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Antimicrobial resistance of aerobic bacteria isolated from the inflamed uterus of cows

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Summary

The aim of this study was to determine the in vitro resistance of aerobic bacteria isolated from the uterine pathological secretion of 312 dairy cows with clinical metritis and clinical endometritis to antibiotics. Animals with pathological discharges from the vagina observable between the 7th and 50th day after parturition were diagnosed clinically per vaginam and per rectum and then swabs from uteri lumen were aseptically collected. Bacteriological examinations of swabs were performed according to commonly accepted rules. Sensitivity to antibiotics was tested by the disk diffusion method and performed according to CLSI (formerly NCCLS) guidelines in Mueller-Hinton agar. The bacteria isolated were mostly Arcanobacterium pyogenes, Escherichia coli, non E. coli Gram-negative rods, Streptococcus species and Staphylococcus species. Strains of Arc. pyogenes were the most susceptible to amoxicillin/clavulanic acid (97.3%), ceftiofur (98%) and bacitracine (96.7%). E. 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%). Streptococcus species were the most susceptible to amoxicillin/clavulanic acid (94.6%), ampicillin (92.3%), norfloxacin (92%), cephapirine (88%), cefoperazone (86.5%), rifaximine (85.7%) and penicillin (84.9%). The highest in vitro activity against Staphylococcus spp. was demonstrated by amoxicillin/clavulanic acid (100%), norfloxacin (100%), neomycin (93.6%) and cefoperazone (85.7%). Arc. pyogenes were the most resistant to oxytetracycline and cloxacillin, E. coli to ampicillin and cephapirin, non-E. coli Gram-negative rods to ampicillin and cephapirin, Streptococcus spp. to neomycin and oxytetracycline, and Staphylococcus spp. to ampicillin.

Keywords: cow, metritis/endometritis, aerobic bacteria, antibiotic sensitivity

Different forms of *metritis/endometritis* have been noted in 14-50 or more per cent of cows (2, 13, 14, 34). The inflammations cause economic losses due to a longer calving interval, costs of extra services and treatment and increased culling rate (8, 13, 17, 18, 23, 25). Sheldon et al. (29) proposed to define postpartum inflammations of the uterus as puerperal metritis, clinical metritis, clinical endometritis and subclinical endometritis. Puerperal metritis (abnormally enlarged uterus, fetid watery red-brown uterine discharge, signs of toxemia, fever > 39.5°C) or clinical metritis (enlarged uterus, purulent discharge in the vagina, no systemic signs) should be diagnosed if it occurs within 21 days postpartum. Clinical endometritis is characterized by the presence of purulent (> 50% pus) uterine discharge detectable in the vagina 21 days or more after parturition, or mucopurulent (pus and mucus) discharge detectable in the vagina after 26 days post partum. Subclinical endometritis (no detectable clinical changes in the uterus) should be diagnosed depending on the percentage of neutrophils in uterine cytology samples (> 18% in days 21-33 or > 10% in days 34-47 post partum).

Factors that predispose cows to develop inflammations of the uterus are dystocia, retained fetal membranes, twins or stillbirths (2-4, 22), abortions (21) and metabolic disorders (26). These conditions facilitate infections of the genital ways with aerobes and anaerobes. *Arcanobacterium pyogenes*, *Prevotella spp.* (formerly *Bacteroides spp.*), *Fusobacterium necrophorum* and *Escherichia coli* are major uterine pathogens (1, 19, 20, 35). Other bacteria, such as *Staphylococcus spp.*, *Streptococcus spp.* or non *E. coli* aerobic Gram-negative rods, have also been isolated as additional flora in connection with major uterine pathogens (15, 16, 32). *Arcanobacterium pyogenes* cooperates with

aerobic bacteria (*E. coli*) and/or with Gram-negative obligate anaerobes such as *F. necrophorum* and *Prevotella spp*. (19, 31). Details on the etiology and pathogenesis of *metritis/endometritis* are well reviewed and described by a number of authors (11, 22, 24, 28, 29). Immunological aspects of the development of uterus inflammations have recently been described by Singh et all. (33). However, the antibiotic sensitivity of bacteria from an inflamed uterus of cows has seldom been tested.

The aim of the study was to determine the *in vitro* resistance of aerobic bacteria isolated from the uterine pathological secretion of dairy cows suffering from clinical *metritis* and clinical *endometritis* to antibiotics.

