*Int.J.Adv.Microbiol.Health.Res.*2018; 2(1):24-35

**International Journal of Advanced Microbiology and Health Research**
**ISSN: 2457-077X. Volume 2, Issue 1 (January to March 2018), PP. 24-35**
**Available online at www.ijamhr.com**

**Original Research Article**

# Clindamycin Resistance among *Staphylococcus aureus* Clinical Isolates in Alexandria

**Ahmed Gaballah[1*], Abeer Ghazal[1]**

[1]Microbiology Department, Medical Research Institute, Alexandria University, Egypt.

**Article Info**

Received 26th February, 2018
Revised 28th February, 2018
Accepted 2nd March, 2018
Published online 13th March, 2018

**Keywords**

- MRSA
- D-test
- Clindamycin resistance
- $MLS_B$

**ABSTRACT**

**Background:** Macrolides, lincosamides, streptogramins-B ($MLS_B$) resistance can be constitutive or inducible.Staphylococcal strains with constitutive $MLS_B$ ($cMLS_B$) phenotype are easily detected using routine disk diffusion technique. Contrarily, inducible $MLS_B$ ($iMLS_B$) phenotypeneeds a special double disk diffusion test, D-testto be detected. Misdiagnosis of $iMLS_B$ can lead to therapy failure with clindamycin. The aim of this study was to detect the prevalence of erythromycin and clindamycin resistance among *Staphylococcus aureus* clinical isolates and to detect the resistance encoding genes *ermA* and *ermC*. **Materials and Methods:** One hundred non-repetitive *S. aureus* isolates were included in this study. Antimicrobial susceptibility was detected to all isolates. The molecular identification of *S. aureus* and methicillin resistance was performed by detecting *femA* and *mecA* genes respectively. $iMLS_B$ was detected by D-test assay. The presence of *ermA* and *ermC* genes was detected by PCR. **Results:** Methicillin resistance was detected in 54% of isolates. Most of isolates (73%) were susceptible to erythromycin and clindamycin. Out of the 27 isolates resistant to erythromycin, 10(37%) showed $cMLS_B$ phenotype. The remaining 17 (63%) isolates resistant to erythromycin but susceptible to clindamycin were clindamycin were classified into 14 (82%) $iMLS_B$ resistance phenotype that showed positive D-test and 3 (18%) macrolide-streptogramin ($MS_B$) resistance phenotype that showed negative D-test. *ermA* and *ermC* were detected among the 27 *S. aureus* isolates resistant to clindamycin and/or erythromycin. Both genes were detected together in 2 isolates (7.5%), *ermA* was detected alone in 5 isolates (18.5 %) and ermC was detected alone in 20 isolates (74%). **Conclusion:** From the previous results, we can conclude that D test is a simple, reliable method to detect clindamycin resistance in erythromycin resistant isolates. Nevertheless, the incidence of true susceptibility to clindamycin among erythromycin resistant *S. aureus* isolates is low.

## INTRODUCTION

*Staphylococcus aureus* (*S. aureus*) is a common human pathogen. It is responsible for a wide range of community and hospital acquired infections including skin and soft tissue infections, respiratory tract infections, bacteremia and infective endocarditis [1]. Antibiotics of the classes Macrolides-Lincosamides-Streptogramin B ($MLS_B$) are interesting for treating such infections particularly with the increasing prevalence of methicillin resistance in staphylococci that renders the organism resistant to all cell wall inhibitors of the family β-lactams [2,3]. Macrolides like erythromycin, Lincosamides like clindamycin and Streptogramin B like quipristin/dafloprístin belongs to different chemical classes of antibiotics that inhibit protein synthesis via binding to 50S ribosomal subunit, thus hindering ribosomal translocation [4]. Because of its perfect pharmacokinetic properties, clindamycin has been frequently used to treat *S. aureus* particularly methicillin resistant *S. aureus* (MRSA). However, the persistent use of clindamycin increases the selection of $MLS_B$ resistant staphylococcal strains [2,5-8].

Staphylococcal resistance to $MLS_B$ can be induced via three molecular mechanisms: target site modification, efflux of the antibiotic and/or drug

modification [9]. In target site modification, methylation of the A2058 residue of the 23S rRNA occurs, which hinders the binding of the $MLS_B$ antibiotics to their target. This resistance mechanism is encoded by erythromycin ribosome methylase (erm) genes and induces cross-resistance to theses antibiotics. Among the 4 main types of erm genes (ermA, ermB, ermC and ermF) in different bacteria; ermA and ermC are the accused $MLS_B$ resistance phenotype in S. aureus [9-11].

$MLS_B$ resistance phenotype can be constitutive or inducible. Staphylococcal strains with constitutive $MLS_B$ resistance ($cMLS_B$) phenotypes are easily detected using routine disk diffusion susceptibility testing [11,12]. On the other hand, strains are showing inducible $MLS_B$ resistance ($iMLS_B$) phenotypes needs the presence of inducing agent for the production of the methylase and exhibit resistance to erythromycin and susceptibility to clindamycin [13]. This makes the detection of $iMLS_B$ not a straight forward task. Nevertheless, a special double-disk diffusion protocol, the D-test, was established for the diagnosis of $iMLS_B$ [14].

Misdiagnosis of $iMLS_B$ may result in therapy failure with clindamycin. However, considering all erythromycin resistant isolates as clindamycin resistant neglects the use of clindamycin to treat infections with truly clindamycin-susceptible staphylococcal isolates [15,16].

In this study we detected the prevalence of erythromycin and clindamycin resistance among S. aureus clinical isolates using disk diffusion technique according to the guidelines of the CLSI [17]. Additionally, the presence of resistance encoding genes ermA and ermC was detected using polymerase chain reaction (PCR).

# MATERIALS AND METHODS

## Bacterial isolates

A total of one hundred non-repetitive *S. aureus* isolates were included in this study. The isolates were obtained from different clinical specimens submitted to the Microbiology laboratory of the Medical Research Institute, Alexandria University. All isolates were identified as Gram positive cocci, catalase and coagulase positive and fermented mannitol in mannitol salt agar.

