Clindamycin resistant S. aureus in Egypt

Phenotypic and genotypic identification of *Staphylococcus aureus* resistant to clindamycin in Mansoura University Children Hospital, Egypt

1*Abouelnour, A., 2Zaki, M. E., 3Hassan R., and 4Elkannishy, S. M. H.

1,2Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt
3Department of Medical Microbiology, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt
4Department of Toxicology, Mansoura Hospital, Mansoura University, Mansoura 35516, Egypt

Abstract:

**Background:** Clindamycin has been a good alternative drug to penicillins in the treatment of infections caused by *Staphylococcus aureus* but resistance to this agent has led to therapeutic failure. Inducible clindamycin resistance in staphylococci carrying *erm* genes may not be detectable by routine disk diffusion test. The objective of this study is to phenotypically detect clindamycin resistance in clinical *S. aureus* isolates and determine the prevalence of *ermA*, *ermB* and *ermC* carriage among these isolates.

**Methodology:** A total of 230 non-duplicate *S. aureus* were isolated from children admitted to Mansoura University Children Hospital during the period January 2016 and June 2017 by conventional microbiology method. *In vitro* antibiotic susceptibility to selected antibiotics including erythromycin was performed by the disk diffusion technique. The ‘D-zone’ test was used to phenotypically detect inducible clindamycin resistance. The presence of *ermA*, *ermB* and *ermC* genes was confirmed by multiplex polymerase chain reaction (m-PCR) assay.

**Results:** One hundred and seven (46.6%) isolates were phenotypically resistant to erythromycin while 109 (47.3%) were methicillin (cefoxitin) resistant *S. aureus* (MRSA). The macrolide-lincosamide-streptograminB (MLS) phenotypes among the erythromycin resistant isolates were 47 (44%) inducible MLS, and 46 (43%) constitutive MLS, while 14 (13.0%) were MS phenotype. Although, the MLS phenotype was more predominant in MRSA (n=60, 56.1%) than MSSA (n=33, 30.7%) while the MS phenotype was more predominant in MSSA (n=9, 8.4%) than MRSA (n=5, 4.6%) isolates, the difference was not statistically significant (p=0.0777). The *ermA* (29.0%, n=31) and *ermC* (18.7%, n=20) were the most prevalent genes carried by the isolates while *ermB* was carried by a few (4.7%, n=5). Forty six (43%) isolates did not carry any detectable *erm* gene.

**Conclusion:** In this study, both inducible and constitutive clindamycin resistance phenotypes were common among *S. aureus* isolates. Although the genetic basis for this may be attributed to carriage of *ermA*, *ermB* and *ermC* genes, a number of the resistant isolates did not carry any of these genes.

**Keywords:** phenotypic, genotypic, macrolide-lincosamide-streptogramin B, *S. aureus*, children,

Identification phénométique et génotypique de *Staphylococcus aureus* résistant à la clindamycine à l’Hôpital Universitaire Mansoura, Égypte

1*Abouelnour, A., 2Zaki, M. E., 3Hassan R., et 4Elkannishy, S. M. H.

1,2Département de pathologie clinique, Faculté de médecine, université Mansoura, Mansoura 35516, Égypte
3Département de microbiologie médicale, Faculté de médecine, université Mansoura, Mansoura 35516, Égypte
43ème département de toxicologie, hôpital Mansoura, université Mansoura, Mansoura 35516, Égypte

Correspondance à: aalaa_abo@yahoo.com

Received July 20, 2019; Revised September 22, 2019; Accepted September 28, 2019
Introduction:

Staphylococcus aureus is a common bacterial pathogen responsible for both community and hospital acquired infections. The infection caused by S. aureus ranges from mild skin and soft tissue to life threatening infections such as septicemia, meningitis and endocarditis (1). The major concern about this pathogen is its ability to acquire resistance to multiple antibiotics. Resistance to penicillin and methicillin has led to the use of alternative antibiotics especially macrolides and lincosamides such as clindamycin. Clindamycin can be given both orally and intravenously and it is effective against soft tissue infections as well as deep penetrating infections such as sepsis (2).

