International Journal of Health Sciences and Research
Vol.10; Issue: 6; June 2020
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

Detection of Inducible Clindamycin Resistance (iMLS$_B$) among the Erythromycin Resistant CONS Isolates in a Rural Tertiary Care Hospital- Need of Time

Abhishek Debnath[1], Reshmi Ghosh[2], Dipayan Ghosh[3]

[1]Dept. of Microbiology, Jawaharlal Nehru Medical College, Wardha, Maharashtra
[2]Research Scholar, Tripura University, Dhaleswar, Agartala, Tripura
[3]State Mission Manager, Tripura Rural Livelihood Mission, A.D.Nagar, S.D.Mission, Agartala, Tripura

Corresponding Author: Abhishek Debnath

ABSTRACT

Introduction: Therapeutic failure of clindamycin has been reported due to various mechanisms that confer resistance to macrolide, lincosamide and streptogramin antibiotics. Because of that, present study was conducted with following aims and objectives.
Aims and Objectives: To detect inducible clindamycin resistance among the erythromycin resistant CONS isolates and correlation with methicillin resistance.
Materials and Methods: 180 CONS strains isolated from clinically significant samples were identified by different conventional methods and Erythromycin resistance was detected by Kirby-Bauer Disk Diffusion method. Methicillin resistance and Inducible and constitutive clindamycin resistance (D test) was detected according to CLSI guidelines.
Results: Among 180 CONS isolates, predominant isolated species were S. epidermidis 75(41.67%), S.haemolyticus 47(26.11%). Out of 180 CONS isolates, 108(60%) showed erythromycin resistance, out of which, 29 (26.85%) isolates showed iMLS$_B$. Among 180 CONS isolates, 119(66.11%) were MRCONS isolates and 61(33.89%) were MSCONS isolates.
Conclusion: D test for detection of inducible clindamycin resistance should be included in the routine clinical laboratories as part of antibiotic susceptibility testing, as it will help physicians in guiding therapy.

Key words: Coagulase negative staphylococci (CONS), inducible clindamycin resistance (iMLS$_B$)

INTRODUCTION

CONS are part of the normal skin flora increasingly recognized as significant nosocomial pathogens, infection often associated with implanted devices, joint prosthesis and different indwelling devices. Although there are about 20 CONS species, they are often considered to be one group. Some species are more immune to commonly used antimicrobial agents than others. Identification to species level can aid in the recognition of outbreaks and in tracking resistance trends. [1-2]

CONS are characterized by an ability to form adhering bacterial film, the biofilm whose formation has been implicated as a factor of virulence. Biofilm is believed to make the micro-organisms more resistant to administered antibiotics and to host defense mechanisms. [3]

CONS became a significant problem as they express methicillin resistance, which involves all β-lactam antibiotics and results in a significant limitation in therapeutic options. Methicillin resistance is associated with the presence of the mecA gene which encodes a penicillin-binding protein

(PBP2a) with altered properties responsible for the observed resistance (Chambers, 1997).Incidence of methicillin resistance in CONS is high, as well as, the accompanying antimicrobial resistance. [4] Among few therapeutic alternatives available for treatment of staphylococcal infections, clindamycin is one of them. [5]

Macrolide (erythromycin), lincosamide (clindamycin) and streptogramins are referred to as the MLS group of antibiotics. Resistance to erythromycin in staphylococci including in CONS especially S.epidermidis, S. haemolyticus, S. hyicus, S. hominis. S. cohnii is usually associated with resistance to other macrolides. Three genes (ermA, ermB, and ermC) encoding methyltransferases responsible of resistance to macrolides (erythromycin), lincosamides (clindamycin) and type B streptogramins (MLSB phenotype) by modification of the ribosomal target site have been found in staphylococci including CONS. The msrA gene displays another mechanism of inducible resistance to erythromycin by encoding an ATP-dependent efflux pump. On the other hand, macrolide efflux is affected by a membrane protein encoded by the mef gene. [6]

