

**PREVALENCE AND MOLECULAR CHARACTERIZATION
OF EXTENDED SPECTRUM BETA-LACTAMASES (ESBLs)
PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE***

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Abstract: The aim of this study was to determine the prevalence of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* as well as genes encoding ESBLs.

Material and methods: A total of 1207 non-repeat isolates of *E. coli* and *K. pneumoniae* were obtained from urine, tracheal aspirate, wound swab and blood from patients hospitalized at the University Clinics in Skopje. ESBL set and E-test were used for phenotypic detection of ESBL-production. Multiplex PCR was used to identify genes for different types of ESBLs in 100 ESBL positive strains (*E. coli*-52 and *K. pneumoniae*-48), randomly selected.

Results: Out of 804 *E. coli* isolates and 403 *K. pneumoniae* isolates, 126 (15.7%) and 125 (31%) isolates were ESBL producers, respectively. The prevalence of ESBL-positive strains of *E. coli* in surgery clinics (42 out of total of 211–19.9%) and *K. pneumoniae* (61 out of a total of 161–37.9%) was higher compared to those in the clinics of internal medicine (84 out of 593–14.2%) and (64 out of 242–26.4%), respectively. Only 87 of ESBL positive isolates could be typed for one or more genes. Among the isolates of *E. coli* and *K. pneumoniae* harbouring a single ESBL gene (39%), *bla*_{SHV}, *bla*_{TEM} and *bla*_{CTX-M} were present in 19.5%, 16% and 3.4% strains, respectively. Two or more genes for ESBL were present in 61% of ESBL isolates; *bla*_{TEM} + *bla*_{SHV} being the most common combination.

Conclusion: The majority of strains harboured two or more ESBL genes and the most common phenotypes were TEM, SHV and CTX-M. Identification of the genes is necessary for the surveillance of their transmission in hospitals.

Key words: ESBL, *Escherichia coli*, *Klebsiella pneumoniae*, prevalence, multiplex PCR.

Introduction

Extended-spectrum beta-lactamases (ESBLs) were first described in the 1980s in *Klebsiella* species and later they have been detected in *E. coli* and other genera of the *Enterobacteriaceae* family. ESBLs are the rapidly evolving group of β -lactamase enzymes which have the ability to hydrolyze all cephalosporins and monobactams, but are inhibited by β -lactamase inhibitors, such as clavulanic acid [1].

ESBLs are undergoing continuous mutation, causing the development of new enzymes showing expanded substrate profiles. At present, there are more than 300 different ESBL variants. TEM (Temoniera) and SHV (sulphydryl variable) were the major types. However, CTX-M type (predominantly hydrolyze cefotaxime) is increasingly becoming important [2]. Variants derived from TEM and SHV enzymes and CTX-M-ESBLs (derived from other sources) are defined as "classical ESBLs". Various β -lactamases conferring wider resistance than their parent types but not meeting the definition for group 2be; e.g., OXA-types (OXA-ESBLs) and mutant AmpC-types (plasmid-borne AmpC-like enzymes, such as DHA), with increased activity against oxyimino-cephalosporins and with resistance to oxacillin and ceftaxime, respectively, were "ESBLs of growing importance" [3].

ESBL are an important cause of transferable multidrug resistance in gram-negative bacteria throughout the world. These bacteria have spread rapidly and have become a serious threat to human health worldwide. Determination of ESBL genes by molecular techniques in ESBL producing bacteria and their pattern of antimicrobial resistance can supply useful data about their epidemiology and risk factors associated with these infections [1, 4, 5].

In Macedonia, few studies have been undertaken to determine the prevalence of ESBL-producing *E. coli* and *Klebsiella pneumoniae* [6, 7, 8]. However, β -lactamases were not molecularly characterized in these studies. Therefore, the present study was carried out to determine the prevalence of ESBL positive strains of *E. coli* and *Klebsiella pneumoniae* in the University Clinics in Skopje as well as genes for the TEM, SHV, CTX-M, OXA and DHA types of ESBL enzymes.

Material and methods

Bacterial isolates

A total of 1207 consecutive non-repeat isolates of *E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*) were obtained from different clinical specimens such as urine, tracheal aspirate, wound swab, blood, etc. over a one-year period

from patients hospitalized at the Surgery and Internal Medicine University Clinics (UC) in Skopje. The isolates were identified on the basis of conventional microbiological procedures.

