BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2019. Vol. **16**(2), p. 221-228

Prevalence of Tetracycline Resistant Genes in *Staphylococcus aureus*Isolates from Surgical Site Infections Egypt

Raghdaa Shrief[1], Reem Mohsen El Kholy[2], Mohamed Annies Rizk[2] and Maysaa El Sayed Zaki[2]

[1]Medical Microbiology and Immunology Department, Mansoura Faculty of Medicine, Egypt.
[2]Clinical Pathology Department, Menoufia Faculty of Medicine, Egypt.

http://dx.doi.org/10.13005/bbra/2739

(Received: 12 June 2019; accepted: 28 June 2019)

The aim of the present study was to investigate the prevalence of tetracycline resistance genes among isolated *S. aureus* from healthcare associatedsurgical site infections. The present study included 350 clinical samples from healthcare associatedsurgical site infections. Identified *S. aureus* strains were subjected to antimicrobial susceptibility testing, detection of methicillin resistance by cefoxitin disc and molecular study of *mec*Aand *tet* genesthat were carried out by polymerase chain reaction and multiplex polymerase chain reaction, respectively. There were high resistance rates of isolated *S. aureus* to gentamicin (71.2%), kanamycin (66.5%) and ceftazidime (41.8%). Resistances to tetracycline, doxycycline and minocycline were 60.6%, 56.5% and 45.3%, respectively. In the comparison between MRSA and MSSA as regards antibiotics resistance, there was a significant increase in resistance to tetracycline, doxycycline, minocycline (P=0.0001) and erythromycin (P=0.01) among MRSA strains compared to MSSA. The tetracycline resistant genes detected were *tet*K(92.3%) and *tet*M(25.2%). Combined genes were detected in 22.3% of *S. aureus*. None of tetracycline isolates had *tet*Lor *tet*Ogene.There was significant higher frequency of *tel*K,*tet*Mand combined genes among MRSA compared to MSSA (P=0.0001). The present study highlights the prevalence of multiple antibiotics resistance among clinical isolates of *S. aureus* associated with healthcare associated infections. The resistance increases among methicillin resistant *S.aureus*. The resistance to tetracycline, minocycline and doxycycline were common. The common genetic basis of the resistance to tetracycline was the *tet*K and *tet*Mgenes.

Keywords: *S. aureus*, *tet* genes, *mec*A.

Staphylococcus is Gram positive bacterium that constitutes a major normal flora on the skin and mucous membrane. The virulent species is *Staphylococcus aureus* (*S. aureus*) that is associated with major hospital acquired infections such as surgical wound infections, sepsis and pneumonia[1].

Antibiotics therapy for *S. aureus* started with methicillin,which is formed of the phenol group of benzylpenicillin provided with two methoxy groups,that was active toward â-lactamase produced by *S.aureus* to penicillin. However, inappropriate use of antibiotics leads to emergence ofmethicillin-resistant *S. aureus* (MRSA) strains. The resistance is mainly related to the expression of penicillin-binding protein (PBP2a). The use of severalclasses of antibiotics resulted in the emergence of multi-resistant MRSA strains

*Corresponding author E-mail: may_s65@hotmail.com

through the mutations in genes coding for target proteins and the acquisition and accumulation of antibiotic resistance genes[2]. The gene associated with methicillin resistance is a mobile heterogenous gene, *mec*A, encoded by a Staphylococcal Cassette Chromosome *mec*(SCC*mec*)[3]. SCC*mec*isformed of *mec*A gene with regulatory elements, a cassette chromosome recombinases (*ccr*) complex and the ûanking direct repeat sequences that contain the integration site sequence for SCC. The classification of SCC*mec*is based upon the combination of *ccr*gene complex and the class of the *mec*gene[4-7].

Other antibiotics that are used for treatment of *S. aureus* especially in skin infections are tetracyclines. Nevertheless, there is an emergence of tetracycline resistance through the acquisition of tetracycline resistance (*tet*) genes and oxytetracycline resistance (*otr*) genes[8]. Tetracycline resistance genes lead to resistance through active efflux pumps, ribosomal protection, and enzyme inactivation with 40 types of different resistant genes[9, 10].

Nowadays MRSA with resistant phenotypes to many antibiotics has emerged in healthcare associated infections. Understanding the molecular resistancemechanisms for these antibiotics is needed to develop active preventive measures for their spread.

