ORIGINAL ARTICLE

PECULIARITIES OF DISTRIBUTION OF ANTIBIOTIC RESISTANT STRAINS OF *E. COLI - E. FAECALIS* ASSOCIATION IN THE UROGENITAL TRACT OF PREGNANT WOMEN

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ABSTRACT

The aim: To investigate the prevalence of antibiotic-resistant strains from the association of *E. coli - E. faecalis* in the urogenital tract of pregnant women.

Materials and methods: Used bacteriological method of sectoral culture of urine on nutrient media and identification of strains to the species. The susceptibility of strains to antibiotics was determined by disk-diffusion method, the interpretation of results - according to current EUCAST recommendations. Statistical processing was performed in MS Excel (Analysis of variance (ANOVA)).

Results: The number of resistant to cefuroxime, cephalexin, nitrofurantoin, norfloxacin and trimethoprim uropathogenic strains of Escherichia coli does not change depending on the monostrain and associated strain of *E. coli* from the urine of pregnant women in Dnipro. It was found that the differences in the number of fosfomycin-resistant uropathogenic strains of *E. coli* depending on the type of culture - the number of fosfomycintolerant urostrains *E. coli* is 2 times higher among the associated strains, compared with monostains of the studied microorganism. The number of uropathogenic strains of *E. faecalis* resistant to ampicillin, trimethoprim, nitrofurantoin and norfloxacin does not change depending on the type of culture isolated from the urine of pregnant women in Dnipro.

Conclusions: The symbiotic type of existence of two representatives of the normal intestinal flora on the uroepithelium of pregnant women may be associated with the development of *Escherichia coli* antibiotic resistance only to fosfomycin compared with monoinfection caused by each of these microorganisms.

KEY WORDS: antibiotic resistance, pregnancy, urogenital tract, urine, fosfomycin-resistans

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INTRODUCTION

Uncontrolled use of ABD in everyday life, the lack of a well-established prescription release in pharmacies, leads to the creation of a reservoir of resistance determinants among pathogenic and, above all, opportunistic bacteria [1-3]. Bacterial resistance to antibiotics is considered one of the main modern problems of clinical microbiology and general therapeutic activity of clinicians. The development of bioengineering and modern biotechnological progress contribute to the discovery and development of new antibacterial drugs (ABD) in the pharmaceutical market in Ukraine and the world, but not all antibiotics are widely used clinically [4, 5]. The problem with antibiotic resistance is primarily that bacteria have a high capacity for phenotypic and genetic variability. They are able to adapt even to those ABD that are considered universal and starting in the treatment of bacterial infections [6, 7]. Bacteriologists and epidemiologists are increasingly registering strains of Escherichia coli and Enterococcus faecalis that become resistant to antibiotics. These microorganisms are opportunistic pathogens for humans and are in close symbiosis with our body, playing a role in the digestive system and the creation of colonization resistance of the gastrointestinal mucosa [2, 8, 9]. Usually these microorganisms are considered to be representatives of the normal intestinal flora, however, some strains of these bacteria are already polyresistant. This has no effect on the breakdown function of nutrients, but when immunoreactivity is impaired, they can alter loci of residence, causing infections such as urinary tract infections (UTI) in pregnant women. Given that Escherichia coli and Enterococcus faecalis are components of the biofilm of the intestinal mucosa, have intercellular contact in symbiosis, are partly represented by multidrug-resistant strains, there is an increased risk of complex superinfections. The study of biofilms and the association of bacteria contributes to the development of certain provisions on their antibiotic sensitivity and contributes to a scientifically sound revision of UTI treatment protocols in pregnant women, taking into account the dose of ABD, the behavior of bacteria in biofilms under different drug concentrations and the establishment of antibiotic resistance [10].

THE AIM

The objectives of the study of the spread of antibiotic-resistant strains from the association of *E. coli - E. faecalis* in the urogenital tract of pregnant women are to study antibiotic resistance to ABD isolates and to establish differences between antibiotic resistance of *E. coli* and *E. faecalis* isolates under conditions of monostrains and association strains.

