



BACTERIOLOGICAL AND MOLECULAR COMPARATIVE STUDY BETWEEN *Staphylococcus aureus* ISOLATED FROM ANIMALS AND HUMAN

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ABSTRACT

Staphylococcus aureus represents a serious health hazard on both animals and humans. The main goal of the present work was to compare between *S. aureus* isolated from animal and human origin. This was achieved through studying the virulence, phenotypic characterization, genotypic testing of *mecA* gene, analysis the antibiotic resistance profile of the recovered *S. aureus* isolates. A total number of 165 samples were collected from clinical mastitic cows and sheep, pus from abscesses collected from septic wounds of infected animals, respectively. Moreover, blood, pus swabs from abscesses and septic wounds, and sputum samples were collected from diseased humans from Assiut Governorate, Egypt. The results revealed that incidence of *S. aureus* isolates recovered from the examined animal samples were 8.33%, 100% and 20%, per, while from human cases (blood, pus and sputum) were 16.67%, 53.49% and 75%, independently. Using Staphaurex kits, the prevalence of coagulase positive *S. aureus* from animal samples reached 8.33%, 100% and 20%, but from the diseased human cases were 16.67%, 53.49% and 75%, respectively. Antimicrobial Sensitivity Testing of the animal isolates were resistant to cefoxitin (15.79%), tetracycline (10.53%), clindamycin and erythromycin (10.53%), while the isolates were sensitive to gentamicin (100%), trimethoprim + sulfamethoxazole and vancomycin (94.74% for each). However, *S. aureus* human isolates were resistant to cefoxitin (75%) and tetracycline (78.57%) and sensitive to vancomycin (100%), ciprofloxacin (89.29%) and trimethoprim+ sulfamethoxazole (82.14%). Out of 47 identified *S. aureus* strains, 3 from animal isolates (15.79%) and 21 from human isolates (75%) proved to be methicillin-resistant (MRSA). Furthermore, one animal isolate (5.26%) and 12 human isolates (42.86%) were multi-drug resistant (MDR). *S. aureus* isolates from animal and humans were subjected to genotypic characterization of *mecA* gene using PCR. All the animal and human isolates were positive for *mecA* gene with a percentage of 100%. The results of this study provide that from both animals and human samples; the isolation rate of *S. aureus* was greater than that of MRSA followed by MDR.

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INTRODUCTION

Staphylococcus aureus is a commensal microorganism and the most virulent of all staphylococci (Jenkins *et al.*, 2015). *S. aureus* strains produce a wide spectrum of protein toxins and virulence factors, which contribute to the pathogenicity of this organism so, pathogenic to both human and

animals (Zecconi and Scali, 2013). In bovines, it is responsible for intra-mammary infection and milk-safety problems, in dairy herds, it is the main etiological agent of contagious clinical/sub-clinical mastitis (Taverna *et al.*, 2007) because it is very well adapted to colonize on the teat skin and teat canal, as milk is a very suitable medium for the growth of many pathogens (Nam *et al.*, 2011).

In human, it causes range of illnesses such as Staphylococcal Scaled Skin Syndrome, toxic shock syndrome and food poisoning by releasing superantigens into the blood stream (Todar, 2005), pneumonia, phlebitis, mastitis, meningitis and deep-seated infections, such as osteomyelitis and endocarditis (Morya et al., 2012), septic arthritis (Edwards and Massey, 2011), in addition to be the major cause of numerous hospital and community acquired infections (Gill et al., 2005). The development of these secondary infections is due to bacterial dissemination from the blood into the surrounding tissues and is associated with significantly increased morbidity and mortality (Edwards and Massey, 2011).

MRSA characteristic phenotype is due to an extra penicillin-binding protein (PBP2a) coded by the *mecA* gene (Rei, 2012). In addition to the phenotypic characterization of bovine *S. aureus* isolates, PCR is valuable in genotypic characterization as in detection of *mecA* gene (McClure et al., 2006).

The purpose of the present study was to investigate phenotypic and genotypic characterization of *S. aureus* isolates recovered from animals and humans. This was achieved by detection of their hemolytic activity, investigating their clumping factor, protein A and capsular polysaccharides using Staphaurex kits, detection of: coagulase enzyme by coagulase test, analysis the antibiotic resistance profile, and genotypic characterization of *mecA* gene using PCR technique of the recovered *S. aureus* isolates.

