MEDICAL SCIENCE MONITOR

EPIDEMIOLOGY

e-ISSN 1643-3750
© Med Sci Monit, 2013; 19: 317-326
DOI: 10.12659/MSM.883898

Received: 2012.09.18
Accepted: 2012.12.18
Published: 2013.05.01

Molecular characterization and drug resistance of *Escherichia coli* strains isolated from urine from long-term care facility residents in Cracow, Poland

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Source of support: This work was supported by a grant from the Ministry of Science and Higher Education (No. N N404 047236). The sponsor provided the funding for the project only

Background: The aim of this study was to assess the prevalence of multidrug-resistant *Escherichia coli* and extended-spectrum β-lactamases (ESBL) pathogens isolated from asymptomatic bacteriuria and urinary tract infections (UTIs), and the relationship between the phylogeny, antimicrobial resistance, and virulence among isolates in residents of 3 long-term care facilities (LTCF) in Krakow, Poland.

Material/Methods: This was point prevalence study and prospective infection control in a group of 217 people. Urine samples were examined with standard microbiological methods and screened for the presence of bla_{CTX-M}, $bla_{SHV,}$ and bla_{TEM}. *E. coli* isolates were screened for 6 common virulence factors (VFs) and classified according to the rapid phylogenetic grouping technique.

Results: Among all the strains tested, 14 isolates (13.9%) expressed ESBL activity. A significant proportion of isolates were resistant to ciprofloxacin (32.7%, n=33). Resistance to trimethoprim/sulfamethoxazole was identified among 45 isolates (44.5%). Independent risk factors for the presence of an ESBL-producing strain were: UTI, urinary and/or fecal incontinence, bedridden, and low values of the Barthel and Katz Indexes. Gene sequencing identified 8 $bla_{CTX-M-15}$, 1 $bla_{CTX-M-3}$, 9 $bla_{TEM-1,}$ and 1 bla_{SHV-12}. Among *E. coli*, no relationship between number of VF genes and phylogeny was found. The most prevalent virulence factor was *fimH* (82.1%).

Conclusions: The findings of this study emphasize the need for further research on the epidemiology of multi-drug resistant organisms (MDRO) and ESBL in LTCF, including transmission patterns, rates of infection, and factors associated with infections. It may be necessary to extend the requirements and precautions to MDRO and ESBL-producers.

Key words: long-term care • extended-spectrum β-lactamase (ESBL) • *Escherichia coli* • urinary tract infections • bacterial virulence factors

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Background

The high incidence of infections and high rates of hospitalization, together with institutionalized long-term care (LTC), highlights the important role of management to the decrease antibacterial susceptibility of organisms cultured from elderly patients. The reservoir for these microorganisms may be the bacterial flora in patients receiving long-term antimicrobial therapy or health care personnel who are colonized with resistant strains or infected with the same pathogens. Multidrug-resistant gram-negative rods (MDR-GNR) are rapidly spreading throughout LTCFs around the world, and antibiotics are also commonly prescribed for older adults residing in LTCFs. There are a number of risks associated with the inappropriate use of antibiotics. There is risk of development of multi-drug antibiotic resistance after exposure [1,2] and risk of drug-related adverse effects. Furthermore, the increased use of antibiotics in LTCFs results in significant costs [3]. The rapid spread of antimicrobial-resistant bacteria through health care institutions is considered a serious medical and public health issue [4]. The intensive use of antibiotics promotes a relatively high prevalence of MDR-GNR and pathogens able to produce extended-spectrum β-lactamases (ESBL) in LTCFs [5,6]. LTCFs have older populations with increased prevalence of resistant bacteria isolated from urinary tract infections (UTI). Resistant isolates are more frequent in long-term care populations than in the general population [5,6]. Preceding colonization with such microorganisms is a risk factor for the development of infection, but the presence of virulence factors in the pathogens is equally important. A wide range of virulence factors (VFs) and a persistence of multidrug resistance can make the treatment of infections challenging. VFs for *Escherichia coli* are widely known. They are essential for the interaction between *E. coli* and its host, as they facilitate colonization, proliferation, and the transition from uncomplicated to severe infections. Knowledge of which VFs are prevalent in specific clinical situations and populations is important for the targeted prevention of *E. coli* infections, as revealed by other epidemiological studies [7–9]. Virulence and resistance may influence the outcome of infections. Older populations in long-term care facilities have an increased prevalence of resistant bacteria isolated from UTIs.

Until now, the prevalence of ESBL-positive strains and drug resistance among LTCF-residents in Poland has not been studied. For this reason, the aim of this study was to assess the prevalence of MDR-GNR and ESBL pathogens isolated from asymptomatic bacteriuria and UTIs. The second purpose of this study was to look for a relationship between the phylogeny, antimicrobial resistance, and virulence among *E. coli* isolates from bacteriuria.

There have been no epidemiological descriptions of the elderly population in Poland. This has resulted in an inability to design feasible infection prevention programs. The proposed methods will be the basis for the development of recommendations for empirical treatment of LTCF residents. The point prevalence study was conducted for cases of asymptomatic bacteriuria and a continuing study is being conducted to measure the rate of infections with ESBL etiology.