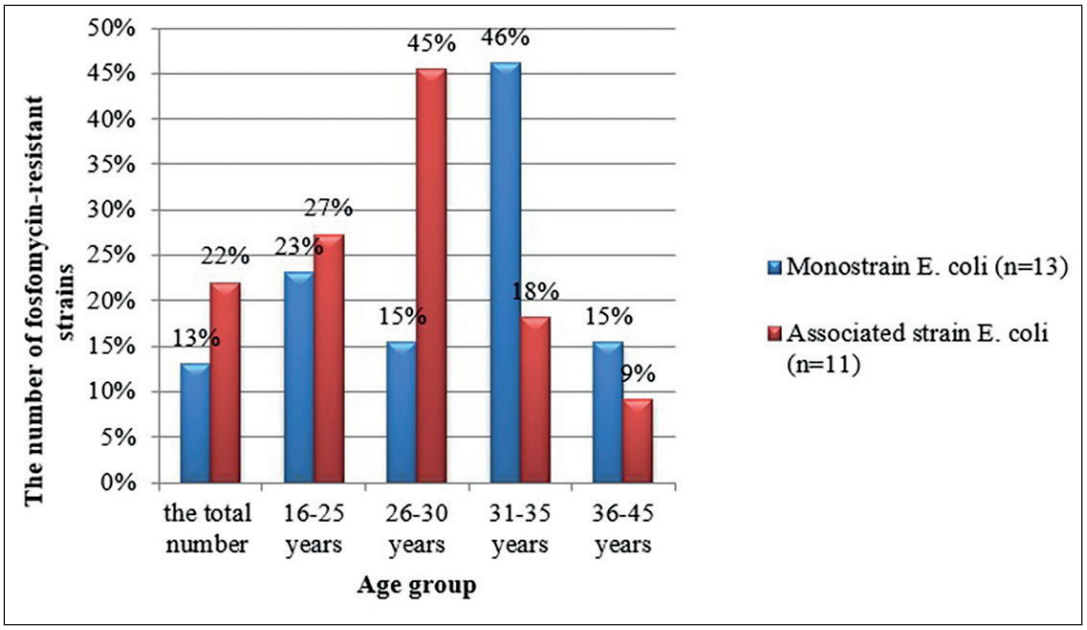

Fig. 1. Frequency of detection of fosfomycin-resistant strains of *E. coli* in women of different ages

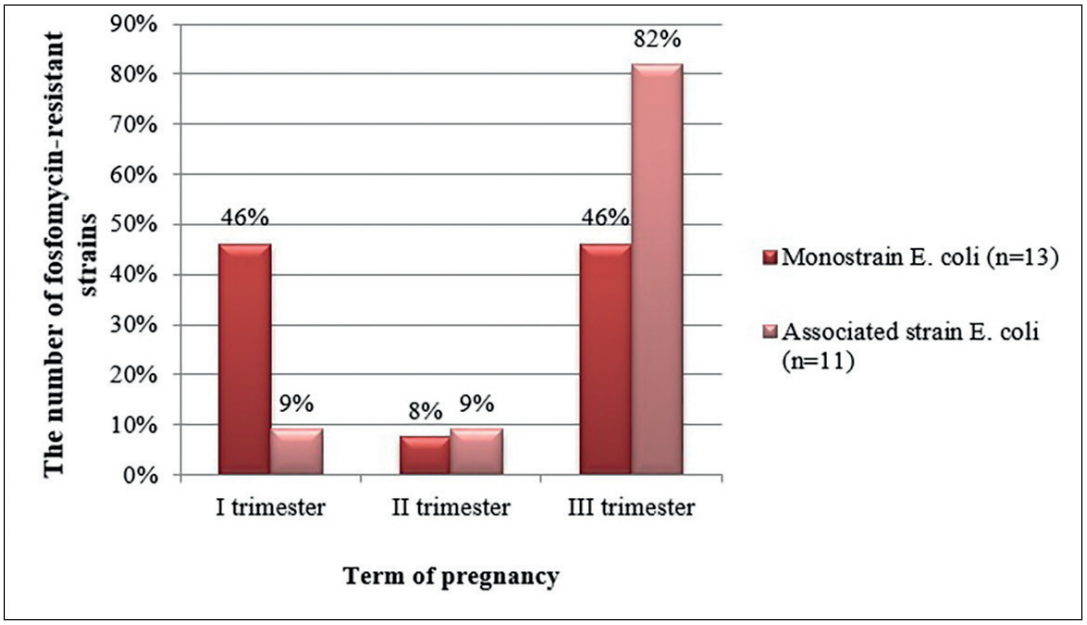

Fig. 2. Frequency of detection of fosfomycin-resistant *E. coli* strains in women of different groups depending on the gestational age

