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Short Communication

ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA FROM DAIRY COWS WITH SUB-CLINICAL ENDOMETRITIS

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SUMMARY

One hundred and forty clear uterine discharge samples (one from each cow) were collected from Jersey and Jersey crossbred cows of Livestock Instructional Farm Complex and Teaching Veterinary Clinical Complex of the university. The aerobic bacteria isolated from sub-clinically endometritic cows showing the characteristic colony, were gram stained and confirmed by standard biochemical tests. The *in vitro* antibiotic sensitivity test with fifteen different antibiotic discs was carried out. Out of 140 samples, 77 showed growth in nutrient broth and incidence of sub-clinical endometritis recorded was 55.0 %. Different species of bacteria isolated from these samples were, *Escherichia coli* (29.03%), *Staphylococcus* species (25.80%), *Bacillus* species (25.80%), *Streptococcus* species (9.67%), *Pseudomonas aeruginosa* (3.22%), *Micrococcus* species (3.22%) and *Salmonella* species (3.22%). The bacteria were most sensitive to Ciprofloxacin, Levofloxacin and Enrofloxacin (87.09, 80.64, 80.64%, respectively) and resistant to Metronidazole, Penicillin and Cloxacillin (96.77, 80.64 and 77.42%), respectively.

Key words: Antibiotic sensitivity, bacteria, cows, sub-clinical endometritis

Microbial disease of the female reproductive tract may be clinical or sub-clinical and is a major concern in dairy cattle production systems (Kasimanikam *et al*., 2013). Microbial infections are highly associated with infertility as they disrupt uterine and ovarian function. Sub-clinical endometritis (SCE) is associated with inflammation of the endometrium that results in a significant reduction in reproductive performance in the absence of signs of clinical endometritis (Herath *et al*., 2009). The non-specific bacterial infection of the reproductive tract in cattle is the main cause of repeat breeding (Dinesh *et al*., 2006). Fertilization failure due to inflammatory exudates causing blockade of oviducts or death of sperms due to toxins produced by bacteria and inflammatory reaction before they reach site of fertilization also results from the non-specific bacterial infections. The most commonly involved organisms are *Escherichia coli, Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Bacillus*, *Pseudomonas*, *Micrococcus* and *Klebsiella* (Gani *et al*., 2008). The infections of uterus caused by bacteria are treated with antibiotics. However, the efficacy of such therapeutic agents needs to be evaluated from time to time due to continuous emergence of drug resistant bacterial strains. Objectives of this study were, to find out the organisms causing SCE in cows and to evaluate their antimicrobial susceptibility to various antimicrobial drugs.

For this study, apparently clear uterine discharge samples from 140 cows were collected at estrus. The animals were restrained properly. The perineal area was washed with 0.01 percent potassium permanganate solution prior to sample collection followed by swabbing with ethyl alcohol. With an assistant parting the vulvar lips, the artificial insemination gun enclosed in sterilized sheath was introduced into the vagina and was guided through the cervix into the body of the uterus by rectal manipulation. The cervix was held with the left hand, the AI gun withdrawn and assistant was asked to aspirate the discharge with the help of a sterile syringe attached to the opposite end of the sheath with a rubber junction. The syringe was then detached; the sheath was withdrawn and wiped with an ethyl alcohol soaked cotton swab. The discharge was then transferred into a sterile screw capped vial containing sterile swabs. The discharge samples were placed upright in the cool box with ice and were transferred to the microbiological laboratory for immediate processing for CST.

For bacteriological culture analysis, the collected discharge samples were inoculated in nutrient broth and incubated for 24-48 hours and turbidity was observed. Samples causing turbidity were considered positive for SCE. Primary isolation from uterine discharge was done by directly streaking it on 5% sheep blood agar plates to obtain discrete colonies. The plates were incubated at 37^0C aerobically and examined for the presence of bacteriological growth after 24-72 hours of incubation. The smears were then prepared from single isolated purified colonies and Gram's staining was followed to study the morphological features of obtained isolates. Further, identification was done by applying the respective biochemical tests and special staining for suspected bacteria using standard procedures.

The Gram negative organisms were grown on different differential and selective media (MacConkey's Lactose Agar and Eosin Methylene Blue). Susceptibility of microorganisms to selected antibiotics was evaluated using the Kirby Bauer disk diffusion method on Mueller-Hinton agar (MHA). Zones of inhibition for individual antimicrobial agents were translated into highly sensitive,

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sensitive and resistant categories. Susceptibility of different isolates to the following antibiotics was tested: Enrofloxacin (10mcg/disc), Ciprofloxacin (5mcg/disc), Ofloxacin (5mcg/disc), Gentamicin (10mcg/disc), Ceftrixone (10mcg/disc),Penicillin (10units/disc), Levofloxacin (5mcg/disc), Oxytetracycline (30mcg/disc), Streptomycin (10mcg/disc), Cephalexin ((30mcg/disc), Cloxacillin (10mcg/disc), Ampicillin (10mcg/disc), Amoxicillin (10mcg/disc), Metronidazole (5mcg/disc) and Tetracycline hydrochloride(30mcg/disc).

