ejpmr, 2021,8(8), 445-449.

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PREVALENCE OF INDUCIBLE CLINDAMYCIN RESISTANCE AMONG STAPHYLOCOCCUS AUREUS ISOLATES FROM VARIOUS CLINICAL SAMPLES

[1]*Dr. Syeda Fahada Zia and [2]Dr. R. Kondal Rao

[1]Assistant Professor, [2]Professor

Department of Microbiology, Mahatma Gandhi Memorial Hospital, Warangal, Telangana, India – 5.

***Corresponding Author: Dr. Syeda Fahada Zia**

Assistant Professor, Department of Microbiology, Mahatma Gandhi Memorial Hospital, Warangal, Telangana, India – 5.

Article Received on 31/05/2021 **Article Revised on 21/06/2021** **Article Accepted on 11/07/2021**

ABSTRACT

Clindamycin is an effective drug to treat methicillin resistant *Staphylococcus aureus* (MRSA). Since Clindamycin and Streptogramin are among the few drugs of choice in the treatment of methicillin resistant S. aureus (MRSA) infections, knowing the resistance to these antibiotics is imperative, reporting *S.aureus* as susceptible to Clindamycin without checking for inducible clindamycin resistance may lead to therapeutic failure. Therefore D-Test is used to screen inducible clindamycin resistance. D-test is a simple disc diffusion test giving high output results. It is used to study the Macrolide Lincosamide Streptogramin resistance (MLSB), both constitutive and inducible as well as macrolide Streptogramin resistance (MSB) in Staphylococcus aureus. The present study was aimed to find out the percentage of *Staphylococcus aureus* having inducible clindamycin resistance (iMLS B) in our geographic area using D-test. Also, we tried to asecertain the relationship between Methicillin-resistant *Staphylococcus aureus* (MRSA) and inducible clindamycin resistance. A total of 200 non-duplicate Staphylococcus aureus isolates from various clinical samples from both outpatients and in-patients were studied. Susceptibility to routine antimicrobial agents was carried our using Kirby Bauer method. Methicillin resistance was detected by oxacillin disc on Mueller Hinton agar (MHA) supplemented with 2% NaCL D- test was performed on all crythromycin-resistant and clindamycin-sensitive *Staphylococcus aureus* strains to detect inducible clindamycin resistance. Among 200 *Staphylococcus* strains, 158 were coagulase positive *Staphylococcus* strains and 42 were coagulase negative *Staphylococcus* strains. 47(23.5%) were found to be MRSA and 21 were D-test positive. Also, MRSA isolates showed both higher inducible resistance and constitutive resistance to clindamycin as compared to Methicillin- sensitive *Staphylococcus aureus* (MSSA). Clindamycin is kept as a reserve drug and is usually advocated in severe MRSA infections depending upon the antimicrobial susceptibility results. Therefore, clinical microbiology laboratory should report inducible clindamycin resistance routinely.

KEYWORDS: Clindamycin, D-test, Erythromycin, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus is responsible for causing a variety of human infections which may range from mild skin infections to life threatening infections. Skin and soft tissue infections (SSTls) are a common manifestation of Staphylococal disease in many community outbreaks, with invasive Staphylococal disease being less common. S*taphylococcus aureus* is a versatile human pathogen causing infections ranging from relatively mild skin and soft tissue infections to life threatening sepsis, pneumonia and toxic shock syndrome. It facilitates disease by its propensityto develop resistance to multiple antibiotics that complicates the treatment well exemplified by Methicillin resistant *Staphylococcus aureus* (MRSA) leaving few therapeutic options. Originally Penicillin was the drug of choice for the treatment of serious *Staphylococcus aureus* infections. The emergence of the resistance to penicillin in *Staphylococcus aureus* was due to the acquisition of plasmid borne genetic elements coding for β lactamase production. The increasing prevalence of methicillin resistance among Staphylococci is an increasing problem and Clindamycin is considered to be one of the alternative agents available to address this issue. Clindamycin belongs to the Lincosamides group of antibiotics, Macrolides, lincosamides and streptogramins (MLS) have traditionally been functionally grouped because they share similar modes of action. However, different mechanisms of resistance to the MLS group have been documented, including intrinsic and acquired resistance. Modifications of the drug target typically consists of alterations (methylation) of the 235 ribosomal RNA, resulting in resistance to all Macrolides, Lincosamides, and group B Streptogramins (i.e, the so-called MLSB phenotype of resistance) but not group A streptogramins. Erythromycin a Macrolide and

