Prevalence of extended spectrum beta-lactamases among Enterobacteriaceae isolated from intrahospital patients in Serbia

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The aim of this study was to determine the prevalence of extended spectrum beta-lactamases (ESBLs) production in hospital strains of Enterobacteriaceae isolated from various clinical specimens (urine, blood and wound swabs) from hospitalized patients at the Military Medical Academy, Belgrade, Serbia. During six months of study, a total of 1034 isolates of Enterobacteriaceae were tested for antimicrobial susceptibility and screened for ESBL production according to standard methods. The overall prevalence of ESBL production in the hospital isolates of Enterobacteriaceae was 57.4%. Among the isolates, minimum frequency of resistance was found for amikacin (25.2%), and maximum for ampicillin (84.5%). The strain resistant to imipenem could not be isolated. Resistance to the tested antibiotics was higher in ESBL producers than non-producers ($P < 0.05$). Among *Escherichia coli* isolates, the prevalence of ESBL production was less than 50% (33.9%). ESBLs were most often produced by isolates of *Serratia* spp. (85.2%) and *Klebsiella* spp. (81.8%). Blood specimens were the most common sources of ESBL-producing isolates (84.0%). These findings might help clinicians in deciding the appropriate empirical treatment for intrahospital patients and emphasize the increasing problem of antimicrobial resistance in Serbia.

Keywords: Antibiotic resistance and susceptibility, Enterobacteriaceae, extended spectrum beta-lactamases, human isolates.

GRAM-NEGATIVE bacterial infections represent a major therapeutic problem because of their importance and abundance[1].

Beta-lactams include the maximum number of antibiotics, and the most used in the clinical practice[2]. Except majority of mycobacteria and intracellular pathogens, most bacteria are susceptible to beta-lactams[3], including penicillin, cephalosporin, carbapenem and monobactam. Carbapenems are beta-lactams with a broad spectrum of activity; in clinical practice, the most commonly used are imipenem and meropenem.

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Some bacteria synthesize beta-lactamases that inactivate the antibiotic or lead to changes in the structure of the drug. These enzymes lead to the development of resistance to penicillin, cephalosporin and carbapenem[4,5]. The expansion of these enzymes, as well as the emergence of new enzymes with a broad spectrum of activity have been recently reported[6].

Many Gram-positive and Gram-negative bacteria have the ability to produce beta-lactamases. Some enzymes are mediated by plasmids, while others are mediated by bacterial chromosomes. Most of the plasmid beta-lactamases are secreted constitutively and have the tendency to be transmitted from one bacterial species to another[7].

Antimicrobial resistance caused by widespread and indiscriminate use of antimicrobial agents has important implications on treatment outcomes and healthcare costs[8]. It is believed that 50–67% of all nosocomial infections are caused by pathogens resistant to antimicrobials[9,10].

Members of the Enterobacteriaceae family usually secrete TEM-1, TEM-2 and SHV-1 beta-lactamases which provide resistance against beta-lactam ring containing antibiotics. These beta-lactamases are commonly found on portable plasmids, but have also been found in the transposons and on bacterial chromosomes. Mutations in these genes may result in the replacement of one or more amino acids in the active part of the enzyme, which leads to an increase in the number of substrates they affect. These mutants are called extended spectrum beta-lactamases (ESBLs)[11]. These beta-lactamases hydrolyse broad-spectrum cephalosporins, i.e. oxiaminocephalosporins (ceftazidime, cefotaxime, ceftriaxone), monobactams, ureidopenicillins, and some of them hydrolyse cephamycin, while having no effect on the carbapenems[12,13]. This leads to frequent reports on the emergence of resistance to these antibiotics in other microorganisms, as well as enterobacteria[14–17]. Very often, isolates that produce these enzymes also show resistance to other groups of antimicorobial agents such as fluoroquinolones, aminoglycosides, tetracycline and co-trimoxazole[18,19].

Till now about 500 different types of beta-lactamases have been reported from clinical isolates[20,21]. ESBLs were detected in the eighties of the last century, first in

Germany and France, and later throughout the world[22–24]. They are practically found in all Enterobacteriaceae and in *Pseudomonas aeruginosa*[25].

