RESEARCHES CONCERNING THE ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM URINARY TRACT INFECTIONS IN VÎLCEA POPULATION, BETWEEN 2010 - 2011

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Abstract
The antibiotic resistance of pathogenic or opportunistic bacteria play a very important role in medical world, being the main concern for future of therapeutic solution. The urinary tract infections are considered the most common infections. The most common causes of urinary tract infections are E. coli strains, from Enterobacteriaceae Family, but another bacterial species might be implicated. For these bacteria the phenomenon of antibiotic resistance increased continuously. In this paper we present the results of investigation about 1090 children and adult patients (Rm. Valcea city, during one year) with symptoms of urinary tract infection, in MondoMedica Medical Laboratory. The samples were investigated by usual methods and bacterial agents were identified. The E. coli strains (43.54% of confirmed urinary infections) were tested through antibiogram for their antibiotic resistance. For tested strains, the bacteria presented high resistance to Ampicillin and Trimethoprim+Sulphamethoxazole, both in children and adult patient. The sensitivity of bacterial strains was higher for Gentamicin.

Keywords: antibiotic resistance, urinary tract infection, E. coli.

1. INTRODUCTION

The Enterobacteriaceae Family includes important bacteria in the background of general microbiology: they are ubiquitous in nature, being found in human and animal gut, plants and soil, water and food; they are saprophytes or pathogens, being used in different systems and scientific bacterial templates; they are frequently used as indicators of sanitary quality of water and food (to show the presence of fecal contamination).

The Enterobacteriaceae, probably the most wide spread bacterial family, contains a large number of genera, some of them with clinical implications.

Escherichia coli, from Enterobacteriaceae Family, is the most common (more than 85%) causative agent of urinary tract infections (UTI) (Davis and Flood, 2011). Multiple contributory factors for urinary infections are known, like the condition of human host, diabetes, innate or acquired immunodeficiencies, the colon malformations, obstructions of urinary tract, pregnancy; these factors increase the susceptibility of host to urinary pathogens. *E. coli* is an opportunistic pathogen; the conditions from human body allow bacteria establishment and proliferation on the intestinal mucosa, causing enteritis (Toma Săcărea, 2006). Sometimes bacteria invade the urinary tract and produce cystitis, pyelonephritis and urethritis. UTI may be asymptomatic or symptomatic, with increased urinary frequency, burning pain and, in severe cases, fever, blood and pus in urine.

The real incidence of urinary tract infection is much higher than it was thought. This fact was obvious when the diagnosis tools were improved, at the same time with intensification of urinary bacteriologic examination; many subclinical and asymptomatic forms of urinary infection were relieved. Diagnosis and location of infection is made by microscopic examination and bacteriologic culture of a urine sample, physical examination of the patient and various radiologic techniques (Davis and Flood, 2011).

Treatment of urinary tract infections includes antibacterial and antiseptic drugs, analgesic, increased fluid intake and good perineal hygiene. Sometimes patients might be taking self-medication, the widely use of broad spectrum antimicrobial agents without recommendation and in inappropriate manner being like a bad practice. This is the main cause for increase the bacterial resistance to

antibiotics. The antibiotic resistance is a progressive phenomenon, caused by selected stress of environmental factors, considered an ecological problem (Aguilar et al., 2010) and medical too.
The judicious use of narrow-spectrum antibiotics, according to antimicrobial mechanisms of action, only after medical recommendation and test the susceptibility of bacteria by antibiogram, enough time to eradicate the infection, is the only way to maintain the relative control of antibiotic resistance phenomenon.

The antibiotic resistance of Enterobacteriaceae, especially of *E. coli*, is one of the main concerns of medical world, because of several possibilities to transfer the resistance factors between different bacterial categories and because of emergence of high resistant bacteria through mutations (Wright, 2010).

