

Capsular Genotypes Distribution and Antibiotic Resistance pattern of Group B *Streptococcus* (GBS) Isolated from Clinical Samples, Tehran, Iran

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Group B *Streptococcus* (GBS) is an opportunistic harmless bacterium which the leading cause of neonatal infections. Our purpose was to determine capsular genotypes distribution and antibiotic resistance pattern of GBS isolated from clinical samples. Two hundred and twenty two GBS strains isolated from clinical samples from different hospitals in Tehran, Iran. After identification by specific cultures and biochemical tests, broth microdilution method was used to determine the minimal inhibitory concentration (MIC) of antibiotics based on standard protocol. The erythromycin-clindamycin double-disk test was used to determine the inducible resistance phenotypes. Capsular genotypes were identified by PCR method. The high rates of antibiotic resistance in GBS were related to gentamycin 89.18%, tetracycline 87.38%, kanamycin 62.16%, clindamycin (67.1%), erythromycin 57.2%, and chloramphenicol 32.8%. All strains were sensitive to vancomycin, penicillin, and ampicillin. Between eleven capsular antigens, serotypes such as III(50.9%) ,V(27.47%) ,Ib(17.76%) ,Ia(15.54%) , Ic (5.85%)were the highest. The genotypes distribution and the patterns of resistance phenotypes of GBS may vary in different areas. Thus, it is required to be considered in each region to work out strategies for prevention. The PCR method is recommended as a rapid and reliable technique for identification and molecular epidemiology study of GBS.

Keywords: Group B *Streptococcus* (GBS), Genotypic characterization, Antibiotic Resistance.

GBS is one of the primary agents of invasive infections such as sepsis and meningitis in neonatal from birth to 4 weeks of age. Also, GBS is a significant pathogen in geriatrics and those with underlying medical disorders¹

Ten to 30% of pregnant women are colonized asymptotically by GBS in their

vagina and rectum. If newborns are exposed to GBS during delivery, close to 50.0% will be colonized and invasive infection will almost increase 2.0%. Routine screening for maternal colonization by GBS is useful for prevention of neonatal infections²⁻⁴

Drugs of choice for treatment of β -hemolytic streptococcal infections are penicillin and ampicillin. Nonsusceptible β -hemolytic *Streptococcus* isolates (i.e., penicillin MICs > 0.12 and ampicillin MICs > 0.25 μ g/mL) are extremely

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rare. So, based on FDA reports (the US Food and Drug Administration), susceptibility testing for treatment of 2-hemolytic streptococcal infections need not to be performed routinely. If antibiotic susceptibility testing is done and each 2-hemolytic streptococcal strain detected to be nonsusceptible should be retested, and reported to an emergency for public health.

Penicillin or ampicillin is recommended for intrapartum prophylaxis against GBS. For at low risk, cefazolin is recommended for anaphylaxis. In case of patients at high risk clindamycin is consumed for anaphylaxis. GBS usually are not resistant to cefazolin, penicillin, and ampicillin, but may be resistant to clindamycin and erythromycin. When a GBS is detected in a pregnant penicillin-allergic woman, inducible clindamycin resistance should be tested by erythromycin and clindamycin, and only clindamycin should be reported. (5) Unfortunately, multi-drug resistant GBS is increasing and there is a little information on the antibiotic resistance patterns against β -lactams, aminoglycosides, and macrolides.

An important target for vaccine strategies against GBS is capsular polysaccharide. Having different chemical and antigenic characters, GBS capsular polysaccharides have been subdivided into eleven serotypes such as Ia, Ib, Ic, II-IX serotypes⁶. Vaccines against infections due to GBS must include the main serotypes associated with disease in different communities⁷. Study of the epidemiological distribution of GBS serotypes may be different based on several aspects, containing studied population, the geographical region, and source of bacterial isolate⁸.

