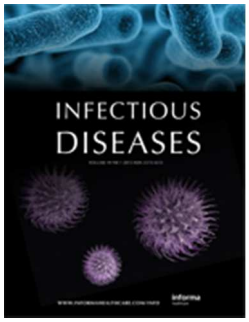

Asymptomatic faecal carriage of ESBL producing Enterobacteriaceae in Hungarian healthy individuals and in long-term care applicants: a shift towards CTX-M producers in the community

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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

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Abstract

Background: Faecal carriage of extended-spectrum beta- lactamase (ESBL) producing Enterobacteriaceae in healthy individuals was examined and compared to previous results obtained in such individuals a few years earlier.

Methods: Faecal samples from 779 individuals screened for employment purposes and from 225 applicants to long-term care (LTC) were screened between November 2013 and May 2014.

Results: The overall rate of fecal carriage was 3.0% (30/1004). The carriage rate was significantly higher in applicants for LTC (5.3% vs. 2.3%; p=0.019). All isolates carried CTX-M ESBLs, with an overwhelming dominance of $bla_{\text{CTX-M-15}}$ (84.4%) in both groups and in both *E. coli* and *Klebsiella pneumoniae*.

Conclusions: The prevalences were comparable to those in the earlier study, but a marked decrease of the diversity of ESBL genes in *E. coli* from the employment screening group was found, suggesting that the ESBL-producing isolates originating from diverse sources are being replaced by highly successful $bla_{\text{CTX-M-15}}$ producing strains.

Introduction

The gut flora of humans and animals is undoubtedly a significant reservoir of plasmids harbouring ESBL genes as well as of ESBL producers [1] and may serve as a source of endogeneous or exogeneous infections [2]. A change in the epidemiology of ESBL producing Enterobacteriaceae took place when CTX-M-type enzymes became prevalent first in the hospital and then in the community setting. Their high ability to disperse locally in the community [3] and to disperse long-range via international travel [4] has been reported. Asymptomatic carriers play an increasingly important role in the epidemiology of ESBL producers. It is, therefore, important to monitor changes in the prevalence, distribution and dynamics of ESBL producers not only in infected patients, but in asymptomatic carriers as well. The aim of the present study was to survey the faecal carriage rate of ESBL producers among healthy individuals and to compare with results obtained in the same population a few years earlier [5].

Material and methods

Stool samples from 779 healthy individuals screened for employment purposes (599 females, 180 males; median age 34 (range 14-61) years) and from 225 applicants to long-term care (LTC) facilities (140 females, 85 males; median age 81 (range 0-103) years) were analysed between November 2013 and May 2014 and compared with results obtained in 2009-2010 in the same population. As the study involved only the samples sent for routine screening purposes and the patients were unidentified, informed consent could not be obtained and and approval by an Ethics Review Board was not required.

To screen for ESBL production, faecal samples were inoculated onto eosin methylene blue agar plates supplemented with 2 mg/l cefotaxime. Confirmation of ESBL phenotype was performed using double disk synergy test (Oxoid, Basingstoke, UK). The isolates were identified by MALDI Biotyper (Bruker, Bremen, Germany) and by species-specific PCRs. Susceptibility to ertapenem, meropenem, imipenem, cefotaxime, ceftazidime, cefepime, ciprofloxacin, co-trimoxazole, colistin, amikacin, gentamicin and tobramycin was tested by disk diffusion according to EUCAST recommendations. All isolates were screened for the genes bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$, amplified genes were sequenced. Five aminoglycoside resistance genes *aac(3')-IIa*, *aac(6')-Ib, aph(3')-Ia*, *ant(2")-Ia*, *ant(3")-Ia*, and class 1 and 2 integrons were sought for by PCRs. Gene cassette arrays were determined by sequencing. For *Escherichia coli* phylogenetic group and the clone O25b-ST131 were determined by PCRs. All technical protocols were described in our earlier publication [5]. Prevalences of isolates or genes were compared by chi-square or Fisher's exact test as appropriate using PaSt 3.0.

