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Antimicrobial Resistance in Clinical Isolates of *Staphylococcus aureus* from Bovine and Human Sources in Egypt

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Abstract: Staphylococcus aureus is an important hospital- and community-associated pathogen that can cause a wide variety of infectious diseases. Methicillin-resistant *Staphylococcus aureus* (MRSA) generally exhibits multiple resistance patterns to antimicrobial drugs. In the present study 19 *S. aureus* isolates (9 from bovine and 10 from human) in comparison with the standard Cowan I strain were investigated using antimicrobial sensitivity test and PCR technology. The collected isolates had polymorphisms of the genes encoding staphylococcal coagulase (*coa* gene) and staphylococci protein A (*spa* gene) and yielded different PCR products in size ranging from approximately 423 to 658 bp and 396 to 462 bp respectively. 47.4% of *S. aureus* isolates and the standard Cowan I strain were positive for both *sea* and *seb* genes. 57.9% of isolates had a high molecular weight plasmid. *S. aureus* isolates showed high resistance to methicillin, followed by oxytetracycline, ampicillin and sulphamethoxazole-trimethoprim. Meanwhile 95% of the examined *S. aureus* isolates were sensitive to vancomycin. All MRSA strains were *mecA* gene positive by PCR. It is clear that MRSA has been described in bovine and it can easily spread between animals and under certain conditions to humans. This study provides important data on current antimicrobial resistance, including methicillin resistance, for a collection of *S. aureus* isolated from Egyptian bovine and humans samples.

Key words: *S. aureus* · Antibiogram sensitivity · MRSA · MSSA · *mec A* - *Coa* and *Spa* genes

INTRODUCTION

Staphylococci often represent part of normal bacterial flora of the skin and mucosal surfaces of the respiratory, upper alimentary and urogenital tracts of mammals and birds. In dairy animals, *S. aureus* still remains one of the most significant organisms associated with clinical and subclinical bovine mastitis worldwide [1].

Because of the widespread use of antibiotics, the resistance profile of microorganisms is increasing among bacterial populations. S. *aureus* have developed resistance mechanisms. Community-acquired MRSA is now increasingly recognized with many of these infections occurring in patients with no prior hospital exposure. These community-based infections have been reported in patients from both rural and urban settings [2]. Consequently, knowledge of community and nosocomial antimicrobial susceptibility profiles of S. *aureus* would prevent abuse of glycopeptides [3]. It is important to isolate and identify the offending strain for appropriate antibiotic therapy to be initiated. The aim of this study was to assess the antimicrobial susceptibility patterns and prevalence of methicillin resistance among *S. aureus* isolates. So the present investigation was planned to investigate: antibiogram sensitivity tests, plasmid containing isolates and gene encoding MRSA among *S. aureus* isolated from Egyptian bovine and humans' samples.

MATERIALS AND METHODS

S. Aureus Isolates: A total of 19 *S. aureus* isolates (5 from cows milk, 2 from buffaloes milk, 2 from cattle septic wounds, 2 from human respiratory infections, 6 from human septic wounds and 2 from human infected urinary tract) collected from cattle, buffaloes and human, as well as Cowan I strain of *S. aureus* obtained from the Namru 3

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Table 1: Types of Primers, Primers Designs and References.

Primer	Primer Design	Product size bp	Reference
Coagulase gene F	5'-ATAGAGATGCTGGTACAGG-3'		Hookey *et al.* [10]
Coagulase gene R	5'-GCTTCCGATTGTTCGATGC-3'	433-638	
SAEA-F	5'-CCTTTGGAAACGGTTAAAACG- 3'		
SAEA-R	5'-TCTGAACCTTCCCATCAAAAAC- 3'	127	Becker *et al.* [11]
SAEB-F	5'-TCGCATCAAACTGACAAACG- 3'		
SAEB-R	5'-GCAGGTACTCTATAAGTGCC- 3'	477	
SPA F	5'-CAAAGATCAACAAAGCGCC- 3'		Annemüller and Zschock [12]
SPA R	5'-CGAAGGATCGTCTTTAAGGC- 3'	412	
MRSA gene F	5'-GGAGACGAGCACTAAAACC-3'		Weller [13]
MRSA gene R	5'-TCGGACGTTCAGTCATT-3'	182	

in Egypt (positive control) were investigated. The collected *S. aureus* isolates were cultured onto bacto-mannitol salt agar "Difco" and identified according to Quinn *et al.* [4].

