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Research Article
Genetic Characterization of Enterotoxigenic Strains of Methicillin-Resistant and Susceptible *Staphylococcus aureus* Recovered from Bovine Mastitis

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Abstract

Background and Objective: The emergence of methicillin-resistant *Staphylococcus aureus* in animals has been an outbreak of mastitis in cattle which represents a great economic loss in milk industry and a serious public health concern worldwide. The objective of this study was to identify the antimicrobial resistance patterns and the prevalence of methicillin resistant and susceptible *Staphylococcus aureus* harboring the classical staphylococcal enterotoxin genes in bovine mastitis cases. **Materials and Methods:** A total of 112 *Staphylococcus aureus* isolates were investigated for antibiotic resistance patterns. Some of the isolates were subjected to genotypic analysis for methicillin-resistant genes (*mecA*) and classical staphylococcal enterotoxin A through E genes. **Results:** Methicillin-resistant *Staphylococcus aureus* was phenotypically detected in 77 (68.7%) of the isolates, while methicillin-susceptible *Staphylococcus aureus* was identified in 35 (31.2%) of isolates, most of the isolates were showed multidrug-resistance to at least one member of the antimicrobial classes. By genotypic analysis, 54.5% phenotypically methicillin-resistant isolates were *mecA* gene positive (methicillin-resistant *Staphylococcus aureus*), while 45.5% of the isolates were *mecA* gene negative (methicillin-susceptible *Staphylococcus aureus*). Variation in the staphylococcal-enterotoxin encoding genes was detected. The 66.7% of methicillin-resistant *Staphylococcus aureus* and 80% of methicillin-susceptible *Staphylococcus aureus* were encoding staphylococcal enterotoxin genes. The most frequently detected staphylococcal enterotoxin genes were the combination of staphylococcal enterotoxin A, D and E representing 50% of methicillin-resistant *Staphylococcus aureus* isolates as well as staphylococcal enterotoxin D representing 75% of methicillin-susceptible *Staphylococcus aureus* isolates. **Conclusion:** The high prevalence of multidrug-resistant and toxigenic methicillin resistant and susceptible *Staphylococcus aureus* strains in bovine milk are considered as a potential threat to public health. Hence, the right use of antibiotic of choice in the line of treatment and control of the bovine mastitis caused by *Staphylococcus aureus* is very crucial in Egypt.

Key words: Methicillin-resistant *Staphylococcus aureus*, methicillin-susceptible *Staphylococcus aureus*, bovine mastitis, *mecA* gene, staphylococcal enterotoxin genes

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of the most pathogenic bacteria is *Staphylococcus aureus* (*S. aureus*) that is associated with contagious bovine mastitis. Pathogenicity of *S. aureus* is mainly related to a combination of toxin-mediated virulence, invasive capacity and antibiotic resistance[1,2]. Virulent S. *aureus* strains include methicillin-resistant *Staphylococcus aureus* (MRSA) strains which have become resistant to most antimicrobial agents including β-lactams, aminoglycosides, macrolides and more recently fluoroquinolones[3]. Therefore, the spread of *S. aureus*, especially MRSA and its control have become a major challenge in Egypt.

The major challenge of *S. aureus* related infections has been the difficulty in treatment due to antimicrobial resistance. The levels of antibiotic-resistant infections in the developing countries, especially Egypt, have increased steadily in the last years as a result of the misuse of antimicrobial agents both in therapy and as growth promoters in food animals[4].

A variety of toxins is produced by some strains of *S. aureus* including staphylococcal enterotoxins (SEs) as superantigens which cause a wide range of infections in humans and animals, including staphylococcal food poisoning and bovine mastitis[5]. The staphylococcal enterotoxins are heat stable exotoxins, include SEA through SEE and SEG through SEJ, being produced sometimes singly and sometimes in combination[6].

