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Original Research Article

EXISTENCE OF VANCOMYCIN RESISTANCE AMONG METHICILLIN RESISTANT *S. aureus* RECOVERED FROM ANIMAL AND HUMAN SOURCES IN EGYPT

Norhan K. Abd El-Aziz[1], Marwa I. Abd El-Hamid[1]*, Mahmoud M. Bendary[2], Asmaa A. El-Azazy[3], Ahmed M. Ammar[1]

[1]Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Sharkia, 44511. [2]Department of Microbiology and Immunology, Faculty of Pharmacy, Port Said University, Port Said, 42511. [3]Abouhamad Veterinary Organization, Ministry of Agriculture, Abou-Hamad, Sharkia, 44661, Egypt

*Corresponding author, E-mail: mero_micro2006@yahoo.com; mero_micro@zu.edu.eg

Abstract: The increased resistance of vancomycin among methicillin resistant *Staphylococcus aureus* (MRSA) has produced a major formidable threat in the therapeutic field. The current study analyzed the vancomycin resistance traits among MRSA isolates recovered from 148 samples of animal and human origins in Sharkia Governorate, Egypt. All staphylococci isolates were examined against 8 antimicrobials and vancomycin minimal inhibitory concentrations (MICs) were then determined among phenotypic vancomycin resistant and intermediate *S. aureus*. Furthermore, all vancomycin-resistant *S. aureus* (VRSA) isolates were exposed to PCR analysis of *mec*A and *van* genes. Herein, 45 of 86 *Staphylococcus* spp. were identified as *S. aureus*, while 41 were coagulase negative staphylococci (CoNS). A higher incidence rate of *S. aureus* was observed in meat products (58.06%), but majority of CoNS isolates were isolated from milk samples (54.54%) with no statistical differences ($P < 0.05$) in the distribution of *S. aureus* and CoNS among all samples. Oxacillin and amoxicillin-clavulanic acid recorded the highest resistance percentages among *S. aureus* (93.33 and 88.89%) and CoNS (75.61 and 87.80%), respectively. Multidrug resistance (MDR) was detected in high proportions of *S. aureus* (64.4%) and CoNS (34.1%). Forty-two of 45 *S. aureus* isolates were MRSA, of which 14 were vancomycin resistant with MIC values ranged from 32-1024 µg/mL. PCR detection of *mec*A and *van* genes in the tested isolates revealed that they were all *mec*A gene positive, while 10 out of them had *van* genes. The *van*B gene was found in 5 isolates with higher MICs (64- 256 µg/mL), while *van*A gene was detected in 4 isolates with MICs of 128-512 µg/mL and only one isolate harbored both *van*A and *van*B genes with MIC value of 1024 µg/mL. According to the upsurge of VRSA prevalence rates, more attentions should be oriented for continuous monitoring of antimicrobial usage with the need for effective drugs against VRSA.

Key words: *S. aureus*; Antibiogram; VRSA; MRSA; *mec*A; *van* genes

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Introduction

Staphylococcus aureus (*S. aureus*) has been documented as a significant cause of a wide range of diseases from wound, skin and bone infections to life threatening pneumonia, devastating septicemia and toxic shock syndrome (1,2). In dairy animals, *S. aureus* is one of the most incriminated pathogens causing clinical and subclinical mastitis worldwide (3). Because of the overuse of antibiotics, there has been a direful increase in the incidence of antibiotic resistant strains, which has complicated the treatment process (1). Penicillin was primarily very effective against most staphylococcal infections, but *S. aureus* began producing β-lactamase enzyme in the mid-1940s, which destroys the penicillin β-lactam ring (4). Later, more than 90% of *S. aureus* strains were penicillin resistant. The increase in this resistance drove the discovery of methicillin drugs, which are semi synthetic penicillin virtually resistant against genetic variations of β-lactamase enzyme. Through evolution, rigorous bacteria those cannot be treated by antibiotics are surviving until the isolation of the first bacterial strain of methicillin resistant *S. aureus* (MRSA) in 1961. Since then, MRSA become a dangerous endemic organism worldwide and listed on the top of the serious problems with a negative impact on public health. MRSA is mediated by the presence of a novel penicillin-binding protein (PBP), PBP-2a, which is expressed by an exogenous *mec*A gene (5, 6).