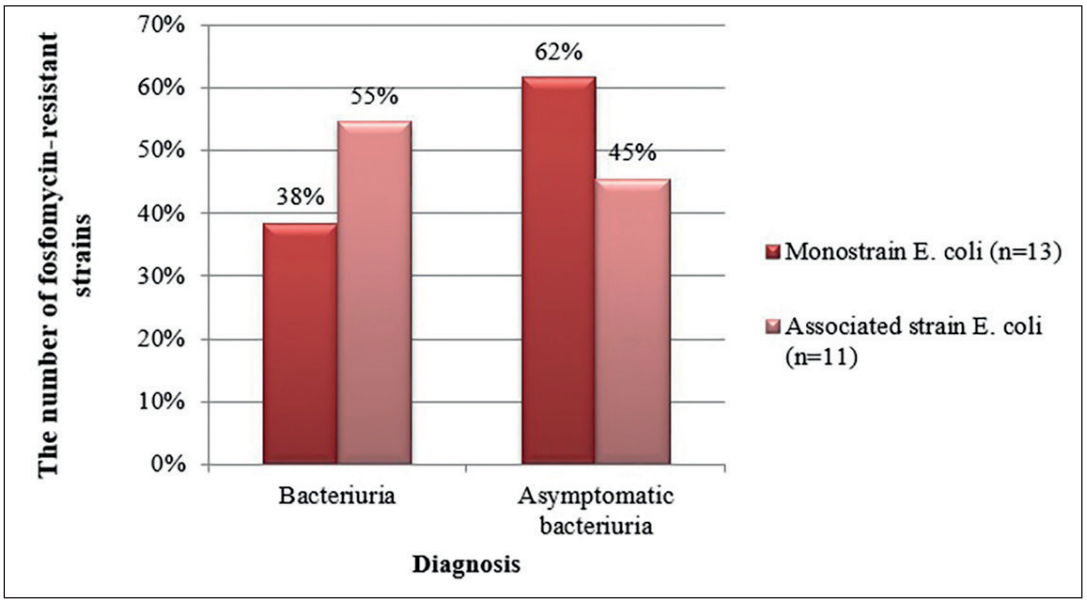

Fig. 3. Frequency of detection of fosfomycin-resistant strains of *E. coli* depending on the diagnosis

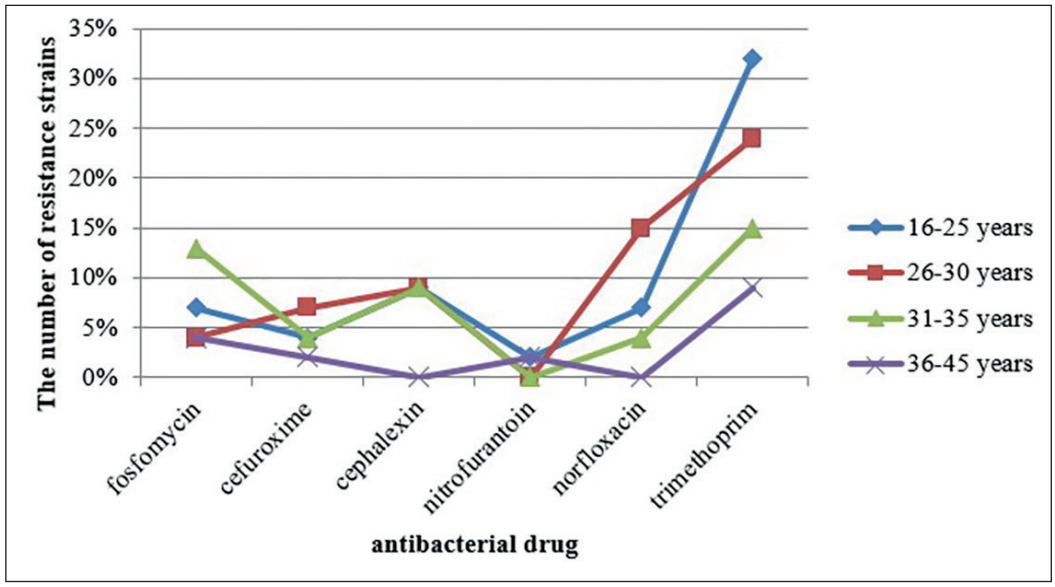

Fig. 4. Frequency of detection of antibiotic-resistant *E. coli* monostrains (n = 45) in different age groups of pregnant women