Little data is available on the epidemiology of MRSA isolates from the bovine origin in Egypt. Therefore, the aim of the current investigation was to study the antimicrobial resistance patterns and the prevalence of methicillin-resistant *Staphylococcus aureus* harboring the classical *SE* genes using multiplex PCR as well as the determination of the predominant enterotoxin in each strain. In Egypt, such information could give an indication of the virulence of MRSA present in bovine mastitis.

MATERIALS AND METHODS

Samples: In total, 230 milk samples were collected from cows (n = 115) and buffaloes (n = 115) with clinical or subclinical mastitis from different farms in Dakahlia and Damietta governorates, Egypt in January, 2016. All milk samples were subjected to bacteriological examination.

Microbiological analysis: *S. aureus* strains were isolated on Baird Parker agar, mannitol salt agar and 5% sheep blood agar (Oxoid). All isolates were identified by conventional tests[7] and coagulase tests (both slide and tube)[8].

Antimicrobial susceptibility test: The disk diffusion assay[9] was performed by using 10 antibacterial disks (Oxoid, England) and interpreted according to NCCLS[10].

Oxacillin agar screen: The oxacillin agar screen was performed as described by Felten *et al.*[11]. The oxacillin resistant isolates were grown within 48 h.

Molecular identification of *mecA* gene and staphylococcal enterotoxin A through E genes using Multiplex Polymerase Chain Reaction (mPCR): The DNA extraction and purification from *S. aureus* isolates (grown in BHI) were performed according to the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) manufacturer's recommendations with modifications. The DNA was amplified using specific primers supplied from Metabion, Germany with the specific profile (Table 1). Separation of the PCR products was done by electrophoresis on 1.5% agarose gel. The fragment sizes were determined by using gelpilot 100 bp DNA ladder (Qiagen, Germany, GmbH). A gel documentation system (Alpha Innotech, Biometra) was used to photograph the gel, followed by analysis of the data through computer software.

RESULTS

Based on cultural and biochemical properties, a total of 112 (48.7%) *S. aureus* isolates were recovered from 230 bovines (cows and buffaloes) mastitis milk samples as shown in Table 2. Oxacillin screening test was performed on all 112 *S. aureus* isolates to detect methicillin-resistant strains. Seventy-seven(77/112, 68.7%) of the tested isolates were positive by the oxacillin screening test and determined to be methicillin-resistant *S. aureus* (MRSA), while other isolates (35/112, 31.2%) without growth on the oxacillin agar screen plate were identified as methicillin-susceptible *S. aureus* (MSSA).

In vitro, the antibiotic susceptibility of all 112 *S. aureus* (77 MRSA and 35 MSSA) isolates against 10 different antimicrobial agents from different antibiotic classes is presented in Table 3. The resistance of 77 MRSA isolates to oxacillin, penicillin and ampicillin (100%, for each) were the

Table 1: Primers sequences, target genes, amplicon sizes and cycling conditions

			Amplification (35 cycles)					
Target genes	Primers sequences	Amplified segment (bp)	Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension	References
SEA	GGTTATCAATGTGCGGGTGG	102	94°C for 5 min	94°C for 30 sec	50°C for 45 sec	72°C for 45 sec	72°C for 10 min	McClure *et al.*[37]
	CGGCACTTTTTTCTCTTCGG							
SEB	GTATGGTGGTGTAACTGAGC	164						
	CCAAATAGTGACGAGTTAGG							
SEC	AGATGAAGTAGTTGATGTGTATGG	451						
	CACACTTTTAGAATCAACCG							
SED	CCAATAATAGGAGAAAATAAAAG	278						
	ATTGGTATTTTTTTTCGTTC							
SEE	AGGTTTTTTCACAGGTCATCC	209						
	CTTTTTTTTCTTCGGTCAATC							
mecA	GTA GAA ATG ACT GAA CGT CCG ATA A	310	94°C for 5 min	94°C for 45 sec	50°C for 45 sec	72°C for 45 sec	72°C for 10 min	Mehrotra *et al.*[38]
	CCA ATT CCA CAT TGT TTC GGT CTA A							