GBS can be subdivided into types Ia, Ib, Ic, II, and III. Almost, 99% of GBS strains can be belonged to one of these five antigen types⁹. In the United States and in some European countries, Ia, II, III and V are generally the most frequently isolates, and VI to IX serotypes are seldom detected^{6, 8, 10-12}. In Brazil, the emerging of Ia, Ib, II, III, IV and V serotypes has been identified in a few studies in South and the Southeast area¹³⁻¹⁸.

So, in this epidemiological study, we examined capsular genotypes of GBS by PCR regarding to antibiotic resistant pattern in clinical isolates by means of disk diffusion and broth dilution.

MATERIALS AND METHODS

GBS detection

In this descriptive study, a total of 222 GBS isolated from clinical samples such as urine, skin and soft tissue, bone, joint, blood, CSF, pleural fluid. Clinical samples were gathered during two years (between June 2013 and May 2015) from Mofid Children hospital, Loghman Hakim hospital and Milad Hospital, Tehran, Iran. Appearance of GBS colonies on blood agar plates were gray-white and tiny colonies with a β -hemolysis halo. Primary identification was hemolytic, gram-positive cocci with catalase-negative reaction. GBS distinction from other streptococci was based on biochemical reactions including resistance to bacitracin and trimethoprim-sulfamethoxazole (SXT), sodium hippurate hydrolysis, and positive CAMP test^{2, 4, 9, 19-22}.

Antimicrobial susceptibility testing Methods

All isolates were tested for ampicillin(10 μ g), cefotaxime, ceftriaxone (30 μ g), clindamycin(2 μ g), chloramphenicol(30 μ g), erythromycin(15 μ g), levofloxacin(5 μ g), penicillin(10 unit), vancomycin (30 μ g), kanamycin (30 μ g), streptomycin(10 μ g), gentamycin(30 μ g) and tetracycline(30 μ g). We used Mueller-Hinton agar (MHA) supplemented by 5% sheep blood for disk diffusion method. For broth dilution method we prepared cation-adjusted Mueller-Hinton broth (CAMHB) supplemented by 2.5% to 5% v/v lysed horse blood (LHB). For McFarland concentration preparation was done. All media were incubated in $35 \pm 2^{\circ}\text{C}$. Five percent of CO_2 during 20 to 24 hours incubation was prepared for disk diffusion method; and for dilution methods was in ambient air condition during 20 to 24 hours incubation time.

Zone of inhibition (mm) was measured and reported as susceptible, intermediate or resistant by according to the CLSI guidelines. By following the guidelines recommended by the CLSI, screening test for detection of inducible clindamycin resistance in GBS was determined using a broth microdilution method. One $\mu\text{g}/\text{mL}$ erythromycin and 0.5 $\mu\text{g}/\text{mL}$ clindamycin in same well was used and minimal inhibitory concentration (MICs) was reported. Any growth was reported as inducible clindamycin resistance and no growth was reported as no inducible clindamycin resistance⁵.

Additionally, to ensure the reproducibility of the MICs for each strain, determinations were repeated three times. *Streptococcus pneumoniae* ATCC 49619 was used as quality control strain. For β -lactamase activity detection, a nitrocefin-based disc procedure (BD Diagnostics) was used. To detection of the inducible resistance phenotypes, erythromycin-clindamycin double-disk test was used²³

Capsular genotypes detection

By AccuPrep Genomic DNA extraction kit (cat.no.k-3032 lot no.1008J, BIONEER) DNA was extracted from all GBS isolates. PCR amplification profile comprised a 300 nM concentration of each oligonucleotide primer (Eurofins MWG Operon); 200 mM (each) deoxynucleoside triphosphates dCTP, dGTP, dATP, and dUTP; 0.125 U of Taq DNA polymerase; and 5.5 mM MgCl₂ (from GENETBIO, prime Taq TM DNA polymerase, URL: www.genetbio.com)