Since the genes for ESBLs are located on the plasmids, they can spread amongst the bacteria; even among different species of Gram-negative bacteria[26].

ESBLs may be the cause of therapy failure in hospitals[27–29]. Their presence in bacterial strains causing hospital outbreaks, especially in intensive care units, oncology departments, burn units and neonatal wards, can have devastating and fatal consequences[30,31]. The Infectious Diseases Society of America included ESBL-producing strains of *Escherichia coli* and *Klebsiella pneumoniae* in a list of six resistant pathogens, causative agents of infectious diseases, for which new therapeutic options are urgently needed[32]. In addition, extensive and expensive infection control measures must be tailored for outbreaks caused by multiresistant Gram-negative pathogens[33].

The frequency of ESBLs shows specific geographic distribution[34]. It is greater in developing countries than in developed countries, because the former have limited access to new antibacterial agents[35]. In fact, it has been noted that the production of ESBLs is somewhat less common in Europe than in Latin America and Asia, but much more frequent than in North America[36]. In Europe, the production of ESBLs is significantly lower in the northern countries compared to those in the south and east (e.g. Serbia)[37].

For detection of ESBLs, as a first step, the sensitivity test with cephopodoxime or cefotaxime and ceftazidime is recommended[38]. The next step is the disc diffusion method using cephalosporin with and without clavulanic acid. Further detection of the enzymes can be continued by molecular methods, which are more sensitive but also more complicated and expensive to perform.

The aim of this study was to determine the prevalence of ESBL production in hospital strains of Enterobacteriaceae isolated from different clinical specimens (urine, blood and wound swabs) from hospitalized patients, with a minimum stay of 4 days, at the Military Medical Academy (MMA) hospital, Belgrade, Serbia. Also, the significance of differences in terms of the frequency of ESBL production among hospital strains of Enterobacteriaceae, as well as between different types of samples was determined. Additionally, the significance of differences in terms of the sensitivity of clinical isolates to other classes of antibiotics was monitored.

Materials and methods

Study settings

This study was conducted in the Medical Microbiology Laboratory of military hospital at MMA, between September 2012 and February 2013.

One thousand thirty-four clinical isolates of Enterobacteriaceae from various clinical specimens were recovered and identified following conventional procedures[39]. These clinical specimens were urine (698 isolates), blood (47 isolates) and wound swabs (289 isolates).

Collection of specimens

Specimens of urine were received into sterile plastic containers and were processed immediately for detection of Enterobacteriaceae.

The specimens of blood were extracted under aseptic conditions and transferred immediately to sterile bottles containing brain heart infusion broth[40].

Specimens from wounds were taken as swabs, placed on transport media and analysed as soon as possible.

Bacterial isolates

All clinical samples were inoculated on blood agar and MacConkey agar. All inoculated plates were incubated aerobically at 37°C for 24 h.

Further identification of the isolates was carried out by a study of their various colonial morphology and biochemical reactions such as coagulase, indole, urease, methyl red, Voges–Proskauer, citrate utilization and sugar fermentation tests as previously described[41] and confirmed by API 20E identification system (bioMerieux, Marcy l'Etoile, France).

Antibiotic susceptibility testing

Susceptibility to beta-lactam and non-beta-lactam antibiotics was performed on Mueller–Hinton agar plates (Oxoid, England) using the Kirby–Bauer disk diffusion method, according to the CLSI guidelines[39].

The antibiotic disks contained ampicillin (10 μg), cefalexin (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), imipenem (10 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), nitrofurantoin (50 μg) and trimethoprim-sulfamethoxazole (25 μg) (Oxoid, England).

Standardized inoculum (0.5 McFarland standard turbidity) of each isolate was spread on Mueller–Hinton agar plates (150 mm in diameter). Then, 8–9 antibiotic disks were placed on each plate at the recommended distance from each other. All plates were incubated aerobically at 37°C for 24 h before the zone sizes were recorded following CLSI guidelines[39].

