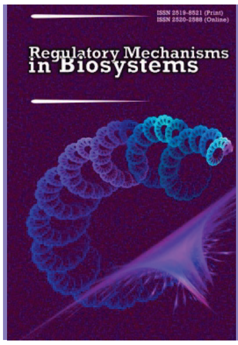

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Microbiological monitoring of antibiotic resistance of strains of *Streptococcus agalactiae* among pregnant women

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Group B *Streptococcus* (GBS) is the causative agent in 2–7% of all urinary tract infections (UTI), including asymptomatic bacteriuria (AB), cystitis, and pyelonephritis. We used the bacteriological quantitative method of sowing urine samples of pregnant women on Columbia agar with 5% lamb blood), Strepto B chromogenic agar and Todd Hewitt broth, identification of GBS strains with determination of sensitivity to bacitracin, hippurate hydrolysis test and CAMP test. Susceptibility to antimicrobial drugs was determined by the disk-diffusion method according to recommendations of the European Committee on Antimicrobial Susceptibility Testing. For the period of 2021, out of 3,356 urine samples of pregnant women, there were 149 samples of the investigated biomaterial with a positive result for GBS (resultancy – 4.4%). It was established that among the studied contingent, 41 strains of GBS were isolated in the first trimester of pregnancy (27.5%), in the second trimester – 59 strains (39.5%), in the third trimester – 49 strains (33.0%). It is established that out of the 149 strains of GBS, 38 strains (25.0%) were resistant to norfloxacin, 45 strains (30.0%) to erythromycin, 41 strains (28.0%) to clindamycin,125 strains (84.0%) to tetracycline, 20 strains (13.0%) to levofloxacin, 3 strains (2.0%) to nitrofurantoin. All strains of *Streptococcus agalactiae* were found to be sensitive to benzylpenicillin. The number of resistant strains of SGB identified from the urine of pregnant women was 47 strains. All resistant strains were resistant to at least three groups of antibacterial drugs, except beta-lactams. The results of the scientific research will allow us to obtain dynamic data on the antibiotic resistance of GBS strains in order to study the rate of development of antibiotic sensitivity of this microorganism. In the future, the research data can be used by scientists when reviewing protocols for the treatment of urinary tract infections in pregnant women.

Keywords: pregnancy; UTI; GBS; antibacterial drugs; bacteriuria; trimester of pregnancy.

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 (Asmat 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 (Balkhi et al., 2018; Guo et al., 2018). *Streptococcus agalactiae*, also known as group B *Streptococcus* (GBS), is part of the resident microflora of the human gastrointestinal and genitourinary tracts (Leclercq et al., 2016; Álvarez-Santás et al., 2018). Is one of many serologically distinct species within the genus *Streptococcus*. It is an encapsulated diplo-streptococcus that causes β-hemolysis on Columbia 5% lamb blood agar, a facultative anaerobe, fastidious to nutrients, negative for catalase and mannitol. It also hydrolyzes sodium hippurate, which is resistant to bacitracin, giving a positive CAMP test (Gizachew et al., 2019).

GBS is an etiological factor in 2–7% of all UTIs, including asymptomatic bacteriuria (AB), cystitis, and pyelonephritis (Balkhi et al., 2018; Zwane et al., 2021). Each year, approximately 6 million births are premature and more than 500,000 newborns die due to prematurity, accounting for 44% of all under-five deaths. The majority of early preterm births are associated with microbial infection, and approximately 10% are due to GBS (Vornhagen et al., 2017). Recto-vaginal colonization of GBS contributes to ascending UTI and can lead to vertical transmission in utero or during delivery, causing life-threatening neonatal infections (Vornhagen et al., 2017; Furfaro et al., 2018; Moraleda et al., 2018).

Recently, considerable effort has been directed toward measuring global rates of GBS colonization, invasive disease, and associated risk factors. GBS-induced bacteriuria among women during pregnancy is a risk factor for late gestational maternal GBS colonization and early-onset neonatal GBS infection (do Nascimento et al., 2019; Santana et al., 2020; Warrier et al., 2022). Worldwide, the incidence of systemic invasive infection 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 & Shane, 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 10^5 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 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 (Jalalifar 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/cytolysin, 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 septicemia (Genovese et al., 2020; Totadhri 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 antibacterial 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 macrolide-lincosamide-streptogramin B (cMLSB) resistance and inducible resistance to macrolide-lincosamide-streptogramin B (iMLSB) (Guo et al., 2018; Dobrut et al., 2022). Streptococci can be resistant to macrolides through the production of ribosomal erm methylases, causing the MLSB phenotype, or through the expression of efflux pumps encoded by genes of the mef(A) class. In the case of MLSB-induced resistance, clindamycin may retain or lose activity depending on the types and levels of expression of erm genes. In the case of efflux-induced resistance, the risk of selection of clindamycin-resistant mutant strains is no higher than for erythromycin-susceptible isolates. When using the disk diffusion test, the induced MLSV phenotype is determined by the presence of a D-shaped flattening of the zone of growth inhibition around the disc with clindamycin versus the disk with erythromycin. In this case, this drug is not recommended for the treatment of severe infections.

