Original paper

Antibiotic resistance features in *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from hospital and community acquired urinary tract infections

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Abstract   A total number of 35 strains (n=23 of *K. pneumoniae* and n=12 of *E.coli*) were isolated in May 2017 from patients with UTI, hospitalized in the National Institute for Cardiovascular Diseases Prof. C.C. Iliescu and from community infections (CA) diagnosed in Central Reference Synevo-Medicover Laboratory from Bucharest. The hospital strains were identified by BD Phoenix and the CA ones by mass spectrometry using MALDI Biotyper. The antibiotic susceptibility was determined by agar disk diffusion (CLSI, 2017) and automated methods (BD Phoenix and Vitek II system). For molecular characterization, all strains were analyzed be using PCR amplification. The investigated strains revealed the presence of tetracycline resistance gene, i.e. tet(A) (67% in *E. coli* and 45% of *K. pneumoniae* strains), tet(D) (8% of *E. coli* and 5% of *K. pneumoniae* strains), carbapenemase genes (*bla*$_{OXA-48}$ in 40% of the *K. pneumoniae* strains); *bla*$_{TEM}$ (25% of *E. coli* strains and 10% of *K. pneumoniae* strains).

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Introduction

Urinary tract infections (UTIs) are one of the most common pathological conditions, with a variety of clinical symptoms, caused by the colonization and invasion of the urinary tract by different microbial species (AL-BADR A. & al [1]). UTIs are the most common infectious diseases in either community or in health-care institutions. UTIs can be uncomplicated or complicated. Uncomplicated UTIs are usually found in sexually active healthy female patients with no history suggesting abnormalities of the urinary tract (GUPTA K. & al [15]), Complicated UTIs are usually found in both women and men of different ages and are often associated with structural or functional urinary tract abnormalities (PALLETT A. & al [27]) (LICHTEN-BERGER P. & al [21]). If not diagnosed or diagnosed too late UTIs will lead to renal failure. The most common uropathogenic Gram-negative bacteria are *E. coli* and *K. pneumoniae* (OBIOGBOLU C.H. & al [25]). Depending on how the infection is acquired, there are two types of UTI, i.e. hospital-acquired (HA) and community-acquired UTIs (CA) (SHALINI MAYA A. & al [31]). In case of HA-UTI the onset of urinary tract infection in patients occurs after 48 hours after admission, whereas CA-UTIs refer to the development of infections before admission to hospital and not within 10 days after the patient exit (SHALINI MAYA A. & al [31], (WAGENLEHNER FME & al [32]). *E. coli* is the most common cause of both hospitals acquired and community UTIs (DIAS NETO J.A. & al [10], NERURKAR A & al [24]). Other common uropathogens encountered in CA-UTIs include *Staphylococcus saprophyticus*, *K. pneumoniae*, and *Citrobacter* sp. (GYANSA-LUTTERODT M. & al [16], BAHADIN J. & al [3]). The etiology of HA-UTI is more diverse and includes a wide range of organisms such as *P. aeruginosa* and *Proteus* spp., which are barely encountered in CA-UTIs (MITTAL R. & al [22]; RONALD A. [30]). CA-UTI is commonly uncomplicated, and risk factors typically include sexual activity and the use of contraception methods (FOXMAN B. [13]; HOOTON T.M. [17]), while HA-UTI is complicated and related to risk factors like catheterization and large-spectrum/excessive antibiotic use (IACOVELLI V. & al [18], CHIN T.L. & al [7]). Several evidence suggests that the treatment of these infections is made more difficult by the rapid emergence of antimicrobial resistance in hospitals and the community (DONKOR E.S. & al [11]; OKEKE I.N. & al [26]), particularly of the enzymatic β-lactam resistance mechanisms, mainly mediated by extended-spectrum β-lactamases (ESBL) (mainly produced by *E. coli* and *K. pneumoniae,* but also by *K. oxytoca, Proteus* sp., *Acinetobacter* spp. and others) responsible for resistance against penicillins, cephalosporins and aztreonam (CANTÓN R. & al [6]). The spread of ESBL-producing bacteria worldwide has become a serious public health being a frequent cause of infection in healthcare centres and in the community: it is estimated that around 50% of non-hospitalised patients are infected with a resistant strain, although it may be difficult to establish boundaries between community and nosocomial cases (YANG Y.S. & al [33]). Although a global problem, antibiotic resistance of uropathogens raises more significance in the developing world, where treatment options are limited.

The aim of this study was to characterize the phenotypic and genotypic resistance profiles of uropathogenic *E. coli* and *K. pneumoniae* strains isolated from HA- and CA- UTIs.

