Microbiological monitoring of antibiotic resistance of strains of *Streptococcus agalactiae* among pregnant women


*City Clinical Hospital No. 9 of the Dnipro City Council, Dnipro, Ukraine
**Oles Honchar Dnipro National University, Dnipro, Ukraine

**Oles Honchar Dnipro National University, Gagarin av., 72, Dnipro, 49023, Ukraine. Tel.: +38-096-225-04-63. E-mail: makinshela12@gmail.com


Introduction

Bacterial resistance to antibacterial drugs (ABD) is an international problem of health care, science, pharmaceuticals and society. By 2050, the WHO predicts the human death rate from infections caused by antibiotic-resistant strains of bacteria at the level of 10 million people per year. The pharmaceutical industry and science are faced with the problem of finding new molecules that could affect the causative agents of bacterial infections, due to the lack of sufficient funding for programs and the rapid development of bacterial resistance to ABD. Today, microbiological laboratories register strains of bacteria that are resistant to the latest generation of ABDs, which were included in the WHO classification “Access, Watch, Reserve – AwaRe”. Antimicrobial resistance is at the forefront of the global problems of national and global health care systems. According to the Centers for Disease Control and Prevention (CDC), there are 2.8 million cases of infections caused by microorganisms resistant to antimicrobial drugs in the United States. In 2019, the CDC found that more than 35,000 people died as a result of developing a systemic infection caused by resistant strains of bacteria (Azarn et al., 2020; Zwane et al., 2021). Urinary tract infection (UTI) occurs in approximately 150 million people worldwide each year and can cause morbidity among infants, women of childbearing age, and elderly patients (Ballgh et al., 2018; Gao et al., 2018). *Streptococcus agalactiae*, also known as group B *Strepto-

Keywords: pregnancy; UTI; GBS; antibacterial drugs; bacteria; trimester of pregnancy.
tion caused by GBS in pregnant women is estimated to be 0.38 per 1000 pregnancies with a fatality rate of 0.2% (Vornhagen et al., 2017; Raabe & Share, 2019; Nguyen et al., 2021).

While *Escherichia coli* is the predominant microorganism found in symptomatic and asymptomatic UTIs, GBS has been isolated in 2.1% to 30% of AB cases in pregnant women. Symptomatic infections such as cystitis and pyelonephritis are less common, with an estimated prevalence of 1.5% and 1–2%, respectively (Lee et al., 2019; Angulo López et al., 2020; Rosenberger et al., 2020). Pregnant women are at risk for UTI, with a prevalence of approximately 2–10%. Reduced immunity promotes the growth of both commensal and non-commensal microorganisms. UTI in pregnant women often manifests as asymptomatic bacteriuria. AB during pregnancy poses a significant risk, as there is a high probability (up to 40%) of progression to acute pyelonephritis, which can cause morbidity and even death of the mother and fetus (Rosana et al., 2020; Mohanty et al., 2021; Balachandran et al., 2022).

Colonization of pregnant women with a high number of GBS colonies continues to be a major risk factor for both the mother and the newborn (Rosenberger et al., 2020; Chelkeba et al., 2022; Warrier et al., 2022). A high colony count is defined as at least 105 (100,000) colony forming units per milliliter (CFU/mL) in the urine. A low colony count is less than 10^6 CFU/mL. A urine culture obtained using an average clean urine sample at 12 to 16 weeks gestation is the standard of care and established diagnostic method for AB. Confirmatory culture of a second sample to control contamination or provisional AB is recommended, although not usually performed. A culture result of at least 10^5 CFU/mL of GBS is considered positive, and lower values indicate anogenital colonization (Rosana et al., 2020; Rosenberger et al., 2020; Chelkeba et al., 2022).

The pathogenic mechanisms underlying acute UTIs due to GBS are related to various virulence factors, including surface-expressed protein adhesion molecules, immune evasion factors, and toxins. The main virulence factors of *S. agalactiae* are capsular polysaccharide, which is involved in virulence and immune evasion, and surface proteins, which are involved in the pathogenesis of GBS and immunization (Jalaliar et al., 2019; Desai et al., 2021; Nguyen et al., 2021). Uropathogenic GBS are able to bind to uroepithelial cells of the urinary bladder and can induce cytokine production by bacterial β-hemolysin/cytolsyn, which induces a strong neutrophilic infiltrate in the bladder and is cytotoxic to uroepithelial cells and may enhance the pathogenicity of *S. agalactiae,* leading it to cause acute UTI. The sialic acid substructures of the capsular polysaccharide, as well as the cathelicidin derived from the host organism, also apparently influence the outcome of *S. agalactiae*-induced UTI (Lee et al., 2019; Desai et al., 2021).

The proliferation of bacteria in pyelonephritis affects the fetus, causing the release of bacterial endotoxins, which initiate the release of toxic inflammatory cytokines, which causes local blood circulation disorders in the placenta. This leads to mid-term abortions, stillbirths, low birth weight and intrauterine growth retardation. Products secreted by the bacteria, such as mucinases and proinflammatory toxins, break down the mucus plug, leading to premature rupture of membranes, which predisposes to preterm labour. In addition, due to the destruction of the mucus plug and the insufficiently developed immunological system of the fetus, bacteria easily colonize and multiply in the fetus, which leads to septicaemia (Genovese et al., 2020; Totadhi et al., 2022).