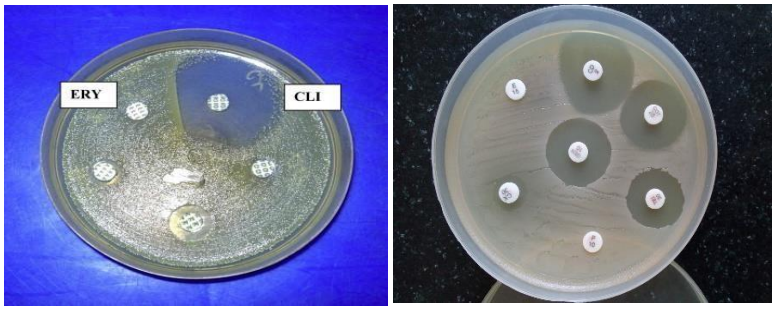
Clindamycin a Lincosamide represent two distinct classes of antimicrobial agents that act by binding to the 50s ribosomal subunit of bacteria to inhibit bacterial protein synthesis. Clindamycin is considered a useful drug in the treatment of skin and soft-tissue infections caused by *Staphylococcus aureus.* It has excellent tissue penetration (except for the central nervous system), accumulates in abscesses, and no dosage adjustments are required in the presence of renal disease. The good oral absorption of clindamycin makes it attractive option for use in outpatients or a follow-up treatment after intravenous therapy. Erythromycin is an effective inducer whereas Clindamycin is a weak inducer. In vitro *Staphylococcus aureus* isolates with constitutive resistance are resistant to both Erythromycin and Clindamycin whereas those with inducible resistance are resistant to Erythormycin and appear sensitive to Clindamycin (iMLS B). Clinically, bacterial strains exhibiting iMLSB have a high rate of spontaneous mutation to constitutive resistance, which could be selected for by use of clindamycin. If Clindamycin is used for treatment of such isolates (iMLS B), selection for constitutive *erm* mutants occurs which may lead to therapeutic failure. In the present study, staphylococcal species isolated from clinical samples were tested with Erythromycin and Clindamycin disk by using "D-test" to find out of the incidence of Staphylococcal species having inducible Clindamycin resistance in our geographic area.

MATERIALS AND METHOD

A Total of 200 non-repetitive isolates of Staphylococcus were taken as sample from various clinical specimens from both outpatient visiting MGM Hospital and in-patient admitted in MGM Hospital. Identification and culture sensitivity of all isolates was done by standard bacteriological procedures following CLSI guidelines. Anti-microbial susceptibility testing was done for all isolates using Kirby Bauer disc diffusion method as per CLSI guidelines. All the S.aureus isolates resistant to erythromycin were taken. Erythromycin (15µg) disc and Clindamycin (2µg) disc were placed 15 mm apart on Mueller Hinton agar plates as per CLSI guidelines and incubated at 370C for 18-24hours. Flattening of zone (D shape) around clindamycin was taken as D-test positive.

OBSERVATION AND RESULTS

The study was conducted at department of Microbiology, Mahatma Gandhi Memorial Hospital (MGM), Warangal during the period February 2017 to July 2017. A Total of 200 non-repetitive isolates of Staphylococcus were taken as sample from various clinical specimens from both outpatient visiting MGM Hospital and in-patient admitted in MGM Hospital. Out of 200 Staphylococcal strains that were isolated from various samples. 158(79%) were coagulase positive Staphylococcus aureus and 42 (21%) were coagulase negative Staphylococcus. It was observed that Staphylococcal infection was more among hospitalized (IPD) 68.5% of the patients. Most of the Staphylococcal strains were isolated from Pus (44%) followed by Wound swabs (16%) and Urine (11.5%). Majority of the patients were in the age group of 21-30 years 57 (28.5%). Male to female ratio is 1.42:1. The number of males were 122 (61.0%) out numbering the female patient's number which was 78 (39.0%) of the total group. Among the 200 Staphylococcal strains Methicillin resistant (MRSA) 47 (23.5%), methicillin sensitive staphylococcus aureus 111 (55.5%). Methicillin resistant coagulase negative Staphylococcus (MRCONS) 7 (3.5%) and 37 (17.5%) were methicillin sensitive coagulase negative Staphylococcus (MSCONS). Majority of the Staphylococcal isolates were MSSA 111 (55.5%) and least number of isolates were MRCONS 7 (3.5%). Most of the MRSA isolates were from hospitalized patients 34 (72.34%).



