Epidemiology and genetic characteristics of extended-spectrum β-lactamase-producing Gram-negative bacteria causing urinary tract infections in long-term care facilities

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**Objectives:** To assess risk factors for acquiring extended-spectrum β-lactamase-producing Gram-negative bacteria (ESBL+ GN) causing urinary tract infections (UTIs) in long-term care facilities (LTCFs).

**Methods:** A prospective case–case–control study was carried out. In the first study, cases were defined as patients harbouring ESBL+ GN, while, in the second study, cases were defined as patients harbouring ESBL-negative (ESBL−) GN. Controls were selected by simple random sampling from patients without GN infection. ESBL determinants were characterized by hybridization, and confirmed by PCR and sequencing.

**Results:** The study involved 297 LTCF patients (99 with ESBL+ GN UTI, 99 with ESBL− GN UTI and 99 without GN infection). ESBL+ GN UTIs were due to Escherichia coli (64%), Proteus mirabilis (25%) and Klebsiella pneumoniae (11%). The CTX-M-type enzymes were the most prevalent (73% of isolates), whereas TEM- and SHV-type ESBLs and AmpC-type enzymes were less prevalent (10%, 2% and 15% of isolates, respectively). Patients with ESBL+ GN UTI were more likely to have a permanent urinary catheter (OR 15, 95% CI 6.9–30.5) and to have received antimicrobial therapy in the previous 30 days (OR 4, 95% CI 1.2–10.9). After adjusting for type, dosage and duration of antibiotic, exposure to ≥7 days of quinolones and third-generation cephalosporins was associated with the highest risk of ESBL+ GN UTI development (OR 7, 95% CI 1.2–40). Independent risk factors for acquiring ESBL− GN UTIs were previous surgical procedures (OR 2, 95% CI 1.1–4) and the presence of a urinary catheter (OR 8, 95% CI 4–16). No specific antibiotics remained a significant risk for ESBL− GN UTI after adjusting for demographic and clinical risk factors.

**Conclusions:** Exposure to ≥7 days of quinolones and third-generation cephalosporins significantly increases the risk of ESBL+ GN UTI. Interventions aimed at improving compliance with antimicrobial stewardship principles should be further developed and implemented in LTCFs.

**Keywords:** Enterobacteriaceae, multidrug-resistance, elderly

**Introduction**

Over the past century, human life expectancy has increased dramatically and infections in the elderly have become a major challenge for physicians due to their high frequency and changing epidemiology. Time spent in long-term care facilities (LTCFs) is an important risk factor for infections in this population. The phenomenon of antibiotic resistance in LTCFs involves both Gram-positive bacteria and Gram-negative bacteria (GN), although in the last few years an increase in the prevalence of multidrug-resistant GN (MDR-GN) has been reported. It is noteworthy that one of the first observations of Escherichia coli harbouring carbapenemases was in patients from LTCFs. O’Fallon et al. reported that the prevalence of MDR-GN among
residents in a 750 bed LTCF increased significantly from 7% in 2003 to 13% in 2005. More than 80% of MDR-GN isolates were resistant to ciprofloxacin, co-trimoxazole and ampicillin/subbac-
tam. A long-term survey carried out in France indicated that the incidence of extended-spectrum β-lactamase (ESBL)-
producing isolates/1000 hospitalization days in LTCFs had increased from 0.07 in 1996 to 0.28 in 2005. Recently, in a 
case–control study performed in Israel, among the E. coli and Klebsiella pneumoniae causing urinary tract infections (UTIs),
the overall rate of ESBLs was 26%. As for the outcome, although the risk of mortality associated with invasive infections due to
ESBL-producing bacteria is still under debate, it has been reported to be particularly severe in the elderly population
because of their poor functional status and the frequent presence of severe comorbidities.

There are only a few studies analysing the epidemiology of ESBL-producing GN (ESBL+ GN) in LTCF residents. Previous
antibiotic therapy and the presence of urinary catheters and other devices are the most frequently reported risk factors. However, the majority of studies did not distinguish between colonization and infections or only focused on colonized patients.