Results and discussion

The overall rate of fecal carriage of ESBL-producing isolates was 3.0% (30/1004). The carriage rate was significantly higher in applicants for LTC than in the employment screening group (5.3%, 12/225 vs. 2.3%, 18/779; p=0.019). Thirty-two isolates produced ESBLs. Only *E. coli* isolates were found in the employment screening group, while in applicants for LTC five persons carried *E. coli*, another five carried *K. pneumoniae*, and further two carried both species simultaneously.

All isolates carried CTX-M type ESBLs with an overwhelming dominance of $bla_{\text{CTX-M-15}}$ (Table I). Diversity of ESBL genes was low. All *K. pneumoniae* isolates carried $bla_{\text{CTX-M-15}}$ (two of them harboured $bla_{\text{SHV-5}}$ and two other $bla_{\text{SHV-110}}$ simultaneously), while six of seven *E. coli* isolates from the LTC group and 14 of 18 isolates from healthy individuals carried $bla_{\text{CTX-M-15}}$. The remaining one and four *E. coli* isolates carried $bla_{\text{CTX-M-1}}$.

Resistance to other antibiotic classes than beta-lactamases was common. Eleven, twelve, eight and 13 of 14 isolates were resistant to ciprofloxacin, co-trimoxazole, gentamicin and amikacin, respectively, in the LTC group, while these numbers were eleven, eleven, six and six of 18 isolates in healthy individuals. This difference in susceptibility to amikacin was also evident when comparing only *E. coli* isolates. Integron carriage rates were comparable. In the LTC group class 1 integrons were detected in four *K. pneumoniae* (three and one with gene cassette arrays *dfrA12-orf*X*-ant(3")-Ib* and *dfrA15-ant(3")-Ia*, respectively) and three *E. coli* (one and two with gene cassette arrays *dfrA7* and *dfrA17-ant(3")-Ie*, respectively). Among *E. coli* isolates from healthy individuals six were class 1 integron carriers with one *dfrA7*, one *dfrA1-ant(3")-Ia*, two *ant(3")-Ia* and two *dfrA17-ant(3")-Ie* gene cassette arrays. Class two integrons were found only in *E. coli*, one from the LTC and two from the employment screening group with a uniform gene cassette array of *dfrA1-sat2-ant(3")-Ia*. The genes *aac(3')-IIa* and *aac(6')-Ib* were more frequently found in the LTC group than in healthy individuals (10/14 vs. 2/18, p=0.001 and 5/14 vs. 0/18, p=0.02, respectively). This applied when comparing only *E. coli* isolates from the two groups (5/7 vs. 2/18, p=0.007 and 3/7 vs. 0/18, p=0.015, respectively). Phylogroup distributions were comparable;

dominance of the pathogenic phylogroup B2 was found in the healthy individuals. All phylogroup B2 isolates in both groups belonged to the *bla*$_{CTX-M-15}$ producer O25b:ST131 pandemic clone.

Compared with the 2009-2010 study [5], the over-all prevalence of ESBL producers was slightly lower. Though the prevalences of the two periods were statistically comparable, there was a significant difference between the overall gene distributions in the two periods as well as between gene distribution in the employment screening group. *K. pneumoniae*, found exclusively in the LTC group in both periods, became uniformly positive for *bla*$_{CTX-M-15}$. Thus, production of SHV enzymes by *K. pneumoniae* became linked to simultaneous production of *bla*$_{CTX-M-15}$. Remarkably, carbapenem (ertapenem) resistance, which appeared in *K. pneumoniae* isolated from inpatients in the same region [6], has not yet emerged.