Table 2: Enzymatic activities of *S. aureus* isolated from milk

	Total (112/230)	
Enzymatic activity	Positive	%
Baired parker agar		
Tellurite	112	100.0
Lecithinase	100	89.3
Mannitol salt agar	112	100.0
Haemolysis on 10% sheep blood agar	112	100.0
Coagulase test		
Slide C.T.	107	95.5
Tube C.T.	102	91.1
Fermentation of sugars		
Glucose	112	100.0
Lactose	112	100.0
Sucrose	100	89.3
Simmons citrate utilization test	95	84.8
Urease activity	99	88.4
Catalase activity	112	100.0

most common finding, followed by tetracycline (91%), clindamycin (77.9%), erythromycin (53.2%) and gentamicin (39%). The highest frequencies of resistance detected by 35 MSSA isolates were those against tetracycline (68.6%), clindamycin (60%), gentamicin (45.7%), ampicillin (40%) and penicillin (34.3%). On the other hand, the resistance to the other antibiotics vancomycin, chloramphenicol and ciprofloxacin were not as high as the other antibiotics either to MRSA or MSSA.

The molecular expression for *mecA* genes revealed that 42 (54.5%) of 77 isolates possessed the resistant genes (*mecA*) while the absence of *mecA* gene was found in 35 (45.5%) of these isolates. Table 4 shows the comparison between the detection of *mecA* gene by PCR and oxacillin screening test to 77 isolates, 42 strains were genotypically *mecA* gene positive and phenotypically resistant to oxacillin, whereas other (35) strains were genotypically *mecA* gene negative and phenotypically oxacillin susceptible.

According to the results of multiplex PCR analysis of 77 isolates, this study revealed about 72.7% (56/77) *S. aureus* isolates, 66.7% (28/42) of MRSA and 80% (28/35) of MSSA, encoding staphylococcal enterotoxin (*SE*) genes (Table 4). The most commonly detected genes were the combination of SEA, SED, SEE (14 isolates) representing 50% of MRSA isolates and SED (21 isolates) representing 75% of MSSA isolates. The remaining isolates harbored combination of *SEA*, *SEE* genes (7 isolates, 25%) and *SED*, *SEE* genes (7 isolates, 25%) of MRSA as well as *SEB*, *SED*, *SEE* genes (7 isolates, 25%) of MSSA. None of the isolates were positive for *SEA*, *SEB*, *SEC* and *SEE* genes alone.

Table 3: Antibiotic susceptibility profiles of MRSA and MSSA isolates

	Staphylococcus aureus (n = 112)					
	MRSA (n = 77)		MSSA (n = 35)		Total (n = 112)	
Antibiotics	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Oxacillin agar screen	77 (100.0)	0.0	0.0	35 (100.0)	83 (74.1)	29 (25.9)
Oxacillin (1 µg)	77 (100.0)	0.0	0.0	35 (100.0)	83 (74.1)	29 (25.9)
Ampicillin (10 µg)	77 (100.0)	0.0	14 (40.0)	21 (60.0)	95 (84.8)	17(15.2)
Penicillin G (10 IU)	77 (100.0)	0.0	12 (34.3)	23 (65.7)	93 (83.0)	19 (17.0)
Tetracycline (30 µg)	70 (91.0)	007 (9.0)	24 (68.6)	11 (31.4)	96 (85.7)	16 (14.3)
Clindamycin (2 µg)	60 (77.9)	17 (22.1)	21 (60.0)	14 (40.0)	81 (72.3)	31 (27.7)
Erythromycin (15 µg)	41 (53.2)	36 (46.8)	13 (37.1)	22 (62.9)	55 (49.1)	57 (50.9)
Gentamicin (10 µg)	30 (39.0)	47 (61.0)	16 (45.7)	19 (54.3)	45 (40.2)	67 (59.8)
Ciprofloxacin (5 µg)	14 (18.2)	63 (81.8)	9 (25.7)	26 (74.3)	22 (19.6)	90 (80.4)
Chloramphenicol (30 µg)	11 (14.3)	66 (85.7)	6 (17.1)	29 (82.9)	16 (14.3)	96 (85.7)
Vancomycin (30 µg)	0.0	71(100.0)	2 (5.7)	33 (94.3)	2 (1.8)	110 (98.2)

