

## Original Article

# Assessment of Clindamycin and Erythromycin Resistance, and Inducible Clindamycin Resistance in *Streptococcus* Group B Isolated from Urinary Samples of Outpatient Women in Tehran

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## Abstract

**Background:** *Streptococcus* group B (GBS) or *Streptococcus agalactiae* is typically associated with neonatal disease and infection in pregnant women. Mortality of GBS sepsis in neonates is over 50% and is particularly high in preterm infants. GBS also causes invasive infection in pregnant and non-pregnant women including urinary tract infection (UTI). Penicillin-derived antibiotics remained as choice drugs for treatment of GBS infection; however, Erythromycin and Clindamycin are useful in cases of allergic to Penicillin. The aim of this study was to investigate the resistance to Erythromycin and Clindamycin, especially inducible Clindamycin resistance, in GBS isolated from urinary samples of women who attended medical offices in Tehran, Iran.

**Materials and Methods:** This study was conducted on 5000 urine samples from Jan. 2011 to Oct. 2012 that 104 GBS were isolated. The isolates were identified as GBS using laboratory criteria. Antimicrobial susceptibility test was done by Erythromycin disk 15µg and Clindamycin disk 2µg for observation inducible resistant D-zone test by double-disk diffusion method with Erythromycin and adjacent Clindamycin.

**Results:** Among the 5000 urine samples 104 (2.08%) were Beta hemolytic GBS. Of the 104 isolated GBS, 22 (21.2%) were resistance, 24 (23%) were intermediate, and 58 (55.8%) were susceptible to Erythromycin; however, 24 (23%) were resistance, 5 (4.8%) were intermediate, and 75 (72.2%) were susceptible to Clindamycin. Of the 22 Erythromycin-resistant isolates, 10 (9.5% in total GBS isolated) displayed the D zone; it means they have inducible Erythromycin resistant to Clindamycin.

**Conclusion:** Various studies in other countries report lower rates of inducible Clindamycin resistance; it indicates the use of more macrolides in the treatment of UTI.

**Keywords:** *Streptococcus* Group B, Inducible Resistance, Erythromycin, Clindamycin, Resistance, UTI

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## Introduction

Group B *Streptococcus* (GBS) or *Streptococcus*

*agalactiae* can be cause of infection in neonates, pregnant women, and non-pregnant adults who those are elderly persons with chronic medical illness<sup>1,2</sup>.

These infections have frequently been seen since 1970s. According to recent studies GBS infections have a high rate in developed countries<sup>3,4</sup>. Group B  $\beta$ -hemolytic *streptococcus* infection is an important cause of early-onset neonatal morbidity and mortality<sup>5</sup>. Mortality of GBS sepsis in neonates is over 50% and is especially high in preterm infants<sup>6</sup>. GBS commonly colonizes the female genitourinary tract (10–40% of pregnant women), which is the usual source of early-onset neonatal infection<sup>5</sup>. Also *Streptococcus agalactiae* is a component of human intestinal and genitourinary microflora<sup>7</sup>. Transmission from a colonized pregnant woman to her neonate occurs via the ascending route during labor and delivery. Administration of intrapartum antimicrobial prophylaxis (IAP) to colonized women has resulted in a striking decline in cases of early-onset and maternal GBS disease<sup>1</sup>. GBS are usually responsible for sepsis and meningitis in the first weeks of life. In spite of regional variations, the incidence for GBS-related neonatal meningitis and sepsis is 0.5-3 per 1000 live births<sup>3</sup>.

GBS is identified as an infectious agent of invasive disease in non-pregnant adults especially those underlying conditions such as diabetes mellitus, malignancy, or liver disease<sup>4</sup>. The incidence of invasive GBS infection in non-pregnant adults has increased four-fold recently up to 4.1-7.2 per 100,000<sup>3</sup>.

GBS also cause urinary tract infections (UTIs), which encompass asymptomatic bacteriuria, cystitis, pyelonephritis, urethritis, and urosepsis. GBS asymptomatic bacteriuria is particularly common among pregnant women; however, those most at risk for cystitis due to GBS are the elderly and immunocompromised individuals. Predisposing factors for GBS UTI may include diabetes mellitus and chronic renal failure<sup>1</sup>. Between 5% and 23% of nonpregnant adults with invasive GBS disease present with a urinary tract infection<sup>8</sup>. Clinical manifestations of GBS infection in adults are numerous and quite varied. Because group B streptococci may colonize skin and mucosal surfaces and may be isolated from infected sites along with other virulent organisms, their role in pathogenesis has often been questioned. However, studies of invasive GBS infection in which the organisms are

isolated from normally sterile sites such as blood or CSF provide direct evidence that group B streptococci are the etiologic agents in many clinical syndromes<sup>8</sup>.

