STUDIES CONCERNING THE INCIDENCE OF ESBL PRODUCING BACTERIA IN URINARY TRACT INFECTIONS

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Abstract
The emergence of antimicrobial resistant strains of pathogenic bacteria has become a great threat to the public health. One of the main risk factor for spreading of resistant bacteria is selective pressure by intense and inadequate antibiotic human therapy and increase the antibiotic resistance phenomenon in bacterial strains from natural sources. Certain bacteria have multidrug resistance to antibiotics by extended spectrum beta lactamases (ESBLs) synthesis, under plasmidial genes or transposable elements control. Resistance to fluoroquinolones and extended spectrum beta lactamases synthesis in E. coli strains limit the therapeutic options for patients with such infections. In this study 1625 urine samples from patients with suspicion of urinary tract infections were collected in the laboratory of MEDAS Medical Centre, from children and adult patients and processed by qualitative and quantitative urine cultures. The E. coli strains isolated from urine samples were tested for presence of ESBLs, 24% from E. coli strains were ESBL producers and justify the interest for this resistance mechanism.

Keywords: antibiotic resistance, urinary tract infection, extended spectrum beta-lactamases.

1. INTRODUCTION

Antimicrobial resistant bacteria have been a global concern and occur both in community and in hospital environment (DebMandal et al., 2011). The emergence of antimicrobial resistant strains of pathogenic bacteria has become a great threat to the public health. One of the main risk factor for spreading of resistant bacteria is selective pressure by intense and inadequate antibiotic human therapy; also, antibiotic usage as an ingredient in animal feeds or in agriculture. It was estimated that the antibiotic market worldwide consumes between 100 and 200 × 10^6 kg (Wise, 2002). Widespread use of antibiotics promotes the spread of antibiotic resistance and this is a major public health problem. This is the reason why is imperative to explore the antibiotic pressure in the environment (Wise, 2002).

The urinary tract infections may be considered in hospital environment as nosocomial infection and in community as common infections. The most common pathogen for urinary tract infection, *E. coli*, developed multiple survival strategies to grow in nutrient deficient environment like urinary tract, for instance aerobactin, cytolysin, hemolysin synthesis. Certain Enterobacteriaceae produce nosocomial (hospital acquired) infections and have multidrug resistance to antibiotics by extended spectrum beta lactamases (ESBLs) synthesis and other mechanisms (Nica et al., 2007). This kind of antimicrobial resistance quickly spread because the responsible genes are plasmidic (and molecular characterization of plasmids is epidemiologically useful) or on the transposable elements (Khadgi et al., 2013). The best known variants of ESBL are TEM (Temoneira) and SHV (Sulphhydryl variable), recently CTX-M (Cefotaximase - Munich) (Burduniuc et al., 2012).

ESBLs bacteria occur both in healthy and sick people. In healthy people ESBLs bacteria is usually not a problem (is called colonisation or carriage), but for sick people presence of these bacteria means infection with more resistant to antibiotics bacteria and makes the infection harder to treat. Beta lactamases are enzymes produced by many types of bacteria, especially in urinary tract infections, wound infections and chest infections. The major risk factors for getting an ESBLs infection are older age, prior antibiotic therapy and long term hospitalization.

Resistance to fluoroquinolones and extended spectrum beta lactamases synthesis in *E. coli* strains limit the therapeutic options for patients with such infections, because bacteria concomitant have resistance to other classes of antibiotics.
Escherichia coli is one of the most studied bacterial group. More than 60 different resistance genes from three classes of resistance integrons (RI) were described for *E. coli*, especially from class 1. In addition to this situation, *E. coli* may be resistant to antibiotics by mutations, because of high frequency of mutation for strains by natural sources (Fluit and Schmitz, 2004). This is the reason why the antibiotic resistance of *E. coli* is very important concern of the medical world.
In perspective, it is known that frequency of ESBLs bacteria varies by geographic region, in Europe, North America and Latin America being about 1-8% for *E. coli* (Jabeen et al., 2005), but in other region being much higher.

2. MATERIAL AND METHOD

In this study were collected 1625 urine samples in sterile containers from patients with suspicion of urinary tract infections, during 8 months (January - August 2012). The samples were collected in the laboratory of MEDAS Medical Centre, from children and adult patients, male and female.
The macroscopic and microscopic observations were made for urine samples, and then the quantitative and qualitative urine culture was made.
Quantitative urine culture was done using the serial dilution method. The samples were diluted in sterile physiological saline solution 1/10, 1/100, 1/1000 and 1/10000. From each dilution 1 ml was inoculated on the surface of two medium: blood agar and AABTL (Bromothymol-blue Lactose Agar) or blood agar and MacConkey medium. The plates were incubated overnight at 37°C and the number of colony forming units per ml (CFU/ml) was established, with formula:

$$\text{CFU/ml} = \text{number of colonies on plate} \times 10 \times \text{urine dilution in plate}$$