R: Resistant, S: Sensitive

Table 4: Incidence of MRSA isolates harbouring classical SE encoding genes (n = 77)

Types of sample	Number of isolates (n)	Oxacillin agar screen (6 µg)	MRSA (*mecA* gene positive)	MSSA (*mecA* gene negative)	SE positive	*mecA* gene +ve and SE positive	*mecA* gene -ve and SE positive	*SEA*	*SEB*	*SEC*	*SED*	*SEE*	*SEA, SEE*	*SED, SEE*	*SEA, SED, SEE*	*SEB, SED, SEE*	Staphylococcal enterotoxins (SE)
Cows milk (n = 39)	4	R	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	R	+	-	+	+	-	-	-	-	-	-	-	+	-	-	*SED, SEE*
	11	R	-	+	+	-	+	-	-	-	+	-	-	-	-	-	*SED*
	7	R	+	-	+	+	-	-	-	-	-	-	-	-	+	-	*SEA, SED, SEE*
	4	R	+	-	+	+	-	-	-	-	-	-	+	-	-	-	*SEA, SEE*
	7	R	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	R	-	+	+	-	+	-	-	-	-	-	-	-	-	+	*SEB, SED, SEE*
Buffaloes milk (n = 38)	3	R	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	R	+	-	+	+	-	-	-	-	-	-	-	+	-	-	*SED, SEE*
	10	R	-	+	+	-	+	-	-	-	+	-	-	-	-	-	*SED*
	7	R	+	-	+	+	-	-	-	-	-	-	-	-	+	-	*SEA, SED, SEE*
	3	R	+	-	+	+	-	-	-	-	-	-	+	-	-	-	*SEA, SEE*
	7	R	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	R	-	+	+	-	+	-	-	-	-	-	-	-	-	+	*SEB, SED, SEE*

DISCUSSION

S. aureus is a major causative bacterium of clinical or subclinical bovine mastitis generates important losses in dairy industries in Egypt[12] and is considered as a risk factor for food poisoning in humans[13]. The high frequency of staphylococcal mastitis (48.7%) in this study confirms the general agreement with other studies supporting the previous postulation that *S. aureus* is the very important factor in bovine mastitis all over the world[4,5,14,15].

MRSA strains have been identified among *S. aureus* strains isolated from bovine milk[16,17], so it is considered an emerging threat to a serious public health hazard. Although the oxacillin disk diffusion test is the least reliable test for detection of resistance to oxacillin in *S. aureus*[18] in comparison to more sensitive and specific oxacillin screening test[19,20], there was a similarity between the results of oxacillin screening and oxacillin disk diffusion tests in the identification of *S. aureus* as MRSA and MSSA. Thus, oxacillin screening test and oxacillin disk diffusion tests have been very useful for the detection of MRSA strains in this study. These results revealed that the MRSA isolates were detected with the high rate (68.7%) in milk samples. Similarly, the high prevalence of MRSA strains was detected by Al-Ashmawy *et al.*[21] (75%) from raw milk in Egypt and Widianingrum *et al.*[4] (50%) from bovine milk in Indonesia. On the contrary, a much lower percentage of MRSA isolation was reported by Intrakamhaeng *et al.*[22] from bovine mastitis cases (19.7%) in Thailand, Kamal *et al.*[12] from raw milk (8.6%) and Ammar *et al.*[23] from examined milk (17.34%) in Egypt. These differences in the MRSA incidence could be contributed to the method of MRSA determination, different national antimicrobial policies and presence of several animals in the same area that facilitate the transfer of genetic material between *S. aureus* strains. Oxacillin and other β-lactam antibiotics have been used as therapeutic agents for staphylococcal mastitis in Egyptian dairy animals and that may be the reason for the emergence of MRSA in the bovine milk. According to *in vitro* susceptibility tests, the majority of MRSA strains exhibited resistance to oxacillin, penicillin, ampicillin, tetracycline, clindamycin and erythromycin. A large percentage of the strains were susceptible to the antibiotics vancomycin, chloramphenicol, ciprofloxacin and gentamicin. Furthermore, most MSSA strains were highly resistant to tetracycline, clindamycin, gentamicin, erythromycin, penicillin and ampicillin but were oxacillin, vancomycin, chloramphenicol and ciprofloxacin susceptible. These findings were similar to the previous studies[24,25]. As well, this coresistance pattern of MRSA strains (β-lactams, tetracyclines, macrolides and aminoglycosides) has been frequently detected by other investigators[26,27]. On the other hand, the most effective antimicrobials against MRSA and MSSA isolates were vancomycin, chloramphenicol and ciprofloxacin according to this study. Interestingly, the emergence of multidrug-resistant MRSA and MSSA strains are due to the misuse of a large number of antimicrobial agents in Egyptian dairy farms. Consequently, the search for newer and more effective antibiotics against this organism as well as effective measures against the unregulated use of antibiotics is necessary for effective treatment of staphylococcal infections and avoiding the development of multidrug resistant strains.

