Comparison of the faecal colonization rates with extended-spectrum beta-lactamase producing enterobacteria among patients in different wards, outpatients and screened medical students

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Abstract

Asymptomatic carriage of extended-spectrum beta-lactamase (ESBL) producers is a risk factor for infection, therefore colonization dynamics are important data to plan infection control. The study investigates faecal colonization with ESBL-producers among inpatients, outpatients and screened medical students and compares the characteristics of ESBL-producers among these groups.

Carriage rates were investigated in 5581 faecal samples; 4343 from inpatients (330, 1397, 619 and 1864 from adult ICUs (intensive care unit), adult non-ICUs, pediatric ICUs and pediatric non-ICUs, respectively), 814 from outpatients and 424 from screening of medical students. ESBL producers were characterized by co-resistance, integrons carried, aminoglycoside resistance genes and ESBL genes. Dynamic regression models were used to identify relationships between combinations of time series of monthly antibiotic consumption, prevalence of carriers and infected patients.

Inpatients, ICU patients and adults showed higher prevalence than outpatients, non-ICU patients or children (7.4%, 9.3% and 12.0% vs 3.1%, 6.1% and 4.1%, respectively). *Klebsiella pneumoniae* was more frequent in ICUs; dominance of CTX-M-15 producers was more marked in adult than in pediatric inpatients. ESBL carriage was shown to be consequence of infection in adults in the time-series analysis; antibiotic consumption had little effect.

Epidemiology of colonization with ESBL-producers was different between pediatric ICU, adult ICU and adult non-ICU patients. In adults, carriage of ESBL-producers seems to be the consequence of infection, especially in ICUs; the main source of colonization is nosocomial acquisition. In contrast, children are less likely to acquire the colonizer strains in the hospital; importation of ESBL-producers by colonized children seems to be significant.

Keywords: asymptomatic carriage; ESBL-producing *E. coli;* ESBL-producing *K. pneumoniae*; antibiotic consumption;

Introduction

Spread of Enterobacteriaceae producing extended spectrum beta-lactamases (ESBLs) is a serious problem, *bla*$_{\text{CTX-M}}$ enzymes being most prevalent worldwide. Prevalence of ESBL-producers depends on patient groups and geographic setting; highest rates were reported in India (≥80%) and China (≥60%), while in Europe and North America rates are 5 to 10% (1). ESBL-producers are often co-resistant to multiple antibiotics such as aminoglycosides, fluoroquinolones and co-trimoxazole. This multidrug resistance was shown to be linked with integrons (2, 3).

ESBL producers may be carried asymptomatically in the faecal flora of humans and animals (4-7); carriage is related to hospitalization and antibiotic consumption (8. 9). The significance of carriers was highlighted both in hospital and in community settings (10). Carriers may serve as a source of endogenous infections or outbreaks and act as resistance gene reservoirs (11, 12). A recent study of our group showed marked differences between dynamics of asymptomatic ESBL carriage in healthy individuals and in people with a hospitalisation history applying for long-term care (13). This inspired the present study investigating the prevalence of faecal colonization with ESBL producers among inpatients of pediatric and adult 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.

Materials and Methods

Samples and isolates

A total of 5581 non-duplicate faecal specimens sent for routine detection of enteric pathogens between October 2010 and February 2013 were used. These originated 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). All samples originated from wards or outpatient clinics of the University of Debrecen (all in a single campus), and the medical students screened were also involved in clinical practices during their training at the same campus. Outbreaks by ESBL producers were not reported during the study.

As the samples used were portions of samples sent for routine diagnostics/screening; the patients themselves or their personal (except sex and age) and clinical data were unavailable for the study. Consequently, informed consent could not be obtained and ethical approval was not necessary (patients remained unidentified and no clinical data of patients has been handled).

A portion of the faecal samples were inoculated directly onto eosin methylene blue agar plates supplemented with 2 mg/l cefotaxime on the day of arrival. Antimicrobial susceptibility was determined by the EUCAST disk diffusion method against ertapenem, meropenem, imipenem, cefotaxime, ceftazidime, cefepime, ciprofloxacin, co-trimoxazole, doxycycline, colistin, amikacin, gentamicin, tobramycin and tigecyclin. All isolates showing decreased susceptibility to at least

one tested cephalosporin were tested for ESBL production by double disk synergy test (Oxoid, Basingstoke, UK). Identification was performed using standard biochemical tests and MALDI Biotyper (Bruker, Bremen, Germany).

