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Characterization of *Staphylococcus aureus* Isolated from Camel and Human samples from Aswan Governorate in Egypt

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ABSTRACT

Aim: The objective of this study was to assess the occurrence and molecular characteristics of *Staphylococcus aureus* isolates among camel and human samples in Aswan.
Materials and Methods: A total of 320 samples: milk, nasal swabs and bus swabs were collected from camels (*n*= 290) and their human contacts (*n*= 30) from Aswan governorate. Disc diffusion test was applied to detect resistant and Methicillin-resistant *Staphylococcus aureus* MRSA isolates. PCR was applied for detection of the virulence genes: *nuc*, *hla*, *pvl*, *mecA*, *vanA*, *vanB* and enterotoxins (*sea*, *seb sec* and *sed*) genes.
Results: 112 Out of 165 she camel milk samples (67.9%), 59 out of 125 camel nasal samples (47.2%), 3 out of 5 worker nasal swabs (60%) and 16 out of 25 abscesses' swabs from human infected wound (64%) were positive for staphylococci. Only 34 out of 190 staphylococci were *S. aureus* (17.9%) and they were tested by PCR of the *nuc* gene. All *S. aureus* isolates were sensitive to vancomycin. The two human isolates were resistant to streptomycin and erythromycin. Among 25 % of *S. aureus* isolated from camel milk samples were resistant to gentamicin and ceftriaxone (each), 50% and 31.2 % were resistant or intermediate resistant to streptomycin and clindamycin respectively. Among *S. aureus* isolated from camel nasal swabs, 18.8% were resistant or intermediate resistant to cefoxitin and ceftriaxone (each). 13 out of 34 *S. aureus* isolates (38.2%) were MRSA. All *S. aureus* isolates were amplified 279 bp of *nuc* gene specific for *S. aureus* strains. While 28, 12 and 11 isolates were harboring *hla*, *mecA* and *pvl* genes respectively. All *S. aureus* isolates were negative for *vanA*, *vanB* and enterotoxins (*sea*, *seb sec* and *sed*) genes.
Conclusion: *S. aureus* and MRSA isolated from camel milk considered a potential health risk for food poisoning.

Key words: Enterotoxin, Camel, *hla*, *mecA* and *pvl* genes, MRSA, *S. aureus*.

INTRODUCTION

In Egypt, cattle, buffaloes and camels are considered as the most important farm animals, giving milk, meat and leathers. Agreeing to FAO databases (2010), total world milk production is around 696.6 million kg comprising 83.3% (580.5 million kg) from cow, 13% (90.3 million kg) buffaloes, 2.2% (15.1 million kg) goat, 1.3 % (9 million kg) sheep and 0.2% (1.6 million kg) camel (Barlowska *et al*., 2011).

Staphylococcus is found as normal bacterial flora. Infection can occur by contact with excretions of animals (Werckenthin *et al*., 2001).

Staphylococcus aureus is a food poisoning m.o (Le Loir *et al*., 2003) food poising is owing to the release of toxins in the food (Wieneke *et al*., 1993).

Staphylococcus aureus enterotoxins (SEs) can exert their actions efficiently. There are 14 distinctive SE types which have comparable structure (Le Loir *et al*., 2003). pathogenicity of *S. aureus* is indomitable by production of staphylococcal toxin such as exofoliatin A, exofoliatin B and Panton-Valentin Leukocidin (PVL) toxins (Lowy, 1998).

MRSA is resistant to wide range of antimicrobials especially methicillin, tetracycline and cephalosporin (EFSA, 2009). The importance of MRSA strains is that in addition to being resistant to methicillin, most strains also are resistant to other beta lactam antibiotics, with the exception of glycopeptides antibiotics (Moses *et al*., 2013). The aim of the study is to characterize *Staphylococcus* species isolated from camel and human sources.

MATERIALS AND METHODS

Samples

Total number of three hundred and twenty samples collected from camel (*n*= 290) and human (*n*= 30) in contact with these animals (Table-1) were collected from

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Aswan governorate (Daraw and Komombo cities) in the period between July 2016 and April 2017. All samples were kept at 4℃ in sterile test tubes and preserved in ice box for microbiological analysis.

