Marmara Pharmaceutical Journal 21/2: 407-411, 2017
DOI: 10.12991/marupj.301167

RESEARCH PAPER

Investigation of inducible clindamycin resistance in methicillin resistant *Staphylococcus aureus* strains

Hayrunisa Hancı, Ahmet Ayyıldız, Hakan İgan

ABSTRACT

Clindamycin is one of the important alternative antibiotics in the therapy of *Staphylococcus aureus* infections. The limited treatment options for MRSA, increaes the importance of the right antibiotic.The major problem of the use of clindamycin for such infections is the presence of inducible clindamycin resistance that can lead to treatment failure. The aim of this study was to investigate the inducible clindamycin resistance of 86 MRSA strains isolated from Research Hospital of Ataturk University. Inducible clindamiycin resistance was detected using D zone test method according to Clinical and Laboratory Standards Institute (CLSI) instructions. Twenty of the strains exhibited the iMLSB, 8 exhibited the cMLSB and 22 exhibited the MS phenotype. The D zone test, which can be done by using erythromycin and clindamycin discs, is a simple disc diffusion test for detection MLSB phenotype and clinical laboratories should report in vitro inducible clindamycin resistance in all *S. aureus* isolates.

Key Words: D zone test, Inducible clindamycin resistance, MRSA,

INTRODUCTION

Staphylococci, which is a member of the normal flora on the skin can cause various infections as opportunistic pathogens (1). Nosocomial and community acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infections are common worldwide (2). The microorganism is responsible for many infections such as skin, soft tissue, eye, ear, bone infections and illness like pneumonia, endocarditis, meningitis and septicaemia (3). Today, perevalance of MRSA varies according to countries, regions and also to the hospitals and services in the same hospital (4). Although, there are many options for control and treatment of staphylococcal infections, the choice of the appropriate antibiotic is important (5).

Macrolide-lincosamide-streptogramin B (MLSB) antibiotics, although chemically different structures have shown similar effects on bacterial protein synthesis. Therefore resistance genes, causing resistance to MLSB antibiotics can lead to the

Hayrunisa Hancı
Atatürk University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 25240, Erzurum, Turkey.

Ahmet Ayyıldız
Ataturk University, Medical Faculty, Department of Medical Microbiology, Erzurum, Turkey.

Hakan İgan
Palandöken State Hospital, Department of Microbiology, Erzurum, Turkey

Corresponding Author:
Hayrunisa HANCI
e-mail: hayrunisa.hanci@hotmail.com

Submitted / Gönderilme: 31.10.2016 **Revised / Düzeltme:** 05.12.2016
Accepted / Kabul: 07.12.2016

development of cross-resistance to any of the other antibiotics of the same group (6). Common mechanisms of acquired resistance to MLSB antibiotics are modification of a target site by ribosomal RNA methylation, enzymatic inactivation and active efflux pump. Target site modification is mediated by the presence of erythromycin resistance methylase (erm) genes. Erythromycin resistance methylase (erm) genes encode methylases. Methylases cause conformational modification of 23S rRNA and lead to reduced binding of MLSB group of antibiotics to the target site in the 50S ribosomal subunit (7). Clindamycin is an alternative for penicillin allergic patients and 100% bioavailable when given orally. It has many advantages like low cost, fewer severe side effects, lack of need for renal adjustments, good tissue penetration and ability to directly inhibit toxin production (3, 5). In treatment of MRSA infections empirical use of clindamycin is common. However, routine in vitro testing for clindamycin susceptibility sometimes fails to detect inducible clindamycin resistance, leading to treatment failure (8, 9). For this reason detecting of inducible clindamiycin resistance is important. Clinical and Laboratory Standards Institute (CLSI) recommends the D zone test (disk diffusion test) or the broth microdilution test using a combination of erythromycin and clindamycin for strains of *S. aureus* that are resistant to erythromycin, but susceptible or intermediate to clindamycin, to detect inducible clindamycin resistance (10).

