Full Length Research Paper

Incidences and antimicrobial susceptibility profile of *Staphylococcus* species isolated from animals in different Qatari farms

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This study was conducted during a six months period (March to August 2008) at the Veterinary laboratory, Department of Animal Resources, Doha, Qatar to determine the incidence of different bacterial genera farm animals in particular the occurrence of *Staphylococcus* species. The samples were from the rectum, milk, feces, nasal, swab of vagina, cloacal - uterin, intestine, liver and pus. Samples were collected from various animals of different regions. A total 318 of 429 samples gave positive results for Gram-negative and Gram-positive bacteria and 90 cultures were identified as *Staphylococcus*. The highest isolates were from camels' milk and none were obtained from specimens of cats and horses. A total of 33 selected *Staphylococcus* isolates were identified to species level and the most common isolated was coagulase-positive *S. aureus* (14), followed by coagulase-negative, *S. lentus* (10), *S. xylosus* (5), *S. sciuri* (2), *S. lugdunensis* (1) and *S. caprae* (1). The antimicrobial susceptibility profiles of these were determined using cephalothin, gentamycin, neomycin, ampicillin, norfloxacin, penicillin, tetracycline and a combination of trimethoprim and sulphamethoxazole. All identified species were sensitive to cephalothin except one strain of *S. aureus.* Most of the isolates showed multiple antimicrobial resistance profiles.

Key words: *Staphylococcus*, farm animals, antibiotic, susceptibility profiles, Qatar.

INTRODUCTION

Each region of the animal body whether the skin, oral cavity, gastrointestinal tract, respiratory tract, or genito-urinary tract are in frequent contact with microorganisms as microflora and pathogens. Gram-positive bacteria like staphylococci and corynebacteria are more common on skin as compared to the flora of the intestines which contains various groups of bacteria among which enterobacteria, such as *Escherichia coli,* and various other anaerobes and Gram-negative species (Quinn et al., 2002). Staphylococci are gram-positive, catalase-positive, oxidasenegative, facultatively anaerobic and are non-motile cocci. The coagulase-positive *Staphylococcus aureus* and *Staphylococcus intermedius*, and the

coagulase-variable *Staphylococcus hyicus* are important pathogens in farm animals and are of particular importance as primary causes of specific diseases such as *S. aureus* (mastitis in ruminants, equine botryomycosis), *S. intermedius* (canine pyoderma) and *S. hyicus* (porcine exudative epidermitis) (Dwight et al., 2004). Although coagulase-negative staphylococci are usually of low virulence and mostly normal skin commensals, some occasionally cause disease in animals and man (Werckenthin et al., 2001; Stepanovic et al., 2001; Morris et al., 2006; Kaliwal et al., 2011).

Antibiotics have been broadly used in farm animals for the purpose of antimicrobial therapy, prophylaxis and growth promotion (Mathew et al., 2007; Waters et al., 2011). This increasing handling of antibiotics has led to a worldwide problem in the development of antibiotic resistance among bacterial populations during recent decades (Shryock and Richwine, 2010). The emergence

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of antibiotic resistance among humans has prompted concerns about the public health implications of antibiotic use in agriculture. Some bacteria have developed resistances to all of the different classes of antibiotics discovered up to date, and the most frequent type of resistance is acquired and transmitted horizontally via mobile genetic elements such as plasmids, transposons and integrons (Tiemersma et al., 2004). The improper antibacterial treatment and overuse of antibiotics for agricultural purposes have contributed to the increased incidence of multiple antibiotic resistances in farm animals (Shryock and Richwine, 2010; Ding and He, 2010).

Staphylococcal infections are frequently treated with antibiotics and consequently resistance to it and or acquired resistance develop (Normand et al., 2000). Currently, medical attention focuses to both coagulase-positive and coagulase-negative staphylococci because they represent a serious therapeutic problem. Moreover, they may develop multi-antimicrobial resistance (Normand et al., 2000; Blahová et al., 2004; Authier et al., 2006). In recent years, much has been written about the emergence of methicillin-resistant *S. aureus* and methicillin-resistant coagulase-negative staphylococci (Normanno et al., 2007; Leonard and Markey, 2008; Springer et al., 2009; Weese, 2010; Huber et al., 2011).

In Qatar, the Department of Animal Resources, Ministry of Environment is in charge of the protection of farm animals. Sheep, goats, cattle, camels, and poultry are an important component of food in Qatar. The Department also controls the spread of various animal diseases and is responsible for the issue of import permits, and oversees exportation, transfer and transit of living organisms and animal products across borders. This study was carried out to draw attention to the level of incidence of bacterial genera in samples of different farm animals collected from different regions in Qatar. The goal is to examine the incidences and antimicrobial susceptibility profiles for *Staphylococcus* species.