Sampling

Milk samples

The camel milk samples were collected in a sterile single use disposable plastic falcon tubes with tightly fitted caps, its volume 15 ml. Latex gloves were worn and complete sanitation of the udder was performed by good washing of the udder with clean water, dried with sterile wipes and finally teats disinfected by dipping for 20-30 second with iodine 0.5% and ethyl alcohol 70% The 3 fore stripping of milk had been discarded and a composite milk sample was collected from all 4 quarters in a single sample tube with a volume 8-10 ml for each tube. The tube was tightly closed as soon as possible and labeled by a number and placed in the tube rack. All collected samples were frozen immediately at -20ºC at the site of collection (Pamel, 2005) until the culture was performed.

Nasal swabs

Total 125 nasal swabs collected under aseptic condition from camel and 5 nasal swabs collected from human (workers) contacting with camels, then were inoculated into sterile tubes containing 2 ml nutrient broth and kept in ice container.

Abscesses

A total 25 pus discharge samples were collected from infected wounds of patients (QENA General Hospital) under aseptic condition, then the swabs were inoculated into sterile tubes containing 2 ml nutrient broth (Oxoid**)** and were kept in ice container.

Isolation and identification of *Staphylococcus aureus* isolates

Each example was refined on mannitol salt agar (Oxoid) and 5% sheep blood agar. All plates were cultured at 37ºC for 18–24 h and inspected for bacterial development. Breaking down for hemolysis as example on sheep blood agar, lecithinase movement on Baird Parker medium (Oxoid), enhanced with egg yolk tellurite emulsion (Forbes et al., 2007). The speculated isolates were gotten and analyzed minutely and oppressed for identification of staphylococci as indicated by Quinn et al. (2002). Likewise, API ® Staph Kit (bioMe'rieux SA, l'Etoile, France) was utilized for affirmation and afterward the strips were perused by the scaled down API instrument and related programming.

Antimicrobial sensitivity testing among *S. aureus* isolates

Mueller-Hinton media (agar and broth) were used for antimicrobial sensitivity testing of *S. aureus* isolates (Bauer *et al*., 1966) using the disc diffusion technique and the following antimicrobial susceptibility discs (HIMEDIA®) cefoxitin (FOX) (30ug), ceftriaxone (SEFO) (30 ug), ciprofloxacin (CIP) (5 ug), clindamycin (DA) (2ug), erythromycin (E) (15ug), gentamicin (CN) (30ug), streptomycin (S) (10 ug) and vancomycin (VAN) (30ug) according to CLSI (2009) and (2016). Also, the disc diffusion technique was adapted using amoxicillin (AMX), oxacillin (OX) and penicillin (P) sensitivity discs (Oxoid) to detect (MRSA) according to Clinical and Laboratory Standards Institute CLSI (2013).

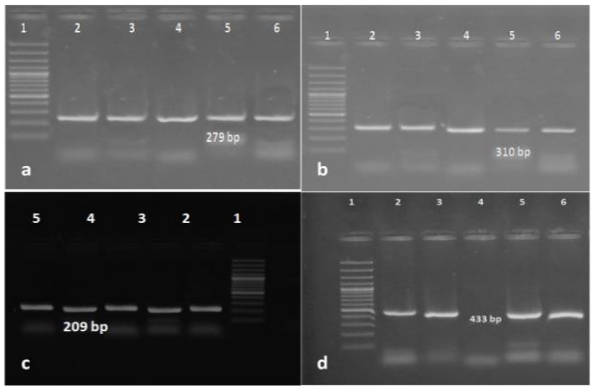

Fig. 1: Agarose gel electrophoresis showing the results of PCR for *S. aureus* isolates.
a): Amplification of 279 bp of *nuc* gene. Lane 1: Marker 100 bp, Lanes 2 and 3: Human abscesses' swabs, Lanes 4 and 5: camel nasal swabs and Lane 6: camel milk sample. b) Amplification of 310 bp of *mecA* gene. Lane 1: Marker 100 bp, Lanes 2 and 3: Human abscesses' swabs, Lanes 4 and 5: camel nasal swabs and Lane 6: camel milk sample. c) Amplification of 209 bp of *hla* gene. Lane 1: Marker 100 bp, Lane 2: camel nasal swab, Lane 3: Human abscess swab and Lanes 4, 5 and 6: camel milk samples. d) Amplification of 433 bp of *pvl* gene. Lane 1: Marker 100 bp, Lanes 2 and 3: camel milk samples, Lane 4: Negative, Lane 5: Human abscess swab and Lane 6: camel nasal swab.

Table 1: Sources and number of the collected samples.

