Short communication

Epidemiology and resistance levels *Enterobacteriaceae* isolates from urinary tract infections expressed as Multiple Antibiotic Resistance (MAR) Indices

ABSTRACT

Aims: To assess the epidemiology of UTIs affecting inpatients and outpatients and the antibiotic resistance levels, expressed as multiple antibiotic resistance (MAR) indices from the isolated species at a tertiary-care hospital in Hungary, during a 10-year study period.
Study design: Retrospective microbiological study.
Place and Duration of Study: 1st of January 2008 - 31st of December 2017 at the University of Szeged, which is affiliated with the Albert Szent-Györgyi Clinical Center, a primary- and tertiary-care teaching hospital in the Southern Great Plain of Hungary.
Methodology: Antimicrobial susceptibility testing (AST) was performed using disk diffusion method and when appropriate, E-tests on Mueller–Hinton agar (MHA) plates. The multiple antibiotic resistance (MAR) index of the isolates was determined.
Results: During the 10-year study period, the Institute of Clinical Microbiology received 21,150 urine samples from outpatient clinics and 19,325 samples from inpatient departments that turned out to be positive for a significant urinary pathogen. Out of the positive urine samples, E. coli represented the overwhelming majority of all positive urine samples. The resistance levels in inpatient isolates were higher than in the outpatient isolates (average MAR indices: 0.347 vs. 0.410, 0.267 vs. 0.435 and 0.318 vs. 0.473 for the *E. coli/Klebsiella*, CES and *Proteae* group, respectively).

Conclusion: As the therapeutic options are becoming increasingly limited in the current antibiotic resistance climate, more effort should be put into the prudent use of antibiotics and the development of novel antimicrobial agents.

Keywords: urinary tract infection, Enterobacteriaceae, Escherichia coli, Klebsiella, CES, Proteae, multiple antibiotic resistance index, antibiotic

1. INTRODUCTION

Urinary tract infections (UTIs) are some of the most common infections in human healthcare, both in community (10–30% of infections in primary healthcare) and nosocomial settings (30–40 %) [1,2]. UTIs are often associated with recurrence, complications and sequelae, leading to a decrease in the quality of life for the affected patients [3]. UTIs are most frequently caused by members of the *Enterobacteriaceae* family (or more recently, the *Enterobacterales* order): typical pathogens include uropathogenic *Escherichia coli* and *Klebsiella* spp., however, the *Proteus-Providencia-Morganella* species (the *Proteae* tribe), *Citrobacter-Enterobacter-Serratia* species (so-called CES pathogens) have now emerged as increasingly relevant Gram-negative pathogens [4-7]. The therapy of UTIs is an increasingly complex challenge for clinicians, due to the increasing levels of antibiotic resistance [8,9]. The emergence of multidrug resistance (MDR) and extensive drug resistance (XDR) in urinary pathogens, together with pre-existing, genetically encoded resistance mechanisms means that these pathogens may be resistant to a broad range of antibiotics [10,11].

Since the beginning of the 21st century, many surveillance reports have been published regarding the resistance trends of Gram-negative bacteria [12]. Nevertheless, the epidemiology and antibiotic susceptibility-patterns of urinary tract pathogens vary greatly by region, and, therefore, the assessment of local data is essential to evaluate trends over time and to reflect on the national situation compared to international data. In addition, the knowledge of the relevant antibiotic susceptibility patterns of the major bacterial pathogens for UTIs is critically important for the optimal choice for antibiotic therapy. With this in mind, the aim of this study was to assess the epidemiology of UTIs affecting inpatients and outpatients and the antibiotic resistance levels, expressed as multiple antibiotic resistance (MAR) indices from the isolated species at the Albert Szent-Györgyi Clinical Center (Szeged, Hungary) retrospectively, during a 10-year study period.

2. MATERIAL AND METHODS

2.1. Study Design, Data Collection

The present retrospective study was carried out using microbiological data collected from the 1st of January 2008 and 31st of December 2017 at the University of Szeged, which is affiliated with the Albert Szent-Györgyi Clinical Center, a primary- and tertiary-care teaching hospital in the Southern Great Plain of Hungary. The Clinical Center has a bed capacity of 1820-beds and annually serves more than 400,000 patients in the region, according to the data of the Hungarian National Health Insurance Fund (NEAK), including GP-level care, all the way to specialized medical interventions [13]. Electronic search in the records of the MedBakter laboratory information system (LIS) for positive urine samples was conducted by the author.

Samples with clinically significant colony counts for the abovementioned bacteria (10^5 < colony forming units [CFU]/mL; however, this was subject to interpretation, based on the information provided on the request forms for microbiological analysis and relevant international guidelines, e.g., presence of underlying conditions in the genitourinary tract) were included in the data analysis. Only the first isolate per patient was included in the study, however, isolates with different antibiotic-susceptibility patterns were considered as different individual isolates [14].

