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Antimicrobial Susceptibility of *Staphylococci* Isolated from Clinical Mastitis in Dairy Cows in Two Regions of Kosovo

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Abstract

This study was undertaken to evaluate antibiotic susceptibility of 26 strains of Coagulase Negative *Staphylococci* (CNS) and 18 strains of *Staphylococcus aureus* isolated from samples of dairy cows with clinical mastitis. The isolates species of staphylococci were identified by using coagulase test (using rabbit plasma) and API Staph system (bioMėrieux SA) which differentiates *S. aurues* from other staphylococci. Isolates were tested for antimicrobial susceptibility on Mueller Hinton agar by dick diffusion method according to the Clinical Laboratory Standards Institute (CLSI/NCCLS). The findings of this study showed that the most effective in vitro drugs for *S. aureus* strains isolated from cows with clinical mastitis were amoxycillin/clavulanic acid (83.3%), tetracycline (77.8%), gentamycin (77.8%) and trimethoprim (66.7%) and for CNS strains trimethoprim (88.5%), amoxicillin-clavulanic acid (84.6%), tetracycline (77%) and gentamicin (69.2%) while the isolates of *S. aureus* and CNS were found to be more resistant to penicillin (55.5% and 53.8%, respectively), streptomycin (50% and 46.1%, respectively) and ampicillin (38.9% and 46.1%, respectively).

Keywords: Antimicrobial susceptibility, staphylococci, clinical mastitis.

1. Introduction

Bovine mastitis is an inflammation of the udder accompanied by physical, chemical and bacteriological changes in milk. Generally, mastitis occurs in two forms which includes clinical and subclinical. In the clinical mastitis all the five cardinal signs of udder inflammation (redness, heat, swelling, pain and loss of milk production) are present, while the sub-clinical form is bereft of any obvious manifestation of inflammation. It is one of the main reasons for economic loss in dairy farming due to the decreased in milk production, spends with medications and medical care, disposal of contaminated milk after treatment and early discarding of sick animals [3]. Clinical mastitis is mostly caused by bacteria. Coagulase-positive *Staphylococcus aureus* is considered a major cause of bovine mastitis. Coagulase Negative Staphylococci (CNS) and *Corynebacterium bovis*, two other highly prevalent pathogens, are historically considered to be of limited importance and are therefore often described as minor pathogens. The impact of CNS is increasing [9], probably because prevalence of major pathogens are decreasing [10]. Mastitis is one of the

major causes of antibiotic use in dairy cows [6]. Approximately 70% of the antimicrobials used in dairy production are for treatment of clinical mastitis [12], but the cure rates for clinical mastitis are not always satisfactory. The efficacy of bovine mastitis treatment depends on the cause, clinical manifestation, antibiotic susceptibility of etiological agent and the efficiency of immunological system. The abusive or incorrect use of antimicrobials has been implicated as the major selective force for the development of resistance [5].

In Kosovo, has no data about the sensitivity and bacterial resistance to antibiotics in clinical mastitis cases, so this study is the first of its kind with a major importance. The purpose of this study was to evaluate the antimicrobial susceptibility profile of strains of *Staphylococcus aureus* and CNS of milk samples from cows with clinical mastitis in two regions in Kosovo.

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2. Material and Methods

From the total of 48 milk samples, 26 strains of coagulase negative *staphylococci* and 18 strains of *Staphylococcus aureus* were obtained from a study in different dairy cows during five months (January - May 2015) in two regions of Kosovo. Quarter milk (inflamed secretion) samples from cows with clinical forms of mastitis were collected aseptically. The teats were cleaned and dipped in a disinfectant and then teat ends were wiped with alcohol swabs and allowed to dry. The first few streams were discarded and then 2-4 of the secretion was collected into sterile tubes. The samples were transported to the laboratory in ice box and processed immediately. Milk samples (0.05ml) were inoculated onto Blood agar (Oxoid, UK) and cultivated at 36 ºC for 24 hours. Suspect colonies were tested for coagulase reaction (which differentiates staphylococci isolates in coagulase positive and coagulase negative staphylococci) and were isolated on Mannitol salt agar, cultivated at 36 ºC for 24 h and identified biochemically using API Staph system (bioMèrieux SA). Isolates were tested for antimicrobial susceptibility on Mueller Hinton agar (HIMEDIA) by dick diffusion method according to the Clinical Laboratory Standards Institute [2]. The following antibiotic disks were tested: penicillin P (10 IU, Oxoid), ampicillin A (10 µg), amoxicillin-clavulanic acid AMC (30 µg, Oxoid), oxytetracycline T (30 µg, BD BBL^{TM}), streptomycin S (10 µg, Liofilchem), cloxacillin CX (5 µg, Liofilchem), gentamicin CN (10 µg, Liofilchem), trimethoprim TM (2.5µg, Liofilchem) and polymyxin B (300 IU, BD BBL^{TM}). During this study we collected data from local veterinarians on antibiotics frequently used for the treatment of clinical mastitis in cows. Antimicrobials such as penicillin-streptomycin (PENSTREP 20/20 administered by parenteral routes), oxytetracycline (ALAMYCIN 100 mg/ml, Limoxin 100, TOPOXY 10, administered by parenteral routes), procain benzylpenicillin-streptomycin sulphate-neomycin sulphate (Mastiquick 5g intramammary injector), colistin sulphate-metampicillin sodium-cloxacillin sodium (Mastidian forte 10 ml intramammary injector) were used most often in cases of clinical mastitis in lactating cows in these regions and very rare sulfadiazine-trimethoprim (Norodine 24 administered by parenteral routes).

