EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) PREVALENCE IN BACTERIA ISOLATED FROM URINE SAMPLES

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Abstract

Urinary tract infections (UTI) due to *E. coli* are the most common infectious diseases. Extended spectrum beta-lactamases (ESBLs) are clinically important because they destroy cephalosporins - antibiotics which are used as first line agents in critically ill patients, including those with intra-abdominal infections, pneumonias and bacteremia. The aim of this study is to present the prevalence of ESBL *E. coli/K. pneumoniae* UTI through a retrospective review of medical records in the period from 2019 to 2023.

This was a retrospective study conducted at the Institute of Public Health (IPH) of the Republic of North Macedonia, in which the prevalence of ESBL *E. coli/ K. pneumoniae* UTI was elaborated covering the period from 2019 to 2023.

The analysis by year at the national level, presented in Table 1, shows that most of the reports were registered in 2019 - 134 or 32.1%, while the smallest number were reported in 2020 - 31 or 7.4%. The analysis of demographic data in terms of gender shows that a cumulatively higher number of reports at the national level was registered among women - 269 or 64.5%. If data from the IPH are analyzed according to this indicator, 46 women (82.1%) and 10 men (17.9%) were registered.

In conclusion, the current study indicated a significant rate of infection with ESBL-producing Gram-negative bacilli in patients with UTI.

Keywords: Prevalence, ESBL *E. coli/ K. pneumoniae*, UTI

Introduction

The prevalence of urinary tract infections (UTI) with extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* UTI is increasing worldwide[1]. Antimicrobial resistance (AMR) results from invasive isolates reported to the Central Asian and European Antimicrobial Resistance Surveillance Network (CAESAR) and the European Antimicrobial Resistance Surveillance Network (EARS-Net) in the 2022 report (data refer to 2021)) show that the majority of isolates (70.0%) were *E. coli* (37.9%), *Staphylococcus aureus* (17.2%) and *K. pneumoniae* (14.9%)[2].

Urinary tract infections due to *E. coli* are the most common infectious diseases. Prolonged UTIs can result in patient morbidity if not adequately treated. The emergence of

the ESBL *E. coli* strain increases the incidence and complication of managing UTI patients. Furthermore, it leads to a long-term hospitalization and increases the cost of treatment. Extended spectrum β-lactamase-producing bacteria harbors ESBL enzymes that hydrolyze β-lactam antibiotics and are associated with the treatment failure of β-lactam antibiotics[3]. ESBLs are enzymes that hydrolyze most penicillins and cephalosporins, including oxyimino-β-lactam compounds (cefuroxime, third- and fourth-generation cephalosporins and aztreonam), but neither cephamycins or carbapenems. Most ESBLs belong to the Ambler class A of β-lactamases and are inhibited by β-lactamase inhibitors (clavulanic acid, sulbactam and tazobactam) and by diazabicyclooctanones (avibactam)[4].

The prevalence of ESBL-positive isolates depends on a range of factors including species, geographic locality, hospital/ward, group of patients and type of infection. Large variations have been reported in different studies[5-8].

ESBL are acquired, class A plasmid-mediated enzymes which hydrolyze and confer resistance to oxyimino - ß-lactams such as the "2nd and 3rd generation" cephalosporins, cefuroxime, cefotaxime, ceftazidime and ceftriaxone being the most important. ESBLs are clinically important because they destroy cephalosporins – antibiotics which are used as first line agents in critically ill patients, including those with intra-abdominal infections, pneumonias and bacteremia[9]. The vast majority of ESBLs are acquired enzymes, encoded by genes on plasmids. The acquired ESBLs are expressed at various levels, and differ significantly in biochemical characteristics such as activity against specific β-lactams (e.g. cefotaxime, ceftazidime, aztreonam). The level of expression and properties of an enzyme, and the co-presence of other resistance mechanisms (other β-lactamases, active efflux, altered permeability) result in the large variety of resistance phenotypes observed among ESBL-positive isolates[10]. ESBL-producing Enterobacterales are resistant to common antibiotics and may require complex treatments. Infections caused by ESBL-producing Enterobacterales can occur both in and outside of healthcare settings. Good hand hygiene and infection prevention practices can help reduce infection risk.

