Prabhu and Chinniah
Brunei International Medical Journal
2025; 21:218-223

Brunei International Medical Journal

Original Article

Open Access

Inducible Macrolide-lincosamide-Streptogramin B (iMLS B) resistance among Streptococci

Kavitha **PRABHU**[1,2] and Terrence Rohan **CHINNIAH**[2*]

Abstract

Introduction: Macrolide and lincosamide resistance among beta-haemolytic streptococci (BHS) and other *Streptococcus* isolates has rarely been described. However, such data are critical to detect and track the emergence of antibiotic resistance. **Materials and Methods**: During the study period of 8 months, all beta-haemolytic streptococci, *Streptococcus pneumoniae* and Viridans streptococci isolated from clinical specimens were included in the study. D zone test was performed by incorporating it in the routine antibiotic susceptibility test for all streptococci isolates by keeping the erythromycin (15 µg) and clindamycin (2 µg) disk 12mm distance according to Clinical Laboratory Standard Institute (CLSI) guidelines. Flattening of the zone of inhibition around clindamycin, adjacent to the erythromycin disk (referred as D zone) is interpreted as D test positive indicating presence of inducible clindamycin resistance (ICR). **Results**: Out of 926 Streptococcal isolates, there were 63 erythromycins resistant and 55 clindamycin resistant isolates were found. Erythromycin resistance was observed in 7.2% (n=4/55) of group A Streptococci, 5.5% (n=42/756) group B Streptococci, 8.7% (n=2/23) of *S. pneumoniae* and 18.5% (n=15/81) of *S. viridans*. There was no erythromycin resistant in group C and group G streptococci isolates. Constitutive clindamycin resistance was found in all four-erythromycin resistant groups; group A (7.2%), 18/756 (2.3%) group B Streptococci, 2/23 (8.69%) *S. pneumoniae*, 5/81 (6.17%) *S. viridans*. Inducible clindamycin resistance was seen in 24/756 (3.7%) of group B Streptococci, 2/81 (2.4%) of Viridians streptococci. **Conclusions**: This study shows that macrolides or lincosamide cannot be used empirically for any streptococci without doing anti-microbial susceptibility and emphasizes the need to incorporate D zone testing in the standard anti-microbial susceptibility tests for streptococci.

Keywords: beta-hemolytic *streptococci*, *S. pneumoniae*, inducible clindamycin resistance, D test, Erythromycin

Author Details:
1 Department of Microbiology, Father Muller Medical College, Mangalore. India
2 Department of Microbiology, Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital, Bandar Seri Begawan, Brunei Darussalam

Correspondence:
Dr Terence Rohan
Chinniahtrctrc64@gmail.com

INTRODUCTION

Penicillin is the recommended first line of treatment for beta haemolytic streptococcal infections. However, macrolides and sometimes lincosamides are recommended as second line options for patients who are allergic to penicillin.[1,2] Development of penicillin resistance in pneumococcus in the 1980s–1990s shifted antibiotic treatment of suspected pneumococcal upper respiratory infections and pneumonia to macrolides. Widespread macrolide use, however, is associated with increased macrolide resistance in *Streptococcus (S) pneumoniae* and clinical failures of macrolide treatment of pneumococcal infections have been reported for lower respiratory tract infections and bacteremia.[3]

The macrolide antibiotics (erythromycin, azithromycin, clarithromycin, and others) and the lincosamide antibiotics (lincomycin and clindamycin) are chemically unrelated but possess many similar biologic properties in terms of mechanisms of action and resistance, antimicrobial activity, and clinical pharmacology.[2]

Erythromycin inhibits RNA-dependent protein synthesis at the step of chain elongation in susceptible prokaryotic organisms. Alteration in the 23S ribosomal RNA of the large 50S ribosomal subunit by demethylation of adenine, is associated with resistance to erythromycin and most other macrolides (M), and sometimes to the lincosamides (L), lincomycin, and clindamycin), and streptogramin type B (S_B) antibiotics. This pattern of resistance, referred to as the MLS_B phenotype, is mediated by the *erm* (erythromycin ribosome methylation) genes on plasmids or transposons on chromosomes, which are self-transferable. This MLS_B resistance phenotype may be constitutive or inducible by subinhibitory concentrations of erythromycin or other macrolides that bring about induction of the methylating enzyme.[2,4]