Characterization of ESBL producers

Species identifications *Klebsiella pneumoniae* and *Escherichia coli* were confirmed by species-specific PCRs; ESBL genes bla_{TEM}, bla_{SHV}, and bla_{CTX-M} were identified by sequencing. Aminoglycoside resistance genes *aac(3')-IIa*, *aac(6')-Ib, aph(3')-Ia*, *ant(2")-Ia, ant(3")-Ia, armA*, *rmtA* and *rmtB*, integrase genes *intl1* and *intl2* were detected by PCR, variable regions of integrons were analyzed by sequencing. *E. coli* phylogrouping and detection of the pandemic O25b-ST131 clone were also performed. These methods have been described in detail in an earlier work (13). Enterovirulence of *E. coli* isolates was assessed by detecting the characteristic virulence genes (14).

Prevalence of ESBL-infected patients and antibiotic consumption

Monthly numbers of ESBL-infected patients were collected from data of the Bacteriology Laboratory and expressed as number of ESBL-infected patients per positive samples, incidence density of ESBL producers per 100 bed-days and proportion of ESBL producers among *Klebsiella spp*. and *E. coli*. Prevalence data were also collected on *K. pneumoniae* and *E. coli* as well as on adult and pediatric patients separately. Monthly antibiotic consumption was calculated as defined daily doses/100 bed-days for the major antibiotic groups (15).

Statistical analysis

Prevalences, distributions of ESBL genes, *E. coli* isolates among the four phylogenetic groups, virulence genes, aminoglycoside modifying enzyme genes, and co-resistance patterns were analyzed by chi square or Fisher's exact test with Bonferroni corrections, as appropriate; association of genes with different characteristics was analyzed using Pearson correlation using PaSt 3.0. (16).

To analyze the relationships between combinations of time series of monthly antibiotic consumption, time series of prevalence of ESBL carriers and time series of prevalence of ESBL-infected patients, dynamic regression models were built using the Pankratz methodology (17). First, pairwise dynamic regressions were performed i) using 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. Then composite models with multiple explanatory variables predicting the dependent variable 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.

Results

Prevalence of carriage of ESBL-producers

Overall prevalence of faecal carriage of ESBL producers among inpatients was 7.4% (323/4343), monthly ranges were between 0.0% (0/126) and 28.1% (25/89). Prevalence of carriage was 3.1% (25/814) and 2.6% (11/424) among outpatients and screened medical students, respectively. When analyzing inpatient subgroups further, adults showed significantly higher carriage rates of ESBL producers (12.0%, 222/1853) than pediatric patients (4.1%, 101/2490; $p<0.001$). When grouping patients by ward type (Figure 1), the highest prevalence was found in rehabilitation (27.1%, 36/133), followed by ICU patients (9.3%, 88/949); lowest prevalences were recorded in non-ICU patients (6.1%, 199/3261); these differences were statistically significant in any pairwise comparison ($p<=0.001$). The prevalences were comparable in screening and outpatient study groups (1.0%-4.0%) as well as in pediatric non-ICU (3.0%, 56/1864), slightly higher in pediatric ICU (6.8%, 42/619) and significantly higher in adult ICU and non-ICU (13.9%, 46/330 and 10.2%, 143/1397; respectively).

Among the 369 ESBL-producing isolates from the 359 patients, 185 *K. pneumoniae*, 179 *E. coli*, two *Proteus mirabilis*, two *Citrobacter brakii* and one *K. oxytoca* isolates were identified; ten inpatients harboured *K. pneumoniae* and *E. coli* simultaneously. In inpatients, proportion of *E. coli* and *K. pneumoniae* was similar, while among outpatients and medical students *E. coli* was more frequent ($p=0.03$). *K. pneumoniae* was more prevalent among adult ICU and rehabilitation

patients than among adult non-ICU patients or among pediatric non-ICU patients ($p<0.001$). In contrast, *E. coli* prevalence was comparable among all groups, excepting between adult and pediatric non-ICUs (Figure 2).