Escherichia coli ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as comparison standards for antimicrobial susceptibility.

Detection of ESBL isolates

The isolates with diameter zones of 10–18 mm for ceftazidime were further screened for ESBL production.

Table 1. Resistance of Enterobacteriaceae clinical isolates to antibiotics. The number of tested isolates; number and percentage of resistant isolates

Antibiotics	Urine	Blood	Wound swabs	Total
Ampicillin	688; 551 (80.1)	47; 44 (94.0)	282; 264 (94.0)	1017; 859 (84.5)
Cefalexin	692; 484 (70.0)	46; 46 (100)	18; 13 (72.2)	756; 543 (71.8)
Ceftriaxone	690; 334 (48.4)	46; 40 (87.0)	283; 204 (71.0)	1019; 578 (56.7)
Ceftazidime	674; 334 (49.5)*	45; 38 (84.0)*	277; 200 (72.2)*	996; 572 (57.4)
Imipenem	670; 0 (0.0)	47; 0 (0.0)	280; 0 (0.0)	997; 0 (0.0)
Gentamicin	693; 365 (53.0)	45; 31 (69.0)	283; 177 (62.5)	1021; 573 (56.1)
Amikacin	675; 156 (23.1)	46; 11 (24.0)	283; 86 (30.4)	1004; 253 (25.2)
Ciprofloxacin	695; 326 (46.9)	45; 14 (31.1)	281; 86 (30.6)	1021; 426 (41.7)
Nitrofurantoin	695; 439 (63.2)	–	–	695; 439 (63.2)
Trimethoprim/sulphamethoxazole	691; 443 (64.1)	47; 38 (81.0)	281; 192 (68.3)	1019; 673 (66.1)

*$P < 0.05$.

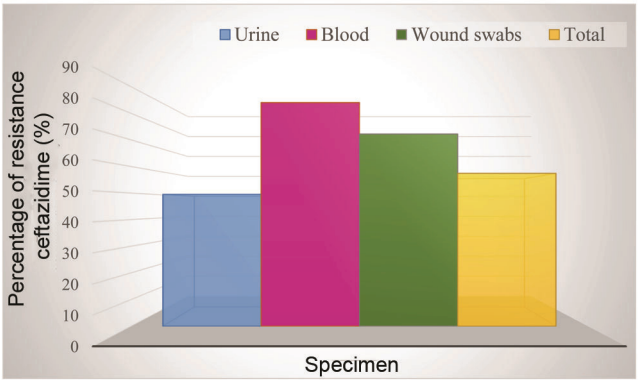

Figure 1. The frequency of extended spectrum beta-lactamases producing Enterobacteriaceae clinical isolates.

Detection of ESBL isolates was performed using the double-disk synergy test (DDST) and confirmed by double-disk diffusion test (DDDT)[39].

DDST was done using amoxicillin/clavulanic acid as beta-lactamase inhibitor. Disks containing ceftriaxone (30 µg), cefotaxime (30 µg) and ceftazidime (30 µg) were placed on Mueller–Hinton agar, streaked with a 0.5 McFarland bacterial suspension of isolates, 30 mm (centre to centre) from the amoxicillin/clavulanic acid (20 µg/10 µg) disk. The plates were incubated at 37°C for 24 h. Synergy between the disks towards the beta-lactamase inhibitor was regarded as presumptive ESBL production[42].

DDDT was used to confirm the results of DDST. Four disks containing third generation cephalosporins with and without clavulanic acid were prepared as follows: ceftazidime (30 µg), ceftazidime + clavulanic acid (10 µg), cefotaxime (30 µg) and cefotaxime + clavulanic acid (10 µg). These disks were placed on Mueller–Hinton agar inoculated with standardized inocula of potential ESBL-producing isolates, at the recommended distance from each other[39]. The plates were incubated aerobically at 37°C for 4 h. The positive result (ESBL production) was defined as 5 mm increase in inhibition zone diameter around combination disks with clavulanic acid versus its standard zone when tested alone. *E. coli* ATCC 25922 was used as the negative control, and *Klebsiella pneumoniae* ATCC 700603 as the positive control.

