

Isolation and Antimicrobial Susceptibility of Bacteria from Dairy Cows with Clinical Endometritis

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Abstract: Clinical endometritis is inflammation of the endometrial lining of the uterus and is associated with delayed uterine involution and poor fertility. Bacterial pathogens are a potential cause when cows fail to conceive on one or more cycles in the same season. Furthermore, emerging bacterial resistance to commonly used antibiotics has been demonstrated. The aim of this investigation was to evaluate the antimicrobial susceptibility of the isolated bacteria from cows with clinical endometritis. In this study, a total of 24 vaginal discharge swabs from cows with clinical endometritis problems were evaluated and the bacterial isolates were identified and measured for resistance to 8 antibiotics most commonly used during bacterial infection. 91.66% percent of the examined cows were positive for bacteriological investigations. Amongst 22 successful isolations, 25% were *S. pyogenes*, 20.8% *Escherichia coli* and 20.8% *Streptococcus* spp. considered frequently associated with fertility problems. Determination of the antibiotic susceptibility pattern of isolates showed that all isolates of *S. aureus* were resistant to ampicillin, oxacillin and vancomycin. *E. coli* showed resistance to sulphamethaxazole (40%), polymixin (100%), tetracycline (100%), oxacillin (40%), gentamycin (40%) and cefoxitin (100%). The isolates of *A. pyogenes* showed resistance to polymixin (66.66%), tetracycline (66.66%), oxacillin (16.66%), gentamycin (50%) and cefoxitin (16.66%). *Klebsiella* spp. showed resistance to only cefoxitin (100%). This study revealed that the clinical endometritis is mainly due to *A. Pyogenes*, *E. coli* and *Streptococcus* spp. This study revealed that the bacteria *A. pyogenes* followed by *Streptococcus* Spp. and *E. coli* are more commonly isolated in clinical endometritis in cows and the drugs cefoxitin and sulphamethaxazole are highly effective.

Key words: Cows • Clinical Endometritis • Bacteria And Antimicrobial Susceptibilities

INTRODUCTION

Clinical endometritis is a common reproductive disorder in female domestic animals with consequences ranging from no effect on reproductive performance to permanent sterility. It affects the general health of animals and adversely affects their reproductive performance [1]. The presence of bacteria in the uterus causes inflammation, histological lesions of the endometrium and delays uterine involution [2]. In addition, uterine bacterial infection or bacterial products suppress pituitary LH

secretion and perturb postpartum ovarian follicle growth and function, which disrupts ovulation in cattle [3, 4]. Thus, clinical endometritis is associated with lower conception rates, increased intervals from calving to first service or conception and more culls for failure to conceive [5]. The uterine infections often cause clinical endometritis.

After parturition, bacteria from the animal's environment contaminate the uterine lumen of most cattle. Infection persists in the uterus of many animals for more than three weeks, with about 15% of dairy cattle having

signs of clinical endometritis. These animals have lower conception rates, take longer to conceive and are more likely to be culled for infertility than unaffected animals. So, endometritis is an expensive condition for vets and farmers to manage [6].

Most bacteria are eliminated from the uterus during the first three weeks after parturition by the physical contraction of the uterus (involution), regeneration of the endometrium and activation of innate immune defenses leading to phagocytosis of bacteria by neutrophils. However, about 15% of dairy cows have clinical signs of uterine disease that persist beyond three weeks post partum. This is termed clinical endometritis and is characterized by purulent material in the uterus, which is usually detectable in the vagina [7].

Antibiotics are one component often used in the treatment of clinical endometritis. For clinicians there is a need of rapid microbiological diagnosis so that adequate treatment of the infection can be performed [8].

The aim of the present study was to determine the most common bacterial species in uterine samples from dairy cows with infertility problems and the antimicrobial susceptibility of isolated bacteria.

MATERIALS AND METHODS

Animals: The study was carried out in small, medium and large sized farms in and around Gondar, during January 2012 to February 2013. The postpartum dairy cows (n=362) were examined once between 21 and 35 days postpartum. Total 24 cows affected with clinical endometritis were selected. None of the cows received any intrauterine or reproductive hormonal therapy for at least 10 days before sampling for this study.

Vaginal Swab Collection and Bacteriological Culture: Specimens included in this study were collected at and around Gondar dairy farms. A total of 362 cows were enrolled in this study. After restraining the animal and securing its tail, the perineal region was washed with soap and water. Sampling for bacteriological examination was performed immediately after diagnosis of clinical endometritis. Vaginal aspirates were collected about 5-10 milliliters from vaginal fornix using sterilized vaginal speculum and cotton swab sticks. The aspirates was poured in sterile test tubes containing sterile Stuart media as transport media and were kept in icebox and delivered for bacteriological culture within 2 hours and processed in the Unit of Veterinary Microbiology laboratory, Gondar University. In the laboratory, the test tubes were left for

1 hour in the incubator at 37 °C, then each aspirate material was streaked on Blood agar and MacConkey agar plates using sterile bacteriological loop. The plates were incubated at 37°C for overnight. After incubation at 37° C for 24 to 48h bacteria were identified based on colonial morphology, Gram's stained smear, hemolysis and biochemical reactions according to the methods described by [9].