MATERIAL and METHODS

This study was conducted for a period from April 2013 to January 2015. Eighty six methicillin resistant *S. aureus* from various clinical samples (52 wound, 16 blood culture, 9 tracheal aspirate, 7 urine, 1 cerebrospinal fluid and 1 ear discharge) from Microbiology Laboratory of Research Hospital of Ataturk University were included in this study. Duplicate isolates from the same patient were excluded. Isolates were identified by conventional methods and Vitek 2 (BioMérieux, France) automated system. Antibiotic susceptibility tests were done by Kirby Bauer disc diffusion method on Mueller–Hinton agar plates using erythromycin, clindamycin, ciprofloxacin, levofloxacin, trimethoprim/sulphametoxazole, linezolid, gentamicin, amikacin and tetracycline discs, methicillin resistance of the isolates were detected by disc diffusion method with 30 μg cefoxitin disc (Oxoid) and inducible clindamiycin resistance was detected by the D zone test according to Clinical and Laboratory Standards Institute (CLSI) recommends (10). *S. aureus* ATCC 43300 was used as quality control strain.

For the D zone test 2 μg clindamycin disc was placed at a distance of approximately 15 mm from 15 μg erythromycin disc on Mueller–Hinton agar plates previously inoculated with 0.5 McFarland bacterial suspensions. After the incubation (18-24 h. at 37 °C.) the diameter of ≤ 13 mm zone of inhibition for erythromycin, ≥ 21 mm for clindamycin and if the flattening of clindamycin zone on the side facing to erythromycin was considered as inducible clindamycin resistance positive (D zone test positive, iMLSB phenotype). If the isolate was erythromycin resistant and clindamycin susceptible and the zone of inhibition showing a circular shape, the isolate was considered to be negative for inducible resistance and the resistance was considered to be due to efflux pump (MS phenotype). Clindamycin and erythromycin resistant isolates were considered to have constitutive resistance (cMLSB phenotype) (11).

RESULTS

Antibiotic susceptibility results of our study showed that the most effective antibiotic was linezolid (100%) and the least effective was gentamicin (25.6%). Table 1 shows the susceptibility results of various antibiotics against MRSA strains.

According to the test results twenty of the strains exhibited the iMLSB, 8 exhibited the cMLSB and 22 exhibited the MS phenotype.

Table 1. Susceptibility results of various antibiotics against MRSA strains.

Antibiotics	Susceptible n (%)	Resistant n (%)
Ciprofloxacin	23 (26.7)	63 (73.3)
Levofloxacin	36 (41.9)	50 (58.1)
Trimethoprim/sulphametoxazole	77 (89.5)	9 (10.5)
Linezolid	86 (100.0)	0 (0)
Gentamicin	22 (25.6)	64 (74.4)
Amikacin	49 (57.0)	37 (43.0)
Tetracycline	40 (46.5)	46 (53.5)
Erythromycin	**36 (41.9)**	**50 (58.1)**
Clindamycin	**58 (67.4)**	**28 (32.6)**
		20 (23.25%) iMLSB **8 (9.30%) cMLSB**

DISCUSSION

Growing multidrug resistance in microorganisms causes serious problems of management and increases the need for new antibiotics. In addition to the cost of treatment, infections caused by resistant microorganisms are increasing morbidity and mortality (12). In our study the susceptibilities of MRSA strains to various antibiotics are investigated by disc diffusion method. As a result, linezolid was the most effective antibiotic (100%) and gentamicin was the least effective one (25.6%). Our findings were consistent with other studies on this aspect (13-15).

Resistance to macrolide, lincosamide and streptogramin B antibiotics results from acquisition of erm gene (16). MLSB resistance can occur as phenotypically, inductable or structural resistance. Clindamycin, a member of MLSB antibiotics is an appropriate choice for skin and soft tissue infections. Clindamycin resistance can be structural or inducible. Structural clindamycin resistance can be an easily recognizable case for both erythromycin and clindamycin resistance. Inducible resistance occurs in the presence of a strong inducer methylase ,such as, erythromycin or azithromycin and it can not be determined by standard test methods without D zone test (17, 18).