Statistical analysis

The results were analysed using chi-square (χ^2) test. *P*-values < 0.05 were considered to be statistically significant. Statistical analysis was done using the software STATISTICA v.10.0 (StatSoft, Inc., USA).

Ethical declaration

This was a laboratory-based study and did not involve any intervention concerning the patients directly.

Results and discussion

The overall prevalence of ESBL production in hospital isolates of Enterobacteriaceae was 57.4% (Table 1). The strains resistant to carbapenem imipenem were not isolated. Among the other classes of antibiotics (other than beta-lactams), the minimum frequency of resistance was found in aminoglycoside amikacin (25.2%) and fluoroquinolone ciprofloxacin (41.7%). The highest frequency of resistance was found for ampicillin (84.5%), trimethoprim/sulphamethoxazole (66.1%) and nitrofurantoin (63.2%) (Table 1). There was a statistically significant difference in the percentage of resistant strains of Enterobacteriaceae to ceftazidime, depending on the clinical specimen (Table 1 and Figure 1).

The frequency of ESPL production was highest in *Serratia* spp., *Klebsiella* spp., *Proteus mirabilis* and *Morganella morgani*, and lowest in *Escherichia coli* (Table 2 and Figure 2).

In case of different clinical specimens, the highest rate of resistance was recorded in strains isolated from the blood (84.0%) (Figure 1). This is in contrast to most studies

in which the highest percentage of ESBL producers was recorded in samples of urine[43–46].

The highest frequency of resistance to gentamicin was found in *Providencia* spp. and *M. morgani*, and the lowest in *E. coli* (Table 3). The highest frequency of resistance to amikacin was also found in *Providencia* spp. and *M. morgani*, and the lowest in *Serratia* spp. and *E. coli* (Table 3).

The highest frequency of resistance to ciprofloxacin was found in *Providencia* spp. and *M. morgani*, and the lowest in *Proteus mirabilis* and *Serratia* spp. (Table 4).

The highest frequency of resistance to trimethoprim/sulphamethoxazole was found in *Serratia* spp. and *Providencia* spp. and the lowest in *E. coli* (Table 5).

The highest frequency of resistance (100%) to nitrofurantoin was found in *Proteus vulgaris*, *Providencia rettgeri* and *M. morgani*, and the lowest in *E. coli* (Table 6).

Penicillins acting on enterobacteria are divided into four groups: aminopenicillins, acilureidopenicillins, carboxypenicillins and amidinopenicillins[3]. The most important aminopenicillins are ampicillin and amoxicillin. Enterobacteriaceae inherently susceptible to these antibiotics are *Salmonella* spp., *Shigella* spp., *E. coli* and *P. mirabilis*. Beta-lactamases degrade both of these antibiotics; so they do not act on the enterobacteria that are producers of class-C beta-lactamases, such as *Klebsiella* spp., *Enterobacter* spp. and indole-positive *Proteus* species. Among clinical strains of *E. coli* (a total of 410

Table 2. Frequency of ESBL producers among Enterobacteriaceae species

Species	No. of tested isolates; no. and percentage of ESBL-producing isolates
Escherichia coli	401; 136 (33.9)
Klebsiella species	66; 54 (81.8)
Enterobacter species	129; 98 (76.0)
Citrobacter species	71; 43 (60.6)
Serratia species	54; 46 (85.2)
Proteus mirabilis	180; 127 (70.6)
Proteus vulgaris	5; 5 (100)
Providencia retgerii	48; 31 (64.6)
Providencia stuartii	13; 7 (54.0)
Morganella morgani	35; 25 (71.4)
Total	1002; 572 (57.1)