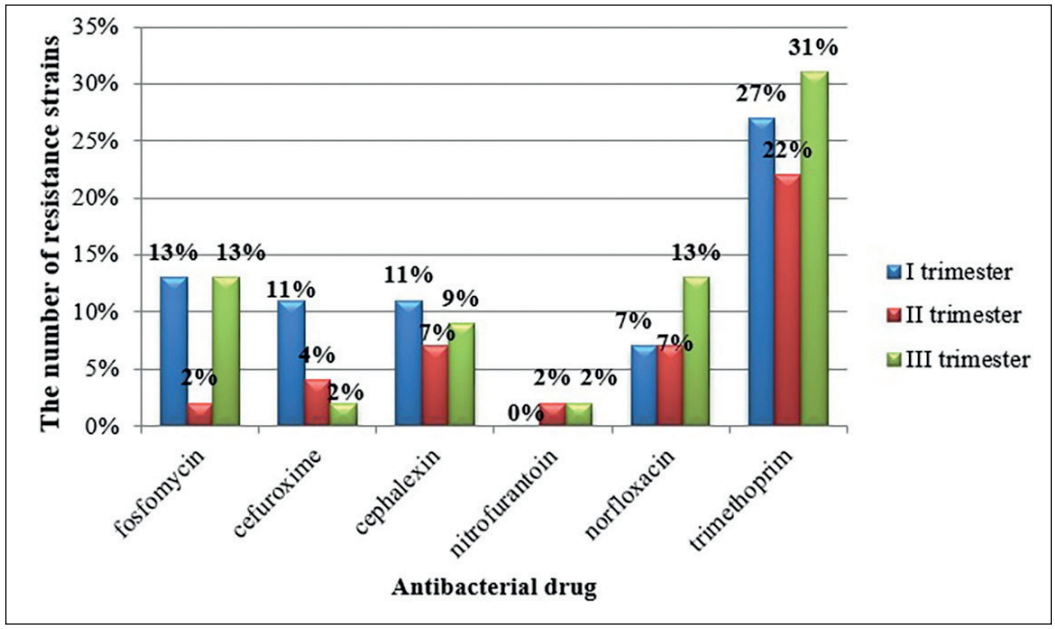

Fig. 5. Frequency of detection of antibiotic-resistant strains of *E. coli* (n = 45) depending on the gestational age

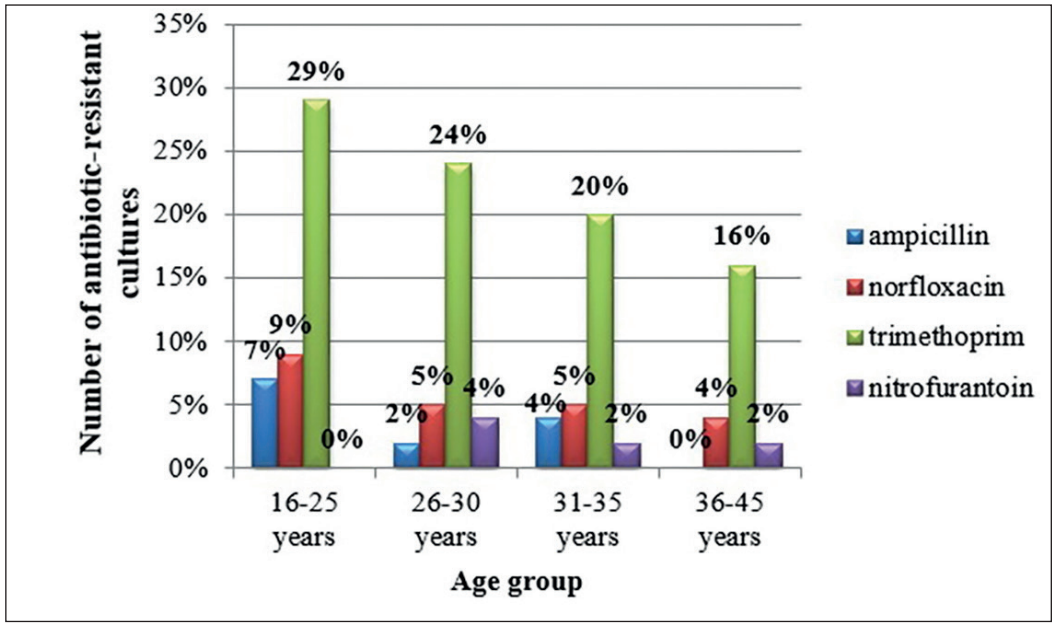

Fig. 6. Frequency of detection of antibiotic-resistant strains of *E. faecalis* (n=55) in different age groups of examined pregnant women