The incidence of MLSB phenotypes varies by geographical regions (19). According to results of a multicenter study conducted in Japan, the incidence of clindamycin resistance, including constitutive and inducible resistance, was approximately 26.7% (451/1688) for methicillin sensitive *Staphylococcus aureus* (MSSA) and 73.8% (482/653) for MRSA. The overall incidence of inducible clindamycin resistance among erythromycin-resistant and clindamycin-susceptible/intermediate isolates was 91%. This study demonstrated a high rate of inducible resistance against clindamycin in MSSA and MRSA isolates in a local population (8). Mokta *et al.* (19) found that in MRSA strains (from Sub Himalayan Region of India) there were 29.62% cMLSB phenotype, 28.39% iMLSB phenotype and 13.58% MS phenotype. Juyal *et al.* (20) (from Garhwal Hills of Uttarakhand, India) demonstrated iMLSB phenotype in 13.3% of MRSA. Sasirekha *et al.*(11) (from Bangalore, India) reported that cMLSB phenotype was 5.22% and iMLSB phenotype was 0.65% in MRSA strains. In another study, among the 297 *S. aureus* isolates, D zone test was positive in 13.46% and negative in 32.65% of the isolates (MS phenotype). Constitutive MLSB phenotype was seen in 24.91% of the isolates (21). Focas *et al.* (22) (from Greece) have reported 15% iMLSB and 47% cMLSB phenotype in MRSA strains between 2002-2004. Schreckenberger *et al.* (23) have conducted a study in two different hospitals in the same region and reported that the incidences of inducible clindamycin resistance were 7% and 12% for MRSA strains. In other studies, done abroad, it has also been reported different results (24, 25).

In studies, different results have also been reported from our country. Şamlıoğlu *et al* (6) from İzmir, Turkey found that cMLSB phenotype was 9%, iMLSB phenotype was 87% and MS phenotype was 4% in all staphylococcal strains. In a different study from Turkey in 404 *S.aureus* strains there were 4.70% cMLSB phenotype, 27.47% iMLSB phenotype and 1.23% MS phenotype (26). Dizbay *et al.* (18) demonsrated that in 65 MRSA strains there were 6.15% iMLSB phenotype, and 64.61% cMLSB phenotype. Tekin *et al.* (7) (from Diyarbakir) have reported 1.7% iMLSB phenotype and 87% cMLSB phenotype in MRSA strains. In a study from Gaziantep, Eksi *et al.* (27) have reported that 6.9% iMLSB phenotype and 39.6% cMLSB phenotype in 101 MRSA strains. Kaskatepe and Yildiz (28) reported that in 79 MRSA strains the incidence of iMLSB, cMLSB and MS phenotypes were respectively 18.9%, 27.9%, 16.5%. Durmaz *et al.* (29) have conducted a study with 38 MRSA and 144 MSSA strains and they reported that MLSB resistance phenotype was found higher in MRSA strains compared to MSSA strains, and this was statistically significant. In our study 20 (23.25%) out of total 86 MRSA isolates were D zone test positive (iMLSB phenotype). There were 8 (9.30%) strains with cMLSB phenotype and 22 (25.58%) strains with MS phenotype. According to these results inducible clindamycin resistance in our hospital is not low.

CONCLUSIONS

The limited treatment options for MRSA strains increaes the importance of the right antibiotic. As seen from the results of many studies, inducible clindamycin resistance may vary by country, region, hospital and service in the same hospital. Therefore, we recommend clinical laboratories to report in vitro inducible clindamycin resistance in *S. aureus* isolates. The D zone test, a simple and important method for detection of MLSB phenotype, prevents the misuse of clindamycin.