## Antimicrobial susceptibility testing

According to clinical and laboratory standards institute (CLSI) guidelines [17], disk diffusion method was used for detection of antimicrobial susceptibility to 11 antimicrobial agents, namely penicillin (P), amoxicillin/clavulanate (AMC), cefoxitin (FOX),ciprofloxacin (CIP), fuscidic acid (FD), co-trimoxazole (SXT), doxycycline (DO), gentamicin (CN), linzeolide (LZD) clindamycin (DA) and erythromycin (E) with the exception of vancomycin which was detected by minimum inhibitory concentration (MIC) method. All culture media and antimicrobial disks were purchased from Oxoid®.

D-test was performed according to the CLSI guidelines [17]. Briefly, erythromycin disk (E) was placed 15 mm (edge to edge) from clindamycin disk (DA) on a Müller Hinton agar plate inoculated with a cotton swab immersed in 0.5 McFarland standard staphylococcal suspension. After overnight incubation at 35ºC, the formation of D-inhibition zone around clindamycin disk with flattening in the area between the two discs indicates $iMLS_B$ phenotype (Figure 1).

## Polymerase Chain Reactions (PCR)

DNA extraction was performed by boiling method. Briefly, 2 to 3 colonies from overnight cultured were suspended in TE buffer containing 0.1% triton X100. Bacterial suspensions were incubated in a boiling water bath for 15 minutes followed by rapid cooling on ice. After centrifugation for 15 minutes at 14,000 rpm in a microfuge, the supernatant was used as a source for bacterial DNA.

## Molecular Identification of MRSA

Multiplex PCR for the detection of the presence of femA gene and mecA was used to confirm the identity of the isolates as *S. aureus* and its resistance to methicillin respectively.

## Molecular detection of erythromycin ribosomal methylation (erm) genes

ermA and ermC genes that encode erythromycin ribosomal methylase enzyme were amplified using specific pair of primers (Table 1). All primers were purchased from Biosearch Technologies (Biosearch Technologies, California, USA).

PCR was performed in 25 µl final volume containing 12.5 µl MyTaq HS Red MixBioLine

(BioLine, London, UK), 10 pmole of each primer and 0.5 µl DNA extract. Amplifications were performed using Veriti Thermal Cycler (Applied Biosystems, California, USA) according to the following protocol, initial denaturation 95ºC for 5 minutes followed by 35 cycles of 95ºC for 15 seconds, annealing for 15 second and extension at 72ºC for 40 seconds followed by final extension at 72ºC for 5 minutes.

## RESULTS

This study included 100 *S. aureus* isolates obtained from different clinical specimens (Figure 2). Most isolates were obtained from wound swabs (40%) followed by nasal swabs (24%).

### Antimicrobial susceptibility testing

All isolates were resistant to penicillin but sensitive to linzeolide and vancomycin. According to susceptibility to cefoxitin, 54% of isolates were diagnosed as MRSA (Figure 3).

### Molecular diagnosis of MRSA

Using multiplex PCR (Figure 4), all isolates included in the current study were diagnosed molecular as *S. aureus* via detection of femA gene. mecA was amplified in only 54% of isolates which was previously diagnosed as MRSA using cefoxitin disk diffusion (100% sensitivity and specificity). The remaining 46 isolates were methicillin susceptible *Staphylococcus aureus* (MSSA).

### $MLS_B$ Resistance profile

Most of isolates (73%) were susceptible to erythromycin and clindamycin. Out of the 27 isolates resistant to erythromycin, 10 (37%) were resistant to clindamycin, which was considered as $cMLS_B$ phenotype. The remaining 17 (63%) isolates resistant to erythromycin but susceptible to clindamycin were clindamycin were classified into 14 (82%) $iMLS_B$ resistance phenotype that showed positive D-test and 3 (18%) macrolide-streptogramin ($MS^B$) resistance phenotype that showed negative D-test (Table 2).

### Molecular detection of ermA and ermC genes

ermA(Figure 5) and ermC (Figure 6) were detected among the 27 *S. aureus* isolates resistant to clindamycin and/or erythromycin. Both genes were detected together in 2 isolates (7.5%), ermA was detected alone in 5 isolates (18.5 %) and ermC was detected alone in 20 isolates (74%) (Table 3).

## DISCUSSION

Although about 30% of the populations are asymptomatic carriers of *S. aureus*, it is capable of generate a wide range of diseases varying from toxin-mediated illness to pyogenic community and hospital acquired infections [21].

*S. aureus* is known to acquire resistance to many classes of antimicrobials including penicillinase stable penicillins like methicillin. Isolates included in this study showed low level of resistance to many classes of antimicrobials including fluoroquinolones (21%), fusidic acid (55%), sulfonamides (12%) aminoglycosides (20%) and tetracyclines (30%). This low resistance levels may be associated with community acquired nature of infection in outpatients presented to the Microbiology department of the Medical Research Institute.

MRSA develops from MSSA upon the acquisition of staphylococcal chromosomal cassette mec (SCCmec) which is known as de novo MRSA [22]. Community acquired MRSA can develop de novo or via person-to-person carriage of hospital strains into the community [23,24]. However, de novo MRSA have generally been characterized as being susceptible to most non-β-lactam agents (other than erythromycin) while those that have spread from the hospital environment into the community often display the multi-drug resistant phenotype dominating among most of hospital-acquired strains [25,26].

Detection of MRSA is achieved via phenotypic and genotypic examination of bacterial isolates. In most cases, phenotypic methods are more feasible than genotypic methods, while genotypic methods usually provide better precision and accuracy [27].

Cefoxitin disk diffusion method was recommended by CLSI for the detection of methicillin resistance [28]. Being a potent inducer of the mecA regulatory system, cefoxitin was shown to be superior to oxacillin in detecting low levels of Methicillin resistance [29]. It shows high degree of sensitivity and specificity in foreseeing the presence of mecA in *S. aureus* [29,30]. In the current study, 54% of isolates were diagnosed as MRSA using both

phenotypic susceptibility testing to cefoxitin and genotypic detection of mecA gene.