Therefore, a case–case–control study was carried out to assess the risk factors for acquiring ESBL+ GN UTIs in LTCFs. The secondary objectives were to characterize the isolates for resistance phenotypes and β-lactam resistance mechanisms, and to investigate the clonality of the isolates.

Methods

Study setting

Approved by the Local Ethics Committee, the study was carried out at two Italian LTCFs. The first one, located in the Hospital of Sant’Angelo Lodigiano, is a 70 bed unit with 500 patient discharges per year. The second one, located in the Hospital of Casalpusterlengo, is a 30 bed unit with 300 patient discharges per year. The majority of LTCF residents suffer from diabetes, cerebrovascular and osteoarticular diseases, and have a urinary catheter; a few of them have a central venous catheter or tracheostomy.

The same infection control measures were applied in both units, according to CDC indications.

Study design

A prospective case–case–control study was carried out to assess potential risk factors for acquiring ESBL+ GN infections in LTCFs. In the first study, cases were defined as patients admitted >72 h before developing a UTI due to ESBL+ GN, while in the second study, cases were defined as those patients admitted >72 h before developing a UTI due to ESBL-negative (ESBL−) GN. Controls were selected by simple random sampling from patients without GN infection who were admitted to the same LTCF, during the same period and with a total length of hospitalization similar to the duration of hospitalization from admission to diagnosis of GN UTI for cases. This approach was chosen because it allowed comparison of the relative contribution of the ESBL production over and above simply having the GN infection. UTI was chosen as a common indicator of infections in elderly residents in LTCFs. Infections caused by isolates producing acquired AmpC β-lactamases, all Proteus mirabilis, were included in the ESBL+ GN infections group, because ESBL- and AmpC-mediated resistance pose unique and similar problems for clinicians.

Data collection

From January 2009 through June 2010, patients corresponding to the study definition were detected by daily inspection of microbiological databases by one dedicated physician. Patients were enrolled only once, during the first diagnosis of GN UTI. Medical records of inpatient admissions, and microbiology and pharmacy databases were reviewed. Data collected at the study enrolment included: patient demographics, previous hospitalization within 1 year, requirement for chronic haemod-
alysis, history of peptic ulcer, cirrhosis, chronic renal failure, bronchopneu-
matic chronic obstructive disease (BPCO), diabetes, neoplasm, and presence of a central venous catheter or urinary catheter. Intensive care unit stay and surgical procedures were analysed if reported within 30 days of study inclusion. A composite score of comorbid illnesses was derived using the Chronic Disease Score for nosocomial infections (CDS-ID). Antibiotics administered during a 30 day period prior to the study enrolment and for ≥48 h were recorded. For the risk-factor analysis, oral and intravenous antibiotic exposure was analysed by individual antibiotics and by classes, and included penicillins, vancomycin, cepha-
lasporins, antibiotics with predominantly anaerobic activity (metronida-
zole and clindamycin), aminoglycosides, quinolones and carbapenems.

All included patients were followed up to discharge or death. Total length of stay (LOS), time at risk for infections and overall mortality were extracted from medical records. For case patients, management of infections, timing and type of antimicrobial therapy, aetiology of the infection, and susceptibility pattern of the bacterial isolate were also recorded.

Definitions

CDC criteria were used to define urinary infections. Time at risk for in-
fection was defined as LOS before the infection diagnosis for cases and total LOS for controls. Crude mortality at 30 days was assessed. Appropri-
ate antimicrobial therapy was defined as the initiation of therapy with ac-

tivity against the GN isolate (according to the results of the antimicrobial
susceptibility pattern) from the day before to 2 days after the positive
clinical culture result.

Microbiological and molecular analysis

All isolates were confirmed for ESBL production by combination disc dif-
fusion testing, according to Clinical and Laboratory Standards Institute
(CLSI) guidelines, using cefotaxime and ceftazidime as indicator mole-

