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Detection of Methicillin Resistant *Staphylococcus aureus* Isolated from Human and Animals in Basra Province / Iraq

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Abstract

During the period from October 2010 to March 2011, two hundred eighty-five specimens were collected from AL-Basra province and surveyed for the occurrence of methicillin resistant *Staphylococcus aureus* (MRSA). Depending on the source of collection, specimen was divided into 6 groups (124 samples of cow milk, 25 samples of cow nasal swabs, 56 samples of sheep nasal swabs, 20 samples of goat nasal swabs, 33 samples of human nasal swabs (obtained from nosocomial infection) and 27 samples of environmental swabs). Totally, *S. aureus* were identified from 72 samples, these consisted of 35/72 (48.61%) isolates from cow milk, 1/72 (1.38%) isolate from cow nasal swabs, 7/72(9.72%) isolates from sheep nasal swabs, 1/72 (1.38%) isolate from goat nasal swabs, 19/72(26.38%) isolates from human nasal swabs and 9/72(12.5%) isolates from environmental swabs, depending on morphological, cultural, microscopical characterization and biochemical tests. The 72 *S. aureus* isolates showed variability in its susceptibility to 18 different antibiotics. In conclusion, this study investigated the presence of methicillin resistant *Staphylococcus aureus* in animals and human samples.

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Introduction

Staphylococcus aureus is bacterium found passively colonizing skin and nasal passages of healthy humans and animals (Rohde, 2011), though this opportunistic pathogen colonizes without causing disease (Davis *et al*., 2004). Nasal carries of *S. aureus* bear an increased risk to become septic, once bacteria gain access to the bloodstream due to breaches in the nasopharyngeal or other mucosal colonized niches (Wertheim *et al*., 2004; Wertheim *et al.*, 2005). The association of owners and veterinarian staff with human healthcare sector (HCS) and animal –related characteristics such as signalment,

antimicrobial, immunosuppressive therapy and surgery were evaluated as putative risk factors using logistic regression (Magalhaes *et al.*, 2010).

Methicillin–resistant *Staphylococcus aureus* (MRSA) has become important acquired pathogen in hospital and also livestock (LA-MRSA) in recent years. MRSA associated with (LA-MRSA) have been reported worldwide in many species (Persoons *et al*., 2009; de Neeling *et al*., 2007; Smith *et al*., 2008). MRSA produce a low affinity penicillin binding protein (PBP2 orPBP2a) in addition to the usual PBPs (Hartman and Tomasz, 1984). Furthermore, MRSA strains are resistant to gentamicin, kanamycin, tobramycin, microldes, tetracycline and fluoroquinolones. Thus, multiple resistance of *S. aureus* strains occurs (Chambers *et al*., 1997; Petinaki *et al*., 2001; Maddox, 2011). For humans, *S. aureus* are important causes of food poisoning, pneumonia,wound infection and nosocomial bacteremia (Horan *et al*.,1988). *S. aureus* also expresses certain virulence factors and due to these virulent determinants, it is tenacious, potentially destructive and shows increasing resistance to antimicrobial agents (Burriel, 1998).

Staphylococcus spp. causes severe disease such as mastitis (Hassan and Yousif 2013), arthritis and urinary tract infection by introducing numerous virulence factors such as extracellular toxins and enzymes into animal species (Waldvogel, 1990). This study intends to detect the methicillin resistant *Staphylococcus aureus* isolated from human and animals in Basra Province / Iraq.

Materials and Methods

Samples collection

Two hundred eighty- five samples were collected from animals, human and environment during the period from October, 2010 to March, 2011 from Basra villages. One hundred and twenty-four milk samples were collected from cow with clinical mastitis and normal milk cow, 25 cow nasal swabs, 56 sheep nasal swabs and 20 goat nasal swabs. Thirty-three samples were collected from human nasal swabs from AL-Basra General hospital in Basra city. Twenty-seven samples were collected from open area of AL-Basra General hospital in Basra city.

Laboratory diagnosis

The specimens were directly inoculated onto mannitol salt agar (MSA) and incubated at 37 ºC for 24 hrs. All colonies from primary cultures were purified by subculture onto MSA medium and incubated at 37ºC for 24- 48 hrs. (Talan *et al.*, 1989). Gram stain were investigated according to Barrow and Feltham, (2003).
Biochemical tests

Free coagulase Test

One ml of 18 hrs culture broth was added to 0.1 ml of human plasma without dilution and incubated at 37 ºC for 4 hrs. The clotting hourly noticed. The appearance of the clotting indicates a positive result comparable to control (Macfaddin, 2000).