High scores of MRSA were observed in Egypt compared to other African and Mediterranean countries (7). The dramatic increase in MRSA prevalence raises the challenge of selecting suitable therapy for MRSA infections because of limited therapeutic options. Glycopeptides such as vancomycin has become the cornerstone for treating MRSA infections over the last twenty years (1).

Conceivably, the excessive usage of vancomycin results in the alarming widespread of its resistance and the existence of two types of glycopeptide resistant *S. aureus*. The first type, vancomycin intermediate resistant *S. aureus* (VISA), is due to cross linked and thickened cell wall matrix that sequesters and limits glycopeptides penetration (8). The second one, vancomycin-resistant *S. aureus* (VRSA) is associated with the acquirement of *van*A operon from *Enterococcus faecalis* by a horizontal gene transfer (9).

The increased prevalence of VRSA strains has augmented the controversy regarding the existing and the future role of vancomycin in treating staphylococcal infections and has stimulated great efforts to understand the mechanisms of its resistance. Though, there are several reports of VRSA worldwide, few cases were recorded in different Governorates of Egypt (10,11). According to earlier studies conducted in Egypt, VRSA strains were recognized with many of their infections occurring in humans, while data on VRSA infections in animals is too limited. Based on this rationale, continuous researches should be done for phenotypic and genotypic detection of VRSA to find the ideal antibiotic therapy and to control VRSA infections. Therefore, it was of a great interest to follow up the resistance to vancomycin in *S. aureus* recovered from animal and human origins in Sharkia Governorate and to determine the molecular characterization of the genes conferring this resistance.

Material and methods

Samples

A total of 148 samples were collected from animal (n=97) and human (n=51) sources. The samples from animal origin were milk (n= 66) from cows with clinical signs of mastitis from various dairy farms in Egypt and meat products (n=31) including sausage (n=7), minced meat (n=15) and burger (n=9), which were randomly collected from different supermarkets in Zagazig city, Sharkia Governorate, Egypt. Clinical samples from human origin comprising pus (n=12), urine (n=9), cerebrospinal fluid (C.S.F) (n=8), sputum and peritoneal fluid (n=7, each) and blood and pericardial fluid (n=4, each) were collected from patients admitted to Zagazig University hospital, Sharkia Governorate, Egypt. All samples were obtained under aseptic conditions and quickly

transferred to the laboratory for further bacteriological investigations.

Isolation and identification of staphylococci

The samples were subjected to standard microbiological techniques for isolation and identification of staphylococci (12). Briefly, Baird Parker agar (Oxoid, UK) with an egg yolk–tellurite emulsion supplement (Oxoid, UK) was used for preliminary isolation of staphylococci which were then subjected to Gram staining, oxidation/fermentation (O/F) test and catalase test. The developed colonies were sub-cultured onto mannitol salt agar medium (Oxoid, UK) to confirm mannitol fermentation. Presumptive *S. aureus* colonies were then identified basing on β-hemolysis on blood agar, production of golden yellow pigments onto milk agar, and tube coagulase test. Biotyping of coagulase negative staphylococci (CoNS) was done using the API 20 Staph identification kit (BioMerieux, Marcy l'Etoile, France). Molecular confirmation of putative *S. aureus* isolates was applied by PCR amplification of the *nuc* gene (447bp) using the primer sequences and PCR conditions described previously (13). The sequences of the primer pairs were 5'-GCGATTGATGGTGATACGGTI-3' and 5'-AGCCAAGCCTTGACGAACTAAAGC-3'. The amplification was carried out in a total of 37 cycles under the following conditions: denaturation at 94°C for 1 min, annealing at 55°C for 0.5 min and extension at 72°C for 1.5 min. The reaction was terminated by a final extension step at 72°C for 3.5 min.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for staphylococci isolates was applied adopting the standardized Kirby-Bauer disc-diffusion procedure (14) using the following antimicrobial discs: amoxicillin/clavulanic acid (AMC, 20/10 μg), oxacillin (OX, 1 μg), vancomycin (VA, 30 μg), clindamycin (DA, 10 μg), gentamicin (CN, 10 μg), ciprofloxacin (CIP, 5 μg), trimethoprim/sulfamethaxole (SXT, 23.75/1.25 μg) and rifampin (RA, 5 μg) (Oxoid, UK). The interpretation of the results was done according to Clinical and Laboratory Standards Institute (CLSI) criteria (15). Staphylococci isolates concomitantly resistant to ≥3 antimicrobial classes were considered as multidrug-resistant (MDR).

