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ANTIBIOTIC RESISTANCE AND EXTENDED SPECTRUM BETA-LACTAMASES (ESBLs) PRODUCTION IN *ESCHERICHIA COLI* STRAINS ISOLATED FROM INTRAHOSPITAL PATIENTS

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Abstract

The main objectives of this work were to determine the prevalence of ESBL-producing *E. coli* and to establish antimicrobial susceptibility pattern of ESBL-producing and non producing strains. The tests were carried out in the laboratory of military hospital, Military Medical Academy (MMA), Belgrade, Serbia, during the second half of 2017 (July-December). Four hundred and fifty-one *E. coli* strains isolated from various clinical specimens were tested. Detection of ESBLs isolates was carried out by the double-disc synergy test and confirmed by double-disc diffusion test. The overall incidence of ESBLs production among *E. coli* isolates was 34%. The highest frequency of ESBLs production was noted in blood isolates (82%), which was statistically significant ($p < 0.05$) compared to the other isolates. The highest susceptibility of *E. coli* strains was expressed to imipenem (100%) and to amikacin (88%), so these antibiotics can be considered antibiotics of choice in the treatment of infections caused by this bacterium. The isolates that produced ESBLs were significantly ($p < 0.05$) more resistant to the classes of antibiotics other than beta-lactams compared to the non-ESBL-producers. These results show very high frequency of ESBLs production in the tested isolates and suggest the urgent introduction of some measures in clinical practice, such as quicker and reliable diagnostic tools, new effective therapies and reasonable use of existing drugs.

Key words: antibiotic resistance, *Escherichia coli*, extended spectrum beta-lactamases, intrahospital patients

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Introduction. One of the most important mechanisms of resistance observed in enterobacteria is the production of extended-spectrum beta-lactamases.

Extended spectrum beta-lactamases (ESBLs) are beta-lactamase enzymes capable of hydrolyzing extended spectrum/third generation cephalosporins (e.g. ceftriaxone and/or ceftazidime) [1].

The presence of ESBLs in enterobacteria renders beta-lactam antimicrobials ineffective, including extended spectrum cephalosporins, necessitating the wider usage of non-beta-lactam agents including ciprofloxacin and amikacin in the treatment of serious infections caused by these pathogens. Nevertheless, ESBL presence may be associated with a phenomenon of antimicrobial coresistance, to both beta-lactam and non-beta-lactam antibiotics [2], significantly reducing therapeutic options available for treatment. Consequently, the duration of hospital stays, cost and mortality rates also increase by over 40%.

Escherichia coli is a part of the normal intestinal microflora, but it is also a common cause of severe infections. It is the most frequent cause of urinary tract infections and blood infections and involved in infections of both community and healthcare origin. This bacterium has become multidrug resistant over time, making infections it causes difficult to treat [3]. The major resistance mechanism expressed by *E. coli* are ESBLs, and it remains the commonest ESBL producing organism worldwide [4]. The ESBL prevalence amongst clinical isolates within institutions varies greatly from country to country. Across Europe there is varying prevalence, with low occurence of 1% and 3% in the Netherlands and Sweden, respectively [5], to as high as 42% in an intensive care unit in France [6].

Extended spectrum beta-lactamase producing *E. coli* is emerging worldwide [7–9]. ESBL-producing *E. coli* is responsible for a similar spectrum of infections as non-ESBL-producers. It is an important cause of urinary infections, intra-abdominal infections, bacteremia, hospital-acquired pneumonia, and wound infections. Bacteremia with an ESBL-producing organism is associated with increased mortality relative to bacteremia with a non-ESBL-producer. Also, there is a significantly increased delay in effective therapy for patients with bacteremia due to ESBL-producers relative to non-ESBL-producers [10].

Often seriously ill patients stand the risk of developing infections caused by ESBL-producing organisms. This is due to prolonged hospital stays and use of invasive medical devices (urinary catheters, endotracheal tubes, central venous lines).

The aims of this study were to determine the prevalence of ESBL-producing strains of *E. coli* isolated from various clinical samples and to establish the antimicrobial susceptibility pattern of ESBL-producing and non producing strains.

Materials and methods. The tests were carried out in the laboratory of military hospital, Military Medical Academy (MMA), Belgrade, Serbia, during the second half of 2017 (July–December).