2.2. Identification of Isolates

10 µL of each un-centrifuged urine sample was cultured on UriSelect chromogenic agar plates (Bio-Rad, Berkeley, CA, USA) with a calibrated loop, according to the manufacturer's instructions and incubated at 37 °C for 24–48 h, aerobically. If the relevant pathogens presented in significant colony count, the plates were passed on for further processing. Between 2008–2012, presumptive phenotypic (biochemical reaction-based) methods and VITEK 2 ID (bioMérieux, Marcy-l'Étoile, France) were used for bacterial identification, while after 2013, this was complemented by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonik Gmbh. Gr., Bremen, Germany) [5-7]. The methodology of sample preparation for MALDI-TOF MS measurements was described elsewhere [15]. Mass spectrometry was performed by the Microflex MALDI Biotyper (Bruker Daltonics, Bremen, Germany) in positive linear mode across the m/z range of 2 to 20 kDa; for each spectrum, 240 laser shots at 60 Hz in groups of 40 shots per sampling area were collected. The MALDI Biotyper RTC 3.1 software (Bruker Daltonics) and the MALDI Biotyper Library 3.1 were used for spectrum analysis.

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed using disk diffusion method and when appropriate, E-tests (Liofilchem, Abruzzo, Italy) on Mueller–Hinton agar (MHA) plates. For the verification of questionable results, VITEK 2 AST (bioMérieux, Marcy-l'Étoile, France) was also used. The interpretation of the results was based on EUCAST breakpoints. *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. The following antibiotics were tested: norfloxacin, ciprofloxacin, levofloxacin, ampicillin, amoxicillin/clavulanic acid, ceftriaxone, cefepime, meropenem, doxycycline, gentamicin, tobramycin, amikacin, fosfomycin, sulfamethoxazole/trimethoprim and nitrofurantoin, which is a total of 15 antibiotics (excluding cases where intrinsic non-susceptibility was present; namely in *Citrobacter-Enterobacter-Serratia* [n=12 antibiotics] and *Morganella-Proteus-Providencia* [n=10 antibiotics]) [5-7]. During data analysis, intermediate-susceptible results were grouped with and reported as resistant. The multiple antibiotic resistance (MAR) index of the isolates was determined using the formula: MAR Index = Number of antibiotics isolate is resistant to/Number of antibiotics tested, as described previously [16]. The MAR index may range between 0.0-1.0.

2.4. Statistical Analysis

Descriptive statistical analysis (including means or medians with ranges and percentages to characterize data) was performed using Microsoft Excel 2013 (Redmond, WA, USA, Microsoft Corp.).

3. RESULTS AND DISCUSSION

During the 10-year study period, the Institute of Clinical Microbiology received 21,150 urine samples from outpatient clinics and 19,325 samples from inpatient departments that turned out to be positive for a significant urinary pathogen. The distribution of *Enterobacteriaceae* isolates among inpatient and outpatient samples is presented in **Table 1.** Out of the positive urine samples, *E. coli* represented the overwhelming majority of all positive urine samples (56.75 ± 4.86% [range: 46.83–65.98%, lowest in 2008, highest in 2010] for outpatients, while 42.29 ± 2.94% [range: 37.19–45.73%, lowest in 2015, highest in 2010] for inpatients), followed by *Klebsiella* spp., members of the *Proteae* tribe and *Citrobacter-Enterobacter-Serratia* species.

Table 1. Species-distribution of urinary pathogens in the *Enterobacteriaceae* family in a tertiary-care hospital in Hungary (2008-2017)

Species	Outpatients (average±SD; %)	Inpatients (average±SD; %)	Outpatients (range; %), highest and lowest years	Inpatients (range; %), highest and lowest years
E. coli	56.7 ± 4.8	42.2 ± 2.9	46.8-65.98 (2008; 2010)	37.2-45.7 (2012; 2011)
K. pneumoniae	8.9 ± 2.3	13.4 ± 2.2	2.9-10.4 (2012; 2011)	9.5-15.3 (2008; 2011)
K. oxytoca	1.4 ± 0.3	1.16 ± 0.2	0.7-1.8 (2008; 2011)	0.7-1.8 (2008; 2011)
CES group (Citrobacter-Enterobacter-Serratia)	5.0 ± 0.9	7.2 ± 1.7	3.6-6.3 (2010; 2017)	3.5-9.6 (2009; 2015)
Proteae group (Morganella-Proteus-Providencia)	2.6 ± 0.1	3.0 ± 0.4	1.9-3.2 (2012; 2017)	2.5-3.8 (2015; 2013)