Similar results were reported by Kumar et al., who found that the prevalence of MRSA was 53.74%.The majority of their Indian MRSA were isolated from pus (37.70%) followed by wound swabs (30.40%) and surgical wound swabs (7.60%). [31] Mansour et al., reported MRSA prevalence of 44.71%. The majority of their Pakistani MRSA isolates were from pus (36.8%) followed by wound Swabs (10.6%) [32]. Dilnessaet al., reported the major source of their Ethiopian MRSA was also pus (20.3%) [33].

femA is a chromosomally encoded factor, occurring naturally in *S. aureus* [34], that is indispensable for the development of high-level methicillin resistance. However, femA does not influence the synthesis of PBP2a [35]. It was shown that *S. aureus* strain with inactivated femA lost the methicillin-resistance trait, and the transduction of femA gene restored the resistance [36,37]. In the current study, femA was detected in all our isolates confirming its identity as *S. aureus*.

Drug selection is important in the treatment of S. aureus infections particularly MRSA. Clindamycin is a good choice recommended for treatment of community acquired MRSA. It is a semi-synthetic derivative of Lincosamine, with superb tissue penetration in lung, pleural fluid and bile. It distributes well into bones and considered an effective antibiotic in treatment of Osteomyelitis [38].

Although some *S. aureus* isolates are susceptible to clindamycin in vitro, they may not be effective in vivo particularly when the strain is resistant to erythromycin. This may be attributed to the presence of $iMLS_B$ phenotype. Strains with $iMLS_B$ resistance demonstrate resistance in vitro to 14- and 15-member macrolides, while showing susceptibility to lincosamides, and streptogramins. On the other hand, strains with $cMLS_B$ resistance phenotype exhibit in vitro resistance to all of these agents [39].

Failure of therapy with clindamycin in $iMLS_B$ phenotype may be due to selection of constitutive resistant bacteria under antibiotic pressure or due to conversion of inducible isolate to a constitutive one. Therefore accurate, susceptibility results are essential for convenient therapy decisions. The presence of $iMLS_B$ can be detected in erythromycin resistant strains by D-test that is simple and reliable [14,40,41]. D-test can distinguish strains that have the genetic potential to develop resistance during therapy from surely susceptible strains to clindamycin. Treating clindamycin susceptible strain without checking their D-test results may result in therapy failure. Contrarily, considering all erythromycin resistant strains as clindamycin resistant would reject a possibly safe and effective treatment [42].

From the results of antimicrobial susceptibility testing to eythromycin and clindamycin as well as the results of the D-test, $cMLS_B$ phenotype was detected in 37% of erythromycin-resistant isolates. $iMLS_B$ resistance phenotype were detected in 82% of erythromycin-resistant clindamycin-susceptible isolates. The remaining 18% were considered $MS_B$ resistance phenotypes that were resistant to erythromycin but truly sensitive to clindamycin with a negative D-test.

Previous studies reported the pattern of the $MLS_B$ resistance among staphylococci; some reports indicated a high frequency of the $iMLS_B$ phenotype, while the others showed increasing prevalence of the $cMLS_B$ phenotype. The true incidence relys on the studied patient population, the geographical region, the hospital characteristics and methicillin susceptibility [43,44].

In this study, it was found that $cMLS_B$ was seen in significantly greater proportion among MRSA as compared to MSSA isolates ($p<0.001$). Studies in India have reported 30% to 64% of the MRSA isolates to be of the $iMLS_B$ phenotype (45). In the present study, 8 (15%) and 6 (11%) out of the 54 MRSA isolates were of $cMLS_B$ and $iMLS_B$ phenotypes respectively. Previous studies showed discrepancies in the incidence of $iMLS_B$ among staphylococci. While some studies showed low incidences ranging from 5.4-19.2% [2,46], others showed higher ratios ranging from 27-74% [11,47].

The occurrence rate of $iMLS_B$ shown by D-test results was 11% in MRSA and 13% in MSSA. In concordance with our results, an earlier study during 2004 reported 19% $iMLS_B$ in MRSA and 19.2% in MSSA [46].

erm genes encode ribosome methylases that confer inducible or constitutive resistance to $MLS_B$, drugs by reducing binding by $MLS_B$ agents to the ribosome [39]. Previous study proved the sensitivity of D-test performed at 15-20mm disk spacing was 100% when correlated with detection of erm and msr genes by PCR [48]. Additionally, it was found that ermC and ermA genes predominated within $MLS_B$ phenotypes [49]. However, some of previous reports demonstrated prevalence of ermA genes [50], other, similar to our study, showed that the ermC gene was more common among *S. aureus* strains [51]. In the present study, most of erythromycin resistant isolates 74% harbored ermC gene, 18.5% harbored ermA gene and 7.5% harbored both genes. Most (60%) of ermC harboring isolates exhibited $iMLS_B$ phenotype.

## CONCLUSION

From the previous results, we can conclude that D-test is a straightforward, decisive method to detect clindamycin resistance in erythromycin resistant isolates which can enable us guiding the use of clindamycin in treating skin and soft tissue infections. Nevertheless, the incidence of true susceptibility to clindamycin among erythromycin resistant *S. aureus* isolates is low.