The widespread use of antibiotics in recent decades has contributed to the growth of resistance to this group of drugs. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), 100% sensitivity of group B streptococci strains corresponds to the expected sensitivity of the EUCAST guideline, therefore GBS strains resistant to benzylpenicillin should be re-identified, and sensitivity to this antibiotic drug determined by the minimum inhibitory concentration method. Isolates of group B streptococci characterized by reduced sensitivity to penicillins are rare. GBS resistance to erythromycin and clindamycin is due to two resistance mechanisms encoded by the erythromycin ribosomal methylase genes, namely erm A and erm B, which encode the methylation process of erythromycin and clindamycin receptor sites in ribosomes. The expression of these genes is defined as the MLSB phenotype, which is associated with cross-resistance to macrolides, lincosamides, and streptogramin B. The MLSB phenotype can be both constitutive macrolides-
against antimicrobial resistance at the regional, national and international levels.

**Materials and methods**

For the year 2021, 3,356 samples of the average portion of urine of pregnant women of Dnipropetrovsk region were examined for the purpose of screening for the presence of AB and symptomatic UTI. The bacteriological method of quantitative culture of urine on Columbia agar with 5% lamb blood (Graso, Poland), Strepto B chromogenic agar (Biomerieux, France) and Todd-Hewitt broth enrichment medium (Biomerieux, France) was used. Primary crops were incubated for 18–24 hours at a temperature of 37 °C. Suspect grey translucent colonies (Biomerieux, France) in order to detect the number of colonies greater than 10^3 CFU/mL, the seeds were incubated at a temperature of 37 °C with subsequent identification of suspicious colonies.

Determination of sensitivity and interpretation of antibiotic patterns of identified S. agalactiae to ABD was performed by the disk diffusion method, according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). To determine the sensitivity of GBS to antibiotics, we used discs with benzylpenicillin (1 U) (Liofilchem, Italy), tetracycline (30 μg) (Pharmaktiv, Ukraine), levofloxacin (5 μg) (Farmaktiv, Ukraine), erythromycin (5 μg) (Farmaktiv, Ukraine), clindamycin (2 μg) (Farmaktiv, Ukraine), gentamicin (15 μg) (Farmaktiv, Ukraine), nitrofurantoin (100 μg) (Himedia, India).

The database with the results of antibioticograms of S. agalactiae strains isolated from the urine of pregnant women was created. After entering the results of the research, statistical data processing was carried out using ANOVA variance analysis in order to find dependence in the obtained data by studying the significance of differences in average values and correlation.

**Results**

In 2021, 149 strains of GBS were isolated from 3,356 urine samples of pregnant women in the Dnipropetrovsk region (4.4%). 69 strains of GBS (61.0%) were isolated from the urine of pregnant women who turned to obstetrician-gynecologists in a women’s consultation for the purpose of registration and periodic examination, 33 strains (30.0%) from women who were admitted to the Department of Pregnancy Pathology, 11 strains (9%) from women who were in the maternity ward.

It was established that among the studied contingent, 41 strains of GBS were isolated in the first trimester of pregnancy (27.5%), 59 strains (39.6%) in the second trimester, 49 strains (32.9%) in the third trimester (correlation coefficient -0.45; P< 0.05; Fig. 1). From the urine of pregnant women under the age of 25, 28 strains of GBS were isolated (18.8%), from women aged 26 to 35 – 87 strains (58.4%), from women over 35 – 34 strains (22.8%) (correlation coefficient –0.10). The number of GBS colonies greater than 10^6 CFU/mL was determined in 28 urine samples (18.8%), less than 10^2 CFU/mL – in 121 urine samples (81.2%). The amount of GBS greater than 10^6 CFU/mL (n = 28) in the 1st trimester of pregnancy was detected in 8 urine samples (28.6%), in the 2nd trimester – in 9 samples (32.1%), in the 3rd trimester – in 11 samples (39.3%) (correlation coefficient –0.98; P < 0.05; Fig. 2).

Among 149 strains of GBS, 38 strains (25.5%) were found to be resistant to norfloxacin, 45 strains (30.2%) to erythromycin, 41 strains (27.5%) to clindamycin, 125 strains (83.8%) to tetracycline, 20 strains (13.4%) to levofloxacin, 3 strains (2.0%) to nitrofurantoin. All strains of GBS were susceptible to benzylpenicillin (Fig. 3).

**Fig. 1. Relationship of the number of GBS (n = 149) strains isolated from the urine of pregnant women and the trimester of pregnancy**

**Fig. 2. Dependence of the number of GBS >10^6 CFU/mL (n = 28) and trimester of pregnancy**

47 GBS strains (31.5%) were found to be resistant to at least three groups of ABD. The number of resistant strains of GBS (n = 47) identified from the urine of pregnant women in the first trimester of pregnancy was 11 strains (23.4%), in the second trimester – 19 strains (40.5%), in the third trimester – 17 strains (36.1%) (correlation coefficient -0.73; P < 0.05; Fig. 4).
Effectiveness of urine tests for the purpose of detecting GBS.