Table 2. Multiple Antibiotic Resistance (MAR) Index of *E. coli and Klebsiella* spp. isolated from urinary tract infections in a tertiary-care hospital in Hungary (2008-2017)

MAR Index (a/b)*	Outpatient isolates Frequency (%)	Inpatient isolates Frequency (%)
0.000	12.1	4.3
0.067	0.2	0.8
0.133	0.3	1.4
0.201	13.4	7.6
0.268	5.3	9.4
0.335	**26.9**	14.6
0.402	14.3	16.8
0.469	8.8	10.2
0.536	6.4	**24.1**
0.603	5.2	6.0
0.670	3.6	1.0
0.737	2.0	0.8
0.804	1.0	1.8
0.871	0.5	1.2
0.938	0.0	0.0
1.000	0.0	0.0

*Number of antibiotics tested: n=15

Boldface: the highest ratio of representative strains; Underlined: highest MAR level in the group

Table 3. Multiple Antibiotic Resistance (MAR) Index of *CES pathogens (Citrobacter-Enterobacter-Serratia)* isolated from urinary tract infections in a tertiary-care hospital in Hungary (2008-2017)

MAR Index (a/b)*	Outpatient isolates Frequency (%)	Inpatient isolates Frequency (%)
0.000	16.3	4.2
0.083	4.6	6.6

0.166	4.2	10.3
0.249	**38.9**	9.4
0.332	14.9	10.1
0.415	3.5	17.8
0.498	10.1	**26.4**
0.581	6.0	14.1
0.664	1.0	8.8
0.747	0.3	1.0
0.830	0.2	0.3
0.913	0.0	0.0
1.000	0.0	0.0

*Number of antibiotics tested: n=12

Boldface: the highest ratio of representative strains; Underlined: highest MAR level in the group

Table 4. Multiple Antibiotic Resistance (MAR) Index of *Proteae (Morganella-Proteus-Providencia)* isolated from urinary tract infections in a tertiary-care hospital in Hungary (2008-2017)

MAR Index (a/b)*	Outpatient isolates Frequency (%)	Inpatient isolates Frequency (%)
0.000	3.2	1.8
0.100	8.6	2.6
0.200	24.9	10.5
0.300	**25.3**	13.7
0.400	17.7	**19.9**
0.500	15.4	16.3
0.600	1.2	12.4
0.700	2.0	12.8
0.800	1.0	6.7
0.900	0.7	3.3
1.000	0.0	0.0

*Number of antibiotics tested: n=10

Boldface: the highest ratio of representative strains; Underlined: highest MAR level in the group

The MAR indices of the different Gram-negative uropathogens are presented in **Tables 2-4.** In all three groups, the resistance levels in inpatient isolates were higher than in the outpatient isolates (average MAR indices: 0.347 vs. 0.410, 0.267 vs. 0.435 and 0.318 vs. 0.473 for the *E. coli/Klebsiella*, CES and *Proteae* group, respectively), additionally, the highest frequency of isolates in the relevant MAR indices were also corresponding to higher MAR-index values (MAR indices: 0.335 vs. 0.536, 0.249 vs. 0.498 and 0.300 vs. 0.400 for the *E. coli/Klebsiella*, CES and *Proteae* group, respectively).

The increasing levels of antimicrobial drug resistance in urinary tract infection corresponds to both local and international changes in treatment guidelines. MDR Gram-negatives leads to poor prognoses and an increased complication rate, mortality rate, especially in nosocomial settings [17]. The therapy of *Proteae* and CES infections is especially challenging due to the various intrinsic resistance mechanisms these pathogens possess [18,19]. In addition, some last-resort drugs used in clinically relevant infections with Gram-negative pathogens, like tigecycline and colistin are not useful in the therapy of *Proteae*-associated pathologies and some beta-lactam antibiotics and colistin are not useful against *Serratia* spp. Intrinsic non-susceptibility to several drug groups severely limits the number of therapeutic alternatives, especially for outpatient care. Nitrofurantoin and

Comment [u1]: **correspond** is the correct spelling

fosfomycin (if susceptibility is confirmed) represents a safe and viable option for the treatment of these infections [9].

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4. CONCLUSION

This study presents a current epidemiological snapshot and resistance levels of *Enterobacteriaceae* associated with of urinary tract infections (UTIs) in Hungary over a long period (2008-2017). To the best of the authors knowledge, this is the first study in Hungary, reporting on the resistance levels of these pathogens using the MAR index. As the therapeutic options are becoming increasingly limited in the current antibiotic resistance climate, more effort should be put into the prudent use of antibiotics and the development of novel antimicrobial agents.

ETHICAL APPROVAL

The study was deemed exempt from ethics review by the Institutional Review Board, and informed consent was not required as data anonymity was maintained.

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