BACTERIOLOGICAL AND THEIR ANTIBIOGRAM STUDIES OF ENDOMETRITIS IN SLAUGHTERED BUFFALOES

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ABSTRACT

The present study was designed to assess the bacterial isolation and their antibiogram from endometritis in slaughtered buffaloes. A total 110 uterine swabs at horn-body junction of slaughtered buffaloes were collected from local abattoir in Junagadh. Out of 110 uterine swabs 56 (50.90%) uterine samples showed the growth of various bacteria and remaining 54 swabs (49.10%) were found to be sterile. Among 56 uterine samples 50 swabs (89.28%) showed single isolate and remaining 06 (10.72%) exhibited mixed infection. The *Escherichia coli* and *Staphylococcus* spp. isolates (24.19%) top the list followed by *Corynebacterium* spp. (17.74%), *Micrococcus* spp. (14.52%), *Fusobacterium* spp. (8.06%), *Pseudomonas* spp. (4.84%), *Bacillus* spp. (4.84%) and *Streptococcus* spp. (1.61%). The antimicrobial susceptibility of these bacterial isolates were showed highest sensitivity against Chloramphenicol (83.9%) followed by Gentamicin (80.6%), Levofloxacin (77.4%), Oxytetracycline (77.4%), Ceftriaxone/ Sulbactam (69.3%), Cefoperazone/Sulbactam (61.2%) and Amoxicillin/Sulbactam (33.9%).

Keywords: *Bubalus bubalis*, buffaloes, antibiogram, bacterial isolates, endometritis

INTRODUCTION

Endometritis is invariably an infectious disease caused by microbes like bacteria, virus, mycoplasma, chlamydia, protozoa and fungi. In majority of cases bacteria take the lead. Uterus remain normally sterile during non-gravid and gravid stages and if bacterial invasion occurs due to any reason it usually results in foetal absorption or abortion (Semambo *et al*., 1991). During the reproductive life of females the uterus has to face the risk of infection particularly at the time of breeding (natural or artificial insemination) followed by parturition (Tibary and Anouassi,

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1997). However, bacterial infections in uterus become ubiquitous around calving (Sheldon *et al*., 2004). The postpartum involution of uterus is considered to be a natural antiseptic process where it takes about 45 to 60 day to clear the infection (Smith and Risco, 2002). The common sources of infection are microorganisms lurking around animal's environment, skin contamination and faecal material in perineal regions. The uterine infection also affect ovarian functions like slower growth of developing ovarian follicles and reduction of plasma estradiol concentration ultimately disturbing the reproductive cycle.

MATERIALS AND METHODS

A total 110 uterine swabs at horn-body junction from slaughtered buffaloes were collected from local abattoir in Junagadh (Gujarat) for bacterial isolation following routine methods. Based on histopathology endometritis cases were diagnosed. These uterine swab samples were inoculated into brain heart infusion (BHI) broth and incubated at 37^{o}C overnight in aerobic and anaerobic environment following routine method. The aliquots of each sample were also plated on BHI, McConkey and blood agar in both aerobic and anaerobic environment and examined every 12 h till 48 h post inoculation for the presence of any turbidity and growth. The growth characteristics of the isolates were recorded. The cultures were then purified by sub-culturing following routine method and refrigerating for further studies. The bacteria isolated both aerobically and anaerobically from endometrium showing the characteristic colony morphology were Gram stained and confirmed by standard biochemical tests such as catalase test, oxidase test, indole test, methyl-red test, Voges-Proskauer test, citrate utilization test and sugar fermentation tests (HiMedia, Mumbai) (Holt *et al*., 1994). All the bacterial isolates obtained were subjected to *in vitro* antibiotic sensitivity test as per Kirby-Bauer (Bauer *et al*., 1966) method. The isolated colonies were suspended in nutrient broth and then incubated for overnight. The sterile cotton swab was dipped in the bacterial suspension and then rolled over the surface of the Muller-Hinton agar medium and covered evenly with the bacterial suspension. Seven different antibiotic discs were placed over the surface of the agar plate. For this purpose, separate Antimicrobial discs (Himedia, Mumbai) *viz*., Amoxicillin/Sulbactam (30/15 mcg), Cefoperazone/Sulbactam (75/30 mcg), Ceftriaxone/ Sulbactam (30/15 mcg), Chloramphenicol (30 mcg), Gentamicin (20 mcg), Levofloxacin (5 mcg) and Oxytetracycline (30 mcg) discs were employed. Zones of inhibition were measured and compared with zone size interpretative table furnished by the CLSI and manufacturer and graded as sensitive and resistant.