Minimum inhibitory concentration of vancomycin (Sigma-Aldrich, USA) was detected against *S. aureus* isolates by broth macrodilution method following CLSI guidelines (15). The interpretive criteria were available in the pertinent CLSI document.

PCR-based detections of methicillin and vancomycin-resistance genes

Genomic DNA was extracted from phenotypic methicillin-resistant and vancomycin intermediate and resistant *S. aureus* isolates using the QIAamp DNA Mini kit (Qiagen, GmbH, Germany) according to the manufacturer's instructions. Oligonucleotide primers for *mec*A gene (a determinant of methicillin resistance), 5′-GTA GAA ATG ACT GAA CGT CCG ATA A-3′ & 5′-CCA ATT CCA CAT TGT TTC GGT CTA A-3′ and vancomycin resistance genes, 5′-CAT GAA TAG AAT AAA AGT TGC AAT A-3′ & 5′-CCC CTT TAA CGC TAA TAC GAT CAA-3′) for *van*A and 5′-GTG ACA AAC CGG AGG CGA GGA-3′ & 5′-CCG CCA TCC TCC TGC AAA AAA-3′ for *van*B were selected according to previous reports (16,17). PCR amplifications were performed with a PTC-100 ™ programmable thermal cycler (MJ Research Inc., Waltham, USA) in a total reaction volume of 50 μl consisting of 25μl of Dream *Taq* TM Green Master Mix (2X) (Fermentas, Inc. Hanover, USA), 1μl of each primer (20 pmole) (Sigma-Aldrich, Co., St. Louis, USA), 5μl template DNA and the volume was completed to 50 μl by nuclease-free water. The amplification conditions for *mec*A gene were performed as following: 94°C for 10 min, followed by 10 cycles of 94°C for 45 s, 55°C for 45 s and 72°C for 75 s and 25 cycles of 94°C for 45 s, 50°C for 45 s and 72°C for 75 s (16). Meanwhile, multiplex PCR amplification was carried out for *van*A and *van*B genes using the following thermal cycling conditions: initial denaturation at 94°C for 5 min, 30 cycles of amplification (denaturation at 94°C for 1 min, annealing at 54°C for 1 min and extension at

72°C for 1 min) and a final extension step at 72°C for 10 min (17). An aliquot of each amplicon (8 µl) was loaded on 1.5% agarose gel (Sigma-Aldrich, Co., St. Louis, MO, USA) containing 0.5 µg/mL ethidium bromide (Sigma-Aldrich, Co., St. Louis, MO, USA). A 100 bp DNA ladder (Fermentas, Inc. Hanover, USA) was used as a molecular weight marker. The amplified DNAs were electrophoresed at 100 V for 60 min on a mini horizontal electrophoresis unit (Bio-Rad, USA). The gel was then visualized and photographed under an UV transilluminator (Spectroline, Westbury, USA). For each PCR experiment, appropriate positive and negative controls were included.

Statistical analysis

Data were analyzed by Chi-square test using Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Corp., Armonk, NY). *P* values of < 0.05 were statistically significant.