MATERIALS AND METHODS

1. Sampling

All the samples were collected under sterile conditions. A total of 165 samples (84 from animals and 81 from humans). The animal samples were collected from clinical mastitis cows' milk (n=40), clinical mastitis sheep's milk (n=14), and pus from septic wounds and abscesses of different animals (n=30). The human samples were collected from diseased human admitted to clinics of Assiut University Hospital in Assiut Governorate, Egypt (blood samples were collected in blood culture bottles), blood (n=13), pus (n=61) septic wounds, abscesses, and sputum (n=7). Then, samples were cultured on nutrient agar for pigmentation and mannitol salt agar then incubated at 37°C for 24h. The grown colonies were examined microscopically.

2. Preparation of the collected samples for isolation and identification of *S. aureus*

Animal quarter milk samples were identified according to NMC (2004).

3. Isolation of *Staphylococcus* spp.

Recovered from the collected samples was done according to Bailey and Scott (1974). The suspected bacterial colonies showing typical colonial appearance were picked up and stabbed into semisolid media and incubated at 37°C for 24 hours for another investigation.

4. Identification and characterization of *S. aureus* isolates (Quinn et al., 2002)

The suspected colonies were inserted into semisolid agar tubes for further identification and preservation of the isolates. They were identified according to diverse biochemical tests including pigmentation, Gram - staining, catalase, coagulase and hemolysis.

- According to Weist et al., (2006), Coagulase positive *S. aureus* (CoPS) was detected by using Staphaurex® kits: (Remel, TSMX7819 and 30859901 ZL30).
- All *S. aureus* isolates were tested for their antimicrobial sensitivity by using agar disk diffusion technique, (CLSI, 2014).

Table 1: The zone diameter interpretive criteria of antimicrobial disks used for *S. aureus* isolates:

Antimicrobial agents	Disk codes	Disk content	Zone diameter interpretive criteria (mm)		
			S	I	R
Cefoxitin	FOX	30 µg	≥ 22	-	≤ 21
Ciprofloxacin	CIP	5 µg	≥ 21	16-20	≤ 15
Clindamycin	DA	2 µg	≥ 21	15-20	≤ 14
Erythromycin	E	15 µg	≥ 23	14-22	≤ 13
Gentamicin	CN	10 µg	≥ 15	13-14	≤ 12
Tetrecycline	TE	30 µg	≥ 19	15-18	≤ 14
Trimethoprim+ Sulphamethoxazole	SXT	25 µg	≥ 16	11-15	≤ 10
Vancomycin	VA	30 µg	≥ 15	-	-

5. Molecular identification of *mecA* gene of *S. aureus* isolates

S. aureus genomic DNA extraction was done according to Bakshi et al., (2005) and Kumar et al., (2010).

- Amplification of the *mecA* gene of *S. aureus* isolates: (Spanu et al., 2004): Each PCR reaction tube was prepared with a total volume of 50 µl reaction mixture; 25 µl of PCR Master Mix (10X), 1-2 µl template DNA (25 µg -50 µg), 0.5 µl of each primer (10 pico mol/reaction of each primer)*

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The used primer is *mecA* gene primer according to **De Neeling et al. (1998)**: as (F) GTTGTAGTTGTCGGGTTTGG and (R) CTTCCACATACCATCTTCTTTAAC, for Amplification target Region of *S. aureus mecA* gene (337bp).

The DNA amplification was performed as

Initial denaturation (94°C for 4 min) then 30 cycles of (denaturation at 94°C for 30s, annealing at

50°C for 30s, extension at 72°C for 1min) then final extension at 72°C for 4min. Negative control reactions without any template DNA were carried out.

The procedure of agarose gel electrophoresis for identification and screening of the PCR products was carried out according to **Sambrook et al., (1989)** using 100 bp DNA ladder (Vivantis- Malaysia).

RESULTS

Table 2: Incidence of *S. aureus* and Coagulase negative *Staphylococci* among the examined animal samples

Type of collected samples	No. of examined samples	<i>Staphylococcus</i> isolates		<i>S. aureus</i>		Coagulase negative <i>staphylococci</i>	
		No.	%	No.	%*	No.	%*
Clinical mastitic cows' milk	40	24	60.00	2	8.33	22	91.67
Clinical mastitic sheep's milk	14	13	92.86	13	100	0	0
Pus	30	20	66.67	4	20	16	80
Total	84	57	67.86	19	33.33	38	66.67

% : was calculated according to the number of examined samples.

%* : was calculated according to the number of positive *Staphylococcus* spp. isolates.

