ESBL-producing multidrug-resistant *Providencia stuartii* infections in a university hospital

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**Objectives:** To investigate the epidemiological and clinical findings of extended-spectrum β-lactamase (ESBL)-producing *Providencia stuartii* infections in a large Italian university hospital.

**Patients and methods:** All consecutive episodes of *P. stuartii* infection that occurred during 1999–2002 were included in the study. For each patient, we recorded the area of hospitalization and drug susceptibility of the *P. stuartii* strains. Patients with ESBL-producing *P. stuartii* infection were considered cases and those with non-ESBL-producing *P. stuartii* infection were used as controls.

**Results:** One hundred and sixteen (52%) out of 223 *P. stuartii* strains collected during the study period were found to be ESBL-producing. On the basis of PCR and DNA sequencing experiments, TEM-52 was identified in 87% of isolates and TEM-72 in 13%. All ESBL-producing *P. stuartii* infections were nosocomially acquired. The prevalence increased from 31% of *P. stuartii* infections in 1999 to 62% in 2002 (*P* = 0.04). All 116 strains were classified as ESBL-producing multidrug-resistant *P. stuartii*, since 88% of the isolates were cross-resistant to ciprofloxacin and amikacin and the other 12% were cross-resistant to ciprofloxacin and gentamicin. At logistic regression analysis, advanced age (*P* < 0.001), previous hospitalization (*P* < 0.01), neoplastic disease (*P* < 0.001) and previous antibiotic therapy (*P* < 0.001) were independent risk factors for the development of ESBL-producing infections.

**Conclusions:** This 4 year surveillance of *Providencia* complaints clearly indicates that infections caused by ESBL-producing multidrug-resistant *P. stuartii* are an emerging problem.

Keywords: Enterobacteriaceae, antibiotic resistance, nosocomial outbreak, *P. stuartii*, ESBLs

**Introduction**

In recent years, bacterial resistance to β-lactam antibiotics has risen dramatically.1,2 Contributing to this increase has been the spread of extended-spectrum β-lactamases (ESBLs).3 Since their first identification at the beginning of the 1980s, ESBL-producing Enterobacteriaceae have spread worldwide by nosocomial routes.4–9 ESBLs comprise a group of active-site serine enzymes that are able to hydrolyse a wide range of β-lactams, including the most recently developed cephalosporins and monobactams, but they are not active against cephemycins and carbapenems.2,10

Most ESBLs found in clinical isolates of Enterobacteriaceae are variants of the original TEM-1 and SHV-1 enzymes in which one or more amino acid substitutions extend the substrate specificities.2 ESBLs occur predominantly in *Klebsiella* species and *Escherichia coli*, but they may also be present in other genera of the family Enterobacteriaceae, such as *Citrobacter*, *Enterobacter*, *Morganella*, *Proteus*, *Providencia*, *Salmonella* and *Serratia*.2,7,11,12 This resistance is particularly difficult to detect in Enterobacteriaceae other than *Klebsiella* spp. and *E. coli* because no criteria for the detection of ESBL production is provided by the NCCLS for these strains.13 Thus for organisms other than *E. coli* and *Klebsiella* spp., production of ESBLs may not be identified until they become widespread in the hospital premises.5,8,9

Treatment of the infections caused by ESBL-producing organisms is difficult, not only because of the resistance to the extended-spectrum cephalosporins themselves, but also because they are often associated with resistance to other antimicrobial agents coded either by the same or different plasmids.2,6,11 The concern caused by the increase in the number of infections due to multidrug-resistant

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(MDR) organisms is ever evolving and constitutes a threat to health-care-centre procedures in many countries.

Strains belonging to the Providencia genus are the most frequently isolated from the urinary tract in hospitalized patients. In addition, Providencia stuartii may be a causative agent of bacteremia unRelated to the urinary tract. Recently, the increasing role of P. stuartii as a nosocomial pathogen in the dissemination of plasmid-mediated resistance has been confirmed, although this species usually preserves its susceptibility to extended-spectrum cephalosporins. The aim of the present study was to describe the emergence of infections caused by ESBL-producing MDR P. stuartii strains in an Italian university hospital over a 4-year period, to identify the patients at higher risk and their clinical outcome. We also examined the antimicrobial susceptibility profiles of the strains of P. stuartii and evaluated whether this resistance was related to antimicrobial use.