Source of samples	Type of samples	Number of samples
Camel	Milk	165
	Nasal swabs	125
Human	Nasal swabs	5
	Abscesses' swabs	25
Total		320

PCR procedure (Sambrook *et al*., 1989)

Polymerase chain response was acted in Biotechnology Center for Services and Research (BCSR) in Faculty of Veterinary Medicine, Cairo University. Qiagen extraction pack for DNA extraction from *S. aureus* was utilized as described by producer manual of Qiagen, Germany. Preliminaries were incorporated by Metabion Company, Germany as referenced in Tables 2 and 3. The DNA groups was distinguished by perception with UV light at frequency 421 nm and contrasted and sub-atomic size marker (Ladder) obtained from Amersco Cleveland Ohio, USA.

RESULTS

Prevalence of *S. aureus* isolates

Table -4 reveals that 112 out of 165 she camels milk samples (67.9%), 59 out of 125 camel nasal samples (47.2%), 3 out of 5 worker nasal swabs (60%) and 16 out of 25 Abscesses' swabs from human infected wounds (64%) were positive for staphylococci.

All staphylococci isolates (n = 190) were tested by coagulase test (slide and tube) and acetoin production to identify *S. aureus* isolates. It is clear that, only 34 isolates were *S. aureus* (17.9%).

Table 2: Primers for toxin genes of *S. aureus* used in multiplex PCR (Mehrotra *et al.*, 2000)

Primers	Nucleotide sequence	bp
Sea: Forward	5` GGTTATCAATGTGCGGGTGG 3`	102 bp
Reverse	5` CGGCACTTTTTTCTCTTCGG 3`	
Seb: Forward	5` GTATGGTGGTGTAACTGAGC 3`	164 bp
Reverse	5` CCAAATAGTGACGAGTTAGG 3`	
sec: Forward	5` AGATGAAGTAGTTGATGTGTATGG 3`	451 bp
Reverse	5` CACACTTTTAGAATCAACCG 3`	
sed: Forward	5` CCAATAATAGGAGAAAATAAAAGG 3`	278 bp
Reverse	5` ATTGGTATTTTTTTTCGTTC 3`	

Table 3: primer sequences of *nuc*, *mec*A, *hla* , *van*A and *van*B genes.

Gene	Primer	Seqence (5' to 3')	Amplicon size(bps)	Reference
nuc	Forward	5'-GCGATTGATGGTGATACGGTT-3'		(Al-Soud, 2019)
	Reverse	5'-CAAGCCTTGACGAACTAAAGC -3'	279bp	
pvl	Forward	5'-GTAAAATGTCTGGACATGATCCAY-3'	433 bp	(Al-Soud, 2019)
	Reverse	5'-GCATCAASTGTATTGGATAGCA-3'		
mecA	Forward	5'-CTTCCACATACCATCTTC-3'		(Tiwari &Sen, 2006)
	Reverse	5'-CTTGTAGTTGTCGGGTTT-3'	310bp	
hla	HLA-1	CTGATTACTATCCAAGAAATTCGATTG	209	(Jarraud *et al*., 2002)
	HLA-2	CTTTCCAGCCTACTTTTTTATCAGT		
*van*A	Forward	5'-CCCCTTTAACGCTAATAGATCAA-3'		(Tiwari &Sen, 2006)
	Reverse	5'-CATGAATAGAATAAAAGTTGCTGCAATA-3'	1030bp	
vanB	Forward	5' -GTGACAAACCGGAGGCGAGGA-3'		(Tiwari &Sen, 2006)
	Reverse	5'-CCGCCATCCTCCTCCTGCAAAAA-3'	433bp	

Table 4: occurrence of *S. aureus* isolates among the samples:

Samples	Number of samples	Staphylococci		*S. aureus*	
		No.	%	No.	%
Camel milk	165	112	67.9	16	14.3
Camel nasal swabs	125	59	47.2	16	27.1
Human nasal swabs	5	3	60	0	0
Human abscesses' swabs	25	16	64	2	12.5
Total	320	190	59.4	34	17.9

Table 5: Antimicrobial sensitivity testing among *S. aureus* isolates

	Antimicrobial discs							
	CIP (%)	Fox (%)	E (%)	S (%)	DA (%)	VAN (%)	CEFO (%)	CN (%)
Human (2 isolates)								
S	50	100	0	0	100	100	50	50
R	50	0	100	100	0	0	50	50
I	0	0	0	0	0	0	0	0
Camel Milk (16 isolates)								
S	87.5	93.8	81.3	50	68.8	100	75	75
R	6.3	6.3	12.5	37.5	18.8	0	25	25
I	6.3	0	6.3	12.5	12.5	0	0	0
Camel Nasal swabs (16 isolates)								
S	100	81.3	100	100	93.8	100	81.3	100
R	0	0	0	0	0	0	6.3	0
I	0	18.8	0	0	6.3	0	12.5	0