Antimicrobial Sensitivity Test: The disk diffusion technique was adapted according to Finegold and Martin [5] using 15 antibacterial disks "Oxoid". The degree of sensitivity of *S. aureus* to the antibacterial agents was determined according to NCCLS [6] and Cheesbrough [7].

DNA Techniques: Plasmid DNA extraction and PCR [8] were performed in the Biotechnology Centre for Services and Research (BCSR) in Faculty of Veterinary Medicine, Cairo University. Extraction of miniprep performed according to Sambrook and Russel [8]. The extracted plasmid was evaluated as visible bands being sized by DNA marker (Hind Ø digest), that measures molecular weight 81-23000 bp (Gibbco). Qiagen extraction kit for DNA extraction from *S. aureus* isolates was used as described by manufacturer manual of Qiagen, Germany. As shown in Table 1 all isolates were characterized by primers synthesized by Metabion Company, Germany as mentioned previously [9]. The presence of specific amplified DNA bands was detected by visualization with UV light at wave length 421 nm and compared with molecular size marker that measures 100-1500 bp "Amersco Cleveland Ohio, USA".

RESULTS AND DISCUSSION

It has been shown that people colonized with *S. aureus* in the nose are at increased risk for developing *S. aureus* infection. It has been estimated that 90% of hospital staff are carriers of *S. aureus* portend serious for the epidemiology and pathogenesis of staphylococcal infections [14].

In the present investigation characterization of 19 *S. aureus* isolates (9 from bovine and 10 from human sources) in comparison with the standard Cowan I strain was performed using the most important conventional biochemical tests and PCR technology. As shown in table (2), all examined isolates had polymorphisms of the gene encoding staphylococcal coagulase at approximately 423 to 658 bp [9]. The evolution of new human and animal pathogenic strains of *S. aureus* has been due to the accumulation of mobile genetic elements (MGE) encoding methicillin resistance and virulence factors into successful lineages [15].

In the current work all examined *S. aureus* isolates (Table 2) had an amplified *spa* gene product at approximately 396-464 bp [9]. Different-sized amplicons were observed by Frénay *et al.* [16] who recorded that the X region of the *spa* gene is stable enough both *in vitro* and *in vivo* to discriminate between different clones. The results in table (2) also show that 4 out of 9 bovine and 5 out of 10 human isolates had staphylococcal enterotoxins. Production of SEA, SEB, SED, SEE and TSST- I by *S. aureus* strains associated with bovine mastitis has been described [17]. Clinical isolates of *S. aureus* often harbor plasmids, 11 out of 19 isolates as well as the Cowan 1 strain had a high molecular weight plasmid [9]. Some MRSA carry large plasmids that encode a multi-drug resistance gene conferring resistance to linezolid, one of the newly licensed drugs for treating multi-drug-resistant *S. aureus* [18].

Antimicrobial sensitivity test of the *S. aureus* isolates recorded high resistance rate to methicillin followed by oxytetracycline and ampicillin (Table 3 & 4). The first MRSA clones in hospitals were detected in 1961, however, MRSA rates were relatively low (1-2%) in most hospitals, until epidemic MRSA emerged [15]. MRSA has only become important in mastitis, after the first report of MRSA in mastitis in 1972 whereas methicillin