## Methods

The study subjects were women patients who presenting to different medical offices and experienced clinical and microbiological assessments for urinary tract infection (UTI) because of symptoms indicating infection or as part of routine patient screening. In this study 5000 urine samples was examined to obtain *Streptococcus agalactiae* between January 2011 and October 2012 in microbiology laboratory of paramedical faculty of Shahid Beheshti University of Medical Sciences in Tehran. The isolates were identified as GBS, if they were: 1) Beta hemolytic catalase-negative, 2) gram-positive cocci resistant both to Trimethoprim Sulphamethoxazole (SXT) and Bacitracin disks on Mueller Hinton blood (MHB) agar medium, 3) positive hippurate hydrolysis test, and 4) positive CAMP test. Antimicrobial susceptibility test was done by Erythromycin disk 15 $\mu$ g and Clindamycin disk 2 $\mu$ g for observation inducible resistant D-zone test by double-disk diffusion method with Erythromycin and adjacent Clindamycin.

Antimicrobial susceptibility test by Erythromycin disk 15 $\mu$ g and Clindamycin disk 2 $\mu$ g (from Rosco company) performed on Mueller–Hinton agar plates with 5% defibrinated sheep blood and incubated for 24 hours in 35°C with 5% CO<sub>2</sub> for all confirmed GBS isolates (104 samples), then interpreted according to the Clinical and Laboratory Standards Institute (CLSI) in 2011. Erythromycin-resistant, Clindamycin-susceptible isolates were appraised for inducible Clindamycin resistance by the D-zone test. For observation inducible resistant D-zone test were used double-disk diffusion method with Erythromycin and adjacent Clindamycin. According to standards of CLSI to detect inducible Clindamycin resistance isolates by disk diffusion method, the D-zone test was performed with a 12mm edge-to-edge spacing between Erythromycin and Clindamycin discs. Appearance of flattened configuration of the Clindamycin zone of inhibition adjacent to the Erythromycin disk shows a positive test.

## Results

Among the 5000 urine samples, 104 (2.08%) were detected as Group B  $\beta$ -hemolytic streptococcus. According to the CLSI recommendations about antimicrobial susceptibility and D zone tests, antimicrobial susceptibility test by Clindamycin and Erythromycin disks was performed for the 104 GBS isolated. 22 (21.2%) isolates were resistance, 24 (23%) isolates were intermediate and 58 (55.8%) isolates were susceptible to Erythromycin. 24 (23%) isolates were resistance, 5 (4.8%) isolates were intermediate and 75 (72.2%) isolates were susceptible to Clindamycin (Table 1). The double disk diffusion test for inducible Clindamycin resistance was performed on all isolates resistant to Erythromycin and susceptible to Clindamycin. Inducible Clindamycin resistance by Erythromycin was displayed by presenting the appearance of a “D” around Clindamycin disk on plate in 10 (9.5% in total GBS isolated) isolates, so these cases showed an positive D zone test (iMLSB phenotype ) (Figure 1). Also resistance to both Erythromycin and Clindamycin disks (cMLSB phenotype) (Figure 2) was indicated in 11 (10.5% in total GBS isolated) isolates and just one (1% in total GBS isolated) case showed a Erythromycin-resistant and Clindamycin-susceptible without D zone (M phenotype) (Figure 3) (Table 2).

## Discussion

Penicillin and Ampicillin are the choice drugs for

treatment of  $\beta$ -hemolytic streptococcal infections<sup>9</sup>. In patients with penicillin allergies or a lack of clinical response, alternative therapies, such as macrolides (e.g., Erythromycin), Lincosamides (e.g., Clindamycin) are often considered for treatment of infections. Group B streptococci are susceptible to Ampicillin, Penicillin, and Cefazolin, but may be resistant to Clindamycin and/or Erythromycin<sup>9-11</sup>. In a research in Tehran in 2010, among the 498 Group B streptococci isolates taken from adult women’s urine cultures, 24.2% and 16.8% isolates were resistance to Erythromycin and Clindamycin, respectively<sup>4</sup>. Resistance frequencies currently range from 6% to 21% for Clindamycin and 12% to 29% for Erythromycin in the United States, while other countries report higher rates<sup>12</sup>.

GBS colonization of the urinary tract in women most likely occurs by an ascending route from the vagina, where GBS can persist asymptotically. Although in treatment of urinary tract infections by GBS is not used Erythromycin and Clindamycin routinely, whereas source of urinary tract infections can be GBS colonization in gastrointestinal or genital tracts, resistance to Erythromycin and Clindamycin will be important<sup>13</sup>.