Characterization of ESBL producer isolates carried

Distribution of ESBL genes in different groups and species are shown in figure 3. The most common gene was $bla_{\text{CTX-M-15}}$ in both species in all groups; in *E. coli* $bla_{\text{CTX-M-1}}$ or, in adult ICU and rehabilitation, $bla_{\text{SHV-12}}$ being the second, while in *K. pneumoniae* $bla_{\text{CTX-M-15}}$ was followed by $bla_{\text{SHV-12}}$ in all inpatient groups. One *K. pneumoniae* isolate harboured $bla_{\text{CTX-M-15}}$ and $bla_{\text{SHV-12}}$ at the same time. Adult inpatients carried $bla_{\text{CTX-M-15}}$ 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 (Figure 3).

Resistance rates to ciprofloxacin and aminoglycosides were significantly higher among adults both in ICUs and non-ICUs than among children, outpatients and screening group ($p<0.001$; Figure 2) . In parallel, *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 aminoglycoside antibiotics was correlated with the presence of $bla_{\text{CTX-M-15}}$ gene ($r=0.42$-0.54; $p<0.001$).

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*. In case of *E. coli* isolates,

resistance rates to ciprofloxacin and aminoglycosides were significantly higher in extraintestinal pathogenic than in commensal strains ($p<0.004$).

Class 1 and 2 integrons were found in 74.5% (275/369) and 4.1% (15/369) of isolates, respectively. Class 1 integrons carriage was significantly higher in *K. pneumoniae* than in *E. coli* (81.1%, 150/185 vs. 68.7%, 123/179; $p=0.006$); and in isolates from adults both in ICU and non-ICU ($p<0.001$) than in isolates from children. Integron carriage was comparable between inpatients and outpatients. A single class 2 integron (*sat2-ant(3")-Ia*) as well as nine different class 1 integrons (Figure 4) were identified.

Distribution of *E. coli* isolates among the four phylogroups is shown in Table 1. Among inpatients, pathogenic phylogroups were more common in adults ($p<0.001$), while phylogroup B1 and A were more frequently found in children ($p<0.001$). Clone ST131 was detected in 24.0% (43/179) of the phylogroup B2 isolates harbouring $bla_{\text{CTX-M-15}}$. Out of the genes characteristic to enterovirulent pathogroups, only an *eae* gene was found in a single phylogroup B1 isolate.

Prevalence of ESBL-infected patients

The rate of ESBL-infected patients per positive samples was 3.2% (monthly rates between 2.1-4.8%). In case of ESBL producing *E. coli* this rate was 1.0% (0.4-2.1% monthly), while ESBL producing *Klebsiella* was found in 2.2% (1.1-3.1% monthly) of positive samples. The proportion of ESBL producers among *E. coli* and *Klebsiella* isolates was 10.2% (3.8-16.5% monthly) and 37.6% (19.6-50.0% monthly), respectively. In adult and in pediatric patients the infection rates were 3.6% (monthly range 2.0-6.0%) and 1.9% (monthly range 0.0-5.2%), respectively.

In adults, the proportion of ESBL producers among *E. coli* was 11.9% (4.5-20.7% monthly), while among *Klebsiella* isolates was 42.5% (20.9-59.3%). In children 4.3% (0.0-12.5%) of *E. coli* and 20.7% (0.0-58.3%) of *Klebsiella* isolates produced ESBLs.

Temporal patterns of carriage dynamics

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 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. Both in ICUs and non-ICUs an increasing trend was observed initially, 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.

Time-series models and Granger causality tests suggested that 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 (Table 2). For *K. pneumoniae*, the effect of infections manifested with longer lags than for *E. coli*; in composite models only the effect of adult 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 (Table 3).