Antimicrobial susceptibility testing

Susceptibility of all isolates was determined by the disk diffusion method on Mueller-Hinton agar (Oxoid, UK) following the zone size criteria as recommended by the Clinical and Laboratory Standards Institute (CLSI). The antibiotic (concentrations in μg) tested included cefixime (5), ceftriaxone (30), amoxicillin-clavulanic acid (20/10), piperacillin (100), piperacillin/tazobactam (100/10), gentamicin (10), ofloxacin (10), ciprofloxacin (5), co-trimoxazole (1.25/23.75), amikacin (30), imipenem (10), cefepime (30), meropenem (10).

ESBL screening and confirmation by phenotypic methods

The isolates showing resistance to one or more 3rd generation cephalosporins were tested for ESBL production by the double disk diffusion test (DDDT) using three pairs of cephalosporins and their combinations with clavulanic acid (ESBL set, Mast Diagnostic). ESBL set contains 3 paired sets of cephalosporins (ceftazidime-CAZ; cefotaxime-CTX and cefpodoxime-CPD) and cephalosporins with clavulanic acid-CA (CAZ + CA; CTX + CA and CPD + CA). The inoculum and incubation conditions were the same as for standard disk diffusion recommendations. A > 5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was designated as ESBL positive.

ESBL E-test

All the isolates positive by ESBL set were further confirmed by the ESBL E-test method (BioMerieux) using strips impregnated with a gradient of different concentrations (0.5–32 $\mu\text{g}/\text{ml}$) of CAZ on one side and on other side different concentration of CAZ (0.064–4 $\mu\text{g}/\text{ml}$) along with a fixed concentration of clavulanic acid (4 $\mu\text{g}/\text{ml}$). The other strip was with gradient of concentration (0.25–16 $\mu\text{g}/\text{ml}$) of CTX at one side and at the other side different concentration of CTX (0.016–1 $\mu\text{g}/\text{ml}$) with a fixed concentration of clavulanic acid (4 $\mu\text{g}/\text{ml}$). The presence of ESBL was confirmed by the appearance of a phantom zone or when CAZ MIC and CTX MIC are reduced $> 3 \log_2$ dilutions in the presence of clavulanic acid as per manufacturer guidelines.

K. pneumoniae ATCC 700603 was used as a positive control (presence of bla_{SHV} gene) and *Escherichia coli* ATCC 25922 was used as a negative control (obtained from American Type Culture Collection, USA), for both phenotypic methods and for multiplex PCR.

Multiplex PCR for TEM, SHV, CTX-M, OXA and DHA genes

A total of 100 ESBL positive strains (*E. coli*-52 and *K. pneumoniae*-48) were randomly selected to detect the presence of genes.

Genomic DNA was prepared from overnight cultures grown on Brain-Heart Infusion Broth (Oxoid, UK) with agitation at 250 rpm at 37 degrees. After centrifugation of 2 ml broth at 14 000 rpm for 10 min, 100 µl distilled water was added to the pellet. After suspension of the pellet, centrifugation was repeated at 14 000 rpm for 5 min. 100 µl distilled water was added to resuspend the pellet. After boiling for 10 min, Eppendorfs were placed on the ice. The upper layer was used as crude DNA.

Suitable primers for two multiplex PCR reactions each targeting different regions were designed. The first multiplex assay (named Set I) was designed to detect TEM, SHV, CTX-M IV group and OXA β-lactamase encoding genes, and the second assay (named Set II) was designed to detect CTX-M I group, CTX-M II group and DHA encoding genes.