Resistance to clindamycin is usually associated with macrolide resistance especially erythromycin (3-7). The responsible mechanisms for macrolide and clindamycin resistance are attributed to two mechanisms (8). The first mechanism is due to the efflux of antibiotics outside the bacterial cell wall before its binding to ribosome. This mechanism is commonly reported in S. aureus and is controlled by msrA gene (9). The other mechanism is called MLSB (macrolide, lincosamide, streptogramin B) which is due to changes on the ribosome affecting the binding site as a result of methylation of the 23S rRNA-binding site of macrolides. This results in resistance to macrolides and is controlled by the ermA, ermB and ermC genes (8, 9).

Clindamycin resistance can be detected phenotypically by two methods. The first method detects constitutive clindamycin resistance (cMLSβ) phenotype by the disk diffusion technique. The other method called the “D-zone” test detects inducible clindamycin resistance (iMLSβ) in susceptible isolates by adding erythromycin (which is a known strong methylase inducer in susceptible strains) to clindamycin disks, which results in appearance of a blunting of the clindamycin inhibition zone (appearing like a letter D) on the margin closest to the erythromycin disk (10).

Published data on the presence of S. aureus strains resistant to clindamycin in Egypt are lacking, however, information on this could have important implications for treatment protocols against staphylococcal infections in the country. The aim of this research therefore is to determine the carriage rate of erm genes responsible for inducible clindamycin resistance among clinical S. aureus isolates in Egypt.

Materials and method:

Study design and subjects

This cross sectional study was conducted on randomly selected children admitted to Mansoura University Children Hospital, Egypt, between January 2016 and June 2017, from whom 230 non-repetitive S. aureus isolates were obtained. The study was approved by Mansoura Faculty of Medicine Institutional Ethical Committee

Specimen collection

The samples for S. aureus isolation were from various clinical specimens such as
blood cultures (n=157), wound samples (n=41), and broncho alveolar lavage (n=32).

**Isolation and identification of S. aureus**

All specimens were inoculated on Sheep blood and MacConkey agar plates, and incubated at 37°C aerobically for 24 hours. Identification of S. aureus was first done by colony morphology on plates, followed by conventional identification methods of Gram stain, catalase activity, and slide and tube coagulase tests.

**Antibiotic susceptibility test**

Antibiotic susceptibility test (AST) was performed on all identified S. aureus isolates (n=230) in accordance with the Clinical and Laboratory Standards Institute (CLSI) agar disk diffusion method on Mueller-Hinton (MH) agar (11) with the following disks (Oxoid, England); erythromycin (15µg), ampicillin-sulbactam (20 µg / 20 µg), amoxicillin-clavulanic acid (20 µg / 10 µg), piperacillin (30µg), ciprofloxacin (5µg), ceftiraxone (5µg), cephradine (30µg), cefotaxime (30µg), ceftazidime (30µg), and ceftoperazone (75µg).

**Detection of methicillin resistance**

Methicillin resistance was detected by the cefoxitin (30µg) disk diffusion test on MH plates which were inoculated with the standardized inoculum of the S. aureus isolates (n=230), and incubated at 37°C for 24 hours. S. aureus with cefoxitin inhibition zone ≤ 21mm were considered MRSA (10).

**Detection of clindamycin resistance**

All S. aureus isolates resistant to erythromycin in the AST (n=107) were evaluated for clindamycin resistance by the disk diffusion method as previously described (10). The isolated colonies were diluted in sterile normal saline to obtain 0.5 McFarland’s standard suspension. The inoculum was aseptically plated over MH agar plate, with both erythromycin (15µg) and clindamycin (2µg) disks placed ~15 mm apart edge to edge, and incubated aerobically at 37°C for 24 hours. Isolates resistant to erythromycin (zone of inhibition <13mm) with flattening of zone (D-zone) around the clindamycin disk (zone of inhibition ≥21mm) were identified as inducible resistance phenotype (Fig 1).

