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Short article

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Pattern of Infection and Antibiotic Activity among *Streptococcus agalactiae* Isolates from Adults in Mashhad, Iran

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Abstract

Background: One of the main causes of sexually transmitted diseases is *group B β- hemolytic streptococci* (GBS) multiplying in the genital tracts. Penicillin is the most common drug for the treatment of infections caused by these bacteria, but in patients suffering from Penicillin allergy, Erythromycin and Clindamycin are used as alternative therapeutic drugs against GBS. Recently, resistance to these drugs has been reported more often. In this study, efforts have been made to determine the prevalence and antibiotic resistance of GBS.

Methods: Modified Christie Atkins Munch-Petersen (CAMP) test was conducted on over 2400 samples of urine and discharge taken from vagina, urethra and prostate. The drug sensitivity was performed by double disk sensitivity tests to Bacitracin, Trimethoprim, and Sulfamethoxazole and then the resistant samples were investigated by E-test to determine the minimal inhibitory concentrations (MICs) value.

Results: Twenty-three vaginal and 10 urethral discharge, 27urine and 6 prostatic secretion samples were GBS positive. The most symbiotic microorganisms with GBS were strains of *Enterococci* (90%), *Staphylococcus saprophyticus* (25%) and *Candida albicans* (6%). The disk diffusion method showed 18 cases with Penicillin resistance (MIC: 1.5 mg/ml).

Conclusion: Taken together, GBS carriers' rate in this study was found 20.65% (8.24% men and 12.4% women). Furthermore, findings showed high-level resistance to Erythromycin and Clindamycin.

Keywords: Antibiotic resistance, Genitourinary system, Minimal inhibitory concentration (MIC), *Streptococcus agalactiae*,

Introduction

Group B β- hemolytic streptococci (GBS) is the main cause of blood infection and Meningitis in infants (1, 2). According to the statistics published by the World Health Organization (WHO), about 15-45% of women are affected by GBS in their genitourinary system (3, 4). Fifty percent of infants become infected before birth or during delivery. In such cases, nearly 1-2% of newborns will develop progressively severe complications such as meningitis (5). In addition, *Streptococcus agalactiae* may cause severe infections in adults. Especially people with background diseases such as diabetes mellitus, malignant tumor, liver and kidney failure, immune deficiency such as acquired immunodeficiency syndrome (AIDS) are at the risk for GBS (6-9). Moreover, GBS increases the risk for sexual diseases; it can multiply in the male reproductive organs, particularly the urethra and prostate and then possibly lead to pneumonia and bacteremia (9). Penicillin is the well-known drug in the treatment of infections caused by GBS. However,

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for people who have an allergic reaction to Penicillin, Erythromycin and Clindamycin are prescribed for patients with GBS infections. Recently, a developing resistance to these drugs has been reported (10). The purpose of this study was to determine the prevalence of *S. agalactiae* in genitourinary system infections and its resistance to Erythromycin and Clindamycin.

Materials and Methods

Samples were obtained from individuals with the genitourinary system infection (aged 15-40 and over) referred to medical diagnostic laboratories in Mashhad (Iran). Human specimens for testing included urine (n=1687), discharge taken from the vagina (n=208), urethra (n=200) and prostate (n=205). At first, epithelial cells (ECs) and white blood cells (WBCs) were counted for all samples. Then, they were streaked over blood agar plates (5% sheep's blood) and incubated into a candle jar. The symbiotic relationship with *Enterococcus*, *Staphylococcus saprophyticus* and *Candida albicans* (11), and the GBS identification from clinical specimens were analyzed using bacitracin, catalase, CAMP and Gram stain tests (12, 13). Moreover, subsequent tests were performed to estimate different resistance levels of GBS to different antibiotics (Ampicillin, Penicillin, Clindamycin, Erythromycin, Amoxiclav, Ceftriaxone, Vancomycin, Amikacin, Gentamicin, Nalidixic acid and Kanamycin).

Clinical isolates of GBS on blood agar were suspended in standard saline inoculating on Mueller-Hinton agar plates. Then, two prepared disks of Trimethoprim and Sulfamethoxazole (Masc Co, UK) were placed on the plates and incubated overnight (14).

In parallel, minimal inhibitory concentration (MIC) values were measured by E-test method and interpreted as susceptible, intermediate or resistant (15). As described for disk diffusion, the inoculation was carried out in the plates, and then strips covered by antibiotic carriers were applied to each plate. After overnight incubation, the MIC was reported at the intersection of growth inhibition zone with the strip.