FOX = Cefoxitin; SEFO = Ceftriaxone; CIP = Ciprofloxacin; DA = Clindamycin; E = Erythromycin; CN = Gentamicin; S = Streptomycin; VAN = Vancomycin

Table -6: Occurrence of MRSA among *S. aureus* isolates

Source of isolates	Number of isolates	MRSA isolates	%
		No	
Camel milk samples	16	5	31.3
Camel nasal swabs	16	6	37.5
Human abscesses' swabs	2	2	100
Total	34	13	38.2

Table 7: Results of PCR among the isolates

Gene	Camel milk samples (n=16)	Camel nasal swabs (n=16)	Human abscesses' swabs (n=2)
nuc	16	16	2
mecA	4	6	2
vanA and *vanB*	-	-	-
hla	12	14	2
pvl	4	5	2
Sea, *seb*, *sec* and *sed*	-	-	-

Antimicrobial sensitivity testing among *S. aureus* isolates

It is clear from Table -5 that all *S. aureus* isolated from camel and human samples were sensitive to vancomycin (100%). The two human isolates were resistant to erythromycin and streptomycin and sensitive to cefoxitin and clindamycin. Among *S. aureus* isolated from camel milk samples, 25% were resistant to gentamicin and ceftriaxone (each), 50% and 31.2% were resistant or intermediate resistant to streptomycin and clindamycin respectively. Among *S. aureus* isolated from camel nasal swabs, 100% were sensitive to ciprofloxacin, erythromycin, streptomycin and gentamicin (each), 93.8% were sensitive to clindamycin and 81.3% were sensitive to cefoxitin and ceftriaxone (each); while 18.8% were resistant or intermediate resistant to cefoxitin and ceftriaxone (each).

Table 6 illustrates that 13 out of 34 *S. aureus* isolates (38.2%) were resistant to amoxicillin, oxacillin and penicillin. The occurrence of MRSA was 100, 37.5 and 31.3% among *S. aureus* isolated from human abscesses, camel nasal and camel milk samples respectively.

Results of PCR

All *S. aureus* isolates were amplified 279 bp of *nuc* gene specific for *S. aureus* strains (Figure 1a), 28, 12 and 11 isolates were harboring *hla*, *mecA* and *pvl* genes; respectively (Table 7 and Figure 1). All *S. aureus* isolates were negative for *vanA*, *vanB* and enterotoxins (*sea*, *seb sec* and *sed*) genes.

DISCUSSION

Camel is a livestock animal in the genus *Camelus* that has a distinctive fatty deposit on its back known as "humps". Camels are working animals provide milk, meat and textiles. Camel milk is considered a meal itself; a nomad can live on only camel milk for almost a month. It inhibits growth of *Clostridium*, *Helicobacter pylori*, *Klebsiella pneumonia Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, etc (Rasheed, 2017). *Staphylococcus aureus* causes food borne disease outbreaks and it is one of the major causes of mastitis.

In the present study 320 samples collected from camel (n= 290) and human (n = 30) in contact with these animals were collected from Aswan governorate in the period between July 2016 and April 2017 and were investigated to detect the occurrence of *Staphylococcus aureus* among the samples. Isolation of colonies on selective culture medium such as mannitol salt agar and Baird–Parker agar for 24–48 h at 37 °C, followed by biochemical testing for suspicious colonies and further confirmation by biochemical tests (Xu *et al.,* 2011). Faller and Schleifer (1981) mentioned that mannitol salt agar and Baird-parker medium are specifically selective media.

In the present study, 112 out of 165 she camels milk samples (67.9%), 59 out of 125 camel nasal samples (47.2%), 3 out of 5 worker nasal swabs (60%) and 16 out of 25 abscesses swabs from human infected wound (64%) were positive for staphylococci. It is clear that only 34 out of 190 staphylococci were *S. aureus* (17.9%) identified from the examined samples according to Quinn *et al.* (2002) and confirmed by amplification of 279 bp of *nuc* gene specific for *S. aureus* strains. Agabou *et al*. (2017) recorded that the rate of *S. aureus* was 53, 50, 44.2, 15.2 and 15% in camels, humans, sheep, horses and cattle nasal samples; respectively. *S. aureus* was detected by Al-Amery *et al*. (2019) in both camel meat (29/200, 14.5%) and in abattoir workers (11/20, 55%).