High levels of GBS resistance to streptomycin are determined by the production of ANT (6) or other enzymes or by ribosomal mutations. In enterococci with a high level of resistance to streptomycin, the synergistic effect between streptomycin and β-lactams is absent (Guo et al., 2018; Dobrut et al., 2022). GBS resistance to kanamycin is associated with the production of APH(3')-1-3 or bifunctional APH (2')-ACC(6) enzymes, which determine the reduction of synergism between kanamycin and amikacin and β-lactams and glycopeptides regardless of MIC levels. The high level of resistance to gentamicin is due to the production of bifunctional APH (2')-ACC(6) enzymes, which determine the reduction of synergism between all aminoglycosides (except streptomycin and arbekacin) and beta-lactams and glycopeptides, regardless of MIC levels (Guo et al., 2018; Dobrut et al., 2022). GBS resistance to tetracycline is most often caused by two mediated genes, tet(M) and tet(O), which are transmitted mainly by transposons (Ali et al., 2020; Khan et al., 2023). The resistance of GBS to fluoroquinolones is due to the presence of mutations in the gyrA and parC genes, therefore, in the bacteriological laboratory, the phenotypic definition of resistance to this group of antibacterial drugs is determined by sensitivity to norfloxacin as a screening test. If the strain is positive, it is necessary to determine the sensitivity separately for each representative of the fluoroquinolone series (Barros, 2021). GBS resistance to nitrofurantoin has not yet been scientifically studied, but researchers and molecular geneticists have identified so-called "hypothetical proteins", helix-turn-helix domain protein and recombinase. Currently, two genes responsible for GBS resistance to nitrofurantoin – nsfA and nsfB – have been reported by world researchers (Guo et al., 2018).

Beta-lactam antibiotics, particularly penicillins and their derivatives, are the drugs of choice for the empiric treatment of GBS-induced urinary tract infections in pregnant women. Therefore, it is important that the level of resistance of this microorganism to beta-lactam drugs does not increase, since it will be difficult to choose a drug to eradicate the beta-lactam-resistant GBS strain, which can cause a urinary tract infection in a pregnant woman, precisely because of the side effects. Intravenous penicillin G is the drug of choice for GBS antibiotic prophylaxis during labour. Penicillin G 5 million units is given intravenously as a loading dose. Ampicillin is an alternative to penicillin G when penicillin G is not available (Santana et al., 2020). Penicillin G and ampicillin should not be used in patients allergic to penicillin. Antibiotic prophylaxis in patients with a history of anaphylaxis, angioedema, respiratory distress, or urticaria after the use of penicillin or cephalosporins is based on the results of antibiotic sensitivity testing. If GBS is sensitive to both clindamycin and erythromycin, clindamycin 900 mg IV every 8 hours is recommended for GBS prophylaxis during labour until delivery. GBS resistance to erythromycin can cause inducible resistance to clindamycin even in the presence of a culture that appears sensitive to clindamycin. For this reason, if the culture is resistant to erythromycin, vancomycin 1 g IV every 12 hours is recommended for GBS prophylaxis (Santana et al., 2020).

Screening the urine of pregnant women for the presence of GBS allows early detection of asymptomatic bacteriuria, obtaining results of the sensitivity of strains to antibacterial drugs, prescribing prophylactic or empiric treatment in order to prevent the development of infectious complications caused by this microorganism (do Nascimento et al., 2019; Santana et al., 2020; Warrier et al., 2022).

Pregnant women are a contingent of the risk group, because pregnancy causes a decrease in the general immunoreactivity of the body, a change in the immunoreactivity of the epithelium of the urinary tract, changes the chemical and physical properties of urine, which can cause an ascending infection. The range of antimicrobial drugs that can be used to treat infections in pregnant women is quite limited, which is due to the side effects of most drugs, in particular, toxicity and effects on the fetus. Therefore, antimicrobial resistance poses a great threat to pregnant women, fetuses and newborns, because drugs that can affect resistant strains have a significant list of side effects (Santana et al., 2020; Warrier et al., 2022).

