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Resistance and Residues of Antibacterial in Dairy Farm and Dairy Production in Al-Hassa Region, Saudi Arabia

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The objective of this study was to assess the antimicrobial susceptibility pattern of bacteria in clinical cases of mastitis and occurrence of antibacterial residues in milk in dairy farms of Al-Hassa region, Saudi Arabia. A 306 milk samples collected from dairy farms, 4.1% showed to contain antibacterial residues. This percentage was even higher in milk collected from supermarkets (9.2%) and from cows with clinical mastitis (17.1%). A total of 30 isolated of *Staphylococci aureus* were recovered from 52 milk samples of mastitic cows. The resistance of these isolates to antibacterials was: penicillin G (73.4%), ampicillin (66.7%), cephaloxin (60 %), erythromycin (53.3%), streptomycin (26.7%), neomycin and tetracycline (20%). It is suggested that treatment of mastitis should be initiated only after conducting antibacterial sensitivity test.

Key words: Cow, resistance, residues, antibacterial, mastitis

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INTRODUCTION

Administration of antibiotic to farm livestock imposed certain hazards to human and animal (Walton, 1988). These hazards may include increase in the resistance of enteric bacteria of animal origin to antibiotics and allergic reaction to some antibiotic. Generally farms with higher antimicrobial usage have been found to have higher residue level in meat or milk and higher proportion of resistant bacteria (Mathew *et al*., 2001; Van Den Bogaard *et al*., 2001). Recent evidence in USA (Erskine *et al.,* 2002). Suggest that an increase in the proportion of susceptibility isolates of mastitis pathogens occurred for many antibiotics and that there was no indication of increased resistance of mastitis isolates to antibacterials that are commonly used in dairy cattle. In this study the antimicrobial susceptibility pattern of bacteria in clinical cases of mastitis was assessed the persistence of antibacterial residues in milk was determined in summer 2005, in Al-Hassa region in Saudi Arabia.

MATERIALS AND METHODS

Collection of milk samples: Milk samples used in this survey were collected at three sources, individual dairy farms, supermarkets and from individual animals with clinical mastitis. Three hundred and eight milk samples were collected for residue determination and 52 samples antibiogram determination.

Screening of antibacterials in milk: For residue screening, Delvotest P Multi Plate Test (Brocades Delyt, The Nether Lands) was used. Specificity and sensitivity of test were previously described (Larocque and Neville, 1986).

Isolation and antibiogram study: For antibiogram study and before sample collection, teats were washed thoroughly and dried with disposable papers towels. Teat ends were cleaned with swabs containing 70% isopropyl alcohol. Samples of milk (0.05 mL) from each quarter were plated on brain-heart infusion agar (Gibco Laboratories, Madison, USA), supplemented with 5% defibrinated sheep blood and 1% yeast extract. Plates were incubated at 37°C and bacterial growth was observed and recorded at 24 h. Primary culture medium were identified tentatively according to colony morphologic features, hemolytic characteristics, Gram-strain reaction and catalase test. Isolates identified presumptively as staphylococci were tested for coagulase production by the tube coagulase method and all coagulase-positive staphylococci were identified using the Viteck Gram-Positive Identification System (Matthews *et al*., 1990) and a streptococcal agglutination system (Streptex. Wellcome Diagnostics, Research Triangle Park, NC). Isolates identified presumptively as streptococci were evaluated initially for growth in 6.5% NaCl, hydrolysis of esculin and sodium hippurate and CAMP reaction. Streptococcal organisms were identified to the species level using the Viteck Gram-Positive Identification System (Jayarao *et al*., 1991). Gram-negative isolates were identified to the species level using the Viteck Gram-Negative Identification System (Viteck System, Inc) and the following biochemical tests: triple sugar iron, urea, oxidasa, motility, indole and omithine decarboxylase. The sensitivities of the strains to, penicillin G, ampicillin, cephaloxin, erythromycin, streptomycin, neomycin, tetracycline, chloramphenicol, methicillin, kanamycin, doxycycline, ceprofloxacin, gentamicin and cloxacillin were tested, using antibiotic sensitivity discs (Oxoid, Unipath, Basingstoke, UK). Interpretation followed criteria recommended in the National Committee for Clinical Laboratory Standards (NCCLS, 1993).

Results were statistically analysed using Student's t-test (Kirkwood, 1988) with a significance level of $p<0.05$.

RESULTS

Three hundred and six milk samples collected from dairy farms, 4.1% showed positive Delvotest P Multi Plate Test (Table 1). This percentage was higher in milk collected from supermarket (9.2%) and significantly ($p<0.05$) higher in milk collected from cows with clinical mastitis (17.1%).

