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Antibiotic Susceptibilities and Serotyping of Clinical *Streptococcus Agalactiae* Isolates

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ABSTRACT

Objective: *Streptococcus agalactiae* (Group B streptococci, GBS) are frequently responsible for sepsis and meningitis seen in the early weeks of life. GBS may cause perinatal infection and premature birth in pregnant women. The aim of this study was to serotype GBS strains isolated from clinical samples and evaluate their serotype distribution according to their susceptibilities to antibiotics and isolation sites.

Material and Methods: One hundred thirty one *S. agalactiae* strains isolated from the clinical samples were included in the study. Of the strains, 99 were isolated from urine, 20 from soft tissue, 10 from blood and 2 from vaginal swab. Penicillin G and ceftriaxone susceptibilities of GBS were determined by the agar dilution method. Susceptibilities to erythromycin, clindamycin, vancomycin and tetracycline were determined by the Kirby-Bauer method according to CLSI criteria. Serotyping was performed using the latex aglutination method using specific antisera (Ia, Ib, II-VIII).

Results: While in 131 GBS strains, serotypes VII and VIII were not detected, the most frequently isolated serotypes were types Ia (36%), III (30.5%) and II (13%) respectively. Serotype Ia was the most frequently seen serotype in all samples. All GBS isolates were susceptible to penicilin G, ceftriaxone and vancomycin. Among the strains, tetracycline, erythromycin and clindamycin resistance rates were determined as 90%, 14.5%, and 13% respectively.

Conclusion: Penicillin is still the first choice of treatment for the infections with all serotypes of *S. agalactiae* in Turkey.

Key Words: *Streptococcus agalactiae*, antibiotic susceptibility, serotype

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Introduction

Infections due to Group B Streptococci (GBS) have frequently been seen since 1970s, especially in developed countries. 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 (1). These microorganisms can commonly colonize in the genital and gastrointestinal system in adults. However, in pregnant women, it can cause perinatal transmission and preterm labor (2). The incidence of invasive GBS infection in non-pregnant adults has increased four-fold recently up to 4.1-7.2 per 100.000. Nine capsular polysaccharide serotypes have been recognized so far: Serotype Ia, Ib, II-VIII. GBS are sensitive to penicillin, and penicillin should be the first choice of treatment following diagnosis. The resistance rates to clindamycin and erythromycin is increasing amongst the strains (3).

The aim of this study was to define the serotypes and the relationship between the serotype, isolation site and antibiotic susceptibility of *S. agalactiae* strains isolated from various clinical specimens.

Material and Methods

The study included 131 *S. agalactiae* strains isolated from the clinical specimens, which were sent to the Bacteriology Laboratory of the Hospital of Erciyes University, Faculty of Medicine and identified by the CAMP test, hippurate hydrolysis and latex agglutination test (Omega, UK). The strains were stored at -20°C in skimmed milk (Oxoid, UK) until the date of the study.

Serotype identification was performed using the latex agglutination method with the Strep-B-Latex kit (Statens Serum Institut, Denmark).

The susceptibilities of GBS for penicillin G and ceftriaxone were studied using the agar dilution method with the recommendations of the CLSI (4). The susceptibilities of the strains to erythromycin, clindamycin, tetracycline and vancomycin were studied and evaluated by the Kirby-Bauer disk diffusion method according to the recommendations of CLSI (5). The phenotypes for macrolide resistance were studied using the D-test method. In the D-test, disks containing erythromycin (15 µg) and clindamcyin (2 µg) were placed 15 to 20 mm apart on an agar plate that had been inoculated with the clinical

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isolate. The formation of a "D"-shaped zone around the clindamycin disk was interpreted as "inducible MLS_B"; the formation of a normal zone of sensitivity was interpreted as "efflux", and the formation of a normal zone of resistance as "constitutional MLS_B" (6). *S. pneumoniae* (ATCC 49619) was used as the quality control strain.