Materials and methods

Setting and study design

The Catholic University Hospital is a 1900 bed university hospital located in Rome, Italy, admitting about 60 000 patients/year. The hospital has medical, surgical and neonatal specialties, as well as intensive care and post-surgical units.

During the study period (January 1999–December 2002), all consecutive episodes of community or nosocomially acquired—according to the Centers for Disease Control (CDC) definition—P. stuartii infection were included in the analysis. In particular, infections were considered to be nosocomial if signs and symptoms of infection became evident >48 h following hospital admission and/or if the patients had been hospitalized during the 2 weeks before the current admission. For each patient, we recorded the area of hospitalization and drug susceptibility of the P. stuartii strains.

The study was observational because the administration of antimicrobial agents and other therapeutic management was controlled by the patients’ physicians and not by the investigators.

Patients with ESBL-producing P. stuartii infection were considered cases and those with non-ESBL-producing P. stuartii infection had the role of controls.

We evaluated the following data in patients with ESBL-producing Providencia strains: age, gender, type of infection (hospital or community acquired), duration of hospitalization, risk factors, clinical findings and outcome. Prognosis was determined with the revised Acute Physiology and Chronic Health Evaluation (APACHE III) system immediately before infection developed.

Clinical cure was defined as the resolution of symptoms and signs of infection.

To establish a relationship between previous hospital use of antibiotics and development of ESBL-producing strains of P. stuartii, we calculated the defined daily dose (DDD) for ciprofloxacin, ceftazidime and amikacin in the hospital during the study period, analysing the hospital pharmacy data. For each ward, mean rates for the study were calculated by dividing the total number of DDs by the total number of patient-days reported over the study period; these were expressed as DDs per 1000 patient-days.

Bacterial strains

The strains evaluated in this study were selected from a consecutive series of P. stuartii isolates obtained from clinical samples in the 4 years of the study. Clinical samples (blood culture, urine, sputum, wound swab, etc.) were processed by conventional methods. The bacterial strains were identified using the VITEK 2 system (bioMérieux Inc., Hazelwood, MO, USA) and/or the Phoenix system (Becton Dickinson Microbiology Systems, Sparks, MD, USA). All non-duplicate P. stuartii isolates were screened for ESBL expression by the double-disc (DD) synergy test as previously described (see below). Strains were collected if the synergy test was positive. Isolates from superficial wounds, ear, throat and swab samples, were those not involved in infections, according to the CDC criteria, and were excluded.

Screening for ESBL production by the DD synergy test

Tests were performed by placing discs of ceftazidime, cefotaxime, ceftriaxone, cefepime and aztreonam (30 mg each) at distances of 20 mm (centre to centre) from a disc containing co-amoxiclav (20 and 10 mg, respectively). A clear-cut extension of the inhibition zone around the extended-spectrum cephalosporin and aztreonam disc towards the co-amoxiclav disc was considered a positive DD synergy test.

Molecular characterization of β-lactamases

Initially, crude preparations of β-lactamases from the isolates were analysed by isoelectric focusing (IEF) with Bio-Rad (Hercules, CA, USA) apparatus on prepared Ampholine PAG plate gels (pH 3.5–9.5, Bio-Rad), as described previously. The identity of the ESBLs was determined by PCR amplification of selected determinants, followed by direct sequencing of the amplicons, as described previously. blaTEM genes were detected using primers 5′-ATGAGTTTCAACATTTCGTT (nt 1–23, numbered from the start of the enzyme-coding region) and 5′-TTCACATTCAATCAGTGAG (nt 861–940). Cycling parameters included 3 min of denaturation at 94°C, followed by 35 cycles of 45 s at 94°C, 1 min at 95°C and 1 min 30 s at 72°C, ending in a final extension period of 10 min at 72°C. Products for sequencing reactions were purified with a PCR Clean Up Kit (Roche Diagnostic Molecular Biochemicals, Monza, Italy), and cloned into a PCR II vector (Advantage PCR Cloning Kit; Clontech, Palo Alto, CA, USA), before analysis on an ABI Prism 377 Sequencer Analyzer (Applied Biosystems, Foster City, CA, USA). DNA sequencing data were analysed with DNASIS software for Windows version 2.1 (Hitachi Software Genetic Systems, San Francisco, CA, USA).