Results

Characterization of Staphylococci species

Staphylococci isolates were identified by conventional bacteriological methods. All the recovered isolates (n=86) grown onto Baird Parker and mannitol salt agar media were Gram positive cocci, non-spore forming, arranged in grape-like clusters, fermentative and catalase test positive; they were identified as staphylococci. Among them, 45 isolates were β-hemolytic on blood agar, produced the characteristic golden yellow pigments on milk agar and tube coagulase test positive, so they were considered as *S. aureus*. Biochemical identification of CoNS (n=41) using API 20 Staph identification kit revealed 5 biotypes; 12 *S. sciuri* (29.27%) from mastitis milk, 12 *S. xylosus* (29.27%) from mastitis milk (n=10) and human (n=2), 10 *S. lentus* (24.39%) from mastitis milk, 4 *S. chromogenes* (9.76%) from mastitis milk (n=3) and meat products (n=1) and 3 *S. simulans* (7.31%) from human (n=2) and mastitis milk (n=1). Further confirmation of *S. aureus* isolates was conducted by PCR amplification of *nuc* gene (Figure 1A).

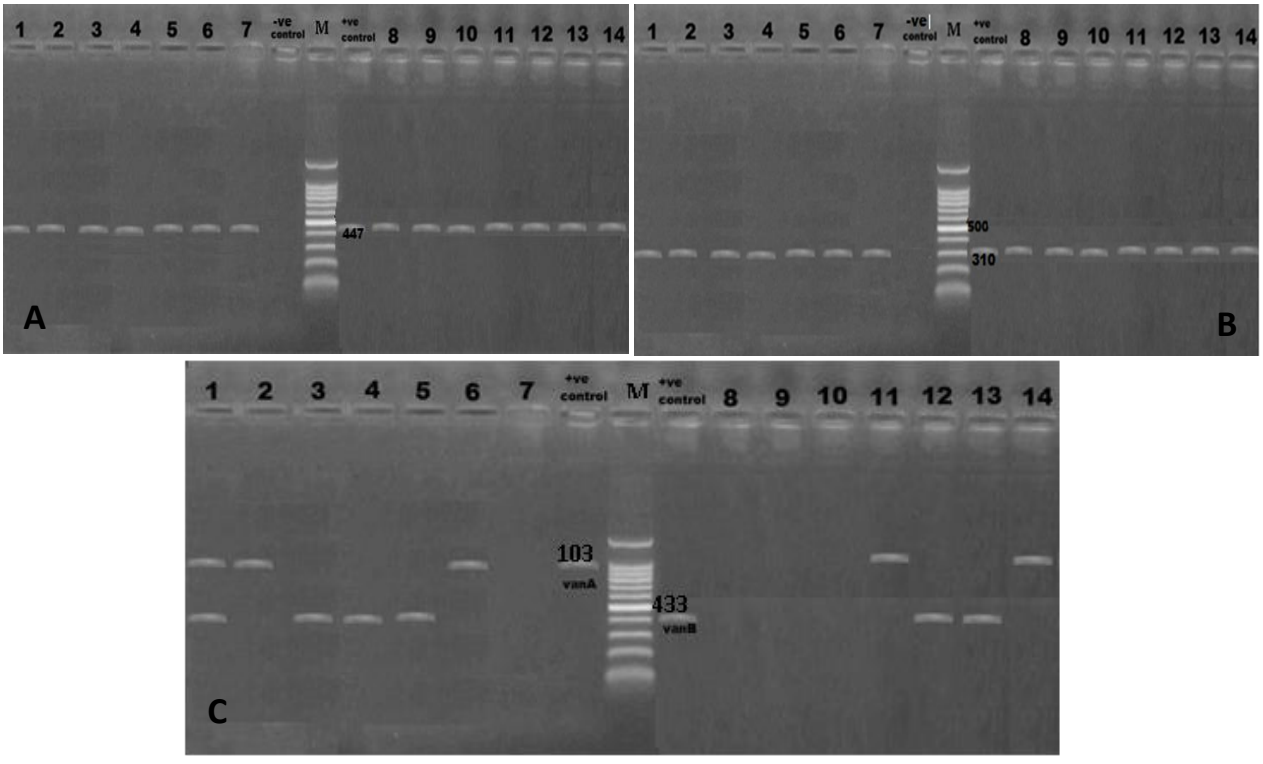

Figure 1: Agarose gel electrophoresis of PCR amplified products of *nuc* (A), *mec*A (B) and *van* (C) genes. Lane M: DNA molecular size marker (100 bp), lanes 1-14: VRSA isolates from minced meat (lanes 1-7), human sources (lanes 8-10), sausage (lanes 11-13) and mastitis milk (lane 14). The size in base pairs (bp) of each PCR product is indicated next to the bands in the +ve control lanes