Material and Methods

Part I. Point prevalence study, PPS

A 1-day prevalence survey (point prevalence study [PPS]) was carried out in October 2009 in 3 LTCFs in Krakow, Poland: 2 residential homes and 1 nursing home. A resident was defined as a person staying in an LTCF during the study day for at least 48 h. A control patient was a person staying in home care (HC), but requiring home visits of a physician. In a nursing home (NH), residents need 24 h/day medical or skilled nurse supervision and intensive health care is provided. In the residential home (RH), residents are unable to live independently and require supervision or assistance with activities of daily living. The study protocol was approved by the Ethics Committee of Jagiellonian University (KBET/57/B/2008), conducted in accordance with the Declaration of Helsinki, and carefully explained to the participants, who then gave their written informed consent. Medical charts were reviewed for patient demographic data, comorbidities, and residency in an LTCF. Medical documentation of residents was analyzed for the presence of chronic diseases and other medical problems. Barthel Index values and Katz Index were obtained from all studied participants. The Barthel Index (BI) is a 10-item measure of disability based on daily activities (e.g., bathing, transfer, dressing, feeding, mobility, stairs, using the toilet, and grooming) [10]. The score corresponds to the sum of all the points obtained, and can range from 0 to 100 points. Lower scores are associated with worse functional status – elderly patients with scores from 0 to 20 are considered to be totally dependent [10]. The Katz Index of Independence in Activities of Daily Living is an instrument used to assess functional status as a measurement of the patient's ability to perform activities of daily living independently [11]. The Index ranks the adequacy of performance in 6 functions: bathing, dressing, toileting, transferring, continence, and feeding. Patients are scored for independence in each of these functions. A score of 6 indicates full function, 4 indicates moderate impairment, and 2 or less indicates severe functional impairment [11]. Physical dependence (mobility) was classified according to a 5-point scale (1 – independent, 2 – independent with falls, 3 – limitations in mobility, 4 – confined to bed, self-changing body position, 5 – confined to bed, dependent). Data on hospital exposure longer than 7 days in the 3 months preceding enrollment and antibiotics taken in the last month were collected. Infections were defined

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according to the McGeer criteria and were detected by trained health personnel in cooperation with a project worker [12].

Part II. Continuing study

Continuing prospective infection control was performed between 1 December 2009 and 30 November 2010, using the standard McGeer definition protocol [12]. Cases of infections were registered by a nurse and accompanying physician. Urine samples were taken only from residents when the symptoms of infections occurred. In the period between enrollment and each follow-up, data on potential factors that would increase the likelihood of MDRO acquisition were also collected (ie, antibiotic and hospital exposure, and presence of infections).

The relationship between types of care, socio-demographic characteristics, probability, and epidemiology of ESBL were analyzed with 2 main groups of statistical techniques. If the numerical parameters (e.g., age, length of stay) were compared with the nominal characteristics (e.g., type of care, form of infection), a simple ANOVA test was used. If the distribution of numerical characteristics did not fit the normal distribution, the nonparametric alternative to ANOVA, the Kruskal-Wallis test, was used. Chi-square (χ^2) frequency tests and a likelihood ratio were used for the contingency of nominal characteristics. *P* values <0.05 were considered statistically significant. All analyses were performed using the SAS JMP 7.01 package (JMP®, Version 7. SAS Institute Inc., Cary, NC, 1989–2007).

Detailed information about epidemiology of infections in the point prevalence study and the continuing study among included LTCFs has been described [13].

Bacterial isolates

Samples of urine were routinely collected (clean-catch method) and cultured by standard quantitative methods at the beginning of the study (PPS). When symptoms of UTI in any enrolled resident occurred throughout the study (continuing study), urine samples were also taken. Isolates were identified using polymerase chain reaction (PCR) with species-specific primers [14,15] or phenotypic methods (API ID 32E,BioMerieux). Strains belonging to the Enterobacteriaceae family were further tested.

Antimicrobial susceptibility

All strains belonging to the Enterobacteriaceae family were tested using disk diffusion antimicrobial susceptibility methods on Mueller-Hinton agar plates according to current guidelines of the European Committee on Antimicrobial Susceptibility Testing (Clinical breakpoints tables v. 1.3; *http://www.eucast.org* v.1.3). Antibiotics used in this study belonged to 6 chemical groups. Antimicrobials included ampicillin (AMP – 10 µg), piperacillin/tazobactam (TZP – 30 µg/6 µg), ceftazidime (CAZ – 10 µg), cefotaxime (CTX – 5 µg), cefepime (FEP – 30 µg), cefuroxime (30 µg)), imipenem (IPM – 10 µg), aztreonam (ATM – 30 µg), ciprofloxacin (CIP – 5 µg), gentamicin (CN – 10 µg), trimethoprim/sulfamethoxazole (SXT – 25 µg), and nitrofurantoin (F – 100 µg).

ESBL activity was detected with a modified double-disk synergy test (DDST) using a combination of cefotaxime (5 µg), ceftazidime (10 µg), cefepime (30 µg), and aztreonam (30 µg) disks, placed 20 mm apart around a disk containing amoxicillin-clavulanate (20 µg/10 µg) [16]. Enhancement of the inhibition zone toward the amoxicillin-clavulanate disks was taken as presumptive evidence of ESBL production. All disks were obtained from Oxoid, Ltd. (Basingstoke, UK). Each isolate was classified as susceptible, intermediate, or resistant using the European Committee on Antimicrobial Susceptibility Testing breakpoints. Criteria for multidrug resistance were defined as resistance or intermediate resistance to 3 or more antimicrobial groups (co-resistance) or 4 or more of the antimicrobials [17].

