

Isolation and Identification of Pathogenic Bacteria from Vaginal Cavity of Sheep and Goats in Iraq

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Abstract

This study intends to explore the prevalence of pathogenic bacteria infected the vaginal cavity of sheep and goats, in addition to determine the normal vaginal bacterial flora in pregnant and non pregnant local breed goat and sheep. Vaginal swabs were collected from 80 cases comprised of 54 sheep and 26 goats that involve 20 and 6 pregnant ewes and goats from Baghdad city during May 2019 to July 2019. These samples were cultivated onto different bacteriological media. The isolated bacteria were identified by different biochemical tests. Mixed bacterial isolates were determined in 65 out of 80 (81.3%) samples. Totally, 9 various microorganisms were isolated from animal's vagina. *E. coli*, *Streptococcus Spp*, *Klebsiella Spp* and *Staphylococcus aureus* were the most common isolated microorganism from vaginal swabs in a percentage of 36, 20, 17.5 and 16.3% respectively. While *Bacillus Spp*, *Proteus Spp*, *Staphylococcus Spp*, *Corynebacterium Spp* and *Salmonella Spp* were the less common isolated microorganisms in a percentages of 10, 10, 7.5, 6.3 and %2.5 respectively. Moreover, the enteric bacteria especially *E. coli* was isolated in the higher incidence rate (44.4%) in pregnant ewes compared to pregnant goats 15.4%. However, in non-pregnant animals, the percentages were 50 and 15% in sheep and goats respectively. Nonetheless, *Proteus Spp* were isolated only from newly birth animals. Meanwhile, *Staphylococcus aureus* were relatively isolated at the same level of incidence at 15%-17% in either pregnant or non-pregnant sheep or goats, while *Streptococcus Spp* were mostly found in pregnant goats and ewes at 50 and 25% respectively. In conclusion, this study approved the isolation of different bacterial spp in various incidence rates from vaginal cavity of pregnant and non-pregnant sheep and goats.

Keywords: Pathogenic bacteria, bacterial flora, vaginal cavity, sheep and goats, vaginal swabs.

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INTRODUCTION

The importance of reproductive efficiency in the sheep is very essential because of the seasonal breeding. Many diseases are classified as specific and non-specific which cause problems in the reproductive system in the sheep. Some of these diseases cause infertility or reduce productivity with clinical signs which may include vaginal discharge. Abortion and infertility can result from infection or from non-infectious causes (El-Arabi et al., 2013).

Genital infections in domestic ruminants are often caused by opportunistic secondary invaders, especially *Escherichia coli* spp that have frequently been isolated in majority from ewes (Sargison et al., 2007 and Manes et al., 2010), goats (Ababneh and Degefa, 2006). 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 (Shallali et al., 2001 and Mshelia et al., 2014). Vaginitis, a common disease of the genital tract of domestic ruminants, is often caused by secondary bacterial invaders, mainly the *E. coli* species (Sargison et al., 2007 and Manes et al., 2010).

These microorganisms caused diseases due to stress and reduction of the immunity of the reproductive system (Al-Dahash and Fathalla 2000). Mavrogianni et al. (2007) found bacteria in the uterus of ewes after lambing and found *E. coli*, *Apyogenes*, *Staphylococci* and *Streptococci* after interference. El-Arabi et al. (2013) isolated normal bacterial flora of vaginal cavity in normal sheep. Vasconcelos et al. (2016) reported that sheep treated with either sponges or intra-vaginal implant containing progesterone (CIDR) have a high prevalence of *E. coli*, probably of fecal source, which are opportunistic agents of bacterial vaginitis. *Staphylococcus aureus* is considered an inhabitant of vulva and vagina and identified as the most common causative agent of purulent vaginitis in ewes (Donders et al., 2002 and Braganna et al., 2017). Manes et al. (2010) isolated *Bacillus* spp., *Corynebacterium* spp., *Escherichia* spp., *Staphylococcus* spp., and *Streptococcus* spp. from the ewe's vagina.

MATERIAL AND METHODS

Collection of samples

For bacterial isolation, 80 vaginal swabs were collected aseptically from 54 sheep and 26 goats from different areas in Baghdad city. All samples were collected using disposable sterile swabs with transport media (Greiner). The out surface of vagina was disinfected by 70% alcohol then the swabs were introduced in vaginal cavities to collect the samples. These swab samples were cultured on different cultural media and incubated at 37°C for 24-48 hrs. All bacterial isolates were identified by cultural characteristic features of colonies on primary and specific media in addition to gram staining and according to the results of biochemical tests. (Quinn et al., 2004).

Cultural media

Different cultural media were used to cultivate the samples including blood agar, MacConky agar, Mannitol salt agar, Eosin Methylene Blue agar, nutrient agar, tetrathionate broth and SS agar and incubated at 37°C for 24-48 hrs.

Biochemical tests

Many different biochemical tests were done to identify the bacterial isolates including catalase test, coagulase test, IMVIC test, TSI test and motility test.