A different efflux system for erythromycin and some other macrolides (but not for lincosamides, or analogues of streptogramin B), called the M phenotype, has been elucidated in erythromycin-resistant strains of *Streptococcus pyogenes*, *Streptococcus pneumoniae,* group C streptococci. That efflux system is encoded by the *mef* (A) gene, is carried on a transposable element, and this resistance does not extend to clindamycin if resistance is due to the M phenotype.[2]

The incidence of macrolide and clindamycin resistance including inducible clindamycin resistance is highly variable from region to region and from country to country.[5-8] Hence, local statistics on this crucial value are important for empiric therapy. Clinical failure of clindamycin therapy has been reported due to inducible Macrolide Lincosamide Streptogramin B (iMLSB) phenotype resistance which cannot be detected by routine in-vitro disk diffusion antibiotic susceptibility testing. Hence, this study was undertaken to estimate the macrolide resistance, clindamycin resistance and inducible clindamycin resistance among Streptococci.

MATERIALS AND METHODS

This prospective study was conducted at the microbiology laboratory at the Department of Microbiology, RIPAS Hospital, Brunei Darussalam, for the duration of eight months. All the streptococci isolated from various clinical specimens were identified by Gram staining, colony morphology, catalase test, commercial latex agglutination kit (Patho DX Strep Remel Inc., USA) and VITEK 2 / VITEK MS (*bioMérieux*) then subjected to routine antibiotic susceptibility testing by modified Kirby Bauer's disk diffusion method on Muller Hinton agar with 5% sheep blood agar plates and interpreted as per Clinical Laboratory Standard Institute (CLSI) guidelines.[9]

D zone test was performed by incorporating it in the routine antibiotic susceptibility test for all streptococci isolates by keeping the erythromycin (15 µg) and clindamycin (2 µg) disk 12 mm distance according to Clinical Laboratory Standard Institute (CLSI) guidelines.[9] Following overnight incubation at 37°C, circular zone of inhibition with a zone size ≥ 19mm around the clindamycin with flattening on the side facing erythromycin disk (D zone), indicated inducible clindamycin resistance (**Figure 1**).

Plates were read and phenotypes were recognised as follows.

1. Constitutive MLSB phenotype – Streptococcal isolates with erythromycin (zone size ≤ 15mm) and

Figure 1: a) Group B Streptococcus isolate showing inducible clindamycin resistance, and b) illustration demonstrating a positive test (E: Erythromycin disc, and C: Clindamycin disk). Black arrow indicate the D zone with flattening.

clindamycin (zone size ≤ 15mm) with circular shape of zone of inhibition around clindamycin.

2. Inducible MLSB (iMLSB) phenotype – Streptococcal isolates showing resistance to erythromycin (zone size ≤ 15mm) while being sensitive to clindamycin (zone size ≥ 19mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disk.

3. M phenotype - Streptococcal isolate exhibiting resistance to erythromycin (zone size ≤ 15mm) while sensitive to clindamycin (zone size ≥ 19mm) and giving circular zone of inhibition around clindamycin disk.[4,10]

Quality control (QC) of the erythromycin and clindamycin discs was performed in triplicates using *S. pneumoniae* ATCC® 49619, according to the standard disc diffusion QC procedure.[9] Additional QC was performed with separate in-house selected *S. agalactiae* strains that demonstrated positive and negative D test reactions. Quality control of commercially obtained media used in the study was done with each shipment/ batch of media. [11] Results were tabulated and analysed statistically.

RESULTS

During this study, a total of 930 Streptococci were isolated from various clinical samples (**Table I**). Among these, 55 were group A beta haemolytic streptococci, 756 group B Streptococci, 10 group C Streptococci, 5 Group G Streptococci, 23 *S. pneumoniae* and 81 Viridans Streptococci.