Polymerase chain reaction (PCR) screening for extended-spectrum β-lactamase genes and sequencing of bla genes

All isolates with ESBL activity (according to DDST) were screened with multiplex-PCR for the presence of bla_{CTX-M}, $bla_{SHV,}$ and bla_{TEM} genes using previously published primers [18]. Bacterial DNA was extracted by the boiling method (10 minutes at 100°C). Relevant positive and negative controls were included in each PCR run. The reaction mixture (25 µl) was amplified using the following conditions: 15 min at 95°C, 30 cycles of 30 s at 94°C, 30 s at 55°C, and 2 min at 72°C, with a final extension of 10 min at 72°C. The amplicon sizes were 445, 593, and 747 bp for bla_{TEM}, bla_{CTX-M}, and bla_{SHV}, respectively. Bands were visualized using the UVP GelDocIT Imaging System after 1.5% TAE-agarose electrophoresis (90 min, 100 mV) with ethidium bromide (BioRad). GeneRuler DNA-ladder 100bp (Fermentas) was used as a size marker. The strains with PCR amplicons positive for any of the previously described *bla* were selected for sequencing. PCR products were purified using a NucleoSpin extract II kit (Macherey-Nagel). Sequencing was performed by an outsourced company (Genomed, Warsaw, Poland). The nucleotide sequences were analyzed with the software available at the National Center of Biotechnology Information (*http://blast.ncbi.nlm.nih.gov/Blast.cgi*).

Virulence factor screening

E. coli isolates from bacteriuria were checked for the presence of selected virulence genes usually associated with the *E. coli* strains responsible for extraintestinal infections: *pap*C and *pap*EF (P fimbriae), *fim*H (type 1 pili), *hly*A (hemolysin), *iut*A (aerobactin), and *sfa* (S and F1C fimbriae) using PCR with previously

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Table 1. Characteristics of studied population at baseline.

Type of care / Characteristics of studied population	Home care N=24 [no/%]		Residential homes N=86 [no/%]		Nursing homes N=107 [no/%]		p-value
Male residents	6	25.0	36	41.9	35	32.7	–
Female residents	18	75.0	50	58.1	72	67.3	
Residents recently hospitalized	6	25.0	20	23.3	8	7.5	<0.001
Urinary incontinence: diapers	6	25.0	23	26.7	39	36.4	<0.001
Permanent catheterization into the bladder	1	4.2	0	0.0	39	36.4	
Stool incontinence	4	16.7	6	7.0	67	62.6	<0.001
Dysphagia	0	0.0	4	4.7	26	24.3	<0.001
Stomach probe	0	0.0	0	0.0	26	24.3	<0.001
Tracheostomy tube	0	0.0	1	1.2	4	3.7	–

published primers [7]. Each VF gene was amplified in a total volume of 25 µl containing 2× Master Mix (A&A Biotechnology, Poland), 0.5 µM primer and DNA. A negative control (containing the same mixture, but with water instead of DNA) was included in each PCR run. The amplification products were separated by electrophoresis in 1.5% agarose gel and visualized using the UVP GelDocIT Imaging System. GeneRuler DNA-ladder 100bp (Fermentas) was used as a size marker. The results were considered positive if the amplification product was of the expected molecular size (*pap*C – 200 bp, *pap*EF – 326bp, *fim*H – 508bp, *hly*A – 1177bp, *iut*A – 300bp, and *sfa* – 410bp). The strains were classified according to the *Escherichia coli* Reference Collection (ECOR) system by the use of the rapid phylogenetic grouping technique described by Clermont et al. [19]. This method is based on a multiplex PCR involving the amplification of 2 genes (*chu*A and *yja*A) and of an anonymous fragment of DNA from *E. coli* (TspE4C2). The results are interpreting as follows: *chu*A and *yja*A positive indicated group B2; *chu*A positive and *yja*A negative indicated group D; *chu*A negative and TspE4C2 positive indicated group B1; and *chu*A negative and TspE4C2 negative indicated group A. Differences were tested using the χ^2 test.

Results

The study was conducted in a group of 217 persons (193 residents of LTCFs and 24 from HC as a control group). Of these residents, 86 (39.6%) stayed at RHs, and 107 stayed at NHs. The studied sample corresponded to 2.6% of the total LTCF population in Poland in 2010 [20]. Among the 217 patients participating in this research, 1 patient quit being in a LTCF, 1 was moved to another facility, and 31 patients died during the study.

The population studied was heterogeneous and significantly diverse with regards to: incidence of hospitalization before study, body weight, problems with maintaining personal hygiene (expressed as urinary/stool incontinence and the Katz Index), and expressed necessity of care (Barthel Index, Table 1). The mean value of the Barthel Index was 75.4 in HC (SD: 27.7, 95% CI: 63.7–87.1), 75.6 in RH (SD: 34.5, 95% CI: 68.2–83.0), and 19.5 in NHs (SD: 17.4, 95% CI: 16.1–22.9). In NHs none of the residents had BI higher than 76 points, and 66.7% of HC patients and 69.8% of RH residents had scores above 76 points. The mean value of Katz Index was 4.4 for HC patients (SD: 2.2, 95% CI: 3.4–5.3), 4.7 for RH residents (SD: 2.1, 95% CI: 4.2–5.1), and 1.3 for NH residents (SD: 1.6, 95% CI: 0.9–1.6). Considerably older patients (aged ≥80) stayed in HC, while younger (age <80) patients were more likely to be residents of RHs. The average age for the population (63.2% female) was 76.2 years in RH (standard deviation SD: 10.5, 95% CI: 73.9–78.4), 76.8 years in NHs (SD: 11.1, 95% CI: 74.6–78.9), and 82.9 in HC (SD: 9.9, 95% CI: 78.7–87.0). The average length of LTCF stay was 6.5 years (SD 5.9; 95%CI 4.1–5.9). Differences were also observed in the physical activity of residents – NH residents were more likely to have limited ability to walk independently. The mean mobility was 2.4 for HC patients (SD: 1.6, 95% CI: 1.7–3.1), 1.9 for RH residents (SD: 1.2, 95% CI: 1.7–2.2), and 3.7 for NH residents (SD: 1.1, 95% CI: 3.5–3.9). Independent mobility (with no restrictions) was characteristic for 12 (50.0%) HC patients, 48 (55.8%) RH residents, and 5 (4.7%) NH residents. There were 5 (20.8%) HC residents who were confined to bed and dependent, 4 (4.7%) in RH, and 33 (30.8%) in NHs.