Isolates resistant to both erythromycin and clindamycin (zone of inhibition<21mm) were identified as constitutive resistance phenotype (cMLSb) and isolates resistant to erythromycin but susceptible to clindamycin (zone of inhibition ≥21mm) with no D-zone were identified as MS phenotype (10).

**Multiplex PCR for macrolide genes:**

DNA extraction of purified colonies of all S. aureus resistant to erythromycin in the AST (n=107) was done by Qiagen extraction kit and kept frozen at -20°C until use. Multiplex PCR was performed for the isolates for detection of ermA, ermB and ermC genes using primers with sequences as shown in Table 1 (9). Amplification was performed in a 25µL PCR mixture (Qiagen) with the use of 50ng DNA template, and 25 pmol/L each, of forward and reverse primers. Thermal cycling was performed as follows; 5 min at 94°C, then 1min at 95°C, 30 sec at annealing temp of 55°C, and 1min at 72°C for 30 cycles, with a final extension at 72°C for 5min.

Ten µL of PCR product was resolved on 2% agarose gel containing 0.5mg/mL ethidium bromide at 90V for 1h and visualized in a gel documentation system. The reaction mix containing all materials except DNA was used as negative control.

![Fig 1: Mueller Hinton agar plate showing positive D-zone test in a Staphylococcus aureus isolate with inducible MLSb phenotype](image)

**Table 1: Genes and the primers sequences used in the study**

| Target gene | Primer sequence | Product size, bp | Reference |
|-------------|-----------------|-----------------|-----------|
| ermA        | F 5′-TAT CTT ATC GTT GAG AAG GGA TT-3′ | 139            | B         |
|             | R 5′-CTA CAC TTG GCT TAG GAT GAA A-3′ |               |           |
| ermB        | F 5′-CTA CCT GAT TGT TGA AGG ATT-3′ | 142            | B         |
|             | R 5′-GTT TAC TCT TGG TTT AGG ATG AAA-3′ |               |           |
| ermC        | F 5′-CTT GTT GAT GAT CAT AAT TTC C-3′ | 190            | B         |
|             | R 5′-ATC TTT TAG CAA ACC CGT ATT C-3′ |               |           |
Results:

A total of 230 non-repetitive S. aureus were isolated from the subjects, mainly from blood cultures (n=157, 68.4%), wound samples (n=41, 17.9%) and broncho-alveolar lavage (n=32, 13.7%). All isolates were resistant to the tested antibiotics to varying degrees, with 109 resistant to cefoxitin, representing MRSA rate of 47.3%, and 107 (46.6%) resistant to erythromycin (Table 2).

Table 2: In vitro antibiotic resistance of Staphylococcus aureus in Mansoura University Children Hospital, Egypt

| Antibiotic disk            | Frequency of resistance (%) |
|----------------------------|----------------------------|
| Ciprofloxacin              | 64 (27.8)                  |
| Ceftazidime                | 44 (19.1)                  |
| Ceftriaxone                | 62 (26.9)                  |
| Ampicillin/Sublactam       | 62 (26.9)                  |
| Cefoperazone               | 38 (16.3)                  |
| Cefotaxime                 | 52 (22.6)                  |
| Cephradine                 | 56 (24.3)                  |
| Piperacillin               | 32 (13.9)                  |
| Amoxicillin/Clavulanic acid| 120 (52.1)                 |
| Erythromycin               | 107 (46.6)                 |
| Cefoxitin                  | 109 (47.3)                 |

The result of the D-zone test shows that 93 of the 107 (86.9%) erythromycin resistant S. aureus isolates were MLSB phenotype (iMLS, 43.9% and cMLS, 43.0%) while 14 (13.1%) were MS phenotype (Table 3).