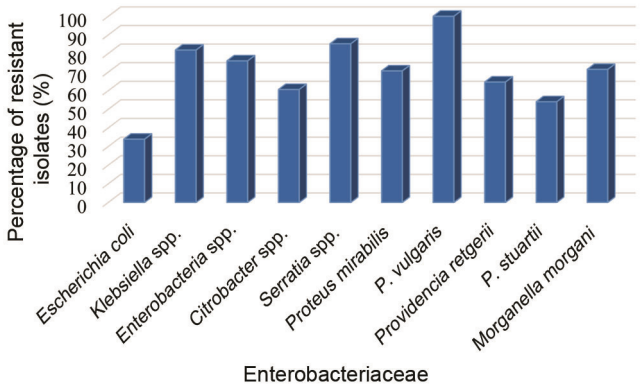

Figure 2. Percentage of resistance to ceftatidime among Enterobacteriaceae clinical isolates.

Table 3. Frequency of resistance to gentamicin and amikacin among Enterobacteriaceae species

Species	No. of tested isolates; no. and percentage of resistant isolates	
	Gentamicin	Amikacin
E. coli	413; 167 (40.4)	401; 48 (12.0)
Klebsiella species	66; 45 (68.2)	66; 18 (27.3)
Enterobacter species	131; 86 (65.7)	130; 30 (23.1)
Citrobacter species	72; 44 (61.1)	71; 28 (39.4)
Serratia species	56; 32 (57.1)	55; 6 (10.9)
P. mirabilis	183; 118 (64.5)	181; 72 (39.8)
P. vulgaris	5; 3 (60.0)	5; 2 (40.0)
P. retgerii	48; 43 (89.6)	47; 21 (44.7)
P. stuartii	12; 10 (83.3)	13; 7 (54.0)
M. morgani	35; 25 (71.4)	35; 21 (60.0)
Total	1021; 573 (56.1)	1004; 253 (25.2)

Table 4. Frequency of resistance to ciprofloxacin among Enterobacteriaceae species

Species	No. of tested isolates; no. and percentage of resistant isolates
E. coli	415; 180 (43.4)
Klebsiella species	66; 33 (50.0)
Enterobacter species	129; 62 (48.1)
Citrobacter species	72; 40 (55.6)
Serratia species	57; 14 (24.6)
P. mirabilis	182; 22 (12.1)
P. vulgaris	5; 3 (60.0)
P. retgerii	48; 38 (79.2)
P. stuartii	12; 10 (83.3)
M. morgani	35; 24 (68.6)
Total	1021; 426 (41.7)

Table 5. Frequency of resistance to trimethoprim/sulphamethoxazole among Enterobacteriaceae species

Species	No. of tested isolates; no. and percentage of resistant isolates
E. coli	410; 236 (57.6)
Klebsiella species	66; 48 (72.7)
Enterobacter species	131; 95 (72.5)
Citrobacter species	72; 48 (66.7)
Serratia species	70; 54 (77.1)
P. mirabilis	184; 123 (66.9)
P. vulgaris	4; 2 (50.0)
P. retgerii	48; 39 (81.3)
P. stuartii	12; 10 (83.3)
M. morgani	35; 26 (74.3)
Total	1032; 681 (66.0)

Table 6. Frequency of resistance to nitrofurantoin among Enterobacteriaceae species

Species	No. of tested isolates; no. and percentage of resistant isolates
E. coli	341; 127 (37.2)
Klebsiella species	32; 25 (78.1)
Enterobacter species	69; 62 (90.0)
Citrobacter species	53; 32 (60.4)
Serratia species	16; 13 (81.3)
P. mirabilis	99; 96 (97.0)
P. vulgaris	4; 4 (100)
P. retgerii	42; 42 (100)
P. stuartii	12; 11 (92.0)
M. morgani	27; 27 (100)
Total	695; 439 (63.2)

isolates), 74.4% of the isolates were resistant to ampicillin, and among clinical strains of *P. mirabilis* (a total of 183 isolates), 79.8% of the isolates were resistant to this drug. As might be expected, ampicillin resistance was more common among *Klebsiella* spp. (from a total of 65 isolates, 90.8% were resistant), *Enterobacter* spp. (from a total of 130 isolates, 98.5% were resistant), *P. vulgaris* (all isolates showed resistance), *P. rettgeri* (95.8% of resistant isolates of 48 tested) and *M. morgani* (97.3% of resistant isolates of 37 tested). Given such a high prevalence of resistance, these antibiotics should not be considered in the empirical treatment of nosocomial infections in the study clinic, even in infections of the urinary tract.