The aim of this study is to present the prevalence of ESBL *E. coli/K. pneumoniae* UTI through a retrospective review of medical records in the period from 2019 to 2023.

Materials and methods
Study design, site and population
This was a retrospective study conducted at the Institute of Public Health (IPH) of the Republic of North Macedonia, in which the prevalence of ESBL *E. coli/ K. pneumoniae* UTI was elaborated covering the period from 2019 to 2023.

The survey included all reported isolates of *E. coli* and *K. pneumoniae* to IPH from all laboratories in North Macedonia through the national system for surveillance of microbiological pathogens/isolates.

An analysis was made according to demographic characteristics, laboratories, time period, type of hospital/ward and AMR. The study was conducted in compliance with the code of ethics to ensure the impossibility of connecting the obtained data with the source/personal data.

Data was obtained from the database of reported isolates from the Epidemiology department of the Institute of Public Health. The data was processed in Microsoft Excel, version 16 for Windows. The quantitative series underwent analysis using measures of central tendency (mean, minimum, maximum values) alongside dispersion measures (standard deviation).

Sample collection and laboratory procedures

Urine samples were cultured on a routine basis by semi-quantitative methods according to standard operating procedures[11] and guidelines from the Institute of Public Health. Urine was collected for diagnostic purposes using sterile urine collection bags (Urinocol®, B. Braun Medical, France) according to the manufacturer's instructions. Each urine specimen was subjected to a routine urinary examination. Urine culture is the gold standard for diagnosing UTI. It is important to iterate that all samples should first be subject to a dipstick testing and/or microscopic examination to look for the presence of nitrites, white blood cells, red blood cells or bacteria.

Specimens were processed routinely using calibrated loops for plating. This method allows CFU/mL findings as well as the isolation of colonies for identification and susceptibility testing. Some of the most utilized media are blood agar and BRILLIANCE UTI AGAR. Brilliance™ UTI Agar (formerly Chromogenic UTI Agar) is a chromogenic medium for the presumptive identification and differentiation of all main microorganisms that cause urinary tract infections (UTIs).

Brilliance UTI Agar contains two specific chromogenic substrates which are cleaved by enzymes produced by *Enterococcus spp.*, *Escherichia* coli and coliforms. In addition, it contains phenylalanine and tryptophan, which provide an indication of tryptophan deaminase activity, indicating the presence of *Proteus spp.*, *Morganella spp.* and *Providencia spp.* It is based on electrolyte deficient CLED medium which provides a valuable non-inhibitory diagnostic agar for plate culture of other urinary organisms, whilst preventing the swarming of *Proteus spp*. The temperature of the plates should be kept between 35 to 37 degrees Celsius with a recommended incubation time of 24 to 48 hours.

One chromogen, X-Gluc, is targeted towards β-glucosidase, and allows the specific detection of enterococci by the formation of blue colonies. The other chromogen, Red-Gal, is cleaved by the enzyme β-galactosidase, which is produced by *Escherichia coli*, resulting in pink colonies. Any uncertainty in identification may be resolved by removing suspect *Escherichia coli* colonies from the plate and performing an indole test using DMACA reagent. Cleavage of both chromogens occurs in the presence of coliforms, resulting in purple colonies.

Results

In the period from 2019 to 2023, by 13 microbiological laboratories in the Republic of Macedonia, 417 reports of an isolated/otherwise proven causative agent of an infectious disease - *Escherichia coli*, ESBL, were reported to the IPH within the national surveillance system; *Klebsiella pneumoniae*, ESBL; and *Klebsiella spp*, ESBLs.

In the examined period, of the total number of reported *Escherichia coli*, ESBL; *Klebsiella pneumoniae*, ESBL; and *Klebsiella spp*, ESBL, by the microbiological laboratory at IPH, 56 isolates or 13.4% were reported.