The general prevalence rate of infections was 22.1/100 residents and general incidence density rate was 2.7/1000 residents per day for both groups (residents and home care patients). The general prevalence rate of ESBL was 4.6/100 in residents with bacteriuria and 0.0/100 in the control group. The incidence density of ESBL-producing isolates was 0.3/1000 residents per day (only in NHs and RH). Of the residents, 22 were taking antibiotics in the month preceding PPS.

Gram-negative rods were identified in 101 cultures of urine: 9 from UTIs and 92 from asymptomatic bacteriuria. Bacteriuria

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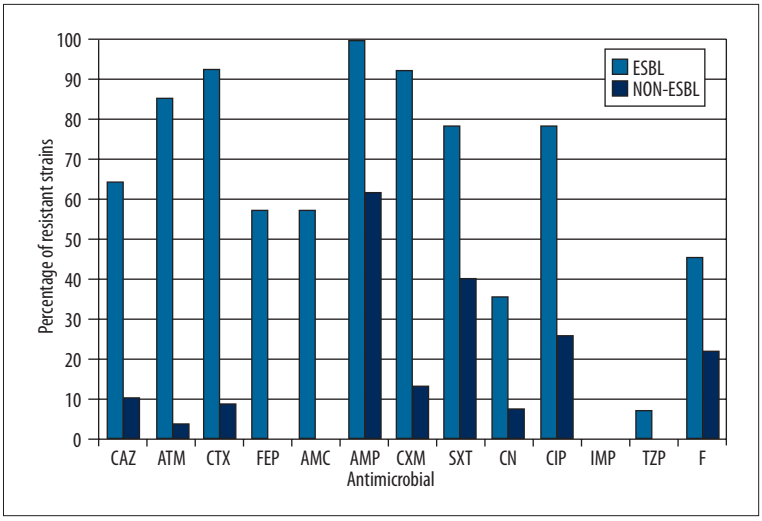

Figure 1. Plot showing % of strains resistant to tested antibiotics according to ESBL activity. (AMP – ampicillin, TZP – piperacillin/tazobactam, CAZ – ceftazidime, CTX – cefotaxime, FEP – cefepime, CXM – cefuroxime, ATM – aztreonam, IPM – imipenem, CN – gentamicin, CIP – ciprofloxacin, SXT – trimethoprim/sulfamethoxazole, F – nitrofurantoin).

was found in 82 (42.5%) residents. The most commonly isolated pathogen was *E. coli* (58.4%, n=59, including infections). Other isolated species included: *Klebsiella pneumonia* (13.9%, n=14), *Proteus* spp. (12.9%, n=13) and others. During the PPS, 1 case of infection was registered.

Accordingly, 60 isolates were ampicillin resistant (58.7% from bacteriuria and 75.0% from UTI) (Figure 1.). Aminoglycoside resistance was present in 11 isolates (10.9%). Resistance to trimethoprim/sulfamethoxazole was identified in 45 isolates (43.5% from bacteriuria and 71.4% UTI). A significant proportion of isolates were resistant to fluoroquinolones, and 33 isolates were resistant to ciprofloxacin (32.7%). The prevalence of pathogens resistant to more than 4 antimicrobials was 16.3% among asymptomatic bacteriuria samples and 33.3% among UTIs. The most common co-resistance pattern was gentamycin and trimethoprim/sulfamethoxazole, present among 9.1% of isolates.

Of the isolates, 14 had ESBL activity (13.9%). ESBL was most prevalent in *E. coli* (n=6) and *Klebsiella* spp. (n=6), but there was 1 *Citrobacter* spp. and 1 *Enterobacter aerogenes* that were also ESBL-positive. There were no ESBL-positive strains among *Proteus* spp., *Morganella morgani, Serratia* spp., or *Providencia* spp. Asymptomatic bacteriuria was not a risk factor for the occurrence of an ESBL-positive strain (p=0.09). Among residents who were confined to bed, the frequency of isolation of ESBL-positive strains was 3 times higher than in residents able to walk independently. The factors independently associated with ESBL were urinary tract infections (OR 5.4, *P*=0.0012), urinary and/or fecal incontinence (OR 13.5, *P*<0.001), being wheelchair-dependent or being confined to bed (OR 6.2, *P*<0.001), low value on the Barthel (OR 3.6, *P*<0.001) and Katz Indexes (OR 2.8, *P*<0.015), and prior hospitalization (OR 3.1, *P*<0.045) (Table 2). The mortality of residents with ESBL-positive strains was significantly higher (OR 4.1, *P*<0.001). Any antibiotic used in the preceding month also remained a significant risk factor (OR 3.2, *P*=0.03) associated with ESBLs.

From the group of LTCF resident isolates, PCR experiments performed on bacterial DNA with primers specific for 3 *bla* genes showed that bla_{CTX-M} was present in 9 strains (75.0%), bla_{TEM} in 9 strains (75.0%), and bla_{SHV} in 1 (7.1%). Among the isolates, 9 (64.3%) were characterized by the presence of a single β-lactamase gene, whereas 5 (35.7%) had a combination of 2 different *bla* genes (bla_{CTX-M} and bla_{TEM}). Furthermore, sequencing of the PCR products revealed the presence of 8 $bla_{CTX-M-15}$ (92.9%) and 1 $bla_{CTX-M-3}$ (7.1%). The only $bla_{CTX-M-3}$ β-lactamase was identified in *Klebsiella oxytoca,* in a sample taken from a case of asymptomatic bacteriuria. All bla_{TEM} were identified as bla_{TEM-1} and 1 bla_{SHV} as bla_{SHV-12}.