Table 1

Primers contained in Set I and Set II assays

Assay	Primers	Primer sequence 5' → 3'	Size (bp)
Set I	CTX-M IV F	GACAAAGAGAGTGCAACGGATG	501
	CTX-M IV F	TCAGTGCATCCAGACGAAA	
	TEM F	AGTGCTGCCATAACCATGAGTG	431
	TEM R	CTGACTCCCCGTCGTGTAGATA	
	OXA F	ATTATCTACAGCAGCGCCAGTG	296
	OXA R	TGCATCCACGTCTTTGGTG	
	SHV F	GATGAACGCTTTCCCATGATG	214
SHV R	CGCTGTTATCGTCATGGTAA		
Set II	CTX-M I F	TCCAGAATAAGGAATCCCATGG	621
	CTX-M I R	TGCTTTACCCAGCGGTCAGAT	
	CTX-M II F	ACCGCCGATAATTCGCAGAT	588
	CTX-M II R	GATATCGTTGGTGGTGCCATAA	
	DHA F	GTGGTGGACAGCACCATTAAA	314
	DHA R	CCTGCGGTATAGGTAGCCAGAT	

Both PCR reactions were performed under identical conditions.

Amplification reactions were performed in a 25 µl volume containing 2.5 µl of 10X PCR reaction buffer with a MgCl₂ (15 mM), 0.5 µl (200 µM) deoxynucleoside triphosphates mix (dNTPs, 10 mM), 1 µl (each) primers (10 pm/µl) with 0,5 µl (5 U/µl) Taq DNA polymerase. Five microlitres of the template DNA preparation was added to the reaction mixture.

Both assays used identical cycling conditions. Reactions were performed in a DNA thermal cycler under the following conditions: denaturation at 94°C for 5 min followed by 30 cycles at 94°C for 1 min, 61°C for 1 min and 72°C for 1 min and a final extension of 72°C for 5 min. After PCR amplification, 10 µl of each reaction was separated by electrophoresis in 1.5% agarose gel. Both assay products were electrophoresed for 1 hour at 100 V in 0,5 × TBE buffer. DNA was stained with ethidium bromide (1 µg/ml) and the gels were imaged under UV light. PCR amplicon size was calculated by comparison to molecular weight size marker (1000 bp DNA ladder). All of the Set I and SET II assays produced single or multiplexed products of the predicted sizes. Methods have been described previously [4, 5, 9, 10, 11].

Statistical analysis

A chi-square test was used to compare the prevalence of ESBL-producing strains between isolates from hospitalized patients in Surgery Clinics and those from Clinics of Internal Medicine. The difference between proportions was used to evaluate the difference between the strains harbouring single ESBL genes and those with two or more ESBL genes. P-value less than 0.05 was considered to be statistically significant ($p < 0.05$).

Results

Out of 804 *E. coli* isolates and 403 *K. pneumoniae* isolates, 126 (15.7%) and 125 (31%) isolates were ESBL producers, respectively.

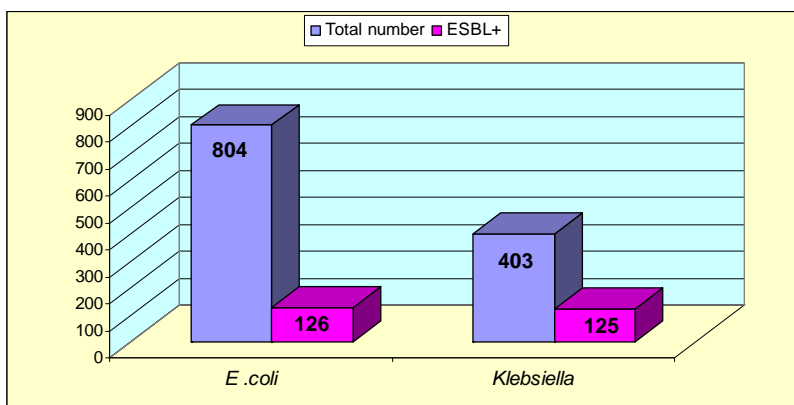


Figure 1 – ESBL – positive strains out of a total number of *E. coli* and *Klebsiella pneumoniae*

In this study, the same number of ESBL positive isolates was detected by both phenotypic methods (DDST and E test).