Catalase Test: A small amount of pure growth was transferred with a wooden stick from MSA into clean slide, then a drop of catalase reagent was added. The evolution of gas bubbles indicates a positive result (Macfaddin, 2000).
Oxidase Test: A filter paper was moistened with several drops of oxidase reagent 1% then a small portion of the colony was removed with a sterile wooden stick rabbed on moistened filter paper. A positive reaction is indicated by the appearance of dark or deep purple color within 10-20 sec. (Macfaddin, 2000).
Nitrophenl-B-D-galactopyranoside (ONPG): Small portion from the colony was mixed with 1 ml of D.W in sterile tube and homogenized then a disc of ONPG was added. The incubation took place at 37 ºC and the results were read after 1- 4-24 hrs. The colourless and yellow color indicates negative and positive results, respectivily (Macfaddin, 2000).
DNase Production Test: Over-night incubated of bacterial isolates were streaked on DNase agar and incubated at 35 ºC for 24hrs. The bacterial growth was flooded with 1N hydrochloric acid (HCl). The appearance of clear zone around of the colonies indicates positive result (MacFaddin, 2000).

Antibiotics susceptibility test

The antibiotic susceptibility testing was done by the agar discs diffusion method as described by Kirby and Bauer, (1966). Three isolated colonies of the same morphological type were selected from the agar plat culture. The top of each colony was touched with a loop and the growth was transferred into a tube containing 5 ml brain heart infusion (BHI) and incubated at 35 ºC for 48 hrs. The turbidity of the actively growing broth culture was adjusted to the 0.5 McFarland standard.
Fifteen minutes post the adjustment sterile cotton swab was dipped into adjusted suspension, then rotated several times and pressed firmly on the side of the tube above the fluid level. The dried surface of a Mueller–Hinton agar (MHA) plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of the inoculums. The predetermined antimicrobial disks were dispensed on to the surface of the inoculated agar plate. Each disk was pressed down individually to ensure complete contact with agar surface. The plates were for 18hrs, at 35 ºC. The resulting zone of inhibition was uniformly circular with confluent lawn of growth. The diameters of the zones of complete inhibition were measured, including diameter of the disk. The size of inhibition zones was interpreted by referring to zone diameter interpretive standard from Bioanalyse sensitivity discs Ankara/Turkey

Results

Isolation and Identification of *Saphylococcus aureus*

Cultural and biochemical examination of 285 samples revealed isolation of 72 S. *aureus* strains. The highest rate of *S. aureus* isolates was observed in human nasal swab19/33(57.57%) followed by environmental swab 9/27(33.33%), cow milk 35/124(28.22%), then sheep nasal swab7/56(12.5%), while goat nasal swab and cow nasal swab where found to have the lowest rate 5% and 4% respectively, with significant

differences (P< 0.05) in the rate of *S.aureus* isolation among the different samples as showed in table 1. The present study revealed that (90.90%) of staphylococcal isolates from human nasal samples were positive to coagulase test followed by environmental swabs (81.48%), cow nasal swabs and goat nasal swabs (80 %), sheep nasal swabs (75%) and while cow milk (65.32%). There were no significant differences (p>0.05) between numbers of isolates in different samples. DNase test revealed that the highest percentage was showed in human nasal swabs were (75.75%), followed by environmental swabs (70.37%), cow milk (33.87%), sheep nasal swabs (26.78%), goat nasal swabs (25%) and cow nasal swabs (24%). There were significant differences (P < 0.05) between isolates of numbers in different samples.

Table (1): Number and percentage of *S. aureus* isolated from different sources

Type of samples	No. of samples	No. S. Aureus	(%)
Cow milk	124	35	(28.22)
Cow nasal swab	25	1	(4)
Sheep nasal swab	56	7	(12.5)
Goat nasal swab	20	1	(5)
Human nasal swab	33	19	(57.57)
Environmental swab	27	9	(33.33)
Total	285	72	(25.26)
$X^2 = 90.563$ (p<0.05)			

In ONPG test the highest percentage in *staphylococcal* human nasal samples were (57.57%), followed by environmental swabs, cow milk, sheep nasal swabs which were (33.33%), (28.22%), (12.5%) respectively, while the lowest percentage observed in goat nasal swabs and cow nasal swabs were (5%and 4%) respectively. There were significant differences (p<0.05) between numbers of isolates in samples.

All staphylococcal isolates from different sources showed catalase positive and oxidase negative (Table 2).