Distribution of MRSA< MSSA, MRCONS, and MSCONS among different samples

Samples	MRSA	MSSA	MRCONS	MSCONS	TOTAL
	No (%)	No (%)	No (%)	No (%)	No (%)
Pus	28 (23.86%)	43 (48.86%)	4 (4.54%)	13 (14.18%)	88 (44.9%)
WoundSwab	7 (21.87%)	17 (53.12%)	9 (0%)	7 (21.87%)	32 (16.0%)
Throat Swab	3 (15.0%)	9 (45.0%)	0 (0%)	8 (40.0%)	20 (10.0%)
Sputum	3 (18.75%)	7 (43.15%)	4 (25.0%)	2 (12.5%)	16 (8.0%)
Blood	1 (4.76%)	9 (42.85%)	1 (3.12%)	8 (38.08%)	21 (10.5%)
Urine	5 (21.73%)	11 (47.82%)	2 (8.69%)	5 (21.75%)	23 (11.5%)
TOTAL	47	111	11	35	200 (100%)

Majority of 93.10% MRSA, 83.86% MSSA and 47.30% MSCONS were isolated from pus sample. Whereas 45.16% MRCONS were isolated from urine sample. Out of 200 Staphylococcal isolates selected for the study 137 (68.5%) isolates were sensitive to both Erythromycin and Clindamycin. The remaining 63 (31.50%) isolates were subjected to D-test. Disk Diffusion induction test / Double disk approximation test: "D-Test" revealed

1) iMLS - 41 (20.50%)
2) cMLS - 15 (7.50%)
3) MS Phenotype – 7 (3.50%)

Out of Staphylococcal isolates selected for the study 137 (68.5%) isolates were sensitive to both erythromycin and Clindamycin. Of the remaining 63 (31.5%) erythromycin resistant isolates which were subjected to D test using double disc approximation test, 41 (65.07%) showed iMLS B Phenotype.