The aim of the present study was to investigate the prevalence of tetracycline resistance genes among isolated *S. aureus* from healthcare associated surgical site infections at Mansoura University Hospitals.

MATERIALS AND METHODS

This study was a cross-sectional study that was carried out in MansouraUniversity hospitals from January 2017 till March 2018.

Specimens Collection

The wound swabs which were submitted to the microbiological laboratory from patients with healthcare associated infections according to CDC definition were included in the study[11]. The study was approved by Mansoura Ethical committee and approvals were obtained from the participant patients.

Wound swabs were obtained from the patients by rolling the swabs over 1cm of the wound for 5 seconds then the swabs were transported in the sterile containers rapidly to the laboratory.

Specimens Processing

Swabs were cultured under aerobic conditions for 24 hours at 37°C. Colonies were identified by Gram stain thenGram positive cocci were identified by catalase,coagulase, and fermentation of mannitol tests[12]. Identified *S. aureus* strains were subjected to antimicrobial susceptibility tests, detection of methicillin resistance by cefoxitin disc and molecular study of *mec*Aand *tet* genes.

Antibiotics Susceptibility Testing by Discs Diffusion Method

The antibiotics susceptibility test was performed by discs diffusion method according to the Clinical Laboratory Standards Institute (CLSI)[13]. The used discs were oxacillin (1 µg), gentamicin (10 µg), amikacin (30 µg),kanamycin (30 µg), erythromycin (15 µg), cefoxitin (5 µg), ceftazidime (30 µg), tetracycline (30 µg), doxycycline (30 µg), and minocycline (30 µg) (Oxoid-Thermal fisher).*S. aureus* ATCC 25923 was used as the control strain.

DNA Extraction

Pure colonies of *S. aureus*were prepared for DNA extraction by boiling method. Briefly, pure colonies were suspended in 100ìlof sterile phosphate buffer and boiled at 100°C for 10 minin water bath thencooled. The suspension was kept frozen at -20°C till amplification procedures[14].

Polymerase Chain Reaction (PCR) for *mec*A Gene

The primers used for *mec*A amplification were listed in table 1. Five microns of the DNA extracted by the boiling method were added to total volume 100ìl of amplification mixtures from Qiagen (Qiagen-Germany) with1ìM of *mec*Aprimers. The amplification conditions were as the following; an initial denaturation step at 94°C for 5 minfollowed by 40 cycles of amplification formed of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 60 sec ending with a final extension step at 72°C for 5 min. After PCR amplification, 5ìl of amplicon were removed and subjected to agarose gel electrophoresis (1.5% agarose, 1× Tris-borate-EDTA buffer, 100 V, 40 min) to estimate the sizes of the amplification products by comparison with molecular marker 1kbp[15].

Multiplex PCR for Amplification of *tet*K, *tet*L, *tet*M *Genes*

Amplification of *tet* genes (*tet*K, *tet*L, *tet*M) were performed using Qiagen PCR master kit (Qiagen). Multiplex PCR was prepared in a final volume of 25µlcontaining 0.5µM of each primer (Table 1). The PCR protocol consisted of an initial denaturation step at 94°C for 5 min then 30 amplification cycles at 94°C for 1 min, 51°C for 1 min and 72°C for 1 min, followed by a final extension step at 72°C for 5 min. Amplified products were analyzed by electrophoresis on 1.5% agarose gel containing 0.5 ìg/ml ethidium bromide and photographed under UV illumination[16].

PCR for *tet*O

The primers used for amplification of *tet*O were listed in table 1. Amplification procedures were similar to the above except that the amplification cycles were 30 cycles at 94°C for 1 min, 57°C for 1min, 72°C for 1 minand a final extension for 72°C for 5 min. The amplified products were visualized as mentioned previously[16].

Statistical Analysis

The statistical analysis was performed using SPSS 22.0 (SPSS Inc., Chicago, Illinois, USA). Descriptive data was expressed as number and percentage. Comparison was performed by Qi-square and P was considered significant if P was < 0.05.

RESULTS

This study recovered 170 isolates of *S.aureus*from 350 wound swabs culture (48.6%) collected during the period of the study (15 months) (Figure 1).