Table 3: Incidence of *S. aureus* and Coagulase negative *Staphylococci* among the examined human samples

Type of the collected samples	No. of the examined samples	<i>Staphylococcus</i> Isolates		<i>S. aureus</i>		Coagulase negative <i>Staphylococci</i>	
		No.	%	No.	%*	No.	%*
Pus	61	43	70.49	23	53.49	20	46.51
Blood	13	12	92.31	2	16.67	10	83.33
Sputum	7	4	57.14	3	75	1	25
Total	81	59	72.84	28	47.46	31	52.54

% : was calculated according to the number of examined samples.

%* : was calculated according to the number of positive *Staphylococcus* spp. isolates.

Results of antimicrobial sensitivity testing of *S. aureus* recovered from the examined samples

All *S. aureus* isolates recovered from animal samples (19 isolates) as well as those recovered from human samples (28 isolates) were tested for their antimicrobial sensitivity.

Table 4: Results of antimicrobial sensitivity testing of *S. aureus* recovered from the examined samples

Animal isolates			Human isolates		
(S)			(S)		
Antibiotic	No	%	Antibiotic	No.	%
Gentamicin	19	100			
Trimethoprim+ sulfamethoxazole	18	94.7 4	Trimethoprim+ sulfamethoxazole	23	82.14
Vancomycin	18	94.7 4	Vancomycin	28	100
Ciprofloxacin	17	89.4 7	Ciprofloxacin	25	89.29
Cefoxitin and clindamycin	16	84.2 1	Clindamycin	22	78.57
(I)			(I)		
Tetracycline	6	31.5 8	Tetracycline	5	17.86
Erythromycin	4	21.0 5	Clindamycin	2	7.14
			Trimethoprim + sulfamethoxazole	2	7.14
(R)			(R)		
Cefoxitin	3	15.7 9	Cefoxitin	21	75
Tetracycline	3	15.7 9	Tetracycline	22	78.57
Clindamycin	2	10.5 3			
Erythromycin	2	10.5 3	Erythromycin	8	28.57

Table 5: Recovery rates of Methicillin Resistant *S. aureus* (MRSA) and Multi-drug resistant (MDR) strains among the isolated *S. aureus* strains.

Sources of the examined samples	No. of <i>S. aureus</i> isolates	MRSA		MDR	
		No.	%	No.	%
Animal	19	3	15.79	1	5.26
Human	28	21	75	12	42.8
Total	47	24	51.06	13	27.66

% was calculated according to the number of total positive organisms

PCR results for molecular detection of *mecA* gene in *S. aureus* strains: all the examined *S. aureus* isolates from both, animals and human, were positive for *mecA* gene (100%). They showed positive *mecA* gene amplification and produced amplicons of average molecular size of 337 bp.

DISCUSSION

S. aureus is one of the most virulent pathogenic *staphylococci*. Although it's a part of the normal flora of human; living on their skin and mucous membranes; in the nose and in the intestines of about 20% of the people (**Frank et al., 2010**).

From bovine mastitis samples, golden-yellow colonies on nutrient agar media, which were Gram-positive cocci, catalase-positive, coagulase-positive, and induced complete and/or incomplete hemolysis on blood agar, were isolated and identified as *S. aureus*, which agrees with **El Behiry et al., (2012)**. *S. aureus* was isolated from 81 diseased human cases admitted to clinics of Assiut University Hospital (AUH), Egypt but, **Mempel et al., (2003)** isolated enterotoxin gene cluster of *S. aureus* from healthy carriers.

A total of 57 (67.9%) *Staphylococci* spp. isolates were recovered from the examined animals, where 24 isolates from clinical mastitis cows' milk samples (60%), 13 from clinical mastitis sheep's milk (92.9%) and 20 pus samples from the animal septic wounds and abscesses (66.7%). The obtained results are higher than that reported by **Kerro and Tareke (2003)** who isolated staphylococci from subclinical mastitis cases (39.2%). Also, staphylococci were isolated from subclinical mastitis in dairy cows by **Banerjee et al., (2002)** with a percentage of 54.9%. On the other hand, a lower incidence of *Staphylococcus* species (28.3%) isolated from milk samples and septic wounds from bovines' dairy farms, was obtained by **El-Jakee et al., (2010)**.