Discussion

Asymptomatic carriage of ESBL producers is considered a risk factor for infection caused by such bacteria (18, 19), therefore the patterns and dynamics of colonization are important input data for control of infections by ESBL producers. In an earlier study, we hypothesized that sources of colonization with ESBL producers may differ for healthy adults and for individuals applying for long-term care probably with an extensive hospitalisation history (13). It is conceivable that colonization of hospitalized patients originates from the hospital microbiota (20-22), but we postulated that ESBL producers of healthy individuals originate partly from diverse environmental sources (4, 13).

The present study started roughly a year later and conducted in the same geographical area seems to support the latter hypothesis, as carriage prevalences of outpatients and of healthy medical students were comparable. The generally higher prevalence of asymptomatic carriage in Asia and Africa (7-9, 23) is also reflected in the somewhat higher prevalence in non-Hungarian medical students, who mainly originate from the Near East and Africa.

Overall rate of faecal carriage of ESBL-producing isolates among inpatients was 7.4%, which is comparable to other European data (7, 10). Colonization with multiple strains was rare. As expected, inpatients showed higher prevalence than outpatients, however, notable differences were found among different inpatient groups. Highest prevalence was found in the rehabilitation wards, paralelling the many reports on long-term care patients (23-25). ICU patients were carriers more frequently than non-ICU patients, and adults showed significantly higher

prevalence than children, even pediatric ICU showed lower prevalence than adult non-ICU wards. The higher prevalence in ICU patients obviously reflects the well-documented high infection prevalence among these patients (26, 27).

The reasons for the lower prevalence among children remains unknown; potential explanations include age-specific lower susceptibility to colonization, their generally lower frequency of hospitalization episodes or pediatrics-specific intervention patterns, which may lead to altered risk of acquisition. It is remarkable, that the difference between pediatric outpatients (probably reflecting the inflow/outflow rate from the community to hospital or vice versa) and pediatric non-ICU was negligible. In contrast, though community carriage in adults was comparable to community carriage among children, but the hospital carriage rate was much higher. In adult inpatients, colonization seem to occur relatively fast, in a Belgian study a median of seven days after hospitalization was reported (22). These findings point to major differences between pediatric and adult patients regarding the dynamics of ESBL transmission in the hospital, and may be interpreted that children may acquire ESBLs in the hospital with lower probabilty than adults or are less susceptible to transient hospital-associated colonization. In adults the major source for colonization as well as infection is the hospital (22), but the dominance of *E. coli* and the diversity of ESBL genes among pediatric carriers suggests the importance of community-based sources. This assumption is further supported by the significantly lower proportion of pathogenic phylogroups among *E. coli* isolates of pediatric non-ICUs as compared to those of adult non-ICUs, which parallels a similar difference between healthy adults and applicants for long-term care (13).

Another strong argument is provided by time-series analysis, clearly demonstrating infections as sources of colonization (rather than vice versa) in case of adults, but not in children.

As among the study subjects only a few carriers had multiple samples and active retesting of carriers was not pursued, the duration of colonization could not be assessed. The difference between carriage rates of adult inpatients and people applying for long-term care in the area serviced by the hospital (13) suggests that a proportion of colonizations may be transient. However, it was demonstrated that people may remain colonized, even in absence of infection, for a long time (28, 29), providing a means for exportation from the ICUs to normal wards, and even further to the community. The ability of spreading among community or family members even in absence of infections has been demonstrated earlier (7, 20, 30, 31). This applies not only for communities with ample access to healthcare which may serve as a continuous source for inflow of ESBL producers, but also in a closed community with little contact with other people or healthcare systems (7).

During the history of ESBL producing bacteria, several major shifts has been observed in species and gene distributions. Initial dominance of bla_{SHV} and bla_{TEM} producing *K. pneumoniae* was overthrown first by pandemic clones of *K. pneumoniae* producing CTX-M ESBLs then CTX-M-15 producing *E. coli* emerged as a major concern, efficiently exporting the problem from the hospital to the community. This is in parallel with shifts in gene epidemiology; initial resistance plasmids carrying bla_{SHV} or bla_{TEM} genes spreading among different strains have

been replaced by stable harbouring of plasmids with lower transfer capacity which carry *bla*$_{CTX}$ genes.

