

Full Length Research Paper

Colonization and antibiotic susceptibility pattern of methicillin resistance *Staphylococcus aureus* (MRSA) among farm animals in Saudi Arabia

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major human pathogen that causes severe morbidity and mortality worldwide. Additionally MRSA is widely spread in different animals. There are a growing number of MRSA cases have been reported in dogs, cats, horses, sheep, and other animals indicating the animal health treat too. To assess the frequency of MRSA among animals in Qassim region, a total of 400 samples were collected from camels, sheep, cows, and goats from 334 *Staphylococci* recovered, 158 (47.3%) were coagulase positive *Staphylococcus*, among them 90 (57%) were MRSA and 68 (43%) were methicillin-sensitive *Staphylococcus aureus* (MSSA). The remaining strains 176 (52.7%) were coagulase negative *Staphylococcus*, including 32 (18.2%) were methicillin-resistant coagulase negative *Staphylococcus* and 144 (81.8%) were methicillin-sensitive coagulase negative *Staphylococcus*. High rate of MRSA and MRCoNS were isolated from camel and sheep while lower rates were observed in goat and cow. Multi drug resistance (MDR) rate among MRSA and MRCoNS isolates was high. MRSA strains are highly prevalent among animals in Qassim region and they may play a potential role of disseminating pathogens between animal and human as well as to the community. Detection of MRSA will be essential for early prevention and control of community acquired infections.

Key words: Prevalence, methicillin resistant *staphylococcus aureus*, multidrug resistance, antibiotic susceptibility.

INTRODUCTION

Pathogenic microorganisms resistant to commonly used antibiotics is a worldwide concern. In this regard, the bacterial pathogen *Staphylococcus aureus* is one of the most important bacteria, particularly its methicillin-resistant strains. After the introduction of methicillin in the 1960s, methicillin resistance was found in strains of *S. aureus* (Grundmann et al., 2006). Soon after, methicillin-resistant *Staphylococcus aureus* (MRSA) in human populations has been reported worldwide becoming a significant cause for both hospital and community associated infections. Currently, MRSA is causing a significant morbidity and motility worldwide (Van Loo et al., 2007).

Occurrence of MRSA in animals has been shown by various previous studies (Walther et al., 2008; Rich and Roberts, 2006; Weese et al., 2006a; O'Mahony et al., 2005; Van Duijkeren et al., 2004). MRSA prevalence

have been increasingly documented in farm or domestic animals including sheep, goats, horses, and cattle as well as in different pet or companion animals such as dogs and cats (Walther et al., 2008; Saleha et al., 2010). Indicating that MRSA has emerged as veterinarian and potentially zoonotic pathogen. The new findings of MRSA among animals have promoted an extensive studies to address the issue of MRSA colonization and transmission to human particularly those with close contact to animals (Khanna et al., 2010).

There are number of studies indicating domestic animals as a potential source of human infections (Grundmann et al., 2006; Weese et al., 2006b; O'Mahony et al., 2005; Seguin et al., 1999; Weese et al., 2006). Similarly, there have some reports indicating the domestic animals were the source of human infections particularly

Table 1. Total number of *staphylococcus* isolated from animals.

Bacterial isolate	No. of isolate	Percentage (%)
<i>Staphylococcus aureus</i>	158	(47.3)
Coagulase negative staphylococcc (CNS)	176	(52.7)
Total	334	(100)

human in close contact with animals either through the nature of their occupations or keeping animal as pet. Thus, it is crucial to understand the presence of MRSA among certain population animals for better preventive measures.

This study was carried out to assess the prevalence level of *Staphylococcus* strains in different life stock animals including; camels, sheep, coats, and cows in Qassim region, Saudi Arabia and to analyze the *in vitro* antimicrobial susceptibility pattern of isolated strains. Findings of this study address the potential reservoir of MRSA infection in human, thus signifying a potential public health importance and global interest on MRSA.

MATERIALS AND METHODS

Study population

From January to April 2010, nasal swabs were collected from 400 different farm animals of various breeds in Qassim regions, Saudi Arabia. The study population included 100 camels, 100 sheep, 100 goats, and 100 cows. All samples (n = 400) were taken from healthy animals.