Clindamycin has several advantages but major barrier in its usage is development of resistance especially inducible resistance with in vitro testing and in vivo during clindamycin therapy leading to therapeutic failure. Hence the prevalence of inducible resistance should be known as it varies by geographical location, bacterial species, methicillin susceptibility and even from hospital to hospital. [5]

Hence the knowledge of prevalence of antibiotic resistance especially methicillin resistance and inducible clindamycin resistance within CONS is highly desirable to prevent therapeutic failure. [5]

The health issues among the rural population in India have seeks the attention of health policy makers and researchers in the present days due to lack of awareness among the rural citizen of India. To know the inducible clindamycin resistance among the rural citizen, health experts need to perform the D test in all the people of rural area. Through these staphylococcal infections especially infections caused by CONS will reduce among the rural people of India.

Hence this study was undertaken with the following aims and objectives.

Aims and Objectives:

The present study was undertaken with the following aim and objectives:-

- To isolate and to identify the species of CONS from clinically significant samples by conventional methods.
- To detect erythromycin resistance among the isolated species of CONS using Kirby Bauer disc diffusion method.
- To detect methicillin resistance among the isolated species of CONS.
- To detect inducible or constitutive clindamycin resistance in the isolated species of CONS.
- To correlate inducible and constitutive clindamycin resistance with methicillin resistance.

MATERIALS AND METHODS

Ethics Committee Approval: The study was conducted after obtaining approval from Institutional Ethics Committee.

Locus of study: Study was carried out in department of Microbiology of Jawaharlal Nehru Medical College and Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha which is a rural tertiary care hospital.

Study design: Cross sectional study.

Study duration: The study was conducted from 29th September, 2016 to 28th September, 2018.

Sample size and source of sample: 180 CONS strains were isolated from clinically significant samples like blood, urine, indwelling catheter, pus and body fluids, received in department of Microbiology and processed according to conventional methods. Samples were inoculated on blood

agar, MacConkey agar and incubated overnight at 37^{0}C.

CONS isolates from blood cultures should be correlated clinically and should be always interpreted with paired blood samples from two peripheral veins. [7] For other samples, to interpret CONS as pathogenic organism, repeated isolation of CONS in two consecutive samples is necessary. [8]

CONS isolates from different clinically significant samples were initially identified by colony morphology, gram staining, catalase and coagulase test (slide and tube method). [9] To exclude Micrococcus and Stomatococcus, Bacitracin (0.04 u) and Furazolidone (100ug) sensitivity were done. [9]

Species identification of CONS was done by battery of biochemical tests such as ornithine decarboxylase test, sugar (mannitol, trehalose, mannose, xylose) fermentation test, phosphatase production, urease activity, nitrate reduction test, pyrrolidonyl arylamidase (PYR) test, acetoin production, novobiocin and polymyxin B (50 unit) sensitivity test etc, according to standard procedure. [10]

Erythromycin resistance was detected by Kirby Bauer Disk Diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines. [11]

Lawn culture was done on Muller Hinton agar plate with broth culture of coagulase-negative Staphylococci strains (turbidity adjusted to 0.5 McFarland standards). [11]

Following antibiotics disc were put on Muller Hinton agar plate like Erythromycin (15µg), Clindamycin (2µg), Cefoxitin (30µg). Plates were incubated at 37^0 C for 16 -18 hours. Next day susceptibility profile of CONS to different antibiotics was noted according to CLSI guidelines. [11]

Methicillin resistance was detected according to CLSI guidelines by using cefoxitin (30 µg) disc [zone of inhibition ≤ 24 mm (resistant-mec A positive) and ≥ 25 mm (sensitive-mec A negative). [11]

