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Antibiotic Susceptibility of Staphylococci Isolated from Bovine Subclinical Mastitis

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Abstract: A total of 365 isolates of staphylococci including 209 *S. aureus* and 156 coagulase negative staphylococci (CNS) isolated from subclinical cases of bovine mastitis in Ahvaz (Iran) were analyzed for their susceptibility to several antimicrobial agents by agar disk diffusion method. Out of 209 isolates of *S. aureus* resistance was detected in 120 (57.42%), 64 (30.62%), 29 (13.88%), 29 (13.88%) and 10 (4.78%) isolates for penicillin, streptomycin, erythromycin, tetracycline and trimethoprim-sulfamethoxazol, respectively. No resistance was detected for gentamicin. Out of 156 CNS isolates resistance was detected in 48 (30.19%), 24 (15.09%), 20 (12.58%), 24 (15.09%) and 9 (5.66%) isolates for penicillin, streptomycin, erythromycin, tetracycline and trimethoprim-sufamethoxazol, respectively, whereas no resistance was detected for gentamicin. Results indicated that these isolates exhibited the highest degree of resistance to penicillin of all antimicrobial agents tested.

Key words: Antibiotic susceptibility, staphylococci, subclinical mastitis

INTRODUCTION

Bovine mastitis is a frequent cause of economic loss in dairy herds. Organisms as diverse as bacteria, yeasts and algae have been implicated as causes of the disease. Although several bacteria can cause this disease, *Staphylococcus aureus* is considered one of the most etiologic agents (Roberson, 1999; Smith, 2001). The role of coagulase-negative staphylococci (CNS) has clearly increased during the last years (Smith, 2001; Wilson *et al.*, 1997). These bacteria can cause mainly subclinical mastitis, but some authors reported high percentage of clinical cases evoked by CNS (Jarp, 1991). Cure rate of *S. aureus* infections are poor after antibiotic treatment (Gruet *et al.*, 2001). Increased resistance of *S. aureus* isolated from Mastitic quarter of cows to several antimicrobial agents has been reported (Myllus *et al.*, 1998; Moroni *et al.*, 2006). The efficacy of bovine mastitis treatment depends on the cause, clinical manifestation, antibiotic susceptibility of etiological agents and the efficiency of immunological system (Mainnowski *et al.*, 2002). The purpose of this study was to determine the *in vitro* activity of antimicrobial drugs against *S. aureus* and CNS isolated from subclinical cases of mastitis, in the Ahvaz, the capital of Khozestan province, Iran.

MATERIALS AND METHODS

This survey was carried out in the Ahvaz during January 2003 to August 2003. Milk samples were taken aseptically from all quarters (n = 791) of 200 bovine udders. For sampling, teat ends were cleaned by alcohol swabs and allowed to dry and the first few streams were discarded, then 5-10 mL of secretions were collected in sterile tubes. Samples were cooled and immediately transported to the laboratory. Bacterial isolation was performed by standard procedures according as described by Tyler and Cullor (2002); briefly 0.01 mL of samples streaked on 5% sheep blood agar and McConkey agar and incubated aerobically at 37°C atmosphere up to 72 h and suspected colonies identified biochemically. A total of 394 strains including 209 *S. aureus* and 156 CNS were isolated and used for antibiotic sensitivity testing.

Susceptibility testing: The isolates were suspended in Brain Heart Infusion broth, according to Kirby Bauer method and the suspension was adjusted to a turbidity equivalent to a 0.5 McFarland standard (Acar and Goldstan, 1996). The lawn cultures were made on Muller Hinton agar and the following disks were used (Beh Lab Teb, Tehran, Iran): penicillin, 10 IU; amoxicillin, 25 µg;

erythromycin, 15 µg; gentamycin, 10 µg; cephalothin, 30 µg; streptomycin, 10 µg. Isolates were categorized as susceptible, intermediate, resistant based upon interpretive criteria developed by the Beh Lab Teb company.