In nucleotide sequences were determined for both strands of PCR amplification products at the Macrogen sequencing facility (Macrogen Inc., Seoul, Korea). Analysis and comparison of nucleotide sequences were carried out using pro-
grams available at the National Center for Biotechnology Information
web interface. Randomly amplified polymorphic DNA (RAPD) genotyping
was performed as previously described, using the decamer primers 1290 and 1254. RAPD patterns were considered to be different when the profiles differed by at least one band. Analysis of the RAPD patterns was performed with Diversity Database fingerprinting software, version 2 (Bio-Rad Laboratories, Hercules, CA, USA). Screening of E. coli isolates as belonging to sequence type (ST) 131 was carried out by the PCR assay described by Clermont et al. Genotyping by
multilocus sequence typing was performed as previously described by Wirth et al. Antimicrobial susceptibility testing was carried out by the disc diffusion test as recommended by the CLSI and categorical assignment was carried out using CLSI breakpoints. The following agents were tested: ertapenem, imipenem, meropenem, cefepime, ceftazidime, cefotaxime, cefoxitin, aztreonam, piperacillin/tazobactam, amoxicillin/clavulanate, ciprofloxacin, levofloxacin, gentamicin and amikacin.

Statistical analysis
Quantitative variables were tested for distribution and compared using the Kruskal–Wallis test. Differences in the group proportions were assessed using the χ² test and Fisher’s exact test. Potential risk factors for ESBL+ GN UTI, ESBL– GN UTI and death were analysed by univariate analysis. Variables with a P value of <0.25 from the univariate analysis were considered for inclusion in the multivariate logistic regression analysis. Results were presented as odds ratios (ORs) and 95% confidence intervals (95% CI). The Cox and Snell and the Nagelkerke R² tests were used to measure the usefulness of the model. The significance level was set at P = 0.05. Statistical analysis was carried out using the software program Intercooled Stata (Stata Statistical Software, release 8.0, College Station, TX, USA).

Results
The demographic and clinical characteristics of the 297 patients included in the study are described in Table 1. The distribution of different enterobacterial pathogens was not significantly different between UTIs due to ESBL+ GN and those due to ESBL– GN. E. coli was the most frequent pathogen (64% in ESBL+ GN and 76% in ESBL– GN), followed by P. mirabilis (25% in ESBL+ GN and 18% in ESBL– GN) and K. pneumoniae (11% in ESBL+ GN and 8% in ESBL– GN). The distribution of types of ESBL genes in isolates from the ESBL+ GN cases is summarized in Table 2. The CTX-M-type enzymes were the most prevalent (73% of isolates) and all belonged to group 1 (either CTX-M-1 or CTX-M-15), except one CTX-M-2 (CTX-M-2 group) and one CTX-M-14 (CTX-M-9 group). TEM-92 enzymes were produced by 10% of isolates, all P. mirabilis. SHV-type ESBLs were produced by two E. coli (SHV-12) and by 11 K. pneumoniae that also co-produced CTX-M-15 and TEM-1 enzymes. An AmpC-type β-lactamase (CMY-16) was detected in 15 P. mirabilis isolates.

Genotyping by RAPD revealed that all CMY-16-producing P. mirabilis exhibited an identical profile corresponding to that shown by the previously described P. mirabilis CMY-16-producing clone circulating in northern Italy. Furthermore, 23 of 61 (38%) of CTX-M group 1-producing E. coli showed an identical RAPD profile and all of them could be assigned to the pandemic virulent clone ST131 by the PCR analysis. The ST assignment was confirmed by multilocus sequence typing analysis for two randomly selected isolates. The susceptibility rates of ESBL+ GN isolates are reported in Table 3.

