



Incidence of Methicillin-Resistant *Staphylococcus aureus* Isolation from Sheep and Goat

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a type of bacteria that is resistant to certain antibiotics including methicillin, oxacillin, penicillin and amoxicillin. Our study investigated the occurrence of *Staphylococcus aureus* in a total of 95 and 86 sheep and goat, respectively from different healthy conditions (apparently healthy, pneumonia, enteritis and pneumoenteritis) using routine bacteriological identification and antimicrobial susceptibility testing. The results showed that *S. aureus* in sheep was detected in 25, 31.25, 43.3 and 20 %, in apparently healthy, pneumonia, enteritis and pneumoenteritis cases, respectively from nasal swabs and 12.5, 20.8, 16.6 and 17.5 %, respectively from fecal swabs. While in goats, *S. aureus* was detected in 25, 26, 15.3, 14.2 % in apparently healthy, pneumonia, enteritis and pneumoenteritis cases, respectively from nasal swabs and 12.5, 8.3, 11.5, 14.2 %, respectively from fecal swabs. Further, the isolates were studied for their antimicrobial susceptibility patterns using 9 antibiotics commonly used. *S. aureus* isolated from nasal or fecal swabs of healthy sheep and goat were 100% resistant to Ampicillin, Amoxycillin and Penicillin, while *S. aureus* isolated from nasal and fecal swabs of sheep with pneumointeritis were 50 % resistant to Methicillin and Oxacillin. All *S. aureus* isolates were 100 % sensitive to Ciprofloxacin and Gentamycin making them the drugs of choice for the treatment of different disease conditions caused by *S. aureus* in sheep and goat.

Key words:

MRSA, sheep, goat, pneumointeritis

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1. INTRODUCTION

Staphylococcus aureus remains one of the most intensively investigated bacterial species in human and animal pathogen, it is an adaptable, opportunistic pathogen with abilities to persist and multiply in a variety of environments and causes a wide scale of diseases (Cucarella et al., 2004). In man, *Staphylococcus aureus* is a common bacterium found on the skin and nasal passages of healthy people. Approximately 25- 40% of the population is colonized with *S. aureus*. It is also a common cause of skin and soft tissue infections (Francois et al., 2005) and sometimes causes severe disease such as pneumonia, bacteremia, meningitis, sepsis, and pericarditis and food poisoning (Gao and Stewart, 2004; von-Eiff et al., 2001). In animals, *S. aureus* bacteria isolated and identified as an important opportunist that can cause

superficial to life – threatening illness in a variety of animal (White et al., 2003). *S. aureus* is one of the genus *Staphylococcus* which are gram positive, non-motile, non-spore forming, facultative anaerobic cocci, it is a bacterium of significant importance because of its capacity to adapt to diverse environmental forms showing multiple antibacterial resistant patterns. It could be resistant to methicillin and other β -lactam antimicrobials and are referred to as methicillin-resistant *S. aureus* (MRSA). Methicillin-resistant *Staphylococcus aureus* are known as a type of bacteria that is resistant to certain antibiotics including methicillin, oxacillin, penicillin, amoxicillin (Wichelhaus et al. 1997). Determination the levels of antibiotics resistant in *S. aureus* strains may serve to character this pathogen and could be used to limit the risks associated the failing in treatment of diseased conditions (Tenover and McGowan, 1996; Acar

and Rostel, 2001). Facing the risk released from MRSA of animal origin is of high significant, the studies showed the widespread occurrence of MARS in animal species and in human with several studies showed transmissions in both directions. Thus, MRSA was of great concern in both veterinary and human medicine as it serious illness in both sets of populations (Saleha and Zunita, 2010). Asymptomatic colonization with MRSA including both nasal and rectal carriage, has been reported in animals, the organism can colonize more than one site. Carrier animals may serve as a reservoir for disease in themselves, and may transmit MRSA to other animals or people (The Center for Food Security and Puplic Health, 2011). This study aimed to isolate and identify *S. aureus* from small ruminant in different healthy condition also to find out the prevalence of MRSA in nasal and fecal swabs. Also to spot light on the antimicrobial phenotype of the isolated MRSA.

2. Materials and Methods

2.1. Sample collection:

Samples were collected from 95 sheep and 86 goats from different localities in Alexandria and El-Behira governorates. Two samples; nasal and fecal swabs, were collected from each animal individually making a total of 362 samples (190 from sheep and 172 from goats). The swabs were transported immediately to the laboratory in nutrient broth at 4°C under complete hygienic condition.