RESULTS

Out of 209 isolates of *S. aureus*, 140 (67%) were resistant to one or more antimicrobial agents tested by this method. There was no resistant strain to gentamicin, whereas 120 (57.42%), 64 (30.62%), 29(13.88%), 29 (13.88%) and 10 isolates (4.78%) were resistant to penicillin, streptomycin, erythromycin, tetracycline and trimethoprim-sufamethoxazol, respectively (Table 1). Out of 156 isolates of CNS, 45 (26.88%) were resistant to one or more antimicrobial agents. There were no resistant to gentamicin, whereas 48 (30.19%), 24 (15.09%), 20 (12.58%), 24 (15.09%) and 9 isolates (5.66%) were resistant to penicillin, streptomycin, erythromycin, tetracycline and trimethoprim-sufamethoxazol, respectively (Table 2).

DISCUSSION

Susceptibility of Staphylococci isolated from bovine intramammary infections to selected antimicrobial agents has been previously reported (Malinowski *et al.*, 2002; Gentilini *et al.*, 2002; Moroni *et al.*, 2006).

Sensitivity to Penicillin predicts susceptibility to other β-lactam antimicrobial agents, e.g., ampicilline. In this study 120 (57.42%) isolates of *S. aureus* were penicillin-resistant. This results is similar to those reported from cows with mastitis in United States (44%) (Bezek, 1998), Argentina (40%) (Gentilini *et al.*, 2002), Poland (66.7%) (Malinowski *et al.*, 2002) and Finland (50%) (Mylus *et al.*, 1998) but higher than reported for *S. aureus* strains isolated from bovine mastitic milk in West Indies (23.6%) (Adesiyun, 1994). Penicillin-resistance found in this study is in agreement with that previously reported for CNS in Argentina (30%) (Gentilini *et al.*, 2002), Finland (37.2%) (Mylus *et al.*, 1998) and Denmark (36.1%) (Aarestrup *et al.*, 1995) but is lower than that reported in study conducted in United States (57%) (Owens *et al.*, 1997).

The *S. aureus* and CNS bacteria were not gentamicin-resistant. This result is similar the results obtained for *S. aureus* in Argentina (3.4%) (Gentilini *et al.*, 2000). Similar results were found in Argentina, Uruguay and United States for CNS (Del Baglivi *et al.*, 1976; Frigerio *et al.*, 1995; Owens *et al.*, 1997).

For erythromycin our findings (Table 1 and 2) showed scarce resistance in *S. aureus* (13.88%) and in CNS (12.58%) similar to the results reported by

Table 1: Sensitivity of *S. aureus* strains (n = 209) from IMI in cows

Antibiotic	No. of resistant	Percentage of resistant
Penicillin	120	57.42
Streptomycin	64	30.62
Erythromycin	29	13.88
Gentamycin	0	0.00
Tetracycline	29	13.88
Trimethoprim-sulfametoxazol	10	4.78

Table 2: Sensitivity of coagulase negative staphylococci strains (n = 156) from IMI in cows

Antibiotic	No. of resistant	Percentage of resistant
Penicillin	48	30.19
Streptomycin	24	15.09
Erythromycin	20	12.58
Gentamycin	0	0.00
Tetracycline	24	15.09
Trimethoprim-sulfametoxazol	9	5.66

Gentilini *et al.* (2000) for *S. aureus* in Argentina. In Finland, Myllus *et al.* (1998) found 2.6 and 11.5% resistance among *S. aureus* and CNS, respectively, while in Sweden, Franklin (1998) reported 1% resistance in *S. aureus*.

Present results regarding tetracycline resistance for *S. aureus* (13.88%) and CNS (15.09%) were similar to those in Finland (Myllus *et al.*, 1998) but higher than the results obtained in Norway (Hofshager *et al.*, 1999).

The results were two fold higher than the 6% reported by Del Baglivi *et al.* (1976) in Uruguay. A possible explanation for this phenomenon could be that for many years tetracycline has been the most widely antimicrobial class used by the farmers to treat any infection.

Both *S. aureus* and CNS were susceptible to trimethoprim-sulfametoxazole that was similar to results reported by Gianneechini *et al.* (2002) in Uruguay.

Antimicrobial susceptibility patterns should be identified for staphylococci, as current susceptibility data are necessary to select appropriate antibiotics for a successful treatment. The high resistance to penicillin emphasizes the importance of identification of Staphylococci when an intramammary infection is present.

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