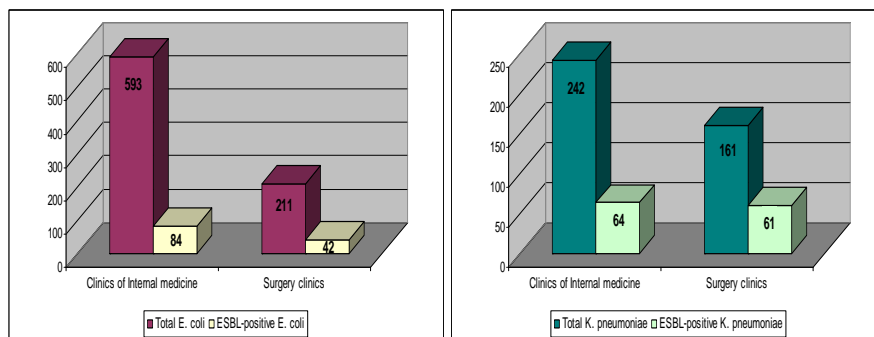


Figure 2 and 3 – Prevalence of ESBL-positive *E. coli* and *K. pneumoniae* in the University Clinics

The prevalence of ESBL-positive strains of *E. coli* and *K. pneumoniae* in Surgery Clinics (42 out of a total of 211–19.9%) and (61 out of total of 161–37.9%) was higher compared to that in the Internal Medicine Clinics (84 out of 593–14.2%) and (64 out of 242–26.4%), respectively. This difference was not statistically significant ($p > 0.05$).

ESBL-positive *K. pneumoniae* strains were more prevalent in Surgery Clinics compared to ESBL-positive *E. coli* strains. This difference was statistically significant ($p = 0.000$), whereas in Internal medicine Clinics the difference in prevalence of ESBL-positive *K. pneumoniae* and *E. coli* strains was not statistically significant ($p = 0.065$).

Amplification profiles of each primer set for the Set I and Set II multiplex assays are shown on the following figures.

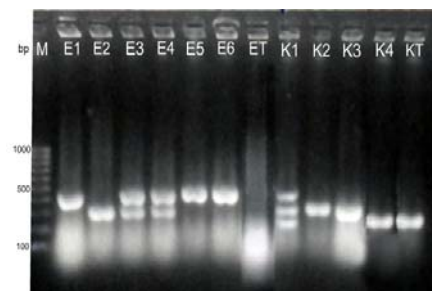


Figure 4 – Multiplex PCR-Set I: M (molecular marker); E1-E6 *E. coli*, ET – negative control; K1-K4 *K. pneumoniae*; KT – positive control E1- TEM (431 bp); E2-OXA (296 bp); E3, E4-TEM, OXA; E5, E6-TEM; K1-TEM, OXA, SHV (214 bp);

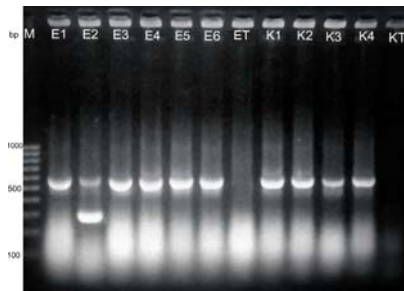


Figure 5 – Multiplex PCR-Set II: M (molecular marker); E1-E6 *E. coli*, ET – negative control; K1-K4 *K. pneumoniae*; KT – positive control E1-CTX-M II (588 bp); E2-CTX-M II, DHA (314 bp); E3-E6- CTX-M II; K1-K4

Both multiplex assays were performed on the same isolates (6 *E. coli* isolates and *E. coli* negative control; 4 *K. pneumoniae* isolates and *K. pneumoniae* positive control). Therefore, the total number and types of ESBL enzymes can be seen in both figures (Fig 4 and Fig 5). For example, E1 isolate has two enzymes: TEM (431 bp) seen in Fig 4 and CTX-M-II (588 bp) seen in Fig. 5.

Table 2

Extended spectrum β -lactamase (ESBL) genotypes in E. coli and K. pneumoniae strains