Altogether, there were 63 erythromycins resistant and 55 clindamycin resistant Streptococcal isolates. Erythromycin resistance was found in 4/55 (7.2%) group A Streptococci, 42/756 (5.5%) group B Streptococci, 2/23 (8.7%) *S. pneumoniae* and 15/81 (18.5%)

Table I: Distribution of type of samples from which streptococci were isolated.

	Group A	Group B	Group C	Group G	*S. pneumoniae*	*S. viridans*
Blood	3	9	-	2	8	32
Pus	24	46	5	-	5	23
Eye	1	3	1	-	-	11
Ear	5	6	1	-	6	-
Throat	17	-	-	2	-	-
Sputum/ETA	2	3	-	-	3	-
CSF	-	-	-	-	1	-
Body fluid	-	2	-	-	-	3
HVS	-	646	-	-	-	-
Urine	-	27	-	-	-	-
Umbilical Cord	3	2	-	-	-	-
Others	-	14	-	-	-	12
Total (N=926)	55	756	7	4	23	81

ETA: endotracheal aspirate CSF: cerebrospinal fluid HVS: high vaginal swab

Table II: Erythromycin and clindamycin resistance phenotypes among clinical isolates of Streptococci.

	Total (n)	Erythro R n (% of total)	cMLSB n (% of total)	iMLSB n (% of total)	M phenotype n (% of total)
Group A	55	4 (7.2%)	4 (7.2%)	0	0
Group B	756	42 (5.5%)	18 (2.3%)	24 (3.7%)	1 (0.1%)
Group C	07	0	0	0	0
Group G	04	0	0	0	0
S. pneumoniae	23	2 (8.7%)	2 (8.%)	0	1 (4.3%)
S. viridans	81	15 (18.5%)	5 (6.17%)	2 (2.4%)	6 (7.4%)
Total	926	63 (6.8%)	29 (3.1%)	26 (2.8%)	8 (0.9%)

S.viridans. There were no erythromycin resistant group C and group G streptococci isolated during our study period. Constitutive clindamycin resistance was found in all four-erythromycin resistant group A (4/55, 7.2%), 18/756 (2.3%) group B Streptococci, 2/23 (8.69%) *S.pneumoniae* and 5/81(6.17%) of *S. viridans*. Inducible clindamycin resistance was seen in 24/756 (3.7%) group B Streptococci, 2/81 (2.4%) Viridians streptococci. There was one group B *Streptococcus* isolate which was intermediate to clindamycin but sensitive to erythromycin. (**Table II**).

DISCUSSION

Clindamycin is an excellent drug for Streptococcal infections, particularly skin and soft tissue infections.[1,2] Macrolide and clindamycin resistance has been rising in streptococci, but data is limited. Inducible clindamycin resistance can develop during the treatment, which can be detected by performing D zone test, which should be done before reporting clindamycin susceptibility. Since the iMLSB resistance mechanism is not recognised by standard antibiotic susceptibility test methods and its prevalence varies according to geographical location, D zone test described in CLSI guidelines has become an imperative part of routine antimicrobial susceptibility test for all clinical isolates of Streptococci and should be included in the routine antimicrobial susceptibility method.

In this study we found 6.8% (n=63) erythromycin resistant Streptococci over the nine months study period. Among these, 29 (3.13%) isolates were clindamycin resistant constitutively and 26 (2.8%) were inducible clindamycin resistance phenotype.

A study from Canada, showed 8% erythromycin resistant and 1% clindamycin resistant Streptococcus Group A isolates including 0.002% (of total isolates) inducible type.[12] In the same study, they observed 17% and 8% erythromycin and clindamycin resistance in group B streptococci, respectively and 6.5% of their group B streptococcal isolates were inducible resistant to clindamycin. Compared to this study, our study showed 7.2% erythromycin resistant group A streptococcal isolates and 7.2% constitutively clindamycin resistant isolates. We did not isolate any inducible clindamycin resistant group A streptococcus isolate in our study.