Screening for Methicillin (Oxacillin) Resistance S. aureus

By using disc diffusion method, 72 S. aureus isolates were tested for susceptibility toward oxacillin (table 3). Total of 25(34.72%) of S. aureus were MRSA which were isolated from this study. The highest percentage of methicillin resistance was in human nasal swabs (42.1%), followed by cow milk (40%) then environmental swabs (33.33%). While sheep nasal swabs, goat nasal swabs and cow nasal swabs were sensitive to methicillin. There were no significant differences (P > 0.05) in the percentage of MRSA was isolated from different samples.

Antibiotic susceptibility testing of *S. aureus* isolates

The susceptibility to ceftriaxone was (100%), to cefotaxime (90.27%), to ampicillin (61.11%), vancomycin (59.72%), carbenicillin (56.94%), oxacillin (34.72%), lincomycin

(38.88%), penicillin (33.33%), The less susceptible results were showed to gentamycin (23.61%), erythromycin (15.27%), doxycycline (11.11%), teicoplanin (9.72%), clindamycin (8.33%), ciprofloxacin (6.94%) and nitrofurantoin, chloramphenicol, tobramycin and azithromycin (0%).There were significant differences (P<0.05) of *S. aureus* isolates between different types of antibiotics Figure (1).

Table (2) Biochemical test of *Staphylococcus aureus* isolates from different sources

Test / Samples	Coagulase + No. (%)	DNase + No. (%)	ONPG – No. (%)	Catalase + No. (%)	Oxidase- No. (%)
Cow milk	81/124 (65.32)	42/124 (33.87)	35/124 (28.22)	124/124 (100)	124/124 (100)
Cow nasal swab	20/25 (80)	6/25 (24)	1/25 (4)	25/25 (100)	25/25 (100)
Sheep nasal swab	42/56 (75)	15/56 (26.78)	7/56 (12.5)	56/56 (100)	56/56 (100)
Goat nasal swab	16/20 (80)	5/20 (25)	1/20 (5)	20/20 (100)	20/20 (100)
Human nasal swab	30/33 (90.90)	25/33 (75.75)	19/33 (57.57)	33/33 (100)	33/33 (100)
Environmental swab	22/27 (81.84)	19/27 (70.37)	9/27 (33.33)	27/27 (100)	27/27 (100)
Average (%)	(78.84)	(42.62)	(23.43)	(100)	(100)
X^2	4.653 (P>0.05)	66.547 (P<0.05)	90.78 (P<0.05)	0	0

Table (3) Susceptibility of *S. aureus* isolates to the methicillin (oxacillin)

Samples	No. of strains	No.MRSA/ strains (%)	No.MSSA/ strains (%)
Cow milk	35	14(40)	21(60)
Cow nasal swab	1	0(0)	1(100)
Sheep nasal swab	7	0(0)	7(100)
Goat nasal swab	1	0(0)	1(100)
Human nasal swab	19	8(42.1)	11(57.9)
Environmental swab	9	3(33.33)	6(66.66)
Total	72	25(34.72)	47(65.28)
X^2		1.165(P>0.05)	27.821(P<0.05)

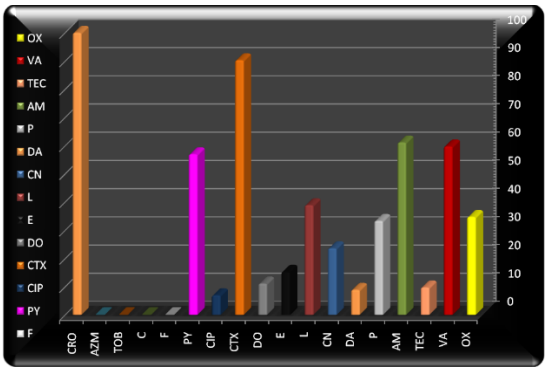

Figure (1). The percentage of antibiotics susceptibility of *S. aureus* isolates.
Ceftriaxone(CRO), azithromycin(AZM), tobramycin(TOB), chloramphenicol(C), nitrofurantoin(F), carbenicillin(PY), ciprofloxacin(CIP), cefotaxime(CTX), doxycycline(DO), erythromycin(E), lincomycin(L), gentamycin(CN), clindamycin(DA), penicillin(P), ampicillin(AM), teicoplanin(TEC), vancomycin(VA), oxacillin(OX).