E. coli, *Klebsiella* spp. and *P. mirabilis* may be susceptible to the first generation of cephalosporins, while *Serratia* spp. and *Enterobacter* spp. are considered to be resistant[3]. Thus, in our hospital isolates of *E. coli* frequency of resistance to cephalexin was 57.1% (out of 359 tested isolates), in *Klebsiella* spp. 87.2% of the isolates were resistant (out of 47 tested), in *P. mirabilis* 76.9% of the isolates were resistant (out of 104 tested), in *Serratia* spp. the frequency of resistance was 86.7% (out of 30 tested isolates) and in *Enterobacter* spp., there 91.9% of the isolates were resistant (out of 74 tested).

With regard third-generation cephalosporins (ceftriaxone, cefotaxime, ceftazidime), enterobacteria without acquired resistance mechanisms were found to be generally sensitive[3]. The most important mechanism of acquired resistance of Enterobacteriaceae to third-generation cephalosporins in hospitals around the world is the production of various ESBLs[47]. The frequency of this resistance mechanism is considered to be higher than those reported due to inconsistencies in reporting and difficulties in proving it. Increased prevalence of ESBL-producing strains of Enterobacteriaceae from all parts of the world has been reported recently[48].

The overall prevalence of ESBL production in this study was 57.4% (Table 1). The overall prevalence of ESBLs produced by enterobacteria in European countries ranged from 1% to 5% in the countries of Northern Europe and from 39% to 47% in Eastern Europe and Turkey[49]. However, a recent study in Germany has recorded ESBL production in 83.6% of human isolates, indicating an alarming increase in this problem in the developed countries[50]. The same trend has also been recorded in other countries around the world[34,51].

The prevalence of ESBL production among clinical isolates of *E. coli* in this study was 33.9% (out of 401 tested isolates, 136 were ESBL producers). In other European countries, the prevalence of ESBL production in strains of *E. coli* ranged from 4.0% to 31.9% (refs 45, 52 and 53).

The prevalence of ESBL production among isolates of *Klebsiella* spp. was 81.8% (out of 66 tested isolates, 54 were ESBL producers). This result far exceeds the values recorded in neighbouring countries, viz. 18.9% in Croatia[54] and 24.3% in FYR Macedonia[55]. The highest prevalence of ESBL production among strains of *Klebsiella pneumoniae* has been observed in China (51%), Turkey (48.8%) and Latin America (47.3%)[47]. Results of the present study are consistent with those from the literature highlighting that prevalence of ESBL strains of *K. pneumoniae* is significantly higher than that of *E. coli*, in the European countries and USA[55–58]. The situation is reversed in the countries of Asia, Africa and South America[43,46,59,60].

Isolates from most other enterobacteria were also ESBLs producers, especially strains of *Enterobacter* spp.[52,61], *Citrobacter* spp.[47], *Serratia* spp.[43,46] and *P. mirabilis*[45,47,62].

In this study, only among the isolates of *E. coli* was the prevalence of ESBL production less than 50%. ESBLs were most often produced by isolates of *Serratia* spp. (85.2%) and *Klebsiella* spp. (81.8%) (Table 2). The isolates of *P. vulgaris* showed resistance in 100% of the cases, but this result must be taken conditionally due to the small number of isolates. This is consistent with the results from other Eastern European countries[63–65], and in contrast with those from Asian countries[59,66].