Incidence of Staphylococcus species in animal and human sources

A total of 86 staphylococcal isolates were recovered from 148 samples (58.10%). Of these, 30.40% (45/148) were *S. aureus* and 27.70% (41/148) were CoNS. It has been observed that 28.87% (28/97) and 33.33% (17/51) of *S. aureus* isolates were recovered from animal and human origins, respectively. Meanwhile, 38.14% (37/97) and 7.84% (4/51) of CoNS isolates were isolated from animal and human samples, respectively. Incidence of *S. aureus* in meat products was 58.06% (18/31) which was higher than milk samples (15.15%, 10/66), but majority of CoNS isolates were isolated from milk samples (36/66, 54.54%). Statistical analysis of our data revealed highly significant differences ($P < 0.05$) in the overall percentage distribution of *S. aureus* and CoNS across mastitis milk, meat products and human samples (Table 1). Incidence of *Staphylococcus* species in diverse samples collected from human and animal origins are illustrated in Table (1).

Table 1: Incidence of *Staphylococcus* species in different samples collected from animal and human origins

Source	Number of analyzed samples (n= 148)	No. of staphylococcal isolates (%)	
		S. aureus	**CoNs**
Mastitis milk	**66**	**10 (.1515)**	**36 (54.54)**
Meat products:	**31**	**18 (.5806)**	**1 (3.23)**
Minced meat	15	9 (60.00)	1 (6.67)
Burger	9	3 (33.33)	0 (0.00)
Sausage	7	6 (85.71)	0 (0.00)
Human:	**51**	**17 (.3333)**	**4 (7.84)**
Pus	12	8 (66.67)	0 (0.00)
Urine	9	1 (11.11)	2 (22.22)
C.S.F	8	2 (25.00)	1 (12.50)
Sputum	7	3 (42.86)	0 (0.00)
Peritoneal fluid	7	3 (42.86)	1 (14.29)
Blood	4	0 (0)	0 (0)
Pericardial fluid	4	0 (0)	0 (0)
Chi-square	-	18.545	42.769
DF	-	2	2
P value*	-	0.000	0.000

CoNS: coagulase negative staphylococci, C.S.F: Cerebrospinal fluid, DF: degree of freedom, *: highly significant.

Antimicrobial susceptibility testing

S. aureus and CoNS were tested against 8 antimicrobial agents (Table 2). *S. aureus* resistance was observed most commonly to oxacillin and amoxicillin-clavulanic acid with percentages of 93.33 and 88.89%, respectively, followed by clindamycin (62.22%). Meanwhile, majority of *S. aureus* showed lower resistance rates against other tested antimicrobials. According to oxacillin/ methicillin susceptibility pattern, majority of the isolates (42/45, 93.33%) were MRSA, while only one isolate (2.22%) was methicillin-sensitive and 2 isolates (4.44%) were methicillin-intermediate. According to vancomycin susceptibility rates (Table 3), 64.44% (29/45) were vancomycin sensitive *S. aureus*, 4.44% (2/45) were VISA, and 31.11% (14/45) were VRSA. Interestingly, all VRSA isolates were MRSA as well as MDR.

Regarding CoNS, resistance was observed most commonly to amoxicillin-clavulanic acid, oxacillin and clindamycin with percentages of 87.80, 75.61 and 58.54%, respectively. Meanwhile, all CoNS exhibited absolute susceptibility (100%) to vancomycin and trimethoprim/sulfamethaxole being the best drugs used for treatment of CoNS infections. Our data showed significant differences in frequency of resistance to several antimicrobials (Table 2) with *P* values < 0.05.