DISCUSSION

Staphylococcus aureus and coagulase negative Staphylococci (CONS) are recognized to be causing nosocomial and community aspired infections in every region of world. Since most of these strains aremultidrug resistance, management of infections is difficult. Clindamycin is kept as reserve drug and is usually advocated in severe MRSA infections depending upon the antimicrobial susceptibility results. For any clinical microbiology laboratory, the differentiation of *crm*-mediated inducible MLS B (iMLS B phenotype) isolates from isolates with *msrA*-mediated (MS phenotype) resistance is a critical issue because of the therapeutic implications of using clindamycin to treat a patient with an inducible clindamycin-resistant *Staphylococcus aureus* isolate. In recent times, clindamycin has become an excellent drug for some Staphylococcal infections, particularly skin and soft tissue infections and as an alternative in penicillin-allergic patients. Of 200 Staphylococcal strains, that were isolated from various samples, 158 (79%) coagulase positive Staphylococcus aureus and 42 (21%) were coagulase negative Staphylococcus, which is comparable with the study conducted by Paul C S *et al* who reported 79.94% *Staphylococcus aureus* and 20.06% CONS. In our study it was observed that Staphylococcal infection was more among hospitalized of the patients (IPD) 137 (68.5%) and from outpatient department (OPD) 63(31.5%). These findings correlate with the report of Patel M *et al* – symbol 71% Staphylococcal strains were health care associated and 25% were community associated. Most of the Staphylococcal strains were isolated from Pus (44%), followed by Wound swabs (16%) and Urine (11.5%). Kumari *et al*. reported 64% Staphylococcus from pus samples followed by 20% blood 4.8% device associated and 3.2% from urine. In the present study, among the 200 Staphylococcal strains Methicillin resistant (MRSA) were 47 (23.5%), methicillin sensitive Staphylococcus aureus were 111 (55.5%), methicillin resistant coagulase negative Staphylococcus (MRCONS) were 7 (3.5%) and 35 (17.5%) were methicillin sensitive coagulase negative Staphylococcus (MSCONS). Predominant isolates were MSSA (55.56%). Ajanta G S *et al* reported 33.64% MRSA and 66.15% MSSA. The prevalence rate of MRSA infection is Kumari et al.1056 study was found to be 26.14% which is in accordance with the reports by Udaya et al 59 (20%) and Mehta et al 60 (32.8%) from India. Krishna B V S *et al*, reported 18.1% MRSA and 81.89% MSSA. **Ð Test:** Of the 200 total Staphylococcus isolates, selected for the study 137 (68.5%) isolates were sensitive to both Erythromycin and Clindamycin. Of the remaining 63 (31.5%) erythromycin resistant isolates which were subjected to D-test using double disc approximation test. 41(65.07%) showed iMLS B Phenotype. In the Study, .851 Staphylococcal isolates were obtained over a period in which 50.52% were ER-resistant. Among the ER- resistant *S.aureus* iMLS B resistance was observed in 24.63%(51/207) similar to that reported by Fiebelkorn *et al.* Jorgensen *et al* and Gadepalli *et al. 74.40%*. Erythromycin resistant isolates were 63 (31.5%) D test performed by placing erythromycin and clindamycin disks at 15mm distance from edge to edge. Interdisk distance of 15mm has been found satisfactory by Ajantha G S *et al*. Fiebelkorn K R *et al* and others. A total of 41 (20.50%) isolates tested positive for iMLS B resistance by D-test. 15 (7.50%) were shown to have a eMLS B and 7 (3.50%) were MS phenotype. The overall incidence of iMLS B in the present study 21.78% is in agreement with Yilmaz G *et al* report. Incidence c iMLS B 15 (7.50%) and MS phenotype 7 (3.50%) is quite low in comparison with other studies.

Comparison of incident of MLS B phenotype among CONS isolates with other studies

Phenotypes	N Pal *et al*		Yilmaz G *et al*		Present	Study
	MRCONS	MSCONS	MRCONS	MSCONS	MRCONS	MSCONS
iMLS B	43.56%	6%	25.70%	19.90%	28.57%	2.85%
cMLS B	48.51%	0	38.30%	10.20%	42.85%	8.57%
MS Phenotype			15.10%	10.70%	28.57%	2.85%

Constitutive resistance is predominant is MRCONS isolates which correlates with other studies.

CONCLUSION

A total of 200 non – duplicate Staphylococcal isolates from various clinical samples from Outpatients and Inpatients were studied. The 200 isolates comprise of 47 (23.5%) MRSA, 111 (55.55%) MSSA, 7 (5.5%) MRCONS and 35 (17.5%) MSCONS were recovered from variety of clinical samples. Inducible MLS B

resistancewas detected by using Disk diffusion induction test "D-test". The incidence of inducible MLS B was found to be 41 (20.5%), constitutive MLS B 15 (7.5%) and 7 (3.5%) isolates were of MS phenotypes. 51.21% of i MLS B were found to be among MRSA followed by MSSA 41.46% least were among MSCONS 2.43%. Both constitutive and inducible resistance phenotypes were found to be significantly higher in MRSA isolates compared to MSSA and CONS. All of i MLS B were susceptible to Teicoplanin 41 (100%) and followed by vancomycin 39 (95.12%). Significantly higher resistance rate was exhibited by cMLS B and by iMLS B towards amoxiclav, ampicillin and gatifloxacin compared to iMLS B and by iMLS B towards ciprofloxacin and Azithromycin compared to MS phenotype. As the D-test is simple, inexpensive and easy to perform it can be included as a part of routine antibiotic susceptibility testing to accurately identify iMLS B and true clindamycin susceptible MS phenotypes.

We described a simple, accurate method to detect inducible clindamycin resistance.