Antibiotics sensitivity testing revealed high resistance rates of isolated *S. aureus* to gentamicin (71.2%), kanamycin (66.5%), Amikacin (52.3%) and ceftazidime (41.8%). Resistances to tetracycline, doxycycline and minocycline were 60.6%, 56.5% and 45.3%, respectively(Table 2).

Antibiotics resistance pattern of the isolates showed that 90isolates (52.9%) were MRSA according to resistance to cefoxitin and/or oxacillin discs.

In the comparison between MRSA and methicillin sensitive *S. aureus* (MSSA) as regards antibiotics resistance, there was a significant increase in resistance to tetracycline, doxycycline, minocycline (P=0.0001) and erythromycin

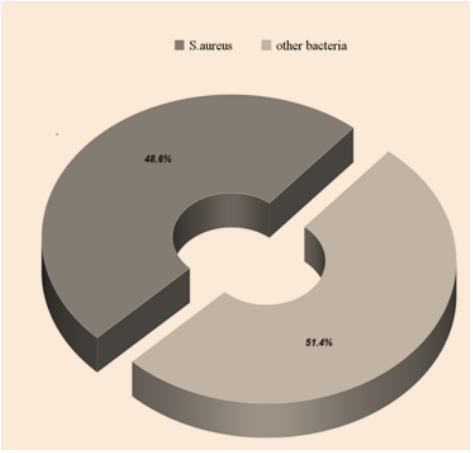

Fig. 1. Frequency of *S. aureus*strains isolated fromhealthcare associated surgical site infections

Table 1. Oligonucleotides sequences and sizes of different primers used in the amplification of *mec*A and *tet* genes

Gene	Primers Sequences	Size
*mec*A	F-AAAATCGATGGTAAAGGTTGGC R-AGTTCTGCAGTACCGGATTTGC	532 bp
*tet*K	F- GTAGCGACAATAGGTAATAGT R- GTAGTGACAATAAACCTCCTA	360 bp
*tet*M	F- AGTGGAGCGATTACAGAA R- CATATGTCCTGGCGTGTCTA	158 bp
*tet*L	F-ATAAATTGTTTCGGGTCGGTAAT R- AACCAGCCAACTAATGACAATGAT	1077 bp
*tet*O	F-AACTTAGGCATTCTGGCTCAC R-TCCCACTGTTCCATATCGTCA	514 bp

(P=0.01) among MRSA strains compared to MSSA (Table 3).

The tetracycline resistant genes detected were *tet*K (92.3%) (Figure 2) and *tet*M (25.2%). Combined *tet*Kand *tet*Mgenes were detected in 22.3% of *S.aureus*resistant to tetracycline(Table 4). None of tetracycline resistant isolates had *tet*Lor *tet*Ogene.There was a significant higher frequency of either *tet*K, *tet*Mor both genes among MRSA compared to MSSA (P=0.0001) (Table 5).

Table 2. Antibiotics resistance pattern of the isolated *S. aureus*from surgical site infections

Antibiotics	*S. aureus*	
	No.	%
Gentamicin	121	71.2%
Kanamycin	113	66.5%
Tetracycline	103	60.6%
Doxycycline	96	56.5%
Cefoxitin	90	52.9%
Oxacillin	89	52.3%
Amikacin	89	52.3%
Minocycline	77	45.3%
Ceftazidime	71	41.8%
Erythromycin	66	38.8%
Total	170	100%

DISCUSSION

Staphylococcus aureus represents a major pathogen that is related to healthcare associated infections. *S. aureus,* especially MRSA and multidrug resistant strains, is a common cause of surgical site infections[17].

In the present study, the etiology of about half of the isolates from healthcare acquired surgical siteinfections were due to *S. aureus*. Previous studies determined the high prevalence rate of *S. aureus* in surgical site infections up to 57%[17, 18]. In several studies from Egypt, *S. aureus*was considered a common pathogen (61%) causing hospital acquired infections(71%)[19, 20].

MRSA represented 52.9% of the isolated *S. aureus*from surgical site infectionsin the present study. This represents a lower prevalence rate of MRSA to previous studies from Nepal, Egypt, Libya andIran[17, 20-22]. This may be attributed to the implantation of active surveillance and monitoring of patients and the rapid diagnostic tests, inaddition to high orientation to preventive and control measures of MRSA during the period of the study in the hospital[23, 24]. Moreover, the difference may be related todifferent clinical samples obtained from the patients, the duration of hospital stay and previous antimicrobial use.