Among *E. coli* isolates, 13 clustered in the ECOR group A, 2 clustered in the B1 group, 31 in the B2 group, and 10 in the D group (Table 3). Of these, 4 ESBL-positive strains belonged to the B2 group, 1 to the D group, and 1 to the A group. VF genes were detected in strains from all of the ECOR groups, and no relationship between the number of VF genes and the ECOR group was found. The most prevalent virulence factor was *fim*H, which occurred in 46 isolates (82.1%). Less frequent was *iut*A, which was found in 32 samples (57.1%). The potential ability of hemolysin production (expressed by the presence of *hly*A gene) was characteristic for 4 strains (7.1%) and 3 of these belonged to the B2 group. The *pap*C gene was found in 16 isolates (28.6%), *pap*EF in 6 (10.7%), and *sfa/foc* was found in 13 (23.2%) strains. *E. coli* able to produce ESBL possessed up to 2 different virulence factors (median: 1.5, range: 0–2), whereas non-ESBL *E. coli* possessed up to 6 (median: 2, range: 0–6). Isolates belonging to the B2 group were more likely to be resistant to fluoroquinolones than those from the A or D group (41.9%, 23.1%, and 30.0%, respectively).

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Table 2. Risk factors for ESBL presence in Long-Term Care Facilities (LTCFs).

Type of care/Characteristics of studied population	ESBL* residents [no/%]		Non-ESBL residents [no/%]		p-value
Male	3	20.0	74	34.1	–
Female	10	80.0	130	65.9	
Type of care	15	100.0	202	93.1	–
RH**	4	26.7	82	37.8	–
NH***	10	66.7	97	44.7	
Infection treated in a hospital	3	20.0	17	7.8	–
Death with infection	5	33.3	11	5.1	<0.001
Hospitalisation before enrollment	5	33.3	28	12.9	0.045
Urinary incontinence: pampers	10	66.7	58	26.7	<0.001
Urinary incontinence: catherization	5	33.3	35	16.1	
Stool incontinence	11	73.3	66	30.4	<0.001
Dysphagia	5	33.3	25	11.5	0.023
Stomach probe	5	33.3	21	9.7	0.008
Tracheotomy tube	2	13.3	3	1.4	0.003
Barthel Index					
0	6	40.0	27	12.4	
1–15	3	20.0	33	15.2	
16–50	3	20.0	54	24.9	0.001
51–75	3	20.0	9	4.1	
76–100	0	0.0	76	35.0	
Katz Index					
0–1	11	73.3	80	36.9	
2–4	3	20.0	30	13.8	0.015
5–6	1	6.7	87	40.1	
Mobility					
Independent, with no restrictions – 1	0	0.0	65	30.0	
Independent, with no restrictions, repeated falls – 2	2	13.3	8	3.7	
Limitation in mobility – 3	3	20.0	74	34.1	<0.001
Lying, self-changing body position – 4	3	20.0	18	8.3	
Lying, dependent – 5	7	46.7	35	16.1	

* ESBL – extended-spectrum β-lactamase; ** RH – residential home; *** NH – nursing home.

Discussion

The presented results are derived from the first Polish study concerning urinary tract infections among long-term care facility residents. The study was performed using 2 independent approaches: a prevalence study (PPS) and an incidence study. These kinds of research programs are carried out in Europe and worldwide [21]

The population of Polish NH and RH residents was slightly different from the population of institutionalized elderly in other countries. The mean age of such residents in Italy is 81 years [22] and 83 in Germany [23]. In Norwegian LTCFs, more that 78% of residents are 81 years or older [24]. The average age of Polish residents was about 5 years younger. Furthermore, the participation of residents confined to bed and residents with low Barthel and Katz Index scores was relatively lower compared to Norwegian or Italian surveys [25]. The use of invasive diagnostic and therapeutic procedures (e.g., catheterization) was also lower in this study than in Italy (18.4% *vs.* 60.6%). The published literature on the epidemiology of ESBLs in LTCFs is not conclusive; for instance, the incidence density of ESBL-positive *Enterobacteriaceae* in our study was not comparable with data from a Swiss tertiary care

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Table 3. *Escherichia coli* isolates used and described during this study – characteristics.

Isolate	β-lactamase(s)	Resistance profile*	ECOR**	Virulence factors
384	CTX-M-15, TEM-1	CAZ ATM CTX FEP AMC AMP CXM SXT CN CIP	B2	*fimH iutA*
369	CTX-M-15	CAZ ATM CTX FEP AMC AMP CXM SXT CIP	B2	*fimH*
359	CTX-M-15, TEM-1	CAZ ATM CTX FEP AMP CXM CN CIP TZP	B2	
79	TEM-1	CAZ ATM CTX AMP CXM CN CIP	A	*fimH*
61	TEM-1	ATM CTX AMP CXM SXT CIP F	B2	*fimH iutA*
71	TEM-1	ATM CTX AMC AMP CXM SXT	D	*fimH iutA*
82		CTX AMP CXM SXT CIP F	B2	*fimH iutA*
375-1		CTX AMP CXM SXT CIP	B2	*fimH iutA*
81		CTX AMP CXM SXT CIP	B2	*fimH iutA*
208		CTX AMP CXM SXT CIP	B2	*fimH iutA*
363		AMP SXT CN CIP	D	*iutA*
76		CAZ AMP SXT	A	*fimH iutA*
77		AMP SXT CN	D	*fimH iutA papC*
355		AMP SXT CIP	B2	*fimH*
362		AMP SXT CIP	B2	*fimH iutA*
386-2		AMP SXT CIP	A	*fimH iutA papEF sfa*
78		AMP CIP	B2	*fimH iutA*
213		AMP SXT	B2	*fimH iutA sfa*
214		AMP SXT	B2	*fimH iutA sfa*
205		AMP SXT	D	*fimH*
284		SXT CIP	D	*fimH iutA*
372		AMP SXT	D	
375-2		SXT CIP	B2	*sfa*
376		AMP CIP	B2	
381		AMP SXT	D	
403		ATM AMP	B2	*fimH iutA sfa*
417		AMP CIP	B2	*fimH iutA papC*
660		AMP CIP	?	
285-2		AMP	B2	*fimH iutA papC papEF*
290		AMP	B2	*fimH papC papEF*
298		AMP	B2	*fimH iutA*
74		SXT	A	*fimH iutA papC*
75		CIP	B2	*fimH iutA*
63		AMP	A	*fimH iutA*
60		AMP	A	*hlyA fimH papC sfa*

