

Original Article

Agreement between azithromycin and erythromycin to determine inducible clindamycin-resistant phenotypes in *Staphylococcus aureus* isolates

Nisha Goyal¹, Narendra Pal Singh¹

¹Department of Microbiology, University College of Medical Sciences & Guru Teg Bahadur Hospital, Delhi, India

***Corresponding author:**

Nisha Goyal, MBBS, MPH, MD,
Department of Microbiology,
University College of Medical
Sciences & Guru Teg Bahadur
Hospital, Dilshad Garden,
Delhi 110095, India

drnishagoyalucms@gmail.com

Epub Ahead of Print: 18 July 2023
Published: 08 June 2024

DOI

10.1055/s-0043-1771244

Quick Response Code:



This article was published by
Thieme Medical and Scientific
Publishers Pvt. Ltd. as online first

ABSTRACT

Objectives: To evaluate agreement in the detection of inducible clindamycin resistance by test method using azithromycin disk with reference method using erythromycin disk and to determine prevalence of inducible clindamycin-resistant phenotypes among *Staphylococcus aureus* isolates.

Materials and Methods: A total of 133 non duplicate isolates of *S. aureus* from clinical samples were included in this prospective study. Agreement between erythromycin (reference method) and azithromycin (test method) disks for detection of inducible clindamycin resistance in *S. aureus* isolates was assessed.

Statistical Analysis: Chi-square test was used for analyzing categorical variables ($p < 0.05$ was considered significant).

Results: The prevalence of inducible clindamycin resistance (iMLS_B) was 15.8%. A 100% agreement was recorded between reference and test methods for detecting inducible clindamycin-resistant phenotypes. For the determination of constitutive resistant phenotypes (cMLS_B) among *S. aureus* isolates, the test method demonstrated an agreement of 94.1%.

Conclusions: The present study demonstrated an agreement in the identification of inducible clindamycin-resistant phenotypes among *S. aureus* isolates by both erythromycin and azithromycin disks.

Keywords: inducible clindamycin resistance, iMLS_B, constitutive-resistant phenotypes, cMLS_B, clindamycin, azithromycin

INTRODUCTION

Staphylococcus aureus is a dominant isolate from the skin and soft tissue infections.^[1] It is linked to both health care-associated infections and community-acquired infections.^[2] In *Staphylococcus* spp., the resistance to penicillin and methicillin was first reported in 1944 and 1961 AD, respectively.^[3] Methicillin resistant *S. aureus* (MRSA) is resistant to all the β -lactam antimicrobial agents. With the rising trend of MRSA isolates, the therapeutic options for staphylococcal infections are severely compromised.^[4] MLS_B family antibiotics are often considered as reserve antibiotics for the treatment of resistant staphylococcal isolates.^[1,5] Though antibiotics under the macrolide group such as erythromycin, azithromycin, clarithromycin, and lincosamides (clindamycin and lincomycin) belong to different classes of antimicrobials, they act by the same mechanism of protein synthesis inhibition by binding to 23S rRNA.^[6] Clindamycin

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, transform, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

©2024 Published by Indian Association of Laboratory Physicians

is a key antimicrobial for the management of staphylococcal infections including both methicillin-susceptible *S. aureus* (MSSA) and MRSA.^[7] Clindamycin is preferred over other macrolides owing to its excellent pharmacokinetic properties including good penetration and distribution into the skin and other soft tissue structures. Furthermore, it has low cost and minimal side effects.^[8,9] Unrestrained use of MLSB antibiotics has resulted in an increased incidence of resistance to these antibiotics particularly clindamycin resistance in staphylococcal isolates.^[10,11] Bacteria can develop resistance to MLSB antibiotics through several mechanisms including modification of target site, macrolide efflux pump, and enzymatic inactivation of antibiotic.^[2]

The *erm* gene is often responsible for target site modification.^[8] Depending on the underlying mechanism for the production of methylase in MLSB, it can be constitutive (cMLSB phenotype) or inducible where methylase production is dependent on the presence of macrolide inducer (iMLSB phenotype).^[12,13] Inducible phenotypes are cross-resistant to other group members of the antibiotics including lincosamides and streptogramin B. Thus, the accurate determination of inducible clindamycin resistance is of paramount significance in the management of staphylococcal infections.