Discussion

Staphylococcus aureus is one of the most commonly identified pathogens in human medicine and is the major cause infections of nosocomial and animals (Boyce *et al*., 1983; Rohde, 2011) and community-acquired infections (Naimi., *et al*., 2003; Said-Salim *et al*., 2003). The present study agreed with a number of studies dealing with human, AL- Saady, (2007), who isolated *S. aureus* from different samples of human in percentage of (66.7%). Abbas, (1989) isolated *S. aureus* from healthy nutrition workers (carriers) in the Hospital of Medical City of Baghdad, the percentage of *S. aureus* isolated from nasal swab range between 18-34%, from hand, 29-55%, from throat swab, 9-18%, while the higher percentage was isolated from old workers, 62-80%. The percentage of *S. aureus* isolated from human in different samples appeared 32.6% at the study of (Saleh, 1990). The causes of this distribution of *S. aureus* in different hosts is due to number of virulence factors which help to colonize, invade and infect different hosts. The present study showed that *S. aureus* are present in human nasal swabs 57.57%, environmental swabs 33.33% and cow milk 28.22%, when compared to other sources, they form low levels isolations. These results are in line with Hanon, (2009), who reported that *S. aureus* was isolated from bovine milk, 48.57% and nasal swab, 52.85%, while the isolation percentage from human was 59.83%, stool 55.2%, urine 86.06% and nasal swab 63.33%.
Seventy-Two *S. aureus* isolates were tested against various antibiotics. The results showed that beta-lactam antibiotic (oxacillin), showed percentage of susceptibility 65.28%. This result is agreement to the study of Hanon, (2009), who mentioned that the percentage of susceptibility of oxacillin was 52.5%. It is also similar to the findings that were found in the study of Omer, (2010) who detected the MRSA in a percentage of (50%) from the buffalo milk. Compared with the present study, low percentage of MRSA were detected in bovine milk by Idbeis, (2010), Farzana *et al*., (2004) and Devriese *et al*., (1997), whom recorded percentage of MRSA were 10.52%, 10%, and 10% respectively.

In the present study, susceptibility of *S. aureus* isolates to vancomycin was 40.28%. Similar finding 5was obtained in the study of Hanon (2009) who recorded that 55% of *S.*

aureus isolates from bovine were sensitive to vancomycin. On the other hand, all *S. aureus* isolates were 100% sensitive to vancomycin (Falcao *et al*., 1999; Santos *et al*., 1999; Panhotra *et al*., 2005; Bendahou *et al*., 2008; AL- Khudheiri, 2008; Idbeis, 2010). AL-Saady, (2007) mentioned that *S. aureus* isolated from human in different samples revealed complete resistance to vancomycin. The antibiotic sensitivity testing reveals that *S. aureus* isolates were sensitive 100% to antibiotics like nitrofurantion, chloramphinicol, tobramycin, and azithromycin for each one. These results are in line with the study of Panhotra *et al*., (2005), who mentioned that the sensitivity to chloramphinicol was 100%. These results are also in agreement with Bendahou *et al*., (2008), who reported that *S. aureus* isolates from raw milk and milk product appeared to be sensitive to tobramycin 95%, nitrofurantion 100%, chloramphinicol 95%. AL-Khudheiri (2008), and Santos *et al*., (1999), stated that *S. aureus* isolates were resistant to chloramphinicol 58.4% and 85%, respectively. The sensitivity of *S. aureus* isolates to gentamycin was 76.39%. A similar finding was obtained by the study of AL-Marsomy (2008), who recorded sensitivity of S aureus isolates from mastitis togentamycin was 76.8%, but the highest sensitivity (100%) was found by the study of (Ismaiel, 1986; Abd-AL-Rahman, 1989 and Bendahou *et al*., 2008).

Staphylococcus. aureus isolates from different sources showed 84.73% susceptibility to erythromycin. This result is in agreement with the study of Ismaiel, (1986), who mentioned that 89% of *S. aureus* being isolated from bovine to have appeared sensitivity to erythromycin. Bendahou *et al*., (2008), on the other hand revealed that *S. aureus* being isolated from raw milk and milk product represented high susceptibility (90%) to erythromycin. Also, Hanon, (2009), reported that sensitivity of *S. aureus* to isolated from human and animals to erythromycin were 25% and 37.5% respectively.

In the present study, *S. aureus* isolates were (38.88%) and (8.33%) resistant to lincomycin and clindamycin, respectively. These results agree with the study of Bratu *et al*., (2005), who found the resistant of *S. aureus* isolated from hospital nursery and maternity units to lincomycin and clindamycin during 1999, 2001 and 2003, were 18%, 15% and 20%, respectively. Hanon, (2009), mentioned that the sensitivity of *S. aureus* to erythromycin was (52.5%) in bovine, but it was (70%) in human. Finally, we conclude that the samples collected from human or animal can be contaminated with drug resistance *S. aureus* which involved in serious health problems.

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