Results

Table 1 shows the frequent GBS infections by gender representing 69.7% of women carrying GBS.

Clinical isolates from different sites distributed among different age groups are summarized in table 2. With routine identification tests, a total of 66 GBS isolates were characterized (27 Urine, 23 Vagina, 9 Urethra and 6 Prostate). The majority were from the collected samples of urine and vaginal discharge demonstrating 40.9% and 34.85%, respectively. Moreover, our findings indicated that specimens taken from the vagina were more infected with GBS in 26-35 years old (48%). Highest rate of GBS presence in urinary tract was reported among individuals over 40 years old (67%). For urethra and prostate secretion, this was ranged from 31 to 40 years old (40%). As shown in table 2, there was an abnormal increase in the number of WBCs and ECs in about 34% of GBS isolates.

It was observed that the most symbiotic interactions were occurring between GBS and *Enterococcus*, 90%, followed by *Staphylococcus saprophyticus*, 25.7% (Table 3).

Table 4 demonstrates sensitivity testing frequencies with Trimethoprim and Sulfamethoxazole. About 27% of infectious samples were resistant to Penicillin at MIC of 1.5 µg/ml. The percentage of GBS resistance to Clindamycin and Erythromycin were 20% and 24.5%, respectively, with the average MIC value of 0.01 µg/ml. Furthermore, the disk diffusion susceptibility to Amikacin, Gentamicin, Nalidixic acid and Kanamycin resulted in 100% resistance for all clinical isolates of GBS fully susceptible to Ampicillin, Amoxiclav and Ceftriaxone.

Table 1. The percentage of GBS carriers by gender.

Gender	Total patients (n)	GBS carrier n (%)
Female	1200	46 (69.7)
Male	1200	20 (30.3)

Table 2. Cell counts in obtained specimens and on site distributions among different age groups

Variable		Vagina (n)	Urine (n)	Urethra (n)	Prostate (n)
Age	15-20	2	3	-	-
	21-25	2	1	2	-
	26-30	6	2	1	2
	31-35	5	2	2	2
	36-40	4	1	2	1
	>40	4	18	3	1
White blood cells (WBCs)	0-5	17	18	3	5
	high	6	9	6	1
Epithelial cells (ECs)	2-4	9	25	4	5
	high	14	2	5	1

Table 3. The culture positivity rate of Group β- hemolytic streptococci (GBS) and its coexistence organisms.

Microorganism	Rate (%)
Enterococcus specie	60 (90)
Staphylococcus aureus	17 (25.76)
Candida species	4 (6.06)

Table 4. Sensitivity testing frequencies with Trimethoprim and Sulfamethoxazole.

Antibiotic	Susceptible (%)	Intermediate (%)	Resistant (%)
Ampicillin	100	0	0
Penicillin	72.7	0	27.3
Clindamycin	80.0	0	20.0
Erythromycin	75.5	0	24.5
Amoxiclav	100	0	0
Ceftriaxone	100	0	0
Vancomycin	86.5	10.5	3
Amikacin	0	0	100
Gentamicin	0	0	100
Nalidixic acid	0	0	100
Kanamycin	0	0	100

Discussion

A growing body of literature reported that the infection rate and the frequency of antibiotic resistance of GBS have increased in adults (16). Taking into account all studies to date, these different incidence rates well correlated with geography, age, gender and collection sites reveal serious reservations about performing the susceptibility test before prescribing any antibiotic therapy (17).

To elucidate the frequent sites in various age and gender groups and resistance rate of GBS, 2400 samples from clinical laboratories in Mashhad, Iran, were studied and patterns of the antibiotic activity were carried out by the disk diffusion susceptibility.

Of the 2400 specimens, vagina indicated a higher proportion of GBS infection (11.05%) among the rest of isolates. In previous studies the frequency of GBS collection from different sites has also predominated in vaginal swabs (15, 18, 19).

Surprisingly comparison of our findings with those from Iran or other countries disclosed that the resistance rate of GBS to Clindamycin and Erythromycin were among ranges (4-43% for Clindamycin and 1.7-46% for Erythromycin) so far recorded (16). We detected lower resistant rate to Clindamycin than those to Penicillin and Erythromycin. Besides, GBS isolates showed full susceptibility against Ceftriaxone providing another alternative option for treating patients especially women with a penicillin allergy in our environment (20).

Our results highlight a rapid screening method for diagnosing GBS in women. In addition, Clindamycin and Ceftriaxone are suggested as alternative antibacterial against GBS.

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