The PCR products were analyzed by gel electrophoresis on 1.5% BIONEER agarose gels in 1X TBE buffer (890 mM of boric acid, 890 mM Tris, 40ml of 0.5 M EDTA, pH 8.0) at 100 V for 60 min. Green loading buffer with DNA stain(Jena Bioscience, Lot:111.034) was used during loading the samples and ladder. The sizes of the PCR products were determined by comparison with the molecular size standard (50bp-1Kb linear scale; low range DNA ladder or 100bp-3Kb linear scale and mid-range DNA ladder, Jena Bioscience). Primer sequences , PCR conditions ,and electrophoresis for Ia,Ib,Ic ,II- IX^{6,13,22}, *S.agalactiae* 16srRNA²⁴ , as described previously.

Statistics

As the study is a descriptive, there is no need to state about statistical methods and software.

RESULTS AND DISSCUSSION

A total of 222 GBS isolates collected from clinical samples (skin and soft tissue18(8.1%), bone10(4.5%),join 13(5.8%),CSF 16(7.2%), pleural fluid29(13.06%), urine 16(7.2%), and vagina107(48.19%)] were investigated for antibiotic susceptibility test. Capsular genotyping and resistance-related genes distribution of GBS were considered by PCR method.

The percentages of resistance to gentamycin (89.18%), tetracycline (87.38%), clindamycin (67.1%), kanamycin (62.16%), erythromycin (57.2%), and chloramphenicol (32.8%) were the highest. All of strains were sensitive to vancomycin, penicillin, and ampicillin. (Fig. 1)

MICs of erythromycin and clindamycin were determined by for all isolates resistant or intermediate to erythromycin. The MICs of erythromycin and clindamycin 23.7% of these isolates were greater than 256 μ g/ml.

Between eleven capsular antigens, serotype III(50.9%) ,V(27.47%), Ib(17.76%), Ia(15.54%) ,Ic (5.85%)were the highest. Four and ninth percent were nontypable. (Figure 2)

Based on our finding, being more than 67.1% of isolates resistant to clindamycin and almost 57.2% resistant to erythromycin, emphasis

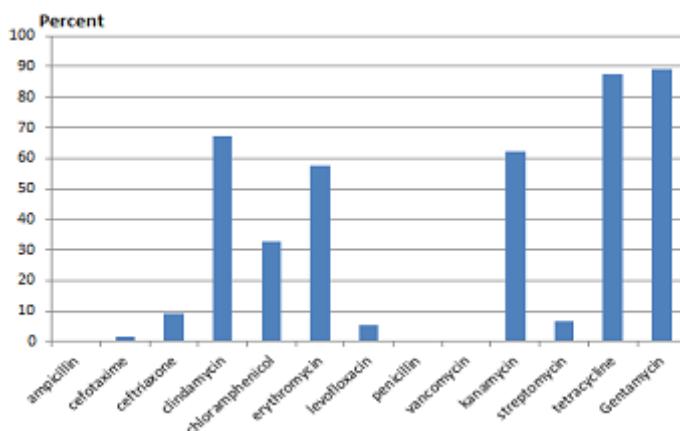


Fig. 1. Distribution of antibiotic resistant among *S. agalactiae* isolated from clinical samples

to do antimicrobial susceptibility testing for GBS strains isolated from clinical specimens. Penicillin and ampicillin were recommended by CLSI 2015 as first-line agents for intrapartum antibiotic prophylaxis. Vancomycin is drug of choices in cases for only women at high risk because of occurring anaphylaxis shock due to penicillin⁵

We detected 87.38% tetracycline-resistance rate among *S. agalactiae* isolates which was lower than other studies from Kuwait (89.5%), Iran (96%), Tunisia (97.3%) and Turkey (100%), and was more than tetracycline-resistance rate in Italy (68.1%) and Japan (46.5%).(25-30) In the current study, resistance to chloramphenicol was (32.8%), which was similar to the other study in Iran (45%), Turkey (44.2%) and Kuwait (30%).(25, 26, 28) The prevalence of drug resistance pattern in GBS can vary among ethnic groups, geographical locations, and the laboratory procedures carried out on the samples.