Table 3: Distribution of macrolide resistance phenotypes among Staphylococcus aureus isolates in Mansoura University Children Hospital, Egypt

| Macrolide resistance phenotype | Staphylococcus aureus isolates | Total (%) | X²  | p value |
|--------------------------------|--------------------------------|-----------|-----|---------|
|                                | MRSA (%)                       | MSSA (%)  |     |         |
| Inducible MLSB                 | 27 (25.2)                      | 20 (18.7) | 47 (43.9) | 3.112 | 0.0777 |
| Constitutive MLSB              | 33 (30.8)                      | 13 (12.2) | 46 (43.0) |      |       |
| Sub-Total                      | 60 (56.1)                      | 33 (30.8) | 93 (86.9) |      |       |
| MS (ERY-R/CLI-S)               | 5 (4.7)                        | 9 (8.4)   | 14 (13.1) |      |       |
| Total                          | 65 (60.8)                      | 42 (39.2) | 107 (100) |      |       |

Ery-R = erythromycin resistant; CLI-S = clindamycin sensitive; MRSA = methicillin resistant Staphylococcus aureus; MSSA = methicillin sensitive Staphylococcus aureus; MLSB = macrolide-lincosamide-streptograminB

Although, the MLSB phenotype was more predominant in MRSA (n=60, 56.1%) than MSSA (n=33, 30.8%) while the MS phenotype was more predominant in MSSA (n=9, 8.4%) than MRSA (n=5, 4.7%) isolates, the difference was not statistically significant (X² = 3.112, p = 0.0777).

Table 4 shows the carriage rates of erm genes among the 107 erythromycin resistant S. aureus isolates, with ermA (29.0%, n=31), ermC (18.7%, n=20) and ermB (4.6%, n=5). One isolate (1.0%) carried both ermA and ermC genes while 14 (13.1%) were MS phenotype (Table 3). Fig 2 shows gel electrophoresis of isolates with amplified ermA and ermC genes, and others with no amplified gene product.

Table 4: Frequency of erm genes in phenotypic macrolide resistant Staphylococcus aureus isolates in Mansoura University Children Hospital, Egypt

| Macrolide resistance genes | Frequency (%) |
|----------------------------|---------------|
| ermA                       | 31 (29.0)     |
| ermB                       | 5 (4.6)       |
| ermC                       | 20 (18.7)     |
| ermA/ermC                  | 1 (1.0)       |
| ermA/ermB/ermC             | 4 (3.7)       |
| No gene                    | 46 (43.0)     |
| Total                      | 107           |
Fig 2: Gel electrophoresis of amplified \textit{erm} genes in some macrolide resistant \textit{Staphylococcus aureus} isolates

Discussion:

The prevalence of MRSA has been increasing worldwide since last decades. This prevalence varies widely across different countries and also in hospitals of same country. In this study, isolated \textit{S. aureus} strains showed high resistance to cefoxitin, representing MRSA rate of 47.3\%, compared to a lower rate of 24\% previously reported in a study from Minia, Egypt. However various studies have reported MRSA rate ranging from 50\% to 68\% (10,12,13).

There was also relatively high resistance to other beta lactam antibiotics such as ampicillin-sulbactam (26.9\%), amoxicillin-clavulanic acid (52.1\%), piperacillin (13.9\%), ceftriaxone (26.9\%), cefazidime (19.1\%), cefotaxime (22.6\%), cephradine (24.3\%), and cefoperazone (16.3\%) in this study. This multidrug resistance among \textit{S. aureus} can be explained by the common association of \textit{mecA} (methicillin gene) with other resistant genes for beta lactamase production, leading to cross resistance to other beta lactam antibiotics. Previous studies have shown such association (14, 15).

Phenotypic resistance to erythromycin was 46.6\% in this study. Similar rates of resistance to erythromycin were reported previously ranging from 46\% to 51.7\% (16-18) whereas lower rates ranging from 15.7\% to 28.4\% were reported by others (19,20). The MRSA isolates in our study had higher rate for both inducible MLS$_B$ (25.2\%) and constitutive MLS$_B$ (30.8\%) phenotypes than the MSSA isolates (though not statistically significant), which agrees with the study of Prabhu et al., who reported 16.6\% cMLS$_B$ phenotype in MRSA and 6.2\% in MSSA (10). Also Gupta et al., reported cMLS$_B$ phenotype in 19\% of the total isolates of which 46\% were MRSA and 10\% were MSSA (8). The phenotype of MLS$_B$ (either constitutive or inducible) may show great variations based on the patient groups in different hospitals and geographical regions. This variation may be attributed to several factors such as usage rate of macrolide antibiotics in different hospitals, age of patients, and source of tested isolates (15).