Among group B Streptococcal isolates, we found 5.5% erythromycin resistant, 2.3% constitutive clindamycin resistant and 3.7% inducible clindamycin resistant isolates. Contrary to what we found, rates of erythromycin resistance and clindamycin resistance were up to 44% and 39%, have been reported amongst clinical group B streptococcal isolates from Taiwan.[7] A study from Malaysia, showed that 22% of Streptococcus Group B were erythromycin resistant and 12% were clindamycin resistant.[13] A Korean study of macrolide resistance amongst beta hemolytic streptococcal isolates from a tertiary hospital also reported a high rate of 37% resistance of group B streptococci to erythromycin, but a reduced rate of 5% resistance of group A streptococcus to erythromycin, and no clindamycin resistance. [8] We also found one group B *Streptococcus* isolate which was sensitive to erythromycin but intermediate to clindamycin, this could be mediated through the presence of Lin B gene.[2] **Table III** shows the comparison of erythromycin and clindamycin resistance among group B isolates, in various studies. Though our study showed comparatively less incidence of erythromycin resistance, all our Streptococcus Group B isolates which were resistant to erythromycin were also resistant to clindamycin either constitutively or inducible.

Prabhu *et al*. from India, found erythromycin

Table III: Comparison of erythromycin and clindamycin resistance in Group B streptococci from various studies.

Authors (years)	Place of study	Erythromycin Resistance (%)	Clindamycin Resistance (%)
Janapatla *et al* (2008) [7]	Taiwan	44	39
Uh *et al* (2007) [8]	Korea	37	0
Desjardins *et al* (2004) [12]	Canada	17	8
Suhaimi *et al* (2017) [13]	Malaysia	22	12
Present study	Brunei	5.5	5.5

resistance in group C streptococci and group G streptococci isolates of 9.4 and 3.1% respectively. Contrary to this, our study there were no Group C and G isolates resistant to erythromycin or clindamycin, but our sample size was small. A study by Megged *et al*, also showed no erythromycin resistance in group C Streptococci.[14] In the same study, erythromycin resistance was found in 19.4% of pneumococcal isolates, 11.9% were clindamycin resistant constitutively and 1.5% were inducible clindamycin resistant.[14] In comparison, our study, showed 8.69% of the pneumococcal isolates resistant to erythromycin and 2% isolates resistant to clindamycin constitutively. Additionally, our study showed 18.5% of Viridians streptococci resistant to erythromycin compared to the study by Ergin *et al.* where it was as high as 27%.[15]

Macrolide and lincosamide resistance are increasing in streptococci including *S. pneumoniae*, and it varies from one geographical area to another. Macrolides or lincosamides cannot be used empirically for any streptococci without doing antimicrobial susceptibility. Reporting Streptococci as susceptible to clindamycin without checking for inducible resistance may result in institution of inappropriate clindamycin therapy. On the other hand, negative results for inducible clindamycin resistance confirm clindamycin susceptibility and provide a very good therapeutic option specially for beta hemolytic streptococci. An accurate susceptibility report is paramount to avoid therapeutic failure due to inducible clindamycin resistance. This emphasizes the need to incorporate D zone testing in the standard antimicrobial susceptibility tests for streptococci. It is a simple, auxiliary, and reliable method to detect inducible clindamycin resistance in routine clinical laboratories without any additional cost or human resources but contributing much to therapeutic management.

CONCLUSION

The prevalence of macrolide and clindamycin resistance differs among β-haemolytic streptococci, Viridans streptococci and *S. pneumoniae*. Altogether, 6.8% of streptococci were erythromycin resistant and 5.93 % were clindamycin resistant. Inducible clindamycin resistance contributed for clindamycin resistance in 2.8% of total isolates. This study shows that macrolides or lincosamides cannot be used empirically for any streptococci without doing antimicrobial susceptibility and emphasizes on need to incorporate D zone testing in the standard antimicrobial susceptibility tests for streptococci. Though we have observed relatively low rate of erythromycin and clindamycin resistance in our study, constant monitoring is required in view of global spread of resistant strains.

