

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

**Epidemiology of faecal carriage of extended-spectrum beta-lactamase producers in
healthy individuals and in different patient populations**

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Epidemiology of faecal carriage of ESBL producers in healthy individuals and in different patient populations

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The Examination takes place at Library of Department of Pharmacology, Faculty of Pharmacy, University of Debrecen, **at 12:00 pm, 13th April 2016.**

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1. Introduction

Improper antimicrobial use is considered as one of the main reasons for selection and transmission of resistant bacteria. Beta-lactams are the most widely used antibiotic classes; however, the spiral of appearance of resistance and subsequent development of newer drugs is a returning characteristic throughout their history. Beta-lactamase production is the most important defense mechanism against beta-lactam antibiotics among members of Enterobacteriaceae family; a major change in resistance was the appearance of extended-spectrum beta-lactamases (ESBLs). ESBLs were first defined as plasmid-mediated beta-lactamases which provide resistance to extended-spectrum cephalosporins and monobactams, and are inhibited in vitro by beta-lactamase inhibitors. Enterobacteriaceae that produce extended-spectrum beta-lactamases ESBLs have become a major problem worldwide, especially since the occurrence and spread of the beta lactamase genes belong to *bla*_{CTX-M} family. Many of the ESBL encoding genes, in contrast to the *bla*_{TEM} and *bla*_{SHV} groups, are linked to epidemic clones and some of them, e.g. the *bla*_{CTX-M-15}-producing *Escherichia coli* O25b:ST131 and *Klebsiella pneumoniae* ST11, ST15 and ST147 clonal complexes, have become pandemic both in the nosocomial and community settings as well as in long-term care (LTC) facilities. The switch from *K. pneumoniae* to *E. coli* as the major ESBL producer species is also linked to the dissemination of *bla*_{CTX-M} genes.

There are marked geographical differences in the proportion of ESBL producing enterobacteria. In Europe the prevalence of bacteria which are resistant to extended-spectrum cephalosporins varies significantly between countries; they are less frequent in the northern European countries and more common in the southern and eastern parts of Europe.

The first detection of ESBL-producing bacteria in Hungary took place in 1996 in clinical isolates of *K. pneumoniae*. Between 2002 and 2003 nosocomial outbreaks caused by *K. pneumoniae* and *K. oxytoca* producing SHV-2a and SHV-5 have been reported, being a result

of dissemination of identical allodemic R-plasmids. Between 1998 and 2004, outbreaks caused by SHV-type ESBL-producing *K. pneumoniae*, were reported exclusively from neonates. In 2005, eruptive and extensive dissemination of CTX-M-15 producing *K. pneumoniae* clones was observed causing both outbreaks and sporadic infection in adult patients across the country. One year later another epidemic clone emerged in various regions of the country. The result from an investigation of fourteen outbreaks caused by ESBL-producing *K. pneumoniae* in Hungary between 2005 and 2008, showed that the outbreaks in neonatal intensive care units (where fluoroquinolone-type antibiotics were not in use), remained polyclonal and strains retained SHV-type ESBLs, while in the adult outbreaks, CTX-M-type ESBL clones (ST15, ST147) were involved. It was also shown that the use of fluoroquinolone type antibiotics was responsible for the expansion of the major clone of ESBL-producing *K. pneumoniae*. There are very little data about the epidemiology of ESBL-producing *E. coli* in Hungary. The most common types of ESBL-producing *E. coli* reported were CTX-M-15 in human and CTX-M-1 in animal isolates.

ESBL producers often exhibit co-resistance to several commonly used antibiotic classes, such as fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole. This high resistance rate against drugs commonly used in the empirical treatment of critically ill patients may result in initial treatment failure, delay of adequate therapy and consequent increased morbidity and mortality rate, higher hospital costs and longer hospital stay. Thus, the treatment options are often very limited in infections caused by these bacteria. Carbapenems have been regarded as the drug of choice for treatment of serious infections caused by ESBL-producing bacteria; however the disadvantage is the potential selection for carbapenem-resistance in ESBL producers and in other Gram-negative bacteria.

2. Objectives

The overall aim was to study the epidemiology of bacteria producing ESBLs in asymptomatic individuals, with no sign of ongoing infection that might be attributed to ESBL-producing bacteria.