## REFERENCES


1. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG, Jr. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical microbiology reviews. 2015 Jul;28(3):603-61. PubMed PMID: 26016486. Pubmed Central PMCID: 4451395.
2. Delialioglu N, Aslan G, Ozturk C, Baki V, Sen S, Emekdas G. Inducible clindamycin resistance in staphylococci isolated from clinical samples. Japanese journal of infectious diseases. 2005 Apr;58(2):104-6. PubMed PMID: 15858290.
3. Rafee Y, Abdel-Haq N, Asmar B, Salimnia T, Pharm CV, Rybak Pharm MJ, et al. Increased prevalence of methicillin-resistant Staphylococcus aureus nasal colonization in household contacts of children with community acquired disease. BMC infectious diseases. 2012 Feb 20;12:45. PubMed PMID: 22348549. Pubmed Central PMCID: 3305649.
4. Tenson T, Lovmar M, Ehrenberg M. The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. Journal of molecular biology. 2003 Jul 25;330(5):1005-14. PubMed PMID: 12860123.
5. Appalaraju B, Jayakumar S. "Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples". The Journal of communicable diseases. 2010 Dec;42(4):263-8. PubMed PMID: 22471195.
6. Colakoglu S, Aliskan H, Turunc T, Demiroglu YZ, Arslan H. [Prevalence of inducible clindamycin resistance in Staphylococcus aureus strains isolated from clinical samples]. Mikrobiyoloji bulteni. 2008 Jul;42(3):407-12. PubMed PMID: 18822883. Klinik orneklerden izole edilen Staphylococcus aureus suslarinda induklenebilir klindamisin direnc prevalansi.
7. Deotale V, Mendiratta DK, Raut U, Narang P. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. Indian journal of medical microbiology. 2010 Apr-Jun;28(2):124-6. PubMed PMID: 20404457.
8. Prabhu K, Rao S, Rao V. Inducible Clindamycin Resistance in Staphylococcus aureus Isolated from Clinical Samples. Journal of laboratory physicians. 2011 Jan;3(1):25-7. PubMed PMID: 21701659. Pubmed Central PMCID: 3118052.
9. Leclercq R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2002 Feb 15;34(4):482-92. PubMed PMID: 11797175.
10. Fomda BA, Peer MA, Zahoor D, Thokar MA, Nasir RA. Phenotypic detection of constitutive and inducible clindamycin resistance in clinical isolates of Staphylococcus aureus and coagulase negative Staphylococcus on routine susceptibility plate. The Journal of communicable diseases. 2010 Mar;42(1):19-26. PubMed PMID: 22468547.
11. Ajantha GS, Kulkarni RD, Shetty J, Shubhada C, Jain P. Phenotypic detection of inducible clindamycin resistance among Staphylococcus aureus isolates by using the lower limit of recommended inter-disk distance. Indian journal of pathology & microbiology. 2008

Jul-Sep;51(3):376-8. PubMed PMID: 18723962.
12. Daurel C, Huet C, Dhalluin A, Bes M, Etienne J, Leclercq R. Differences in potential for selection of clindamycin-resistant mutants between inducible erm(A) and erm(C) Staphylococcus aureus genes. Journal of clinical microbiology. 2008 Feb;46(2):546-50. PubMed PMID: 18077631. Pubmed Central PMCID: 2238126.
13. Lina G, Quaglia A, Reverdy ME, Leclercq R, Vandenesch F, Etienne J. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrobial agents and chemotherapy. 1999 May;43(5):1062-6. PubMed PMID: 10223914. Pubmed Central PMCID: 89111.
14. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci. Journal of clinical microbiology. 2003 Oct;41(10):4740-4. PubMed PMID: 14532213. Pubmed Central PMCID: 254362.
15. Siberry GK, Tekle T, Carroll K, Dick J. Failure of clindamycin treatment of methicillin-resistant Staphylococcus aureus expressing inducible clindamycin resistance in vitro. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2003 Nov 1;37(9):1257-60. PubMed PMID: 14557972.
16. Drinkovic D, Fuller ER, Shore KP, Holland DJ, Ellis-Pegler R. Clindamycin treatment of Staphylococcus aureus expressing inducible clindamycin resistance. The Journal of antimicrobial chemotherapy. 2001 Aug;48(2):315-6. PubMed PMID: 11481309.
17. Clinical and Laboratory Standards Institute. 2013. Performance standards for antimicrobial susceptibility testing; 25th informational supplement. CLSI document M100-S25 Clinical and Laboratory Standards Institute, Wayne, PA.
18. Vandenbroucke-Grauls CM, Kusters JG. Specific detection of methicillin-resistant Staphylococcus species by multiplex PCR. Journal of clinical microbiology. 1996 Jun;34(6):1599. PubMed PMID: 8735132. Pubmed Central PMCID: 229076.
19. Nawaz MS, Khan SA, Khan AA, Khambaty FM, Cerniglia CE. Comparative molecular analysis of erythromycin-resistance determinants in staphylococcal isolates of poultry and human origin. Molecular and cellular probes. 2000 Oct;14(5):311-9. PubMed PMID: 11040095.
20. Frye JG, Jesse T, Long F, Rondeau G, Porwollik S, McClelland M, et al. DNA microarray detection of antimicrobial resistance genes in diverse bacteria. International journal of antimicrobial agents. 2006 Feb;27(2):138-51. PubMed PMID: 16427254.
21. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. Journal of medical microbiology. 2007 Mar;56(Pt 3):342-5. PubMed PMID: 17314364.
22. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant Staphylococcus aureus (MRSA). Proceedings of the National Academy of Sciences of the United States of America. 2002 May 28;99(11):7687-92. PubMed PMID: 12032344. Pubmed Central PMCID: 124322.
23. Chambers HF. The changing epidemiology of Staphylococcus aureus? Emerging infectious diseases. 2001 Mar-Apr;7(2):178-82. PubMed PMID: 11294701. Pubmed Central PMCID: 2631711.
24. Charlebois ED, Perdreau-Remington F, Kreiswirth B, Bangsberg DR, Ciccarone D, Diep BA, et al. Origins of community strains of methicillin-resistant Staphylococcus aureus. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004 Jul 1;39(1):47-54. PubMed PMID: 15206052. Pubmed Central PMCID: 2567833.
25. Eguia JM, Chambers HF. Community-acquired Methicillin-resistant Staphylococcus aureus: Epidemiology and Potential Virulence Factors. Current infectious disease reports. 2003 Dec;5(6):459-66. PubMed PMID: 14642185.
26. Tacconelli E, Venkataraman L, De Girolami PC, EM DA. Methicillin-resistant Staphylococcus aureus bacteraemia diagnosed at hospital admission: distinguishing between community-acquired versus healthcare-