Material and methods

Cows showing pathological discharges from the vagina between the 7th and 50th day after parturition were diagnosed clinically *per vaginam* and *per rectum* and then swabs for bacteriological examinations were collected by the authors of this study. The diagnosis was made according to Sheldon et al. (29). The swabs were collected aseptically from the lumen of the uterus of 312 cows that showed puerperal metritis (7 cows), clinical metritis (106 cows), and clinical endometritis (199 cows). The material was collected by means of transcervical, sterile, disposable catheters (Equivet uterine culture swab cat. No 290055 Jorgen Kruuse A/S Denmark). The swabs were inserted into vials containing a commercial transport medium, cooled and transported to the laboratory of the National Veterinary Research Institute. Bacteriological examinations of the collected material were performed according to commonly accepted rules as described previously (16).

The antimicrobial sensitivity of *Arc. pyogenes*, *E. coli*, non *E. coli* Gram-negative rods, *Streptococcus spp.* and *Staphylococcus spp.* to different antibiotics was tested by the disk diffusion method and performed according to CLSI guidelines in Mueller-Hinton agar. The following antibacterial agents (Oxoid) were used: penicillin (P; 10 i.u), amoxicillin with clavulanic acid (Amc; 30 μg), ampicillin (Amp; 10 μg), cloxacillin (Ob; 5 μg), cefoperazone (Cfp; 30 μg), cephapirin (Cpr; 30 μg), Ceftiofur (Eft; 30 μg), streptomycin (S; 10 μg), neomycin (N; 30 μg), gentamycin (Cn; 10 μg), oxytetracycline (Ot; 30 μg) and rifaximin (Rax; 40 μg), lincomycin (My; 15 μg), bacitracin (B; 10 μg), norfloxacin (Nor; 10 μg) and marbofloxacin (Mar; 10 μg). *Staphylococcus aureus* ATCC 25 923 and *Escherichia coli* ATCC 25 922 were the control strains. Interpretation of the test results: sensitive (S), intermediate sensitive (I) and resistant (R) were based on CLSI criteria.

Results and discussion

Different species of aerobic bacteria were isolated from the uterine swabs of 112 cows diagnosed with puerperal/clinical *metritis* and 179 cows with clinical *endometritis*. Bacteriological examinations of swabs from 1 cow with clinical metritis and from 20 cows with clinical endometritis were negative. *Arcanabacterium pyogenes* in pure culture or in combination with other bacteria species (*E. coli*, *Streptococcus spp*., *Staphylococcus spp*., non E. coli Gram-negative rods) was the most frequently isolated, both from *metritis* and *endometritis* cases. Another group consisted of *Escherichia coli* and other Gram-negative aerobic bacteria, such as *Proteus spp*., *Klebsiella spp*., *Kluyvera spp*., *Pantoea spp*., *Enterobacter spp*. and *Pseudomonas spp*. *Escherichia coli*, as the only aerobic bacteria found in the material taken from the uteri of 17 cows (15%) with clinical *metritis* before the 21st day post partum. *Streptococcus* or *Staphylococcus* and other bacteria species were usually accompanied by *A. pyogenes* and *E. coli.*

According to Sheldon et al. (27) and Williams et al. (34) the bacteria isolated belonged to obligate uterine pathogens (*A. pyogenes*, *E. coli*), potential uterine pathogens (non-haemolytic streptococci) and opportunistic bacteria (*Klebsiella spp., Proteus spp*. and coagulase-negative staphylococci). Königsson et al. (19) reported that *Escherichia coli*, alpha-haemolytic streptococci, *Fusobacterium necrophorum*, *Arcanobacterium pyogenes*, *Bacteroides spp.*, *Pasteurella spp.*, *and Proteus spp.* were predominant bacteria in cows with endometritis resulting from retained fetal membranes. Our results were close to the above reports in the cases of aerobic bacteria, and agreed with other authors (10, 16, 35). The presence of *E. coli* and LPS in *lochia* early postpartum favor the development of uterine infections by *A. pyogenes* and Gram-negative anaerobes in later phase (6).

Results of bacteriological examinations of the material taken from the uteri of cows with clinical metritis/endometritis were negative in 10.7% of cases. It can be partially explained by the conditions of our bacteriological examinations, which were aerobic only. However, other authors, who examined uterine swabs both in aerobic and anaerobic conditions, also noted lack of bacteria in some cases of endometritis (5, 35). It is possible that such cases were probably caused by a viral infection (12).

The resistance of the most frequently isolated aerobic bacteria to antibiotics is presented in Table 1. *Arcanobacterium pyogenes* was the most susceptible to amoxicillin/clavulanic acid (97.3%), ceftiofur (98%) and bacitracine (96.7%). Less active in vitro were: cefoperazone (20.3% of resistant strains), neomycin (25.3%), lincomycin (22.8%) and norfloxacin (24.3%). Other authors (32) found that, *in vitro*, *A. pygenes* was the most sensitive to ampicillin, enrofloxacin, cephalotin, lincomycin, neomycin, penicillin, novobiocin, gentamycin, chloramphenicol and tertracycline. Sheldon et al. (28) reported the highest activity of cefalosporins (cefquinome, cephapirin, ceftiofur) and enrofloxacin against *A. pyogenes*. However, Farca et al. (10) stated that this species was the most resistant to oxytetracycline, enrofloxacin, lincomycin/spactinomycin and amicacin.