Positive by PCR for ESBL genes	Number amplified		
	<i>E. coli</i> (n = 46)	<i>K. pneumoniae</i> (n = 41)	Total N = 87 (%)
A. Single ESBL gene	17	17	34 (39%)
bla _{TEM} only	11	3	14 (16%)
bla _{SHV} only	4	13	17 (19,5%)
bla _{CTX-M} only	2	1	3 (3.4%)
B. Two or more ESBL genes	29	24	53 (61%)
bla _{TEM} + bla _{SHV}	8	6	14 (16%)
bla _{TEM} + bla _{CTX-M}	6	3	9 (10.3%)
bla _{SHV} + bla _{CTX-M}	0	2	2 (2.3%)
bla _{OXA} + bla _{CTX-M}	7	2	9 (10.3%)
bla _{TEM} + bla _{SHV} + bla _{CTX-M}	3	2	5 (5.7%)
bla _{TEM} + bla _{OXA} + bla _{CTX-M}	4	2	6 (6.9%)
bla _{OXA} + bla _{CTX-M} + bla _{DHA}	1	0	1 (1.1%)
bla _{TEM} + bla _{SHV} + bla _{OXA} + bla _{CTX-M}	0	7	7 (8%)
Not determined by PCR	6	7	13 (14.9%)

Genomic DNA isolated from 100 phenotypic confirmed ESBL-producing organisms (*E. coli*-52 and *K. pneumoniae*-48) were subjected to PCR using 7 pairs of primers. Only 87% of ESBL positive isolates could be typed for one or more genes. Among the isolates harbouring single ESBL gene (39%), bla_{SHV}, bla_{TEM} and bla_{CTX-M} were present in 19.5%, 16% and 3.4% strains of *E. coli* and *K. pneumoniae*, respectively. There were no strains harbouring a single OXA and DHA type beta-lactamase. The equal number of strains of *E. coli* and *K. pneumoniae* had a single ESBL gene, although TEM and SHV types of ESBL were frequently found in *E. coli* (11/17) and *K. pneumoniae* (13/17), respectively.

Two or more genes for ESBL were present in 53 (61%) of the 87 ESBL typeable isolates, bla_{TEM} + bla_{SHV} being the most common combination (16%),

followed by $bla_{TEM} + bla_{CTX-M}$ and $bla_{OXA} + bla_{CTX-M}$ (10.3% each). Eight strains of *E. coli* and 4 strains of *K. pneumoniae* harbour 3 genes for ESBL. The most common combination was $bla_{TEM} + bla_{OXA} + bla_{CTX-M}$ (6.9%). Only 7 strains of *K. pneumoniae* (8%) and none of *E. coli* harbour 4 genes for ESBL $bla_{TEM} + bla_{SHV} + bla_{OXA} + bla_{CTX-M}$. There is a difference between strains harbouring two or more genes (61%) compared to those with a single ESBL gene (39%). This difference is statistically significant ($p = 0.024$).

There were 13 strains (14.9%) tested positive for ESBL using phenotypic tests, but negative with PCR. The negative amplification in these isolates may be due to other ESBL genes, which could not be detected by these particular Set I and Set II.

Table 3

Genes detected in isolates either alone or in various combinations

	<i>E. coli</i>	<i>Klebsiella</i>	Total
bla_{TEM}	32	23	55
bla_{SHV}	15	30	45
bla_{OXA}	12	11	23
$bla_{CTX-M-I}$	16	8	24
$bla_{CTX-M-II}$	23	19	42
$bla_{CTX-M-IV}$	0	0	0
bla_{DHA}	1	0	1

Overall, bla_{TEM} was present in 63.3% (55/87) and bla_{SHV} in 52% (45/87) isolates either alone or in combinations. OXA and DHA type beta-lactamases were present in combination with other enzymes. Bla_{OXA} was present in 26.4% (23/87) isolates only in combination with other genes. Bla_{DHA} was present in only one strain of *E. coli* in combination with two other genes. Multiplex PCR was designed to detect three different genes encoding CTX-M enzymes ($bla_{CTX-M-IV}$ 621 bp – detected with Set I) and the second assay (Set II) was designed to detect $bla_{CTX-M-I}$ and $bla_{CTX-M-II}$. $Bla_{CTX-M-I}$ was always co-present with $bla_{CTX-M-II}$ in 27.6% (24/87) isolates, while $bla_{CTX-M-II}$ was present in 18 strains without $bla_{CTX-M-I}$. Overall, $bla_{CTX-M-II}$ was present in 48% (42/87) isolates, either alone or in combinations. There were no strains harbouring $bla_{CTX-M-IV}$.