Coagulase is one of the diagnostic enzymes produced by *S. aureus*. It was used to indicate their virulence. However, after performing coagulase and Staphaurex kits, the present study showed that, from the 81 examined diseased human samples, 59 isolates were staphylococci spp. (72.8%). Out of 12 (92.3%) positive staphylococci from human blood, only 2 were *S. aureus* (16.7%), out of 43 (70.5%) positive staphylococcal pus samples from human septic wound and abscesses, only 23 were *S. aureus* (53.5%) and out of 4 (57.1%) positive staphylococcal sputum samples, only 3 were positive (75%). So, the total *S. aureus* isolates from the present work was 28 isolates (47.5%) and the coagulase negative staphylococci (CoNS) were 31 isolates (52.5%) represented as; 20

isolates from the pus samples (46.5%), 10 isolates from the blood samples (83.3%) and 1 isolate from the sputum samples (25%). These findings were higher than those recorded by **El-Jakee et al., (2008)** who estimated staphylococci from the urine of infected urinary tract, septic wounds and nasal swabs from humans (36%). In Uganda, out of 97 participants, 28 of them were nasal carriers of *S. aureus* (28.8%) as mentioned by **Abimana et al. (2019)**. This finding was higher than that achieved by **Suelam et al., (2012)**, who isolated *S. aureus* from pus samples with an incidence of 36.4%; Moreover, the *S. aureus* isolation rate from various clinical specimens of out-patients attending Abia State University Teaching Hospital Abia, Nigeria was 24.5% as reported by **Nsofor et al., (2016)**.

Using Stafaurex kit and coagulase test, confirmed that out of the 57 *Staphylococcus* spp. isolates from the examined animal samples, only 19 were coagulase positive staphylococci CoPS 33.3%. The clinical mastitis cows' milk, only 2 were CoPS (8.3%), and 22 were CoNS (91.7%). The clinical mastitis sheep's milk, all the isolates were CoPS (100%). From the pus samples, only 4 isolates were confirmed as CoPS (20%) and 16 were CoNS (80%). This was higher than what was achieved by **De Almeida et al., (2011)** who isolated *S. aureus* from the milk of dairy mastitis sheep with an incidence of 29%. A higher incidence of *S. aureus* was recorded from mastitis cows' milk in a percentage of 23.4%, by **Tesfaye (2014)**.

Higher results were found by **El-Jakee et al., (2008)** from mastitis cows' milk (22.7%) and buffaloes (16%), but from the cattle septic wounds was (22%). **Akram et al., (2013)** isolated *S. aureus* from milk samples of cows and buffaloes affected with mastitis (31.9%). A recent study by **Negash (2015)** reported that the occurrence of *S. aureus* in clinical mastitis (73.3%) was higher than that in the subclinical mastitis (42%).

In the present study, the obtained CoNS was 66.7%, which was higher than that obtained by **Bradley et al., (2007)**, and **Nam et al., (2010)** who reported CoNS with percentages of 15%, and 40.7%, of mastitis cows' milk, respectively.

PCR was used for detection of *mecA* gene of *S. aureus* as a rapid diagnostic tool offers a specific, and, sensitive, alternative to traditional susceptibility testing; this agrees with **Chu et al. (2012)**. This coincides with result obtained by **Helal et al., (2015)** who recovered MRSA from

both; animals and humans' samples collected from Al-Fayoum, Giza, BeniSuef and Cairo Governorates, Egypt and with **Kim et al., (2003)** who reported *mecA* gene in forty two isolates and with **Momtaz and Hafezi (2014)** who revealed that 80.3% of *S. aureus* isolates from superficial and surgical wounds of patients in hospitals were possessed *mecA* gene.

The present study revealed that the 19 *S. aureus* isolated from animal samples were examined by the antimicrobial sensitivity test, and revealed high sensitivity against gentamicin (100%). This finding agreed with that of **Negash (2015)** who reported that all animal isolates were found susceptible to gentamicin (100%). **Abo-Zaid and Bahout (1993)** estimated that gentamicin was the antibiotic of choice in the treatment of 86.7% of the mastitis cows. **Akram et al., (2013)** reported that gentamicin was effective in the treatment of bovine mastitis with an incidence of 80.4%.

In addition to Gentamicin, the animal isolates were sensitive to trimethoprim+sulfamethoxazole and vancomycin (94.7% for each), ciprofloxacin (89.5%) and ceftiofur and clindamycin (84.2%), and was highly resistant to ceftiofur and tetracycline with a percent of 15.8%, and clindamycin and erythromycin 10.5% each, but were intermediate sensitive to tetracycline (31.6%) and erythromycin (21.05%). These results indicated the need to use prophylactic treatment and control measures of mastitis for heifers and cows as confirmed by **Castelani et al., (2015)**. Also, a higher resistance of ceftiofur (42.7%) and tetracycline (77.4%) were reported by **Negash (2015)**.