Specific aims were as follows:

- 1) To evaluate the prevalence of ESBL-producing Enterobacteriaceae among applicants for LTC facilities and individuals screened for employment eligibility purposes and to compare the prevalence of faecal carriage and characteristics of these enzymes in these two groups (paper I).
- 2) To survey the faecal carriage rate of ESBL producers among healthy individuals in the same geographic area a few years later and to compare the prevalence and characteristics of ESBL producers to our previous results (paper III).
- 3) To assess dynamics of ESBL carriage in patients by investigating the prevalence of faecal colonization with ESBL producers among pediatric and adult inpatients of different wards or intensive care units (ICUs), as well as among outpatients and screened medical students and comparing the characteristics of ESBL producers in these populations (paper II).

3. Materials and Methods

3.1. Samples and isolates

We investigated three batches of samples. The first batch (paper I), contained 1,640 faecal samples sent for screening for enteric pathogens from two groups of asymptomatic individuals investigated between March 2009 and April 2010. Samples originated from 1,109 individuals (300 males and 809 females with a median age of 34 years, range 15–68 years) being screened for employment eligibility purposes (e.g. jobs at hospitals, kindergartens and food-processing plants) and 531 individuals (218 males and 313 females with a median age of 75 years, range 0–100 years) who needed to be screened for enteric pathogens prior to admittance to LTC facilities.

The second batch (paper II) of samples included 5581 non-duplicate faecal specimens which were sent for routine diagnostics from October 2010 to February 2013, originating from 4343 inpatients (adult ICU, n=330; adult non-ICU, n=1397; pediatric ICU, n=619); pediatric non-ICU, n=1864; rehabilitation, n=133), 814 outpatients and 424 screened medical students (223 foreign and 201 Hungarian students).

The third batch (paper III), contained stool samples from 1004 healthy individuals from the same population as the first batch; 779 from individuals screened for employment purposes (599 females, 180 males; median age 34 (range 14-61) years) and 225 from applicants for long-term care (LTC) facilities (140 females, 85 males; median age 81 (range 0-103) years)) between November 2013 and May 2014.

With the exception of international medical students included in our second batch, the vast majority of individuals in our studies were located in North-Eastern Hungary from the EU regions of Northern Hungary and Northern Great Plain.

Faecal samples were inoculated directly onto eosin methylene blue agar plates supplemented with 2 mg/l cefotaxime. All colonies with different morphology were further identified by

means of biochemical tests; enterobacterial isolates were then processed further. Antimicrobial susceptibility to different antibiotic groups, were determined by the EUCAST disk diffusion method. All isolates showing a decreased susceptibility to at least one third generation cephalosporin or to cefepime were tested for ESBL phenotype using double disk synergy test and then re-identified using a MALDI Biotyper.

In the case of isolates displaying an ESBL phenotype, DNA was extracted by heating a loopful of bacterial cells in 200 µl TE buffer; at 98°C for 15 minutes. For sequencing, PCR products were then purified with a PCR purification kit, following the manufacturer's instructions. The resulting sequences were compared with known sequences using CLC DNA Workbench software and the Basic Local Alignment Search Tool (BLAST, NCBI).

3.2. Identification of ESBL genes and aminoglycoside resistance genes and characterization of integrons

The *bla*_{TEM} , *bla*_{SHV} , *bla*_{CTX-M} genes were detected by PCR and further were identified by sequencing. The aminoglycoside resistance genes *aac*(3')-IIa (*aacC2*) , *aac*(6')-Ib (*aacA4*), *aph*(3')-Ia (*aphA1*) , *ant*(2')-Ia (*aadB*) , *ant*(3')-Ia (*aadA1*) , *armA* , *rmtA* and *rmtB* were sought for. The detection of class 1 and class 2 integrons was performed by PCR assays and the amplification and sequencing of the variable regions were performed, using newly designed internal primers when necessary. Sequences were assembled in the CLC DNA Workbench (CLC Bio, Aarhus, Denmark), and gene cassettes were identified using the BLAST. Identification of class 1 and class 2 integrons with variable regions of the same size was performed by restriction analysis using at least two enzymes for each cassette array, including *EcoRI*, *Hind III*, *MseI* and *RsaI* .

3.3. Phylogenetic analysis of *E. coli* isolates

Phylogenetic groups of *E. coli* were determined using the multiplex PCR. The pandemic O25b-ST131 clone was screened in the isolates of the phylogenetic group B2 with a PCR-based assay.