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Table 3 continued. *Escherichia coli* isolates used and described during this study – characteristics.

Isolate	β-lactamase(s)	Resistance profile*	ECOR**	Virulence factors
270		F	B2	*hlyA fimH papC sfa*
271		CIP	AA	*fimH*
278		AMP	B2	*fimH papC*
374		AMP	B2	*fimH*
405		AMP	B2	
406		AMP	B1	*iutA*
437		CAZ	D	*fimH iutA sfa*
667		AMP – LACK OF DATA***	?	
423			B2	*fimH iutA*
430			A	*fimH*
441-2			B2	*fimH papC papEF sfa*
633				
62			D	*fimH iutA papC*
70			A	*fimH iutA papC*
73			A	*fimH iutA papC*
88			B2	*hlyA fimH papC sfa*
281			B2	*fimH papC*
282			A	
287-2			A	*fimH*
292			B2	*hlyA fimH iutA papEF sfa*
297			A	*fimH iutA*
352			B2	*iutA sfa*
353			B1	*fimH papC papEF sfa*
357			D	*fimH*

* Antimicrobials: AMP – ampicillin; TZP – piperacillin/tazobactam; CAZ – ceftazidime; CTX – cefotaxime; FEP – cefepime; CXM – cefuroxime; ATM – aztreonam; IPM – imipenem; CN – gentamicin; CIP – ciprofloxacin; SXT – trimethoprim/sulfamethoxazole; F – nitrofurantoin. ** The strains were classified according to the ECOR system by the use of the rapid phylogenetic grouping technique described by Clermont et al. (Clermont 2000). This method is based on a multiplex PCR involving the amplification of two genes (chuA and yjaA) and of an anonymous fragment of DNA from E. coli (TspE4C2). the results are interpreting as follows: chuA and yjaA positive: group B2; chuA positive and yjaA negative: group D; chuA negative and TspE4C2 positive: group B1; chuA negative and TspE4C2 negative – group A. *** It was impossible to grow the strain after freezing.

hospital (0.7/1000 patients per day) [26]. This suggests the need for further multicentre studies involving a larger number of residents over the spread of the studied group of microorganisms.

The most commonly isolated pathogen was *E. coli* (58.4%, n=59, including infections), similarly to other studies, for hospital patients, patients with CAUTI, and outpatients [27].

MDRO are a serious problem in health care institutions such as hospitals and LTCFs. Resistance determined by ESBL-synthesis is one of the most important resistance mechanisms. Patients colonized with ESBL-positive strains and MDRO are considered as reservoirs and are potential sources of infection. It is important to note that colonization often precedes an infection, and about one-fourth of colonized residents may develop an

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infection with the same factor [17,28]. The prevalence of bacteriuria in residents without chronic long-term indwelling catheters reaches 25–50% for women and 15–40% for men [5], and approximately 40% of nosocomial infections originate in the urinary tract [29]. In this study, bacteriuria was found in 42.5% of residents, and infection developed in 4.6%.

In this study, residents infected with ESBL-positive strains were 2.6 times more frequently transferred from LTCFs to hospitals and treated under hospital conditions. Their attendance at hospital units requires special rules for isolation, common for patients with MDROs (contact isolation). The genes responsible for drug resistance are frequently located on plasmids, which contributes to their fast spread [30]. This study identified a relatively high prevalence of ESBL-positive strains among residents of LTCFs. Another Polish survey, conducted in 13 Polish hospitals, found a 11.1% prevalence of ESBL-positive pathogens [31], which is consistent with our study. The identification of ESBL types revealed the very common occurrence of CTX-M-15 β-lactamase, as far as TEM-1, whereas SHV appeared rarely (only 1 case). The rapid increase in the prevalence of CTX-M producers cannot be questioned and it is the most prevalent ESBL in other countries [32].

Predictors of ESBL-positive strains found by other authors include permanent urinary catheter, pressure sores, and any recent invasive procedures [33,34]. Other strong risk factors that increased the likelihood of ESBL, like being wheelchair-dependent or confined to bed, and low scores on the Barthel or Katz Indexes, have not yet been investigated. Our studies indicate the significance of this element in a long-term care.

Resistance to gentamicin and trimethoprim/sulfamethoxazole frequently occurs among ESBL-positive isolates [17,30,35–37]. Our study confirmed that 35.7% of ESBL-positive strains were resistant to both of these antibiotics. The majority of the studied isolates were resistant to commonly prescribed antibiotics, including ampicillin, trimethoprim/sulfamethoxazole, and ciprofloxacin. This data raises serious concern about the therapeutic options available for physicians treating LTCF residents. The prevalence of pathogens resistant to more than 4 antimicrobials was 16.2%, which is less than in the O'Fallon report from Boston LTCFs [17]. Resistance to piperacillin/tazobactam, which are counted as broad-spectrum antimicrobials, was very low (1.4%). Piperacillin-tazobactam was the most active agent for out-patient urinary isolates in Turkey [37].