Metisilin dirençli *Staphylococcus aureus* suşlarında indüklenebilir klindamisin direncinin araştırılması

ÖZET

Klindamisin, *Staphylococcus aureus* enfeksiyonlarının tedavisinde önemli alternatif antibiyotiklerden biridir. MRSA tedavisinde seçeneklerin sınırlı olması doğru antibiyotik seçiminin önemini artırmaktadır. Klindamisinin bu enfeksiyonlarda kullanılmasındaki en önemli sorun, tedavinin başarısızlığına sebep olabilen indüklenebilir klindamisin direncinin varlığıdır. Bu çalışmanın amacı Atatürk Üniversitesi Araştırma Hastanesi'nden izole edilen 86 MRSA suşunda indüklenebilir klindamisin direnci varlığının araştırılmasıdır. İndüklenebilir klindamisin direnci varlığı Clinical and Laboratory Standards Institute (CLSI) önerileri doğrultusunda D zon test yöntemi kullanılarak saptandı. Suşlardan 20'sinde iMLSB, 8'inde cMLSB ve 22'sinde MS fenotipi görüldü. D zon test MLSB fenotipinin saptanmasında eritromisin ve klindamisin antibiyotik disklerini kullanarak yapılabilen basit bir disk difüzyon testidir ve klinik laboratuvarlar tüm *S. aureus* izolatlarında indüklenebilir klindamisin direncini bildirmelidir.

Anahtar Kelimeler: D zon test, indüklenebilir klindamisin direnci, MRSA

REFERENCES

1. Shittu A, Oyedara O, Abegunrin F, Okon K, Raji A, Taiwo S, Ogunsola F, Onyedibe K, Elisha G. Characterization of methicillin-susceptible and resistant staphylococci in the clinical setting: a multicentre study in Nigeria. BMC Infect Dis 2012; 12:286.
2. Vural A, Afşar İ, Kurultay N, Demirci M. Comparison of disk diffusion, oxacillin agar screening, microdilution and pbp2a latex agglutination tests for detection of methicillin resistance in *Staphylococcus aureus*. Ankem Derg, 2011;25:145-9.
3. Farooq S, Saleem M. Prevalence of constıtutıve and inducıble clindamycin resıstance among clinical isolates of *Staph aureus* in Kashmir valley: a hospital based study. J Evolution Med Dent Sci 2016;5:828-31.
4. Uyanik MH, Yazgi H, Bilici D, Özden K, Karakoç E. Detection of macrolide-lincosamide-streptogramin B resistance in nosocomial *Staphylococcus aureus* strains. Ankem Derg 2009;23:66-70.
5. Mittal V. Kishore S, Siddique ME. Prevalence of inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* detected by phenotypic method: A preliminary report. J Infect Dis Immun 2013;51:10-2.
6. Şamlıoğlu P, Ece G, Atalay S, Köse Ş. Macrolide-lincosamide-streptogramin B (MLSB) resistance phenotypes in staphylococci isolated from clinical samples. Ankem Derg 2012;26:116-9.
7. Tekin A, Dal T, Deveci Ö, Tekin R, Atmaca S, Dayan S. Assessment of methicillin and clindamycin resistance patterns in *Staphylococcus aureus* isolated from a tertiary hospital in Turkey. Infez Med 2013;2:111-6.
8. Shoji K, Shinjoh M, Horikoshi Y, Tang J, Watanabe Y, Sugita K, Tame T, Iwata S, Miyairi I, Saitoh A. High rate of inducible clindamycin resistance in *Staphylococcus aureus* isolates-A multicenter study in Tokyo, Japan. J Infect Chemother 2015;21:81-3.
9. Lewis JS 2nd, Jorgensen JH. Inducible clindamycin resistance in Staphylococci: should clinicians and microbiologists be concerned? Clin Infect Dis 2005;40:280-5.
10. CLSI. Performance standards for antimicrobial susceptibility testing; twentysecond informational supplement. M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
11. Sasirekha B, Usha MS, Amruta JA, Ankit S, Brinda N, Divya R. Incidence of constitutive and inducible clindamycin resistance among hospital-associated *Staphylococcus aureus*. 3 Biotech 2014;4:85–9.
12. Sancak B. *Staphylococcus aureus* and antibiotic resistance Mikrobiyol Bul 2011;45: 565-76.
13. Doğan M, Feyzioğlu B, Baykan M. The change of antibiotic resistance in *S. aureus* strains within ten year periods. Abant Med J 2014;3:237-41.
14. Güngör S, Uzun BK, Yurtsever SG, Baran N. Antibiotic resistance in *Staphylococcus aureus* strains isolated from blood cultures. Ankem Derg 2012; 26:171-5.
15. Haznedaroğlu T, Öncül O, Hoşbul T, Çavuşlu Ş, Baylan O, Özyurt M. Methicillin resistance in *Staphylococcus aureus* strains isolated from hospitalized patients: Three-year trend. TAF Prev Med Bull 2010;9:585-90.
16. Lall M, Sahni AK. Prevalence of inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. Med J Armed Forces India 2014;70:43-7.
17. Mert Dinç B, Karabiber N, Aykut Arca E. Macrolide-lincosamide-streptogramin b (MLSB) resistance and fusidic acid susceptibility of methicillin resistant *Staphylococcus aureus* (MRSA) strains isolated from clinical samples. Türk Hij Den Biyol Derg 2009;66:89-94.
18. Dizbay M, Günal O, Ozkan Y, Ozcan Kanat D, Altuncekic A, Arman D. Constitutive and inducible clindamycın resistance among nosocomially acquired staphylococci. Mikrobiyol Bul 2008;42:217-21.
19. Mokta KK, Verma S, Chauhan D, Ganju SA, Singh D, Kanga A, Kumari A, Mehta V. Inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* from Sub Himalayan Region of India. J Clin Diagn Res 2015;9:20-3.
20. Juyal D, Shamanth AS, Pal S, Sharma MK, Prakash R, Sharma N. The prevalence of inducible clindamycin resistance among staphylococci in a tertiary care hospital - a study from the garhwal hills of uttarakhand, India. J Clin Diagn Res 2013;7:61-5.
21. Sida H, Chauhan B, Pethani J, Patel L, Shah P. Inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus*. Natl J Med Res 2015;5:268-71.
22. Fokas S, Fokas S, Tsironi M, Kalkani M, Dionysopouloy