Reflecting this, CTX-M-15 producer isolates were predominant in all patient groups, however, this dominance was less marked in case of pediatric patients, regardless of ward type. An overall increase in the importance of the *bla*$_{CTX\text{-}M\text{-}15}$ gene was observed during the study period both in *K. pneumoniae* and *E. coli*. The worldwide observation of the increase in the importance of *E. coli* (6, 32, 33), is also reflected in these data. The prevalence of *K. pneumoniae* dropped significantly in pediatric ICUs during the study period and this species remained dominant only in adult ICUs and in the rehabilitation wards.

Colonization with *K. pneumoniae* is clearly a nosocomial acquisition; ESBL-producing *K. pneumoniae* is usually highly clonal and show extensive co-resistance (34, 35). In this study, colonization rates with ESBL-producing *K. pneumoniae* were statistically preceded by infection rates in the time-series analysis; infection rates explained colonization rates better and the effects were shown over longer time lags than in case of *E. coli*. ESBL-producing *E. coli* is abundant in the community worldwide (36, 37) as well as in the geographical area of the study (13). Consequently, these findings can be interpreted that the typically nosocomial *K. pneumoniae* is decreasing, and *E. coli*, which now also may originate from the community besides nosocomial sources, slowly outcompetes *Klebsiella*. This is supported by the higher diversity of ESBL genes in *E. coli*, again pointing to multiple sources for these bacteria, similarly to ESBL colonization found in an isolated rural community with little access to healthcare (7). A similar shift in

species and increasing in the dominance of CTX-M-15 in colonized long-term care applicants was observed in the same geographical area (Ebrahimi et al, 2016, Infectious Diseases, Accepted for publication).

Patterns of integron carriage was different between species as well as between patient groups. *K. pneumoniae* carried integrons more frequently than *E. coli*, as well as isolates colonizing adults compared to those from children. This is in line with the more extensive co-resistance of *K. pneumoniae*; carbapenem resistance and aminoglycoside resistance by rRNA methylation appeared only in *K. pneumoniae*, and the aminoglycoside modifying enzyme genes of major clinical importance *aac(3)-IIa* and *aac(6')-Ib* was more frequent in *K. pneumoniae* as well. Differences in co-resistance was more marked between the two species in case of pediatric patients. These data, though indirectly, also points to differences in epidemiology of carriage in case of the two species.

Though it was shown that infection prevalence with ESBL producers is enhanced by consumption of 3rd generation cefalosporins and fluoroquinolones (36, 38, 39), the effect of antibiotic consumption was very limited on either colonization or infection with ESBL producers in this study. However, it should be kept in mind that the study was started in a period characterized by intensive carbapenem consumption (40) and when prevalence of infections due to ESBL-producers was decreasing. We believe that carriage rates reported hereby represent a burden largely independent of the present-day antibiotic usage. This carriage burden arises from the summation of influx-derived slow increase in case of *E. coli*, especially in

case of pediatric patients, and a slow decrease of hospital-acquired carriage events in case of *K. pneumoniae*.

In summary, epidemiology of colonization with ESBL-producers was different between pediatric, adult ICU and adult non-ICU patients. In adults, the carriage of ESBL producers seems to be the consequence of infection, especially in ICUs and rehabilitation wards; the major source for colonization is nosocomial acquisition. In contrast, children are less likely to acquire the colonizer strains in the hospital than adults; importation of ESBL producers by colonized children seems to be significant.

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Disclosure

All authors report no conflicts of interest relevant to this article.

References

1. Livermore D.M. (2012) Current epidemiology and growing resistance of gram-negative pathogens. *Korean J Intern Med* **27**:128–42.

2. Machado E., Cantón R., Baquero F., Galán J.C., Rollán A., Peixe L., Coque T.M. (2005) Integron content of extended-spectrum-beta-lactamase-producing *Escherichia coli* strains over 12 years in a single hospital in Madrid, Spain. *Antimicrob Agents Chemother* **49**:1823-29.

3. Sompolinsky D., Nitzan Y., Tetry S., Wolk M., Vulikh I., Kerrn M.B., Sandvang D., Hershkovits G., Katcoff D.J. (2005) Integron-mediated ESBL resistance in rare serotypes of *Escherichia coli* causing infections in an elderly population of Israel. *J Antimicrob Chemother* **55**:119-22.

