

ANTIMICROBIAL SUSCEPTIBILITY OF STAPHYLOCOCCI ISOLATED FROM CLINICAL MASTITIS IN DAIRY COWS IN KOSOVO

A. HAMIDI and D. SYLEJMANI*

University of Prishtina, Faculty of Agriculture and Veterinary, Str. Bill Clinton, n.n., 10000, KOSOVO

Abstract

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This study was carried out 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 using coagulase test (using rabbit plasma) and API Staph system (bioMérieux SA) which differentiates *S. aureus* from other staphylococci. Isolates were tested for antimicrobial susceptibility on Mueller Hinton agar by dick diffusion method according to the Clinical Laboratory Standards Institute. The findings of this study showed that the most effective *in vitro* antibiotics for *S. aureus* isolates, isolated from cows with clinical mastitis were amoxicillin/clavulanic acid (83.3%), tetracycline (77.8%), gentamycin (77.8%) and trimethoprim (66.7%) and for CNS isolates trimethoprim (88.46%), amoxicillin (84.6%), tetracycline (77%) and gentamycin (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). Antimicrobial susceptibility tests are important for the selection of the most effective antimicrobial agent for treatment of bovine mastitis caused by staphylococci (*Staph. aureus* and CNS). Continuous monitoring for antimicrobial susceptibility of Staphylococci isolated from dairy cows with clinical mastitis is recommended as an important component of efficiently and prudent antimicrobial use practices.

Key words: Antimicrobial susceptibility/ staphylococci/ bovine mastitis/ Kosovo.

Introduction

Bovine mastitis is an inflammation of the udder associated with physical, chemical and bacteriological changes in milk. Generally, mastitis occurs in two forms which includes clinical and subclinical form. 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 characterised with obvious manifestation of inflammation. It is one of the main factors for economic losses in dairy farming due to the decrease in milk production, costs for medications, disposal of contaminated milk after treatment and early discard of diseased animals (Gentilini et al., 2002). Clinical mastitis is caused by bacteria and coagulase-positive *Staphylococcus aureus* is consid-

ered 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 (Pyörälä and Taponen, 2009), probably because prevalence of major pathogens are decreasing (Sampimon et al., 2009). Mastitis is one of the major causes imposing the antibiotic use in dairy cows (Mitchell et al, 1998). Approximately 70% of the antimicrobials applied in dairy production are used for treatment of clinical mastitis (Thomson et al., 2008), but the cure rates for clinical mastitis are not always satisfactorily. The efficacy of bovine mastitis treatment depends on the cause, clinical manifestation, antibiotic susceptibility of etiological agent and the efficiency of immunological system. The abu-

*Corresponding author: driton.sylejmani@uni-pr.edu

sive or incorrect use of antimicrobials has been implicated as the major selective force for the development of resistance (Levy, 2002).

In Kosovo, there are no data about the sensitivity and bacterial resistance to antibiotics in 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 dairy cows with clinical mastitis in two regions in Kosovo.

Materials and Methods

From the total of 48 milk samples, 26 isolates of Coagulase Negative *Staphylococci* and 18 isolates of *Staphylococcus aureus* were obtained from cows originating from different dairy farms during a period of five months (from January to June 2015) in two regions of Kosovo. Milk samples from each inflamed udder quarters of cows with clinical mastitis were collected aseptically. The teats were cleaned and dipped in a disinfectant and finally wiped with alcohol swabs and dried. The first few streams were discarded and then following leaksof the secretion were collected into sterile tubes. The samples were transported to the laboratory in ice box and processed immediately. Milk samples (0.05 ml) were inoculated onto Blood agar (Oxoid, UK) and cultivated at 36°C for 24 hours. Suspect colonies were tested for coagulase reaction (which differentiates coagulase positive staphylococci from coagulase negative staphylococci) and were isolated on Mannitol salt agar, cultivated at 36°C for 24 h and identified biochemically using API Staph system (bioMérieux, SA). Isolates were tested for antimicrobial susceptibility on Mueller Hinton agar (HIMEDIA) by disk diffusion method according to CLSI/NCCLS (Clinical Laboratory Standards Institute, 2012). The antimicrobial resistance of isolates was performed using following antibiotic disks: penicillin P (10

IU, Oxoid), ampicillin A (10 µg), amoxycillin/clavulanic acid AMC (30 µg, Oxoid), oxytetracycline T (30 µg, BD BBL™), streptomycin S (10 µg, Liofilchem), cloxacillin CX (5 µg, Liofilchem), gentamycin CN (10 µg, Liofilchem), trimethoprim TM (2.5 µg, Liofilchem) and polymyxin B (300 IU, BD BBL™). During this study the data were collected from local veterinarians for most frequent used antibiotics 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), procainbezilpenicillin/streptomycin sulphate/neomycin sulphate (Mastiquick 5 g intramammary injector), colistin sulphate/metampicillin sodium/cloxacillin sodium (Mastidian forte 10 ml intramammary injector) comprise the most used antibiotics for the treatment of clinical mastitis in lactating cows in these regions and very less frequent sulfadiazine/trimethoprim (Norodine 24 administered by parenteral routes).