Clindamycin resistance is usually attributed to the carriage of any of \textit{ermA}, \textit{ermB} or \textit{ermC} genes. The association of MRSA with clindamycin resistance can be attributed to acquisition of one of these genes via transposition to become part of the SCC\textit{mec} cassette chromosomes containing the methicillin resistant (\textit{mecA}) genes (16). In our study, \textit{ermA} and \textit{ermC} genes were the two genes frequently carried by the \textit{S. aureus} isolates, while a few isolate carried the \textit{ermB} gene. This is similar to the report of a study in Denmark over a 30 year period (1959–1988) by Westh et al., which showed high carriage rates of \textit{ermA} and \textit{ermC} genes among the \textit{S. aureus} isolates but no \textit{ermB} gene detected (21). Similarly, a multicenter study of 24 European University Hospitals in the year 2000 reported high prevalence of \textit{ermA} (64.0\%) and low prevalence of \textit{ermC}.
Clindamycin resistant S. aureus in Egypt

Afr. J. Clin. Exper. Microbiol. 2020; 21 (1): 30 - 35

(19.5%) but much lower prevalence of ermB (0.6%) genes among 851 S. aureus isolates (22). A more recent study on animal staphylococci showed a similar pattern with carriage rate of 55.6% ermA, 30.6% ermC and 11.1% ermB genes (23). These are similar to the findings in our study.

Conclusion:

From this study, we can conclude that clindamycin resistance is common among S. aureus isolates in Mansoura Children University Hospital, Egypt both inducible and constitutive types carrying mostly ermA and ermC genes that may be partly responsible for these phenotypes. Although, we did not find statistically significant association of clindamycin with methicillin resistance, nevertheless, we recommend routine mandatory testing of all S. aureus isolates for inducible clindamycin resistance in this hospital.