In the current study, most strains were *mecA* positive oxacillin-resistant *S. aureus*. On the other hand, there were some strains *mecA* negative oxacillin-resistant *S. aureus*. This indicated the presence of incompatibility between the detection of methicillin resistance phenotypically using oxacillin screening test and the absence of *mecA* gene in some MRSA strains. This finding is in accordance with Garcia-Alvarez *et al.*[28], who identified phenotypic MRSA isolates without *mecA* gene. This may be attributed to the presence of PCR inhibitors or other physical factors that may have compromised the sensitivity of PCR in the detection of *mecA* gene[29]. Also, methicillin resistance may be occurred by other mechanisms other than the expression of *mecA* gene[28] or instability of the *mecA* gene in strains with the loss of *mecA*[30]. Furthermore, the discovering of phenotypically resistant of some *mecA* negative isolates to β-lactam antimicrobial agents may be correlated to a less common type of resistance because of overproduction of β-lactamase or the presence of altered penicillin-binding protein (PBP) not related to 2a or 2'[31]. It is also possible that the *mecA* negative oxacillin-resistant *S. aureus* may have *mecA* alleles which could not be detected by the primers used in this study. However, the previous studies showed that *mecA* gene-positive isolates are not necessary to be associated with its expression to oxacillin resistance by the conventional phenotypic methods because there are some determinants, such as the *mecR1-mecI*, *BlaI* and *fem* genes, that control the expression of oxacillin resistance[30]. Furthermore, the cost and work load of PCR to detect the *mecA* gene have prevented their broad use in a clinical microbiology laboratory. Thus, regardless the presence or absence of *mecA* gene by PCR, the detection of resistance to oxacillin and β-lactams offers an interesting new approach to the rapid characterization of *S. aureus* as MRSA or MSSA.

Overall, there is a serious health risk of MRSA and MSSA caused by the production of staphylococcal enterotoxins as superantigens, stimulating T-lymphocytes to release

cytokines and T-cell proliferation[32]. Thus, a multiplex PCR-based diagnostic protocol was used in this study to detect the genes for staphylococcal enterotoxins A to E. About 72.7% of *S. aureus* isolates were enterotoxigenic in this research. Similar results were observed by El-Bagoury[33] and Bystron *et al.*[34], who recorded 75 and 66% toxigenic strains of S. *aureus* isolates, respectively. Moreover, this study detected the high prevalence (66.7%) of MRSA isolates encoding staphylococcal enterotoxin genes. This result is consistent with other researchers who found 82.4% of MRSA isolates harboring *SE* genes[19]. Interestingly, the most commonly detected genes were the combination of *SEA*, *SED*, *SEE* genes in MRSA isolates and *SED* gene in MSSA isolates in this research. MRSA isolates encoded *SED* as the most predominant enterotoxin genes were observed by other researchers in mastitis isolates[35]. These classical genes, *SEA*, *SEB*, *SED* and *SEE* are more frequently detectable genes from MRSA and MSSA isolates from mastitis milk[36]. The *SEC* gene could not be detected by this study in comparison to the previous studies[36]. This finding highlights a potential risk for consumers because MRSA and MSSA of animal origin may contaminate foods and represent a source of potential infection and intoxication in humans.

