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Screening Conjunctival Bacterial Flora and Antibiogram Tests in Cattle

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Abstract: This field survey was conducted to monitor microbial flora in conjunctiva and compare antibiogram test. Conjunctival swabs from both eyes of 365 cattle that were in different breeds, ages and sexes were sampled. Antibiogram tests were performed using the disc diffusion method. Microorganisms were not isolated from 402 of total 730 conjunctival samples (55%), whereas they were isolated from 328 conjunctival samples (45%). Of these, 352 samples were contaminated with bacteria (99.4%) and 2 samples were contaminated with fungi (0.6%). The sensitivity of bacteria to different antibiotics was different. Isolated *Staphylococcus sp*. were highly resistant to antibiotics. Danofloxacin, gentamicin and kanamycin were sensitive whereas amoxicillin/clavulanic acid and ampicillin/sulbactam were moderately sensitive. Most bacteria were resistant to penicillin G and cefaperazon.

Key words: Conjunctiva, bacteria, antibiogram, screening

INTRODUCTION

Conjunctivitis is the inflammation of bulber or palpebral conjunctiva[1]. It is characterized with erithem, hyperemia, chemozis, leukocyte infiltration, folliculitis, blepharospazm in eyelid, photophobia and tears in different composition[2-4]. Conjunctivitis can be broadly categorized according to duration of inflammation, composition of tear and predisposition and etiological factors. Regardless of category of conjunctivitis, ethologic diagnosis is the basic of the efficient treatment[3].

Conjunctival swab or scraping or biopsy techniques are used for diagnosis of microbial conjunctiviti[3]. Following isolation and identification of microorganism, antibiogram results help us to chose the most effective antibiotics. As a replacement therapy, antiseptics, astringents and vasoactive agents can be administered as well[2-4]. This study was designed to screen the conjunctival bacteria flora and evaluate antibiogram responses in cattle.

MATERIALS AND METHODS

Animal and sample collection: The study material was 365 cattle: 0-6 months old 24 calves (1 crossbred female, 16 Brown Swiss (9 male and 7 female) and 7 Holsteins (3 female and 4 male)], 6-12 months old 91 calves [4 crossbred (1 female and 3 male), 50 Brown Swiss (23 female and 27 male) and 37 Holsteins (17 female and 20 male)], 12-24 months old 49 heifers [3 crossbred, 21 Brown Swiss, 22 Holsteins and 3 Simmental], 12-24 months old 23 steers [2 crossbred, 10 brown Swiss, 10 Holsteins and 1 Simmental], 24 months or older 172 cows [6 crossbred, 109 brown Swiss and 57 Holsteins) and 6 bulls [3 brown Swiss, 2 Holstein and 1 Simmental].

Sterile cotton swabs were used to obtain scrapping from both eyes of 365 cattle. The scrapping were placed into the Brain Heart Infusion Buyyon (BHIB) tubes and covered with corkscrew for storage at 4°C. The samples were incubated under the optimal conditions in order to isolate and identify causing pathogens.

Isolation and identification: In order to determine the microorganisms from the scrapping, general and selective agars such as Blood Agar Base, Chocolate Agar, MacConkey Agar, EMB Agar, Saboraud Dextrose Agar (SDA) added with %5 sheep blood were used. All agars were incubated for 24-72 h at 37°C, under aerobic and microaerophilic conditions. Colonies were then subjected to classic methods including gram coloring, colony morphology and biochemical tests for identification. Biochemical efficiency was measured using oxidase, catalase, urease, tube coagulase, nitrate reduction, indol and citrate tests.

Antibiogram test and antibiotic discs: Amoxicillin/clavulanic acid (20/10 μg), ampicillin/sulbactam (10/10 μg), gentamicin (10 μg), kanamicin (10 μg), danofloxacin (5 μg), penicillin G (10 μg),

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oxytetracyline (30 μg) and cefoperazon (30 μg) provided by Becton-Dickinson and Bioanalyse companies were used in order to determine the sensitivity of the isolated and identified microorganisms to antibiotics.