References

1. Projan, S. J., and Novick, R. P. The molecular basis of pathogenicity. In: Crossley K. B., and Archer, G. L. (eds). The staphylococci in human diseases. New York, Churchill, Livingstone: 1997: 55–81.
2. Bradley, J. R. Newer antistaphylococcal agents. Curr Opin Paediatr. 2005; 17: 71-77
3. Daurel, C., Huet, C., Dhalluin, A., et al. Differences in potential for selection of clindamycin-resistant mutants between inducible erm(A) and erm(C) Staphylococcus aureus genes. J Clin Microbiol. 2008; 46 (2): 546–550
4. Patil, N., Mali, U., Kulkarni, S., Ghorpade, M., and Mane, V. Detection of inducible clindamycin resistance among clinical isolates of Staphylococcus aureus in a tertiary care hospital. Int J Curr Microbiol App Sci. 2014; 127: 689-694
5. Lim, J. A., Kwon, A. R., Kim, S. K., Chong, Y., Lee, K., and Choi, E. C. Prevalence of resistance to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in a Korean hospital. J Antimicrob Chemother. 2002; 49: 489–495
6. Gadepalli, R., Dhanaw, B., Mohanty, S., Kapil, A., Das, B. K., and Chaudhry, R. Inducible clindamycin resistance in clinical isolates of Staphylococcus aureus. Indian J Med Res. 2006; 123: 571 - 573
7. Angel, M. R., Balaji, V., Prakash, J., Brahmadathan, K. N., and Mathews, M. S. Prevalence of Inducible clindamycin resistance in Gram positive organisms in a tertiary care center. Indian J Med Microbiol. 2008; 26: 262-264
8. Gupta, V., Datta, P., Rani, H., and Chander, J. Inducible clindamycin resistance in Staphylococcus aureus: a study from North India. J Postgrad Med. 2009; 55: 176-179
9. Martineau, F., Picard, F., Lansac, N., Menard, C., Roy, P. H., Ouellette, M., and Bergeron, M. G. Correlation between the Resistance Genotype Determined by Multiplex PCR Assays and the Antibiotic Susceptibility Patterns of Staphylococcus aureus and Staphylococcus epidermidis: Antimicrobial Agents and Chemother. 2000; 44: 231-238
10. Prabhu, K., Rao, S., and Rao, V. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. J Lab Phys. 2011; 3: 25-27
11. Clinical and Laboratory standards Institute (CLSI). Performance standards for Antimicrobial Susceptibility Testing; Twenty Second Informational Supplement, M100-S22, Wayne, 2012
12. Lewis, M. T., Gales, A. C., and Sader, H. S. Frequency of occurrence and antimicrobial susceptibility patterns for pathogens isolated from Latin-American patients with a diagnosis of pneumonia: results from the antimicrobial surveillance program (1998). Diagn Microbial Infect Dis. 2000; 37: 63–74.
13. Barisic, Z., Babic-Erceg, A., Borzic, E., et al. Urinary tract infections in South Croatia: aetiology and antimicrobial resistance. Int J Antimicrob Agents. 2003; 2: 61–64
14. Bashir, A., Mujahid, T. Y., and Jehan, N. Antibiotic resistance profile: isolation and characterization of clinical isolates of staphylococci from patients with community-acquired skin infections. Pak J Pharm Sci. 2007; 20: 299–304
15. Ojulong, J., Mwambu, T. P., Jalooba, M., Bwanga, F., and Kaddu-Mulindwa, D. H. Relative prevalence Methicillin Resistant Staphylococcus aureus and its susceptibility pattern in Mulago Hospital, Kampala, Uganda. Tanzania J Health Research. 2009; 11 (3): 149–153.
16. Leclercq, R. Staphylococcus resistant to antibiotic therapy. Annales Francaises d'anestheise et de Reanimation. 2002; 21: 375–383
17. Lyali, K. S., Gupta, V., and Chhina, D. Inducible clindamycin resistance among clinical isolates of Staphylococcus aureus. J Mahatma Gandhi Inst Med Sci. 2013; 18: 112-115
18. Sande, S. V. Phenotypic detection and incidence of inducible clindamycin resistance among Staphylococcus aureus from tertiary care hospital. Int J Adv Med. 2015; 2 (3): 264-268.
19. Ajanta, G. S., Kulkarni, R. D., Shetty, J., Shukhada, C., and Jain, P. Phenotypic detection of Inducible clindamycin resistance among S. aureus isolates by using the lower limit of recommended inter disk distance. Indian J Pathol Microbiol. 2008; 51 (3): 376 - 378.
20. Goyal, R., Singh, N. P., Manchanda, V., and Mathur, M. Detection of clindamycin susceptibility in macrolide resistant phenotypes of Staphylococcus aureus. Indian J Med Microbiol. 2004; 22: 251-254
21. Westh, H., Houpha, D. M., Vuurt, J., and Rosdahl, V. T. Prevalence of erm gene classes in erythromycin resistant Staphylococcus aureus strains isolated between 1959 and 1988. Antimicrob Agents Chemother. 1995; 39 (2): 369–373. doi:10.1128/acid.39.2.369
22. Schlotz, F. J., Sadurski, R., Krav, A., Boos, M., Geisel, M., Köhler, K., Verhoej, J., and Fluit, C. Prevalence of macrolide-resistance genes in Staphylococcus aureus and Enterococcus faecium isolates from 24 European university hospitals. J Antimicrob Chemother. 2000; 45: 891-894
23. Ishibashi, E. O., and Beshri, A. Characterization of antibiotic resistance and species diversity of staphylococci isolated from apparently healthy farm animals. Afr J Clin Expz Microbiol. 2019; 21 (4): 289-298