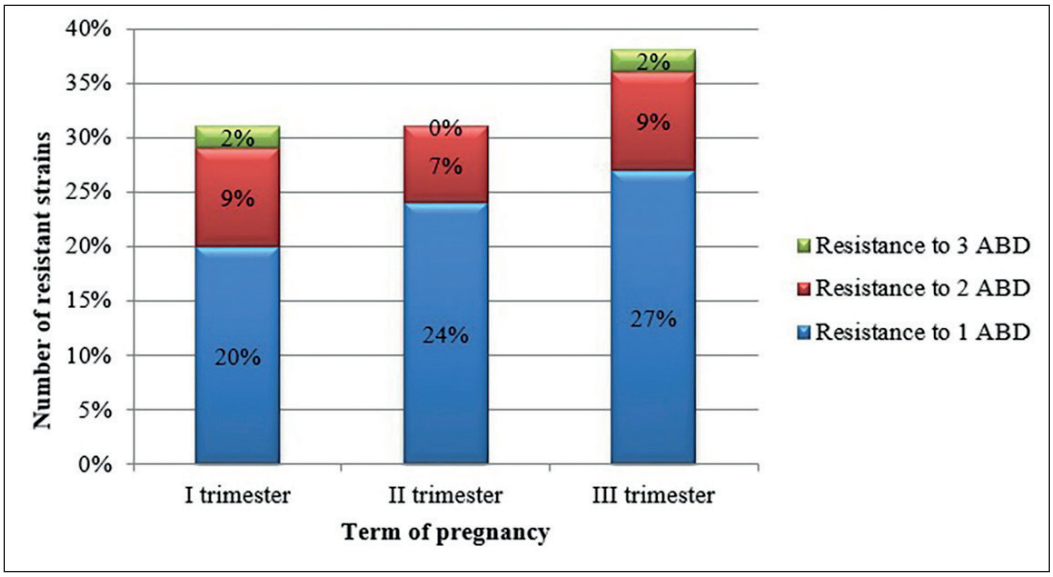

Fig. 7. Frequency of detection of mono- and polyresistant strains of *E. faecalis* (n=55) in women of the examined groups at different stages of pregnancy

MATERIALS AND METHODS

250 urine samples (v = 100 ml) were taken from 250 women for the study. Selection was performed in sterile disposable urine containers. Delivery of biomaterial to the laboratory was not more than 2 hours. Primary culture of urine for sterility was performed by the method of sector cultures on agar Columbia with 5% sheep blood (Grasko, Poland) and 0.25% glucose broth. Petri dishes and tubes with plates on lamellar and liquid nutrient media were incubated at 37 ° C for 18-24 hours. The next day we studied the morphological, tinctorial properties of selected strains, put biochemical tests to identify E. coli and E. faecalis. Incubation of tubes with nutrient media was performed at 37 ° C for 18-24 hours. On the third day, the results of biochemical tests were taken into account, the susceptibility of selected strains of microorganisms was determined by the disk-diffusion method in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The results of antibiotic-ograms of selected strains of E. coli (n = 100), E. faecalis (n = 100) and association E. coli-E. faecalis (n = 50) from the urine of pregnant women were selected for the study. Statistical data processing was performed using ANOVA analysis of variance to find the dependence in the obtained data by studying the significance of differences in averages and correlations.

RESULTS

According to the results of analysis of variance ANOVA growth retardation zones for each antibacterial drug monostrain *E. coli* and its strain from the association *E. coli - E. faecalis* found a significant difference in the two samples of growth retardation zones to fosfomycin - the criterion of significance (P-value) is <0.05, which indicates the presence of statistically significant differences. The criterion of reliability of individual samples of growth retardation zones to cefuroxime, cephalexin, nitrofurantoin, norfloxacin and trimethoprim of two strains of *Escherichia coli* is P> 0.1, which indicates the absence of differences in them.

According to the results of analysis of variance ANOVA growth retardation zones for each antibacterial drug mono strain *E. faecalis* and its strain from the association *E. coli-E. faecalis*, no differences were found in the respective samples - criterion P> 0.1 (for ampicillin, trimethoprim, nitrofurantoin and norfloxacin).

It was found that the number of fosfomycin-resistant mono strains of *E. coli* isolated from the urine of pregnant women is 13 cultures (13% of 100 strains studied), and associated strains of *E. coli* - 11 cultures (22% of the 50 studied strains), which in 1,7 times more.