Discussion

In recent years, the problem of increasing resistance to antibiotics has threatened the entire world. Production of beta-lactamase, which hydrolyses and inactivates beta-lactam antibiotics has been one of the most important resistance

mechanisms of many bacterial species, mainly in the *Enterobacteriaceae* family. Resistance to an extended spectrum beta-lactams among gram-negative pathogens is increasingly associated with ESBLs.

The correct detection of ESBL producing microorganisms is a challenge for the laboratories, requiring not only phenotypic tests, but also genotypic tests for all genes associated with beta-lactamase production. *K. Pneumoniae* and *E. coli* are the most prevalent among ESBL-producing microorganisms, confirming international multicentric studies [1, 5, 12, 13].

The use of three distinct substrates in the combined disk tests increased the sensitivity of the test and cefotaxime and cefpodoxime performed the best. Tofteland et al. [14] recommended the use of cefpodoxime alone or a combination of cefotaxime and ceftazidime as preferred substrates for ESBL detection. We also performed an E test to detect the presence of ESBL and MIC to two cephalosporins. All ESBL isolates had MIC > 8 µg/ml to cefotaxime and more than 90% had MIC > 2 to ceftazidime; the majority had a high level of resistance to both drugs.

The SENTRY Antimicrobial Surveillance Program showed that ESBL-producing *K. pneumoniae* isolates were more prevalent in Latin America (45.5%), followed by the Western Pacific region (24.6%), Europe (22.6%), the United States (7.6%) and Canada (4.9%) [15]. In Asia the prevalence of ESBL-producing *K. pneumoniae* and *E. coli* vary from 5% in Japan to 20–50% in other countries. In Europe, the prevalence of these organisms varies from country to country (3% in Sweden to 34% in Portugal) [16, 17, 18]. In this study only data from hospitalized patients in the University Clinics in Skopje have been shown. Although *E. coli* strains were more frequently isolated than *K. pneumoniae* strains, the production of ESBLs was more often present in *K. pneumoniae*. In our study, the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* had means of 15.7% and 31% respectively.

The prevalence of ESBL-producing bacteria was increasing every year especially in tertiary hospitals. The major risk factors are long term exposure to antibiotics, prolonged ICU stay, nursing home residency, severe illness, instrumentation or catheterization, length of hospitalization (more than 48 hours of hospitalization), etc. [19]. In our study, there was a difference (although not statistically significant) in the prevalence of ESBL-producing *E. coli* and *K. Pneumoniae* in surgical clinics compared to the one in clinics of internal medicine. ESBL-positive *K. pneumoniae* strains were more prevalent in surgery clinics compared to ESBL-positive *E. coli* strains. This difference was statistically significant.

Using specific primers for TEM, SHV, CTX-M, OXA and DHA only 87 (87%) of 100 ESBL positive isolates could be typed for one or more genes.

The negative amplification in the remaining isolates may be due to the presence of other ESBL genes, which we did not explore further.

In the present study, 39% and 61% of 87 ESBL typeable isolates harbour a single ESBL gene and two or more genes for ESBL, respectively. TEM and SHV types of ESBL were frequently found in *E. coli* (11/17) and *K. pneumoniae* (13/17), respectively. bla_{SHV} and bla_{TEM} were also co-present with other genes. Overall, bla_{TEM} was present in 57.3% (51/87), predominantly in *E. coli* (32/51) and bla_{SHV} in 49.4% (44/87), predominantly in *K. pneumoniae* (29/44) isolates, either alone or in combinations.

$bla_{CTX-M-I}$ was always co-present with $bla_{CTX-M-II}$ in 27.6% (24/87) isolates, while $bla_{CTX-M-II}$ was present in 17 strains without $bla_{CTX-M-I}$. Overall, $bla_{CTX-M-II}$ was present in 47% (41/87) isolates, either alone or in combinations.

In other studies, two or more ESBL genes were present in 57.3% typeable isolates. The bla_{CTX-M} was the most common and was present either alone or in combination with other ESBL types (13). Pournaras *et al.* reported an 87% prevalence of CTX-M enzyme among ESBL producers in a tertiary care hospital in Greece [20]. In a multi-centric study from Russia, CTX-M gene was reported in 35.9% of *E. coli* and 34.9% of *K. pneumoniae* ESBL strains [21]. In a nationwide survey in Italy, CTX-M producing strains were reported with remarkably variable rates among the centres (1.2–49.5% of the ESBL producers) [22].