2. MATERIAL AND METHOD

The urine samples were collected in sterile containers from 1090 patients, children and adult, male and female, as suspected cases of urinary tract infections, during 12 months.
The *quantitative urine culture* was done using a calibrated loop (the inside diameter 5mm). Samples were inoculated on blood agar, AABTL (Bromothymol-blue Lactose Agar) and MacConkey media. The inoculated plates were incubated overnight at 37°C and the number of colony forming units per ml (CFU/ml) was established, with formula:

CFU/ml = number of colonies on plate x 100

The significant bacteriuria was defined as culture of a single bacterial species with concentration 10^5 (or more) CFU/ml. Such samples were further operated for identification of bacterial strain of uropathogens (*qualitative urine culture*). For identification of pathogens were effected Gram reaction and biochemical features, with MIU (motility - indole - urea), TSI (triple – sugar - iron agar) and MILF (motility – indole – lysine decarboxylase - phenylalanine deaminase) media.
The antimicrobial susceptibility of bacterial strains was determined by standard disk diffusion method (Bauer et al., 1996). The Mueller-Hinton agar plates (4 mm depth) were used for inoculation of tested bacterial strains and placement of antibiotic disks onto the medium surface. After 24 hours at 37°C, the growth inhibition zones were measured and the susceptibility level of bacteria was established: sensitivity, intermediate sensitivity or resistance. The following antibiotics were used: Ampicillin (AM), Amoxicillin/Clavulanic acid (Augmentin, BAN), Azithromycin (AZ), Trimethoprim+Sulphamethoxazole (Co-trimoxazole, SXT), Cefaclorum (CEF), Cephalexin (CN) Chloramphenicol (C), Ciprofloxacin (CIP), Colistin (CS), Ceftriaxone (CRO), Gentamicin (GM), Levofloxacin (LE), Nalidixic acid (NA), Nitrofurantoin (FM), Norfloxacin (NX), Ofloxacin (OFX), Penicillin (P), Ceftazidime (CAZ), Ampicillin/Sulbactam (SAM), Cefuroxime (CXM). The results were interpreted according to CLSI (formerly NCCLS) 2000.

3. RESULTS AND DISCUSSIONS

Among 1090 urine samples, from children and adult patients, male and female, 519 (48%) were positive (confirmed urinary tract infections, figure 1). The female subjects with urinary infections were more than male ones (81%, figure 2), and the children under 12 years represented just 18.30% among infected patients. Other studies about the incidence of urinary tract infections reveal that women are more susceptible than men for these infections (Kashef et al., 2010).
The main pathogen in uncomplicated urinary tract infections is *E. coli* (Nielubowicz and Mobley, 2010; Kashef et al., 2010); 43.54% of urinary infection cases were determined by *E. coli* strains. Other pathogens were *Enterococcus, Proteus, Klebsiella, Pseudomonas, Enterobacter, Staphylococcus* (figure 3), some of them in significant percentages.
Number of urinary tract infections with *E. coli* was higher for women than for men (85%), like general incidence of urinary infections.