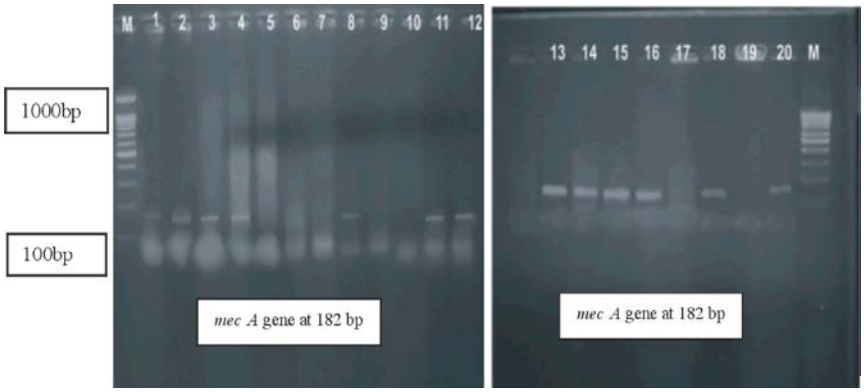

Photo (1): Agarose gel electrophoresis showing the result of amplification of mec A gene (182 bp). M: DNA molecular weight marker (100 bp. ladder), Lane 1: Cows milk (Positive for mec A gene), Lane 2: Cows milk (Positive for mec A gene), Lane 3: Cows milk (Positive for mec A gene), Lane 4: Cows milk (Positive for mec A gene), Lane 5: Cows milk (Negative for mec A gene), Lane 6: Buffaloes milk (Negative for mec A gene), Lane 7: Buffaloes milk (Negative for mec A gene), Lane 8: Bovine septic wounds (Positive for mec A gene), Lane 9: Bovine septic wounds (Negative for mec A gene), Lane 10: Human respiratory infection (Negative for mec A gene), Lane 11: Human respiratory infection (Positive for mec A gene), Lane 12: Human septic wounds (Positive for mec A gene), Lane 13: Human septic wounds (Positive for mec A gene), Lane 14: Human septic wounds (Positive for mec A gene), Lane 15: Human septic wounds (Positive for mec A gene), Lane 16: Human septic wounds (Positive for mec A gene), Lane 17: Human septic wounds (Negative for mec A gene), Lane 18: Human infected urinary tracts (Positive for mec A gene), Lane 19: Human infected urinary tracts (Negative for mec A gene), Lane 20: Cowan-1 standard strain (Positive for mec A gene).

Table 2: Characteristics of S. aureus isolated from bovine and humans' samples.

			Bovine samples					
				Toxins gene Mol. wt.				
Strains	Strain type	Source of the isolates	Coa. gene Mol. wt. 423 to 658	A-127	B-477	Spa gene Mol. wt. 396-464bp	Mec. A gene Mol. wt. -182	Plasmid
1	MRSA	Cows milk	630	-ve	-ve	396	+ve	+ve
2	MRSA	Cows milk	658	-ve	+ve	418	+ve	+ve
3	MRSA	Cows milk	423	+ve	+ve	464	+ve	-ve
4	MRSA	Cows milk	658	-ve	-ve	422	+ve	+ve
5	MSSA	Cows milk	658	+ve	+ve	430	-ve	-ve
6	MSSA	Buffaloes milk	658	-ve	-ve	452	-ve	-ve
7	MSSA	Buffaloes milk	428	-ve	-ve	428	-ve	+ve
8	MRSA	Cow septic wounds	432	-ve	-ve	452	+ve	-ve
9	MSSA	Cow septic wounds	456	+ve	+ve	452	-ve	+ve
			Human samples					
10	MSSA	Human nasal swabs	658	-ve	+ve	448	-ve	+ve
11	MRSA	Human nasal swabs	658	+ve	-ve	448	+ve	+ve
12	MRSA	Human septic wounds	448	-ve	-ve	452	+ve	-ve
13	MRSA	Human septic wounds	608	-ve	+ve	452	+ve	+ve
14	MRSA	Human septic wounds	484	-ve	-ve	418	+ve	-ve
15	MRSA	Human septic wounds	484	-ve	-ve	448	+ve	-ve
16	MRSA	Human septic wounds	658	-ve	+ve	462	+ve	+ve
17	MSSA	Human septic wounds	428	+ve	-ve	452	-ve	+ve
18	MRSA	Human urine	642	-ve	-ve	418	+ve	+ve
19	MSSA	Human urine	518	-ve	-ve	448	-ve	-ve
20	MRSA	Cowan-1	642	+ve	+ve	448	+ve	+ve

Table 3: Results of antibiogram of bovine MRSA and MSSA strains.