The analysis by year at the national level, presented in Table 1, shows that most of the reports were registered in 2019 - 134 or 32.1%, while the smallest number were reported in 2020 - 31 or 7.4%. If the reports from the IPH are analyzed, it is noted that they were reported in the period 2019-2021 (Table 1).

The analysis of demographic data in terms of gender showed that a cumulatively higher number of reports at the national level was registered among women - 269 or 64.5%. If data from the IPH are analyzed according to this indicator, 46 women (82.1%) and 10 men (17.9%) were registered.

Regarding the representation of gender by the causative agent, a higher number and percentage of females had *Escherichia coli*, ESBL (n=228; 69.1%) and *Klebsiella spp*, ESBL (n=7; 53.8%), while a greater number and percentage of males had *Klebsiella pneumoniae*,

ESBL (n=40; 54.1%). The representation of female gender in the positive samples from IPH for *Escherichia coli*, ESBL was 84.9% (n=45).

Table 1. Annual number of isolates

| Bacterial species (ESBL)	2019			2020			2021			2022			2023		
	Labs (n)	Isolates (n)	Isolates from IPH (n;%)	Labs (n)	Isolates (n)	Isolates from IPH (n;%)	Labs (n)	Isolates (n)	Iolates from IPH (n;%)	Labs (n)	Isolates (n)	Iolates from IPH (n;%)	Labs (n)	Isolates (n)	Iolates from IPH (n;%)
E. coli	8	120	46 (38,3%)	5	25	5 (20,0%)	6	44	2 (4,5%)	5	75	/	8	66	/
K. pneumonie	2	7	/	3	5	2 (40,0%)	1	4	/	4	36	/	3	22	/
Klebsiella spp	1	7	/	1	1	/	2	3	1 (33,3%)	1	1	/	1	1	/
Total	8	134	46 (34,3%)	5	31	7 (22,6%)	6	51	3 (5,9%)	5	112	/	8	89	/
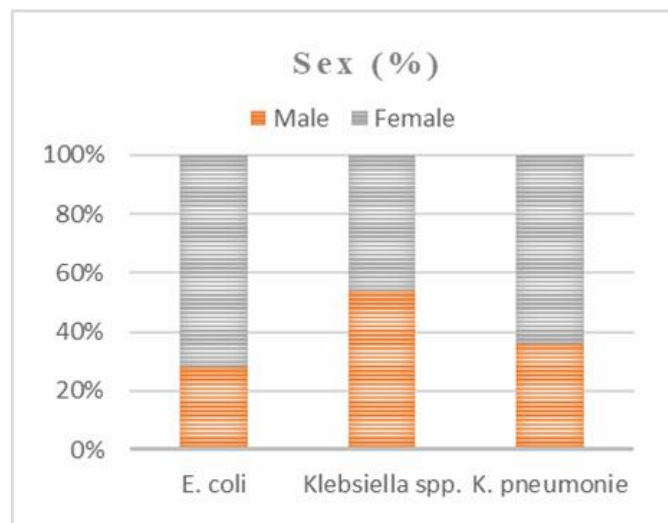
Fig.1. Percentage of isolates by patient sex,
by bacterial species, 2019-2023

The analysis of data in relation to age showed that the cumulative largest number of reports at the national level was registered among people over the age of 65 – 204 or 51.4%. If the data from the National Health Service are analyzed according to this indicator, 22 people over the age of 65 (40.0%) were registered.