3.4. Genetic Relatedness

For the first batch of samples (paper I), the epidemiological relationship was analyzed by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) with ERIC2 and ERIC1R primers and pulsed-field gel electrophoresis (PFGE). Plugs were prepared, macrorestriction was performed using XbaI in a CHEF DRIII machine in 1% SeaKem Gold agarose at 14° C. Electrophoresis was performed at 6 V/cm, with a reorientation angle of 120°, and switch times were ramped between 2 and 64 s for 20 h for both species. Gels were stained with ethidium bromide and visualized under UV light. Banding patterns were analyzed with Fingerprinting II software. The threshold for probable relatedness was set at >85% similarity. The Simpson index of diversity (D) was calculated to assess diversity.

3.5. Statistical analysis

Prevalences were compared by means of the χ^2 test or the Fisher exact test as appropriate. Age distribution in the different groups was compared by means of the Kolmogorov-Smirnov test. The distribution of genes coding for ESBLs, aminoglycoside modifying enzyme genes and co-resistance patterns were all analyzed by χ^2 test, and the pairwise comparisons derived were adjusted with the Bonferroni correction. The association of genes with each other or with different characteristics was analysed using the Pearson correlation. PaSt v3.0 was used to perform the statistical tests.

Time series data of monthly antibiotic consumptions, prevalence of ESBL carriers and prevalence of ESBL-infected patients were used in different combinations to build dynamic regression models (paper II) using the Pankratz methodology. First, pairwise dynamic regression models were built to assess the relationship between a) variables characterizing

ESBL-infected patients as explanatory and ESBL carriage rates as dependent variables, ii) these two sets of variables tested in reverse order, iii) using consumption of different antibiotics as explanatory variables against ESBL carriage rates or iv) against variables characterizing ESBL-infected patients. Later models with multiple explanatory variables were constructed using v) rates of infection with ESBL-producing *E. coli* and *K. pneumoniae* as separate explanatory variables and vi) rates of infection in pediatric and adult patients as separate explanatory variables. Causal relationship between two time-series was assessed using Granger causality tests. Time-series analysis was performed in the software environment Eviews 3.1.

4. Results

4.1. Prevalence of ESBL-producer carriage

The overall prevalence of ESBL carriers among individuals screened for enteric pathogens in 2009-2010 and in 2013-2014 (first and third batches) was comparable (3.7 and 3.0%, respectively). The LTC groups carried ESBL-producers significantly more frequently than the employment screening groups (7.2% vs. 2.0%, $p < 0.001$ and 5.3% vs. 2.3%, $p = 0.019$ respectively).

In the first batch, among the 63 ESBL-producing isolates from the 60 patients, the species found were *E. coli* (43 isolates), *K. pneumoniae* (18 isolates), *Klebsiella oxytoca* (1 isolate) and *Proteus mirabilis* (1 isolate). Three individuals, all from the LTC group, harboured multiple ESBL-producing isolates simultaneously. Among the LTC group, the same proportion of *E. coli* and *K. pneumoniae* isolates was found, while among individuals on employment screening only *E. coli* isolates occurred.

In the same population of individuals sampled in 2013-14 (third batch), altogether 32 isolates producing ESBLs were found. Similarly to the first batch, the same proportion of *E. coli* and *K. pneumoniae* occurred in the LTC group and only *E. coli* isolates were found in the employment screening group.

Prevalence of faecal carriage of ESBL-producers in the second batch was 7.4% among inpatients which was significantly higher than outpatients and screened medical students (3.1% and 2.6%, respectively; $p < 0.001$). Among inpatient subgroups, adults showed significantly higher carriage rates of ESBL producers than pediatric patients (12.0% vs. 4.1%; $p < 0.001$). The highest prevalence was found in rehabilitation wards (27.1%), followed by ICU patients (9.3%); lowest prevalences found in non-ICU patients (6.1%). These differences were statistically significant in any pairwise comparison ($p \leq 0.001$). The prevalence rates were comparable in medical screening students and outpatient study groups

(1.0%-4.0%) as well as in pediatric non-ICU (3.0%), however significantly higher, in pediatric ICU (6.8%) and more significantly higher in adult ICU and non-ICU (13.9% and 10.2%; respectively). Among the screened medical students non-Hungarian medical students showed higher prevalence rate than Hungarian students ($p=0.049$). The rate of carriage of ESBL producers was not affected by the region where patients lived, by their gender or by their age in any of the comparisons.