Inducible and constitutive clindamycin resistance in erythromycin resistant (zone size ≤13mm) CONS was detected by D test according to CLSI guidelines. [11] In this test, erythromycin (15µg disc) and clindamycin (2 µg disc) were placed at a distance of 15 mm edge to edge on a Muller Hinton agar plate already inoculated with test strain(turbidity adjusted to 0.5 McFarland standard) and incubated over night at 37^0 C. D test results were interpreted as per CLSI guidelines. [11]

MS Phenotype: CONS isolates exhibiting resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin (zone size ≥ 21mm) and giving circular zone of inhibition around clindamycin was labelled as having MS phenotype. [11]

Inducible MLS (iMLS$_B$) Phenotype: CONS isolates showing resistance to erythromycin (zone size ≤ 13mm) while being sensitive to clindamycin (zone size ≥ 21mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having inducible clindamycin resistance phenotype. [11]

Constitutive MLS (cMLS$_B$) Phenotype: This phenotype was labelled for those CONS isolates which showed resistance to both erythromycin (zone size ≤13mm) and clindamycin (zone size ≤14mm) with circular shape of zone of inhibition if any around clindamycin. [11]

RESULTS

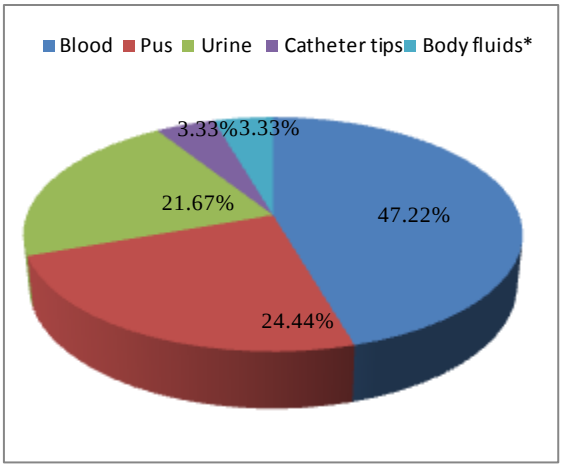

Figure 1. Sample wise distribution of CONS (n=180).

Body fluids include [CSF (n=1), Ascitic fluid (n=2), Pleural fluid (n=3)].

Figure 1 shows sample wise distribution of CONS isolates from clinically significant samples. Among 180 CONS isolates, 85(47.22%) isolates were from blood samples, 44(24.44%) isolates from pus samples, 39(21.67%) isolates from urine samples, 6(3.33%) isolates from catheter tip samples and 6(3.33%) isolates from body fluids respectively.

Table 1. Species distribution of CONS isolates (n=180).

Species	No of CONS isolates (n=180)
S.epidermidis	75(41.67%)
S.haemolyticus	47(26.11%)
S. schleiferi	20(11.11%)
S.lugdunensis	18(10%)
S.saprophyticus	11(6.11%)
S.xylosus	4(2.22%)
S.intermedius	3(1.67%)
S.warneri	1(0.55%)
S. hominis	1(0.55%)

Table 1 shows species distribution of CONS isolates. Among 180 CONS isolates, predominant isolated species were S. epidermidis 75(41.67%), S.haemolyticus 47(26.11%), S. schleiferi 20(11.11%) and S.lugdunensis 18(10%). Least commonly isolated CONS species were S.saprophyticus 11(6.11%), S.xylosus 4(2.22%), S.intermedius 3(1.67%), S.warneri 1(0.55%) and S. hominis 1 (0.55%).

Table 2. Erythromycin resistant CONS and MLS$_B$ phenotypes (n=180).