4. Smet A., Martel A., Persoons D., Dewulf J., Heyndrickx M., Herman L., Haesebrouck F., Butaye P. (2010) Broad-spectrum beta-lactamases among Enterobacteriaceae of animal origin: molecular aspects, mobility and impact on public health. *FEMS Microbiol Rev* **34**:295–316.

5. Guenther S., Ewers C., Wieler L.H. (2011) Extended-spectrum beta-lactamases producing *E. coli* in wildlife, yet another form of environmental pollution? *Front Microbiol* **2**:246.

6. Maciuca I.E., Williams N.J., Tuchilus C., Dorneanu O., Guguianu E., Carp-Carare C., Rimbu C., Timofte D. (2015) High Prevalence of *Escherichia coli*-Producing CTX-M-15 Extended-Spectrum Beta-Lactamases in Poultry and Human Clinical Isolates in Romania. *Microb Drug Resist [Epub ahead of print]*.

7. Woerther P.L., Burdet C., Chachaty E., Andremont A. (2013) Trends in human fecal carriage of extended-spectrum beta-lactamases in the community: toward the globalization of CTX-M. *Clin Microbiol Rev* **26**:744–58.

8. Tian S.F., Chen B.Y., Chu Y.Z., Wang S. (2008) Prevalence of rectal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* among elderly people in community settings in China. *Can J Microbiol* **54**:781–5.

9. Luvsansharav U.O., Hirai I., Nakata A., Imura K., Yamauchi K., Niki M., Komalamisra C., Kusolsuk T., Yamamoto Y. (2012) Prevalence of and risk factors associated with faecal carriage of CTX-M beta-lactamase-producing Enterobacteriaceae in rural Thai communities. *J Antimicrob Chemother* **67**:1769–74.

10. Miró E., Mirelis B., Navarro F., Rivera A., Mesa R.J., Roig M.C., Gómez L., Coll P. (2005) Surveillance of extended-spectrum beta-lactamases from clinical samples and faecal carriers in Barcelona, Spain. *J Antimicrob Chemother* **56**:1152–5.

11. Pallecchi L., Bartoloni A., Fiorelli C., Mantella A., Di Maggio T., Gamboa H., Gotuzzo E., Kronvall G., Paradisi F., Rossolini G.M. (2007) Rapid dissemination and diversity of CTX-M extended-spectrum beta-lactamase genes in commensal *Escherichia coli* isolates from healthy children from low-resource settings in Latin America. *Antimicrob Agents Chemother* **51**:2720–5.

12. Damjanova I., Tóth A., Pászti J., Jakab M., Milch H., Bauernfeind A., Füzi M. (2007) Epidemiology of SHV-type beta-lactamase-producing *Klebsiella* spp. from

outbreaks in five geographically distant Hungarian neonatal intensive care units: widespread dissemination of epidemic R-plasmids. *Int J Antimicrob Agents* **29**:665-71.

13. Ebrahimi F., Mózes J., Mészáros J., Juhász Á., Kardos G. (2014) 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**:239–49.

14. Persson S., Olsen K.E.P., Scheutz F., Krogfelt K.A., Gerner-Smidt P. (2007) A method for fast and simple detection of major diarrhoeagenic *Escherichia coli* in the routine diagnostic laboratory. *Clin Microbiol Infect* **13**:516–24.

15. Monnet D.L. (2006) ABC Calc – Antibiotic consumption calculator (MS Excel application).V3.1.Copenhagen (Denmark). Statens Serum Institut.

16. Hammer Q., Harper D.A.T., Ryan P.D. (2001) Paleontological statistics software package for education and data analysis. Palaeontological Association.

17. Pankratz A. (1991) Front Matter. Forecasting with dynamic regression models. John Wiley & Sons, Inc.

18. Ruppé E., Lixandru B., Cojocaru R., Büke C., Paramythiotou E., Angebault C., Visseaux C., Djuikoue I., Erdem E., Burduniuc O., El Mniai A., Marcel C., Perrier M., Kesteman T., Clermont O., Denamur E., Armand-Lefèvre L., Andremont A. (2013) Relative fecal abundance of extended-spectrum-beta-lactamase-producing

Escherichia coli strains and their occurrence in urinary tract infections in women. *Antimicrob Agents Chemother* **57**:4512–7.