According to the antibiogram results, *S. aureus* and CoNS of human origin (82.35 and 50%) were more multi-drug resistant as compared to those recovered from animal origin (53.57 and 32.43%), respectively

Preliminary screening of vancomycin resistance

The MIC values of vancomycin were determined against all phenotypic vancomycin resistant (n=14) and intermediate (n=2) *S. aureus* isolates with the disc diffusion method. The results showed that 14 *S. aureus* isolates (87.5%) were resistant to vancomycin with MIC values ranged from 32 to 1024 µg/mL, which were classified as VRSA in accordance with the breakpoints published by CLSI. Interestingly, the highly resistance for vancomycin was observed by 7 *S. aureus* from minced meat samples. One of these isolates had MIC value of 1024 µg/mL and the other six isolates had MIC values ranged from 64 to 512µg/mL.

Table 2: Antimicrobial resistance in *S. aureus* and coagulase negative staphylococci isolates from animal and human sources

Antimicrobial agent	Number of resistant *S. aureus* isolates (%)			Total (45)	*P* value	Number of resistant CoNS isolates (%)			Total (41)	*P* value
	Mastitis milk (10)	Meat products (18)	Human (17)			Mastitis milk (36)	Meat products (1)	Human (4)		
AMC	8 (80.00)	18 (100.00)	14 (82.35)	40 (88.89)	0.157	31 (86.11)	1 (100.00)	4 (100.00)	36 (87.80)	0.680
OX	10 (100.00)	17 (94.44)	15 (88.24)	42 (93.33)	0.490	27 (75.00)	1 (100.00)	3 (75.00)	31 (75.61)	0.680
VA	1 (10.00)	10 (55.56)	3 (17.65)	14 (31.11)	0.015	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1.00
DA	3 (30.00)	14 (77.78)	11 (64.71)	28 (62.22)	0.043	20 (55.56)	1 (100.00)	3 (75.00)	24 (58.54)	0.534
CN	1 (10.00)	2 (11.11)	4 (23.53)	7 (15.56)	0.522	0 (0.00)	0 (0.00)	1 (25.00)	1 (2.44)	0.010
CIP	3 (30.00)	0 (0.00)	10 (58.82)	13 (28.89)	0.001	7 (19.44)	1 (100.00)	1 (25.00)	9 (21.95)	0.164
SXT	1 (10.00)	10 (55.56)	3 (17.65)	14 (31.11)	0.015	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1.00
RA	0 (0.00)	4 (22.22)	5 (29.41)	9 (20.00)	0.181	17 (47.22)	1 (100.00)	2 (50.00)	20 (48.78)	0.588

AMC: amoxicillin-clavulanic acid, OX: oxacillin, VA: vancomycin, DA: clindamycin, CN: gentamicin, CIP: ciprofloxacin, SXT: trimethoprim/sulfamethaxole, RA: rifampin, CoNS: coagulase negative staphylococci.

Table 3: Multi-drug resistance profile of recovered staphylococci isolates

No. of antimicrobials to which the isolates were resistant	No of staphylococci isolates (%)					
	S. aureus (45)			CoNS (41)		
	Milk (10)	Meat products (18)	Human (17)	Milk (36)	Meat products (1)	Human (4)
3	0 (0.00)	5 (27.78)	9 (52.94)	9 (25.00)	0 (0.00)	1 (25.00)
4	0 (0.00)	7 (38.89)	2 (11.76)	2 (5.56)	1 (100.00)	0 (0.00)
5	0 (0.00)	2 (11.11)	3 (17.64)	0 (0.00)	0 (0.00)	1 (25.00)
6	1 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

CoNS: coagulase negative staphylococci

PCR detection of mecA and van genes

All 14 MDR-VRSA isolates (3 from human and 11 from animal origins) were screened by PCR detection of *mec*A and *van* genes. The results indicated that all selected isolates were positive for *mec*A gene (Figure 1B).

Furthermore, PCR amplification of *van* genes confirmed their possession among 10 *S. aureus* isolates only (71.4%); however, the other 4 *S. aureus* isolates (28.8%) were *van* genes negative. Data revealed that *van*B and *van*A genes were found in 5 and 4 isolates (35.7 and 28.6%, respectively) and they were detected simultaneously in one isolate (7.1%). Bands with approximate size of 310 bp were detected for *mec*A gene (Figure 1B), while *van*A and *van*B genes were observed at 1030 and 433 bp, respectively (Figure 1C). Interestingly, *van*A gene was detected in isolates with MICs ranging from 128 to 512 µg/mL, where *van*B gene was observed in isolates with MICs ranging from 64 to 256 µg/mL. In addition, the only isolate gave positive results for both *van*A and *van*B genes exhibited a high MIC value (1024 µg/mL). Characterization of all VRSA isolates elicited from the study is illustrated in Table (4).