CONCLUSION

These data indicate the bovine milk represents a potential risk of multidrug-resistant and toxigenic *S. aureus* either MRSA or MSSA in Egypt. Accordingly, the line of treatment with the prudent use of antibiotics and control of the bovine mastitis caused by *S. aureus* is very crucial to prevent the emergence and spread of multidrug-resistant *S. aureus* strains in Egypt. In addition, further detailed analysis of functional genomics is now warranted to get a better understanding of enterotoxin activity and virulence of *S. aureus* in Egypt.

SIGNIFICANCE STATEMENT

This study discovers the high prevalence of multidrug-resistant and toxigenic methicillin resistant and susceptible *Staphylococcus aureus* strains in bovine mastitis. Such information can give an indication of the virulence of methicillin resistant and susceptible *Staphylococcus aureus* present in bovine mastitis as well as a potential threat of bovine milk to public health. This study will help the researchers to uncover the critical areas of the misuse of the antibiotic in the dairy farms on the emergence of multidrug-resistant MRSA and MSSA strains. Thus, the significance of the searching for newer and more effective antibiotics against this pathogen besides effective measures against the unregulated use of antibiotics is essential for effective treatment of staphylococcal infections and avoiding the development of multidrug resistant strains.

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REFERENCES

1. Argudin, M.A., M.C. Mendoza and M.R. Rodicio, 2010. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxin, 2: 1751-1773.
2. Petersson-Wolfe, C.S., I.K. Mullarky and G.M. Jones, 2010. *Staphylococcus aureus* mastitis: Cause, detection and control. Publication No. 404-229. Virginia Cooperative Extension, USA., pp: 1-7.
3. Van Loo, I., X. Huijsdens, E. Tiemersma, A. de Neeling and N. van de Sande-Bruinsma *et al*., 2007. Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. Emerg. Infect. Dis., 13: 1834-1839.
4. Widianingrum, D.S., S. Windria and S.I.O. Salasia, 2016. Antibiotic resistance and methicillin resistant *Staphylococcus aureus* isolated from bovine, crossbred Etawa goat and human. Asian J. Anim. Vet. Adv., 11: 122-129.
5. Wang, D., L. Zhang, X. Zhou, Y. He and C. Yong *et al*., 2016. Antimicrobial susceptibility, virulence genes and randomly amplified polymorphic DNA analysis of *Staphylococcus aureus* recovered from bovine mastitis in Ningxia, China. J. Dairy Sci., 99: 9560-9569.
6. Al-Shangiti, A.M., C.E. Naylor, S.P. Nair, D.C. Briggs, B. Henderson and B.M. Chain, 2004. Structural relationships and cellular tropism of staphylococcal superantigen-like proteins. Infect. Immun., 72: 4261-4270.
7. Iurlina, M.O. and R. Fritz, 2004. Microbiological quality of Port Salut Argentino cheese stored at two temperature treatments. LWT-Food Sci. Technol., 37: 739-748.
8. Mackie, T.J., 1996. Mackie & McCartney Practical Medical Microbiology. International Student 14th Edn., Chapter 11. Churchill Livingstone, USA.
9. Finegold, S.M. and W.J. Martin, 1982. Bailey and Scott's Diagnostic Microbiology. 6th Edn., The C.V. Mosby Company, St. Louis.
10. NCCLS., 2002. Performance standards for antimicrobial disk susceptibility tests. Document M100-S12, National Committees for Clinical Laboratory Standard, Pennsylvania.