The number of fosfomycin-resistant strains of *E. coli* depending on the type of culture and age group of pregnant women is presented in Fig. 1.

According to the results of correlation analysis, there is no dependence (coefficient is 0) between the number of fosfomycin-resistant *E. coli* monostains and age, but there is an inverse relationship (coefficient -0.6) between the number of fosfomycin-resistant associated strains of *E. coli* and age that indicates a decrease in the number of such strains depending on the increase in the age category of pregnant women. It was found that among pregnant women aged 16-25 years, the number of fosfomycin-resistant monosteps of *Escherichia coli* isolated from urine is 3 strains (23% of 13 resistant strains), associated *E. coli* - 3 strains (27% of 11 resistant strains). Among pregnant women aged 26-30 years, the number of fosfomycin-resistant monostrains of *Escherichia coli* isolated from urine is 2 strains (15% of 13 resistant strains), associated *E. coli* - 5 strains (45% of 11 resistant strains).

Among pregnant women aged 31-35 years, the number of fosfomycin-resistant monostrains of *E. coli* isolated from urine is 6 strains (46% of 13 resistant strains), associated with *E. coli* - 2 strains (18% of 11 resistant strains).

Among pregnant women aged 36-45 years, the number of phosphomycin-resistant monostrains of *Escherichia coli*

isolated from urine is 2 strains (15% of 13 resistant strains), associated *E. coli* - 1 strain (9% of 11 resistant strains).

Thus, the largest number of fosfomycin-resistant monostrains of *Escherichia coli* (45%) is observed among pregnant women aged 26-30 years, and associated strains of *E. coli* (46%) - among pregnant women aged 31-35 years.

The number of fosfomycin-resistant strains of *E. coli* excreted in the urine, depending on the type of culture and trimester of pregnancy is presented in Fig. 2.

Correlation analysis showed no relationship (coefficient 0) between the number of fosfomycin-resistant *E. coli* monostrains and gestational age, but there is a straightforward relationship (coefficient 0.8) between the number of fosfomycin-resistant *E. coli* strains of pregnancy and trimester pregnancy that is indicating an increase in the number of such strains depending on the increase in gestational age.

It was found that the number of fosfomycin-resistant uropathogenic *Escherichia coli* strains in the first and third trimesters of pregnancy is 6 strains (46% of 13 resistant strains), in the second trimester - 1 strain (8%).

The number of fosfomycin-resistant associated strains of *E. coli* in the first and second trimesters of pregnancy is 1 strain (9% of 11 cultures), in the third trimester - 9 strains (82%).

The number of fosfomycin-resistant strains of *E. coli* isolated from the urine of pregnant women, depending on the type of culture and diagnosis is presented in Fig. 3.

The number of fosfomycin-resistant monostrains of *E. coli* isolated from the urine of pregnant women with bacteriuria is 5 strains (38% of 13 cultures), associated strains - 6 cultures (55% of 11 strains).

The number of fosfomycin-resistant monostrains of *E. coli* isolated from the urine of pregnant women with asymptomatic bacteriuria is 8 strains (62% of 13 cultures), associated strains - 5 cultures (45% of 11 strains).

The distribution of antibiotic-resistant monostrains of *E. coli* depending on the antibacterial drug and age group is presented in Fig. 4

It was found that among fosfomycin-resistant monostrains of *E. coli* (n = 13) isolated from the urine of pregnant women, there is no correlation (coefficient - 0.06) between increasing age and the number of resistant isolates to this ABD, of which the largest number - 6 strains (13% of the 45 isolates) were isolated among pregnant women aged 31-35 years.

Among resistant *Escherichia coli* to cefuroxime (n = 8), there is a negative correlation (coefficient of -0.6) between the number of such strains and age, namely the number of resistant strains decreases depending on the growth of age among pregnant women.

The correlation between the age of pregnant women and the number of resistant urostrains of *E. coli* to cephalexin (n = 12) is -0.7. It was found that among women aged 16-35 years the number of such isolates is distributed evenly (4 strains) between each age group, except for pregnant women aged 36-45 years, among which no resistant to *Escherichia coli* strain was isolated.

The number of *Escherichia coli* resistant to nitrofurantoin is 2 strains isolated from pregnant women of I and IV age groups.

There is a negative correlation between the number of norfloxacin-resistant urostrains of *E. coli* (n = 12) and the age of pregnant women, that is the number of resistant strains decreases with age. The largest number of such strains (7 cultures (15% of 45 isolates) was isolated from the urine of pregnant women aged 26-30 years).