MRSA strains in the present study were identified by phenotypic characters and molecular methodsfor detection of *mec*A gene. In agreement

Table 3. Comparison of antibiotics resistance pattern between MRSA and MSSA strains

Antibiotics	MRSA(No.=90)		MSSA(No.=80)		P value
	No.	%	No.	%	
Amikacin	50	55.6%	39	48.7%	P=0.3
Kanamycin	56	62.2%	57	71.3%	P=0.1
Gentamicin	65	72.2%	56	70%	P=0.4
Tetracycline	90	100%	13	16.3%	P=0.0001
Minocycline	70	77.8%	7	8.7%	P=0.0001
Doxycycline	84	93.3%	12	15%	P=0.0001
Ceftazidime	38	42.2%	33	41.3%	P=0.5
Erythromycin	45	50%	21	26.2%	P=0.01

Table 4. Frequency of *tet*genes among *S. aureus* strainsresistant to tetracycline as detected by multiplex PCR

Gene	Frequency	
	No.	%
*tet*K	95	92.3%
*tet*M	26	25.2%
Combined *tet*K,*tet*M	23	22.3%
Total	103	100%

with our study, the molecular method for detection of *mec*A gene is an accurate and gold standardtool[25, 26]. MRSA strains have resistance to methicillin and other semi-synthetic penicillinase resistant â-lactam antibiotics. The *mec*A gene is transferred horizontally and known to encode for a specific binding protein termed penicillin-binding protein 2a that has low affinity for â-lactam antibiotics which leads the complete synthesis of the bacterial cell wall without interruption by the â-lactam antibiotics[27,28].

There were high resistance rates of isolated *S. aureus* to aminoglycosides and ceftazidime. Previous studies demonstrated multiple drug resistance among isolated *S. aureus* from healthcare associated infections[20]. The high resistance to aminoglycosides among isolated *S. aureus* was similar to previous reports[20, 26, 29]. The resistance pattern of isolated *S.aureus* depends upon the antibiotics policy in different institutions and infection control practices that lead to distribution diversity of antibiotics resistance genes.

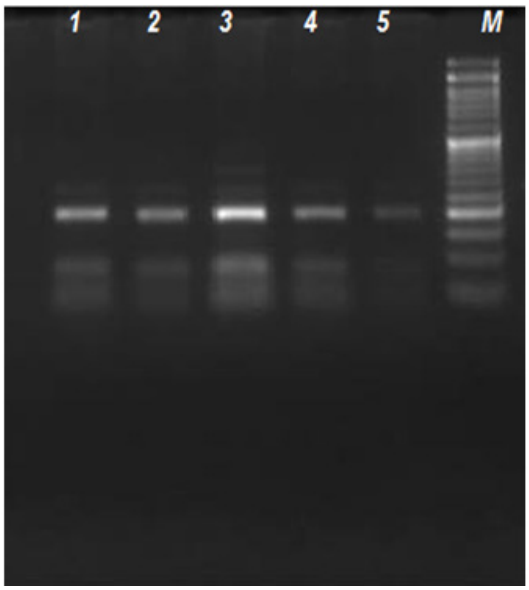

Fig. 2. Positive samples for *tet*Kgene as detected by multiplex PCR.
M: Molecular marker, 1-5: Positive samples

In the present study, resistances to tetracycline, doxycycline and minocycline were 60.6%, 56.5% and 45.3%, respectively with significant increase in resistance to tetracycline, doxycycline, minocycline(P=0.0001) and erythromycin (P=0.01) in MRSA isolates compared to MSSA. These findingsarein agreement with previous reports[16,19]. However, the resistance rate for tetracycline is higher than rates reported from the United States and Canada which have been reported to be 15.6% and 14.8%, respectively[30]. Again, these findings reflect the difference in antibiotics policy between different geographic countries.

There are several mechanisms that lead to the development of tetracyclines resistance. One of these mechanisms is by the acquisition of *tet*Kgene that leads to active efflux of divalent metal ion tetracycline from the bacterial cells[31]. Another mechanism for tetracyclines resistance is mediated by *tet*Mgene. This gene leads to tetracycline resistance through protection of the ribosome by the production of a ribosome protection protein thatdislodges tetracycline from the ribosome freeing it from the inhibition by the drug[32].