Erythromycin resistant (n=108)	iMLS$_B$ (n=29)	Constitutive MLS$_B$ (n=51)	MS Phenotype (n=28)
60%	26.85%	47.22%	25.92%

Abbreviations: iMLS$_B$- Inducible clindamycin resistance, Constitutive MLS$_B$- Constitutive clindamycin resistance.
iMLS$_B$ - Erythromycin-R, Clindamycin-S, D test-Positive
Constitutive MLS$_B$ - Erythromycin-R, Clindamycin-R
MS Phenotype - Erythromycin-R, Clindamycin-S, D test- Negative

Table 2 shows distribution of MLS$_B$ phenotypes among erythromycin resistant CONS isolates. Out of 180 CONS isolates, 108(60%) showed erythromycin resistance, out of which, 29 (26.85%) isolates showed iMLS$_B$, 51(47.22%) isolates showed Constitutive MLS$_B$ and 28(25.92%) isolates showed MS Phenotype.

Table 3. Distribution of MRCONS and MSCONS among CONS isolates (n=180)

	CONS (n=180)	MRCONS (n=119)	MSCONS (n=61)
Percentages	180(100%)	119(66.11%)	61(33.89%)

Table 3 shows distribution of MRCONS and MSCONS isolates among CONS isolates. Among 180 CONS isolates, 119(66.11%) were MRCONS isolates and 61(33.89%) were MSCONS isolates.

Table 4. Correlation of MRCONS and MSCONS with MLS$_B$ phenotypes.

CONS (n-180)	iMLS$_B$	Constitutive MLS$_B$	MS Phenotype
MRCONS(n=119)	23(19.33%)	44(36.97%)	13(10.92%)
MSCONS (n=61)	6(9.83%)	7(11.47%)	15(24.59%)
Total (n=180)	29	51	28

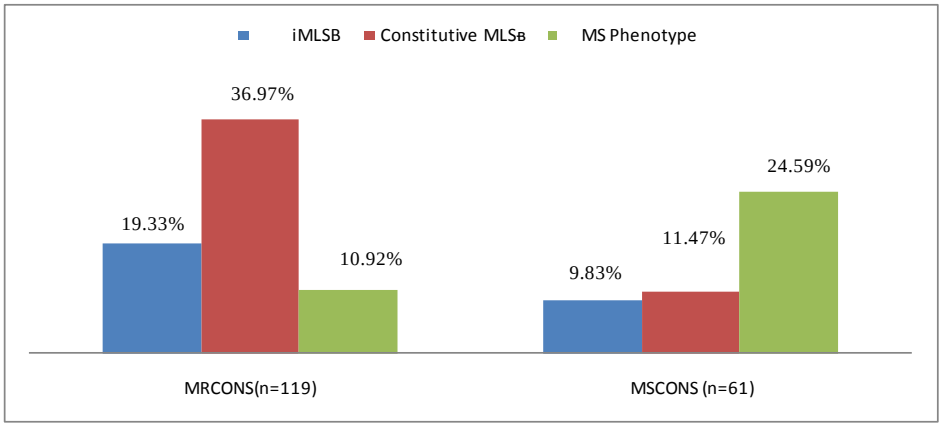

Figure 2. Correlation of MRCONS and MSCONS with MLS$_B$ phenotypes.

Table 4 shows correlation of MRCONS and MSCONS with MLS$_B$ phenotypes. Out of 180 CONS isolates, 119 isolates were MRCONS and 61 isolates were MSCONS. Out of 119 MRCONS isolates, 23(19.33%) isolates showed iMLS$_B$ phenotype, 44(36.97%) isolates showed constitutive MLSв phenotype and 13(10.92%) isolates were having MS Phenotype. Out of 61 MSCONS isolates, 6(9.83%) isolates showed iMLS$_B$ phenotype, 7(11.47%) isolates showed constitutive MLSв phenotype and 15(24.59%) isolates showed MS Phenotype.

So from the table, it was observed that iMLS$_B$ phenotype was more among MRCONS as compared to MSCONS.