Antimicrobial Susceptibility Testing: The antimicrobial resistance patterns of the isolates were determined using the Kirby Bauer disk diffusion technique [10]. The disks were impregnated with the following antibiotics: tetracycline, ampicillin, oxacillin, polymyxin, sulphamethoxazole, cefoxitin, gentamycin and vancomycin. Isolated colonies of the same morphologic type were inoculated into 5ml of a nutrient broth incubated at 37°C for 5 hours until a visible turbidity appeared. The turbidity was compared to the 0.5 McFarland standards.

Mueller Hinton agar was used as plating medium. 15 minutes after the plates were inoculated, antibiotic impregnated discs were applied to the surface of the inoculated plates with sterile forceps.

All discs were gently pressed down onto the agar with forceps to ensure complete contact with the agar surface; the plates were inverted and then aerobically incubated for 24 hours at 37°C.

The diameters of the zones of complete inhibition were measured to the nearest whole millimeter using zone inhibition scale. Zones of inhibition for individual antimicrobial agents were translated into susceptible and resistant categories by referring the recommended NCCLS interpretative standards.

Retrospective data were compiled on the type of antibiotics used to treat clinical endometritis and other infectious diseases in the area. Specifically the antibiotics used to treat clinical endometritis cases were gathered from Gondar town veterinary clinic records.

RESULTS

In total 24 cows with clinical endometritis were found and sampled at 40-60 days postpartum. Twenty two (91.66%) swabs were found positive bacteriologically which were identified phenotypically based on colony morphology (Table 1) biochemical characteristics. The remaining 02(8.33%) showed no bacterial growth. The bacteria which were isolated are listed in Table 2. A total of 22 isolates were identified from the positive swabs.

Table 1: Colony characteristics of the isolated bacteria

Organism	Colony characteristics
<i>Staphylococcus aureus</i>	Distinctly white colour and lemon yellow colour in blood agar were studied separately.
<i>Streptococcus spp</i>	Small, white, hard drew drop like colonies with hemolysis in blood agar were situated.
<i>Escherichia coli</i>	Red colonies surrounded by a precipitation zone
<i>Actinomycespyogenes</i>	Tiny colonies surrounded by a narrow zone of complete hemolysis gram positive pleomorphic rods.
<i>Klebsiella spp</i>	Typical pink to red coloured colonies were verified as K lebsiella
<i>Campylobacter fetus</i>	Small, round, slightly raised, smooth, translucent colonies with a "dewdrop" appearance.

Table 2: Bacterial isolates from postpartum clinical endometritis of dairy cows

Bacteria isolated	Number of isolates	Percentage (%)
<i>S. aureus</i>	3	12.5
<i>Streptococcus Spp.</i>	5	20.8
<i>Klebsiella Spp.</i>	2	8.3
<i>E. coli</i>	5	20.8
<i>C. fetus</i>	1	4.2
<i>A. Pyogenes</i>	6	25.0
Total	22	91.7

The most frequently isolated bacteria was *A. pyogenes*06 (25%) followed by *Streptococcus Sp.* and *E. coli* (20.8% each), *S. aureus* (12.5%), *Klebsiella Spp.* (8.3%) and *C. fetus* (4.2%).

The result of antimicrobial susceptibility tests showed that the all isolates of *S. aureus* were resistant to ampicillin, oxacillin and vancomycin. The susceptibility pattern of *E. coli* showed resistance to sulphamethaxazole (40%), polymixin (100%), tetracycline (100%), oxacillin (40%), gentamycin (40%) and cefoxitin (100%).

The isolates of *A. pyogenes* showed resistance to polymixin (66.66%), tetracycline (66.66%), oxacillin (16.66%), gentamycin (50%) and cefoxitin (16.66%). *Klebsiella Spp.* showed resistance to only cefoxitin (100%) and susceptible for all the tested antimicrobials. The isolates of *Streptococcus Spp* and *C. fetus* were susceptible for all the tested antimicrobials (Table 3).

DISCUSSION

The uterine lumen is sterile before parturition and if bacterial invasion occurs, there is usually resorption of the fetus or abortion [11]. During or shortly after parturition, microorganisms from the animal's environment, skin and feces contaminate the uterine lumen [12]. The flora of the postpartum uterus has been shown to be quite variable and the results of one sample may not give a full picture of the infection status [9]. However, intrauterine bacterial infection does not even necessarily present as a clinical manifestation of disease; this is dependent on the immune status of the host [12].