Another study summarized 50%, 14.9% and 11.7% ESBL rates for TEM, SHV and CTX-M type beta lactamases, respectively. TEM and CTX-M type ESBL were observed in 72.7 and 22.7% of *E. coli* isolates, respectively. SHV type ESBL was frequently found in *K. pneumoniae* (53%) isolates [1].

To conclude, phenotypic methods are only screening methods for detection of ESBLs in a routine laboratory. The prevalence of ESBL-producing *E. coli* and *K. pneumoniae* had means of 15.7% and 31%, respectively. The prevalence of ESBL-producing *E. coli* and *K. pneumoniae* in surgical clinics was higher compared to that in clinics of internal medicine.

The genotypic methods help us to confirm the genes responsible for ESBL production. The majority of our strains harboured two or more ESBL genes and the most common phenotypes were TEM, SHV and CTX-M. We used a multiplex PCR for the detection of bla_{TEM} , bla_{SHV} , bla_{CTX-M} , bla_{OXA} , and bla_{DHA} genes in ESBL-producing *E. coli* and *K. pneumoniae*. This method provided an efficient, rapid differentiation of ESBLs. The correct identification of the genes involved in ESBL-mediated resistance is necessary for the surveillance and epidemiological studies of their transmission in hospitals. Appropriate antibiotic policy and infection control measures in hospital settings are crucial to overcome the problems associated with infections by ESBL-producing strains.

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Резиме

ПРЕВАЛЕНЦА И МОЛЕКУЛАРНИ КАРАКТЕРИСТИКИ НА *ESCHERICHIA COLI* И *KLEBSIELLA PNEUMONIAE* ШТО ПРОДУЦИРААТ БЕТА ЛАКТАМАЗИ СО ПРОШИРЕН СПЕКТАР (ESBLs)

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Апстракт: Цел на студијата е да се одреди преваленцата на *E. coli* и *K. pneumoniae* кои продуцираат ESBL, како и гените што ги кодираат овие ензими.

Материјал и методи: Вкупно 1 207 соеви на *E. coli* и *K. pneumoniae* (*Kp*) беа изолирани од урина, трахеален аспират, рана, крв од пациенти хоспитализирани во Универзитетските клиници во Скопје. Осетливоста кон анти-микробни агенси беше одредена со диск дифузионен метод, а ESBL сетот и E-тестот беа применети за фенотипска детекција на продукцијата на ESBL. За идентификација на гените за различни типови на ESBL беше применет Multiplex PCR (полимеразна верижна реакција) кај вкупно 100 соеви кои продуцираат ESBL (*E. coli*-52 и *Kp*-48), селектирани по случаен избор.

Резултати: Од 804 изолати на *E. coli*, 126 (15,7%) продуцираа ESBL, а од 403 изолати на *Kp*, 125 (31%) продуцираа ESBL. Преваленцата на ESBL-позитивните соеви на *E. coli* (42 од вкупно 211–19,9%) и на *Kp* (61 од вкупно 161–37,9%) од хируршките клиници беше повисока споредена со преваленцата на клиниките по интерна медицина (84 од вкупно 593–14,2% соеви на *E. coli* и 64 од вкупно 242–26,4% соеви на *Kp*).

Кај 87 ЕСБЛ-позитивни соеви беа детектирани еден или повеќе гени. Од соевите кај кои беше детектиран по еден ген (39%), *bla_{SHV}* генот беше детектиран кај 19,5%, *bla_{TEM}* кај 16% и *bla_{CTX-M}* кај 3,4% од соевите. Два и повеќе гени беа детектирани кај 61% од ESBL-позитивните изолати. Комбинацијата на *bla_{SHV}* и *bla_{TEM}* беше најчеста.

Заклучок: Повеќето соеви вклучени во студијата содржеа два и повеќе гени и најчести беа TEM, SHV и CTX-M типовите на ESBL. Идентификација на гените кои кодираат синтеза на ESBL е неопходна за следењето на нивната трансмисија во болничка средина.

Клучни зборови: ESBL, *Escherichia coli*, *Klebsiella pneumoniae*, преваленца, Multiplex PCR.

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