Tab. 1. The resistance (%) of aerobic bacteria isolated from the uterus of cows to antibiotics

Antibiotics	Species									
	Arc. pyogenes		*E. coli*		Other Gram-negative		*Streptococcus*		*Staphylococcus*	
	n	%	n	%	n	%	n	%	n	%
P	110	31.8	–	–	–	–	73	15.1	31	51.8
Amc	111	2.7	35	4.5	21	28.6	74	5.4	31	0
Amp	82	36.6	35	62.9	16	62.5	52	7.7	8	75.0
Ob	108	47.2	–	–	–	–	65	32.3	18	22.2
Cpr	61	37.7	14	50.0	7	42.8	50	12.0	29	20.5
Cfp	89	20.3	59	18.6	20	5.0	59	13.5	28	14.3
Eft	22	0	22	22.7	–	–	6	33.3	–	–
S	–	–	30	33.3	–	–	–	–	–	–
N	111	25.3	67	14.9	21	0	74	67.5	31	6.4
Cn	–	–	25	4.0	–	–	–	–	–	–
Ot	111	64.0	67	28.3	21	19.1	73	48.0	30	23.3
Rax	60	36.6	33	3.0	15	13.3	21	14.3	4	25.0
My	22	22.8	–	–	–	–	–	–	–	–
B	30	3.3	–	–	–	–	–	–	21	0
Nor	66	24.3	34	0	16	0	25	8.0	–	–
Mar	–	–	10	0	14	21.4	–	–	–	–

Explanation: – not examined

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 (32), as well as to cefquinome and enrofloxacin (27) or ceftiofur (7) and resistant to oxytetracycline, enrofloxacin, lincomycin/spactinomycin and amicacin (10).

Streptococcus species were the most susceptible to amoxicillin/clavulanic acid (94.6%), ampicillin (92.3%), norfloxacin (92%), cephapirine (88%), cefoperazone (86.5%), rifaximine (85.7%) and penicillin (84.9%). The highest in vitro activity against *Staphylococcus spp*. was demonstrated by amoxicillin/clavulanic acid (100%), norfloxacin (100%), neomycin (93.6%) and cefoperazone (85.7%). Less active were cephapirin (79.3%) or cloxacillin (77.8%) and rifaximin (75%). Farca et al. (10) stated that *Streptococcus* and *Staphylococcus* isolated from uterine swabs were resistant to oxytetracycline, enrofloxacin, lincomycin/spectinomycin and amicacin. Other authors found that gentamycin, enrofloxacin, cephalotin and chloramphenicol were the most active against these species (32).

It should be emphasized that *A. pyogenes* was the most resistant to oxytetracycline and cloxacillin, *E. coli* to ampicillin and cephapirin, non-E. coli Gram-negative rods to ampicillin and cephapirin, *Streptococcus spp*. to neomycin and oxytetracycline, and *Staphylococcus spp.* to ampicillin. On the other hand the examined aerobic bacteria were the most sensitive to amoxicillin/clavulanic acid, ceftiofur and to norfloxacine. However, apart from the above presented data, the knowledge on the susceptibility of anaerobic bacteria to antibiotics also has a great significance for the selection of drugs to treat metritis and endometritis. Sheldon et al. (27) found that cefalosporins (cefquinome, cephapirin, cephapirin/mecillinam, ceftiofur) had low MIC values for anaerobic bacteria: *Fusobacterium necrophorum* and *Prevotella melaninogenicus*. Cohen et al. (5) reported high susceptibility of *Bacteroides spp.* to clindamycin and metronizadole, and Silva and Lobato (32) demonstrated highest sensitivity of this species to chloramphenicol, lincomycin and tetracycline.

Literature reports that amoxicilin/clavulanic acid is also highly active against anaerobic bacteria such as *Bacteroides spp*., *Prevotella spp*., and *Fusobacterium spp*. (9).

In conclusion, amoxicillin/clavulanic acid is one of the most active *in vitro* against aerobic bacteria isolated from inflamed uteri of cows and therefore could be more frequently applied in the treatment of clinical metritis/endometritis in cows apart from cefalosporins,

oxytetracycline or rifaximin. However, the usefulness of quinolones could also be considered.

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