Out of total 140 clear uterine discharge samples from repeat breeder cows, 77 (55.0 %) yielded bacteria and 63 were bereft of any growth. The different bacterial species isolated from 77 positive samples were *Escherichia coli* (29.03%), *Staphylococcus* species (25.80%), *Bacillus* species (25.80%), *Streptococcus* species (9.67%), *Pseudomonas aeruginosa* (3.22%), *Micrococcus* species (3.22%) and *Salmonella* species (3.22%). Maximum isolates were of *Escherichia coli, Bacillus* species and *Staphylococcus* species (29.03, 25.80 and 25.80%, respectively). Only few bacteriological studies have been carried out in cases of SCE, with no conclusive results (Baranski *et al*., 2012). In a few cases, *Arcanobacterium pyogenes* and *Staphylococcus aureus* were also detected in the uteri (Baranski *et al*., 2012). In cows with SCE, *Truperella pyogenes* is a frequently isolated pathogen (Sens and Heuwieser, 2013). These bacterial species are frequently isolated from bovine reproductive tract and are supposed to be opportunistic contaminants (Williams *et al*., 2005). Sens and Heuwieser (2013) discussed the role of α- haemolytic *Streptococci* as a potential uterine pathogen in case of SCE.

Similar findings were reported by Sahadev *et al*. (2017) where the most common single aerobic bacterial isolate cultured from cows with SCE in their study was *Escherichia coli* in 45.71%. The other most commonly recovered bacterial isolates were *Staphylococcus* (42.86%), *Proteus* spp. and *Enterobacter* spp. (5.71% each). Barman *et al*. (2013) reported that non lactose fermenting organisms belonging to family Enterobacteriaceae were the most common isolates in cows with SCE. The other common bacteria isolated were *E. coli*, *Streptococcus*, *Staphylococcus* spp., *Pseudomonas* spp. and *Bacillus* spp. Sunita *et al*. (2015) recorded that *Staphylococcus aureus* isolates were the highest amongst total isolates (38.88 %) followed by *E. coli* spp. (36.11 %), *Streptococcus* spp. (33.33 %), *Enterobacter* spp. (22.22 %), *Proteus* spp. (18.05 %) and *Pseudomonas* spp. (16.67 %). Several authors reported *Staphylococcus* spp. as the most frequently isolated bacteria from the female genital tract with mild uterine infection or repeat breeding (Palanisamy *et al*., 2015) whereas *E. coli* was reported to be in maximum frequency by Bhat *et al*. (2014).

Isolation of pathogenic bacteria during late postpartum period is highly suggestive of endometrial inflammation and when such condition is not associated with clinical endometritis, it is highly possible that cow is suffering from SCE. A swab is the most accurate means of obtaining samples for identification of the specific and non-specific bacteria that cause infection (Bonnett *et al*., 1993). A true uterine culture should be taken from the uterus without contamination by extraneous bacteria.

From the 15 different antimicrobial drugs (Table 1) used, bacteria were highly sensitive to Ciprofloxacin, Enrofloxacin and Levofloxacin (81.82, 77.42 and 76.62%, respectively), whereas resistant to Metronidazole, Cloxacillin and Penicillin (96.55, 80.52 and 73.77%, respectively). In our study, gram positive and Gram negative bacteria were found highly sensitive to Fluoroquinolone group of antibacterial drugs. Specifically, Ciprofloxacin was the most effective and Metronidazole, β-lactum antibiotics were the least effective for both Gram positive and Gram negative pathogens. The use of proper antibiogram is required as a necessity, in the context of variation factors concerning the frequency of different involved bacterial species, and their susceptibility to different antimicrobial products. Gani *et al*. (2008) found Ciprofloxacin as one of the most effective antimicrobial agent against *Staphyloccocal* uterine infections in dairy cows. Cows diagnosed with SCE had prolonged days open and a reduced probability of conception at first AI compared with cows without SCE (Barlund *et al.,* 2008). The antibiogram could be useful to the practitioner in choosing the most efficient antibacterial products.

This study revealed that sub-clinical endometritis in cows is mainly caused by *Escherichia coli*, *Staphylococcus* species, *Bacillus* species and the Fluoroquinolone group of antibiotics is highly effective.

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Table 1: Sensitivity of various bacteria isolated from sub-clinically endometritic cows to different antibacterial drugs

Antibiotic	Highly Sensitive (++)		Sensitive (+)		Resistant	
	n	%	n	%	n	%
Ciprofloxacin (n=77)	63	81.82	9	11.68	5	6.94
Levofloxacin (n=62)	48	77.42	10	16.12	4	6.45
Enrofloxacin (n=77)	59	76.62	13	16.88	5	6.49
Gentamicin (77)	45	58.44	20	25.97	12	15.58
Tetracycline (n=16)	9	56.25	7	43.75	0	0
Cephalexin (n=58)	15	25.86	16	27.58	27	46.55
Ofloxacin (n=77)	10	12.99	59	76.62	8	10.39
Ampicillin (59)	7	11.86	27	45.76	25	42.37
Oxytetracycline (n=61)	7	11.47	27	44.26	27	44.26
Streptomycin (n=62)	5	8.06	41	66.13	16	25.80
Ceftriaxone (n=55)	2	3.64	25	45.45	28	50.90
Ampicillin (55)	2	3.64	25	45.45	28	50.90
Cloxacillin (n=61)	2	3.27	14	22.95	45	73.77
Penicillin (n=77)	2	2.59	13	16.88	62	80.52
Metronidazole (58)	0	0	2	3.45	56	96.55

++ (Highly sensitive), + (Sensitive)

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