Similarly, in *E. coli* from the LTC group the gene *bla*$_{CTX-M-15}$ became slightly more frequent (16/21 vs. 6/7), and became predominant in healthy individuals as well, but this difference was not statistically significant. In the earlier study five of 22 isolates and in the present study 14 of 18 isolates carried *bla*$_{CTX-M-15}$ (Table I). This change was paralleled by increased resistance to ciprofloxacin and amikacin together with more frequent carriage of *aac(3')-IIa* and *aac(6')-Ib*, co-resistances and genes commonly associated with *bla*$_{CTX-M-15}$ [7,8]. There was s significant difference in *bla*$_{CTX-M-15}$ carriage between the LTC group and the employment screening group in the first period [5] but not in the second period. The similarity between the two groups in the present study suggests a scenario where the hospital based CTX-M-15 producers have been established in the non-hospitalized group, i.e. in the community.

Several studies report the emergence of *bla*$_{CTX-M-15}$ producing *E. coli* in infections both in the hospital and in the community setting [9,10]. Assuming, as in the earlier study, that individuals in the LTC group are highly likely to have an extensive history of hospitalization in contrast to healthy individuals, the present study reports a direct observation of this emergence in asymptomatic carriers. This is underlined by the marked decrease of the diversity of ESBL genes in *E. coli* from the healthy individuals, where out of the six *bla*$_{CTX-M}$ enzymes representing all four major groups found in 2009-2010, only the *bla*$_{CTX-M-15}$ and *bla*$_{CTX-M-1}$ from group 1 remained in 2013-2014. This suggests that the ESBL-producing isolates originating from diverse sources [11-16], are being replaced by highly successful *bla*$_{CTX-M-15}$ producing

strains. This epidemiological shift is confirmed by the replacement of commensal phylogroups by phylogroup B2 and is in parallel with the increase of the proportions of $bla_{CTX\text{-}M\text{-}15}$ producers in both *E. coli* and *K. pneumoniae* isolated from inpatients from the same geographical area [6].

Whether the community is the source or the target for these strains is debated. In some populations the importance of importation to the hospital has been shown [17-19], while previous hospitalization [20] as well as long-term care [5], are risk factors. In the geographical area of the study, exportation of $bla_{CTX\text{-}M\text{-}15}$ producing (phylogroup B2) *E. coli* isolates from the hospital to the community followed by spread within the community seems to take place, as suggested by the scenario outlined above.

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References

1. Woerther PL, Angebault C, Jacquier H, Hugede HC, Janssens AC, Sayadi S, et al. Massive increase, spread, and exchange of extended spectrum beta-lactamase-encoding genes among intestinal Enterobacteriaceae in hospitalized children with severe acute malnutrition in Niger. Clin Infect Dis 2011;53:677-85.

2. Damjanova I, Tóth A, Pászti J, Jakab M, Milch H, Bauernfeind A, et al. 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 2007;29:665-71.

3. Valverde A, Grill F, Coque TM, Pintado V, Baquero F, Cantón R, et al. High rate of intestinal colonization with extended-spectrum-beta-lactamase-producing organisms in household contacts of infected community patients. J Clin Microbiol 2008;46:2796-9.

4. Van der Bij AK, Pitout JD. The role of international travel in the worldwide spread of multiresistant Enterobacteriaceae. J Antimicrob Chemother 2012;67:2090-100.

5. 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 2014;60:239-49.

6. Ebrahimi F, Mózes J, Monostori J, Gorácz O, Fésűs A, Majoros L, et al. Comparison of the faecal colonization rates with extended-spectrum beta-lactamase producing enterobacteria among patients in different wards, outpatients and screened medical students. (submitted).

7. Hansen DS, Schumacher H, Hansen F, Stegger M, Hertz FB, Schønning K, et al. Extended-spectrum beta-lactamase (ESBL) in Danish clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*: prevalence, beta-lactamase distribution, phylogroups, and co-resistance. Scand J Infect Dis 2012;44:174-81.

8. Pitout JD, Hanson ND, Church DL, Laupland KB. Population-based laboratory surveillance for *Escherichia coli*-producing extended-spectrum beta-lactamases: importance of community isolates with bla_{CTX-M} genes. Clin Infect Dis 2004;38:1736-41.