Material and Methods

1. Bacterial Strains

In this study, a total number of 35 strains (n=23 of *K. pneumoniae* and n=12 of *E. coli*) were isolated in May 2017 from patients with UTIs, hospitalized in the National Institute for Cardiovascular Diseases Prof. C.C. Iliescu and from community infections (CA) in Central Reference Synevo-Medicover Laboratory from Bucharest. The HA strains were identified by BD Phoenix and the CA ones by mass spectrometry using MALDI Biotyper. All selected strains exhibited multidrug resistance (MDR) and extended spectrum β-lactamases (ESBL).

2. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility profiles for the *K. pneumoniae* and *E. coli* strains were determined by agar disk diffusion (CLSI, 2017) and automated methods (BD Phoenix and Vitek II system).

3. Molecular investigation

The resistance genes (carbapenemases, ESBLs, aminoglycosides, quinolones and tetracyclines) were investigated by simplex and multiplex PCR, using a reaction mix of 20 µl (PCR Master Mix 2x, Thermo Scientific) containing 1 µl of bacterial DNA extracted using the alkaline extraction method. For this purpose, 1-5 colonies of bacterial cultures were suspended in 1.5 ml tubes containing 20 µl solution of NaOH 0.05M (sodium hydroxide) and SDS 0.25% (sodium dodecyl sulphate) and heated on a thermo block at 95°C for 15 min. for the permeabilization of bacterial cell wall. The following step was the addition of 180 µl of TE buffer (TRIS+EDTA) 1X and centrifugation at 13000 rpm for 3 min., and the supernatant was used as the DNA template for the polymerase chain reaction (PCR) on a Corbet Thermal Cycler. The primers used for the detection of carbapenemase resistance genes bla_{OXA-48}, bla_{TEM} and tetracycline resistance genes tet(A), tet(B), tet(C), tet(D) and PCR conditions are presented in the Tables no. 1, 2, 3, 4.

The resulting PCR products were subjected to electrophoretic migration on a 1.5% agarose gel, followed by the visualization of amplicons after staining with ethidium bromide (7 µg/ml) under UV trans illuminator. DNA bands of each amplicon were compared with specific molecular weight 1000-bp DNA marker (Table 1).

Table 1. Reaction components used in the PCR experiments

Concentration						Final volume
Primer F&R	Mgcl$_2$	DNTP	DNATaq-pol	Reaction buffer	DNA template	
0.5 µM	1.2 mM	2 µM	0.2 U	1x	10x	20 µl

Table 2. The amplification program for carbapenems resistance genes

Gene	Amplification program				
	Initial denaturation	Numbers of cycle	Denaturation in each cycle	Annealing	Extension primer
bla$_{OXA-48}$	95°C for 5 min	30	95°C, 30 sec	55°C, 40 sec	72°C, 5 min
bla$_{TEM}$	95°C for 5 min	35	95°C, 30 sec	59°C, 40 sec	72°C, 10 min

Table 3. The amplification program for tetracycline resistance genes

Genes	Amplification program				
	Initial denaturation	Number of cycle	Denaturation in each cycle	Annealing	Extension primer
tet(A) tet(B)	94°C for 5 min	25	94°C, 30 sec	61°C, 30 sec	61°C, 7 min
tet(C) tet(D)	94°C for 5 min	25	94°C, 30 sec	60°C, 30 sec	68°C, 7 min

Table 4. Primer sequences and amplicons for selected genes

The gene	Primer	Nucleotide sequence	Amplification size(bp)	References
bla$_{OXA-48}$	*bla*$_{OXA-F}$ *bla*$_{OXA-R}$	5´GCGTGGTTAAGGATGAACAC -3' 5´CATCAAGTTCAACCCAACCG -3'	438	(OKEKE I.N. & al [26])
bla$_{TEM}$	*bla*$_{TEM-F}$ *bla*$_{TEM-R}$	5'-ATGAGTTTTCAACATTTTCG-3' 5'-TTACCAATGCTTAATCAG TG-3'	861	(FOXMAN B. [13])
tet(A)	*tet*$_{A-F}$ *tet*$_{A-R}$	5'-GCGCGATCTGGTTCACTCG-3' 5'-AGTCGACAGYRGCGCCGGC-3'	164	(AMINOV R. & al [2])
tet(B)	*tet*$_{B-F}$ *tet*$_{B-R}$	5'-TACGTGAATTTATTGCTTCGG-3' 5'-ATACAGCATCCAAAGCGCAC-3'	206	
tet(C)	*tet*$_{C-F}$ *tet*$_{C-R}$	5'-GCGGGATATCGTCCATTCCG-3' 5'-GCGTAGAGGATCCACAGGACG-3'	207	
tet(D)	*tet*$_{D-F}$ *tet*$_{D-R}$	5'-GGAATATCTCCCGGAAGCGG-3' 5'-CACATTGGACAGTGCCAGCAG-3'	187	

Results and Discussion

Antimicrobial Susceptibility Profiles of the UTI isolates

In this study, the resistance profiles of 35 strains (23 *K. pneumoniae* and 12 *E. coli*) isolated from patients with CA- and HA-UTIs were investigated by phenotypic and genotypic approaches.