RESULTS AND DISCUSSION

Out of 110 uterine genitalia, 89 (81%) exhibited varying degree of inflammatory changes with infiltration of different inflammatory cells in the endometrium whereas remaining 21 (19%) uterine samples did not show any significant infiltration / inflammatory changes on histopathology.

The bacterial cultural studies were carried out on all the 110 uterine swabs collected during the present study. Fifty six (50.90%) uterine samples showed the growth of various bacteria and remaining 54 swabs (49.10%) were found to be sterile including apparently normal

based on histopathology (Table 1). Out of the 56 uterine samples 50 swabs (89.28%) showed single isolate and remaining 06 (10.72%) exhibited mixed infection. The cultural isolates comprised of *Staphylococcus* spp. (11.82%), *Escherichia* coli (8.18%), *Micrococcus* spp. (8.18%), *Corynebacterium* spp. (7.27%), *Fusobacterium* spp. (4.55%), *Pseudomonas* spp. (2.73%), *Bacillus* spp. (1.82%), *Streptococcus* spp. (0.91%) and mixed infections consisted of two different types of isolates in different combinations (*E. coli* + *Corynebacterium* spp. - 2.73%, *E. coli* + *Staphylococcus* spp. - 1.82% and *E. coli* + *Bacillus* spp. - 0.91%).

As mentioned above, a total of 62 bacterial culture were isolated from uterine swab samples including 06 mixed infection (Table 2). The *Escherichia coli* and *Staphylococcus* spp. isolates (24.19%) top the list followed by *Corynebacterium* spp. (17.74%), *Micrococcus* spp. (14.52%), *Fusobacterium* spp. (8.06%), *Pseudomonas* spp. (4.84%), *Bacillus* spp. (4.84%) and *Streptococcus* spp. (1.61%).

In respect of *Staphylococcus* isolates our present finding supported the observations recorded by a number of workers both in India and abroad in past (Ahmed and Bhattacharya, 2005; Prajapati *et al*., 2006; Gani *et al*., 2008; Behera *et al*., 2015) who too registered a *Staphylococcus* infection in major number of isolation studies in maximum number of cases in their own study. The prevalence of *E. coli* infection in present study is also well compared with *Staphylococcus* uterine infection and were in agreement with the findings of a number of workers who in their studies also registered higher prevalence of *E. coli* in their respective studies (El-Sakkar *et al*., 2008; Yilmaz *et al*., 2012; Udhayavel *et al*., 2013; Barman *et al*., 2013; Takamtha *et al*., 2013; Mshelia *et al*., 2014). In contrast, higher percentage of *A. pyogenes* (Moges *et al*., 2013; Sayyari *et al*., 2012); *Salmonella* (Samatha and Babu, 2013), respectively were reported in their study.

There appears to be hardly any unanimity in respect of different uterine bacterial isolates causing uterine infection in different states of India or foreign countries reported time to time by different workers. Absence of unanimity is supposed to be influenced by a number of factors like sample size, bacterial contamination of surrounding environment especially during parturition, degree / stage of uterine involution, retention of fetal membranes, immune status, abortion, premature birth, dystocia, induction of parturition, bacterial contamination of the uterus, laceration or lesions of the uterus, cervix, vagina and vulva, uterine inertia, lack of exercise, injury during insemination, unhygienic conditions, lactational stress, animal health status and different husbandry practices followed, variations in agro-climatic conditions, etc. We fully endorse the views put forth by a number of earlier workers (Azawi, 2010; Sayyari *et al*., 2012; Behera *et al*., 2015; Sarkar *et al*., 2016).

Overall antimicrobial susceptibility of these bacterial isolates were showed highest sensitivity against Chloramphenicol (83.9%) followed by Gentamicin (80.6%), Levofloxacin (77.4%), Oxytetracycline (77.4%), Ceftriaxone/ Sulbactam (69.3%), Cefoperazone/Sulbactam (61.2%) and Amoxicillin/Sulbactam (33.9%). The antibiotic sensitivity pattern of bacterial isolates are presented in Table 3.