Correlation analysis of the data showed that there is a strong negative relationship between the number of trimethoprim-resistant *Escherichia coli* and the age group of pregnant women. The correlation coefficient is -0.9. The number of such strains for women aged 16-25 years is 14 strains (32% of 45 cultures), aged 26-30 years - 11 cultures (24%), aged 31-35 years - 7 strains (15%) and aged 36- 45 years - 4 strains (9%).

The distribution of antibiotic-resistant monostrains of *E. coli* depending on the antibacterial drug and the trimester of pregnancy is presented in Fig. 5.

According to the results of correlation analysis, it was found that the number of fosfomycin-resistant monostains of *E. coli* (coefficient is 0) does not change with increasing gestational age, but from 45 strains 6 isolates (13%) were excreted in the urine of pregnant women in the first and third trimesters.

There was a negative correlation (coefficient of -1) of the number of *Escherichia coli*-resistant strains to cefuroxime and cephalexin depending on the increase in gestational age, namely, the larger the trimester of pregnancy, the less resistant strains to these cephalosporins are released. The number of *E. coli* urostrains resistant to cefuroxime in the first trimester is 5 cultures (11% of 45 resistant isolates), in the second trimester - 2 cultures (4%), in the third trimester - 1 culture (2%).

The correlation between the number of norfloxacin-resistant *Escherichia coli* strains and trimesters of pregnancy was identified as straightforward (coefficient is 1) - the larger the trimester, the greater the number of resistant *E. coli* monostrains (I trimester - 3 strains (7% of the total), III trimester - 6 strains (13%)).

There is a straight line correlation (coefficient is 1) in the number of trimethoprim-resistant *E. coli* monostrains depending on the growth of the gestational age, that is the larger trimester of pregnancy, the more allocated resistant strains to this ABD. The number of resistant to trimethoprim *E. coli* in the first trimester is 12 cultures (27% of 45 resistant isolates), the second trimester - 10 cultures (22%), the third trimester - 14 cultures (31%).

The distribution of antibiotic-resistant urostrains of *E. faecalis* (n = 55) depending on the age group of pregnant women and ABD is presented in Fig. 6

According to the results of correlation analysis, an inverse relationship (correlation coefficient is -0.8) was found between the number of ampicillin-resistant *E. faecalis* monostrains and the age of pregnant women, which indicates a decrease in the number of ampicillin-resistant strains depending on age. The number of ampicillin-resistant

E. faecalis isolated from the urine of pregnant women aged 16-25 years is 4 strains (7% of 55 resistant strains), from 26-30 years - 1 strain (2%), from 31-35 years - 2 isolates (4%).

The relationship between the number of norfloxacin-resistant *E. faecalis* urostrains and the age of pregnant women is a reverse linear trend (correlation coefficient is -0.8), indicating a decrease in the number of norfloxacin-resistant enterococcal strains depending on age. The number of norfloxacin-resistant cultures of fecal enterococci isolated from the urine of pregnant women aged 16-25 years is 5 strains (9% of 55 isolates), from 26-30 years - 3 strains (5%), from 31-35 years - 3 strains 5%), from 36-45 years - 2 cultures (4%).

There is a rapid trend and an inverse correlation (coefficient of -0.9) between the number of trimethoprim-resistant *E. faecalis* monostrains and the age of pregnant women, indicating a clear tendency to decrease trimethoprim-resistant enterococcal urostrains depending on the increase in the age of pregnant women. The number of trimethoprim-resistant urocultures of *E. faecalis* isolated from the urine of women aged 16-25 years is 16 strains (29% of 55 resistant isolates), from 26-30 years - 13 strains (24%), from 31-35 years - 11 cultures. 20%), from 36-45 years - 9 strains (16%).

There was no correlation (coefficient is -0.3) between the number of resistant strains to nitrofurantoin and the age of pregnant women. The number of resistant to nitrofurantoin strains of *E. faecalis* isolated from the urine of pregnant women aged 26-30 years - 2 strains (4%), from 31-35 years - 1 strain (2%), from 36-45 years - 1 isolate (2 %).

The frequency of isolation of antibiotic-resistant monostrains of *E. faecalis* depending on the trimester of pregnancy and the number of ABP to which antibiotic tolerance has been registered is presented in Fig. 7.