associated strains. The Journal of antimicrobial chemotherapy. 2004 Mar;53(3):474-9. PubMed PMID: 14762054.
27. Farahani A, Mohajeri P, Gholamine B, Rezaei M, Abbasi H. Comparison of different phenotypic and genotypic methods for the detection of methicillin-resistant Staphylococcus aureus. North American journal of medical sciences. 2013 Nov;5(11):637-40. PubMed PMID: 24404541. Pubmed Central PMCID: 3877436.
28. Clinical laboratory standards institute (CLSI). performance standards for antimicrobial disk susceptibility testing. Wayne, PA, USA.2015.
29. Swenson JM, Tenover FC, Cefoxitin Disk Study G. Results of disk diffusion testing with cefoxitin correlate with presence of mecA in Staphylococcus spp. Journal of clinical microbiology. 2005 Aug;43(8):3818-23. PubMed PMID: 16081917. Pubmed Central PMCID: 1233887.
30. Demir T, Coplu N, Esen B. Comparative analysis of phenotypic and genotypic detection of methicillin resistance among Staphylococcus aureus. Indian journal of pathology & microbiology. 2016 Jul-Sep;59(3):314-7. PubMed PMID: 27510667.
31. Sachin Kumar SB. Increasing trend of methicillin-resistant Staphylococcus aureus in Jaipur, Rajasthan, India. African Journal of Microbiology Research. 2016;10:1417-21.
32. Kanaan Mansoor SBT, Ali Shariq, Munam Yousufi, Sumaira Ahmed, Badar Jahan Farooqi. Prevalence and antimicrobial susceptibility pattern of Clindamycin in MRSA isolates of patients in a tertiary care hospital. European Journal of Biotechnology and Bioscience. 2015;3:17-9.
33. Dilnessa T, Bitew A. Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus isolated from clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. BMC infectious diseases. 2016 Aug 09;16:398. PubMed PMID: 27506613. Pubmed Central PMCID: 4977752.
34. Hurlimann-Dalel RL, Ryffel C, Kayser FH, Berger-Bachi B. Survey of the methicillin resistance-associated genes mecA, mecR1-mecI, and femA-femB in clinical isolates of methicillin-resistant Staphylococcus aureus. Antimicrobial agents and chemotherapy. 1992 Dec;36(12):2617-21. PubMed PMID: 1362343. Pubmed Central PMCID: 245516.
35. Li X, Xiong Y, Fan X, Feng P, Tang H, Zhou T. The role of femA regulating gene on methicillin-resistant Staphylococcus aureus clinical isolates. Medecine et maladies infectieuses. 2012 May;42(5):218-25. PubMed PMID: 22609268.
36. Kobayashi N, Wu H, Kojima K, Taniguchi K, Urasawa S, Uehara N, et al. Detection of mecA, femA, and femB genes in clinical strains of staphylococci using polymerase chain reaction. Epidemiology and infection. 1994 Oct;113(2):259-66. PubMed PMID: 7925664. Pubmed Central PMCID: 2271538.
37. Maidhof H, Reinicke B, Blumel P, Berger-Bachi B, Labischinski H. femA, which encodes a factor essential for expression of methicillin resistance, affects glycine content of peptidoglycan in methicillin-resistant and methicillin-susceptible Staphylococcus aureus strains. J Bacteriol. 1991 Jun;173(11):3507-13. PubMed PMID: 2045371. Pubmed Central PMCID: 207965.
38. Martinez-Aguilar G, Hammerman WA, Mason EO, Jr., Kaplan SL. Clindamycin treatment of invasive infections caused by community-acquired, methicillin-resistant and methicillin-susceptible Staphylococcus aureus in children. The Pediatric infectious disease journal. 2003 Jul;22(7):593-8. PubMed PMID: 12867833.
39. Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. Antimicrobial agents and chemotherapy. 1999 Dec;43(12):2823-30. PubMed PMID: 10582867. Pubmed Central PMCID: 89572.
40. Somily AM, Babay HA. Superiority of D-zone Testing Method over Standard Method to detect Rnducible Resistance in Gram Positive Bacteria: a Prospective Surveillance from a Teaching Hospital in Saudi Arabia. International journal of health sciences. 2008 Jul;2(2):8-16. PubMed PMID: 21475484. Pubmed Central PMCID: 3068738.
41. Steward CD, Raney PM, Morrell AK, Williams PP, McDougal LK, Jevitt L, et al. Testing for induction of clindamycin resistance in erythromycin-resistant isolates of Staphylococcus aureus. Journal of clinical microbiology. 2005 Apr;43(4):1716-21.

PubMed PMID: 15814990. Pubmed Central PMCID: 1081368.
42. Sanchez ML, Flint KK, Jones RN. Occurrence of macrolide-lincosamide-streptogramin resistances among staphylococcal clinical isolates at a university medical center. Is false susceptibility to new macrolides and clindamycin a contemporary clinical and in vitro testing problem? Diagnostic microbiology and infectious disease. 1993 Mar-Apr;16(3):205-13. PubMed PMID: 8477574.
43. Positive MKTpoicrag, 2011;5:38-40. cfvcsJoCaDR.
44. Frank AL, Marcinak JF, Mangat PD, Tjhio JT, Kelkar S, Schreckenberger PC, et al. Clindamycin treatment of methicillin-resistant Staphylococcus aureus infections in children. The Pediatric infectious disease journal. 2002 Jun;21(6):530-4. PubMed PMID: 12182377.
45. Shantala GB SA, Rao RK, Vasudeva, Nagarathnamma T. Detection of inducible , diffusion cricioSabtd, 5:35-7. itJoCaDR.
46. Navaneeth BV. A preliminary in vitro study on inducible and constitutive clindamycin resistance in Staphylococcus aureus from a South Indian tertiary care hospital. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2006 Mar;10(2):184-5. PubMed PMID: 16290013.
47. Matthew V. N. O'Sullivan YC, Fanrong Kong, Xianyu Zeng and GwendolynL. Gilbert. Influence of disk separationdistance on accuracy of the disk approximation atest for detection of InducibleClindamycin resistance in, spp. S, 4072-76 JoCM.
48. Jorgensen JH, Crawford SA, McElmeel ML, Fiebelkorn KR. Detection of inducible clindamycin resistance of staphylococci in conjunction with performance of automated broth susceptibility testing. Journal of clinical microbiology. 2004 Apr;42(4):1800-2. PubMed PMID: 15071055. Pubmed Central PMCID: 387617.
49. Otsuka T, Zaraket H, Takano T, Saito K, Dohmae S, Higuchi W, et al. Macrolide-lincosamide-streptogramin B resistance phenotypes and genotypes among Staphylococcus aureus clinical isolates in Japan. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2007 Mar;13(3):325-7. PubMed PMID: 17391391.
50. Bemer P, Juvin ME, Corvec S, Ros A, Drugeon H. Correlation of agar dilution and VITEK2 system for detection of resistance to macrolides, lincosamides and pristinamycin among Staphylococcus aureus and Staphylococcus epidermidis: association with genotypes. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2005 Aug;11(8):656-61. PubMed PMID: 16008619.
51. Gherardi G, De Florio L, Lorino G, Fico L, Dicuonzo G. Macrolide resistance genotypes and phenotypes among erythromycin-resistant clinical isolates of Staphylococcus aureus and coagulase-negative staphylococci, Italy. FEMS immunology and medical microbiology. 2009 Jan;55(1):62-7. PubMed PMID: 19076222.