Antibiotic resistance of the isolated and identified bacteria was assessed using the disc agar diffusion test according to NCCLS standards as described by Kirby-Bauer[5-6]. To do so, selected colonies were placed into tubes containing Triptic Soy Broth. The tubes were incubated for 5-6 h at 37°C. Following incubation, with standard # 5 of Mc Farland, colony forming unit was determined if it was equal to $1x10^8$ per milliliter.

Colonies were sprinkled to the surface area of Mueller Hinton Agar adjusted suspensions blurriness by sterile swabs. Standard antibiotic discs were accommodated in 120x120 mm petri discs at 1 cm distance from edges and 3-4 cm away from each other. The agars were placed into sterlizators for incubation for 18-24 h at 37°C. After the incubation, the zone diameters surrounding the discs were measured. The zone diameters were evaluated by comparing the values (M2 A4) in NCCLS[6].

RESULTS

Different isolated and identified microorganisms from both eyes of 365 cattle are listed in Table 1. Of samples, microorganisms were isolated from 354 samples, which were as follow: *Streptococcus* sp., 86 (24.2%); *Staphylococcus* sp., 54 (15.2%); *Moraxella* sp., 49 (14%); *Escherichia coli*, 40 (11.3%); *Neisseria* sp., 35 (10%); *Bacillus* sp., 26 (7.3%); *Corynebacterium* sp., 21 (6%); *Pseudomonas* sp., 17(5%); *Micrococcus* sp., 8 (2.2%); *Klebsiella pneumoniae*, 7 (2%); *Haemophilus* sp., 2 (0.5%); *Acinetobacter* sp., 2 (0.5%); *Proteus vulgaris*, 1 (0.2%); and *Candida* sp., 2 (0.5%). It was also found that 12 samples infected by *S. aureus* were coagulase positive, whereas 42 samples infected by *S. aureus* were coagulase negative. Among samples infected by *Streptococcus* sp., 42 were 42 α-hemolytic streptococci, 19 were β-hemolytic streptococci and 25 were non-hemolytic streptococci.

Table 1: The distribution of the isolated and identified microorganisms.

Microorganis	The number of microorganism	Isolationrate(%)
Staphylococcus sp.	54	15.2
S. aureus	12	(22.2)
KNS	42	(77.8)
Streptococcus sp.	86	24.2
alpha hem streptococci	42	(48.8)
beta hem streptococci	19	(22.1)
non hem streptococci	25	(29.1)
Micrococcus sp.	8	2.2
Corynebacterium sp.	21	6.0
Bacillus sp.	26	7.3
Moraxella sp.	49	14.0
Haemophilus sp.	2	0.5
Acinetobacter sp.	2	0.5
Neisseria sp.	35	10.0
Escherichia coli	40	11.3
Pseudomonas sp.	17	5.0
Proteus vulgaris	1	0.2
Klebsiella pneumoniae	7	2.0
Enterobacter sp.	4	1.1
Candida sp.	2	0.5
Total	354	100.0

Table 2 summarizes the antibiotic sensitivity of isolated and identified gram-positive bacteria. There were differences in sensitivity and resistance to different antibiotics. Especially, *Staphylococcus* sp. was resistant to most of antibiotics. Moreover, penicillin G and cefaperazon were the least sensitive antibiotics.

Table 2: Antibiotic sensitivity test for the isolated and identified gram-positive bacteria

Antibiotics	Amx/Clv		Gentam		Pen. G		Amp/Slb		Oxytet		Danoflox		Cefoper		Kanam	
Microorganism	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Staphylococcus sp. (54)	32	22	37	17	5	49	30	24	13	41	42	12	29	25	31	23
Streptococcus sp. (86)	78	8	81	5	83	3	72	14	85	1	86	0	60	26	84	2
Micrococcus sp. (8)	8	0	8	0	7	1	8	0	8	0	8	0	5	3	6	2
Corynebacterium sp. (21)	21	0	19	2	21	0	21	0	11	10	21	0	4	17	13	8
Bacillus sp. (26)	24	2	20	6	26	0	26	0	6	20	26	0	14	12	26	0
Moraxella sp. (49)	43	6	47	2	23	26	41	8	45	4	49	0	28	21	47	2
Haemophilus sp. (2)	2	0	2	0	0	2	2	0	2	0	2	0	0	2	2	0
Acinetobacter sp. (2)	2	0	2	0	0	2	2	0	2	0	2	0	2	0	2	0
Neisseria sp. (35)	33	2	35	0	20	15	35	0	27	8	35	0	16	19	35	0
Escherichia coli (40)	36	4	38	2	0	40	32	8	16	24	33	7	25	15	37	3
Pseudomonas sp. (17)	10	7	15	2	0	17	6	11	3	14	15	2	17	0	12	5
Proteus vulgaris (1)	1	0	1	0	0	1	1	0	0	1	1	0	1	0	0	1
Klebsiella pneumoniae (7)	5	2	7	0	0	7	7	0	1	6	7	0	7	0	5	2
Enterobacter sp. (4)	4	0	4	0	0	4	4	0	2	2	4	0	3	1	4	0