19. Ben-Ami R., Schwaber M.J., Navon-Venezia S., Schwartz D., Giladi M., Chmelnitsky I., Leavitt A., Carmeli Y. (2006) Influx of extended-spectrum beta-lactamase-producing enterobacteriaceae into the hospital. *Clin Infect Dis* **42**:925–34.

20. Tham J., Odenholt I., Walder M., Andersson L., Melander E. (2013) Risk factors for infections with extended-spectrum beta-lactamase-producing *Escherichia coli* in a county of Southern Sweden. *Infect Drug Resist* **6**:93–7.

21. Friedmann R., Raveh D., Zartzer E., Rudensky B., Broide E., Attias D., Yinnon A.M. (2009) Prospective evaluation of colonization with extended-spectrum beta-lactamase (ESBL)-producing enterobacteriaceae among patients at hospital admission and of subsequent colonization with ESBL-producing enterobacteriaceae among patients during hospitalization. *Infect Control Hosp Epidemiol* **30**:534–42.

22. Schoevaerdts D., Bogaerts P., Grimmelprez A., de Saint-Hubert M., Delaere B., Jamart J., Swine C., Glupczynski Y. (2011) Clinical profiles of patients colonized or infected with extended-spectrum beta-lactamase producing Enterobacteriaceae isolates: a 20 month retrospective study at a Belgian University Hospital. *BMC Infect Dis* **11**:12.

23. Yoo J.S., Byeon J., Yang J., Yoo J.I., Chung G.T., Lee Y.S. (2010) High prevalence of extended-spectrum beta-lactamases and plasmid-mediated AmpC

beta-lactamases in Enterobacteriaceae isolated from long-term care facilities in Korea. *Diagn Microbiol Infect Dis* **67**:261–5.

24. Lim C.J., Cheng A.C., Kennon J., Spelman D., Hale D., Melican G., Sidjabat H.E., Paterson D.L., Kong D.C., Peleg A.Y. (2014) Prevalence of multidrug-resistant organisms and risk factors for carriage in long-term care facilities: a nested case-control study. *J Antimicrob Chemother* **69**:1972–80.

25. Tinelli M., Cataldo M.A., Mantengoli E., Cadeddu C., Cunietti E., Luzzaro F., Rossolini G.M., Tacconelli E. (2012) Epidemiology and genetic characteristics of extended-spectrum beta-lactamase-producing Gram-negative bacteria causing urinary tract infections in long-term care facilities. *J Antimicrob Chemother* **67**:2982–7.

26. Sader H.S., Farrell D.J., Flamm R.K., Jones R.N. (2014) Antimicrobial susceptibility of Gram-negative organisms isolated from patients hospitalized in intensive care units in United States and European hospitals (2009-2011). *Diagn Microbiol Infect Dis* **78**:443–8.

27. Benner K.W., Prabhakaran P., Lowros A.S. (2014) Epidemiology of infections due to extended-spectrum beta-lactamase-producing bacteria in a pediatric intensive care unit. *J Pediatr Pharmacol Ther* **19**:83–90.

28. Tham J., Walder M., Melander E., Odenholt I. (2012) Duration of colonization with extended-spectrum beta-lactamase-producing *Escherichia coli* in patients with travellers' diarrhoea. *Scand J Infect Dis* **44**:573–7.

29. Tängdén T., Cars O., Melhus A., Löwdin E. (2010) Foreign travel is a major risk factor for colonization with *Escherichia coli* producing CTX-M-type extended-spectrum beta-lactamases: a prospective study with Swedish volunteers. *Antimicrob Agents Chemother* **54**:3564–8.

30. Rodríguez-Baño J., López-Cerero L., Navarro M.D., Díaz de Alba P., Pascual A. (2008) Faecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli*: prevalence, risk factors and molecular epidemiology. *J Antimicrob Chemother* **62**:1142–9.