Staphylococci aureus showed maximum sensitivity to doxycycline (83.3%) followed by ceprofloxacin (80%), kanamycin (80%), gentamicin (76.6%), cloxacillin (76%),chloramphenicol (70%), methicillin (66.7%) and tetracycline (56.7%). The isolates showed maximum

Table 1: Rate of antibacterial residues detected by Delvotest P Multi Plate Screening test in dairy farm and dairy products in Al-Hassa region

Sources of sample tested	No. of samples	Percent with inhibitory activity
Individual dairy farms	106	4.1
Supermarkets	150	9.2
Cows with clinical mastitis	52	17.1*
Total	308	8.7

*Significant difference ($p<0.05$)

Table 2: Antibiogram of isolates of *Staphylococci aureus*, recovered from cows with clinical mastitis (n = 30)

Antimicrobial agents	Concentration (μg) of agent used	Response of isolates		
		Sensitive (%)	Intermediate sensitive (%)	Resistance (%)
Ampicillin	10	10.0	23.3	66.7*
Penicillin G	10	13.3	13.3	73.4*
Methicillin	5	66.7	20.0	13.3*
Kanamycin	30	80.0	16.6	3.4
Streptomycin	10	6.6	66.7	26.7*
Tetracycline	30	56.7	23.3	20.0
Erythromycin	15	20.0	26.7	53.3*
Cephaloxin	30	13.3	26.7	60.0*
Chloramphenicol	30	70.0	30.0	-
Neomycin	30	13.3	66.7	20.0
Ceprofloxacin	5	80.0	20.0	-
Gentamicin	30	76.6	20.0	3.4
Doxycycline	30	83.3	16.4	-
Cloxacillin	10	76.0	13.0	1.0

*Significant difference ($p<0.05$)

resistance to penicillin G (73.4%) followed by ampicillin(66.7%), cephaloxin (60%), erythromycin (53.3%), streptomycin (26.7%), neomycin and tetracycline (20%) (Table 2).

DISCUSSION

The occurrence of residues in milk in many countries has been documented (Dorgan, 1982; Egan *et al*., 1985; Kaneene and Miller 1992; Sato *et al*., 2004). The primary source of antibiotic residues in milk arises from intra mammary treatment and prophylaxis of mastitis and failure to with hold milk for appropriate withdrawal period after treatment (Egan *et al*., 1985; Walton, 1988). There is also suspicion that antibiotics are being added directly to the milk to retard spoilage. Although, we were unable to obtain information regarding the extend of such practice in dairy farms, we suspected their use to be common. The presence of antibiotics in milk is objectionable both for public health, reasons and for their deleterious effects in the manufacture of cultured dairy products (Walton, 1988; Sato *et al*., 2004).

A total of 30 isolates were recovered from 52 milk samples obtained from animals with clinical mastitis. On the basis of anaerobic growth and after biochemical tests all strains were considered to be *Staphylococcus aureus* (Baird-Parker, 1974). High prevalence of clinical and subclinical staphyloccal mastitis in dairy herds and presence of *Staphylococcus aureus* in raw milk from mastitic animals have been reported world-wide (Juneja and Pal, 1975; Gudding, 1980; Sigh and Baxi, 1980; Abbar *et al*., 1986; Teale, 2002; Jayarao and wolfgang, 2003; Sato *et al*., 2004).

Numerous reports have been published on antimicrobial susceptibility in *Staphylococci aureus* from dairy cattle which collected from individual quarter milk samples from cows with clinical mastitis (Devriese, 1980; Nagaraja and Chengappa, 1998; De Oliveria *et al*., 2000; Erskine *et al*., 2002; Sato *et al*., 2004). Such studies have attributed antimicrobial resistance mainly to antibiotics commonly used for mastitics therapy (Watts *et al*., 1995; Rossitto *et al*., 2001).

High susceptibility to ciprofloxacin could be related to the fact that the drug is newly introduced in Saudi veterinary market, doxycycline is not yet available as veterinary preparation, kanamycin, gentamicin and cloxacillin are not used routinely in mastitis therapy and chloramphenicol is limited now to treatment of eye infection when there is no alternative antibiotic. Levels of resistance to β-lactom antibiotics (penicillin G, ampicillin and cephaloxin), amino glycoside (streptomycin and neomycin), macrolide (erythromycin) and tetracycline was due to the fact that these antibiotics are extensively used for mastitis therapy. The presence of β-lactom and streptomycin-resistant staphylococci in bovine mastitis milk was reported elsewhere (Holmberg, 1975; Abbar *et al*., 1986; Erskine *et al*., 2002; Makovec and Ruegg, 2003; Sato *et al*., 2004).

Samples submitted for diagnosis of clinical mastitis cases tend to exhibit more antimicrobial resistance, probably because cases that are refractory to treatment are more likely to be cultured in an attempt to identify the causative agent and its antimicrobial resistance traits (Aarestrup *et al*., 1998). Milk can act as a source for dissemination of antibiotic-resistant staphylococcus (Lacey, 1973). Therefore, it is advisable to initiate treatment of mastitis only after conducting antibiotic sensitivity test. Controlling of occurrence of antibiotic residues in milk maybe one mean of minimizing the occurrence of mastitis antibiotic-resistant staphylococcus (Walton, 1988).

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