These data accompany with the other results are useful to define the importance of emergence and transmission of antibiotic resistance genes in GBS for supporting the decision of rational and economical program on specific antibiotics usage to treat GBS infections.

The multiplex PCR assay described in this work provides a simple tool for GBS capsular genotyping. We identified five common capsular antigens such as III(50.9%), V(27.47%), Ib(17.76%), Ia(15.54%) , Ic (5.85%) were the highest, a hypothesis consistent with findings by a variety of groups^{6, 8, 11, 13, 20-22} The majority of isolates belonged to capsular antigens III, which is mostly involved in neonatal infections in human. The

present findings, capsular antigens III is reported to be the most common GBS serotype around the world³¹⁻³⁴ Also, we observed the capsular antigens of GBS at Ardabil, Tabriz, Kerman was different from capsular antigens of GBS reported from central Iran^{31, 36-39}

This difference in distribution could be attributed to vary in materials and methods of research, geographical region, profile of the population being studied, and source of the bacterial isolates .Even subtle regional differences in demographic data gathering can affect GBS serodistribution within the same country.

The second capsular antigens which we identified with high level in GBS isolates was capsular antigens V. This serotype able to cause invasive infections in neonates and adolescents may be considered an emerging public health concern^{8, 25, 40, 41}

Other treatments for penicillin-allergic patients are clindamycin and erythromycin. Sad to say, erythromycin and clindamycin resistance have been enhanced in some countries.

All of differences related to the prevalence of genes related to antibiotic resistance may be explained by the different policies in the use of antimicrobials in different regions. The recognition of resistant strains in this study advance a proposal that these agents should be apply with precaution in the prophylaxis or remedy of GBS infection and that intermittent supervision is, therefore, needed to consider a proper therapeutic option in patients with allergy to β -lactam agents, necessary.

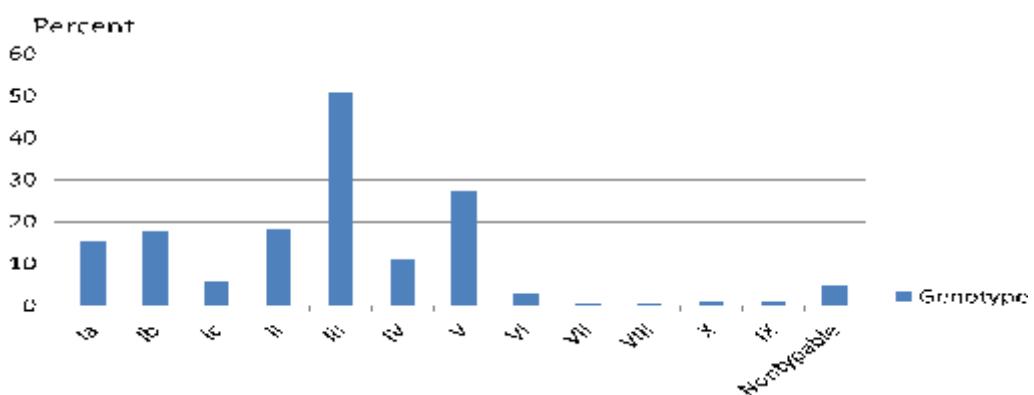


Fig. 2. Distribution of capsular genotyping among *S. agalactiae* isolated from clinical samples

We conclude that GBS isolates represent various capsular antigens and resistant patterns relate to the origin of the sample, the studied population characteristics, different pattern of antibiotic consumption, and the geographical location. Dissemination and emergence of antibiotic-resistant GBS against erythromycin, tetracycline, and aminoglycoside are warning. Doing epidemiological studies and screening method for detection of MDR-GBS and appropriate drug prescriptions is recommended annually. PCR method particularly well adapted for GBS capsular antigens typing and detection of genes associated to resistance in large-scale epidemiological studies.

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