It was with the aim of obtaining reliable data on antimicrobial resistance of GBS that we conducted these scientific studies, which made it possible to establish a starting point in dynamic scientific studies of this problem for further contribution to the scientific and practical component of the fight 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. Suspicious grey translucent colonies with β-hemolysis on Columbia agar, pink colonies on chromogenic Strepto B agar were subjected to identification with the determination of sensitivity to bacitracin, the ability to hydrolyze hippurate, CAMP test and using a GP card to the VITEK 2 compact 15 microbiological analyzer. The enrichment medium was seeded on Strepto B chromogenic agar (Biomeriuex, France) in order to detect the number of *S. agalactiae* isolates less 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), norfloxacin (10 μg) (Farmaktiv, Ukraine), erythromycin (15 μg) (Farmaktiv, Ukraine), clindamycin (2 μg) (Farmaktiv, Ukraine), tetracycline (30 μg) (Pharmaktiv, Ukraine), levofloxacin (5 μg) (Pharmaktiv, 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 10^5 CFU/mL was determined in 28 urine samples (18.8%), less than 10^5 CFU/mL – in 121 urine samples (81.2%). The amount of GBS greater than 10^5 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).

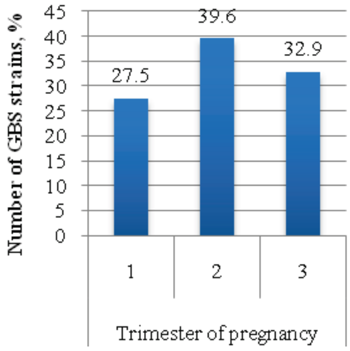

Fig. 1. Relationship of the number of GBS (n = 149) strains isolated from the urine of pregnant women and the trimester of pregnancy

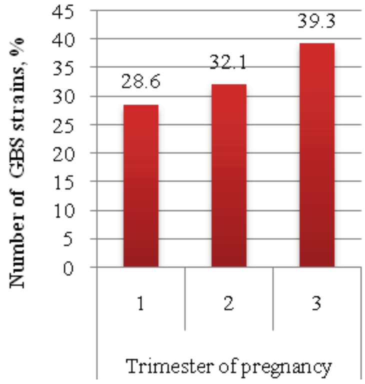

Fig. 2. Dependence of the number of GBS >10^5 CFU/mL (n = 28) and trimester of pregnancy

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).

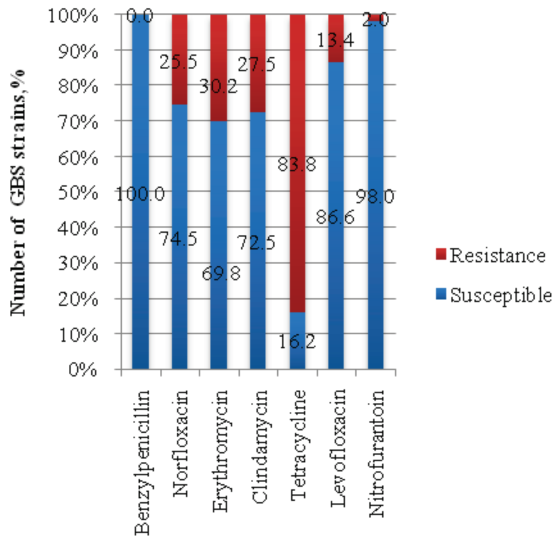

Fig. 3. Percentage of antibiotic-resistant GBS strains (n = 149) isolated from urine of pregnant women

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).

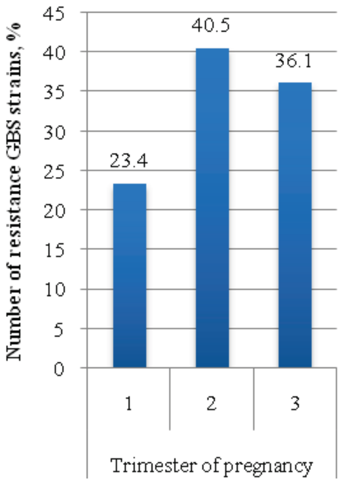

Fig. 4. Dependence of the number of resistant GBS strains (n = 47) isolated from the urine of pregnant women and the trimester of pregnancy

The number of resistant 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 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 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 (Zwane 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 Turkish colleagues who studied the identical problem.

It was established that 30% of the strains of GBS isolated from the urine of pregnant women of the Ukraine were resistant to erythromycin. Polish researchers established that 22% of GBS strains were resistant to this antibacterial drug (Dobrut et al., 2022). In China, 63% of GBS strains were resistant to erythromycin, in the USA – 39.5% of such strains (Mohanty et al., 2021). Other researchers from China (Suzhou) found that 63% of GBS strains were resistant to this antibacterial drug (Guo et al., 2018). In Spain, 81% of strains of GBS isolated from the urine of pregnant women were resistant to erythromycin (Álvarez-Santás et al., 2018). Turkish researchers found that 10% 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.

It was established that 28% of the strains of GBS isolated from the urine of pregnant women tested in Ukraine were resistant to clindamycin. 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 (Dobrut 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 nitrofurantoin.

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 β-lactam antibiotics and in 98% of cases – nitrofurantoin 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.

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