[1]Amx/Clv = Amoxicillin/Clavulanic acid (20/10 μg); Gentam Gentamicin (10 μg); Pen. G Penicillin G (10 μg); Amp/Slb=Ampicillin/Sulbactam (10/10 μg); OxytetOxytetracyline (30 μg); Danoflox=Danofloxacin (5 μg); Cefoper =Cefoperazon (30 μg); Kana=kanamicin (10 μg). S=sensitive; R=Resistant

[2]The numbers in parantheses indicate the samples from which bacteria were isolated

DISCUSSION

Determination of the locally effective antibiotics and the incidences of the pathogenic microorganisms bases for administering ocular antibiotics[3]. Because the isolation and identification of the causing agent may require a longer time and could be expensive, large spectrum antibiotics are commonly applied in ocular treatment. However, many studies concluded that use of effective antibiotics after antibiogram for isolation and identification of causing microorganisms increases likelihood of eradication of problem. Thus, empirical treatment may not result in effective cure[3].

Simple extraoculer infections are generally responsive to the antibiotic administration. However, it is necessary to make antibiogram tests or to determine the pathogen bacteria in severe infections and cases attempted to be cured by empirical method[3].

The limited number of studies is available for herd surveillance with respect to conjunctival flora and effective antibiotic to use against infectious bovine keratoconjunctivitis. Thus, recent surveys focus on screening and consequently herd-basis approach. Sarma *et al*[7]. isolated mostly *Streptococcus* sp. and lesser extent, *Pasteurella haemolytica*, *Corynebacterium bovis*, *Micrococcus* sp. and *Candida* sp. from conjunctival flora 27 cows in England. In this study, chloramphenicol was reported to be the most effective antibiotic. Turnes and Albuquerque[8] conducted a similar study in France and isolated *Moraxella bovis* (82.3%) from conjunctival flora of 16 cows with infectious bovine keratoconjunctivitis. In another research, Dietz *et al*[9].isolated chalamydia sp. 103 (66%) among 156 cows' conjunctival samples and lesser extent, *Moraxella bovis*, *Neisseria catarrhalis*, *Corynebacterium bovis* and *Clamida* sp. In a large-sample epidemiological study conducted in Australia[3], it was shown that gram-positive bacteria predominated (54.4%). Other bacteria were *Corynebacterium* sp. (27.4%), *Moraxella nonliquefaciens* (26.9%), *Neisseria catarrhalis* (10.5%), *Acinetobacter spp.* (8.0), *Moraxella bovis* (6.5%), *Coliform* sp. (6.5%) and *Bacillus* sp. (1.3%).

In present study determined a high ratio of gram-positive bacteria (*streptococci* and *staphylococci*), but not mycoplasma and anaerobic bacteria from the conjunctival swab samples. On the basis of the antibiogram test, it was found that isolated Staphylococcus spp. were highly resistant. Danofloxacin, gentamicin and kanamycin were sensitive whereas amoxicillin/clavulanic acid and ampicillin/sulbactam were moderately sensitive. Most bacteria were resistant to penicillin G and cefaperazon.

CONCLUSIONS

Bacteria were highly variable and had great virulence. Antibiogram results also ascertained the necessity of selecting effective antibiotics. Thus, isolation, identification and determination of virulence of bacteria were crucial for selection of proper antibiotics. Moreover, localization, pharmacological, pharmaceutical and toxicological effects of microorganisms should be assessed for effective ocular antibiotherapy.

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