Regarding the representation of age by the causative agent, the highest number and percentage of people over the age of 65 had *Escherichia coli*, ESBL (n=150; 47.8%), *Klebsiella spp*, ESBL (n=11; 84.6 %) and *Klebsiella pneumoniae*, ESBL (n=43; 61.4%). The representation of people over the age of 65 in the positive samples from the IPH for *Escherichia coli*, ESBL was 38.5% (n=20).
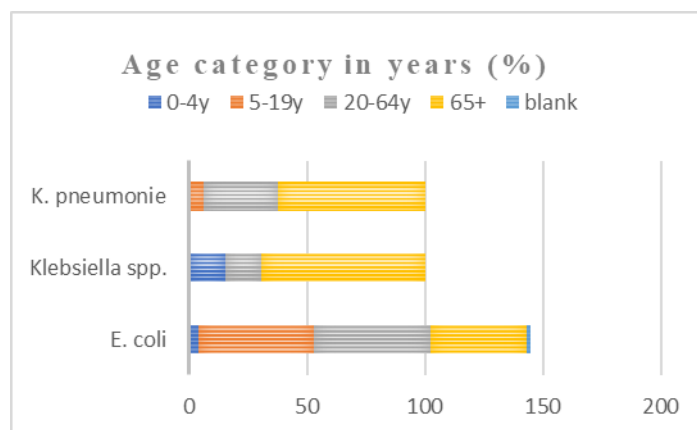

Fig. 2. Percentage of isolates by age group,
by bacterial species, 2019-2023

The analysis of data in relation to the place of residence of patients with a positive result showed that cumulatively the largest number of reports at the national level was registered among people from Skopje - 180 or 43.2% and Bitola - 103 or 24.7%. If the data from the IPH is analyzed according to this indicator, all registered persons with a positive result lived in Skopje.

In terms of the representation of the place of residence by the causative agent, the largest number and percentage of people from Skopje were observed to have *Escherichia coli, ESBL (n=143; 43.3%) and Klebsiella pneumoniae*, ESBL (n=34; 72.3 %), while *Klebsiella spp*, ESBL (n=11; 76.9%) was mostly registered in Bitola.

If the origin of the samples is analyzed, of the total number of reported isolates (n=417), 245 or 58.8% were from hospitalized patients, while the rest were from outpatients (sent by a family doctor, gynecologist…). Of the samples from hospitalized patients, the largest part were from Sistina Clinical Hospital - 113 or 27.1% and Bitola Clinical Hospital - 101 or 24.2%. If data from the National Health Service are analyzed according to this indicator, 50 of the positive samples or 89.3% were sent by a family doctor.
In terms of the representation of the origin of the samples by the causative agent, the largest number and percentage of persons from hospitalized patients were found to have *Escherichia coli*, ESBL (n=172; 52.1%) and *Klebsiella pneumoniae*, ESBL (n=63; 85, 1%).

The analysis of data in relation to the month in which a positive result was obtained showed that the cumulative largest number of applications at the national level was registered in September - 70 or 16.8% and November - 51 or 12.2%. If data from the IPH is analyzed according to this indicator, the majority of those registered were in September and November - after 9 and April - 8.

In terms of the representation of the month of a positive result by the causative agent, the highest number and percentage of people was observed in September for *Escherichia coli*,

ESBL (n=51; 15.5%) and for *Klebsiella pneumoniae*, ESBL (n=16; 21 .6%), while *Klebsiella spp*, ESBL (n=4; 30.8%) was mostly registered in October. The representation by month in the positive samples from the IPH for *Escherichia coli*, ESBL was highest in October – 17.0% (n=9).
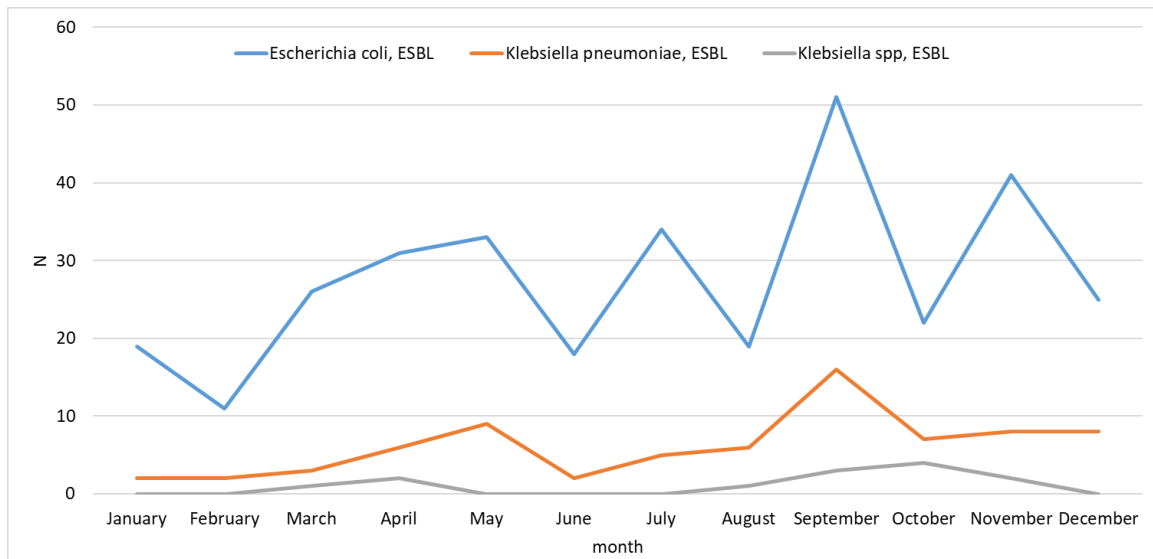