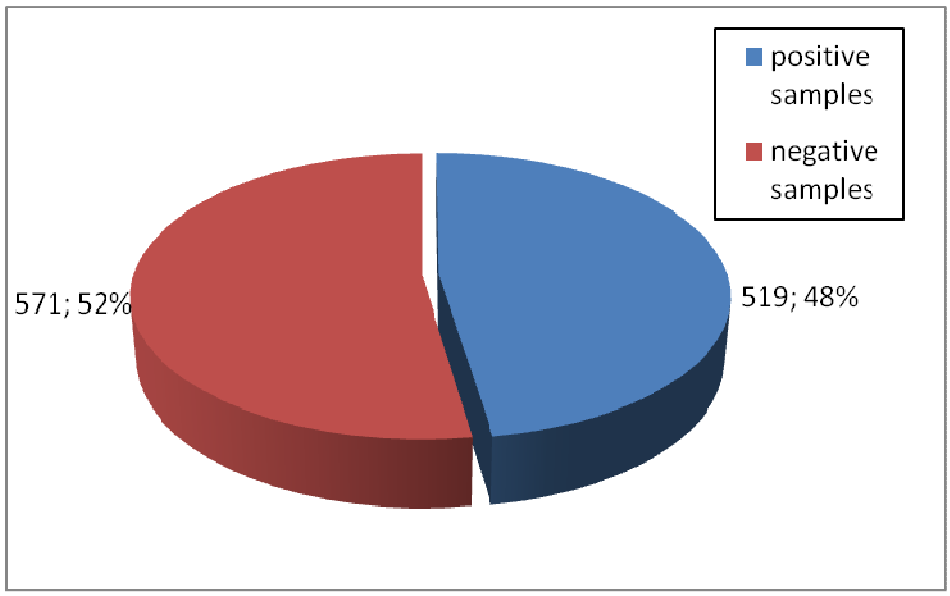

Figure 1. Incidence of urinary tract infections in studied samples

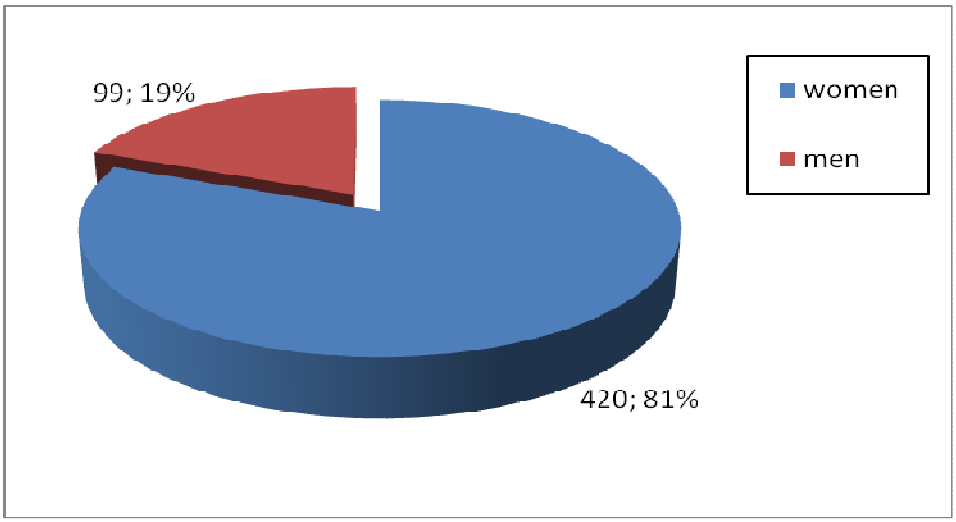

Figure 2. Incidence of urinary tract infections depend on gender of patients

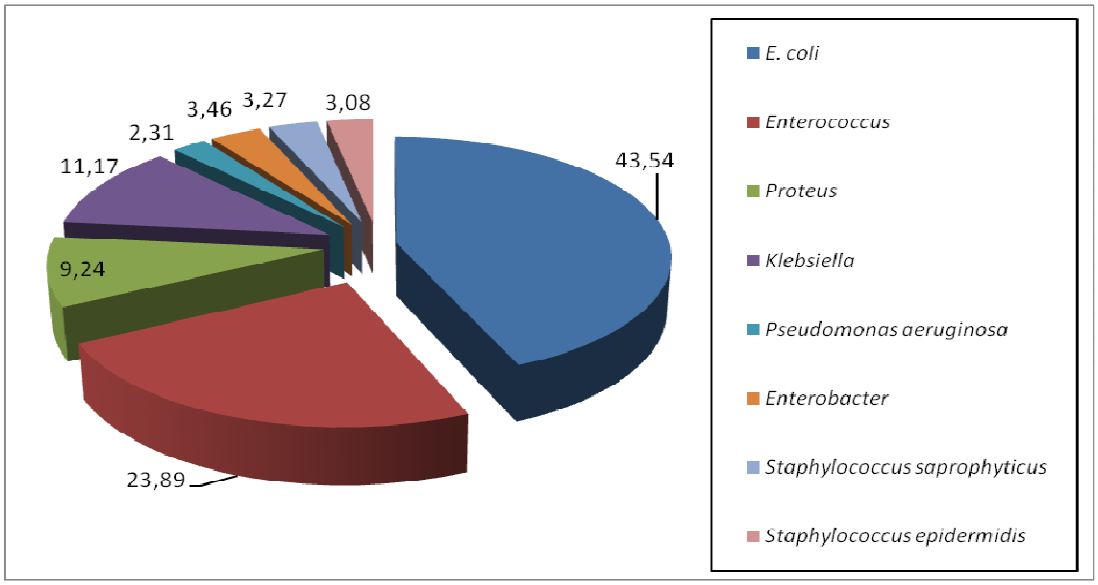

Figure 3. Incidence of urinary tract infections depend on type of pathogen

The strains of *Escherichia coli* isolated from urinary tract infections were tested to 14 antibiotics and the level of susceptibility was established (figure 4). In Figure 4 are presented the number of *E. coli* strains resistant to each antibiotic. These bacteria presented the highest resistance to Ampicillin and Trimethoprim+Sulphamethoxazole (Kashef and colleagues illustrate that, too) and lower resistance to Chloramphenicol.