11. Felten, A., B. Grandy, P.H. Lagrange and I. Casin, 2002. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): A disk diffusion method with cefoxitin and moxalactam, the vitek 2 system and the MRSA-screen latex agglutination test. J. Clin. Microbiol., 40: 2766-2771.
12. Kamal, R.M., M.A. Bayoumi and S.F.A. Abd El Aal, 2013. MRSA detection in raw milk, some dairy products and hands of dairy workers in Egypt, a mini-survey. Food Control, 33: 49-53.
13. Kadariya, J., T.C. Smith and D. Thapaliya, 2014. *Staphylococcus aureus* and staphylococcal food-borne disease: An ongoing challenge in public health. BioMed Res. Int. 10.1155/2014/827965.
14. Sospedra, I., R. Marin, J. Manes and J.M. Soriano, 2012. Rapid whole protein quantification of staphylococcal enterotoxin B by liquid chromatography. Food Chem., 133: 163-166.
15. Ahmady, M. and S. Kazemi, 2013. Detection of the enterotoxigenic genes (SEI, SEJ) in *Staphylococcus aureus* isolates from bovine mastitis milk in the West Azerbaijan of Iran. Comp. Clin. Pathol., 22: 649-654.
16. Pilla, R., V. Castiglioni, M.E. Gelain, E. Scanziani, V. Lorenzi, M. Anjum and R. Piccinini, 2013. Long-term study of MRSA ST1, t127 mastitis in a dairy cow. Vet. Record Case Rep., Vol. 1, No. 1. 10.1136/vetreccr.100510rep.
17. Hummerjohann, J., J. Naskova, A. Baumgartner and H.U. Graber, 2014. Enterotoxin-producing *Staphylococcus aureus* genotype B as a major contaminant in Swiss raw milk cheese. J. Dairy Sci., 97: 1305-1312.
18. Knapp, C.C., M.D. Ludwig, J.A. Washington and H.F. Chambers, 1996. Evaluation of Vitek GPS-SA card for testing of oxacillin against borderline-susceptible staphylococci that lack mec. J. Clin. Microbiol., 34: 1603-1605.
19. Chambers, H.F., 1997. Methicillin resistance in staphylococci: Molecular and biochemical basis and clinical implications. Clin. Microbiol. Rev., 10: 781-791.
20. Kalhor, H., L. Shariati, M. Validi, M.A. Tabatabaiefar and M.R. Nafisi, 2012. Comparison of agar screen and duplex-PCR methods in determination of Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains isolated from nasal carriage. Afr. J. Microbiol. Res., 6: 3722-3726.
21. Al-Ashmawy, M.A., K.I. Sallam, S.M. Abd-Elghany, M. Elhadidy and T. Tamura, 2016. Prevalence, molecular characterization and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolated from milk and dairy products. Foodborne Pathog. Dis., 13: 156-162.
22. Intrakamhaeng, M., T. Komutarin, K. Pimpukdee and W. Aengwanich, 2012. Incidence of enterotoxin-producing MRSA in bovine mastitis cases, bulk milk tanks and processing plants in Thailand. J. Anim. Vet. Adv., 11: 655-661.
23. Ammar, A.M., A.M. Attia, M.I. Abd El-Hamid, I.M. El-Shorbagy and S.A. Abd El-Kader, 2016. Genetic basis of resistance waves among methicillin resistant *Staphylococcus aureus* isolates recovered from milk and meat products in Egypt. Cell. Mol. Biol., 62: 7-15.
24. Kumari, D.N., V. Keer, P.M. Hawkey, P. Parnell, N. Joseph, J.F. Ricardson and B. Cookson, 1997. Comparison and application of ribosome spacer DNA amplicon polymorphisms and pulsed-field gel electrophoresis for differentiation of methicillin-resistant *Staphylococcus aureus* strains. J. Clin. Microbiol., 35: 881-885.