According to the results of correlation analysis of the dependence of the number of *E. faecalis* urostrains resistant to 1 ABD (n = 39) and the gestational age, a strong straightforward relationship was established (correlation coefficient is 1), which indicates a rapid increase in the number of such strains. Among pregnant women, the number of resistant to 1 ABD enterococci in the first trimester of pregnancy is 11 cultures (20% of 55 isolates), in the second trimester - 13 strains (24%), in the third trimester - 15 strains (27%).

The number of resistant to 2 ABP monostrains of *E. faecalis* (n = 14) among pregnant women in the first trimester of pregnancy is 5 cultures (9% of 55 isolates), in the second trimester - 4 strains (7%), in the third trimester - 5 strains (9%).

The number of multidrug-resistant urostrains of *E. faecalis* isolated from pregnant women in the first trimester is 1 culture (2%), in the third trimester - 1 strain (2%).

DISCUSSION

Ramos et al. established that of the 50 strains of *Escherichia coli* isolated from the urine of pregnant women from Sweden, 2 strains (4%) are resistant to cephalexin, 2 strains (4%) - to norfloxacin, 25% - to trimethoprim. The authors found that from the urine of pregnant women from Uganda, the number of E. coli strains resistant to cephalexin is 12 strains (21%), to norfloxacin - 13 strains (23%), to trimethoprim - 49 strains (88%). From the urine of pregnant women in Vietnam, researchers isolated 4 strains (9%) of Escherichia coli resistant to cephalexin, 15 strains (36%) to norfloxacin, 29 strains (70%) to trimethoprim [11]. Comparing the obtained data of own research it is established:

- the number of cephalexin-resistant strains of *E. coli* (12 strains) corresponds to the data obtained from women from Vietnam, lower than the number of such strains isolated from women from Uganda, and higher than the number of strains isolated from the urine of women from Sweden;
- the number of *E. coli* strains resistant to norfloxacin (12 strains) is higher than the number of such strains excreted in the urine of pregnant women from Sweden, and lower than the number of norfloxacin-resistant strains of *Escherichia coli* excreted in the urine of women from Uganda and Vietnam;
- the number of trimethoprim-resistant strains of *E. coli* (36 strains) correlates with the obtained data of the authors - resistance of uropathogenic *Escherichia coli* to this antibacterial drug has high values.

Ballesteros-Monrreal et al. found that among pregnant women in Sonora (Mexico) isolated 50 strains of uropathogenic *E. coli*, of which 4% are resistant to fosfomycin, 82% - to cefuroxime, 40% - to nitrofurantoin, 56% - to norfloxacin, 64% - to trimethoprim [12]. In comparison with own data, it is established:

- the number of fosfomycin-resistant *Escherichia coli* (n = 13) isolated from the urine of pregnant women in Dnipro is 9% higher than among such a contingent of the state of Sonora (Mexico);
- the number of cefuroxime-resistant growths of *E. coli* is 10 times lower among pregnant women in Dnipro;
- the number of nitrofurantoin-resistant urostrains of *Escherichia coli* is 38% lower than the data obtained among pregnant women in the state of Sonora;
- the number of norfloxacin-resistant urostrains *Escherichia coli* is 44% lower than the results obtained in the state of Sonora;
- the number of trimethoprim-resistant urostrains *E. coli* is 2 times higher, but there is a high resistance to this ABD.

CONCLUSIONS

Thus, the results of the study of antibiotic resistance strains of the association of *E. coli - E. faecalis* partially confirmed the hypotheses:

1. The number of resistant to cefuroxime, cephalexin, nitrofurantoin, norfloxacin and trimethoprim uropathogenic strains of *E. coli* does not change depending on the monostep and associated strain of *Escherichia coli* from the urine of pregnant women in Dnipro.
2. It was found that the differences in the number of fosfomycin-resistant uropathogenic strains of *E. coli* depending on the type of culture - the number of fos-

fomycintolerant strains of *Escherichia coli* is 2 times higher among the associated strains, compared with monostrains of the studied microorganism.

3. The amount of uropathogenic strains of *E. faecalis* resistant to ampicillin, trimethoprim, nitrofurantoin and norfloxacin does not change depending on the type of culture isolated from the urine of pregnant women in Dnipro.

Thus, the symbiotic type of existence of two members of the normal intestinal flora on the uroepithelium of pregnant women may be associated with the development of *Escherichia coli* antibiotic resistance only to fosfomycin compared with monoinfection caused by each of these microorganisms.

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Conflict of interest:

The Authors declare no conflict of interest.

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