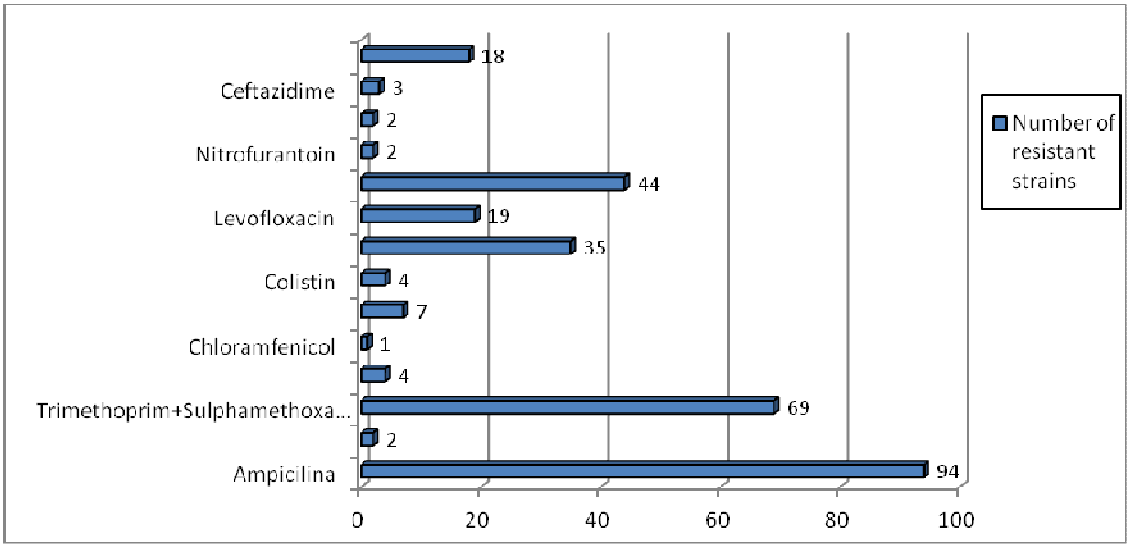

Figure 4. Resistance of E. coli strains isolated from urinary tract infections

4. CONCLUSIONS

This study confirmed higher incidence of urinary tract infections with *E. coli* relative to other pathogens. Women get UTIs more often than men because of their anatomy, the percentage of positive cases being four times higher. The antibiotic resistance was determined to more drugs, especially for *E. coli*. The higher resistance was to Ampicillin and Trimethoprim – Sulphamethoxazole. Because the urinary infections are frequent infections in community, the antibiotic resistance phenomenon continue to be the main concern of medical world.

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