Collection and culturing of nasal swabs

Sterile, individually warped cotton-tipped swabs were used for nasal swabbing of the different animal. Swabs were inserted into the anterior nares and rubbed very well by rotating 5 times over the inner well of nasal septum by trained person. Swabs were then stored at stored at 4°C until processing, which occurred within 24 h of collection. Swabs collected were cultured on Mannitol Salt agar (selective medium for *S. aureus*) by streaking as per the conventional technique. The culture plates were incubated at 37°C for 24 to 48 h.

Identification of *S. aureus*

The suspected *Staphylococcus* colonies-yellow colonies showing Mannitol fermentation and non-yellow colonies (mannitol negative) were selected and subject to Gram staining and sub-cultured into nutrient agar slopes. The isolates showing Gram-positive cocci in clusters were subjected to coagulase test (Staphase Oxoid) by slide and test tube technique.

Identification of MRSA by a chrom agar plate method

For the identification of the MRSA among the isolates of *S. aureus*, ChromID MRSA (selective media) (OXOID) was used. All cultures showing bright blue colored growth were taken as MRSA positive

strains, while all others are recorded as MSSA strains. As controls, all strains were also inoculated on MH Agar and incubated simultaneously. Further, methicillin-resistance was confirmed through penicillin binding protein 2a (PBP2a) latex agglutination test (PBP20 Test Kit, Oxoid, Hants, UK).

Antibiotics susceptibility testing

All nasal isolates of *S. aureus* were subjected to *in-vitro* anti-microbial testing method on Muller-Hinton agar, using fresh nutrient broth culture and antibiotic discs (OXOID) as previously described (Khadri and Alzohairy, 2010). Briefly, the zone of inhibition around the discs were measured and interpreted as sensitive, moderately sensitive, and resistant using the interpretation chart supplied by the antibiotic disc manufacturers (OXOID). The disk-diffusion method (Oxoid, Basingstoke, UK) was used to determine the susceptibility of all isolates to oxacillin, methicillin, gentamicin, vancomycin, rifampicin, ciprofloxacin, co-trimoxazole, fusidic acid, and tetracycline, according to Clinical and Laboratory Standards Institute (CLSI-2005) guidelines *S. aureus* ATCC 26923 was used as a standard control strain.

RESULTS

Based on the identification methods used, a total of 334 Staphylococcal strains were isolated from different animal. Out of them, 158 (47.3%) strains were coagulase positive staphylococci (CPS) and 176 (52.7%) isolates were coagulase negative staphylococci (CNS) as illustrated in Table 1. Among the (CPS) strains, 90 (57%) were MRSA and 68 (43%) were Methicillin-Sensitive *Staphylococcus aureus* (MSSA). The remaining isolates (CNS) were divided into 70 (39.7%) as Methicillin-Resistant Coagulase Negative *Staphylococcus* (MRCoNS) and 106 (60.3%) as Methicillin-Sensitive Coagulase Negative *staphylococcus* (MSCoNS).

MRSA in camel

A total number of 96 *staphylococcus* isolates were recovered from camels. Out of them, 54 (56.2%) were coagulase positive *staphylococcus* and 42 (43.7%) were coagulase negative *staphylococcus*. Among the total number of bacterial strains obtained from animals, 32 (35.5%) isolates were MRSA and 22 (32.3%) isolates were methicillin-sensitive *Staphylococcus aureus* (MSSA). The coagulase negative *Staphylococcus* strains sub divided into methicillin-resistant coagulase negative

Table 2. Total distribution of *Staphylococcus* in animals.

Animal	Total no.	<i>S. aureus</i>	Coagulase-negative <i>Staphylococcus</i> (CNS)
Camels	96	54	42
Sheep	88	38	50
Cows	72	36	36
Goats	78	30	48
Total	334	158	176

Table 3. Frequency of MRSA and MSSA in animals.

Specimen	Frequency of MRSA and MSSA			
	MRSA (n=90)	MRSA (%)	MSSA (n=68)	MSSA (%)
Camel	32	35.5	22	32.3
Sheep	26	28.9	12	17.6
Cow	14	15.5	22	32.3
Goat	18	20	12	17.6
Total	90	100	68	100

Table 4. Frequency of MRCoNS and MSCoNS in animals.