Table 1: Sequence and characteristics of PCR primers included in this study

| Gene | Primer | Sequence 5'-3' | Ta ºC* | Amplicon size(BP) | reference |
|---|---|---|---|---|---|
| *femA* | Fem A F | CTTACTTACTGCTGTACCTG | 52 | 686 | [18] |
| | Fem A R | ATCTCGCTTGTTATGTGC | | | |
| *mecA* | mecA F | TGGCTATCGTGTCACAATCG | 52 | 304 | [18] |
| | mecA R | CTGGAACTTGTTGAGCAGAG | | | |
| *ermA* | *ermA* F | 5'ACGATATTCACGGTTTACCCACTTA3' | 51 | 610 | [19] |
| | *ermA* R | 5'AACCAGAAAAACCCTAAAGACACG3' | | | |
| *ermC* | *ermC* F | 5'AGTACAGAGGTGTAATTTCG3' | 54 | 520 | [20] |
| | *ermC* R | 5'AATTCCTGCATGTTTTAAGG3' | | | |

*Ta → annealing temperature

Table 2:$MLS_B$ resistance profile using antimicrobial susceptibility with D-test results.

| Resistance Phenotype | Erythromycin susceptibility | Clindamycin susceptibility | D-test result | No of isolates |
|---|---|---|---|---|
| Susceptible | S | S | Not applicable | 73 |
| $cMLS_B$ | R | R | Not applicable | 10 |
| $iMLS_B$ | R | S | Positive | 14 |
| $MS_B$ | R | S | Negative | 3 |

Table 3: Association between methicillin resistance and $MLS_B$ resistance phenotype among *S. aureus* isolates.

| $MLS_B$ resistance phenotype | MRSA | MSSA | Total |
|---|---|---|---|
| Susceptible | 38 | 35 | 73 |
| $cMLS_B$ | 8 | 2 | 10 |
| $iMLS_B$ | 6 | 8 | 14 |
| $MS_B$ | 2 | 1 | 3 |
| Total | 54 | 46 | 100 |

Table 4: Association between $MLS_B$ resistance phenotypic and genotypic patterns.

| Phenotypic resistance patterns | Molecular resistance patterns | | | Total |
|---|---|---|---|---|
| | ermA (%) | ermC(%) | ermA/ermC (%) | |
| $cMLS_B$ | 2 (7.5) | 6 (22) | 2 (7.5) | 10 (37) |
| $iMLS_B$ | 2 (7.5) | 12 (44.5) | 0 (0) | 14 (52) |
| $MS_B$ | 1 (3.5%) | 2 (7.5) | 0 (0) | 3 (11) |
| Total | 5 (18.5) | 20 (74) | 2 (7.5) | 27 (100%) |

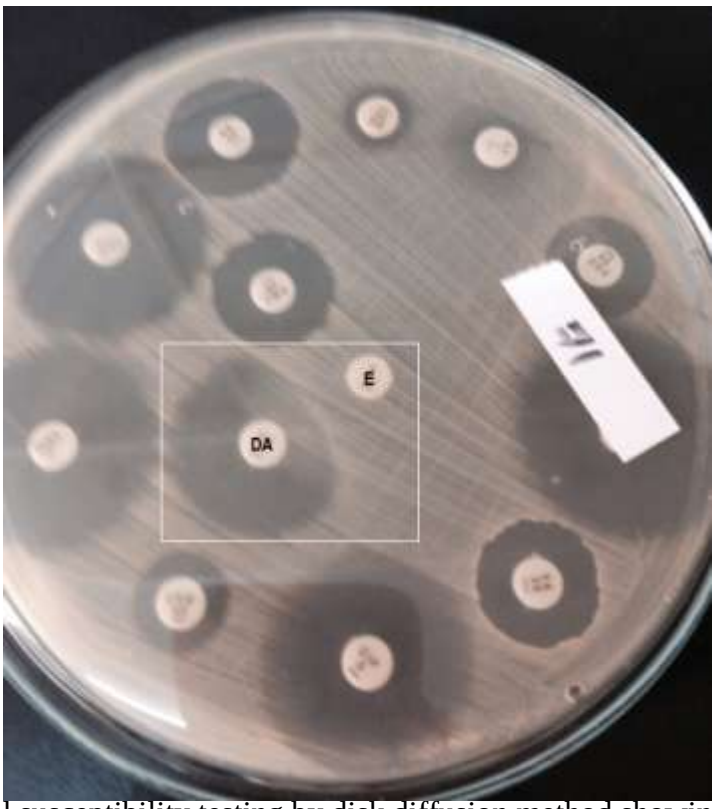


Figure 1: Antimicrobial susceptibility testing by disk diffusion method showing D-zone of inhibition around clindamycin (DA) with flattening side between DA and erythromycin (E) disk.