	MRSA					MSSA			
Source	Mastitic milk				Septic wound	Mastitic milk			Septic wound
Strain No.	1	2	3	4	8	5	6	7	9
Most resistant antibiotic	OX, OT, AMC, AML	AMP, CX, CD, OX, ON, SXT	TN, OT, OX, E	AMP,AML, CB, OX	AMP, CF, OX	AZ, CF, OT, SXT	AMP, CB, CD, ON	AML, CX, E, OT, SXT	AMC, AZ, CD, OT, SXT
Most intermediate antibiotic	AMP, CB, CX	CF, AZ, CB	AML, CB, CX	ON, SXT, TN, CX	CD, OT, SXT, CB, CX	CB, CX	AML, CX	CB	AML, AMP, CB, CX
Most sensitive antibiotic	AZ, CF, CD, E, ON, SXT. TN, VN	AML, AM C, E, OT, TN, VN	AMC, AM P, AZ, CF, CD, ON, SXT, VN	AMC, AZ, CF, CD, E, OT, VN	AML, AMC, AZ, ON, E, VN, TN	AML, AMC, AMP, CD E, OX, ON, VN TN	AMC, AZ, CF, E, OX, OT, SXT, VN, TN	AMC, AMP, AZ, CF, CD, OX, ON, TN, VN	CF, E, OX, ON, TN, VN

AML: Amoxicillin, AMC: Amoxicillin / clavulanic, AMP: Ampicillin, AZ: Azithromycin, CB: Cefoperazone, CX: Cefotaxime, CF: Ciprofloxacin, CD: Clindamycin, E: Erythromycin, OX: Methicillin (Oxacillin), ON: Ofloxacin, OT: Oxytetracycline, SXT: Sulphamethoxazole-Trimethoprim, TN: Tobromycin and VN: Vancomycin .

Table 4: Results of antibiogram of human MRSA and MSSA strains.

	MRSA								MSSA		
Source	Cowan-1 strain	Respiratory infection	Septic wound					Urinary infection	Respiratory infection	Septic wound	Urinary infection
Strain No.		11	12	13	14	15	16	18	10	17	19
Most resistant antibiotic	OX, OT,TN, AML, E, ON	AZ, OX, SXT, TN	CD, OX, ON, OT	AMP, CX, CF, OT, SXT	AML,OX, E, SXT, TN	AMP, OX, OT, ON	AML, AZ, OX, SXT	CD, OX, SXT	AML, AMP, CB, OT	AMC, AMP, ON, OT	AML, AMP, CF, E
Most inte mediate antibiotic	AMP,CB, CF,SXT	OT, ON, CF, AMP, AML	TN	OX, AML	VN, CD	AML, AMC, CB, CF	-	AML, AMP, OT	SXT	CF, E	OT, SXT
Most sensitive antibiotic	CX, AMC, AZ, CD, VN	AMC, CB, CX, CD, E, VN	AML, AMC, AMP, AZ, CB, CX, CF, E, SXT, VN	AMC, AZ, CB, CD, E, ON, TN, VN	AMC, AMP, AZ, CB, CX, CF, ON, OT	AZ, CX, CD, E, SXT, TN, VN	AMC, AMP, CB, CX, CF, CD, E, ON, OT, TN, VN	AMC, AZ, CB, CX, CF, E, ON, TN, VN	AMC, AZ, CX, CF, CD, E, OX, ON, TN, VN	AML, AZ, CB CX, CD, OX, SXT, TN, VN	AMC, AZ, CB, CX, CD, OX, ON, TN, VN

(cloxacillin)-resistant *Staphylococcus aureus* isolates were obtained by Devriese *et al.* [19] from cows (2.2%) and their antibiotic resistance characteristics were similar to those of the methicillin-resistant strains which have been described from human sources. Five out of 9 (55.6%) *S. aureus* isolated from bovine were MRSA (Table 3). A higher prevalence of MRSA among *S. aureus* isolated from mastitis cases is described [20]. We found that 95% of the isolates were sensitive to vancomycin while one human isolate showed an intermediate resistance to it. The first intermediate-level vancomycin resistant *S. aureus* (VISA) were described in Japan in 1997 [21]. The acquisition of transferable glycopeptide resistance *(vanA)* in *S. aureus* has also been reported [22]. All MRSA isolates were *mec*A gene positive by PCR (Photo 1). Vannuffel *et al.* [23] used a multiplex PCR: 310-bp and 686-bp regions of the *mec*A gene and *fem*A genes, respectively, were amplified to identify susceptible (lacking *mec*A) and resistant (carrying *mec*A*)* staphylococci and to differentiate *S. aureus* (*fem*A+) from coagulase-negative staphylococci (*fem*A-).

It could be concluded that antibiogram clarifying the developed resistance of *S. aureus* strains to commonly used antibiotics ensuring that the right use of antibiotic of choice is very important in line of treatment and control of the infections caused by *S. aureus* especially MRSA strains.

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