Ukraine. A direct relationship between the number of identified strains of GBS and the growth of the trimester of pregnancy was revealed. It was established that the age of pregnant women does not affect the effectiveness of urine tests for the purpose of detecting GBS.

The number of strains of GBS (n = 47) isolated from the urine of pregnant women under the age of 25 was 7 strains (14.9%), from 26 to 35 years – 26 strains (55.3%), 36 years and older – 14 strains (29.8%) (correlation coefficient -0.37).

Discussion

GBS is the etiological factor of asymptomatic bacteriuria and symptomatic UTI in 4.4% of cases among the pregnant women tested in Ukraine. A direct correlation was established between the number of identified strains of GBS and the growth of the trimester of pregnancy was revealed. It was established that the age of pregnant women does not affect the effectiveness of urine tests for the purpose of detecting GBS.

The number of GBS less than 10^5 CFU/mL (n = 121, 81.2%), which were isolated from the urine of pregnant women, indicates colonization of the anogenital locus or asymptomatic bacteriuria in 3.6% of cases.

In 1% of cases, GBS is the etiological factor of UTI.

A direct correlation was established between the number of GBS more than 10^5 CFU/mL (n = 28) and the growth of the trimester of pregnancy, which is caused by a decrease in the immune reactivity of the uroepithelium of pregnant women and an increased level of colonization of the urogenital tract by GBS.

In 100% of cases, it is appropriate to use beta-lactam drugs to eradicate GBS as a factor in asymptomatic and symptomatic bacteriuria in pregnant women.

Resistance to norfloxacin of GBS urostams isolated from the urine of pregnant women of Ukraine was found in 25% of cases, to levofloxacin – in 13% of cases. In South Africa 100% of GBS strains were sensitive to ciprofloxacin (Zwané et al., 2021). In China 48.1% of GBS were resistant to fluoroquinolones (Mohanty et al., 2021). Turkish researchers found that 22.5% of S. agalactiae strains isolated from the urine of pregnant women were resistant to ofloxacin (Baba & Aydın, 2016). Thus, the data obtained by us indicate that the resistance of GBS to fluoroquinolones correlates with the data of Chinese and American researchers.

The number of resistant strains of GBS (n = 47) isolated from the urine of pregnant women and the trimester of pregnancy

![Graph](image)

Dependence of the number of resistant GBS strains (n = 47) isolated from the urine of pregnant women and the trimester of pregnancy

Researchers from United Arab Emirates found that 42% of GBS strains were resistant to clindamycin (Balachandran et al., 2022), and in Poland 18% of GBS strains were resistant to this antibacterial drug (Dobrat et al., 2022). In China, 44.4% of GBS strains isolated from the urine of pregnant women were resistant to clindamycin, in the USA – 26.4% of such strains (Mohanty et al., 2021). Spanish researchers found that 80% of the strains of GBS isolated from the urine of pregnant women were resistant to clindamycin (Álvarez-Santás et al., 2018). Turkish researchers found that 5% of S. agalactiae strains isolated from the urine of pregnant women were resistant to this drug (Baba & Aydın, 2016). Thus, the data obtained by us indicate that the resistance of GBS to erythromycin correlates with the data of Polish and American researchers.

In Ukraine, 84% of the GBS strains isolated from the urine of the pregnant women tested were resistant to tetracycline. In the USA, 80.4% of GBS strains isolated from the urine of pregnant women were resistant to this drug (Mohanty et al., 2021). Chinese researchers found that 74.1% of GBS strains were resistant to tetracycline (Guo et al., 2018). Thus, the data obtained by us indicate that the resistance of GBS to tetracycline correlates with the data of Chinese and American researchers.

It was established that 2% of GBS urostams isolated from pregnant women were resistant to nitrofurantoïn.

Conclusions

GBS was found to be the etiological factor of asymptomatic bacteriuria and symptomatic GBS in 4.4% of cases among pregnant women of the Dnipropetrovsk region. A direct correlation was established between the number of GBS more than 10^5 CFU/mL and the progression of the trimester of pregnancy, which is caused by a decrease in the immune reactivity of the uroepithelium of pregnant women and an increased level of colonization of the urogenital tract by GBS. In 100% of cases it is appropriate to use beta-lactam antibiotics and in 98% of cases – nitrofurantoïn for the treatment of infection of the genitourinary system of pregnant women caused by GBS. The rate of resistance of GBS urostams to fluoroquinolones, erythromycin, clindamycin, and tetracycline correlates with the results of studies in other countries.

Special thanks to Olenna Finkova, Director General of the Municipal non-commercial enterprise “City Clinical Hospital No. 9” of the Dnipro City Council, for the strong funding of the bacteriological laboratory. Special thanks to the bacteriological doctors of the bacteriological laboratory of the Municipal non-commercial enterprise “City Clinical Hospital No. 9” of the Dnipro City Council for the bacteriological research.

The authors declared no conflicting interests.

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