Among the 369 ESBL-producers from the 359 patients, the most common species were *K. pneumoniae* and *E. coli* (185 and 179 isolates, respectively), followed by two *Proteus mirabilis*, two *Citrobacter brakii* and one *K. oxytoca* isolate. Ten individuals among inpatients harboured *K. pneumoniae* and *E. coli* simultaneously. In inpatients, proportion of *E. coli* and *K. pneumoniae* was similar (46.0% vs. 52.9%), while among outpatients and medical students *E. coli* was more frequent (64.0% vs. 32.0%; $p=0.024$ and 90.9% vs. 9.1%, $p<0.001$, respectively). *K. pneumoniae* was more prevalent among adult ICU (75.5% vs. 24.5%; $p<0.001$) and rehabilitation patients (75.0% vs. 22.2%; $p<0.001$). In contrast, *E. coli* prevalence was significantly higher among pediatric non-ICU ($P=0.030$).

4.2. Characterization of ESBL producing isolates

In the first batch, out of the 63 ESBL-positive isolates 50 harboured a *bla*_{CTX-M} gene; the majority of which were *bla*_{CTX-M-15} (68.0%). Other *bla*_{CTX-M} types were found only in *E. coli* isolates. In the LTC group *bla*_{CTX-M-15} was found more frequently than in the employment screening group (68.3% vs. 22.7%, respectively; $p<0.001$). In *E. coli* from the LTC group, *bla*_{CTX-M-15} gene was found in 76.2% of the isolates, while in the employment screening group only in 22.7%.

In the same population of individuals sampled in 2013-14 (the third batch), all isolates carried CTX-M type ESBLs, with the dominance of *bla*_{CTX-M-15} (84.4%). Diversity of ESBL genes was lower than the first batch. All *K. pneumoniae* isolates carried *bla*_{CTX-M-15}, while 85.7% of

E. coli isolates from the LTC group and 77.8% from the employment screening group carried *bla*_{CTX-M-15}. Comparing with the first batch, *bla*_{CTX-M-15} in *E. coli* from the employment screening group in 2013-14, became significantly more frequent (p=0.001), however no significant differences found between *E. coli* in the LTC group for both batches.

In the second batch, the most common gene was also *bla*_{CTX-M-15} in both major species and in all groups. Adult inpatients carried *bla*_{CTX-M-15} more frequently than children, regardless of ward type (p<0.001). Diversity of ESBL genes in *E. coli* was higher in children than in adults.

4.3. Resistance patterns and aminoglycoside resistance genes

In 2009-2010 (first batch) *K. pneumoniae* was resistant to all the tested antibiotics, except for carbapenems and colistin, and it carried *aac(6')-Ib* gene. In case of *E. coli* there was marked variability in the patterns of resistance to ciprofloxacin, amikacin, tobramycin and trimethoprim-sulfamethoxazole (p=0.048 to p<0.002) between isolates from individuals on employment screening and applying for LTC admission. In contrast to *K. pneumoniae*, isolates resistant to all non-beta-lactam antibiotics (except colistin) were not found in *E. coli* from either group. In the *E. coli* isolates, the presence of the *bla*_{CTX-M-15} gene correlated positively with ciprofloxacin, amikacin and tobramycin resistance.

All isolates were negative for *ant(2')-Ia*, *armA*, *rmtA* and *rmtB* genes. In *E. coli* from the LTC group, *aac(6')-Ib* and *aac(3')-IIa* were frequently found. In the case of *E. coli* from the employment screening group, *ant(3')-Ia* was more frequent than other genes.

In the consecutive study of the same population in 2013-14 (third batch), resistance to amikacin, co-trimoxazole, ciprofloxacin and gentamicin was found in 13, 12, 11 and 8 of the 14 isolates in the LTC group respectively, while these numbers were 6, 11, 11 and 6 of the 18 isolates in the employment screening group. The difference in susceptibility also showed when comparing only *E. coli* isolates; six and all of the seven isolates in the LTC group were

ciprofloxacin and amikacin resistant, respectively, while eleven were ciprofloxacin resistant and six were amikacin resistant of the 18 *E. coli* isolates from the employment screening group. Similarly to the first batch, all isolates were negative for *ant(2')-Ia*, *armA*, *rmtA* and *rmtB* genes. The genes *aac(3')-IIa* and *aac(6')-Ib* were more dominant in the LTC group than in the employment screening group. This was also evident when comparing only *E. coli* isolates from the two groups.