Determination of MICs using the Epsilometer test

The MICs of amikacin, amoxicillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, aztreonam, cefalothin, cefotaxin, cefepime, ceftriaxone, ceftazidime, cefotaxime, imipenem, piperacillin, piperacillin/tazobactam, gentamicin and ciprofloxacin for DD-positive isolates were determined by a gradient diffusion method (Epsilometer test, Etest; AB Biodisk, Solna, Sweden), which was performed in accordance with the manufacturer’s instructions. Susceptibility tests were performed from a bacterial inoculum the turbidity of which was equivalent to that of a 0.5 McFarland turbidity standard. The suspension was used to inoculate Mueller–Hinton agar plates by swabbing them with cotton. The plates were incubated for 18 h in air at 35°C. MIC was interpreted as the point of intersection of the inhibition ellipse with the Etest strip edge. Susceptibility was determined according to NCCLS breakpoints as follows: imipenem, gentamicin: MICs ≤4 mg/L; amoxicillin, co-amoxiclav, ampicillin/sulbactam, aztreonam, cefalothin, ceftaxin, cefepime, ceftriaxone: MICs ≤8 mg/L; ciprofloxacin: MIC ≤1 mg/L; and amikacin, piperacillin, piperacillin/tazobactam: MICs ≤16 mg/L. The quality control strains E. coli ATCC 25922, E. coli ATCC 35218, Pseudomonas aeruginosa ATCC 25783 and Klebsiella pneumoniae ATCC 700603 were included in all sessions. ESBL-producing P. stuartii isolates that were cross-resistant to ciprofloxacin (breakpoint, ≥4 mg/L) and at least one of the two aminoglycosides tested [i.e. amikacin (breakpoint, ≥64 mg/L) or...
ESBL-producing \textit{P. stuartii} infections

gentamicin (breakpoint, $\geq 16$ mg/L) or both were defined as ESBL-producing MDR isolates.

\textit{Strain typing by repetitive extragenic palindromic sequence-based PCR (REP-PCR)}

Genomic DNA from the strains was obtained as described previously.\textsuperscript{19} An aliquot (4 $\mu$L) of each preparation was subjected to REP-PCR using the following primers: REP1R-1 (5'-IIIICGICGICATCIGGC) and REP2-1 (5'-ICGICTTATCIGGCTTAC). Reactions were carried out under conditions already described.\textsuperscript{19} Following amplification, PCR products were analysed by electrophoresis in a 1.5% agarose gel, subsequently stained with ethidium bromide. DNA fingerprinting similarity was assessed with Dendron software (version 2.0; Solltech, Inc., Iowa City, IA, USA). Strains with a coefficient of similarity ($S_{\text{DA}}$) of 0.9 or greater were considered identical.

\textit{Statistical analysis}

Contingency data were analysed by the two-tailed $\chi^2$ test or Fisher’s exact test, and continuous data by the Student’s $t$-test. Significance testing of differences in proportions was done using the exact test, and continuous data by the Student’s $t$-test. Multivariate analysis was utilized to determine what risk factors, demonstrated by univariate analysis, were independently significant. All statistical analysis was performed using the software program Intercooled Stata, version 6, for Windows (Stata Corporation USA). Two-tailed tests of significance at the $P < 0.05$ level were used to determine statistical significance.

\textbf{Results}

In the study period, out of 262,364 hospitalized patients, \textit{P. stuartii} was isolated in 223 patients from the following specimens: urine in 193 patients (87%), blood in 23 (10%) and respiratory tract secretions in seven (3%).

The overall hospital incidence of \textit{P. stuartii} infection was 0.008 per 1000 hospital admissions/year and it remained constant over the study period.

One hundred and sixteen (52%) out of 223 \textit{P. stuartii} strains collected during the study were found to be DD-positive. The $\beta$-lactamases produced by these ESBL-producing strains were characterized by IEF and PCR. All the strains showed a single IEF band with a pl compatible with the presence of a TEM enzyme (pl 6.0). On the basis of PCR and DNA sequencing experiments, TEM-52 was identified in 101 (87%) and TEM-72 in 15 (13%) DD-positive isolates.