Sixteen *S. aureus* (14.3%) isolates were identified from camel milk samples. Milk is an important food because it contains numerous important nutrients including proteins, vitamins, and minerals. On the other hand, *Staphylococcus aureus* is the most common microorganism incriminated in staphylococcal food poisoning because it is considered a principal contaminant of raw milk (Asao *et al*., 2003; Silva *et al.,* 2003). Among human staphylococci two *S. aureus* isolates were identified from abscesses swabs. The coagulase positive *Staphylococcus aureus* was frequently involved in suppurative infection approximately 30% of the human population is infected with *S. aureus* (Tong *et al.,* 2015). Recently Hamdy *et al*. (2019) determined as risk factors for infection with MRSA. *S. aureus* microorganisms have the ability to produce the enterotoxins which make a risk factor on public health (Wu *et al*., 2016).

From our data it is clear that the two human isolates were resistant to erythromycin and streptomycin and sensitive to clindamycin and cefoxitin. Among *S. aureus* isolated from camel milk samples, 25% were resistant to gentamicin and ceftriaxone (each), 50% and 31.2% were resistant or intermediate resistant to streptomycin and clindamycin; respectively. Among *S. aureus* isolated from camel nasal swabs, 18.8% were resistant or intermediate resistant to cefoxitin and ceftriaxone (each). Meanwhile all isolated from camel and human were sensitive to vancomycin (100%). El-Jakee *et al.* (2011) concluded that 95% of the examined *S. aureus* isolated from bovine and human sources were sensitive to vancomycin. About 27% (8/29, camel) and 54% (6/11, human) were identified as vancomycin resistant *S. aureus* (Al-Amery *et al*., 2019).

MRSA is a hospital associated strains (31Redwan *et al*., 2016). In the present study methicillin resistant *S. aureus* isolates were detected in 13 out of 34 *S. aureus* isolates (38.2%), 2 from human abscesses (100%), 6 from camel nasal (37.5%) and 5 from camel milk (31.3%) samples respectively. Nine MRSA isolates (7.6%) were identified from sheep and camels (Agabou *et al*., 2017).

In the present study molecular detection by multiplex PCR was used for detection of genes for enterotoxins of *S. aureus*. In latest years, new types of SEs have been reported by Riva *et al*. (2015). All our isolates were negative for production of enterotoxins *sea, seb, sec* and *sed* genes. Using RPLA, 10 out of 25 *S. aureus* isolated by El-Jakee *et al*. (2015) from bovine and human sources were found to be toxigenic with an incidence of 40%.

Fisher *et al*. (2018) showed that by using PCR, 28 (82.5%), 12 (35.3%) and 11 (32.4%) isolates were harboring *hla*, *mecA* and *pvl* genes; respectively. El-Jakee *et al.* (2010) detected *mec A* gene MRSA recovered from bovine and human sources.

Alpha-hemolysin (Hla), is the major cytotoxic agent released by *S. aureus* and is encoded by the *hla* gene. The toxin is causing rupture, cell death and it can cause lethality in a wide variety of animals (Monecke *et al*., 2014).

Resistance genes have been detected as virulence markers in many different microbes (Hossam *et al.,* 2016;

Elhariri *et al.,* 2017; Elhariri *et al*., 2017; Saad *et al.,* 2017). With high incidence in different animals (Khalifa *et al.,* 2014; Abdel-Moein *et al.,* 2017; Elhariri *et al.,* 2017).

In the present study 11 isolates were harbor *pvl* gene were identified in camel milk (*n* = 4, 25%), camel nasal (*n* = 5, 31.3%) samples and human abscesses (*n* = 2, 100%). Panton-Valentine-leukocidin is produced by about 2% of *S. aureus* strains (Abu Al-Soud, 2019). Among *S. aureus* isolates, Panton–Valentine- Leukocidin isolates belonging to ST80- Methicillin-resistant *S. aureus* -IV and ST152-Methicillin-sensitive *S. aureus* were identified in camels (*n* = 3, 13%) and sheep (*n* = 4, 21.1%) as recorded by Agabou *et al*. (2017), they investigated that some *S. aureus* isolates can cross the species barrier.

Conclusion

This study detected *S. aureus* from raw milk and demonstrated that Methicillin-resistant *Staphylococcus aureus* (MRSA) in nasal carriage of camel and milk samples. Public health concern is highly reflected in this study. This considered a potential risk for food poisoning by raw milk consumption.

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