The plates were incubated at 35 ºC for 24 hours. The zone of inhibition around each disc was measured and the interpretation was made as per the zone size interpretation chart provided by the disc manufacturer.

2. Results and Discussion

The tested isolates of *Staphylococcus aureus* and CNS within the period of five months and the results for susceptibility testing of antimicrobial agents are presented in Table 1 and 2. In this study strains of *Staphylococcus aureus* were found to be highly sensitive to amoxicillin (83.3%), followed by tetracycline (77.8%), gentamicin (77.8%) and trimethoprim (66.7%). However, these isolates were resistant to penicillin (55.5%), streptomycin (50%), followed by ampicillin (38.9%), polymyxin B (33.3%) and cloxacillin (27.8%). None *S. aureus* isolate was resistant to amoxicillin/clavulanic acid.

Coagulase-negative staphylococci were sensitive mostly to trimethoprim (88.5%) amoxicillin-clavulanic acid (84.6%), followed by tetracycline (77%), gentamicin (69.2%), cloxacillin (42.3%) and polymyxin B (42.3%). CNS strains were more resistant to penicillin (53.8%), streptomycin (46.1%), ampicillin (46.1%), followed by cloxacillin (38.5%), polymyxin B (30.8%). None CNS isolate was resistant to trimethoprim and amoxicillin-clavulanic acid (Table 2).

Table 1. Antibiotic sensitivity of *Staphylococcus aureus* strains isolated from clinical cases of mastitis

		S		I		R		
No.	**Antibiotic**	No.	%	No.	%	No.	%	**Total**
1	Amoxicillin-clavulanic acid	15	83.3	3	16.7	-	-	18
2	Tetracycline	14	77.8	2	11.1	2	11.1	18
3	Cloxacillin	9	50	4	22.2	5	27.8	18
4	Gentamicin	14	77.8	3	16.7	1	5.5	18
5	Trimethoprim	12	66.7	4	22.2	2	11.1	18
6	Penicillin	5	27.8	3	16.7	10	55.5	18
7	Streptomycin	7	38.9	2	11.1	9	50	18
8	Ampicillin	6	33.3	5	27.8	7	38.9	18
9	Polymyxin B	7	38.9	5	27.8	6	33.3	18

Table 2. Antibiotic sensitivity of CNS strains isolated from clinical cases of mastitis

		S		I		R		
No.	**Antibiotic**	No.	%	No.	%	No.	%	**Total**
1	Amoxicillin-clavulanic acid	22	84.6	4	15.4	-	-	26
2	Tetracycline	20	77	5	19.2	1	3.8	26
3	Cloxacillin	11	42.3	5	19.2	10	38.5	26
4	Gentamicin	18	69.2	6	23.1	2	7.7	26
5	Trimethoprim	23	88.5	3	11.5	-	-	26
6	Penicillin	6	23.1	6	23.1	14	53.8	26
7	Streptomycin	10	38.5	4	15.4	12	46.1	26
8	Ampicillin	8	30.8	6	23.1	12	46.1	26
9	Polymyxin B	11	42.3	7	26.9	8	30.8	26

The antimicrobial susceptibility test carried out in present study indicated the existence of susceptibility and resistance of *Staphylococcus aureus* and CNS strains to some of the antimicrobials. The majority of authors have noted the increase in the resistance to antibiotics of bacteria, mostly staphylococci, isolated from mastitis. Since penicillin has been extensively used along with streptomycin for treating clinical mastitis, it may led to the development of high resistance in *S. aureus* and CNS against these antibiotics. In Finland, the proportion of *S. aureus* strains resistant to at least one antimicrobial drug increased from 36.9% in 1988 to 63.6% in 1995, and that of CNS from 26.6% to 49.7% and multiresistance also increased [7,8]. Studies from Finland and the UK reported high prevalence of penicillin-resistant *S. aureus* 52.1% and 56% [1,8] and in Turkey, resistance to penicillin was detected in 62.5% strains, to amoxicillin-clavulanic acid in 2.9%, to cloxacillin in 22.1%, to neomycin in 30.9%, oxytetracycline in 31.6%, and to trimethoprim-sulphamethazole in 37.5% [4]. In Croatia, 88% of *S. aureus* strains were resistant to penicillin, 81% to ampicillin, only 4% to cloxacillin and resistance was not detected to cefoperazone and amoxicillin-clavulanic acid [13]. The findings of this study showed that the most effective in vitro drugs for *S. aureus* strains isolated from cows with clinical mastitis were amoxicillin-clavulanic acid, tetracycline, trimethoprim and gentamicin, and for CNS strains trimethoprim, amoxicillin, tetracycline and gentamicin. This may be due to their least frequent use (or not using as is the case with amoxicillin-clavulanic acid) in the study area in treatment of clinical mastitis, resulted into no more development of resistance to these antimicrobials during the observation period. The results of the present study shows that *S. aureus* and CNS isolates were found to be more resistant to penicillin (55.5% and 53.8%), streptomycin (50% and 46.1%), ampicillin (38.9% and 46.1%) followed by cloxacillin and polymyxin B. Compared with *S. aureus*, CNS are more often resistant to several antibiotics [11].

3. Conclusions

The results of the present study demonstrated the development of resistance to frequently used antimicrobials (especially penicillin and streptomycin) in bovine clinical mastitis. Therefore, isolation and identification of mastitis pathogens and antimicrobial susceptibility testing are essential to control the disease and achieve effective therapy. The findings of our study will contribute to provide about the level of sensitive and resistance of these bacteria isolates to different antibiotics, especially to veterinarians in veterinary practice for efficient use of them, and to promote management program for monitoring of bacterial strains, their sensitivity and resistance to antibiotics not only in cases of bacterial infections that occur in animals but also and in humans.

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5. References

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