Since the clinical significance of inducible Clindamycin resistance among all  $\beta$ -hemolytic streptococci is unclear, it may not be necessary to perform this induction test on all isolates that are Erythromycin resistant and Clindamycin susceptible; however, all isolates from invasive infections should be tested. When a Group B *streptococcus* is isolated from a pregnant woman with severe Penicillin allergy,

**Table 1:** Result of antimicrobial susceptibility test by Clindamycin and Erythromycin disks.

Number of samples detected as Group B $\beta$ -hemolytic streptococcus	Erythromycin (%)			Clindamycin (%)		
	susceptible	intermediate	resistance	susceptible	intermediate	resistance
104 samples	55.8%	23%	21.2%	72.2%	4.8%	23%

**Table 2:** Result of The double disk diffusion test for inducible Clindamycin resistance (phenotypes).

Number of samples detected as Group B $\beta$ -hemolytic streptococcus	iMLSB phenotype (%)	cMLSB phenotype (%)	M phenotype (%)
104 samples	9.5%	10.5%	1%



Figure 1. iMLS phenotype (D Test Positive)



Figure 2. cMLS phenotype

Clindamycin and Erythromycin should be tested and reported<sup>9</sup>.

Recognition of Macrolide–Lincosamide–Streptogramin B (MLS<sub>B</sub>) phenotype was identified by a disc diffusion method<sup>14</sup>. The constitutive MLS<sub>B</sub> (cMLS<sub>B</sub>) phenotype is associated with high resistance to Erythromycin and Clindamycin, in our study it obtained 10.5% in total GBS isolated. The inducible MLS<sub>B</sub> (iMLS<sub>B</sub>) phenotype is associated with resistance to Erythromycin and susceptibility to Clindamycin, with antagonism between the Erythromycin and Clindamycin discs (with positive D zone test), in our study it obtained 9.5% in total GBS isolated. The M phenotype is determined by resistance to Erythromycin, susceptibility to Clindamycin and no antagonism between the two discs (without D zone test), in our study it obtained 1% in total GBS isolated. Resistance to Erythromycin and inducible Clindamycin resistance show limitation of Clindamycin usage when there is a resistance to Erythromycin<sup>15</sup>.

Various studies in other countries report different prevalence of inducible Clindamycin resistance, for



Figure 3. M phenotype

example, in Turkey in 2003 of 156 isolates *S. agalactiae* collected, 28 isolates (18%) expressed the iMLS phenotype and 7 isolates (4.5%) expressed the cMLS phenotype<sup>10</sup>. In Canada in 2004, Among the 338 GBS isolates tested, 55 (17%) and 26 isolates (8%) were resistant to erythromycin and clindamycin, respectively. Of the 55 erythromycin-resistant isolates, 26 isolates (8%) had constitutive MLS<sub>B</sub> resistance and 22 isolates (6.5%) had an inducible MLS<sub>B</sub> resistance phenotype<sup>16</sup>. between 1997 and 1999 in two Health Authority Areas in Móstoles and Granada, Spain of 221 *Streptococcus agalactiae* isolated, 185 isolates (83.7%) were susceptible to erythromycin and azithromycin and 191 isolates (86.4%) were susceptible to miocamycin and clindamycin and 23 isolates (10.4%) had a constitutive MLS<sub>B</sub> phenotype and 7 isolates (3.2%) had an inducible phenotype<sup>17</sup>. In Massachusetts in the USA between January 2002 and April 2003, of the 200 isolates of GBS, 44 isolates (22%) were resistant to erythromycin. 32 isolates (16%) were resistant to erythromycin but susceptible to clindamycin. Of these isolates, 21 isolates (10.5%) had increased clindamycin resistance upon induction with erythromycin as determined by the D test and There were 11 erythromycin-resistant, clindamycin-susceptible isolates which did not have inducible resistance to clindamycin in this test<sup>18</sup>. In Poland in 2010, among 169 GBS isolates detected, 27 isolates (16%) were resistant to erythromycin and 17(10%) resistant to clindamycin. 17 isolates (10%) isolates had a cMLS<sub>B</sub> phenotype and 7 isolates (4%) isolates had an iMLS<sub>B</sub> phenotype<sup>19</sup>. In July 2009, in a laboratory in Louisiana, USA, performed routine susceptibility testing of GBS isolates. Between 1 July 2009 and 31 December 2010, 544 GBS isolates were identified,

with 283 (52%) and 178 (33%) demonstrating Erythromycin and Clindamycin resistance, respectively. The highest reported rate of inducible Clindamycin resistance was 11%<sup>20</sup>. In this way, prevalence of inducible Clindamycin resistance in *S. agalactiae* in other studies were 2% in New Zealand in 2004, 2% in Australasia in 2005, 2.4% in France in 2008 and 10.3% in Ireland in 2011<sup>10,16-19</sup>.

## Conclusion

Various studies in other countries report lower rates of inducible Clindamycin resistance. Also rate of resistance to Erythromycin and Clindamycin in Group B Streptococcus isolates was relatively high in this research. These results are may be due to use of more macrolides in the treatment of UTI in our country.

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