Blood agar is used to grow fastidious organisms requiring many nutrients and growth factors. This medium admits to grow Gram positive bacteria, too. Selective media (AABTL and MacConkey) permit important differentiation between Gram negative bacilli. After colony growth was estimated the level of bacteriuria; investigation result being designated as positive or negative depending on this. The significant bacteriuria was defined as cultures of a single bacterial species with concentration 10^5 (or more) CFU/ml. Positive samples were further investigated for identification of bacterial uropathogens.
Qualitative urine culture was performed and the determination of causative bacterial strains was made by Gram reaction and biochemical features, with MIU (motility - indole - urea), TSI (triple – sugar - iron agar), MILF (motility – indole – lysine decarboxylase - phenylalanine deaminase), malonate broth and Simmons citrate media. The strains identified as *E. coli* were isolated and tested for antimicrobial susceptibility.
The antimicrobial susceptibility of bacterial strains was determined by standard disk diffusion method (Bauer et al., 1996). The inoculum was placed on Mueller-Hinton agar (4 mm depth medium); after bacterial strains inoculation and placement of antibiotic disks onto the medium surface plates were incubated minimum 18 hours at 35 - 37°C. The growth inhibition zones were measured and the susceptibility level of bacteria was established.
Because the common disk diffusion method cannot furnish enough data for determination of resistance level, the double – disc synergy test was used to detection one of the antimicrobial resistance mechanisms of *E. coli*, the presence of extended spectrum beta lactamases; the increased prevalence of Enterobacteriaceae producing ESBLs creates a great need for laboratory testing methods to identify the presence of these enzymes in clinical isolates (Bradford, 2001). This test used a beta lactamases inhibitor (usually Clavulanic Acid, 10μg/disc) in association with Cephalosporine (Cefotaxime 30μg/disc or Ceftazidime, Ceftriaxon). Although most ESBLs confer

resistance to one or more beta lactam antibiotics, bacterial enzymes does not always increase enough the minimum inhibitory concentration to be called resistant by standards (Bradford, 2001).
Disc with Cefotaxime and disc with Amoxicillin + Clavulanic Acid combination were placed on the inoculated surface of medium at 25 – 30 mm away. After incubation period were observed the growth inhibition zones. If the bacterial strain was ESBL producer, the zone of either cephalosporin is expanded by the clavulanate and are obvious synergy images between two discs as ”champagne cork” (Burduniuc et al., 2012).
Because the variation of ESBLs, the double – disc synergy test cannot be standardized. For instance, for TEM and SHV variants the synergy is more obvious with Ceftazidime and for CTX-M types the synergy is more obvious with Cefotaxime (Livermore and Brown, 2001).

3. RESULTS AND DISCUSSIONS

Among 1625 urine samples, from children and adult patients, male and female, 525 (32%) were positive (confirmed urinary tract infections, figure 1). The main pathogen in uncomplicated urinary tract infections is *E. coli* (Nielubowicz and Mobley, 2010; Kashef et al., 2010); 71.42% of urinary infection cases were determined by *E. coli* strains. Other pathogens were *Enterococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Enterobacter*, *Morganella*, *Acinetobacter*, *Staphylococcus* (figure 2).