- To use D test routinely in all microbiologic laboratories.
- Not to apply clindamycin in patients with infections caused by inducible resistant.

Staphylococcus aureus

- To avoid switch therapy from erythromycin to clindamycin

The prevalence of iMLS B may change over time with the emergence of strains with different sensitivity patterns. So periodic surveys should be performed. We conclude that it is important for laboratories to be aware of the local prevalence of iMLSB isolates. On the basis of their data they can choose whether or not to perform the D-test routinely.

REFERENCES

1. Lim J.A., Kwon A.E., Kim S.K., Chong Lee K., Choi E.C. Prevalence of resistance to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in Korean hospital. *J Antimicrob Chemother,* 2002; 49: 489–495.
2. Lina G., Quaglia A., Reverdy, Leclercq R., Vandenesch, Etienne Distributon of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. *Antimicrob Agents Chemother,* 1999; 43: 1062–1066.
3. Drinkovic D., Fuller E.R., Shore K.P., Holland D.J., Ellis Pegler Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. *J Antimicrob Chemother,* 2001; 48: 315–316.
4. Steward Christine D., Raney Patti M., Morell Allison. Testing for induction of clindamycin resistance in erythromycin resistant isolates of *Staphylococcus aureus*. *J Clin Microbiol,* 2005; 43: 1716–1721.
5. Patel Mukesh, Waites Ken B., Moser Stephen A., Cloud Gretchen, Hoesley Craig. Prevalence of inducible clindamycin resistance among community and hospital associated *Staphylococcus aureus* isolates. *J Clin Microbiol,* 2006; 44: 2481–2484.
6. Delialioglu Nuran, Aslan Gonul, Ozturk Candan, Baki Vildan, Sen Sebahat. Inducible clindamycin resistance in staphylococci isolated from clinical samples. *J Infect Dis,* 2005; 58: 104–106.
7. Leclercq Roland. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin Infect Dis,* 2002; 34: 482–492.
8. Fiebelkorn K.R., Crawford S.A., McElmeel M.L., Jorgensen J.H. Practical disc diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. *J Clin Microbiol,* 2003; 41: 4740–4744.
9. Hamilton-Miller J.M.T., Shah Patterns of phenotypic resistance to the macrolide-lincosamide-ketolide- streptogramin group of antibiotics in staphylococci. *J Antimicrob Chemother,* 2000; 46: 941–949.
10. Schreckenberger Paul, Ilendo Elizabeth, Ristow Kathryn. Incidence of constitutive and inducible clindamycinresistance in *Staphylococcus aureus* and coagulase – negative staphylococci in a community and a tertiary care hospital. *J.Clin Microbiol,* 2004; 42: 2777–2779.
11. Yilmaz Gurdal, Aydin Kemalettin. Detection and prevalence of inducible clindamycin resistance in staphylococci. *J Med Microbiol,* 2007; 56: 342–345.
12. Gadepalli Ravisekhar, Dhawan Benu, Mohanty Srujana, Kapil Arti. Inducible clindamycin resistance in clinical isolates of *Staphylococcus aureus*. *Indian J Med Res,* 2006; 123: 571–573.
13. Sanchez, Flint, Jones Occurrence of macrolide-lincosamide-streptogramin resistance 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. *Diagn Microbiol Infect Dis,* 1993; 16: 205–213.
14. Goyal R., Singh N.P., Manchanda V., Mathur M. Detection of clindamycin susceptibility in macrolide resistant phenotypes of *Staphylococcus aureus*. *Indian J Med Microbiol,* 2004; 22: 251–254.
15. Lewis J.S., Jorgensen J.H. Inducible clindamycin resistance in staphylococci: should clinicians and microbiologists be concerned? *Clin Infect Dis,* 2005; 40: 280–285.
16. Rao G.G. Should clindamycin be used in treatment of patients with infections caused by erythromycin resistant staphylococci? *J Antimicrob Chemother,* 2000; 45: 715.
17. Levin T.P. Potential clindamycin resistance in clindamycin susceptible, erythromycin resistant *Staphylococcus aureus*: report a clinical failure.

Antimicrob Agents Chemother, 2005; 49: 1222–1224.

18. Siberry G.K., 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: 1257–1260.