The 116 ESBL-producing isolates were obtained from 103 (89%) subjects with urinary tract infections and from 13 (11%) with bacteremia. All these infections were nosocomially acquired. The first case of infection caused by a \textit{P. stuartii} strain possessing an ESBL enzyme occurred in an ICU in April 1999. Since then, the prevalence of ESBL-producing \textit{P. stuartii} infections has increased from 31% (17/55) in 1999 to 32% (56/173) in 2002 ($P = 0.04$) (Figure 1). Among 116 patients with ESBL-producing \textit{P. stuartii} infection, 31 patients (27%) were hospitalized in medical wards, 41 (35%) in ICUs and 44 (38%) in surgical wards ($P = \text{not significant}$). All the patients admitted to medical and surgical wards, as well as 26 out of 41 ICU patients, were infected by \textit{Providencia} strains producing TEM-52. The remaining 15 ICU patients, all admitted in 2000 only, had infections caused by strains producing TEM-72.

In order to assess an epidemiological link among the isolates, all strains were analysed by REP-PCR. Typing analysis identified three different REP-PCR profiles (data not shown). All the 101 isolates producing TEM-52 were included in two of the profiles, whereas the remaining 15 isolates, all producing TEM-72, were in the third. These findings suggested the dissemination of three \textit{P. stuartii} clones.

The results of antimicrobial susceptibility tests of 116 ESBL-producing multiresistant \textit{P. stuartii} isolates are summarized in Table 1. All isolates showed very similar resistance profiles, characterized by low MICs.

\textbf{Table 1. In vitro antimicrobial susceptibility of 116 ESBL-producing multidrug-resistant \textit{P. stuartii} isolates}

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC range</th>
<th>MIC$<em>{50}$/MIC$</em>{90}$</th>
<th>No. susceptible (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>$\beta$-Lactams</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amoxicillin</td>
<td>$\geq 32$</td>
<td>$\geq 32$/$\geq 32$</td>
<td>0 (0)</td>
</tr>
<tr>
<td>co-amoxiclav</td>
<td>16–32</td>
<td>32/32</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ampicillin/sulbactam</td>
<td>$\geq 32$</td>
<td>$\geq 32$/$\geq 32$</td>
<td>0 (0)</td>
</tr>
<tr>
<td>aztreonam</td>
<td>0.5–4</td>
<td>0.5/4</td>
<td>116 (100)</td>
</tr>
<tr>
<td>cefalothin</td>
<td>$\geq 32$</td>
<td>$\geq 32$/$\geq 32$</td>
<td>0 (0)</td>
</tr>
<tr>
<td>cefotaxine</td>
<td>2–23</td>
<td>2/23</td>
<td>59 (51)</td>
</tr>
<tr>
<td>cefepime</td>
<td>1–23</td>
<td>4/23</td>
<td>63 (54)</td>
</tr>
<tr>
<td>ceftriaxone</td>
<td>2–23</td>
<td>8/23</td>
<td>59 (51)</td>
</tr>
<tr>
<td>cefazidime</td>
<td>2–23</td>
<td>8/23</td>
<td>50 (43)</td>
</tr>
<tr>
<td>imipENEM</td>
<td>$\leq 0.25$</td>
<td>0.5/1</td>
<td>116 (100)</td>
</tr>
<tr>
<td>piperacillin</td>
<td>$\geq 128$</td>
<td>$\geq 128$/$\geq 128$</td>
<td>0 (0)</td>
</tr>
<tr>
<td>piperacillin/tazobactam</td>
<td>2–8</td>
<td>2/8</td>
<td>116 (100)</td>
</tr>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amikacin</td>
<td>1–64</td>
<td>$\geq 64$/$\geq 64$</td>
<td>14 (12)</td>
</tr>
<tr>
<td>gentamicin</td>
<td>1–232</td>
<td>1/16</td>
<td>102 (88)</td>
</tr>
<tr>
<td>Quinolones</td>
<td>4–232</td>
<td>$\geq 32$/$\geq 32$</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