Specimen	Frequency of MRCoNS and MSCoNS			
	MRCoNS (n=32)	MRCoNS (%)	MSCoNS (n=144)	MSCoNS (%)
Camel	8	25	34	23.6
Sheep	14	43.75	36	25
Cow	4	12.5	32	22.2
Goat	6	18.75	42	29.2
Total	32	100	144	100

staphylococcus 8 (19%) (MRCoNS), and Methicillin-sensitive coagulase negative *staphylococcus* 34 (81%) were (MSCoNS) as illustrated in Tables 3 and 4. *Staphylococci* strains isolated from nasal cavity of camels showed a high rate of multi-drug resistance (MDR). As shown in Tables 7, The majority of MDR strains were methicillin-resistant *staphylococcus* isolates when compare to methicillin sensitive *staphylococcus* isolates both in coagulase positive *staphylococcus* and coagulase negative *staphylococcus*, the total 18 number of MDR strains were obtained from camel among these 14 were MRSA and 4 were MRCoNS isolates display a multidrug resistant pattern.

MRSA in sheep

Only few studies have reported the prevalence of MRSA in sheep. In this study, 88 *Staphylococcus* isolates were recovered from sheep. Out of them, 38 (43.2) isolates were coagulase positive *Staphylococcus* and 50 (56.8) isolates were coagulase negative *Staphylococcus* (Table

2). Among the coagulase positive *Staphylococcus*, 26 (28.9%) were MRSA and 12 (17.6%) were MSSA (Table 3). Out of 32 coagulase negative *Staphylococcus*, 14 (43.75%) were methicillin-resistant coagulase negative *Staphylococcus* (MRCoNS) and 36 (25%) were methicillin-sensitive coagulase negative *Staphylococcus* (MSCoNS) (Table 4). Among sheep isolates, only 8 (23.5%) strains were multi-drug resistance MRSA strains and 6 (42.8%) strains were multi-drug resistance MRCoNS (Table 7).

MRSA in cows

72 *Staphylococcus* isolates were recovered from cows. Out of them, 36 (50%) were coagulase positive *Staphylococcus* and 36 (50%) were coagulase negative *Staphylococcus*. Among the coagulase positive *Staphylococcus* 14 (15.5%) were MRSA and 22 (32.3%) were MSSA (Table 3). Out of 36 coagulase negative *Staphylococcus* 4 (12.5%) were methicillin-resistant coagulase negative *Staphylococcus* (MRCoNS) and 32

Table 5. Antibiotic resistance pattern of MRSA and MSSA.

Antibiotic	Percent of isolates' resistance to antibiotics			
	MRSA (n=90)	MRSA (%)	MSSA (n=68)	MSSA (%)
Penicillin	90	(100)	50	(73.5)
Oxacillin	90	(100)	00	(00)
Erythromycin	76	(84.4)	30	(44.1)
Cephalothin	70	(77.8)	36	(52.9)
Ciprofloxacin	50	(55.5)	18	(26.5)
Tetracycline	54	(60)	28	(41.2)
Gentamycin	72	(80)	24	(35.3)
Co-Trimoxazole	74	(82.2)	40	(58.8)
Vancomycin	0	(00)	0	(00)

(22.2%) were methicillin-sensitive coagulase negative *Staphylococcus* (MSCoNS) (Table 4). Multi-drug resistance rate among cows isolates was relatively low compare to the other animals with only 6 (17.6) MRSA isolates and 2 (14.2) MRCoNS isolates (Table 7).

MRSA in goats

Thirty isolates recovered from goats were determined as *S. aureus*, and another 48 isolates as coagulase-negative *Staphylococci*. Through the oxacillin screening agar, 26 strains were determined as methicillin-resistant isolates including 18 isolates (20%) as MRSA and 6 (18.8%) isolates as coagulase-negative (MRCoNS) (Table 3 and 4). On the other hand, 54 goat isolates were methicillin sensitive including 12 (17.6) isolates determined as coagulase-positive *Staphylococcus* and 42 (29.2%) isolates as coagulase-negative *Staphylococcus* (Tables 3 and 4). Among MRSA isolates, only 6 (17.6%) strains were multidrug resistant and 2 (14.2%) strains were multidrug resistance MRCoNS strains (Table 7).