On the other hand, 28 *S. aureus* isolates from the diseased human cases were tested for antimicrobial sensitivity, and showed high sensitivity to vancomycin (100%), ciprofloxacin (89.3%) and trimethoprim+ sulfamethoxazole (82.1%), and clindamycin (78.6%), where *S. aureus* isolates were highly resistant to tetracycline (78.6%). Also, 5 isolates showed intermediate sensitivity was recorded against tetracycline (17.9%) and clindamycin and trimethoprim + sulfamethoxazole with a percentage of 7.1% for each. The obtained results revealed that *S. aureus* isolated from diseased human was highly resistant to ceftiofur with an incidence of 75%, a higher resistance to ceftiofur (100%) was reported by **Davoodabadi et al., (2016)**, while lower resistance (46.4%) was recorded by **Abimana et al., (2019)**.

The study obtained results reflect the extent of misuse of antibiotics in the treatment of bacterial infection in both animals and humans, which may lead to antibiotic resistance. That agrees with **Frieden (2013)** who stated that the animal, the environment and the infected persons play a vital role in the spread of the bacteria and also with **Apparao et al., (2009)** who mentioned that the antibiotic selected for the treatment of mastitis has often been based on results of *in-vitro* susceptibility testing.

The present study concluded that 3 out of 19 *S. aureus* isolates were found to be MRSA strains (15.79%) isolated from animal samples. Higher rates were recorded in diseased human, where 21 out of 28 *S. aureus* isolates were found to be MRSA strains (75%). So, the total number of MRSA isolates was 24 out of 47 isolated from all the examined samples in the present study. It was such a higher rate than recorded by **Nsofor et al., (2016)** (38.5%). MRSA was detected among *S. aureus* hospital-acquired (HA) infections in Italy (41%) as revealed by **Monno et al., (2003)**. On contrary, a very lower rate was detected among secondary school students, where 2.04% of the students were found to be MRSA carriers (**Habeeb et al., 2014**). MRSA was detected in 32% of the Egyptian outpatients while it was found in 25% of the Saudi Arabian outpatients (**Abou Shady et al., 2015**), 47% in India and 90% in Latin American hospitals (**CDDEP, 2019**), 28.6% (genotypically MRSA) in Uganda (**Abimana et al., 2019**).

Regarding the results of MRSA detected in animal samples (15.85%), higher rates (36.6%) were recorded by **Tesfaye (2014) and Helal et al., (2015)** who recovered MRSA among *S. aureus* isolated from clinical mastitis cases (36.6%) and sheep abscess and bumble-foot samples (100%) and mastitic milk samples 77.7%, depends on the context itself.

MDR *S. aureus* is a complicated problem and is difficult to be eliminated due to the presence of numerous antibiotics resistance genes (**Anitha et al., 2016**). The obtained results showed that 1 (5.3%) out of 19 *S. aureus* strains from the examined animal isolates were found to be multidrug-resistant (MDR) (5.26%). In contrast, higher rates were recorded by **Kim et al., (2003) and Negash (2015)** from animal cases with a percentage of 51% and 45.3%, respectively. In addition, 12 out of 28 *S. aureus* strains were found to be MDR strains (42.9%). However, MDR isolation rate was higher in strains recovered from diseased human than that of the examined animals, showing resistance to three or more antimicrobial agents as cited by **Intrakamhaeng and Komutarin (2012)**.

The clinical administration of antibiotics, against the pathogenic bacteria has gradually stopped due to emergence of MDR bacterial strains including *S. aureus* (Efuntoye *et al.*, 2011).

CONCLUSION

From the aforementioned results, there was a significant increase in *S. aureus* isolation rate in the examined clinical mastitis sheep's milk compared with examined clinical mastitic cows' milk and pus samples of different animals. *S. aureus* strains were highly resistant to cefoxitin and tetracycline followed by clindamycin and erythromycin, which were isolated from infected animals. In diseased human, the strains were highly resistant to tetracycline and cefoxitin, resulting in methicillin-resistant *S. aureus* (MRSA) problem. Also, gentamicin, trimethoprim+ sulfamethoxazole, and vancomycin were the most effective antibiotics that can be used in the treatment of *S. aureus* infections in field animals, while in diseased human, the most suitable antibiotics for treatment of *S. aureus* infections were vancomycin, ciprofloxacin and trimethoprim+ sulfamethoxazole. On the other hand, *S. aureus* is susceptible to vancomycin and ciprofloxacin so, both drugs are the most frequent drug of choice to treat a variety of *S. aureus* infectious diseases for humans.

Declaration of Competing interest

On behalf of all authors, I hereby declare that no conflict of interest may interfere with the publication of the manuscript.

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