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Prevalence and genetic diversity of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* in nursing homes in Bavaria, Germany

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ABSTRACT

Main goal of this study was to determine the prevalence and molecular epidemiology of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae among 156 nursing home residents in Bavaria and to compare the results with healthy individuals from the Bavarian community. Intestinal colonisation by ESBL-producing *Escherichia coli* was detected in 23 nursing home residents (14.7%) using MacConkey agar supplemented with cefotaxime (1 mg/L) for screening and the combined disc method for ESBL confirmation. Antimicrobial susceptibility testing revealed co-resistance to ciprofloxacin in 86.9% of the ESBL-producers. All isolates harboured CTX-M-ESBL with CTX-M-15 (65.2%) and CTX-M-27 (21.7%) as the most common types. Moreover, 16 isolates (69.6%) could be assigned by PCR-typing to the epidemic clonal lineage *E. coli* O25b-ST131. Further typing by rep-PCR and XbaI-macrorestriction with subsequent pulsed-field gel electrophoresis, respectively, revealed that two or more residents shared the same ESBL-producing *E. coli* clone in four nursing homes. In conclusion, we could show a high prevalence of ESBL-producing *E. coli* in Bavarian nursing homes (14.7%) compared to the healthy population (6.3%). Although the prevalence of ESBL-type CTX-M-15 in *E. coli* was similar in nursing home residents (65.2%) and healthy individuals (46%) the presence of *E. coli* O25b-ST131 clones differed substantially (69.6% and 14.2%, respectively). Furthermore, this study demonstrates that a person-to-person transmission or a common source of infection for ESBL-producing microorganisms may occur in these facilities. Therefore, basic hygiene measures should be assiduously implemented to prevent the further spread of these multidrug-resistant bacteria.

1. Introduction

Fecal carriage of extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* has been reported from many regions of the world with high prevalence rates in India, China and Thailand (20–70%) but significantly lower rates in Europe (5–6%) (Geser et al., 2012; Lübbert et al., 2015; Nicolas-Chanoine et al., 2013). A recent study investigating healthy individuals ($n = 3344$) from Bavaria in Germany revealed that 6.3% are carriers of ESBL-producing *E. coli* (Valenza et al., 2014).

Little is known about the prevalence of these multidrug-resistant bacteria in particular populations such as nursing home residents. A recent study investigating 240 residents from 11 nursing homes in Hesse, Germany, revealed that 22 (9.2%) were carriers of ESBL-producing *E. coli*. Moreover, 45.8% of these ESBL-producing *E. coli* were assigned to the epidemic clonal lineage O25b-ST131, which represents worldwide the major cause of serious multidrug-resistant *E. coli* infections (Johnson et al., 2010). Interestingly, no association between colonisation and gender, previous antibiotic treatment or previous hospitalisation could be detected (Arvand et al., 2013).

Here we describe the results of determination of the prevalence and molecular characterisation of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae among 156 nursing

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home residents in Bavaria, Germany. Furthermore, we assessed the risk factors for ESBL carriage in nursing home residents.

2. Materials and methods

2.1. Study design

A total of 156 residents from 31 Bavarian nursing homes were included in the study. The above mentioned nursing homes were located in five different areas of Bavaria (Upper Bavaria, $n = 16$; Upper Palatinate, $n = 6$; Central Franconia, $n = 5$; Upper Franconia, $n = 2$; Lower Franconia, $n = 2$).

Sixty-nine percent of participants were female and the median age was 84.0 years (range, 47–103 years). A fecal sample for determination of ESBL carriage and a completed questionnaire for assessment of risk factors for ESBL carriage were collected for each subject between October 2013 and March 2014.

2.2. Phenotypic detection of ESBL-producing isolates and antimicrobial susceptibility testing

All 156 fecal samples were investigated for presence of ESBL-producing Enterobacteriaceae using MacConkey agar with cefotaxime (1 mg/L) as previously described (Valenza et al., 2014). All colonies with different phenotypes were identified to the species level using API E strips (bioMérieux, Nürtingen, Germany), and ESBL production was confirmed by the combined disc method (Mast Diagnostica, Rheinfeld, Germany) using cefotaxime and ceftazidime with and without clavulanic acid. Susceptibility testing for 16 antimicrobial substances (cefotaxime, ceftriaxone, ceftazidime, cefepime, cefpodoxime, cefoxitin, ertapenem, imipenem, aztreonam, amikacin, gentamicin, tetracycline, fosfomycin, ciprofloxacin, nalidixic acid and co-trimoxazole) was carried out by disk diffusion technique (Oxoid Ltd, Basingstoke, UK). Results of all antimicrobials tested were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (http://www.eucast.org/clinical_breakpoints) except for results of nalidixic acid, which were interpreted as recommended by the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2010).