DISCUSSION

The increasing incident and frequency of CONS and increasing problem of methicillin resistance among staphylococci have led to renewed our interest to treat such infections by using clindamycin therapy and for treatment of such kind of infections, Clindamycin is a very good alternative to vancomycin.' It's tolerability, cost, good absorption and easy tissue penetration which makes it an essential and extraordinary good option for treatment of patient. [12] But, in clindamycin treatment one important issue is failure during therapy which is mainly caused by inducible resistance phenotypes. Without relevant antibiotic susceptibility testing, a therapeutic decision is not possible and this is the place where the D-test becomes essential and significant. [5]

In the present study, 47.22% CONS isolates were from blood samples, 24.24 % isolates were from pus samples, 21.67% isolates were from urine samples and 3.33% isolates were from catheter tip samples. This study correlates with the study done by Sadhvi Parashar et al. [13] where 45.95% CONS isolates were from blood samples, 15.6% isolates were from pus samples and 19.46% isolates were from urine samples.

In the present study, predominant isolated species were S. epidermidis (41.67%), S.haemolyticus (26.11%), S. schleiferi (11.11%) and S.lugdunensis (10%). This is in accordance to study done by Badampudi et al. [14] where predominant isolated species were S. epidermidis (40%), S. haemolyticus (26%) and S. schleiferi (13%).

In present study, among 180 CONS isolates, 108(60%) showed resistance to erythromycin, out of these, percentage of inducible clindamycin resistance (iMLS$_B$), constitutive clindamycin resistance (constitutive MLSв) and MS phenotype were found to be 26.85%, 47.22% and 25.92 % respectively. These findings correlates with a study done by Bansal et al. [5] where 18%, 26% and 22% of CONS isolates were iMLS$_B$, constitutive MLSв and MS phenotype respectively.

In present study, among 119 MRCONS isolates, 19.33%, 36.97% and 10.92% isolates showed iMLS$_B$, constitutive MLSB resistance and the MS phenotype respectively. These findings correlates with a study done by Bansal et al. [5] where 25.8%, 51.7% and 12.4% of MRCONS isolates showed iMLSB, constitutive MLSв and MS phenotype respectively. In the present study, among 61 MSCONS isolates, 9.83%, 11.47% and 24.59% isolates were iMLS$_B$, constitutive MLSB and the MS phenotype respectively. These findings correlate with a study done by Bansal et al. [5] where 13.7%, 11.8% and 27.3% of MSCONS isolates were iMLS$_B$, constitutive MLSв and MS phenotype respectively.

Inducible clindamycin resistance was significantly higher in MRCONS isolates as compared to MSCONS. This finding correlates with a study done by Bansal et al. [5] where he also found higher incidence of inducible clindamycin resistance in MRCONS.

Another study done by Lim et al. [15] reported higher rate of inducible and constitutive clindamycin resistance in MRCONS isolates compared to MSCONS isolates where 30% and 33% of MRCONS isolates were iMLS$_B$ and constitutive MLSв respectively and 21% and 9% of MSCONS

isolates were iMLS$_B$ and constitutive MLS$_B$ respectively.

Different variations in the prevalence of iMLS$_B$, constitutive MLS$_B$ and MS phenotype is seen which could be explained by differences in antibiotic susceptibility pattern in various geographical areas and varying drug subscription habit of physicians. [16]

CONCLUSION

For detection of inducible and constitutive clindamycin resistance in CONS, D test is simple cost effective, reliable and has high sensitivities and specificities and can be introduced in day to day practice. Hence implementation of D test routinely in all the hospital clinical microbiological laboratories should have been considered by all hospital authorities for reporting inducible and constitutive clindamycin resistance, followed by prevents therapeutic failure of clindamycin which will help physicians to treat infections caused by staphylococcal isolates especially CONS.