In the present study, the tetracycline resistant genes detected were *tet*K(92.3%) and *tet*M (25.2%). Combined genes were detected in 22.3% of *S. aureus*, while none of the clinical isolates was positive for *tet*L and *tet*O genes. Similar previous studies have reported that *tet*K gene is the predominant gene of tetracycline resistance in

Table 5. Distribution of *tet* genes among MRSA and MSSAstrains as detected by multiplex PCR

Gene	MRSA(No.=90)		MSSA(No.=80)		P value
	No.	%	No.	%	
*tet*K	81	90%	14	17.5%	P=0.0001
*tet*M	22	24.4%	4	5%	P=0.0001
Combined *tet*K and *tet*M	22	24.4%	1	1.2%	P=0.0001

clinical isolates of *S. aureus* followed by *tet*M[16, 33]. However, other studies have documented that *tet*M is the predominant gene followed by *tet*K gene[8, 34]. Similar to many reports, *tet*L and *tet*O were not detected in any of the isolates in the present study[16, 34].

Both *tet*K and *tet*Mgenes in the present workpredominated in MRSA compared to MSSA. This is in accordanceto previous findings[16, 33].On contrary, a literature has reported that *tet*Mis more frequent in MRSA isolates[8].

These findings demonstrated that both *tet*M and *tet*K genes either single or combined have an important role in tetracycline resistance among MRSA strainsin agreement with the previous report of Schmitz *et al.*[35]. Similar to many reports, *tet*L and *tet*Owere not detected in any of the isolates in the present study, indicating that these genes have no role in tetracycline resistance in our isolates[34, 36].

The predominance of *tet*M in tetracycline remittance MRSAmay be attributed to previous exposure to tetracycline that leads to excision and transfer of the genetic element Tn*916* carrying *tet*M gene[37]. This theory may explain the relative less frequency of this gene among our isolates as the prescription of tetracycline is not common in our hospitals to *S. aureus*. On the other hand, *tet*K is transmitted through a small 4.4 kb plasmid known as pT181 from resistant strains in the absence of selective pressure[38].

CONCLUSION

The present study highlights the prevalence of multiple antibiotics resistance among clinical isolates of *S.aureus* associated with healthcare associated infections. The resistance increases among methicillin resistant *S.aureus*. The resistance to tetracycline, minocycline and doxycycline were common. The common genetic basis of the resistance to tetracyclines was the *tet*Kand *tet*Mgenes.

Studying the mechanisms of antibiotics resistance is a mandatory tool for implementation of preventive and control measures to combat the resistant bacteria and reduce the risk of healthcare associated infections.

REFERENCES

1. DeLeo FR, Chambers HF. Reemergenceof antibiotic-resistant *Staphylococcus aureus* in the genomics era. *J Clin Invest.* 2009; **119**(9):2464–74.
2. Stapleton PD, Taylor PW. Methicillin resistance in *Staphylococcus aureus*: mechanisms and modulation. *Sci Prog*. 2002; **85**(1):57-72.
3. Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome:genomic island SCC. *Drug Resist Updat.* 2003; **6**(1):41–52.
4. Hisata K, Kuwahara-Arai K, Yamanoto M, Ito T, Nakatomi Y, Cui L,Baba T, Terasawa M, Sotozono C, Kinoshita S, Yamashiro Y, Hiramatsu K. Dissemination of methicillin-resistant staphylococci amonghealthy Japanese children. *J Clin Microbiol.* 2005; **43**(7):3364–72.
5. Kwon NH, Park KT, Moon JS, Jung WK, Kim SH, Kim JM, Hong SK, Koo HC, Joo YS, Park YH. Staphylococcal cassette chromosome *mec*(SCCmec) characterization andmolecular analysis for methicillin-resistant *Staphylococcus aureus*and novel SCCmec subtype IVg isolated from bovine milk in Korea.*J Antimicrob Chemother.* 2005; **56**(4):624–32.
6. Milheirico C, Oliveira DC, de Lencastre H. Multiplex PCR strategyfor subtyping the staphylococcal cassette chromosome *mec* type IVin methicillin-resistant *Staphylococcus aureus*: 'SCCmec IV multiplex'.*J Antimicrob Chemother.* 2007; **60**(1):42–48.
7. Shore A, Rossney AS, Keane CT, Enright MC, Coleman DC. Sevennovel variants of the staphylococcal chromosomal cassette *mec* in methicillin-resistant *Staphylococcus aureus* isolates from Ireland. *Antimicrob Agents Chemother.* 2005; **49**(5):2070–83.
8. Hui-Ling OngM, HoWY, Ng W-W,Chew C-H. High prevalence of *tet*M as compared to *tet*Kamong methicillin resistant *Staphylococcus aureus* (MRSA) isolates from hospitals in Perak, Malaysia. *Jundishapur JMicrobiol*. 2017; **10**(6) 1-6.
9. Roberts MC. Update on acquired tetracycline resistance genes. *FEMS Microbiol Lett*. 2005; **245**:195–203.
10. Hedayatianfard K, Akhlaghi M, Sharifiyazdi H. Detection of tetracycline resistance genes in bacteria isolated from fish farms using polymerase chain reaction. *Vet Res Forum* 2014; **5**: 269-275.
11. Tsai DM, Caterson EJ. Current preventive measures for health-care associated surgical site