Erythromycin (14-membered) and azithromycin (15-membered) macrolides are strong inducers of methylase synthesis. However, spiramycin (16-membered macrolides), clindamycin (lincosamides), and streptogramin B antibiotics are only weak inducers of methylase synthesis. In the presence of the iMLSB phenotype, weaker inducers such as clindamycin may appear active on standard antibiotic susceptibility tests but may lead to therapeutic failure. As per Clinical and Laboratory Standards Institute (CLSI) guidelines, the erythromycin–clindamycin disk approximation test (D test) should be used to determine the presence of inducible clindamycin resistance due to the presence of *erm* gene in erythromycin-resistant isolates.^[14] Azithromycin belongs to the same class of macrolide group of antibiotics. The variation in the basic structure of macrolide resulted in the formation of azalides, a macrolide subclass, that includes azithromycin, clarithromycin, and roxithromycin.^[15] Macrolides, 14- or 16-member ring structures, consist of carbon and oxygen, whereas azalides, 15-member ring structures, also contain nitrogen in addition to carbon and oxygen. This variation in the molecule resulted in an enhanced pharmacokinetic property.

In this study, we used the antibiotic discs of azithromycin and erythromycin for the detection of inducible clindamycin resistance and evaluated the agreement in the detection of inducible clindamycin resistance by test method using azithromycin disk with reference method using erythromycin disk. Our secondary objective was to determine the prevalence of inducible clindamycin-resistant phenotypes among *S. aureus* isolates in our high-volume tertiary care center.

MATERIALS AND METHODS

A prospective study was conducted over a period of 6 months in the bacteriology division of the microbiology department of a tertiary care hospital in Delhi. This study included 133 non duplicate isolates of *S. aureus* from clinical samples of pus aspirates, body fluids, respiratory tract, and genital tract samples, received in our laboratory for routine bacterial identification and antibiotic susceptibility testing. No separate clinical sample was collected for the purpose of this study.

All clinical samples were inoculated on 5% sheep blood agar, MacConkey agar without crystal violet (HiMedia, India), and incubated at 37°C aerobically for 24 hours. The isolated cream to golden yellow colonies with or without hemolysis on 5% sheep blood agar were further identified by using standard microbiological techniques. The antibiotic susceptibility test was performed using modified Kirby Bauer's disk diffusion method and interpreted according to CLSI guidelines (CLSI M100, 2022).^[14] To detect inducible clindamycin resistance in *S. aureus* isolates, latest CLSI guidelines were followed; 15 µg erythromycin disk and 2 µg clindamycin disk were spaced 15 to 26 mm apart on Mueller–Hinton agar at 35°C T 2°C, ambient air for 16 to 18 hours.^[14]

The detection of inducible clindamycin resistance using the erythromycin disk was considered as the reference test for the same. To determine the agreement between erythromycin (reference method) and azithromycin (test method) disks for inducible clindamycin resistance in *S. aureus* isolates, azithromycin disk (15 µg) was placed 15 to 26 mm apart from clindamycin disk (2 µg) on the same Mueller–Hinton agar plate on the opposite side of erythromycin disk such that clindamycin disk was placed in the center of the imaginary line joining the erythromycin and azithromycin disks (Figure 1). This ensured the uniformity of distribution of factors that could have influenced the reading of inducible clindamycin resistance using either of the two disks. HiMedia (India) antibiotic disks were used for antibiotic susceptibility testing. Clindamycin resistance was detected as:

1. Inducible-resistant phenotypes (iMLSB): Resistant to erythromycin and having a clindamycin zone ≤21 mm with a D-shaped zone.
2. Constitutive-resistant phenotypes (cMLSB): Resistant to both erythromycin and clindamycin.
3. MS phenotype: Isolates resistant to erythromycin and susceptible to clindamycin without D-zone.

For interpretation of inducible clindamycin resistance by azithromycin disk, the CLSI guidelines for interpretation of inducible clindamycin resistance by erythromycin disk were percolated.^[14]

Statistical data were analyzed using IBM Statistical Package of Social Sciences (SPSS) software version 20 for Windows

software. The chi-square test was used for analyzing categorical variables ($p < 0.05$ was considered significant).

RESULTS

During the study period, 133 *S. aureus* isolates were collected prospectively. (Table 1) shows the agreement between the detection of iMLSB, cMLSB, and MSSA phenotypes by testing clindamycin in combination with erythromycin (reference method) and azithromycin (test method) disks. Twenty-one *S. aureus* isolates that expressed inducible clindamycin resistance (iMLSB) when tested with the reference method also demonstrated the same with the test method. A 100% agreement was recorded between reference and test methods for detecting inducible clindamycin-resistant phenotypes. To determine constitutive resistance phenotypes (cMLSB) among *S. aureus* isolates, the test method missed one isolate and demonstrated an agreement of 94.1% for the cMLSB phenotype identification. For the determination of susceptibility to individual macrolide antibiotics, 45 *S. aureus* isolates that were susceptible to erythromycin were also susceptible to azithromycin.