It has been shown that the number of VFs is proportional to its pathogenic potential [38]. FimH protein recognizes its receptor on uroplakin, which is disseminated on the surface of uroepithelium [9]. It acts as an adhesin but also as an invasin, because it is necessary to initiate the response signalling pathways of the host, leading to internalization of *E. coli* [9,39]. Its high frequency shows that it is important in the early stage of urinary tract infection [9]. The prevalence of the *fimH* gene, which is an adhesin important in the early phase of UTI, was lower than in studies conducted by Narciso et al. [9]. P fimbriae are the second common virulence factor of uropathogenic *Escherichia coli*, which plays an important role in the pathogenesis of ascending UTIs and pyelonephritis in humans [40,41]. In our study we show that *E. coli* isolates from bacteriuria are very often equipped in such fimbriae, as the *papC* gene was found in 28.6% isolates. S fimbriae and F1C fimbriae are implicated in the process of UTI as they show binding to epithelial and endothelial cells [40,42]. The S fimbriae are also associated with *E. coli* strains that cause sepsis, meningitis, and ascending UTIs [40]. *Sfa/foc* was found in 23.2% of isolates, which is also a large proportion of the isolates. The reason for the lower prevalence of *hlyA* in this population than in uroseptic strains could be that those strains came from asymptomatic bacteriuria cases [7]. The studied strains did not possess high levels of virulence, as the selection may favor strains of intermediate resistance.

Conclusions

This study shows that the prevalence of ESBL-producing rods isolated from urine samples from LTCF residents was lower then in other similar studies published recently. The findings derived from this study emphasize the need for further research on epidemiology of MDRO and ESBL in LTCFs, including transmission patterns, rates of infection, and factors associated with infections. It may be necessary to extend the requirements and precautions to MDR-GNR and ESBL-producers. Several potential limitations should be considered in the interpretation of data presented here. First, the number of patients included was very low (only 193 residents from 3 LTCFs). This was due to mistrust on the part of residents (who had to agree to participate) and the insufficiently enthusiastic attitude of personnel. Therefore, the number of isolates was relatively low. The control group was also very small, as these patients also had to agree to participate in the study. However, this is the first Polish surveillance conducted in LTCFs, and further research should be done because it might benefit physicians in Poland in the treatment of LTCF patients. In addition, to develop recommendations for treatment, an extended study is recommended.

Acknowledgements

We would like to thank to all the physicians and nurses from the LTCFs who agreed to participate in this research. We would like to thank to Ms. Anna Kumorek, MSc, for her support in some statistical data analysis.

The work presented here was carried out in collaboration between all authors. All authors have seen and approved the manuscript.

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Partial results shown in this publication were presented at 2 conferences in as a poster (Eurobiotech, Cracow 2011 and VI International Scientific Conference for Students and PhD Students "Youth and Progress of Biology", Lviv 2010).

References:

1. Gaynes R, Weinstein RA, Chamberlain W, Kabins SA: Antibiotic resistant flora in nursing home patients admitted to the hospital. Arch Intern Med, 1985; 145: 1804–7
2. Muder RR, Brennen C, Drenning SD et al: Multiply antibiotic-resist gram-negative bacilli in a long-term-care facility: a case-control study of patient risk factors and prior antibiotic use. Infect Control Hosp Epidemiol, 1997; 18: 809–11
3. Loeb M, Brazil K, Lohfeld K et al: Optimizing antibiotics in residents of nursing homes: protocol of a randomized trial. BMC Health Serv Res, 2002; 2: 17
4. Spellberg B, Guidos R, Gilbert D et al: The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Clin Infect Dis, 2008; 46: 155–64
5. Nicolle LE: Urinary infections in the elderly: symptomatic or asymptomatic? Int J Antimicrob Agents, 1999; 11: 265–68
6. Nicolle LE: Resistant pathogens in urinary tract infections. J Am Geriatr Soc, 2002; 50: 230–35
7. Johnson JR, Stell AL: Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis, 2000; 181(1): 261–72
8. Johnson JR: Epidemiological considerations in studies of adherence. In: Doyle RJ, Ofek I (ed), Adhesion of microbial pathogens: methods in enzymology. Orlando, FL: Academic Press, 1995; Vol 253: 167–78
9. Narciso A, Nunes F, Amores T et al: Persistence of uropathogenic *Escherichia coli* strains in the host for long periods of time: relationship between phylogenetic groups and virulence factors. Eur J Clin Microbiol Infect Dis, 2012; 31: 1211–17
10. Mahoney FI, Barthel DW: Functional evaluation: the Barthel Index. Md State Med J, 1965; 14: 61–65
11. Wallace M, Shelkey M: Hartford Institute for Geriatric Nursing. Katz Index of Independence in Activities of Daily Living (ADL). Urol Nurs, 2007; 27: 93–94
12. McGeer A, Campbell B, Emori TG et al: Definitions of Infection for Surveillance in Long-term Care Facilities. Am J Infect Control, 1991; 19: 1–7
13. Wójkowska-Mach J, Gryglewska B, Czekaj J et al: Infection control: point prevalence study versus incidence study in Polish long-term care facilities in 2009-2010 in the Małopolska Region. Infection, 2013; 41(1):1–8
14. Liu Y, Liu C, Zheng W et al: PCR detection of *Klebsiella pneumoniae* in infant formula based on 16S–23S internal transcribed spacer. Int J Food Microbiol, 2008; 125: 230–35
15. Maheux AF: Analytical comparison of nine PCR primer sets designed to detect the presence of *Escherichia coli/Shigella* in water samples. Water Res, 2009; 43: 3019–28
16. Drieux L, Brossier F, Sougakoff W, Jarlier V: Phenotypic detection of extended-spectrum β-lactamase production in Enterobacteriaceae: review and bench guide. Clin Microbiol Infect, 2008; 14: 90–103
17. O'Fallon E, Pop-Vicas A, D'Agata E: The emerging threat of Multidrug-Resistant Gram-Negative Organisms in Long-Term Care Facilities. J Gerontol A Biol Sci Med Sci, 2009; 64A: 138–41
18. Monstein HJ, Ostholm-Balkhed A, Nilsson MV et al: Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in *Enterobacteriaceae*. APMIS, 2007; 115(12): 1400–8
19. Clermont O, Bonacorsi S, Bingen E: Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol, 2000; 66(10): 4555–58
20. Demographic yearbook of Poland 2010. Central Statistical Office, Warsaw, 2010: 474–75, 506–8; 75–76, 128–30, 167; 146
21. Moro ML, Jans B, Cookson B, Fabry J: The burden of healthcare-associated infections in European long-term care facilities. Infect Control Hosp Epidemiol, 2010; 31: 59–62

