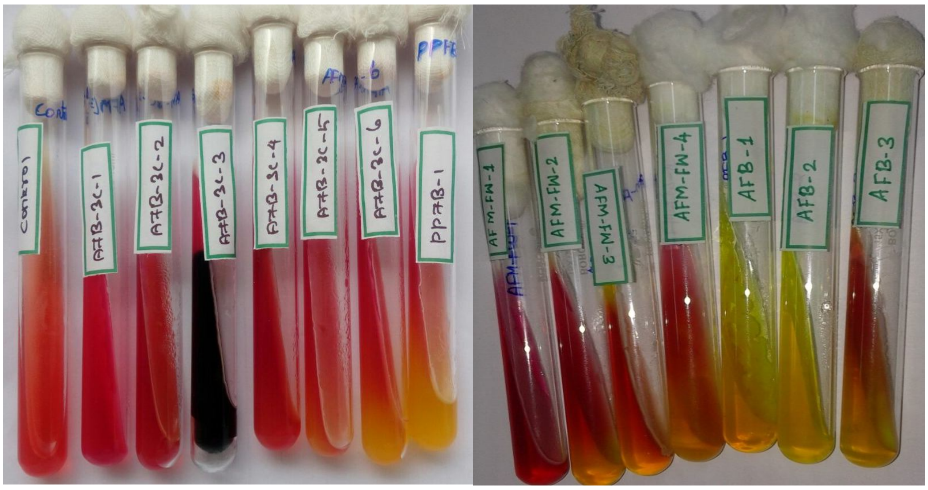

Fig 8: Triple sugar test of female goat vaginal swab in different reproductive stages

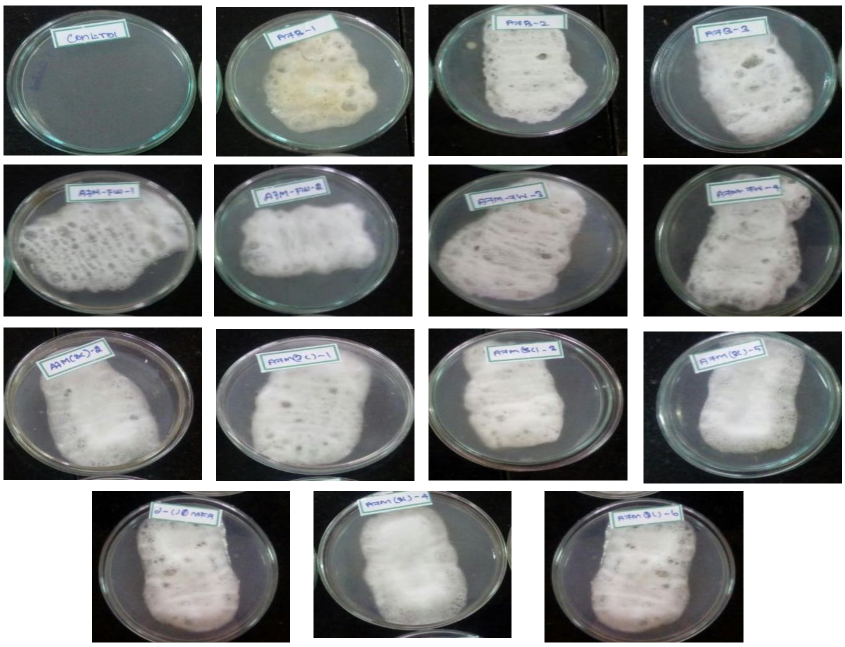

Fig 9: Catalase test of female goat vaginal swab in different reproductive stages

Table 1: Identification of the microbes present in the female goat vaginal swab

Culture	Indole	Methyl red	Voges proskauer's	Citrate agar	Urease	TSI	Catalase	Gram staining	Identification of microbes
Control	-	-	-	-	-	-	-	-	
AFM-FW-1	-	+	+	+	-	R	+	-	*Serratia marcescens*
AFM-FW-2	-	+	+	-	-	RS/YB	+	-	*Serratia marcescens*
AFM-FW-3	+	+	+	+	-	Y/R/YB	+	-	*Serratia marcescens*
AFM-FW-4	-	+	+	+	-	RS/YB	+	-	*Klebsiella oxytoca*
AFB-1	-	+	+	+	-	Y	+	-	*Serratia marcescens*
AFB-2	-	+	+	+	-	Y	+	-	*Serratia marcescens*
AFB-3	-	+	+	-	-	R	+	-	*Serratia marcescens*
AFB-3C-1	-	+	-	+	+	R	+	-	*Providencia rettgeri*
AFB-3C-2	-	+	-	+	-	R	+	-	*Providencia staurtil*
AFB-3C-3	-	+	-	+	+	R/B	+	-	*Proteus mirabilis*
AFB-3C-4	-	-	-	+	+	R	+	-	*Providencia rettgeri*
AFB-3C-5	-	-	-	+	+	R/YB	+	-	*Providencia rettgeri*
AFB-3C-6	-	+	-	+	+	R/YB	+	-	*Citrobacter freundili*
PPFB	-	+	-	-	+	R/YB	+	-	*E.coli*

CONCLUSION

The healthy female goats from SIFS, Bharathidasan University, Tiruchirappalli – 620 024**,** were chosen to isolate the microbial strains present in the urine and vaginal fluid profiles of female goats at different reproductive stages and these microbes can play an vital role to produce volatiles compounds. Bacterial isolates were obtained from vaginal fluid of healthy female goat of different reproductive status. . Bacteria isolated from vagina were *Serratia masrcescens*, E. coli, Klebsiella oxytoca, Proteus mirabilis, Providencia stuartil, *Providencia rettgeri* and Citrobacter freudilli. No mycoplasmas were observed. In addition, no bacterial isolates were detected in urine of goats. These findings stress that bacteria present in urine can escape detection by culture-dependent methods. Therefore, the current study suggests to carry out the methods to prove the presence of non-culturale bacteria.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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