Figure 2 depicts the distribution of iMLSB, cMLSB, and MS phenotypes among *S. aureus* isolates as determined by reference and test methods. The prevalence of inducible clindamycin resistance (iMLSB) was 15.8%. Susceptibility to either of the macrolide antibiotic of erythromycin or azithromycin was 33.8%.

DISCUSSION

Azithromycin, the first azalide, has a more extensive spectrum of activity, better tolerability, and an improved drug interaction profile compared with erythromycin.^[15] The addition of nitrogen in azalides makes azithromycin a dibasic molecule (erythromycin is made up of carbon and oxygen only and monobasic in nature) which greatly enhances its pharmacokinetic distribution, sustained release of drugs in the tissues, with their slower elimination rate from our body. Azithromycin has also a unique dosing regimen and postantibiotic effect.^[16]

Table 1: Agreement between detection of iMLSB, cMLSB, and MS *Staphylococcus aureus* phenotypes by testing clindamycin in combination with erythromycin (reference method) and azithromycin (test method) disks ($n = 133$)

<i>Staphylococcus aureus</i> phenotype	Reference method	Test method
Inducible-resistant phenotypes (iMLSB)	21	21/21 (100%)
Constitutive-resistant phenotypes (cMLSB)	17	16/17 (94.1%)
MS phenotype	50	27/50 (54%)

The prevalence of inducible clindamycin resistance varies markedly across different countries and sometimes among hospitals in the same country. The present study reported 15.8% inducible clindamycin resistance among *S. aureus* isolates. Our findings are in line with the studies.^[17,18] In our research, we observed a 100% agreement for the determination of inducible clindamycin resistance between the reference test using erythromycin and the test method using azithromycin. Azap et al in their study also reported a 100% concordance in the detection of inducible clindamycin resistance by azithromycin instead of an erythromycin disk.^[19] Furthermore, it is important to mention that azithromycin use has been shown to possess a higher potential for induction of resistance if compared with exposure to other macrolides such as erythromycin and clarithromycin.^[8,16]

Resistance against macrolide antibiotics is increasing worldwide. In several severe infections caused by *S. aureus*

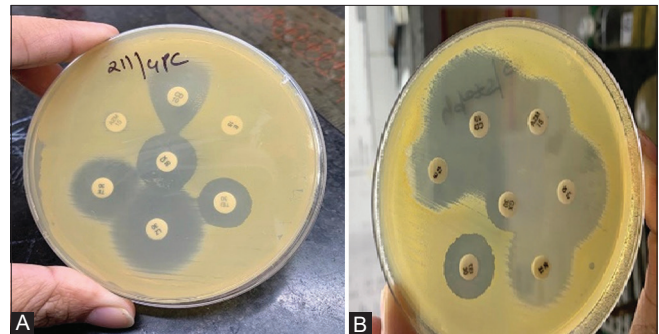


Figure 1: Inducible-resistant phenotypes (iMLSB) and constitutive-resistant phenotypes (cMLSB) among *Staphylococcus aureus* isolates as determined by testing clindamycin in combination with erythromycin (reference method) and azithromycin (test method) disks on same Mueller–Hinton agar plate ($n = 133$). (A) Inducible-resistant phenotypes (iMLSB) and (B) Constitutive-resistant phenotypes (cMLSB) among *Staphylococcus aureus* isolates as determined by testing clindamycin in combination with erythromycin (reference method) and azithromycin (test method) disks on same Mueller–Hinton agar plate.

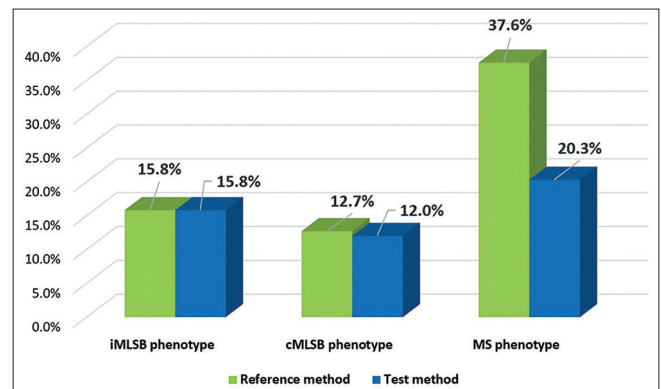


Figure 2: Distribution of iMLSB, cMLSB, and MS phenotypes among *Staphylococcus aureus* isolates as determined by reference and test methods ($n = 133$).

such as sepsis, pneumonia, osteomyelitis, and other serious invasive infections, the presence of even a small risk for the emergence of resistance to clindamycin should defer the clinician to its therapeutic use in the management of that particular case. This risk can be easily determined by a positive D-test result.