2.2. Isolation and identification of *Staphylococcus aureus*:

Samples were incubated in nutrient broth at 37°C for 24h. 1 ml of each sample was streaked on Baird parker's agar plates supplemented with egg yolk tellurite. Plates were incubated at 37°C for 24- 48 hours, suspected colonies (black, shiny with narrow white margin and surrounded by clear zone extending into the opaque medium). For confirmation, suspected colonies were picked up and streaked on mannitol salt agar medium (yellow colonies) and further, were sub-cultured on blood agar plates and incubated at 37°C for 24- 48 hours. Brain heart infusion agar purified isolates were subjected to Gram staining and biochemical testing according to Harrigan (1998).

2.3. Antimicrobial susceptibility testing

The Antimicrobial susceptibility testing by using disc diffusion method was carried out according to the Clinical laboratory standards Institute (CLSI, 2012). The following antibiotics were used: β . Lactam antimicrobials: Methicillin (ME; 5 μ g),

Ampicillin (A; 10 μ g), Cloxacillin (OB; 5 ug), Oxacillin (OX; 1 μ g), Amoxycillin (AML; 25 μ g), Amoxycillin/Clavulanic acid (AMC; 20 μ g) and Pencillin (P; 10 IU). Aminoglycosides: Gentamycin (G;10 μ g) and Fluoroquinolones; Ciprofloxacin (CIP; 5 μ g) (Koneman et al., 1992; Rocha et al., 2014).

3. RESULTS

3.1. Incidence of *Staphylococcus aureus* in nasal and fecal swabs from sheep and goats in different health conditions:

Staphylococcus aureus isolated from sheep and goats in different healthy conditions gave typical characteristics of gram positive cocci appearing as clusters that were +ve catalase, -ve coagulase and gave white golden yellow pigmentation. The prevalence of *Staphylococcus aureus* in apparently healthy sheep and goats in were 37.5% and 6.25%, respectively, in pneumonic cases were 52.1% and 34.3%, respectively, in enteric cases were 59.9% and 26.9%, respectively, while in pneumoenteric cases were 37.5% and 28.5%, respectively as shown in Table (1).

3.2. Coagulase activity of *Staphylococcus aureus* isolated from sheep and goats:

Positive coagulase activity of *Staphylococcus aureus* isolated from sheep and goats Table (2) in apparent healthy conditions was 66.6% and 0%, respectively. In Pnemonic animals it was 44% and 48.4%, respectively, in enteric ones were 66.6% and 71.4%, respectively, while in Pnemoenteric conditions were 66.6% and 40%, respectively.

3.3. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from nasal and fecal swabs from sheep and goats in different health conditions

Antimicrobial susceptibility was observed in *S. aureus* isolates from all samples from nasal and fecal swabs. As shown in Table (3), complete resistance was noted for Amoxycillin, Amoxycillin/Clavulanic acid and Pencillin. While *S. aureus* isolated from nasal and fecal swabs from sheep with pneumointeritis were 50 % resistant to methicillin and oxacillin. All *S. aureus* isolates were 100 % sensitive to ciprofloxacin and gentamycin. Multiple antibiotic resistances were detected in all tested isolates. Nasal and fecal isolates from goat with pneumonia showed variability in their resistance to methicillin and oxacillin.

Table (1): Incidence of *Staphylococcus aureus* in nasal and fecal swabs from sheep and goats in different health conditions

Source	No. of Sheep samples	<i>S. aureus</i> +ve			No. of Goat samples	<i>S. aureus</i> +ve		
		Nasal No (%)	Fecal No (%)	Total No (%)		Nasal No (%)	Fecal No (%)	Total No (%)
Apparently Healthy	24	6 (25)	3 (12.5)	9 (37.5)	16	4 (25)	2 (12.5)	6 (6.25)
Pneumonia	96	30 (31.25)	20 (20.8)	50 (52.1)	96	25 (26)	8 (8.3)	33 (34.3)
Enteritis	30	13 (43.3)	5 (16.6)	18 (59.9)	26	4 (15.3)	3 (11.5)	7 (26.9)
Pneumoenteritis	40	8 (20)	7 (17.5)	15 (37.5)	34	5 (14.2)	5 (14.2)	10 (28.5)
Total	190	57 (30)	35 (18.4)	92 (48.4)	172	38 (21.9)	18 (10.4)	56 (32.3)

Table (2) Coagulase activity of *Staphylococcus aureus* isolated from nasal and fecal swabs from sheep and goats