In the second batch, resistance rates to ciprofloxacin and aminoglycosides were significantly higher among adults both in ICUs and non-ICUs than among children, outpatients and screened students ($p < 0.001$). The genes *aac(3')-IIa* and *aac(6')-Ib* were more frequent in adults ($p < 0.001$), while in children *aph(3')-Ia* was found more frequently ($p < 0.001$). Resistance to ciprofloxacin and to aminoglycoside antibiotics correlated with the presence of *bla_{CTX-M-15}* gene. *K. pneumoniae* showed significantly higher resistance to all antibiotics tested than *E. coli* ($p < 0.001$), ertapenem resistance and *rmtA* was detected only in *K. pneumoniae* from inpatients. The genes *aac(3')-IIa*, *aac(6')-Ib* and *ant(3'')-Ia* were also significantly more frequent in *K. pneumoniae* ($p < 0.001$). In case of *E. coli* isolates, resistance rates to ciprofloxacin and aminoglycosides were significantly higher in extraintestinal pathogenic than in commensal strains ($p < 0.004$).

4.4. Characterization of integrons

In the first batch, class 1 integrons were found more frequently than class 2 integrons ($p < 0.001$). The carriage rate of class 1 integrons was significantly higher in the *E. coli* from the employment screening group than from the LTC group. The majority of *K. pneumoniae* carried a class 1 integron with a single *ant(3')-Ib* gene. Seven and two different gene cassette arrays (*dfrA1-sat2-ant(3'')-Ia* and *sat2-ant(3'')-Ia*) were identified amongst the class 1 and 2 integrons, respectively.

Overall carriage rate of class 1 and 2 integrons in the same population group in 2013-14 (third batch), was 40.6% and 9.4% respectively. Class 1 integrons were detected in four *K. pneumoniae* and three *E. coli* from the LTC group, while in six of *E. coli* isolates from the employment screening group.

In the second batch, class 1 and 2 integrons were found in 74.5% and 4.1% of isolates, respectively. Carriage of class 1 integrons was significantly more frequent in *K. pneumoniae* than in *E. coli* ($p=0.006$); and in isolates from adults than in isolates from children both in ICU and non-ICU (79.6% and 85.8% vs 53.5% and 61.4%, respectively; $p=0.018$ to $p<0.001$). Rates of integron carriage were comparable between inpatients and outpatients.

4.5. Phylogrouping of *E. coli* isolates

Phylogenetic analysis in the first batch showed that the majority of the isolates belonged to group A (46.5%). Group B2, B1 and D were found for 25.6%, 23.3% and 4.6% of the strains, respectively. The commensal *E. coli* phylogroup A was more frequent in the employment screening group ($p = 0.021$), while the pathogenic phylogroup B2 was more frequent in the LTC applicants ($p=0.016$). All phylogroup B2 isolates belonged to the *bla*_{CTX-M-15} producer O25b:ST131 pandemic clone.

In the employment screening group for the same population studied in 2013-14 (third batch), phylogroup B2 became significantly more dominant than in the first batch (44.4% vs. 9.1%; $p=0.025$), while in the LTC group, phylogroup D was found more frequently (42.9% vs. 4.8%; $p=0.038$). All phylogroup B2 isolates in both groups belonged to the *bla*_{CTX-M-15} producer O25b:ST131 pandemic clone.

Among inpatients (second batch), pathogenic phylogroups B2 and D were more common in adults, ($p<0.001$), while phylogroup B1 and A were more frequently found in children ($p<0.001$). We observed lower proportion of pathogenic phylogroups among pediatric non-

ICU as compared to adult non-ICU. Clone ST131 was detected in 95.6% of the phylogroup B2 isolates harbouring *bla*_{CTX-M-15}.

4.6. Genetic diversity (paper I)

The result of ERIC-PCR and PFGE (D=0.99 and 0.96, respectively) showed that the genetic diversity among *E. coli* isolates was high. The diversity of *E. coli* between the two groups was comparable both with ERIC-PCR and PFGE analysis (D=0.99 vs. 0.99 and D=0.94 vs. 0.95, respectively). Isolates in the same cluster were not uniform considering ESBL genes and carriage of integrons. In contrast, *K. pneumoniae* isolates were markedly less diverse, both with ERIC-PCR and PFGE (D=0.58 and 0.31, respectively); a PFGE clone containing 15 of the 18 isolates was detected. Notably, one of the distinct isolates was the carbapenem nonsusceptible isolate.