Table 4: Description of 14 VRSA isolates elicited from the study

VRSA isolate No.	Source	Results of disc diffusion method			Vancomycin		*mec*A gene	*Van* genes
		S	I	R	MIC (µg/mL)	MBC (µg/mL)		
1	Minced meat	CIP	CN	AMC, OX, VA, DA, SXT, RA	1024	>1024	+	*van*A, *van*B
2	Minced meat	CIP	CN, RA	AMC, OX, VA, DA, SXT	256	512	+	*van*B
3	Human	CIP	RA	AMC, OX, VA, DA, CN, SXT	64	128	+	-
4	Mastitis milk	RA	-	AMC, OX, VA, DA, CN, CIP , SXT	128	256	+	*van*A
5	Sausage	CIP	CN, RA	AMC, OX, VA, DA, SXT	64	64	+	*van*B
6	Minced meat	CIP, RA	-	AMC, OX, VA, DA, CN, SXT	64	128	+	*van*B
7	Human	CN, CIP, RA	DA	AMC, OX, VA, SXT	32	64	+	-
8	Human	CN, CIP	-	AMC, OX, VA, DA, SXT, RA	32	64	+	-
9	Minced meat	CIP, RA	CN	AMC, OX, VA, DA, SXT	128	256	+	*van*A
10	Minced meat	CIP	CN, RA	AMC, OX, VA, DA, SXT	512	1024	+	*van*A
11	Sausage	CIP, RA	CN	AMC, OX, VA, DA, SXT	64	128	+	*van*B
12	Sausage	CIP, RA	CN	AMC, OX, VA, DA, SXT	128	256	+	*van*A
13	Minced meat	CIP	CN, RA	AMC, OX, VA, DA, SXT	256	512	+	*van*B
14	Minced meat	CIP	DA, CN, RA	AMC, OX, VA, SXT	64	128	+	-

S: sensitive, I: intermediate, R: resistant, AMC: amoxicillin-clavulanic acid, OX: oxacillin, VA: vancomycin, DA: clindamycin, CN: gentamicin, CIP: ciprofloxacin, SXT: trimethoprim/sulfamethaxole, RA: rifampin, MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration, +: positive, -: negative

Discussion

Multidrug resistant MRSA is the uppermost cause of community acquired and nosocomial infections worldwide, being associated with high mortality rates. Vancomycin has been suggested as the best drug for treatment of such infections (18). The increase in vancomycin resistance among MDR and MRSA strains has been supposed as a formidable threat in the therapeutic fields (19). Therefore, this study has made an attempt to explore the vancomycin resistance among MRSA isolates originated from human and animal sources (mastitis milk and meat products) for the first time in Egypt.

In the current study, staphylococci isolates were identified phenotypically by conventional microbiological methods as anecdotally reported (20). Further confirmation of all *S. aureus* isolates was conducted based on PCR detection of *nuc* gene as declared previously (13). Identification of the recovered CoNS isolates using API 20 Staph identification kit revealed 5 biotypes which were *S. sciuri*, *S. xylosus*, *S. lentus*, *S. chromogenes* and *S. simulans* with *S. sciuri* and *S. xylosus* being predominant (29.27%, each). They were mostly

from milk samples of cows suffering from mastitis. On the contrary, *S. chromogenes*, *S. haemolyticus*, *S. epidermidis* and *S. simulans* were accounted for 81.3% of all CoNS milk isolates in a recent scientific literature in Belgium (21).