infections: a review.*Patient Saf Surg.* 2014; **8**: 42-55.

12. Reisner BS, Woods GL, Thomson RB Jr, Larone DH, Garcia LS, Shimizu RY. Specimen processing. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolker RH (eds). *Manual of Clinical Microbiology*. 7th ed Washington DC, USA; 1999. pp. 64–104.
13. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing; M100-24: 2014. CLSI 24th Informational Supplement, Wayne PA, USA.
14. Fatholahzadeh B, Hashemi FB, Emaneini M, Aligholi M, Nakhjavani FA, Kazemi B. Detection of Vancomycin Resistant Enterococci (VRE) isolated from urinary tract infections (UTI) in Tehran, Iran. *Daru J Pharm Sci.*2006; **14**: 141–145.
15. Meshref AA, Omer MK. Detection of (*mec*A) gene in methicillin resistant *Staphylococcus aureus* (MRSA) at Prince A/RhmanSidery hospital , Al-Jouf, Saudi Arabia. *JMed. Genet. Genomics* 2011; **3**(3):41–45.
16. Khoramrooz SS, Dolatabad SA, Dolatabad FM, Marashifard M, Mirzaii M, Dabiri H, Haddadi A, Rabani SM, Shirazi HRG, Darban-Sarokhalil D. Detection of tetracycline resistance genes, aminoglycoside modifying enzymes, and coagulase gene typing of clinical isolates of *Staphylococcus aureus* in the Southwest of Iran.*Iran J Basic Med Sci.* 2017; **20**(8):912-919.
17. Upreti N, Rayamajhee B, Sherchan SP, Choudhari MK, Banjara MR. Prevalence of methicillin resistant *Staphylococcus aureus*, multidrug resistant and extended spectrum â-lactamase producing gram negative bacilli causing wound infections at a tertiary care hospital of Nepal. *Antimicrob Resist Infect Control*, 2018; **7**: 121-131.
18. Bhowmik D, Chetri S, Paul D,Chanda DD, Bhattacharjee A. Detection and molecular typing of methicillinresistant *Staphylococcus aureus* from northeastern part of India. *Med J Armed Forces India* 2019; **75**: 86 -89.
19. Ahmed EF, Gad GFM, Abdalla AM, Hasaneen AM, Abdelwahab SF. Prevalence of methicillin resistant *Staphylococcus aureus* among Egyptian patients after surgical interventions. *Surg Infect*. 2014; **15**: 404–411.
20. Abdel-Maksoud M, El-Shokry M, Ismail G, Hafez S, El-Kholy A, Attia E, Talaat M. Methicillin-Resistant *Staphylococcus aureus*recovered from healthcare- and community-associated infections in Egypt. *Int J Bacteriol*. 2016; 2016: 5751-785.
21. Ghenghesh KS, Rahouma A, Tawil K, Zorgani A, Franka E. Antimicrobial resistance in Libya: 1970–2011. *Libyan J Med*. 2013; **27**(8):1–8.
22. Jafari-Sales A, Farhadi F, Ezdiyadi M, Tarbiat-Nazloo D. Study of antibiotic resistance pattern in methicillin-resistant *Staphylococcus aureus* isolated from clinical samples of hospitals in Tabriz – Iran. *Int J BioMed Public Health.* 2018; **1**(2):71-75.
23. Chowers MY, Paitan Y, Gottesman B S, Gerber B, Ben-Nissan Y, Shitrit P. Hospital-wide methicillin-resistant *Staphylococcus aureus* control. Program: a 5-year follow-up. *Infect ControlHosp Epidemiol*. 2009; **30**(8): 778–781,
24. Hadler JL, Petit S, Mandour M, Cartter ML. Trends in invasive infection with methicillin-resistant *Staphylococcus aureus*, Connecticut, USA, 2001–2010. *Emerg Infect Dis*. 2012; **18**(6):917–924,
25. Choi SM, Kim S-H, Kim H-J, Lee D-G, Choi J-H, Yoo J-H, Kang J-H, Shin W-S, Kang M-W. Multiplex PCR for the detection of genes encoding aminoglycoside modifying enzymes and methicillin resistance among *Staphylococcus* species. *J Korean Med Sci.* 2003; **18**(5):631–636.
26. Yadegar A, Sattari M, Mozafari NA, Goudarzi GR. Prevalence ofthe genes encoding aminoglycoside-modifying enzymes and methicillinresistance among clinical isolates of *Staphylococcus aureus* in Tehran, Iran. *Microb Drug Resist.* 2009; **15**(2):109–13.
27. Hartman BJ, Tomasz A. Low-affinity penicillin-binding protein associated with beta-lactam resistance in *Staphylococcus aureus*. *J Bacteriol.* 1984; **158**(2):513–6.
28. Zapun A, Contreras-Martel C, Vernet T. Penicillin-binding proteins and beta-lactam resistance. *FEMS Microbiol Rev.* 2008; **32**(2):361–85.
29. Hauschild T, Sacha P, Wieczorek P, Zalewska M, Kaczyñska K, Tryniszewska E. Aminoglycosides resistance in clinical isolates of *Staphylococcus aureus* from a University Hospital in Bialystok, Poland. *Folia Histochem. Cytobiol* 2008; **46**: 225-228. 15:129-132.
30. Diekema D, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, Beach M and the SENTY Participants Group. Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe and the Western Pacificregion for the SENTRY Antimicrobial Surveillance Program, 1997–1999. *Clin Infect Dis*. 2001; **32**:S114-S132.
31. Jin J, Guûanti AA, Bechhofer DH, Krulwich TA. *Tet*(L) and *tet*(K) tetracycline-divalent metal/H+ antiporters: characterization of

