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Karbala International Journal of Modern Science 3 (2017) 259–266
http://www.journals.elsevier.com/karbala-international-journal-of-modern-science/

Prevalence and antibiotic resistance pattern of certain types of bacterial flora in uterine ewe's samples

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Received 3 April 2017; revised 6 August 2017; accepted 9 August 2017
Available online 12 September 2017

Abstract

A study was carried out in abattoirs to identify certain bacterial flora in a number of samples collected from ewes' uteri with detection of phenotypic and genotypic antibiotic resistance patterns of the identified bacteria. The study was done in the South of Iraq during a period that started in February and ended in March 2015. Seventy-nine samples were collected randomly and aseptically, examined grossly, cultured using standard bacteriological techniques and examined for antibiotic resistance. Thirty-one isolates were reported belong to the following bacteria with resistance percentages accordingly: *Escherichia coli* 41. 94% (No: 13), *Klebsiella* spp. 29.03% (No: 9), *Enterobacter* spp. 16.13% (No: 5), *Pseudomonas aeruginosa* 6.45% (No: 2) and *Proteus* spp. 6.45% (No: 2). Results revealed that 100% (No: 31) of isolates were resistant to oxacillin while resistance to both ampicillin and tetracycline appeared in 93.64% (No: 30), 41.92% (No: 13) of isolates respectively, moreover there was for some extent resistance to ceftriaxone 9.68% (No: 3), while the isolates were highly susceptible to cefamandole and gentamicin. The isolates were also examined to determine the presence of bla_{SHV} genes by PCR assay which showed that 12.9% of isolates harbored this gene. This study contributes to a better knowledge about identified bacterial species inhabiting the uterus of ewes and exerting a significant and distinct antimicrobial resistance pattern.

Keywords: Flora; bla_{SHV} genes; Ewes; Uteri

1. Introduction

The normal uterus is a clean environment in contrast to the vagina, which contains numerous opportunistic secondary invaders that can invade uterus of ewes and behave in a different manner from normal vaginal flora to a pathogenic agents [1,2]. An effective control of postpartum contamination of the uterus provides the opportunity to improve both fertility and general health condition of ewes and other ruminant animals; however, the uterus of ruminants is usually contaminated with different microflora in the immediate postpartum period [3].

It has been reported that goats have many microflora in their genitalia and recorded that these microflora are usually harmless but certain predisposing factors such

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Peer review under responsibility of University of Kerbala.

http://dx.doi.org/10.1016/j.kijoms.2017.08.002

as trauma or another infection can drive them to a pathogenic state and disease causing agents [4].

There are also other pathogens which are harmless in normal situation but can be converted to a more pathogenic form when favorable conditions are available in the reproductive tract such bacteria as coliform bacteria and other non-specific bacteria under stressful conditions, may cause genital infection that usually leads to reproductive failure in dairy cows, sheep and goats [5,6,7]. Different types of bacteria have been isolated from the genitalia of the doe and these include (*Staphylococci*, *Streptococci*, *Actinomyces*, *Pseudomonas*, *Escherichia coli*, *Mycoplasma* and *Brucella*) species [8].

The importance of studying such microorganisms is related to diseases caused by them due to reduction of the immunity of the reproductive system [9]. Several studies mentioned that there were several types of bacteria found in the reproductive tract [10–13]. Several studies have been made in Iraq which focused on isolation of certain microorganisms behaving as an opportunistic bacteria in uteri of different animals [14–16].

Many researchers indicated that the reproductive system contains normal flora [17–21].

Beta-lactamases are enzymes that are major causes of bacterial resistance to the beta-lactam family of antibiotics such as penicillins, cephalosporins, cephamycins, and carbapenems. These enzymes catalyze the hydrolysis of the amide bond of four membered beta-lactam ring and render the antibiotic inactive against its original cellular target, the cell wall transpeptidase. On the basis of their primary structure, beta-lactamases are grouped into four classes A, B, C, and D enzymes. Enzymes of classes A, C, and D have serine at the active site, whereas the class B enzymes are zinc-metallo enzymes. Extended-spectrum beta-lactam antibiotics have widely been used for treatment of serious Gram-negative infections.

However, bacterial resistance has emerged due to production of extended-spectrum beta-lactamases (ESBLs). These enzymes are capable of hydrolyzing extended-spectrum beta-lactam antibiotics such as penicillins, cephalosporins along with a monobactam (aztreonam) but are inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. ESBLs are derived from genes for the Narrow spectrum beta-lactamases (TEM-1, TEM-2, or SHV-1) by mutations that alter the amino acid configuration around the enzyme active site. They are typically encoded by plasmids that can be exchanged readily between bacterial species. These enzymes are most commonly produced by the members of the *Enterobacteriaceae*, especially *E. coli* and *Klebsiella*. To date, more than 350 different natural ESBL variants are known that have been classified into nine distinct structural and evolutionary families based upon their amino acid sequence comparisons such as TEM, SHV, CTX-M, PER, VEB, GES, BES, TLA, and OXA [22–25].

Modern biological techniques for detection of antibacterial resistance have been rarely applied to study of the distribution of resistance. This study aimed to identify certain flora resident in the uterus of ewes and study some phenotypic traits and occurrence rate of bla_{SHV} genes among the identified bacteria.

2. Materials and methods

2.1. Samples collection

A total of seventy-nine uteri were collected from ewes immediately after slaughtering, and instantly transported to the laboratory in sterile polythene bags. The uterine sample collection processes were compatible to standard techniques described [26,27]. The surface of the uterus was sterilized by shearing the uterine wall with a preheated surgical blade then the uterine wall was lanced with another sterile blade and a sterile swab stick was inserted and rolled over into the uterine lumen to collect bacteriological samples.

2.2. Isolation and identification

Samples were cultured on selective and differential MacConkey agar (Merck, Germany) and the isolates were identified based on their ability to ferment lactose. Cultural and morphological characteristics of colonies such as the shape, size, consistency and color and Gram's stain had been performed; moreover, the biochemical tests such as IMViC tests (indol production, Methylred, Voges-Proskauer and Citrate utilization), catalase, oxidase, phenyldeaminase and motility were performed by which different bacteria have been identified [28,29].

2.3. Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed using a disk diffusion method described by Bauer et al. (1966) [30] using Mueller-Hinton agar (Oxoid, U.K.). Six antibiotic disks containing Ampicillin (10 μg), Ceftriaxone (30 μg), Cefamandole (30 μg), Gentamycin (30 μg) Oxacillin (1 μg) and tetracycline (30 μg) have been used. The susceptibility breakpoints for all antimicrobials were recommended by Clinical and Laboratory Standards Institute CLSI [31].