Bacterial isolates were taken from urine, blood, wounds, sperm, urethra,

vagina and cervix. Given the smaller number of isolates of sperm, urethra, vagina and cervix, they were considered in common as "other" samples.

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 *E. coli* isolates was carried out by investigation of their various colonial morphology and biochemical reactions such as coagulase, indole, urease, methyl red, Voges–Proskauer, citrate utilization and sugar fermentation tests as described in KONEMAN et al. [11] and confirmed by API 20E identification system (bioMerieux, Marcy l'Etoile, France).

In this way, a total of 451 isolates of *E. coli* were collected. Out of these, 341 isolates were from urine (75.61%), 11 from blood (2.44%), 63 isolates from wound swabs (14%) and 36 isolates from other specimens (7.95%).

The susceptibility of *E. coli* isolated strains to beta-lactam and non-beta-lactam antibiotics was tested on Mueller-Hinton agar plates (Oxoid, England) by the Kirby-Bauer disc diffusion method [12]. The following antibiotic discs were used: 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 plate. Then onto each plate, 8 to 9 of antibiotic discs were placed at the recommended distance from each other. All plates were incubated aerobically at 37 °C for 24 h. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls for antimicrobial susceptibility.

The isolates with diameter zones of 10–18 mm for ceftazidime were further tested for ESBLs production. Detection of ESBLs isolates was performed by the double-disc synergy test – DDST and confirmed by double-disc diffusion test – DDDT [12]. DDST was done using amoxicillin/clavulanic acid as beta-lactamase inhibitor. Discs 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) disc. The plates were incubated at 37 °C for 24 h. Synergy between the discs towards the beta-lactamase inhibitor was regarded as presumptive ESBLs production. In order to confirm the results of DDST the DDDT was used. Four discs 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). The discs were placed on Mueller-Hinton agar inoculated with standardized inoculums of potential ESBLs-producing isolates [12]. The plates were incubated at 37 °C for 24 h. The positive result (ESBLs production) was defined as 5 mm increase in inhibition zone diameter around combination discs with clavu-

lanic acid versus its standard zone when tested alone. *Escherichia coli* ATCC 25922 was used as negative control.

Chi-square test was used to analyze the obtained results using the software statistical package STATISTICA v. 10.0, StatSoft, Inc. P-values less than 0.05 were considered to be statistically significant.

Results and discussion. Penicillins that affect enterobacteria are divided into four groups: aminopenicillins, acylureidopenicillins, carboxypenicillins and amidinopenicillins [13]. The most important aminopenicillins are ampicillin and amoxicillin. After oral administration, amoxicillin achieves two times higher serum concentrations while its spectrum of action is the same as ampicillin. *Escherichia coli* is innate susceptible to ampicillin and amoxicillin. Among the *E. coli* hospital strains, 72.1% of the isolates were resistant to ampicillin (Table 1). Given this high frequency of resistance, these antibiotics are not taken into account in the empirical therapy of hospital infections in investigated hospital, even in the case of urinary tract infections. At the level of Europe, the resistance of *E. coli* strains to aminopenicillins ranged from 35.8% in Finland, to 78% in Bulgaria [14].

According to the literature, *E. coli* can be sensitive to the first-generation cephalosporins [13]. In this investigation, the frequency of resistance of *E. coli* isolates to cefalexin was 55% (Table 1).

When it comes to the third-generation cephalosporins (ceftriaxone, cefotaxime) enterobacteria without acquired resistance mechanisms are generally sensitive. The most important mechanism of the acquired resistance of enterobacteria to the third-generation cephalosporins in hospitals around the world is the production of various extended-spectrum beta-lactamases [15].