Herein, *S. aureus* was isolated from animal and human origins with percentages of 28.87 and 33.33%, respectively. Incidence of *S. aureus* in meat products was 58.06% which was higher than mastitis milk (15.15%). Abd El-Hamid and Bendary (22) recorded a lower percentage of *S. aureus* in human subjects (26.67%). In previous studies, the isolation percentages of *S. aureus* in mastitis milk samples were 23 and 24%, respectively (23,24) which were higher than the frequency documented in the current study. Meanwhile, another study carried out in Egypt reported a lower incidence rate of *S. aureus* in milk samples from cases of cow mastitis (10.94%) (22). Additionally, a lower incidence rate of *S. aureus* (10%) was previously found in meat products by Ammar and coauthors (25). The differences in incidence rates among studies are interrelated to sampling, geographic area, environmental factors and the detection methods.

Resistance to commonly used drugs is an alarming emergent concern in human and veterinary fields. Considering the sequential changes in the burden of the resistance problem, detecting its determinants is important for managing control efforts (26). In the present study, the results of the antimicrobial susceptibility testing showed that *S. aureus* resistance was observed most commonly to oxacillin exhibiting 93.33% MRSA isolates, followed by amoxicillin-clavulanic acid (88.89%) and clindamycin (62.22%). In contrast, 37% resistance to amoxicillin-clavulanic (27) and 20% resistance to clindamycin (19) have been previously reported. In addition, twenty one (72.41%) of 29 *S. aureus* strains were MRSA as declared by Hasan and joint authors (19). This variation in resistance might be correlated to the type of antimicrobial agent recommended for treatment in various geographical areas.

Regarding CoNS, the antimicrobial resistance was observed most commonly to amoxicillin-clavulanic acid (87.80%), followed by oxacillin (75.61%), while all the recovered isolates showed 100% susceptibility to vancomycin and trimethoprim-sulfamexazole. The previous findings were consistent with a recent previous study (26), where CoNS showed significant increasing temporal trends in oxacillin and amoxicillin-clavulanate resistance. Moreover, a previous work carried out in Pakitsan revealed that their CoNS isolates exhibited high resistance levels to oxacillin (70.3%) and amoxicillin (74.8%), but low resistance rates were observed against ciprofloxacin (35.2%), amoxicillin/clavulanate (32.8%), clindamycin (16.3%) and vancomycin (2.6%), which was in harmony with our results indicating that ciprofloxacin, clindamycin and vancomycin are effective agents for treatment of CoNS infections (28).

Moreover, antibiogram results revealed that MDR pattern was pronounced in majority of *S. aureus* and CoNS isolates of human origin (82.35 and 50%) comparing to those recovered from animal origin (53.57 and 32.43%), respectively, which is consistent with another study in India (29). This may be attributed to that the extensive use of antibiotics in Egypt has rendered the frequently used antibiotics fully ineffective in treatment of staphylococcal infections.

A myriad of studies have focused on vancomycin resistance among MDR-MRSA isolates (18,19) revealing the necessity for effective and new drugs against MRSA. In this study, 31.11% (14/45) of *S. aureus* isolates were categorized as VRSA with MIC values ranged from 32-1024 µg/mL, they were all MDR and MRSA as was indicated by the presence of *mec*A gene. The highly resistance for vancomycin was observed in 7 *S. aureus* from minced meat samples. PCR amplifications exhibited the possession of the tested *van* genes among 10 *S. aureus* isolates only (71.4%); *van*B and *van*A genes were detected in 5 and 4 VRSA isolates with percentages of 35.7 and 28.6%, respectively and they were detected simultaneously in one isolate (7.1%). Previously, a similar level of VRSA was

observed in Egypt as 32% of *S. aureus* isolates (commonly from minced meat) were vancomycin resistant, 87.50% out of them possessed the *van* genes with a high predominance of *van*B (50%) (25). In contrast, all examined *S. aureus* isolates were vancomycin sensitive in another study in Pakistan (30).

Conclusion

Our study has focused on the great emergence of VRSA among isolated MRSA from mastitis milk, meat products and human sources as a first report at least in Egypt. In light of the results, a major fraction of VRSA was detected among recovered MRSA isolates indicating the unawareness and undiscerning usage of the broad-spectrum antibiotics and thereby revealing the need for novel and effective antibiotics against MRSA.

Conflict of interest

None of the authors have any conflict of interest to declare.

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