RESULT AND DISCUSSION

In this study, the results were revealed that nine bacterial isolates were identified in various percentages in sheep and goats. The isolated microorganisms are including *E. coli*; *Staphylococcus aureus*; *Staphylococcus* spp; *Salmonella* spp; *Streptococcus* spp; *Proteus* spp; *Bacillus* spp; *Klebsiella* spp and *Corynebacterium* spp. (Table 1). These results agree with previous researchers Manes et al., 2010 and Al-Zubaidi et al., 2013. These researchers approved the isolation of many bacteria from vaginal cavity of sheep and goats and found the impact factors of the isolated bacteria on the occurrence of infertility and problems of the reproductive system. Moreover, Al-Delemi (2005) stated that several bacterial types presented in the female genital system. Meanwhile, Zaid, (2009) reported the isolation of 12 different types of bacteria from the reproductive tract and approved the probability

of presence of more than one type of bacteria in the same swabs. However, many studies shown that these bacteria were normal residents of the anterior vagina in does and ewes (Bukar-Koloet al.,2007; Mshelia et al., 2014).

E.coli was the first dominant bacteria in ewes and goats and occurred at 44.4% and 15.4% respectively, moreover, *Klebsiella* was isolated as a second dominant bacterium.

These results agree with previous reresearch (Al-Hilli and Ajeel, 2015), who stated that *E.coli* was the dominant isolates from vaginal swabs of ewes and *Klebsiella* was the second dominant. Additionally, El-Arabi et al., (2013)

mentioned that *E.coli* and *Streptococcus spp* were the predominant bacteria.

The results of the current study revealed the isolation of different percentages of microorganisms in pregnant and non-pregnant ewes and does table (2 & 3). These results are compatible with other researcher (Martins et al., 2009), who stated that the alteration on microflora was due to changes of vaginal environment such as pH modifications during estrus and pregnancy.

The presence of these microorganisms in the vagina as we isolated in this study may cause bacterial vaginitis following the exposure to any factors causing depression of the immunity system

Table 1. The percentage of bacterial isolates from vaginal swabs of sheep and goats

Bacterial isolates (nine isolates) from vaginal cavity	Sheep		Goat		Total no. of isolates out of (80 sample)	
	No. of isolates out of (54 samples) (%)		No. of isolates out of (26 samples) (%)			(%)
<i>E.coli</i>	24	44.40%	4	15.40%	28	35%
<i>Staphylococcus aureus</i>	9	16.60%	4	15.40%	13	16.3
<i>Staphylococcus spp</i>	2	3.70%	4	15.40%	6	7.50%
<i>Salmonella spp.</i>	1	1.90%	1	3.80%	2	2.50%
<i>Streptococcus Spp.</i>	6	11.10%	10	38.50%	1	620%
<i>Proteus spp</i>	3	5.60%	5	19.20%	8	10%
<i>Bacillus spp</i>	3	5.60%	5	19.20%	8	10%
<i>Klebsiella spp.</i>	10	18.50%	4	15.40%	14	17.50%
<i>Corynebacterium spp</i>	3	5.60%	2	7.70%	5	6.25%
No growth	10	18.50%	5	19.20%	15	18.80%

Table 2. The percentage of bacterial isolates from vaginal swabs of pregnant and non- pregnant ewes

Bacterial isolate (nine isolates)from vaginal cavity	Sheep No. of isolates out of				Total no. of isolates out of (54 samples)(%)	
	Pregnant (20) samples%		Non-Pregnant (34) samples%			
<i>E.coli</i>	7	%35	17	%50	24	%44.4
<i>Staphylococcus aureus</i>	3	%15	6	%17.6	9	%16.6
<i>Staphylococcus spp</i>	1	%5	1	%2.9	2	%3.7
<i>Salmonella spp.</i>	—	.	1	%2.9	1	%1.9
<i>Streptococcus Spp.</i>	5	%25	1	%2.9	6	%11.1
<i>Proteus spp</i>	—	.	3	%8.8	3	%5.6
<i>Bacillus spp</i>	—	.	3	%8.8	3	%5.6
<i>Klebsiella spp.</i>	2	%10	8	%23.5	10	%18.5
<i>Corynebacterium spp</i>	—	.	3	%8.8	3	%5.6
No growth	5	%25	5	14.7	10	%18.5

Table 3. The percentage of bacterial isolates from vaginal swabs of pregnant and non- pregnant does

Bacterial isolate (nine isolates) from vaginal cavity	goats				Total no. of isolates out of (26samples)	
	No. of isolates out of				()	
	Pregnant (6) samples %		Non -Pregnant (20) samples %			
<i>E.coli</i>	1	16.70%	3	15%	4	15.40%
<i>Staphylococcus aureus</i>	1	16.70%	3	15%	4	15.40%
<i>Staphylococcus</i> spp	2	33.30%	2	10%	4	15.40%
<i>Salmonella</i> spp.	–	.	1	5%	1	3.80%
<i>Streptococcus</i> spp.	3	50%	7	35%	10	38.50%
<i>Proteus</i> spp	–	.	5	25%	5	19.20%
<i>Bacillus</i> spp	–	.	5	25%	5	19.20%
<i>Klebsiella</i> spp.	–	.	4	20%	4	15.40%
<i>Corynebacterium</i> spp	–	.	2	10%	2	7.70%
No growth	1	16.70%	4	20%	5	19.20%

of the animals and affect the fertility status and this idea also mentioned by Bukar *et al.*, (2007).

In conclusion, this study approved the isolation of different bacterial spp in various incidence rates from vaginal cavity of pregnant and non-pregnant sheep and goats. Totally, 9 various microorganisms were isolated from animals vagina. According to our conclusion we recommended to follow hygienic precautions in intra vaginal implantation to minimize the contamination and avoid the infection with secondary bacterial invaders in addition to reduce the chance of infertility.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during

this study are included in the manuscript and/or the Supplementary Files.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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