2.3. Characterisation of resistance genes and bacterial strain typing

Relevant β-lactamase genes ($bla_{\text{TEM-like}}$, $bla_{\text{SHV-like}}$, $bla_{\text{CTX-M-1-2-9-group}}$) and plasmid mediated quinolone resistance (PMQR) genes (*qnrA, qnrB, qnrS, aac(6′) 1b-cr*) were identified by PCR and sequencing as previously described (Valenza et al., 2014; Eller et al., 2013; Gröbner et al., 2009).

Genetic diversity of all ESBL-producing *E. coli* was analysed by the semi-automated repetitive sequence-based (rep-PCR) DiversiLab® System (bioMérieux) and Pulsed field gel electrophoresis (PFGE) after restriction with XbaI enzyme with interpretation of genetic relationship according to the criteria of Tenover et al. (Tenover et al., 1995). Furthermore, determination of *E. coli* phylogenetic groups (Clermont et al., 2000) and allele-specific PCR for multilocus sequence type (ST) 131 (Clermont et al., 2008; Blanco et al., 2009) were also performed. To compare the typing results, 211 ESBL-producing *E. coli* from healthy individuals of a previous study in Bavaria (Valenza et al., 2014) were further investigated for presence of multilocus sequence type (ST) 131 by PCR.

2.4. Assessment of risk-factors for ESBL-carriage and statistical analyses

All subjects included in this study were asked to fill in a questionnaire on risk factors for ESBL carriage. The study questionnaire enquired the residents' age, gender, antibiotic treatment or hospitalisation in the previous 12 months, level of care, contact with domestic or production animals, meat consumption per week and sharing of toilet facilities with other residents.

Statistical analyses were performed with SPSS, version 21.0. Correlation between every single factor and ESBL carriage was analysed by chi-square test or Fisher's exact test. Furthermore, a stepwise logistic regression model was used.

3. Results

The presence of cefotaxime-resistant *Enterobacteriaceae* was detected in 31 of 156 nonreplicate fecal samples (*E. coli*, $n = 23$; *Enterobacter cloacae*, $n = 2$; one *Citrobacter koseri*, *Hafnia alvei*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Morganella morganii* and *Proteus mirabilis* each). Phenotypic ESBL production was confirmed in each cefotaxime-resistant *E. coli* isolate and excluded in all non *E. coli* Enterobacteriaceae isolates. Therefore, the rate of fecal carriage of ESBL-producing Enterobacteriaceae in the population of this study was 14.7%. Apart from cefotaxime resistance, the 23 ESBL-positive *E. coli* isolates were additionally resistant to further antibiotics, e.g. 86.9% of them were resistant to ciprofloxacin, 52.0% to trimethoprim-sulfamethoxazole and 13.0% to fosfomycin. In contrast, no isolate showed resistance to carbapenems.

Table 1
Comparison ESBL-producing *E. coli* isolates from nursing home residents ($n = 23$) and healthy individuals of the Bavarian community ($n = 211$).

Characteristics of the ESBL-producing *E. coli*	Rate (%) in the population of nursing home residents	Rate (%) in the population of healthy individuals[a]
Ciprofloxacin resistance	20/23 (86.9)	76/211 (36.0)
CTX-M-15	15/23 (65.2)	97/211 (46.0)
CTX-M-27	5/27 (21.7)	8/211 (3.8)
CTX-M-1	2/23 (8.7)	51/211 (24.2)
CTX-M-14	1/23 (4.3)	31/211 (14.7)
Phylogroup A	3/23 (13.0)	85/211 (40.3)
Phylogroup B2	18/23 (78.2)	31/211 (14.7)
O25b-ST131	16/23 (69.6)	30/211 (14.2)
O25b-ST131 + CTX-M-15	11/23 (47.8)	16/211 (7.6)
O25b-ST131 + CTX-M-15 + phylogroup B2 + ciprofloxacin resistance	11/23 (47.8)	10/211 (4.7)

[a] Results on ESBL-types and phylogenetic groups were published previously (Valenza et al., 2014)

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