4.7. Temporal patterns of carriage dynamics

Among patients (in the second batch), trend analysis of monthly prevalences revealed two stages within the study period. Between October 2010 and October 2011 carriage rates showed increasing tendency, while during the second part (from November 2011 to February 2013) it oscillated around a level; this applies both to *E. coli* and *K. pneumoniae*. Prevalence in adults showed an increasing trend in both stages, but in pediatric patients the initial rise was followed by a decreasing tendency. An increasing trend was observed initially both in ICUs and non-ICUs, followed by a decrease in the ICUs, while by oscillation around a level in non-ICUs. The proportion of CTX-M producers among *K. pneumoniae* steadily increased throughout the study, but among *E. coli* the initial decrease was followed by an increasing trend both in colonized and infected individuals.

According to time-series models and Granger causality, infected patients are more likely to be sources for carriage than carriers for infections; carriage of *K. pneumoniae* Granger-caused infection only in adults, while carriage of *E. coli* only in children. For *K. pneumoniae*,

the effect of infections manifested with longer lags than for *E. coli*; in composite models only the effect of adults and *K. pneumoniae* infections was significant on ESBL carriage. *K. pneumoniae* infections predicted carriage in adults as well as in children, the effect of *E. coli* infections was only significant in adult carriage.

5. Discussion

At the time we began our studies, almost no data available on asymptomatic carriage of ESBLs in Hungary. In our first study (2009-10), we found that the prevalence of ESBL-producers among healthy individuals in the employment screening group was low (2.0%), which was comparable to other European studies conducted on healthy individuals. However, higher prevalence in the LTC group observed (7.2%), and the rate was closer to the prevalences reported in our hospitalized patients (7.4%) and in other studies examining hospitalized asymptomatic carriers. The higher rate in the LTC group is likely to reflect the effect of previous hospitalizations since the average age was higher in this group, and their application for long-term care indicates poor health in the majority of them. This is also supported by the fact that ESBL carriage may last for several months. The carriage rate in the employment screening group corresponds to the actual rate in the community. Most of these individuals work in hospitals, food processing industries and day-care units, where there is a high transmission risk of pathogens. These colonized individuals may act as a source of ESBLs for clinical strains through horizontal gene transfer, or as direct source of ESBL-producers, consequently may become initiators of outbreak (e.g. a nurse in a hospital ward).

We found high diversity of carried ESBL genes in the employment screening group. *K. pneumoniae*, which was absent in the employment screening group, showed higher co-resistance, as expected. Co-resistance profile was more similar to that of *K. pneumoniae*, in *E. coli* from the LTC group than in *E. coli* from the employment screening group. This was also reflected in distribution of the aminoglycoside modifying enzyme gene patterns with more frequent carriage of *aac(6')-Ib* gene associated with *bla_{CTX-M-15}* in the LTC group, but the dominance of *ant(3')-Ia* in *E. coli* isolates from the employment screening group, which is in agreement with other studies which reported the *ant(3')-Ia* as a frequently found gene in the intestinal *E. coli* of healthy individuals and animals as well. These results suggest the

ESBL-producing isolates in the LTC group originating from the hospital microbiota, whereas the colonization of individuals in the employment screening group may also originate from different environmental sources. Besides the exportation of hospital-derived strains either through colonized patients after hospital discharge or through hospital workers, the source for colonization with *bla_{SHV}* may be the horizontal gene transfer from exported *K. pneumoniae* isolates, whereas for *bla_{CTX-M}* other than *bla_{CTX-M-15}*, livestock and animal-derived food, companion animals, wild animals, surface water, drinking water are also potential sources, either by direct transfer of the resistant bacteria or by the horizontal transfer of resistance plasmids to human commensal *E. coli*. The assumption of diverse environmental sources for colonization with ESBL-producers in the employment screening group is supported by high frequency of *bla_{CTX-M-1}* genes in the employment screening group which is in accordance with high frequency of the same gene in *E. coli* isolates of animal origin in Hungary, of water birds in Poland, of food processing animals and raw milk in Switzerland, also by frequent occurrence of *bla_{CTX-M-2}*, *bla_{CTX-M-8}*, *bla_{CTX-M-14}* and *bla_{CTX-M-32}* in *E. coli* isolates of food animals, pointing to a possible zoonotic dissemination of ESBL-producing *E. coli*.

In spite of the small change in the prevalence of ESBL-producers between the studies, significant differences were found in the gene distribution in the employment screening group. The high gene diversity in the employment screening group in 2009-10 disappeared in 2013-14 and high percentage of *bla_{CTX-M-15}* was found in both groups. In the employment screening group the prevalence of *bla_{CTX-M-15}* increased significantly, and almost all other *bla_{CTX-M}* genes disappeared.