The overall incidence of ESBLs production among *E. coli* isolates in this

Table 1

Resistance of hospital strains of *Escherichia coli* to antibiotics; number of tested, number and (%) of resistant strains

Antibiotic	Urine	Wound swabs	Blood	Others[1)]	Total
Ampicillin	336; 239 (71.1)	63; 55 (87.3)	11; 11 (100)	35; 16 (46.0)	445; 321 (72.1)
Cephalexin	337; 187(55.5)	11; 7 (64.0)	11; 11 (100)	29; 7 (24.1)	388; 212 (55.0)
Ceftriaxone	337; 98 (29.1)	60; 30 (50)	11; 9 (82.0)	36; 2 (5.5)	444; 139 (31.3)
Ceftazidime	**327; 98 (30.0)***	**63; 29 (46)***	**11; 9 (82.0)***	**5; 1 (20)***	**406; 137 (34.0**)
Imipenem	324; 0 (0)	62; 0 (0)	11; 0 (0)	5; 0 (0)	402; 0 (0)
Gentamicin	339; 127 (37.5)	63; 32 (51.0)	11; 8 (73.0)	36; 2 (5.5)	449; 169 (38.0)
Amikacin	327; 37 (11.3)	63; 9 (14.3)	11; 2 (18.2)	3; 0 (0)	404; 48 (12.0)
Ciprofloxacin	341; 141 (41.3)	63; 31 (49.2)	11; 8 (73.0)	34; 3 (9.0)	449; 183 (41.0)
Nitrofurantoin	341; 127 (37.2)	–	–	26; 6 (23.1)	367; 133 (36.2)
Trimethoprim/ Sulfamethoxazole	337;186 (55.2)	62; 41 (66.1)	11; 9 (82.0)	35; 10 (29.0)	445; 246 (55.3)

[1)] urethral, vaginal and cervical swabs, and sperm

$^*p < 0.05$

study was 34% (Table 1). Recent studies showed an increasing trend of ESBLs production in *E. coli* strains [14]. The lowest frequency of ESBL production in Europe is recorded in countries of northern and western Europe (Iceland 4.2%; Norway 5.6%; Netherlands 6.4%; Denmark 6.6%; Finland 6.9%). Serbia, with Bulgaria (41.6% of ESBLs production) belong to the countries with the highest frequency of ESBL-producing *E. coli* strains, unlike other countries in the region (Slovenia 12.5%; Croatia 14.7%; Romania 23.4%).

The frequency of ESBLs production in *E. coli* strains was the highest in hemoculture isolates (82%), and significantly less in isolates from wound swabs, urine and other specimens (Fig. 1). There was a statistically significant difference at level of $p < 0.05$, in terms of the frequency of ESBLs production in strains isolated from blood compared to the isolates from other samples (Table 1).

No resistance to carbapenem – imipenem was found in any isolate or sample. The same result was obtained in other European countries [16,17]. This may be attributed to the fact that carbapenems are very expensive broad-spectrum antimicrobial agents usually prescribed for serious infections. More so, carbapenems administration is parenteral and is less likely to be abused. Contrary to that, in the USA each year approximately 600 deaths result from infections caused by carbapenem-resistant *Klebsiella spp.* and carbapenem-resistant *E. coli* [18].

E. coli strains have exhibited good sensitivity to aminoglycosides. There was 38% of isolates resistant to gentamicin and 12% of isolates were resistant to amikacin (Table 1). This is in line with literature data which state that amikacin is still effective in many strains resistant to gentamicin [13]. Despite the long-term use of amikacin in investigated hospital, the sensitivity of tested strains to this antibiotic is preserved.

The frequency of resistance of *E. coli* to ciprofloxacin was relatively high, 41%.

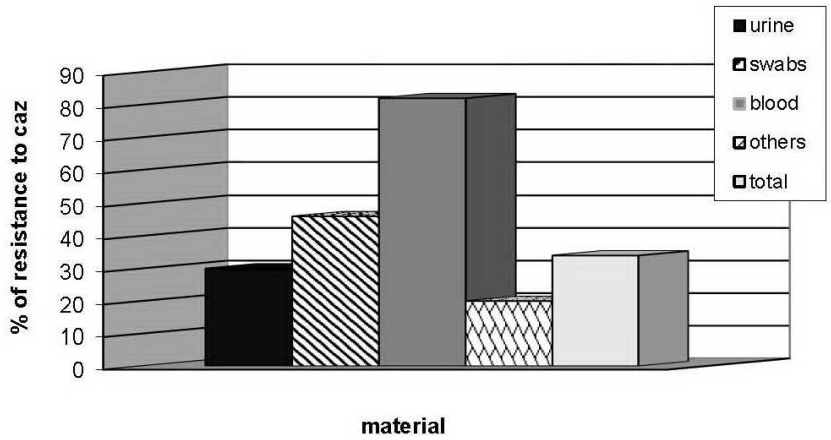

Fig. 1. Percentage of resistance of clinical strains of *E. coli* to ceftazidime (caz)