M. Prevalence of inducible clindamycin resistance in macrolide-resistant *Staphylococcus spp*. Clin Microbiol Infect 2005;11:337-40.

23. Schreckenberger PC, Ilendo E, Ristow KL. Incidence of constitutive and inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci in a community and a tertiary care hospital. J Clin Microbiol 2004;42:2777-9.
24. Siberry GK, Tekle T, Carroll K, Dick J. Failure of clindamycin treatment of methicillin-resistant *Staphylococcus aureus* expressing inducible clindamycin resistance in vitro. Clin Infect Dis 2003;37:1257-60.
25. Saxena S, Singh T, Dutta R. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* at a tertiary care hospital: implications for clinical therapy. J Commun Dis 2012;44:97-102.
26. Aydeniz Ozansoy F, Cevahir N, Kaleli I. Investigation of macrolide, lincosamide and streptogramin B resistance in *Staphylococcus aureus* strains isolated from clinical samples by phenotypical and genotypical methods. Mikrobiyol Bul 2015;49:1-14.
27. Eksi F, Gayyurhan ED, Bayram A, Karsligil T. Determination of antimicrobial susceptibility patterns and inducible clindamycin resistance in *Staphylococcus aureus* strains recovered from southeastern Turkey. J Microbiol Immunol Infect 2011;44:57-62.
28. Kaskatepe B, Yıldız S. Determination of inducible clindamycin resistance in staphylococci strains isolated from clinical samples. Turk J Pharm Sci 2014;11:317-22.
29. Durmaz S, Kiraz A, Toka Özer T, Perçin D. Macrolide-lincosamide-streptogramin B resistance phenotypes in *Staphylococcus aureus*. Eur J Gen Med 2014;11:217-20.