Table 1. Demographic and clinical characterization of 198 residents of LTCFs with UTIs due to ESBL+ and ESBL– GN compared with 99 randomized controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (%), n=99</th>
<th>ESBL+ GN (%), n=99</th>
<th>P</th>
<th>ESBL– GN (%), n=99</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years ± SD</td>
<td>84 ± 8</td>
<td>81 ± 9</td>
<td>0.01</td>
<td>82 ± 9</td>
<td>0.11</td>
</tr>
<tr>
<td>Female sex</td>
<td>31 (31)</td>
<td>26 (26)</td>
<td>0.43</td>
<td>21 (21)</td>
<td>0.10</td>
</tr>
<tr>
<td>Previous hospitalizations a</td>
<td>5 (5)</td>
<td>93 (94)</td>
<td>&lt;0.01</td>
<td>83 (84)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CDS-ID score ± SD</td>
<td>1.1 ± 1.5</td>
<td>1.6 ± 1.7</td>
<td>&lt;0.01</td>
<td>1.1 ± 1.8</td>
<td>0.87</td>
</tr>
<tr>
<td>Peptic ulcer</td>
<td>—</td>
<td>4 (4)</td>
<td>0.04</td>
<td>5 (5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>—</td>
<td>6 (6)</td>
<td>0.02</td>
<td>3 (3)</td>
<td>0.11</td>
</tr>
<tr>
<td>BPCO</td>
<td>—</td>
<td>8 (8)</td>
<td>&lt;0.01</td>
<td>9 (9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1 (1)</td>
<td>12 (12)</td>
<td>0.02</td>
<td>11 (11)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Neoplasm</td>
<td>—</td>
<td>2 (2)</td>
<td>0.15</td>
<td>2 (2)</td>
<td>0.15</td>
</tr>
<tr>
<td>Urinary catheter</td>
<td>17 (17)</td>
<td>79 (80)</td>
<td>&lt;0.01</td>
<td>66 (66)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Dialysis</td>
<td>—</td>
<td>2 (2)</td>
<td>0.19</td>
<td>1 (1)</td>
<td>0.35</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>8 (8)</td>
<td>25 (25)</td>
<td>&lt;0.01</td>
<td>18 (18)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Previous surgery b</td>
<td>19 (19)</td>
<td>41 (41)</td>
<td>&lt;0.01</td>
<td>35 (35)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ICU admission b</td>
<td>—</td>
<td>2 (2)</td>
<td>0.15</td>
<td>1 (1)</td>
<td>0.31</td>
</tr>
<tr>
<td>Central venous catheter</td>
<td>2 (2)</td>
<td>4 (4)</td>
<td>0.65</td>
<td>3 (3)</td>
<td>0.65</td>
</tr>
<tr>
<td>Antibiotic therapy b</td>
<td>6 (6)</td>
<td>31 (31)</td>
<td>&lt;0.01</td>
<td>—</td>
<td>0.05</td>
</tr>
</tbody>
</table>

ESBL+ GN, ESBL-producing Gram-negative bacteria; ESBL– GN, ESBL-negative Gram-negative bacteria; SD, standard deviation; CDS-ID score, Chronic Disease Score for nosocomial infections; BPCO, bronchopneumonic chronic obstructive disease; ICU, intensive care unit.

aAt least one in the previous year.

bWithin 30 days.
The risk was higher in patients with ≥7 days of antibiotic exposure (OR 11, 95% CI 4.1–45.2; P = 0.001).

Compared with patients with ESBL– GN UTI, these patients had a longer duration of hospital stay before the diagnosis of UTI (17 versus 9 days; OR 1.1 per 1 day longer; P = 0.04).

Multivariate analysis
Logistic regression analysis identified previous antibiotic therapy (OR 4, 95% CI 1.2–10.9; P = 0.02) and presence of a urinary catheter (OR 15, 95% CI 6.9–30.5; P < 0.01) as independent risk factors for acquiring ESBL– GN UTIs. The model correctly identified 82% of cases (R² = 0.63). Exposure, within 30 days of UTI, to ≥7 days of quinolones and third-generation cephalosporins was associated with the highest risk of ESBL+ GN UTI development (OR 7, 95% CI 1.2–40; P = 0.02), after adjusting for type, dosage and duration of antibiotic. The model correctly classified 83% of cases (R² = 0.66).

Risk factors for ESBL– GN UTIs

Univariate analysis
Table 1 shows the results of univariate analysis. Compared with controls, patients with ESBL– GN UTIs were more likely to have been hospitalized in the previous year, to have had surgical procedures in the previous 30 days, to suffer from chronic renal failure, diabetes, BPCO and peptic ulcer, and to have a permanent urinary catheter.

Multivariate analysis
Logistic regression analysis identified previous surgical procedures (OR 2, 95% CI 1.1–4; P = 0.04) and the presence of a urinary catheter (OR 8, 95% CI 4–16; P < 0.01) as independent risk factors for acquiring ESBL– GN UTIs. The model correctly identified 74% of cases (R² = 0.49). No specific antibiotics were a significant risk for UTIs after adjusting for demographic and clinical risk factors.