Take Home Message

- The prevalence of macrolide and clindamycin resistance differs among various streptococci.
- Group A β-haemolytic streptococci which were resistant to erythromycin were also resistant to clindamycin constitutively.
- Group B β-haemolytic streptococci isolates which were resistant to erythromycin were also resistant to clindamycin either constitutively or inducible.
- Antimicrobial susceptibility test for erythromycin and clindamycin should be performed on all streptococci.
- D test must be incorporated in the antibiotic susceptibility testing of any streptococci to rule out inducible clindamycin resistance.

Declarations

Ethical Declaration

This was a retrospective study which was conducted following the standard as set in the Declaration of Helsinki.

Disclosure and Conflict of Interest

The authors declare that they have no conflicts of interest and no financial disclosures relevant to this case report.

Acknowledgments

The authors would like to acknowledge the laboratory staff, Clinical Microbiology of RIPAS Hospital for doing the technical work.

References

1. Stevens DL, Bisno AL, Chambers HF, E. Dellinger EP, Goldstein EJ et al. Practice guidelines for the Diagnosis and Management of Skin and Soft Tissue Infections: 2014 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2014:59: e10–e52.
2. Sivapalasingam S, Steigbigel NH. Macrolides, Clindamycin, and Ketolides. In Mandell, Douglas, and Bennett's Principles and Practice of Infectious diseases 8th ed. Elsevier Churchill Livingstone 2015: 29:358-76.
3. Schroeder MR, Stephens DS. Macrolide resistance in Streptococcus pneumoniae. Front. Cell. Infect. Microbiol. 2016; 6:98.
4. Leclercq R: Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin Infect Dis, 2002;34:482-92.
5. Leclercq R, Courvalin P. Resistance to macrolides and related antibiotics in Streptococcus pneumoniae. Antimicrob Agents Chemother. 2002;45:2727-34.
6. Prabu D, Menon T. Genotyping of erythromycin resistant group C & G streptococci isolated in Chennai, south India. Indian J Med Res. 2013;137:164–8.
7. Janapatla RP, Ho YR, Yan JJ, Wu HM, Wu JJ. The prevalence of erythromycin resistance in group B streptococcal isolates at a University Hospital in Taiwan. Microb Drug Resist. 2008;14:293–7.
8. Uh Y, Hwang GY, Jang IH, Kwon O, Kim HY, Yoon KJ. Antimicrobial susceptibility patterns and macrolide resistance genes of beta-hemolytic group streptococci in a tertiary Korean hospital. J Korean Med Sci. 2007;22:791–4.
9. Clinical laboratory standard institute Performance standards for antimicrobial susceptibility testing; twenty second informational supplement 2016.M100-S28;32:67.
10. Mei L. Castor, Cynthia G. Whitney, Kathryn Como-Sabetti. Antibiotic Resistance Patterns in Invasive Group B Streptococcal Isolate. Infect Dis Obstet Gynecol. 2008; Article ID 727505, 5 pages.
11. Clinical Laboratory Standard Institute (CLSI) 2012. Quality control for commercially prepared microbiological culture media, approved standard third edition. 2012; 24:13-21.
12. Desjardins M, Delgaty KL, Ramotar K, Seetaram C, Toye B. Prevalence and Mechanisms of Erythromycin Resistance in Group A and Group B Streptococcus: Implications for Reporting Susceptibility Results. J Clin Microbiol 2004; 42:5620-3.
13. Suhaimi MES, Desa MNM, Eskandarian N, Pillay SG, Ismail Z, Neela VK, Masri SN, Nordin SA. Characterization of a Group B Streptococcus infection based on the demographics, serotypes, antimicrobial susceptibility and genotypes of selected isolates from sterile and non-sterile isolation sites in three major hospitals in Malaysia. J Infect Public Health. 2017;10:14-21.
14. Megged O, Assous M, Weinberg G, Schesinger Y. Inducible clindamycin resistance in beta hemolytic Streptococci and Streptococcus pneumoniae. IMAJ 2013;15
15. Ergin A, Ercis S, Hascelik G Macrolide resistance mechanisms and invitro susceptibility patterns of viridians group streptococci isolated from blood cultures. J. Antimicrob. Chemother 2006;57:139-41.