Transparency declarations

None to declare.

22. Brusaferro S, Regattin L, Silvestro A, Vidotto L: Incidence of hospital-acquired infections in Italian long-term-care facilities: a prospective six-month surveillance. J Hosp Infect, 2006; 63: 211–15
23. Engelhart ST, Hanses-Derendorf L, Exner M, Kramer MH: Prospective surveillance for healthcare-associated infections in German nursing home residents. J Hosp Infect, 2005; 60: 46–50
24. Eriksen HM, Koch AM, Elstrøm P et al: Healthcare-associated infection among residents of long-term care facilities: a cohort and nested case-control study. J Hosp Infect, 2007; 65: 334–40
25. Moro ML, Mongardi M, Marchi M, Taroni F: Prevalence of long-term care acquired infections in nursing and residential homes in the Emilia-Romagna Region. Infection, 2007; 35: 250–55
26. Frankhauser C, Zingg Q, Francois P et al: Surveillance of extended-spectrum-beta-lactamase-producing *Enterobacteriaceae* in a Swiss Tertiary Care Hospital. Swiss Med Wkly, 2009; 139: 747–51
27. De Francesco MA, Ravizzola G, Peroni L et al: Urinary tract infections in Brescia, Italy: etiology of uropathogens and antimicrobial resistance of common uropathogens. Med Sci Monit, 2007; 13(6): BR136–44
28. Ben-Ami R, Schwaber MJ, Navon-Venezia S et al: Influx of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* into the hospital. Clin Infect Dis, 2006; 42: 925–34
29. Tenke P, Kovacs B, Johansen TE et al: European and Asian guidelines on management and prevention of catheter-associated urinary tract infections. Int J Antimicrob Agents, 2008; 31: S68–78
30. Livermore DM: β-Lactamases in Laboratory and Clinical Resistance. Clin Microbiol Rev, 1995; 8: 557–84
31. Empel J, Baraniak A, Literacka E et al: Molecular survey of beta-lactamases conferring resistance to newer beta-lactams in *Enterobacteriaceae* isolates from Polish hospitals. Antimicrob Agents Chemother, 2008; 52(7): 2449–54
32. Livermore DM, Canton R, Gniadkowski M et al: CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother, 2007; 59(2): 165–74
33. Mendelson G, Hait V, Ben-Israel J et al: Prevalence and risk factors of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in an Israeli long-term care facility. Eur J Clin Microbiol Infect Dis, 2005; 24: 17–22
34. Wiener J, Quinn JP, Bradford PA et al: Multiple antibiotic-resistant *Klebsiella* and *Escherichia coli* in nursing homes. JAMA, 1999; 281: 517–23
35. March A, Aschbacher R, Dhanji H et al. Colonization of residents and staff of a long-term-care facility and adjacent acute-care hospital geriatric unit by multiresistant bacteria. Clin Microbiol Infect, 2010; 16: 934–44
36. Kurtaran B, Candevir A, Tasova Y et al: Antibiotic resistance in community-acquired urinary tract infections: prevalence and risk factors. Med Sci Monit, 2010; 16(5): CR246–51
37. Yilmaz N, Agus N, Yurtsever SG et al: Prevalence and antimicrobial susceptibility of *Escherichia coli* in outpatient urinary isolates in Izmir, Turkey. Med Sci Monit, 2009; 15(11): PI61–65
38. Picard B, Sevali Garcia J, Gouriou S et al: The Link between Phylogeny and Virulence in Escherichia coli Extraintestinal Infection. Infect Immun, 1999; 67: 546–53
39. Wang H, Min G, Glockshuber R et al: Uropathogenic *E. coli* adhesin-induced host cell receptor conformational changes: implications in transmembrane signaling transduction. J Mol Biol, 2009; 392: 352–61
40. Bien J, Sokolova O, Bozko P: Role of Uropathogenic *Escherichia coli* Virulence Factors in Development of Urinary Tract Infection and Kidney Damage. Int J Nephrol, 2012; 681473
41. Plos K, Connell H, Jodal U et al: Intestinal carriage of P fimbriated *Escherichia coli* and the susceptibility to urinary tract infection in young children. J Infect Dis, 1995, 171: 625–31
42. Marre R, Kreft B, Hacker J: Genetically engineered S and F1C fimbriae differ in their contribution to adherence of *Escherichia coli* to cultured renal tubular cells. Infect Immun, 1990, 58: 3434–37

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