Fig. 3. Analysis of the data in relation to the month

If data is analyzed in relation to the reported resistance to antibiotics at the national level, of the total number of reported isolates for *Escherichia coli*, ESBL; *Klebsiella pneumoniae*, ESBL; and *Klebsiella spp*, ESBL (n=417), data on AMR was present in 153 reports or 36.7%.

Of the reports with AMR data, 107 or 69.9% were from hospitalized patients, while 46 or 30.1% were from outpatients. *Escherichia coli*, ESBL with 119 reports or 77.8% was present in the largest number of reports with AMR data, of which 76 or 63.9% were from hospitalized patients.

If data are analyzed in relation to the reported resistance to antibiotics, of those reported by the IPH, of the total number of reported isolates for *Escherichia coli*, ESBL; *Klebsiella pneumoniae*, ESBL; and *Klebsiella spp*, ESBL (n=56), no data on AMR were found in any report.

Of the total number of reported isolates for *Escherichia coli*, ESBL; *Klebsiella pneumoniae*, ESBL; and *Klebsiella spp*, ESBL (n=417), 18 or 4.3% were found in patients with two or more causative agents at the same time. All 18 reports were for hospitalized patients, and 3 of them had data on AMR.

Discussion

Over the past decades, ESBL producing Gram-negative bacilli, especially *E. coli* and *K. pneumoniae*, have emerged as serious pathogens in both hospital-acquired and community-acquired infections worldwide. Identification of ESBL-producing organisms is a major challenge in the clinical setting and, due to selective pressure caused by the heavy use of extended-spectrum cephalosporins, gaps in effective infection control measures, and the affinity of these enzymes for different substrates, epidemics are increasing.

The results suggest that females are more at risk of developing infection from uropathogens, which is due to their anatomical structure.

In terms of age, the most common uropathogens were found to be associated with the 65+ (57.6%) and 20-64 (31.96%) age groups. These results agree with other studies in which

the prevalence of ESBL production was higher in the age group of 21 - 40 years. In addition, the practice of self-medication, which is high in this age group, may further contribute to the higher prevalence[12-13].

A higher prevalence of ESBL production was observed in *E. coli* isolates (79%), followed by *K. pneumoniae*, contrary to the findings of another study of 365 *E. coli* isolates where 33 (9.0%) were producers of ESBL[14].

A study conducted on samples taken from urinary tract infections of patients showed that 60.9% of ESBL producing *E. coli* indicated an extremely high prevalence of resistant pathogenic *E. coli*.

ESBLs of *E. coli* and *K. pneumoniae* isolated from humans are the most common cause of infections, especially in countries with underdeveloped health systems[15]. Currently, the increase in ESBL-producing Enterobacteriaceae is a global health concern due to high antibiotic resistance and limited treatment options, cephalosporins providing selective pressure for emergence of new ESBL variants[16].

In conclusion, the current study indicated a significant rate of infection with ESBL-producing Gram-negative bacilli in patients with UTI.

Conflict of interest statement. None declared.

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