The results of present study are more or less in agreement with the findings of El-Kader and Shehata (2001) who reported that sensitivity of bacterial isolates with ciprofloxacin and gentamycin were the best of the used antibiotics.

Table 1. Overall bacterial isolates from uterine swabs.

Bacterial isolates	No of samples (n=110)	Percentage
Staphylococcus spp.	13	11.82
Escherichia coli	9	8.18
Micrococcus spp.	9	8.18
Corynebacterium spp.	8	7.27
Fusobacterium spp.	5	4.55
Pseudomonas spp.	3	2.73
Bacillus spp.	2	1.82
Streptococcus spp.	1	0.91
Mixed infection	6	5.45
Absence of growth	54	49.09

Table 2. Total bacterial isolates including mix infection.

Bacterial isolates	No of bacterial isolates (n=62)	Percentage
Escherichia coli	15	24.19
Staphylococcus spp.	15	24.19
Corynebacterium spp.	11	17.74
Micrococcus spp.	9	14.52
Fusobacterium spp.	5	8.06
Pseudomonas spp.	3	4.84
Bacillus spp.	3	4.84
Streptococcus spp.	1	1.61

Table 3. Antibiotic sensitivity pattern of bacterial isolates from endometritis.

Bacterial isolates	Various antibiotic discs used													
	Ceftriaxone/ Sulbactam (30/15 mcg)		Cefoperazone/ Sulbactam (75/30 mcg)		Amoxicillin/ Sulbactam (30/15 mcg)		Levofloxacin (5 mcg)		Chloramphenicol (30 mcg)		Gentamicin (20 mcg)		Oxytetracycline (30 mcg)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Escherichia coli (15)	15	100	15	100	08	53.3	10	66.6	13	86.6	06	40.0	15	100
Staphylococcus spp. (15)	07	46.6	11	73.3	02	13.3	10	66.6	15	100	13	86.6	10	66.6
Corynebacterium spp. (11)	11	100	07	63.6	05	45.5	10	90.9	10	90.9	11	100	06	54.5
Micrococcus spp. (9)	09	100	05	55.5	04	44.4	08	88.8	08	88.8	09	100	08	88.8
Fusobacterium spp. (5)	00	00	00	00	00	00	05	100	05	100	05	100	05	100
Pseudomonas spp. (3)	00	00	00	00	02	66.6	02	66.6	00	00	03	100	03	100
Bacillus spp. (3)	01	33.3	00	00	00	00	02	66.6	00	00	02	66.6	00	00
Streptococcus spp. (1)	00	00	00	00	00	00	01	100	01	100	01	100	01	100
Total bacterial isolate (62)	43	69.3	38	61.2	21	33.9	48	77.4	52	83.9	50	80.6	48	77.4

On the other hand Zahid (2004) noted that highest number of their isolates were sensitive to neomycin and doxycyline. El-Sakkar *et al.* (2008) reported that isolated microorganisms were highly sensitive to florafincol. The highest number of isolates were sensitive to Ceftriaxone and least sensitive to Chloramphenicol as reported by Udhayavel *et al.* (2013). In recent studies Moges *et al.* (2013) reported that all the isolates of *S. aureus* were resistant to ampicillin, oxacillin and vancomycin and *E. coli* showed resistance to polymixin, tetracycline and cefoxitin. The antimicrobial susceptibility of *E. coli* was highest against Pefloxacine, Ofloxacin, Amoxycillin-clavulanate, Ciprofloxacin, Gentamycin and Streptomycin, whereas antibiotic sensitivity of *S. aureus* showed the highest susceptibility against Ampicillin (Mshelia *et al*., 2014).

The resistance of these organisms to the commonly used antibiotics might be attributed to large scale and indiscriminate use of these antibiotics over a long period of time (Arora *et al*., 2000; Mohanty *et al*., 1992). Rational use of antibiotics is the key approach to improve the antibiotic performance and tackling antimicrobial resistance. The efficacy of antimicrobial activities are influenced by many factors like bacterial status (susceptibility and resistance, tolerance, persistence, biofilm), antimicrobial concentrations and host factors like impact on gut micro-biota (Li *et al*., 2017).

Based on present study it was concluded that most common bacteria involved were *E. coli* and *Staphylococcus* which were highly sensitive to Chloramphenicol.

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