The plates were incubated at 35°C 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.

Results

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 isolates of *Staphylococcus aureus* were found to be highly sensitive to amoxycillin (83.3%), followed by tetracycline (77.8%), gentamycin (77.8%) and trimethoprim (66.7%). Intermediate sensitivity was to ampicillin, polymyxin B, cloxacillin, trimethoprim, gentamycin, penicillin, amoxycillin, tetracycline and streptomycin.

However these isolates were resistant to penicil-

Table 1
Antibiotic sensitivity of *Staphylococcus aureus* isolates, from clinical cases of mastitis

No.	Antibiotic	S		I		R		Total
		No.	%	No.	%	No.	%	
1	Amoxycillin/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	Gentamycin	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 isolates from clinical cases of mastitis

No.	Antibiotic	S		I		R		Total
		No.	%	No.	%	No.	%	
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	Gentamycin	18	69.2	6	23.1	2	7.7	26
5	Trimethoprim	23	88.46	3	11.54	-	-	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

lin (55.5%), streptomycin (50%), followed by ampicillin (38.9%), polymyxin B (33.3%) and cloxacillin (27.8%). None of the *S. aureus* isolates was resistant to amoxicillin/clavulanic acid.

Coagulase Negative Staphylococci were sensitive mostly to trimethoprim (88.46%) amoxicillin/clavulanic acid (84.6%), followed by tetracycline (77%), gentamycin (69.2%), cloxacillin (42.3%) and polymyxin B (42.3%) (Table 2.)

Intermediate sensitivity was registered for polymyxin B, ampicillin, penicillin, gentamycin, tetracycline, trimethoprim, cloxacillin, amoxicillin/clavulanic acid, and streptomycin.

CNS isolates were more resistant to penicillin (53.8%), streptomycin (46.1%), ampicillin (46.1%), followed by cloxacillin (38.5%), polymyxin B (30.8%). None of the CNS isolates was resistant to trimethoprim and amoxicillin/clavulanic acid (Table 2).

Discussion

The antimicrobial susceptibility test carried out in present study indicated the existence of susceptibility and resistance of *Staphylococcus aureus* and CNS isolates to some of the antimicrobials. The majority of the authors have reported an increase of the bacterial resistance to antibiotics, and mostly for staphylococci, isolated from mastitis. Since penicillin has been extensively used along with streptomycin for treating clinical mastitis, it may possibly have led to the development of high resistance in *S. aureus* and CNS against these antibiotics. In Finland, the proportion of *S. aureus* isolates 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 multi resistance also increased (Myllys et al., 1998 and Pitkälä et al., 2004). Studies from Finland and the UK reported high prevalence (52.1% and 56%) of penicillin-resistant *S. aureus* (Bradley et al., 2007 and Pitkälä et al., 2004) 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% (Turutoglu et al., 2006). In Croatia, 88% of *S. aureus* isolates were resistant to penicillin, 81% to ampicillin, only 4% to cloxacillin and resistance was not shown to cefoperazone and amoxicillin/clavulanic acid (Benić et al., 2003). The findings of this study showed that the most effective *in vitro* drugs for *S. aureus* isolates, isolated from cows with clinical mastitis were amoxicillin/clavulanic acid, tetracycline, trimethoprim and gentamycin, and for CNS strains trimethoprim, amoxicillin, tetracycline and gentamycin. This may be due to their low frequent use (or not using as is the case with amoxicillin/clavulanic acid) in the study area in treatment of clinical mastitis, which seems to not have impacted the development of resistance to these antimicrobials during the observation period.

The results of the present study show 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 (Taponen and Pyörälä, 2009). The results of our study are approximately in line with the findings of these authors. Also, the results of the present study demonstrated the development of high level of resistance to frequently used antimicrobials in bovine clinical mastitis.

Data of this study will contribute:

- to reflect some of the most important bacteria isolated from clinical mastitis in cows,
- to provide information about the level of sensitivity and resistance of these bacteria to antibiotics,
- to instruct the veterinarians in veterinary practice for efficient use of antibiotics in such cases of clinical mastitis in cows,
- to encourage other researchers in my country to deal with this problem as in animals as well as in humans,
- to suggest the importance of implementation of systematic use of antimicrobials after in vitro antimicrobial sensitivity test prior to their use in treatment of mastitis, 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.

Conclusions

Continuous monitoring for antimicrobial susceptibility of Staphylococci isolated from dairy cows with clinical mastitis is recommended as an important component of efficiently and prudent antimicrobial use practices.

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