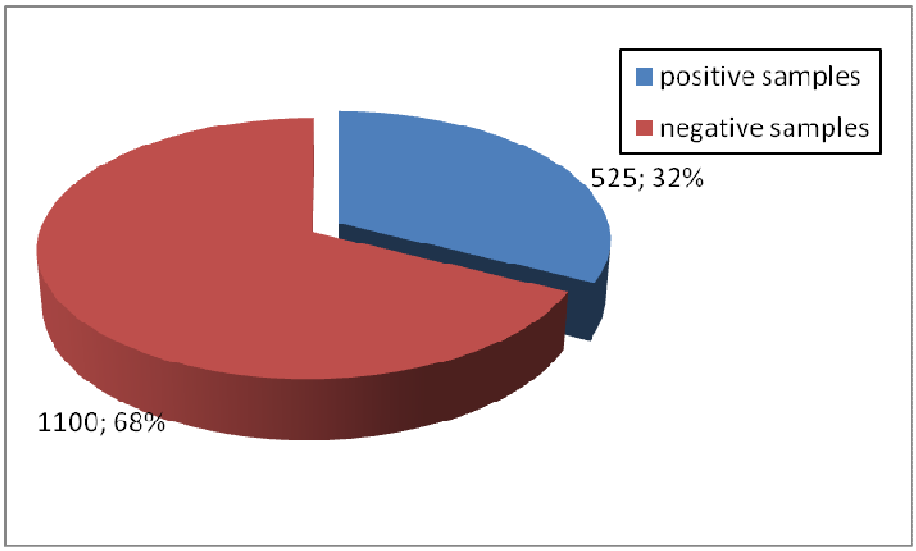

Figure 1. Incidence of urinary tract infections in studied samples

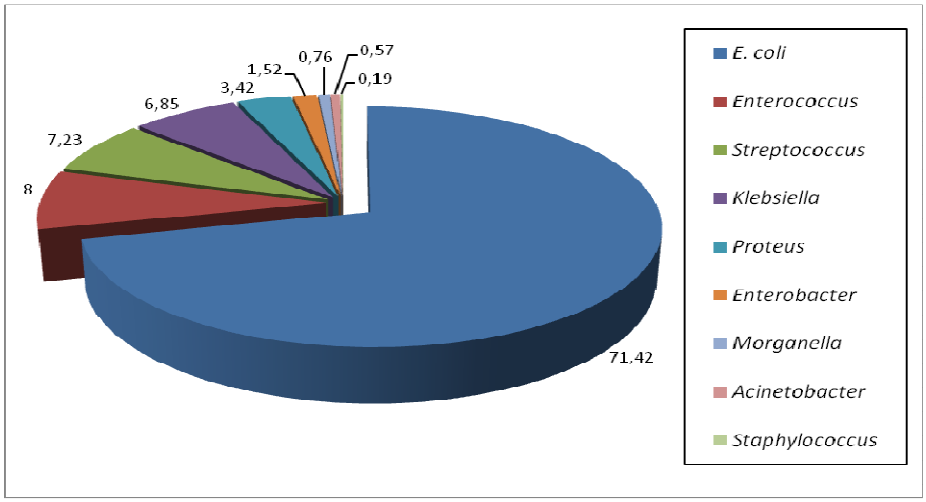

Figure 2. Incidence of urinary tract infections depend on type of pathogen

The female subjects with urinary infections were more than male ones (368 cases, representing 70.09%). Among these, 268 cases (73.02%) were infections with *E. coli* (figure 3). Other studies about the incidence of urinary tract infections reveal that women are more susceptible than men for these infections (Kashef et al., 2010).
Some of female patients were pregnant women. It is known that the incidence of urinary tract infection is higher in this period of woman life. Among 1003 urine samples collected from women (284 samples from pregnant ones), 368 were positive for bacteriuria, just 38 being from pregnant women (figure 4).