Source	<i>S. aureus</i> +ve		+ve Coagulase activity			
	sheep	Goat	Sheep		Goat	
			Nasal No (%)	Fecal No (%)	Nasal No (%)	Fecal No (%)
Apparent healthy	9	6	4 (44.4)	2 (22.2)	0	0
Pneumonia	50	33	15 (30)	7 (14)	10 (30.3)	6 (18.1)
Enteritis	18	7	7 (38.8)	5 (27.7)	3 (42.8)	2 (28.5)
Pneumoenteritis	15	10	5 (33.3)	5 (33.3)	2 (20)	2 (20)
Total	92	56	31 (33.6)	19 (20.6)	15 (26.8)	10 (17.8)

4. DISCUSSION

Different parts of animal body as skin, oral cavity, digestive system, respiratory tract often come in contact with microorganisms. Staphylococci are one of them; it may inhabit normally on skin, and may invade other systems of the animal causing diseases (Quinn et al., 2002). The coagulase – positive *Staph aureus* are important pathogens in farm animals and are of particular importance as primary cause of specific diseases (Dwight et al., 2004). So, antibiotics broadly used in farm animals for treatment of different diseases, and for prophylaxis and growing purposes (Waters et al., 2011), misusing of antibiotics led to a wild world problem due to development of resistant among bacterial populations (Shryock and Richwine, 2010). *Staph. aureus* clinically generates ability of resistant to Methicillin and other β -lactam antibiotics, Methicillin-resistant *S. aureus* emerged in recent decades to become a leading cause of infection worldwide, colonization with

MRSA predisposes to infection and facilitates transmission of the pathogen; (Bonggoo et al., 2011), Table (1) illustrated that, a total of 190 and 172 of sheep and goat samples in different healthy conditions (12 sheep / 8 goat apparently healthy, 48 sheep / 48 goat have pneumonia, 15 sheep / 13 goat suffering from enteritis, 20 sheep / 17 goat have pneumoenteritis) *S. aureus* was recorded in relation to types of swab (nasal – fecal / animal) as follow: *S. aureus* in apparently healthy, pneumonia, enteritis, pneumoenteritis cases of sheep were 25, 31.2, 43.3, 20 %, respectively, in nasal swabs and 12.5, 8.3, 20.8, 16.6 %, respectively, in fecal swabs, while in goat were 25, 26, 15.3, 14.2 %, respectively, in nasal swabs and 12.5, 8.3, 11.5, 14.2 %, respectively, in fecal swabs. there was a difference in the occurrence of *S. aureus* in both species as a total, in sheep *S. aureus* were 30, 18.4 %, respectively, while in goat were 21.9, 10.4 %, respectively.