multiple catalytic modes and a mutagenesis approach to diûerences in their eûux substrate and coupling ion preferences. *J Bacteriol.* 2002; **184**(17):4722–32.

32. Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev.* 2001; **65**(2):232–60.
33. Jones CH, Tuckman M, Howe AY, Orlowski M, Mullen S, Chan K, Bradford PA. resistance genes among *Staphylococcus aureus* isolates from phase 3 clinical trials of tigecycline for complicated skin and skin structure infections. *Antimicrob Agents Chemother*. 2006; **50**: 505-510.
34. Lim KT, Hanifah YA, Yusof M, Thong KL. *erm*A, *erm*C, *tet*Mand *tet*K are essential for erythromycin and tetracycline resistance among methicillin-resistant *Staphylococcus aureus* strains isolated from a tertiary hospital in Malaysia. *Indian J Med Microbiol*. 2012; **30**:203-207.
35. Schmitz FJ, Krey A, Sadurski R, Verhoef J, Milatovic D, FluitAC; European SENTRY Participants. Resistance to tetracycline and distribution of tetracycline resistance genes in European *Staphylococcus aureus* isolates.*J Antimicrob Chemother*. 2001; **47**(2): 239-240.
36. Trzcinski K, Cooper BS, Hryniewicz W, Dowson CG. Expression of resistance to tetracyclines in strains of methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother*. 2000; **45**:763-770.
37. Su YA, He P, Clewell DB. Characterization of the *tet*(M) determinant of Tn916: evidence for regulation by transcription attenuation. *Antimicrob Agents Chemother.* 1992; **36**(4):769–778.
38. Villedieu A, Diaz-Torres ML, Hunt N, McNab R, Spratt DA, Wilson M, Mullany P. Prevalence of tetracycline resistance genes in oral bacteria. *Antimicrob Agents Chemother.* 2003; **47**(3):878–882.