The best sensitivity to aminoglycosides was shown by *E. coli* and *Serratia* spp.; 40.4% of the isolates of *E. coli* and 57.1% of the isolates of *Serratia* spp. were resistant to gentamicin, while 10.9% of the isolates of *Serratia* spp. and 12.0% of *E. coli* strains were resistant to amikacin (Table 3). This is in line with data from the literature, which show that amikacin is still effective in many strains resistant to gentamicin[3]. The frequency of resistance to gentamicin was highest among isolates of *Providencia* spp. (83.3–89.6%) and *M. morgani* (71.4%). In case of amikacin, the percentage of resistant isolates was 44.7–54.0 in *Providencia* spp. and 60 in *M. morgani*. High prevalence of resistance to amikacin was observed in *P. mirabilis* (39.8%), while the strains of *Klebsiella* spp. and *Serratia* spp. were relatively well-sensitive to this drug, in spite of the high prevalence of ESBL

production (Table 2). Despite the application of amikacin for many years in the study hospital, the overall sensitivity of enterobacteria to this antibiotic has been preserved (25.2% of resistant isolates). A good sensitivity of enterobacteria to amikacin is also recorded in other regions of the world[46,51,67].

The susceptibility to ciprofloxacin was shown by the isolates of *P. mirabilis* (12.1% of resistant isolates) and *Serratia* spp. (24.6% of resistant isolates; Table 4). Similar results have been obtained in neighbouring Croatia[52]. The highest frequency of resistance to ciprofloxacin was observed in *M. morgani* (68.6%) and *Providencia* spp. (79.2–83.3% of resistant isolates). High prevalence of resistant *E. coli* strains (43.3%) was observed in these samples.

The lowest frequency of resistance to trimethoprim/sulphamethoxazole was found in isolates of *E. coli* (57.6%) and *Citrobacter* spp. (66.7%), and highest in isolates of *Serratia* spp. (77.1%), *P. rettgeri* (81.3%) and *M. morgani* (74.3%) (Table 5).

The isolates of *Proteus* spp., *Providencia* spp. and *M. morgani* showed nearly 100% resistance to nitrofurantoin, which is in accordance with their innate resistance[3]. Significant sensitivity to nitrofurantoin was found only in *E. coli* (37.2% of resistant isolates) and *Citrobacter* spp. (60.4% of resistant isolates) (Table 6). Taking into account the inherent sensitivity, applying this antibiotic only to isolates of *E. coli* and *Citrobacter* spp. from the urine seems valid, because the clinical response of susceptible strains (90% of clinical isolates) can only be expected in those cases[3]. Other enterobacteria almost always have been found to be resistant. Considering that the response to this drug is less satisfactory and requires long-term therapy, it remains an alternative antibiotic more than the drug of first choice[68].

The results of the present study show that the antibiotic of choice in the treatment of serious infections caused by Enterobacteriaceae is imipenem. This has been confirmed by many other studies as well[45,69,70].

Statistical comparison of frequency of resistance to antibiotics other than beta-lactams between hospital isolates that produced ESBLs and non-producing strains was performed in this study. There was a statistically significant higher prevalence of resistance ($P < 0.05$) to gentamicin, amikacin, ciprofloxacin, nitrofurantoin and trimethoprim/sulphamethoxazole in all tested isolates of Enterobacteriaceae that produced ESBLs in comparison to non-producers.

Conclusion

ESBLs were found in all species of Enterobacteriaceae isolated from various clinical specimens from intrahospital patients (urine, blood and wound swabs).

ESBLs were significantly more in *Klebsiella* spp. and *Serratia* spp. (81.8% and 85.2% respectively), and rarely occurred in isolates of *E. coli* (33.9%).

The tested isolates that produced ESBLs were significantly more resistant to other classes of antibiotics (other than beta-lactams). This does not include enterobacteria which are inherently resistant to certain classes of antibiotics.

Following imipenem, the clinical isolates of Enterobacteriaceae showed highest sensitivity, i.e. lowest frequency of the resistance to aminoglycoside amikacin, where the overall prevalence of 25.2% of resistant isolates was observed.

The results of this study showed that the prevalence of ESBL-producing Enterobacteriaceae isolates from intrahospital patients was very high and was among the highest in Europe. This emphasizes the need for urgent interventions in terms of introducing a stringent control of antibiotic usage, strict application of hygienic measures and increasing awareness among health professionals and the general public.

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