The β-lactam drugs are one of the most effective drugs for the UTI treatment. The ESBL-producing bacteria, with inactivation of a wide range of β-lactam drugs especially cephalosporins and monobactams, cause treatment failure and increase healthcare costs. Therefore, the study of β-lactams resistance mechanisms is important. It seems that the emergence and spread of these bacteria are due to prolonged hospitalization, increased consumption of β-lactam antibiotics (especially ceftazidime), use of catheters, and experimental treatments against antibiotic resistant strains (EJAZ H & al [12]). The PCR *screening* revealed the presence of the β-lactamase encoding genes (*bla*$_{OXA-48}$ was present in 40% of the *K. pneumoniae* isolates) and of *bla*$_{TEM}$ (25% of *E. coli* and 10% of *K. pneumoniae* strains) and of tetracycline resistance genes: tet(A) (67% of *E. coli* and 45% of *K. pneumoniae* strains) and tet(D) (8% of *E. coli* and 5% of *K. pneumoniae* investigated strains. The OXA-48carbapenemase was first described in *K. pneumoniae* epidemic isolates from Turkey and then in several European countries, such as France and Belgium. Recently, it has also been identified in enterobacterial isolates recovered from non-European countries, such as Lebanon, Tunisia, Senegal, Morocco, Israel, and India (GOREN MG & al [14]; KTARI S & al [19]; LASCOLS C & al [20]; MOQUET O & al [23]; POIREL & al [29]). In *E. coli* strains, the most prevalent gene responsible for β-lactams resistance was *bla*$_{TEM}$

(13%). Extended-spectrum TEM β-lactamases are derived from TEM-1 and TEM-2 enzymes. TEM-1 is the most important β-lactamase in Gram-negative bacteria. Both TEM-1 and TEM-2 are capable of hydrolyzing penicillins and first generation cephalosporins, but are not able to degrade oxyimino – cephalosporins (BONOMO RA & al [5]; PITOUT JD & al [28]). A study from Iran reported a prevalence of 59.2% for ESBLs production in *K. pneumoniae* and *E. coli* strains, while in Japan and USA the prevalence rate was reported to be of 40 and 44% respectively (PITOUT JD & al [28]; COQUE TM & al [9]). Recently, a study performed by Baicus et al reported that he carbapenem resistance genes of *K.* pneumoniae most frequently detected in Romanian patients were OXA 48 (73,77% of cases), while 13,11%, of isolates presented the combinations bla_{OXA-48} and bla_{NDM} (BAICUS & al [34]).The analyzed strains showed increase of resistance to antibiotics from other classes indicating the presence MDR phenotypes. From the non β-lactam antibiotics, tetracyclines registered the highest resistance rates, the *K. pneumoniae* strains carrying the tet(A) (62%) and followed by the tet(D) (7%) gene while, the *E. coli* strains harboured less frequently, both tet(A) (28%) and tet(D) (3%). Tetracyclines and other antibiotics have been frequently used to treat diseases, but unfortunately repeated use of these compounds has resulted in the development of resistant strains (CHOPRA I & al [8]). A study performed in Iran on UTIs *K. pneumonia*e isolates has also demonstrated a high resistance rate to tetracyclines (50%) (BOKAEIAN M & al [4]). As the investments for development of new antibiotics remain insufficient in the scope of drug development while resistance trends are increasing alarmingly, clinicians need to apply new therapeutic strategies to fight infections (i.e. synergistic drug combinations) (UNLU [35]).

Conclusion

Urinary tract infections are among the most common bacterial infections, with high costs for the medical care. *K. pneumoniae* and *E. coli* uropathogens have been shown to be the most prevalent bacterial strain isolated from patients hospitalized or diagnosed in ambulatory from Bucharest, Romania. The high rates of resistance threaten the efficiency of the antibiotic treatment. Therefore, the discovery of new appropriate treatments as well as the improvement of the prevention methods are required. This study shows the high prevalence of resistance genes, in particularly of $bla_{-OXA-48}$, bla_{TEM} and tet(A). Therefore, continuous surveillance is essential to monitor the occurrence and dissemination of these resistant microorganisms in both hospitals and community.

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