MATERIALS AND METHODS

Collection of samples

This study was conducted during a six month period (March to August 2008) at the Veterinary laboratory, Department of Animal Resources, Doha, Qatar. Different samples including rectum, milk, feces, nasal, and other samples collected from various animals of different regions in Qatar were sent to the Veterinary lab for routine examining (Table 1).

Culturing and identification of samples

Samples were cultured using different culture methods: mannitol fermentation, MacConky, Muller-Hinton, hektoen enteric and xylose lysine deoxycholate. For further identification, the cultures were examined using standard methods: colony morphology, Gram stain and biochemical tests with catalase, coagulase, oxidase, citrate,

indole, triple sugar iron and urease. For staphylococci species identification, API staph system (biomerieux) was used.

Antibiotics susceptibility test

All 33 which were identified to species level of coagulase-positive and coagulase-negative *Staphylococcus* sp. were subjected to antimicrobial testing method on Muller-Hinton agar, using fresh nutrient broth culture and antibiotic discs (OXOID) as described in the standard manual. Briefly, the zone of inhibition around the discs were measured and interpreted as sensitive and resistant using the interpretation chart supplied by the antibiotic disc manufacturers. The disk – diffusion method was used to determine the susceptibility of all selected isolates to cephalothin, gentamycin, neomycin, ampicillin, norfloxacin, penicillin, tetracycline and a combination of trimethoprim and sulphamethoxazole.

RESULTS

Collection of samples and bacterial identification

During the six months, the laboratory received 429 samples collected from different farm animals. A total of 318 were positive for Gram-negative and Gram-positive bacteria. *Escherichia* spp. was the highest number isolated (154) followed by *Staphylococcus* (90), *Salmonella* (30), *Klebsiella* (17), *Pseudomonas* (14) and *Corynebacterium* (4).

90 cultures were identified as *Staphylococcus* species. The highest number was isolated from camels (37) and there was no isolates from cats and horses. A total of 33 staphylococci were identified to the species level with API Staph (API staph- biomerieux). The most common species isolated were *S. aureus* (14), followed by *S. lentus* (10), *S. xylosus* (5), *S. sciuri* (2), *S. lugdunensis* (1) and *S. caprae* (1). Table 1 shows the total number of *Staphylococcus* / total number of bacterial isolated from different animal samples from Qatari farms during the six months. The highest isolated number of *Staphylococcus* was from camels and the lowest were from deers while no isolates was from horses and cats. Most of the isolated bacteria were *Escherichia* spp. followed by *Staphylococcus* while *Corynebarterium* was the least. This indicates that gram negative bacilli constituted the majority of bacteria in the Veterinary laboratory.

Antibiotics susceptibility test

Table 2 shows the resistant profiles of *Staphylococcus* spp. The percentage of resistant of the isolates per the antibiotics varied from 3 to 66.66%. The most frequent resistance was to penicillin and tetracycline (66.66%) followed by ampicillin, while all the isolates were susceptible to cephalothin with one strain of *S. aureus* was resistance of 7%. No isolates were resistant to norfloxacin and a combination of trimethoprine and sulphamethoyazole. Resistance to *β*-Lactam antibiotics

Table 1. Total number of *Staphylococcus* / total number of bacterial isolated from different animals from Qatari farms.

Parameter	Camel	Goat	Sheep	Cattle	Horse	Dog	Cat	Bird	Deer	Rabbit	Total
Rectum	0/11	0/11	0/0	0/3	0/0	0/0	0/0	0/0	0/1	3/10	3/36
Milk	15/56	4/21	5/12	3/16	0/13	0/0	0/0	0/0	0/0	0/0	27/118
Feces	1/45	2/7	2/6	0/6	0/0	2/8	0/5	1/9	1/6	0/0	9/92
Nasal	6/14	2/7	0/5	0/3	0/0	0/0	0/0	0/0	0/0	4/8	12/37
Others*	15/63	6/13	1/3	2/7	0/0	3/4	0/0	12/56	0/0	0/0	39/146
Total	37/189	14/59	8/26	5/35	0/13	5/12	0/5	13/65	1/7	7/18	90/429

*Vagina swab, oral swab, cloacal- uterin swab, intestine sample, buccal swab, liver sample, and pus sample.

Table 2. Antimicrobial resistance profiles of selected *Staphylococcus* spp. Isolated from animal samples.