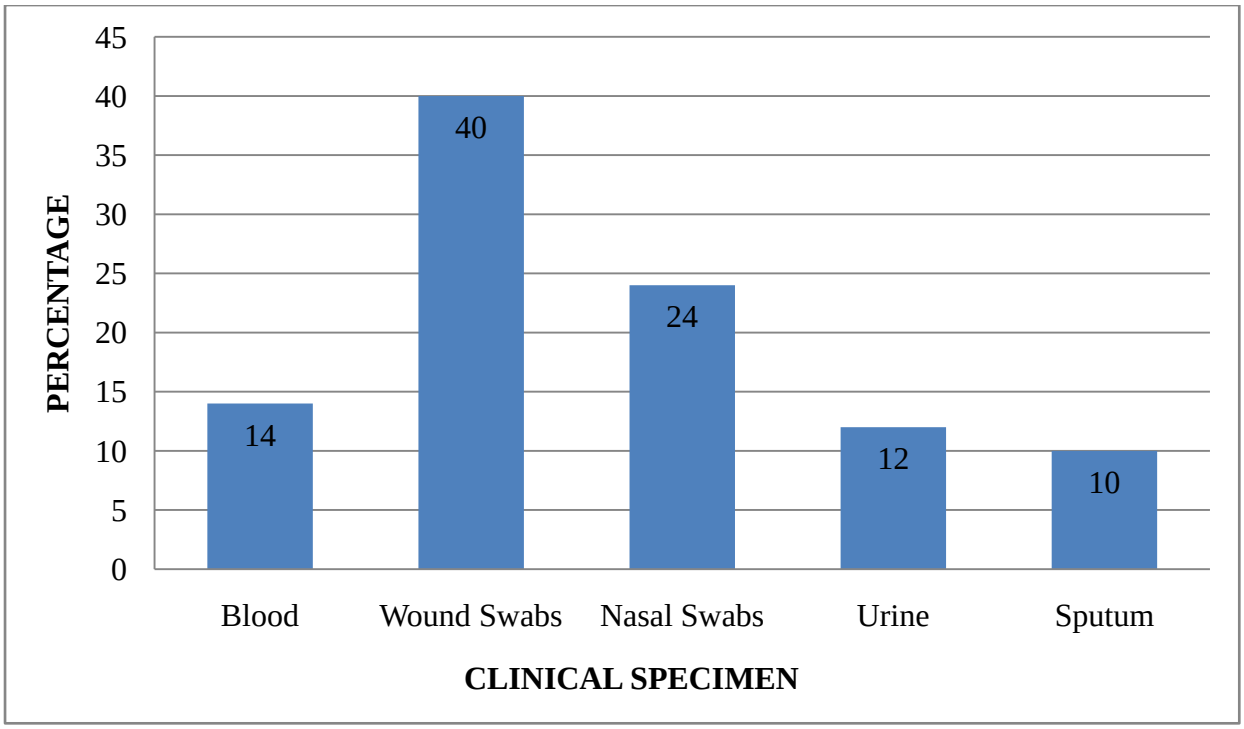


Figure 2: Distribution of *S. aureus* isolates among clinical specimen

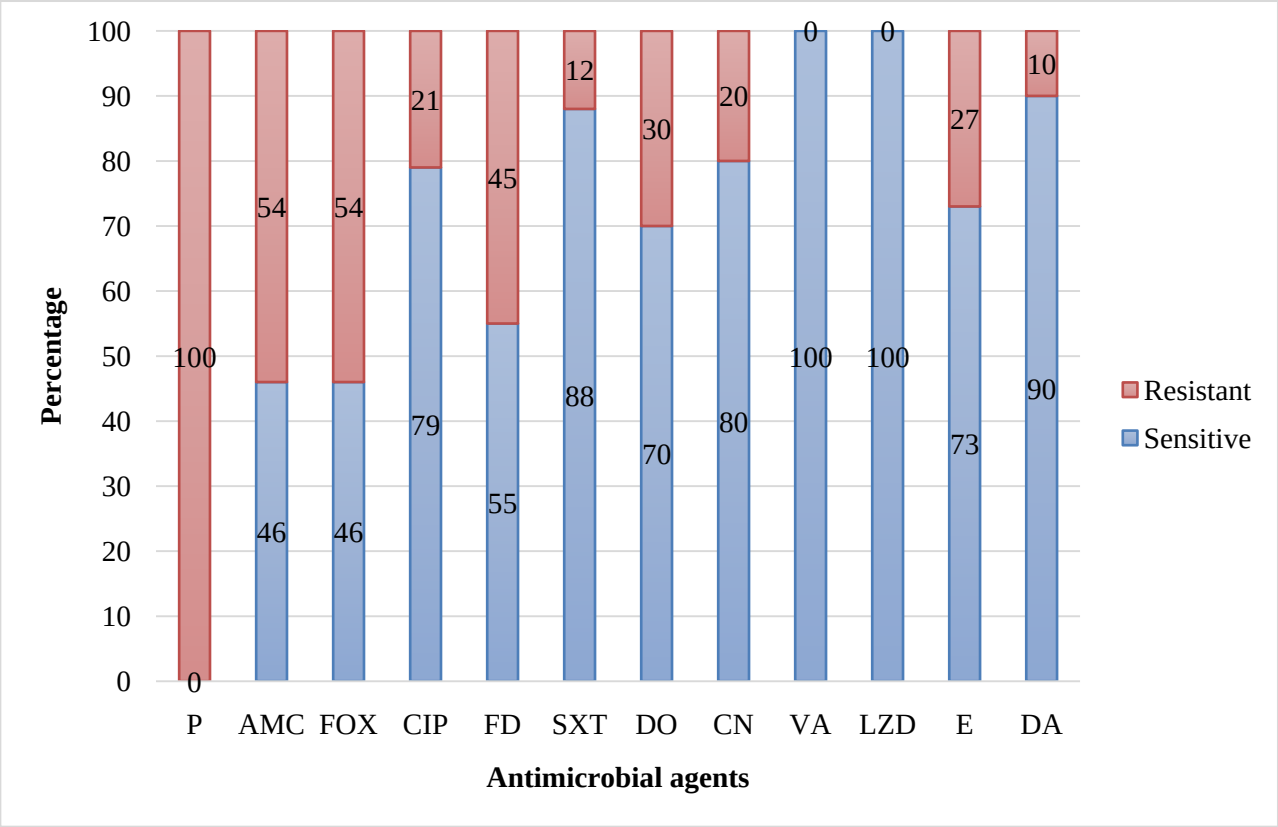


Figure 3: Antimicrobial susceptibility results

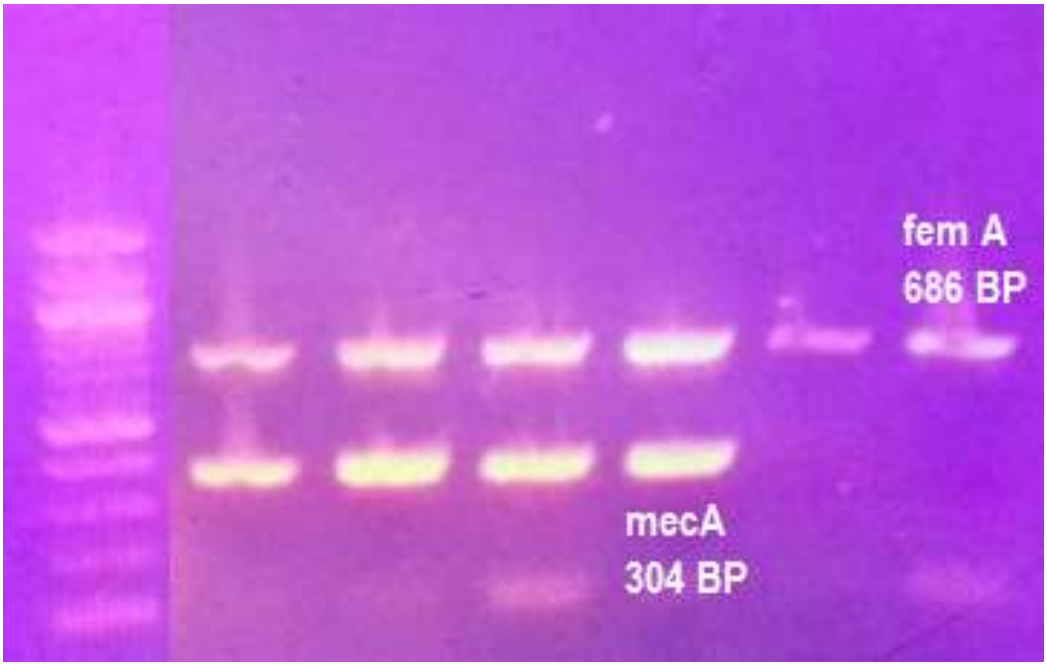


Figure 4: Ethidium bromide stained agarose gel showing the amplification bands of femA (686 BP) and mecA (304 BP)

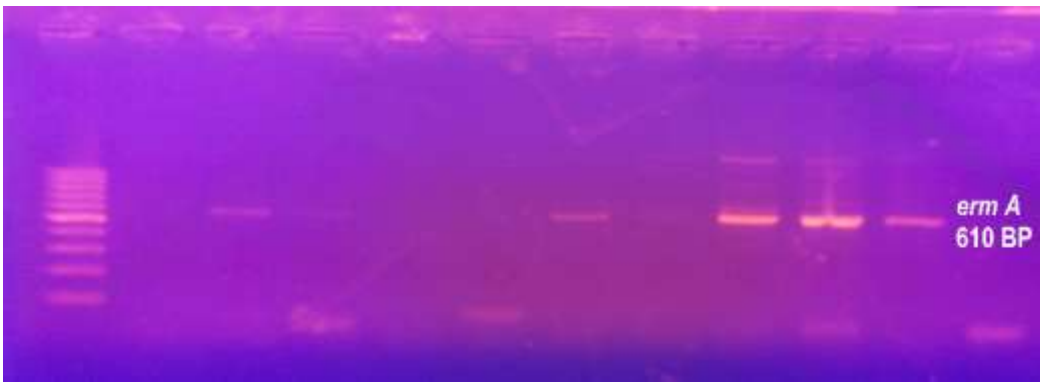


Figure 5:Ethidium bromide stained agarose gel showing the amplification bands of ermA (610 BP)

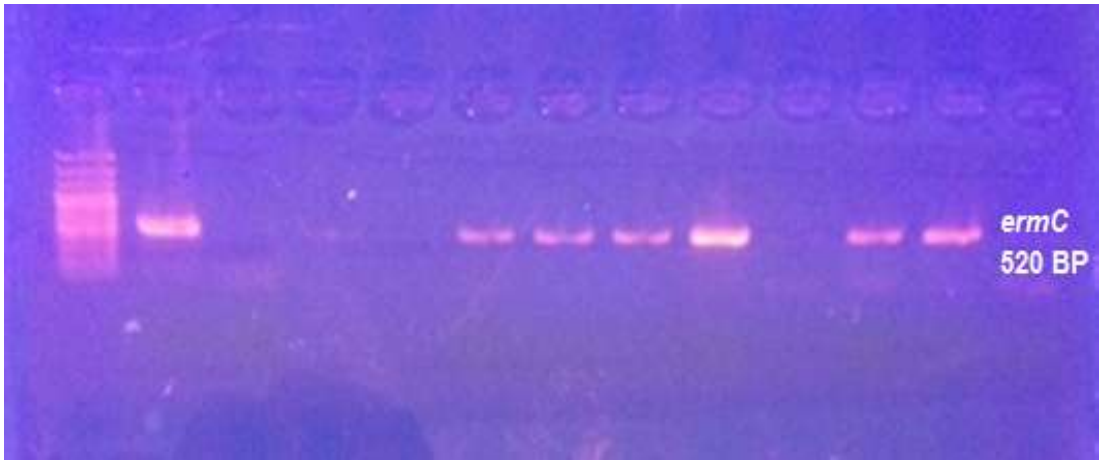


Figure 6:Ethidium bromide stained agarose gel electrophoresis showing the amplification bands of ermC (520 BP)

**Corresponding Author:** Ahmed Gaballah,
Microbiology Department, Medical Research Institute,
Alexandria University, Alexandria, Egypt.
E-mail: Ahmed.gaballah@alexu.edu.eg

**How to cite this article:**
Gaballah A, Ghazal A. Clindamycin Resistance among *Staphylococcus aureus* Clinical Isolates in Alexandria. Int. J. Adv.Microbiol.Health.Res., 2018; 2(1):24-35.
**Source of Financial Support:** Nil, **Conflict of interest:** Nil.