This change was paralleled by the high frequency of ciprofloxacin and amikacin resistance, and high carriage of *aac(6')-Ib* and *aac(3')-IIa*, similar to other studies where this co-resistance and these genes are commonly associated with *bla_{CTX-M-15}*. These results point to the emergence of *bla_{CTX-15}* producing *E. coli* in asymptomatic individuals (i.e. individuals in

the employment screening group), this suggests that ESBL-producers which were characterized by different colonization sources are being replaced by highly successful *bla*_{CTX-M-15} producing strains. A possible explanation for this shift is that there is a high frequency of phylogroup B2 in the employment screening group and is in parallel with the dominance of *bla*_{CTX-M-15} in hospitalized patients in 2010-13 both in *E. coli* and *K. pneumoniae* which points to an exportation of pathogenic ST131 clone carrying *bla*_{CTX-M-15} from hospital to the community and its spread within the community. This epidemiological shift took place in the UK a decade earlier.

Adult inpatients in 2010-13 showed significantly higher prevalence than paediatrics patients. These carriers may be a source of exportation of ESBLs from one ward to other wards or from the hospital to other hospitals through patient exchange as well as to the community with patients discharged and may also spread further within households. Among outpatients and screened medical students the prevalences were comparable to the employment screening groups, reflecting the less probable hospitalization history among outpatients and students.

The differences in the distribution of ESBL genes among adults and children may be explained by different probable source of colonization. Most of the ESBL carriers from paediatrics represent patterns similar to employment screening group in 2009-10, and they are more likely to be imported to the hospital rather than acquired in a hospital-based manner. It is a conceivable hypothesis that the ESBL producing isolates were already present in intestinal flora of children before admission into hospital and there is lower chance that they are nosocomially acquired than in case of adults. In adults most carriers seemed to be of hospital-based colonization, similar to ESBL colonization among the LTC group in 2009-10 and in 2013-14. This was also supported by time-series analysis where infections Granger-caused colonization in case of adults but not in case of children. This assumption is supported by low frequency of pathogenic phylogroups among *E. coli* from pediatric non-ICU in

comparison with adult non-ICU, as was the case for healthy individuals in 2009-10. Based on time-series analysis, carriage rate of ESBL-producing *K. pneumoniae* is influenced more strongly by infection rates and the effect of infections manifested with longer time lags than in case of *E. coli*. Time-series analysis also demonstrated that adults carriage of ESBL-producers seems to be the consequence of infection, especially in ICUs. We found higher rate of integron carriage in *K. pneumoniae* isolates from adult patients which indicates that these integrons are widely present in ESBL-producers and may be considered as an important factor in development of multidrug resistant strains.

In conclusion, a clear difference between dynamics of community-based and hospital-based colonization was found, as suggested by differences in carrier species, ESBL gene diversity and co-resistance patterns. The basis of carriage in the community seems to be exportation from the hospital, which is characterized by establishment of hospital-derived clones in the healthy carriers and replacement of the diverse environment-derived strains. Colonization of children seems to derive more from environmental sources, emphasizing the importance of community transmission from family members or from healthy individuals in daycare or in food processing especially in their case.

6. Summary

In summary, our results denote the importance of the intestinal tract as a reservoir for ESBL-producers both in the community and in the hospital settings. Besides being able to serve as outbreak sources directly, they may also act as resistance gene reservoirs. The study draws attention to the different epidemiology of colonization with ESBL-producers in both hospital and community. Sources of colonization in the employment screening group in 2009-10, outpatients, screened medical students and pediatric patients in 2010-13, seem to involve environment-based besides nosocomial sources. In contrast, it was shown that colonization precedes infection in case of adults and they are more likely to acquire colonizer strains in the hospital. Intestinal colonization by these organisms among pediatric patients, can be a source for influx of ESBL determinants into the hospital settings and can represent a risk factor for colonization or as well as infection of other hospitalized patients and health workers. We found a marked decrease of diversity in ESBL genes among the employment screening group in 2013-14. This suggests that ESBL-producers originating from different sources in 2009-10, have been replaced by highly successful *bla*_{CTX-M-15} producing *E. coli*. This was in line with our observation of the increase in the importance of *bla*_{CTX-M-15} gene in both *E. coli* and *K. pneumoniae* in 2010-13. The reason might be related to the exportation of *bla*_{CTX-M-15} producing *E. coli*, with majority belonged to phylogroup B2, from hospital to the community, and consequently further spread within the community.