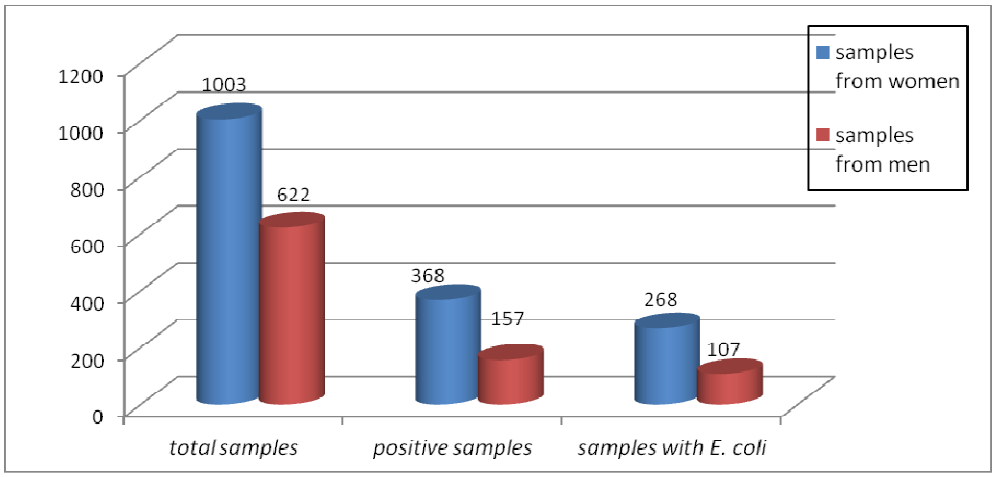

Figure 3. Incidence of urinary tract infections depend on gender of patients

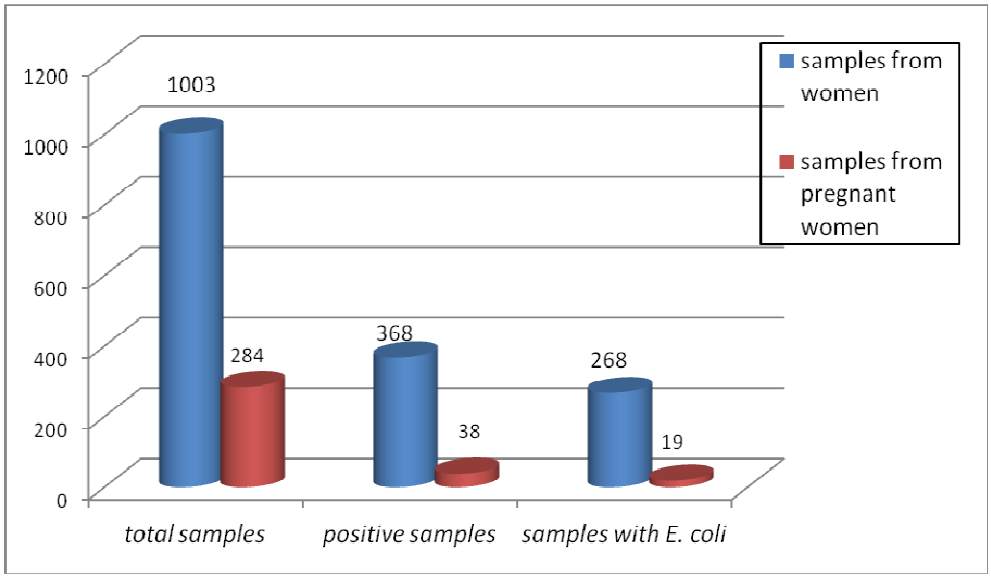

Figure 4. Incidence of urinary tract infections depend on gender of patients

The strains of *Escherichia coli* isolated from urinary tract infections were tested to cephalosporin (Cefotaxime) and combination beta lactamic antibiotic + beta lactamases inhibitor (Amoxicillin + Clavulanic acid) and the presence of ESBLs was established. Among all bacterial strains just 17% were ESBL producers (90 strains), but 24% from *E. coli* strains were ESBL producers (figure 5).
This study revealed different percentages for incidence of ESBL producers *E. coli* strains in male and female patients. Among 90 strains of ESBL producers *E. coli* isolated from urinary tract infections, 54 were isolated from women (20.14% from *E. coli* strain in women) and 36 were isolated from men (33.64% from *E. coli* strain in men).

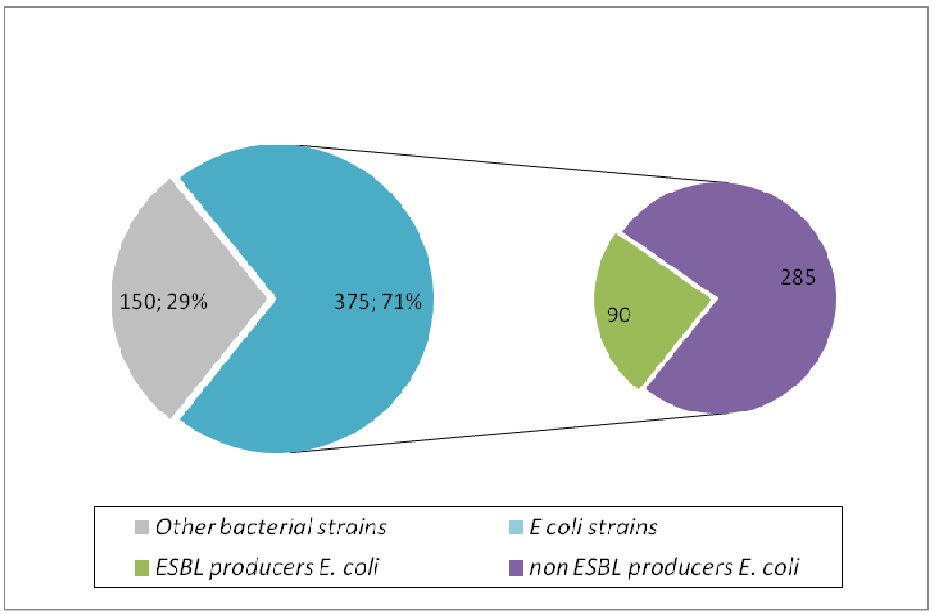

Figure 5. Incidence of ESBL producer bacterial strains

While this study revealed 24% ESBL producers from *E. coli* isolated bacterial strain from urinary tract infections, other study indicate just 5.3% for this kind of infection and 15% for intestinal carriage (Burduniuc et al., 2012) or more than 30% percentage of ESBL producers from Enterobacteriaceae family (Jabeen et al., 2005).

4. CONCLUSIONS

The incidence of urinary tract infections were 32% for studied samples, the most frequent implicated type being *Escherichia coli* (71.42% from all positive samples). This study revealed that women are more susceptible than men for these infections, because of their anatomy and physiological conditions. Among all *E. coli* isolated strains 24% were ESBL producers, most probably CTX-M types.

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