Antimicrobial therapy appropriateness and overall mortality
Thirteen (13%) and seven (7%) patients in the ESBL+ GN and ESBL– GN groups died, respectively. Inappropriate antimicrobial therapy was not a significant risk factor for mortality in patients with ESBL+ GN UTI nor in those with ESBL– GN UTI (OR 2.7, 95% CI 0.4–17.6 and OR 1.1, 95% CI 0.3–4, respectively).

Discussion
Our case–case–control study performed in two Italian LTCFs documented that exposure to ≥7 days of quinolones and third-generation cephalosporins and the presence of a permanent urinary catheter were independent risk factors for acquiring ESBL– GN UTIs, whereas previous surgical procedures and the presence of a urinary catheter were independent risk factors for acquiring ESBL+ GN UTIs.

The evidence of the association between previous antibiotic therapy and infection due to ESBL+ GN has been extensively documented in hospitalized patients.30,31 Our study demonstrated, for
the first time, the role of exposure to quinolones and third-generation cephalosporins among residents of LTCFs. Importantly, the selection of controls among patients without GN infections and coming from the source population (i.e. LTCF) allowed the assessment of the relative contribution of ESBL production over and above simply having the GN infection, and avoided underestimation of the association. Our results also suggest that the phenomenon might be dose related. In patients with a duration of quinolone and third-generation cephalosporin exposure <7 days, the increase in the risk of ESBL+ GN UTIs was not significant. Interestingly, antibiotic exposure was not independently associated with ESBL+ GN UTIs. This result supports the hypothesis that antibiotic therapy may facilitate the selection and outgrowth of antibiotic-resistant strains. It could be hypothesized that patients carrying GN, when exposed to antibiotics, become colonized by MDR strains and develop subsequent infections.\(^{32,33}\)

In contrast, those not recently exposed are more likely to develop infections due to susceptible strains. These results further underscore the need for a special effort to develop and implement education programmes aimed at improving antibiotic-prescribing practices in LTCFs, where the rate of inappropriate prescription of antibiotics might be as high as 75%.\(^{34,35}\)

Many studies showed that the presence of a urinary catheter, because of the formation of biofilms, is a risk factor for UTIs due to GN, regardless of the resistance pattern.\(^{36}\)

Our study has potential limitations. First, this is a case-control study, although data were collected prospectively from clinical charts and by direct interview of all included patients. Recall bias regarding previous exposure to antibiotics or visits to ambulatory clinics, as well as selection bias, might have occurred. However, to improve the study design we selected controls independently of their exposure status and from the source population. Second, the role of previous colonization with MDR-GN has not been assessed and we could not analyse its role as a risk factor for ESBL+ GN UTIs. Third, we cannot rule out cross-transmission among patients through the hands of healthcare workers; nevertheless, a lower ESBL-producing Enterobacteriaceae transmission rate was documented in LTCFs, compared with acute care facilities.\(^{37}\)

Lastly, this study was performed at two LTCFs located in the same Italian region and therefore the generalizability to other LTCFs might be limited.

Our study might have important clinical implications in identifying LTCF patients at high risk of having UTI due to ESBL+ GN. The possibility of predicting the probability of having an ESBL+ GN UTI based on a simple review of the patient’s medical records and of recent antibiotic therapy might be particularly useful in settings where there is less availability of diagnostic testing. The choice of appropriate therapy would reduce the excessive usage of broad-spectrum antibiotics and duration of treatment.

Finally, our study further emphasizes the need for multidisciplinary action to promote awareness among the scientific community about the complex therapeutic management of infections in the elderly. Importantly, the risk factors for ESBL+ GN UTIs documented by our analysis would be modifiable through improvements to antimicrobial stewardship as well as clinical practice for device maintenance in LTCFs. Since antibiotic therapy has been confirmed as an independent risk factor for antibiotic-resistant bacteria also in non-acute settings, LTCF physicians should strongly reinforce compliance with antimicrobial stewardship principles. Moreover, since infections have a strong impact on the quality of life of the elderly population, all infection control measures need to be implemented in LTCFs.\(^{38}\)

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**Transparency declarations**

None to declare.

**References**


ESBL infections in LTCF residents