DISCUSSION

The presence of MRSA in animals and humans is of public health concern. Where the multi-drug resistant MRSA strains disseminate from animal to human and vice versa in Community (DeNeeling et al., 2007; Smith et al., 2008; Persoons et al., 2009). Several reports shows the presence of MRSA in a variety of domestic species including dogs (Pak et al., 1999; van Duijkeren et al., 2004), cats (Bender et al., 2005) horses (Anzai et al., 1996; Hartmann et al., 1997), sheep (Goni et al., 2004) pigs (Voss et al., 2005) and chickens (Lee, 2003) leading to an upsurge of reports and interest in MRSA colonization and infection in animals. Screening the prevalence of MRSA will be of much use in early prevention and control of community acquired infections. Hence, this study was carried out to address the prevalence of MRSA among domestic animals in Qassim region, Saudi Arabia

and to describe the antibiotics susceptibility pattern of these isolates collected from January to April, 2010.

Of the isolates tested in this study, coagulase positive *Staphylococcus* accounted for 47.3% where as coagulase negative *Staphylococcus* accounted for 52.7%. In camels, this study is the first to report an unexpected high rate of MRSA colonization in Saudi Arabia reaching up to 35.5%. However, colonization rate was lower in sheep (28.8%), goats (20%), and cows (15.5%). Different pattern was observed with the occurrence rate of MRCoNS colonization with sheep being the highest (43.7%) followed by camels (25%). The similar report on the emergence of MRSA in animals was also reported in different parts of world from Pakistan (Farzana et al., 2004), Korea (Moon et al., 2007), and Hungary (Juhasz-Kaszanyitzky et al., 2007), except the colonization of MRSA in camel. In another study, several isolates from cows, goats, and sheep were isolated and compared by (Ben Zakour et al., 2008b). Our findings are also supported by those of above reports.

Antibiotic susceptibility profile of the isolates tested in this study demonstrated high resistant of MRSA strains to penicillin and oxacillin followed by erythromycin, Co-trimoxazole, gentamycin, and cephalothin. Resistant rate of MRSA strains was higher than those sensitive to methicillin (Table 5). This phenomena was reported else where (Tahnkiwale et al., 2002). Similarly, the resistant rate of MRCoNS strains was higher than those sensitive to methiicillin MSCoNS (Table 6). On the other hand, all tested strain in this study were sensitive to vancomycin.

The highest rate of multidrug resistant MRSA strain was observed in isolated recovered from camel (41.1%). Strains isolated from sheep, cows, and goats had a closely similar rate (23.5%, 17.6%, and 17.6% respectively). The pattern of multidrug resistant is different among MRCoNS strains with sheep isolates being the highest (42.8%) followed by camels, cows, and goats (28.5, 14.2 and 14.2% respectively). These findings go in agreement with previously reported studies (Goni et al., 2004; Quddoumi et al., 2006; Stastkova et al., 2009). While another study described evaluation of MRSA

Table 6. Antibiotic resistance pattern of MRCoNS and MSCoNS.

Antibiotic	Percent of isolates' resistance to antibiotics			
	MRCoNS (n=32)	MRCoNS (%)	MSCoNS (n=144)	MSCoNS (%)
Penicillin	32	100	96	66.7
Oxacillin	32	100	00	00
Erythromycin	24	75	50	34.7
Cephalothin	22	68.7	58	40.3
Ciprofloxacin	12	37.5	30	20.8
Tetracycline	20	62.5	38	26.4
Gentamycin	10	31.2	18	12.5
Co-Trimoxazole	26	81.2	62	43
Vancomycin	0	00	0	00

Table 7. Distribution of Multi-drug resistant MRSA and MRCoNS in animals.

Specimen	Multi-drug resistant MRSA and MRCoNS			
	MRSA (n=34)	MRSA (%)	MRCoNS (n=14)	MRCoNS (%)
Camel	14	41.1	4	28.5
Sheep	8	23.5	6	42.8
Cow	6	17.6	2	14.2
Goat	6	17.6	2	14.2
Total	34	100	14	100

infection and colonization in household pets, and transmission of MRSA between animals and humans (Baptiste et al., 2005).

In conclusion, this study confirms that staphylococci are one of the most common pathogens associated with animals. Over 26% of tested strains were confirmed to be MRSA with high multidrug resistant rate in isolates recovered from camels. It must be emphasized that there is a need to monitor the presence of MRSA in animals similar to that being done in humans so as to prevent further spread of MRSA in both sets of populations.

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