Table 2

Antibiotic resistance of clinical strains of *Escherichia coli* which were resistance to ceftazidime (ESBL-producers)

Antibiotic	Urine	Wound swabs	Blood	Others[1)]	Total
Gentamicin	101; 80 (79.0)	30; 23 (77.0)	9; 8 (89.0)	1; 0 (0)	141; 111 (79.0)*
Amikacin	99; 28 (28.0)	31; 9 (29.0)	9; 2 (22.2)	1; 0 (0)	140; 39 (28.0)*
Ciprofloxacin	101; 86 (85.2)	30; 23 (77.0)	9; 8 (88.9)	1; 0 (0)	141; 117 (83.0)*
Nitrofurantoin	102; 62 (61.0)	–	–	–	102; 62 (61.0)*
Trimethoprim/ Sulfamethoxazole	102; 89 (87.2)	30; 25 (83.3)	9; 9 (100)	1; 0 (0)	142; 123 (87.0)*

[1)] urethral, vaginal and cervical swabs, and sperm
*$p < 0.05$

Table 3

Antibiotic resistance of clinical strains of *Escherichia coli* which were susceptible to ceftazidime (non-ESBL-producers)

Antibiotic	Urine	Wound swabs	Blood	Others[1)]	Total
Gentamicin	224; 45 (20.1)	34; 9 (26.5)	2; 0 (0)	6; 0 (0)	266; 54 (20.3)*
Amikacin	223; 7 (3.1)	34. 0 (0)	2; 0 (0)	6; 0 (0)	265; 7 (2.6)*
Ciprofloxacin	224; 44 (17.0)	34; 9 (26.5)	2; 0 (0)	6; 0 (0)	266; 53 (20.0)*
Nitrofurantoin	224; 60 (27.0)	–	–	1; 1 (100)	225; 61 (27.1)*
Trimethoprim/ Sulfamethoxazole	221; 87 (39.4)	34; 14 (41.2)	2; 0 (0)	6; 0 (0)	263; 101 (38.4)*

[1)] urethral, vaginal and cervical swabs, and sperm
*$p < 0.05$

E. coli strains were significantly more susceptible to nitrofurantoin (36.2% of resistant isolates). Given the innate sensitivity, this drug can be successfully applied only in *E. coli* isolates from urine, because it is the only case where the clinical response in sensitive strains is possible to expect [13].

E. coli isolates from blood were significantly more resistant to other antibiotic classes (aminoglycosides – amikacin and gentamicin, ciprofloxacin, trimethoprim/sulfamethoxazole), compared to the isolates from urine, wound swabs and genital system samples (Table 1).

In Tables 2 and 3, the frequencies of resistance to antibiotics other than beta-lactams in *E. coli* isolates that produced ESBLs in relation to isolates that did not produce them, are shown. There was statistically significant higher incidence ($p < 0.05$) of resistance to gentamicin, amikacin, ciprofloxacin, nitrofurantoin and trimethoprim/sulfamethoxazol in the ESBL-producers compared to the non-producing strains. Tetteh [19] noted the significant differences in antibiotic resistance between ESBL-producers and non-ESBL-producers among *E. coli* and *K. pneumoniae* strains for cefuroxime, gentamicin and amikacin.

Conclusions. ESBLs were found in 34% of *E. coli* strains isolated from different clinical samples from intrahospital patients. The isolates that produced

ESBLs were significantly more resistant to the classes of antibiotics other than beta-lactams.

The resistance of the tested bacterium to carbapenem – imipenem was not recorded. After imipenem, the hospital isolates of *E. coli* showed the best sensitivity, i.e. the lowest incidence of resistance, to aminogycoside amikacin.

This investigation showed that the frequency of ESBLs production in bacteria strains isolated from hospitalized patients in examined hospital is very high and is among the highest in Europe. Urgent work is needed to develop quicker, cost-effective and reliable diagnostic tools as well as new effective therapies. Also, there is a need for more reasonable use of existing drugs and introduction of more effective infections control measures.

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