31. Valverde A., Grill F., Coque T.M., Pintado V., Baquero F., Cantón R., Cobo J.(2008) High rate of intestinal colonization with extended-spectrum-beta-lactamase-producing organisms in household contacts of infected community patients. *J Clin Microbiol* **46**:2796–9.

32. Coque T.M., Novais A., Carattoli A., Poirel L., Pitout J., Peixe L., Baquero F., Cantón R., Nordmann P. (2008) Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. *Emerg Infect Dis* **14**:195–200.

33. Pitout J.D.D., Church D.L., Gregson D.B., Chow B.L., McCracken M., Mulvey M.R., Laupland K.B. (2007) Molecular epidemiology of CTX-M-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. *Antimicrob Agents Chemother* **51**:1281–6.

34. Damjanova I., Tóth A., Pászti J., Hajbel-Vékony G., Jakab M., Berta J., Milch H., Füzi M. (2008) Expansion and countrywide dissemination of ST11, ST15 and

ST147 ciprofloxacin-resistant CTX-M-15-type beta-lactamase-producing *Klebsiella pneumoniae* epidemic clones in Hungary in 2005--the new 'MRSAs'? *J Antimicrob Chemother* **62**:978–85.

35. Webster D.P., Young B.C., Morton R., Collyer D., Batchelor B., Turton J.F., Maharjan S., Livermore D.M., Bejon P., Cookson B.D., Bowler I.C. (2011) Impact of a clonal outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in the development and evolution of bloodstream infections by *K. pneumoniae* and *Escherichia coli*: an 11 year experience in Oxfordshire, UK. *J Antimicrob Chemother* **66**:2126–35.

36. Kang C.I., Wi Y.M., Lee M.Y., Ko K.S., Chung D.R., Peck K.R., Lee N.Y., Song J.H. (2012) Epidemiology and risk factors of community onset infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* strains. *J Clin Microbiol* **50**:312–7.

37. Nakayama T., Ueda S., Huong B.T.M., Tuyen le D., Komalamisra C., Kusolsuk T., Hirai I., Yamamoto Y. (2015) Wide dissemination of extended-spectrum beta-lactamase-producing *Escherichia coli* in community residents in the Indochinese peninsula. *Infect Drug Resist* **8**:1–5.

38. Tóth A., Kocsis B., Damjanova I., Kristóf K., Jánvári L., Pászti J., Csercsik R., Topf J., Szabó D., Hamar P., Nagy K., Füzi M. (2014) Fitness cost associated with resistance to fluoroquinolones is diverse across clones of *Klebsiella pneumoniae* and may select for CTX-M-15 type extended-spectrum beta-lactamase. *Eur J Clin Microbiol Infect Dis* **33**:837-43.

39. Aldeyab M.A., Harbarth S., Vernaz N., Kearney M.P., Scott M.G., Darwish Elhajji F.W., Aldiab M.A., McElnay J.C. (2012) The impact of antibiotic use on the incidence and resistance pattern of extended-spectrum beta-lactamase-producing bacteria in primary and secondary healthcare settings. *Br J Clin Pharmacol* **74**:171-9.

40. Mózes J., Ebrahimi F., Gorácz O., Miszti C., Kardos G. (2014) Effect of carbapenem consumption patterns on the molecular epidemiology and carbapenem resistance of *Acinetobacter baumannii*. *J Med Microbiol* **63**:1654–62.

Figure legends

Figure 1. Prevalence of ESBL producing isolates among different groups. ICU: intensive care unit.

Figure 2. Proportions of ESBL-producing *E. coli* and *K. pneumoniae* in the different study groups and number of isolates resistant to different antibiotics. ICU: intensive care unit, erta R: ertapenem resistant, cip R: ciprofloxacin resistant, sxt R: co-trimoxazole resistant, tig R: tigecycline resistant, amik R: amikacin resistant, gen R: gentamicin resistant.

Figure 3. Distribution of ESBL genes in different groups in *E. coli* and *K. pneumoniae*. ICU: intensive care unit.

Figure 4. Gene cassette arrays of class I integrons in *E. coli* and *K. pneumoniae* isolated from different study groups. ICU: intensive care unit

List of abbreviations

ESBL; extended spectrum beta-lactamase

ICU; intensive care unit