Antibiotic tested	*S. aureus* (*n* =14)	*S. lentus* (*n* =10)	*S. xylosus* (*n* = 5)	*S. sciuri* (*n* = 2)	*S. lugdunensis* (*n* = 1)	*S. caprae* (*n* = 1)	Percent of all isolates' resistance to antibiotic
Cephalothin	1	0	0	0	0	0	(3%)
Gentamycin	2	3	0	0	0	1	(18.18%)
Neomycin	1	4	2	0	1	0	(24.24)
Norfloxacin	0	6	1	1	0	1	(27.27%)
Trimethoprim and Sulphamethoxazole	0	8	2	1	0	1	(36.36%)
Ampicillin	3	9	1	1	1	0	(45.45%)
Penicillin	11	7	2	2	0	0	(66.66%)
Tetracycline	7	9	3	2	0	1	(66.66%)

was: 78.5% to penicillin, 50% to tetracycline and 21.4% to ampicillin.

The highest numbers of coagulase-negative staphylococci species were resistant to tetracycline (78.9%) with no resistant to cephalothin. Table 2 shows multiple antimicrobial resistance profiles for the other antibiotics.

DISCUSSION

The results of the analysis were expected since the isolated number of Gram-negative bacteria, *Escherichia, Salmonella, Klebsiella* and *Pseudomonas* species was higher than Gram-positive bacterial genera, *Staphylococcus* and *Corynebacterium*; are known to be part of the normal microfolra and some can cause disease such as mastsides in cattle (Getahun et al., 2008; Holmes and Zadoks, 2011). These results are in agreement with other studies which indicated that the commonly isolated genera were *Staphylococcus*, *Escherichia* and *Salmonella* (Van Duijkeren et al., 2004; Alzohairy, 2011). This study focuses on *Staphylococcus* because of its presence in several habitats including humans, animals and food and results in a wide range of diseases which easily spread among animals and, under certain conditions they infect humans by skin to skin contact, or by contact with excretions which contain staphylococci. Moreover, staphylococci may spread via animal products such as non-pasteurized milk.

S. aureus is the most important coagulase-positive pathogen due to a combination of toxin-mediated virulence, invasiveness and antibiotic resistance (LeLoir et al., 2003). In this study, 42.4% from the selected strains were identified as *S. aureus* and 57.6% were as coagulase-negative staphylococci, with five species identified as *S. lentus, S. xylosus, S. sciuri, S. lugdunensis* and *S. caprae* (Table 2). The API Staph-system provides

a rapid and accurate method for identifying the examined species. The study indicated that staphylococci was isolated most were from camels milk. This is in agreement with the findings by Kivaria et al. (2007), Getahun et al. (2008), Olde et al. (2008) and Alzohairy (2011).

The prevalence and degree of antimicrobial resistance in veterinary medicine are increasing worldwide (Werckenthin et al., 2001; SØrum and Sunde, 2001; Levy and Marshall, 2004). The dissemination of antimicrobial resistant staphylococci is presenting a challenge to both human and animal health professionals. Antimicrobial profiles of selected staphylococci isolated in this study were multi-antimicrobial drug resistant for the antimicrobial agents used. The antimicrobial susceptibility tests carried out in this study indicated the high resistance of *Staphylococcus* species to tetracycline and penicillin (66%) followed by ampicillin (45%). The resistance of staphylococci to these *β*-lactams antibiotics may be attributed to the production of *β*-lactamase, an enzyme that inactivates penicillin and closely related antibiotics and this may be probably explained by a horizontal transfer of antibiotic resistance gene from the resistant bacterium to another bacterium normally susceptible to this antibiotic. Moreover, this could be associated with the predominant use of penicillin for treatment of animal diseases; this result agrees with other results regarding the increase in incidence of *β*-lactam antibiotics resistance (Schwarz et al., 1998; Alekshun and Levy, 2000). A high incidence of antimicrobial resistance among coagulase-negative staphylococci was also reported by Mártonová et al. (2008). Also, this results indicate that the highest degree of staphylococci of all the tested antimicrobial agents were susceptible to cephalothin followed by gentamycin and neomycin. Cephalothin is used in both dogs and cats to treat a variety of bacterial infections (Authier et al., 2006).

Based on the present and limited study data, attention should be paid to the occurrence of multi-drug resistant staphylococci and other microorganisms in farm and domestic animals continuously evaluated within veterinary and public health aspects. Further, studies are required to investigate the impact of the high multi-drug resistant staphylococci prevalence on human and animal health in Qatar.

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