25. Fadel, H.M. and J. Ismail, 2016. Occurrence and zoonotic importance of methicillin-resistant *Staphylococcus aureus* in raw milk and some dairy products at Ismailia City, Egypt. Zagazig Vet. J., 43: 95-104.
26. Franco, A., H. Hasman, M. Iurescia, R. Lorenzetti and M. Stegger *et al*., 2011. Molecular characterization of spa type t127, sequence type 1 methicillin-resistant *Staphylococcus aureus* from pigs. J. Antimicrobial Chemother., 66: 1231-1235.
27. Lozano, C., H. Gharsa, K. Ben Slama, M. Zarazaga and C. Torres, 2016. *Staphylococcus aureus* in animals and food: Methicillin resistance, prevalence and population structure. A review in the African continent. Microorganisms, Vol. 4, No. 1. 10.3390/microorganisms4010012.
28. Garcia-Alvarez, L., M.T. Holden, H. Lindsay, C.R. Webb and D.F. Brown *et al*., 2011. Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: A descriptive study. Lancet Infect. Dis., 11: 595-603.
29. Shrestha, N.K., M.J. Tuohy, G.S. Hall, C.M. Isada and G.W. Procop, 2002. Rapid identification of *Staphylococcus aureus* and the *mecA* gene from BacT/ALERT blood culture bottles by using the lightcycler system. J. Clin. Microbiol., 40: 2659-2661.
30. Sakoulas, G., H.S. Gold1, L. Venkataraman, P.C. DeGirolami, G.M. Eliopoulos and Q. Qian, 2001. Methicillin-resistant *Staphylococcus aureus*: Comparison of susceptibility testing methods and analysis of *mecA*-positive susceptible strains. J. Clin. Microbiol., 39: 3946-3951.
31. Grema, H.A., Y.A. Geidam, G.B. Gadzama, J.A. Ameh and A. Suleiman, 2015. Methicillin Resistant *Staphyloccus aureus* (MRSA): A review. Adv. Anim. Vet. Sci., 3: 79-98.
32. Fey, P.D., B. Said-Salim, M.E. Rupp, S.H. Hinrichs and D.J. Boxrud *et al*., 2003. Comparative molecular analysis of community-or hospital-acquired methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother., 47: 196-203.
33. El-Bagoury, A.M., 1992. Incidence and public health importance of food poisoning causative organism in milk and some dairy products in Kaliobia Governorate. Ph.D. Thesis, Faculty of Veterinary Medicine, Benha University, Egypt.
34. Bystron, J., K. Kosek-Paszkowska and J. Molenda, 2001. Occurence of enterotoxigenic staphylococci in raw milk. Medycyna Weterynaryjna, 57: 645-648.

35. Carfora, V., A. Caprioli, N. Marri, D. Sagrafoli and C. Boselli *et al*., 2015. Enterotoxin genes, enterotoxin production and methicillin resistance in *Staphylococcus aureus* isolated from milk and dairy products in central Italy. Int. Dairy J., 42: 12-15.
36. Ashraf, A.A.E.T., M.A. Ahmad, A.E.H.S. Mokhtar, I.E.H. Fatma and S.S.S. Heba, 2016. Molecular characterization for some virulence and antibiotic resistance genes of *Staphylococcus aureus* isolated from dairy cattle's subclinical mastitis in El-Sharkia Governorate. Benha Vet. Med. J., 30: 219-230.
37. McClure, J.A., J.M. Conly, V. Lau, S. Elsayed, T. Louie, W. Hutchins and K. Zhang, 2006. Novel multiplex PCR assay for detection of the *Staphylococcal virulence* marker panton-*Valentine leukocidin* genes and simultaneous discrimination of methicillin-susceptible from-resistant *Staphylococci*. J. Clin. Microbiol., 44: 1141-1144.
38. Mehrotra, M., G. Wang and W.M. Johnson, 2000. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1 and methicillin resistance. J. Clin. Microbiol., 38: 1032-1035.