These results were lower than those reported by Anna-Katarina *et al.*, (2012) who sampled feces and nasal liquid of 48 animals (28 goats, 20 sheep). It was observed that the presence of *Staphylococcus* spp. in 75% of both sheep and goats could be demonstrated. Emikpe *et al.*, (2009) investigated 60 apparently healthy West African Dwarf goats. The nasal swab from each goat was analyzed using standard methods, *Staphylococcus aureus* were the second dominant bacteria after *E. coli*. Al-Thani and Al-Ali, (2012) could isolate *S. aureus* from sheep at 2 out of 6 fecal samples and couldn't detected in nasal swabs while in goat could isolate *S. aureus* at 2 out of 7 samples of nasal swabs and 2 out of 7 samples of fecal swabs. This difference between our results and other results is due to bad hygienic conditions, immune status of the animals and stress factors (weather, diseases, poor management) as reported by Anna-Katarina *et al.*, (2012) and Emikpe *et al.*, (2009). All *S. aureus* isolates were catalase positive while Table (2) cleared that the total coagulase positive *S. aureus* in sheep were 33.6 % in nasal swabs and 20.6 % in fecal swabs, while in goat were 26.8 % in nasal swabs and 17.8 % in fecal swabs. It is recorded that coagulase positive *S. aureus* were in nasal swabs more than fecal swabs. Al-Thani and Al-Ali, (2012) detected coagulase positive *S. aureus* of fecal and nasal swabs in 43% of the isolates. LeLoir *et al.*, 2003 mentioned that, *S. aureus* is the most important coagulase-positive pathogen due to a combination of toxin-mediated virulence, invasiveness and antibiotic resistance. By studying the resistance pattern of coagulase-positive *S. aureus* isolated from nasal swabs and fecal swabs of sheep and goat in different healthy cases (Table 3), it is noticed that positive coagulase *S. aureus* isolated from nasal or fecal swabs in different healthy conditions of sheep and goat were 100% resistant to Ampicillin, Amoxicillin, Pencillin, while, *S. aureus* isolated from nasal and fecal swabs in pneumointeritis of sheep and goat were 50% resistant to Methicillin and Oxacillin. Ciprofloxacin as a drug of Aminoglicosides group and Gentamycin as a drug of Fluoroquinolones group were drugs of choice in treatment of different diseases caused by *S. aureus*. Alzohairy (2011) reported 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, he recovered 176 (52.7%) coagulase-negative *Staphylococcus* and 158 (47.3%) coagulase-positive *Staphylococcus*, among them 90 (57%) were MRSA and 68 (43%) were methicillin-sensitive *Staphylococcus aureus* (MSSA). Al-Thani and Al-Ali, (2012) found that 3 out of 14 and 11 out of 14 strains of *S. aureus* were resistant to Ampicillin and penicillin, respectively. 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 as reported by Schwarz *et al.*, (1998); Alekshun and Levy (2000). While Haythem Gharsa *et al.*, (2012) detected MRSA in only 3% of nasal swabs of sheep. Klevens *et al.*, (2006) identified that the prevalence of MRSA among clinical *S. aureus* isolates vary between below 1% and above 40% worldwide. Cuteri *et al.*, (2004) characterized 32 (14.34%) from animals and 53 (63.8%) from men strains of *S. aureus* and showed resistance to methicillin. Silva *et al.*, (2008) investigated *Staphylococcus aureus* from apparently healthy cattle in the State of Paraiba, Brazil, in relation to resistance to 21 antimicrobial agents. Among the 46 isolates obtained, resistance to penicillin was most frequent, followed by resistance to streptomycin, tetracycline, erythromycin and kanamycin/neomycin. All isolates were susceptible to gentamicin and methicillin and only six isolates were susceptible to all the drugs tested. With respect to the antibiotics, multi-resistant isolates were common. Islam *et al.*, (2007) presented that the antibiotic resistant pattern of the coagulase-positive *S. aureus* showed 83.33% isolates to be resistant to Penicillin-G.

On the other hand, the results demonstrated that gentamicin, spiramicin, ciprofloxacin, oxacillin, oxytetracycline and streptomycin might be used for the treatment of *S. aureus* infection. In conclusion, the prevalence of infection with *S. aureus* in sheep was higher than in goats in different healthy conditions. Further, high prevalence of MRSA in sheep and goats of different healthy conditions was detected raising concerns about the role of these animals as reservoirs of MRSA, which is considered to be of great concern in both veterinary and human health. Moreover, apparently

healthy animals should be taken in consideration as a possible reservoir of MRSA.

5. REFERENCES

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3.4. Table (3): Antimicrobial resistance pattern of coagulase positive *Staphylococcus aureus* isolated from nasal and fecal swabs from sheep and goats in different health conditions:

Species	Health condition	Antimicrobial resistance						
		Methicillin ME (5 µg)	Ampicillin A (10 µg)	Cloxacillin OB (5 ug)	Oxacillin OX (1 µg)	Amoxycillin AML (25 g)	Amoxycillin/ Clavulanic acid AMC (20 µg)	Pencillin P (10 IU)
Sheep (N)	Apparent healthy	100	100	50	100	100	100	100
Sheep (F)	Apparent healthy	100	100	100	100	100	100	100
Sheep (N)	Pneumonia	50	50	100	50	100	100	100
Sheep (F)	Pneumonia	50	100	50	100	100	100	100
Goat (N)	Pneumonia	100	100	100	50	100	100	100
Goat (F)	Pneumonia	50	100	100	100	100	100	100
Sheep (N)	Enteritis	100	100	100	50	100	100	100
Sheep (F)	Enteritis	100	100	100	100	100	100	100
Goat (N)	Enteritis	0	100	100	100	100	100	100
Goat (F)	Enteritis	50	100	100	100	100	100	100
Sheep (N)	Pneumoenteritis	50	100	100	50	100	100	100
Sheep (F)	Pneumoenteritis	50	100	100	50	100	100	100
Goat (N)	Pneumoenteritis	50	100	50	50	100	100	100
Goat (F)	Pneumoenteritis	50	100	100	50	100	100	100

N; nasal sample, F; fecal sample.

Ciprofloxacin and Gentamycin were not included in the table as all the isolates were 100 % sensitive to them.

Antibiotic sensitivity test carried out on 28 samples from sheep and goats (nasal and fecal swab sample)