9. Hu F, O'Hara JA, Rivera JI, Doi Y. Molecular features of community-associated extended-spectrum-β-lactamase-producing *Escherichia coli* strains in the United States. Antimicrob Agents Chemother 2014;58:6953-7.

10. Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Claeys G, et al. Characterization of extended-spectrum beta-lactamases produced by *Escherichia coli* isolated from hospitalized and nonhospitalized patients: emergence of CTX-M-15-producing strains causing urinary tract infections. Microb Drug Resist 2010;16:129-34.

11. De Boeck H, Miwanda B, Lunguya-Metila O, Muyembe-Tamfum JJ, Stobberingh E, Glupczynski Y, et al. ESBL-positive *Enterobacteria* isolates in drinking water. Emerg Infect Dis 2012;18:1019-20.

12. Geser N, Stephan R, Hächler H. Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. BMC Vet Res 2012;8:21.

13. Literak I, Dolejska M, Janoszowska D, Hrusakova J, Meissner W, Rzyska H, et al. 2010. Antibiotic-resistant *Escherichia coli* bacteria, including strains with genes encoding the extended-spectrum beta-lactamase and QnrS, in waterbirds on the Baltic Sea Coast of Poland. Appl Environ Microbiol 2010;76:8126-34.

14. Tacão M, Moura A, Correia A, Henriques I. Co-resistance to different classes of antibiotics among ESBL-producers from aquatic systems. Water Res 2014;48:100-7.

15. Tóth A, Juhász-Kaszanyitzky É, Mag T, Hajbel-Vékony G, Pászti J, Damjanova I. Characterization of extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* strains isolated from animal and human clinical samples in Hungary in 2006-2007. Acta Microbiol Immunol Hung 2013;60:175-85.

16. Wieler LH, Ewers C, Guenther S, Walther B, Lübke-Becker A. Methicillin-resistant staphylococci (MRS) and extended-spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae in companion

animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. Int J Med Microbiol 2011;301:635-41.

17. Ben-Ami R, Schwaber MJ, Navon-Venezia S, Schwartz D, Giladi M, Chmelnitsky I, et al. Influx of extended-spectrum beta-lactamase-producing enterobacteriaceae into the hospital. Clin Infect Dis 2006;42:925-34.

18. Bert F, Larroque B, Paugam-Burtz C, Dondero F, Durand F, Marcon E, et al. Pretransplant fecal carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae and infection after liver transplant, France. Emerg Infect Dis 2012;18:908-16.

19. Harris AD, McGregor JC, Johnson JA, Strauss SM, Moore AC, Standiford HC, et al. Risk factors for colonization with extended-spectrum beta-lactamase-producing bacteria and intensive care unit admission. Emerg Infect Di*s* 2007;13:1144-9.

20. Doi Y, Park YS, Rivera JI, Adams-Haduch JM, Hingwe A, Sordillo EM, et al. Community-associated extended-spectrum β-lactamase-producing *Escherichia coli* infection in the United States. Clin Infect Dis 2013;56:641-8.

Table I. Comparison of the prevalence of ESBL and *bla*$_{CTX-M-15}$ in the present study with an earlier study from the same population in the same region [5]

	2009-10 [reference 5]	present study	probability (P)
Prevalence of ESBL in LTC	38/531(7.2%)	12/225 (5.3%)	NS
Prevalence of ESBL in employment screening group	22/1109 (2.0%)	18/779 (2.3%)	NS
Proportion of *bla*$_{CTX-M-15}$ producers among ESBL producers	34/63 (54.0%)	27/32 (84.4%)	0.003
Proportion of *bla*$_{CTX-M-15}$ producing *K. pneumoniae*	11/18	7/7	NS
Proportion of *bla* $_{CTX-M-15}$ producing *E. coli* in LTC group	16/21	6/7	NS
Proportion of *bla* $_{CTX-M-15}$ producing *E. coli* in healthy individuals	5/22	14/18	<0.001

LTC: Long-term care; NS: not significant