REFERENCES

1. Davenport DS, Massanari RM, Pfaller MA, et al. Usefulness of a test for slime production as a marker for clinically significant infections with coagulase-negative staphylococci. J Infect Dis. 1986; 153: 332 – 339.
2. Christensen GD, Simpson WA, Bisno AL, et al: Adherence of slime-producing strains of Staphylococcus epidermidis to smooth surfaces. Infect Immune 1982; 37: 318 – 326.
3. Go¨ tz, F. (2002). Staphylococcus and biofilms. Mol Microbiol 43, 1367–1378.
4. Lucía E. Alcaráz, Sara E. Satorres, Rodolfo M. Lucero, Olga N. Puig de Centorbi. Species identification, slime production and oxacillin susceptibility in coagulase-negative staphylococci isolated from nosocomial specimens. Braz. J. Microbiol. 2003; 34(1):45-51.
5. Bansal N, Chaudhary U, Gupta V. Prevalence of inducible clindamycin resistance in clinical isolates of coagulase negative staphylococci at a tertiary care hospital. Ann Trop Med public health 2012; 5(5): 427-30.
6. M T Parker & BI Duerden. Topley & Wilson. Principles of Bacteriology, Virology and Immunity. 8thed. London, UK: Edward Arnold by Butler and Tanner; 1990: 162-181.
7. Maj Puneet Bhatt, Capt Kundan Tandel , Maj Alina Singh , M. Mugunthan , Col Naveen Grover , Brig A.K. Sahni . Species distribution and antimicrobial resistance pattern of Coagulase-negative Staphylococci at a tertiary care centre. Medical Journal Armed Forces India 72 (2016) 71–74.
8. Washington CW Jr, Stephen DA, William MJ et al. Koneman"s color Atlas and Textbook of diagnostic Microbiology, in Gram positive cocci.Ch 12; 6th ed, Lippincott Williams and Williams, USA, 2006; 661-62.
9. Baird.D. - Chapter -11 Staphylococcus-cluster forming gram positive cocci in: Colle. J. G., Fraser A.G., Marmion B.P., Simmons A. editors Mackie &McCartney practical Medical Microbiology 14th ed. Edinburg: Churchill Livingstone; 245-261, 1996.
10. Washington CW Jr, Stephen DA, William MJ et al. Koneman"s color Atlas and Textbook of diagnostic Microbiology, in Gram positive cocci.Ch 12; 6th ed, Lippincott Williams and Williams, USA, 2006; 623-71.
11. Clinical and Laboratory Standards Institute .Performance standards for antimicrobial susceptibility testing; Twenty second informational supplement. Wayne, PA: CLSI; 2012: CLSI document M100-S22, 32(3).
12. Yilmaz G, Aydin K, Iskender S, Calyan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. J med Microbiol. 2007; 56: 342-45.
13. Sadhvi Parashar . Significance of Coagulase Negative Staphylococci with Special Reference to Species Differentiation and Antibiogram. Indian Medical Gazette - July 2014.
14. Vijayasri Badampudi, S.S, Surya Kirani K.R.L, Gunti R. Speciation and biofilm production of coagulase negative Staphylococcal isolates from clinically

significant specimens and their antibiogram. JKIMSU. 2016; 5(2): 69-78.
15. Hwan Sub Lim, Hyukmin Lee,Kyoung Ho Roh, Jong Hwa Yum, Dongeun Yong, Kyungwon Lee, and Yunsop Chong. Prevalence of Inducible Clindamycin Resistance in Staphylococcal Isolates at a Korean Tertiary Care Hospital. Yonsei Med J Vol. 47, No. 4, 2006.
16. Rodrigues Perez LR, Caierao J, Souza Antunes AL, Alves d'Azevedo P. Use of D test method to detect inducible clindamycin resistance in coagulase negative staphylococci (CONS). Braz J Infect Dis. 2007; 11:186-88.

How to cite this article: Debnath A, Ghosh R, Ghosh D. Detection of inducible clindamycin resistance (iMLS$_B$) among the Erythromycin resistant CONS isolates in a rural tertiary care hospital- need of time. Int J Health Sci Res. 2020; 10(6):12-18.

******