Table 3: Antimicrobial susceptibility pattern of bacteria isolated from vaginal discharge of clinical endometritis cow

Bacteria isolated	Total No.	S/R	Antimicrobial agents tested							
			SX	Pb	T	A	Ox	G	Cn	Va
<i>S. aureus</i>	3	S	3(100)	3(100)	3(100)	0	0	3(100)	3(100)	0(0)
		R	0	0	0	3(100)	3(100)	0	0	3(100)
<i>Streptococcus Spp.</i>	5	S	5(100)	5(100)	5(100)	5(100)	5(100)	5(100)	5(100)	5(100)
		R	0	0	0	0	0	0	0	0
<i>Klebsiella Spp.</i>	2	S	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	0	2(100)
		R	0	0	0	0	0	0	2(100)	0
<i>E. coli</i>	5	S	3(60)	0	0	5(100)	3(60)	3(60)	0	5(100)
		R	2(40)	5(100)	5(100)	0	2(40)	2(40)	5(100)	0
<i>C. fetus</i>	1	S	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
		R	0	0	0	0	0	0	0	0
<i>A. pyogenes</i>	6	S	6(100)	2(33.33)	2(33.33)	6(100)	5(83.33)	3(50)	5(83.33)	6(100)
		R	0	4(66.66)	4(66.66)	0	1(16.66)	3(50)	1(16.66)	0

SX=sulphamethaxazole, Pb=polymixin, T= tetracycline, A=ampicillin, Ox=oxacillin, G= gentamycin, Cn= cefoxitin, Va=vancomycin

In the present study 22 (91.66%) swabs were found positive bacteriologically. The most frequently isolated bacteria was *A. pyogenes*, 06 (25%) followed by *Streptococcus* Spp. and *E. coli* (20.8% each), *S. aureus*(12.5%), *Klebsiella* Spp. (8.3%) and *C. fetus* (4.2%). Similarly *E.coli* and *Klebsiella* spp, *Staphylococcus* spp, *Bacillus*spp, *Pseudomonas* spp, *Proteus* spp and *Streptococcus* spp are more commonly isolated in endometritis in cows [13-17].

According to Sheldon *et al.* [18] and Williams *et al.* [19] the bacteria isolated belonged to obligate uterine pathogens (*A. pyogenes*, *E. coli*), potential uterine pathogens (non *hemolytic* *Streptococci*) and opportunistic bacteria (*Klebsiella* spp., *Proteus* spp. and *coagulase-negative staphylococci*).

Escherichia coli isolates were the most susceptible to norfloxacin (100%), marbofloxacin (100%), rifaximin (97%), gentamycin (96%) and amoxicillin/clavulanic acid (95.5%). Other Gram-negative bacteria were the most sensitive to norfloxacin (100%), neomycin (100%) and cefoperazon (95%). Rifaximin (86.7%) and oxytetracycline (80.9%) were also active against these bacteria. According to other scientific papers, *E. coli* strains from the uterus were the most sensitive to chloramphenicol, enrofloxacin, gentamycin and polymixin B [20], as well as to cefquinome and enrofloxacin [18] or ceftiofur [21] and resistant to oxytetracycline, enrofloxacin, lincomycin/spactinomycin and ampicillin [22].

It was found that out of 4 aerobic bacteria *Bacillus* spp. and *Escherichia coli* are more or less effective against 7 antibiotic agents [23]. On the other hand, amoxicillin, oxytetracycline and ciprofloxacin are moderate to highly sensitive to the all of isolates [24]. In contrast, penicillin and sulphamethoxazole are only sensitive to the Gram positive and Gram negative bacteria, respectively. Similar, observations were recorded earlier by [25]. Antibiotic sensitivity test was performed only in aerobic condition.

Arcanobacterium pyogenes and *E. coli* with many others like *Staphylococcus* spp., *Streptococcus* spp. and *Proteus* spp. are frequently isolated from cows with retained placenta and cows with clinical endometritis[26]. Our results indicated that *A. pyogenes* and *E. coli* were mainly present in diseased postpartum uteri. *Escherichia coli* could penetrate the epithelial layer of endometrium and colonize the subepithelial region of endometrium. It could be suggested that bacterial contamination is mainly *E. coli* in uterus after parturition as shown by the high prevalence of *E. coli* in cows in the control group. The *E. coli* penetration and colonization in the

endometrium might favor the development of uterine infection by other highly pathogenic organisms such as *A. pyogenes* and *F. necrophorum*[27].

CONCLUSION

This study revealed that the bacteria *A. pyogenes* followed by *Streptococcus* Spp. and *E. coli* are more commonly isolated in clinical endometritis in cows and the drugs cefoxitin and sulphamethaxazole are highly effective.

Recommendations: Clinical endometritis is the most common cause of infertility in cows and make an important contribution to economic losses in the dairy industry as they delay uterine involution, prolong the time until first oestrus, increase the number of services per conception and consequently prolong the inter calving interval. Therefore, the availability of these data is also very important to set-up strategies that can assist in preventing and controlling clinical endometritis, by and large in improving veterinary services of the studied area.

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