Results

Of the 131 *S. agalactiae* strains, 99 (75.6%) were isolated from the urine, 20 (15.3%) from the wound, 10 (7.6%) from blood, and 2 (1.5%) from vaginal swab specimens. The serotypes of the strains are presented in Table 1. There was no serotype VII or VIII. The most common serotypes were serotype Ia (36%), serotype III (30.5%) and serotype II (13%), respectively. As seen in Table 1, while serotype III could not be found in specimens except the blood culture, almost 40% of the isolated ones from the urine culture were serotype III. The serotypes of the isolates in terms of resistance for antibiotics are presented in Table 2. The highest resistance rate to erythromycin and clindamycin was found in serotype III and V. The highest resistance rate to tetracycline was in serotype Ia and III. $MIC_{50,}$ MIC_{90} values and MIC ranges of the strains for GBS are shown in Table 3. All the strains were found to be susceptible to penicillin G and ceftriaxone. According to the D-test results, constitutional MLS_B phenotype was found in 15 out of 19 (79%) erythromycin-resistant GBS strains; inducible MLS_B phenotype was found in 2 (10.5%), and efflux phenotype was found in 2 (10.5%). When the resistance mechanisms of the isolates for macrolide were assessed according to serotype distribution, the structural and inducible MLS_B resistance phenotype was seen mostly in serotype III and V, whereas the efflux phenotype was seen only in serotype III.

Discussion

S. agalactiae is one of the most important pathogens in newborn sepsis and meningitis, and has a high mortality and morbidity in spite of the current antibiotic treatment in newborn. Pregnancy-related GBS infections cause serious infections both in the mother and the neonate during labor or the early postpartum period. The mortality rate for newborns due to GBS infection during the first three months after birth is 0.5-3 per 1000 live births (1). The invasive *S. agalactiae* infections in adults is in the form of puerperal infections or infections with underlying immune-compromising conditions such as alcoholism, diabetes mellitus, malignancy or HIV. In adults with these conditions, the infection spectrum consists of pneumonia, bacteremia, endocarditis, urinary system infection, skin and soft tissue infections, and osteomyelitis (7). Serotype definition in these strains can be made by various methods including immunoprecipitation, enzyme immunoassay, co-agglutination, immunoelectrophoresis, capillary precipitation, latex agglutination, fluorescence microscopy and inhibition ELISA. Molecular typing methods can be used in the epidemiological studies; however, they are not chosen for serotype definition as they are very expensive and time-consuming. We used the latex agglutination method. Nine capsular polysaccharide serotypes of GBS have been known so far: serotype Ia, Ib, II-VIII. Based on the US and European data, 86%-90% of the clinical isolates are serotype Ia, II, III and V (8, 9). Serotype VI and VIII were reported as the most common GBS strains in Japan (10, 11). Recently, Zeng et al. (12) developed separate multiplex PCR-based reverse line blot-hybridization assays to identify molecular serotypes of GBS and isolated mainly the serotypes III, Ia, V, Ib and II. In Chile and Senegal, the most commonly isolated serotypes were III, Ib, V and Ia in pregnant women and neonates using the latex agglutination method (13, 14). In Turkey, while the most commonly isolated serotypes were Ia, II, III in Istanbul (15), serotype III, 1b and V were the most common serotypes in the Tokat region (16). We could not find any strain of serotype VII and VIII, and the most common serotypes in our study were serotype Ia (36%), III (30.5%), II (13%), V (13%), Ib (3%), IV (1.5%), and VI (1.5%), respectively. Two isolates could not be serotyped with the available antisera.