CONCLUSIONS

The majority of previous research has focused on identifying inducible clindamycin resistance among clinical isolates using erythromycin disk only. To the best of our knowledge, the present study is the first report from India that has demonstrated an agreement in the identification of inducible clindamycin resistance phenotypes among *S. aureus* isolates by both erythromycin and azithromycin disks.

Authors' Statement

The manuscript has been read and approved by all the authors and the requirements for authorship as required have been met, and that each author believes that the manuscript represents honest work.

Authors' Contribution

N.G. and N.P.S. contributed to conception and design, acquisition of data, or analysis and interpretation of data; drafting the article; final approval of the version to be published.

Source of Funding

None.

Conflict of Interest

None declared.

REFERENCES

- Saffar H, Rajabiani A, Abdollahi A, Habibi S, Baseri Z. Frequency of inducible clindamycin resistance among gram-positive cocci in a tertiary hospital, Tehran, Iran. *Iran J Microbiol* 2016;8: 243–248
- Kishk RM, Anani MM, Nembr NA, Soliman NM, Fouad MM. Inducible clindamycin resistance in clinical isolates of staphylococcus aureus in Suez Canal University Hospital, Ismailia, Egypt. *J Infect Dev Ctries* 2020;14:1281–1287
- Adhikari RP, Shrestha S, Barakoti A, Amatya R. Inducible clindamycin and methicillin resistant *Staphylococcus aureus* in a tertiary care hospital, Kathmandu, Nepal. *BMC Infect Dis* 2017;17:483
- Mama M, Aklilu A, Misgna K, Tadesse M, Alemayehu E. Methicillin- and inducible clindamycin-resistant *Staphylococcus aureus* among patients with wound infection attending Arba Minch Hospital, South Ethiopia. *Int J Microbiol* 2019;2019:2965490
- Mahesh CB, Ramakant BK, Jagadeesh VS. The prevalence of inducible and constitutive clindamycin resistance among the nasal isolates of staphylococci. *J Clin Diagn Res* 2013;7: 1620–1622
- Steward CD, Raney PM, Morrell AK, et al. Testing for induction of clindamycin resistance in erythromycin-resistant isolates of *Staphylococcus aureus*. *J Clin Microbiol* 2005;43:1716–1721
- 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. *J Clin Microbiol* 2003;41:4740–4744
- Woods CR. Macrolide-inducible resistance to clindamycin and the D-test. *Pediatr Infect Dis J* 2009;28:1115–1118
- Firth A, Prathapan P. Azithromycin: the first broad-spectrum therapeutic. *Eur J Med Chem* 2020;207:112739
- Lim JA, Kwon AR, Kim SK, Chong Y, Lee K, Choi EC. 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
- Drinkovic D, Fuller ER, Shore KP, Holland DJ, Ellis-Pegler R. Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. *J Antimicrob Chemother* 2001;48 :315–316
- Mokta KK, Verma S, Chauhan D, et al. Inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* from Sub Himalayan Region of India. *J Clin Diagn Res* 2015;9: DC20–DC23
- Leclercq R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin Infect Dis* 2002;34:482–492
- Clinical and Laboratory Standards Institute. Performance Standard for Antimicrobial Susceptibility Testing, 22nd ed. Wayne, United States: CLSI; 2022
- Amsden GW. Erythromycin, clarithromycin, and azithromycin: are the differences real? *Clin Ther* 1996;18:56–72, discussion 55
- Matzneller P, Krasniqi S, Kinzig M, et al. Blood, tissue, and intracellular concentrations of azithromycin during and after end of therapy. *Antimicrob Agents Chemother* 2013;57: 1736–1742
- Majhi S, Dash M, Mohapatra D, Mohapatra A, Chayani N. Detection of inducible and constitutive clindamycin resistance among *Staphylococcus aureus* isolates in a tertiary care hospital, Eastern India. *Avicenna J Med* 2016;6:75–80
- Saxena S, Singh T, Rakshit P, Dutta R, Gupta RK. Prevalence of inducible clindamycin resistance in *Staphylococcus aureus* at a tertiary care hospital: implications for clinical therapy. *Int J Curr Microbiol Appl Sci* 2014;3:720–725
- Azap OK, Arslan H, Timurkaynak F, Yapar G, Oruç E, Gagir U. Incidence of inducible clindamycin resistance in staphylococci: first results from Turkey. *Clin Microbiol Infect* 2005;11: 582–584

How to cite this article: Goyal N, Singh NP. Agreement between azithromycin and erythromycin to determine inducible clindamycin-resistant phenotypes in *Staphylococcus aureus* isolates. *J Lab Physicians*. 2024;16:160-3. doi: 10.1055/s-0043-1771244