Registry number: DEENK/36/2016.PL
Subject: Ph.D. List of Publications

Candidate: Fatemeh Ebrahimi
Neptun ID: A9M4HX
Doctoral School: Doctoral School of Pharmaceutical Sciences

List of publications related to the dissertation

1. **Ebrahimi, F.**, Mózes, J., Mészáros, J., Juhász, Á., Majoros, L., Szarka, K., Kardos, G.:
Asymptomatic faecal carriage of ESBL producing Enterobacteriaceae in Hungarian healthy individuals and in long-term care applicants: A shift towards CTX-M producers in the community.
Infect. Dis. "Accepted by Publisher" p. 1-12, p. 2016.
DOI: <http://dx.doi.org/10.3109/23744235.2016.1155734>
IF:1.495 (2014)*
2. **Ebrahimi, F.**, Mózes, J., Mészáros, J., Juhász, Á., Kardos, G.: Carriage Rates and Characteristics of Enterobacteriaceae Producing Extended-Spectrum Beta-Lactamases in Healthy Individuals: Comparison of Applicants for Long-Term Care and Individuals Screened for Employment Purposes.
Chemotherapy. 60 (4), 239-249, 2015.
DOI: <http://dx.doi.org/10.1159/000375407>
IF:1.288 (2014)

*Due to the change of the journal name the old journal (*Scandinavian Journal of Infectious Diseases* (ISSN:003-5548)) impact factor value is used until the 2015th impact factor values published.





List of other publications

3. Mózes, J., **Ebrahimi, F.**, Gorácz, O., Miszti, C., Kardos, G.: Effect of carbapenem consumption patterns on the molecular epidemiology and carbapenem resistance of *Acinetobacter baumannii*.

J. Med. Microbiol. 63, 1654-1662, 2014.

DOI: <http://dx.doi.org/10.1099/jmm.0.082818-0>

IF:2.248

4. Mózes, J., Szűcs, I., Molnár, D., Jakab, P., **Ebrahimi, F.**, Szilasi, M., Majoros, L., Orosi, P., Kardos, G.: A potential role of aminoglycoside resistance in endemic occurrence of *Pseudomonas aeruginosa* strains in lower airways of mechanically ventilated patients.

Diagn. Microbiol. Infect. Dis. 78 (1), 79-84, 2014.

DOI: <http://dx.doi.org/10.1016/j.diagmicrobio.2013.09.015>

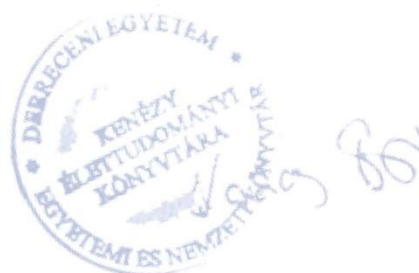
IF:2.457

Total IF of journals (all publications): 7,488

Total IF of journals (publications related to the dissertation): 2,783

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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Other paper related to the dissertation

Ebrahimi F, Mózes J, Gorács O, Fésűs A, Majoros L, Szarka K, Kardos G. Comparison of the faecal colonization rates with extended spectrum beta-lactamase producing enterobacteria among patients in different wards, outpatients and screened medical students. *Microbiol Immunol* (submitted, referees asked for a minor revision).

Conferences

Ebrahimi F, Mózes J, Kardos G. Changes in the distribution of asymptotically carried ESBL-producing enterobacteria and their ESBL genes among healthy individuals. Hungarian Society for Microbiology, Budapest, Hungary (July 8-10, 2015).

Ebrahimi F, Mózes J, Kardos G. Prevalence and integron carriage in ESBL producers isolated from faecal samples of inpatients and outpatients. Hungarian Society for Microbiology, Keszthely, Hungary (October 15-17, 2014).

Ebrahimi F, Mózes J, Kardos G. Characterisation of ESBL-producing enterobacter in stool samples of individuals screened for enteric pathogens. 4th Central European Forum for Microbiology, Keszthely, Hungary (Oct 16-18, 2013).

Ebrahimi F, Mózes J, Kardos G. Prevalence of ESBL-producing enterobacteria in stool samples of asymptomatic individuals. Spring Wind Conference, Sopron, Hungary (May 31-June 2, 2013).

Ebrahimi F, Mózes J, Kardos G. Prevalence of ESBL-producing enterobacteria in patients with diarrhoea. European Medical Students' Conference, Debrecen, Hungary (Oct 19-22, 2012).