Intrapartum penicillin is recommended for prevention of perinatal infections, as there is a risk of infection transmission from the GBS-colonized mother to the neonate. Erythromycin

Table 1. Serotype distribution of *S. agalactiae* strains according to the isolation sites (n:131)

Serotype	Urinary tract infection n (%)	Soft tissue infection n (%)	Bloodstream infection n (%)	Vaginal colonization n (%)	Total n (%)
Ia	33 (33.4)	9 (45)	4 (40)	1 (50)	47 (36)
Ib	2 (2)	2 (10)	-	-	4 (3)
II	9 (9.1)	5 (25)	2 (20)	1 (50)	17 (13)
III	39 (39.4)	-	1 (10)	-	40 (30.5)
IV	1 (1)	1 (5)	-	-	2 (1.5)
V	13 (13.1)	2 (10)	2 (20)	-	17 (13)
VI	1 (1)	-	1 (10)	-	2 (1.5)
VII	-	-	-	-	0 (0)
VIII	-	-	-	-	0 (0)
Nontypable	1 (1)	1 (5)	-	-	2 (1.5)

Table 2. Serotype distribution of resistant *S. agalactiae* strains (n:131)

Serotype	Number	Antibiotic		
		Erythromycin n (%)	Clindamycin n (%)	Tetracycline n (%)
Ia	47	1 (2.1%)	1 (2.1%)	40 (85%)
Ib	4	-	-	4 (100%)
II	17	1 (5.8%)	1 (5.8%)	14 (82.3%)
III	40	14 (35%)	12 (30%)	39 (97.5%)
IV	2	-	-	2 (100%)
V	17	3 (17.6%)	3 (17.6)	16 (94%)
VI	2	-	-	2 (100%)
VII	0	-	-	-
VIII	0	-	-	-
Nontypable	2	-	-	1 (50%)
Total	131	19 (14.5)	17 (13)	118 (90)

Table 3. MIC ranges, MIC_{50} and MIC_{90} values of *S. agalactiae* strains to penicillin G and ceftriaxone (n:131)

Antibiotic	MIC (µg/ml)		
	MIC range	MIC_{50}	MIC_{90}
Penicillin G	<0.01-0.06	<0.01	0.03
Ceftriaxone	<0.01-0.25	0.03	0.03

and clindamycin are preferred in the event of penicillin allergy. Although there is no reported penicillin resistance in GBS isolates responsible for the neonatal invasive infections in recent studies, resistance to erythromycin was reported as 7%-46%, and to clindamycin as 3%-43% (17, 18). All *S. agalactiae* isolates reported from Turkey were found to be susceptible to penicillin, ampicillin, vancomycin, ceftriaxone, chloramphenicol and ofloxacine. The rates of resistance to erythromycin, clindamycin and tetracycline were found to be 7%-24.5%, 9%-19.4%, and 81.6%-91%, respectively (15, 16, 19, 20). In our study, while all GBS isolates were found to be susceptible to penicillin, ceftriaxone and vancomycin, 14.5% were found to be resistant to erythromycin and 13% were found to be resistant to clindamycin. In a previous study in the same region, erythromycin resistance was reported as 7% (19). Therefore, it was seen that erythromycin resistance in our region had doubled. It was reported that *ermB*, *ermA*, *ermTR* and *mefA* genes were responsible for erythromycin and clindamycin resistance (18, 21, 22). As the PCR method was not used in this study, macrolide resistance genes could not be identified; however, the D-test was performed on the resistant strains to define the resistance phenotypes. The structural resistance was found to be 79%, the inducible resistance 10.5% and the efflux-related resistance 10.5%. It was reported that erythromycin and clindamycin resistance was more prevalent in serotypes V and III (22). We also found erythromycin and clindamycin resistance to be more prevalent in serotypes III and V. Tetracycline was the antimicrobial agent with the highest rate (90%) of resistance among the tested antibiotics. Similar results were reported in other studies. Tetracycline resistance is considered to be related to the *tetM* gene (22).

In conclusion, there is no problem regarding resistance to penicillin G, ceftriaxone and vancomycin in GBS strains in our region, and penicillin is still the first choice of treatment in GBS infections. As it was reported that the resistance rates of strains for erythromycin and clindamycin is increasing, these antibiotics should not be preferred, except for beta-lactam allergy. Studies showed that long-term prevention can be maintained with the use of conjugated polysaccharide vaccine for GBS-related maternal infections, still-births and neonatal infections (23). Further epidemiological studies on serotype distribution are required to assess the value of the vaccine in our country.

Conflict of Interest

No conflict of interest was declared by the authors.

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