SUSCEPTIBILITY LIMITS: The Minimal Inhibitory Concentration (MIC) limits were defined according to NCCLS breakpoints\textsuperscript{13} as follows: piperacillin, gentamicin: MICs $\leq 4$ mg/L; amoxicillin, co-amoxiclav, ampicillin/sulbactam, aztreonam, cefalothin, cefotaxime, ceftriaxone,! cefazidime, cefotaxine: MICs $\leq 8$ mg/L; ciprofloxacin: MIC $\leq 1$ mg/L; and amikacin, piperacillin, piperacillin/tazobactam: MICs $\leq 16$ mg/L.
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Table 2. Demographic data of 223 patients with *P. stuartii* infections. Univariate and logistic regression analysis of risk factors for the development of ESBL-producing strains

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>ESBL-producing strains n = 116 (52%)</th>
<th>Non-ESBL-producing strains n = 107 (48%)</th>
<th>Z</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years) ± S.D.</td>
<td>52 ± 12</td>
<td>40 ± 18</td>
<td>&lt;0.001*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Length of hospital stay (days)</td>
<td>34 ± 19</td>
<td>18 ± 16</td>
<td>0.002*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Previous hospitalization</td>
<td>39 (34)</td>
<td>13 (12)</td>
<td>&lt;0.001b</td>
<td>14.29 (3.55–18.20)</td>
<td>–</td>
</tr>
<tr>
<td>Previous UTI (&gt;2)</td>
<td>81 (70)</td>
<td>35 (33)</td>
<td>0.04b</td>
<td>1.32 (0.99–1.75)</td>
<td>–</td>
</tr>
<tr>
<td>Neoplastic disease</td>
<td>57 (49)</td>
<td>11 (10)</td>
<td>&lt;0.01b</td>
<td>4.77 (2.65–8.61)</td>
<td>–</td>
</tr>
<tr>
<td>Previous antibiotic therapy</td>
<td>109 (94)</td>
<td>39 (36)</td>
<td>&lt;0.001b</td>
<td>2.57 (1.99–3.32)</td>
<td>–</td>
</tr>
<tr>
<td>Quinolones</td>
<td>91 (78)</td>
<td>30 (28)</td>
<td>&lt;0.001b</td>
<td>2.79 (2.03–3.84)</td>
<td>–</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>73 (63)</td>
<td>23 (21)</td>
<td>&lt;0.001b</td>
<td>2.92 (1.98–4.31)</td>
<td>–</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>65 (56)</td>
<td>21 (20)</td>
<td>&lt;0.001b</td>
<td>2.85 (1.88–4.42)</td>
<td>–</td>
</tr>
<tr>
<td>Logistic regression analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>–</td>
<td>–</td>
<td>3.87</td>
<td>&lt;0.001</td>
<td>1.06 (1.03–1.10)</td>
</tr>
<tr>
<td>Previous hospitalization</td>
<td>–</td>
<td>–</td>
<td>3.35</td>
<td>&lt;0.01</td>
<td>18.10 (4.43–28.45)</td>
</tr>
<tr>
<td>Previous antibiotic therapy</td>
<td>–</td>
<td>–</td>
<td>4.64</td>
<td>&lt;0.001</td>
<td>20.22 (5.68–71.86)</td>
</tr>
<tr>
<td>Quinolones</td>
<td>–</td>
<td>–</td>
<td>4.23</td>
<td>&lt;0.001</td>
<td>9.93 (3.43–28.76)</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>–</td>
<td>–</td>
<td>3.49</td>
<td>&lt;0.01</td>
<td>8.35 (2.54–27.46)</td>
</tr>
<tr>
<td>Neoplastic diseases</td>
<td>–</td>
<td>–</td>
<td>3.34</td>
<td>&lt;0.001</td>
<td>15.23 (3.09–74.99)</td>
</tr>
</tbody>
</table>

UTI, urinary tract infections. *Student’s t*-test. χ² test.

of aztreonam (0.5–4 mg/L) with moderately high or elevated MICs (2–32 mg/L) of extended-spectrum cephalosporins. Overall, imipenem and piperacillin/tazobactam proved to have the strongest activity, inhibiting 100% of the isolates. Gentamicin and cefotaxim were effective against 88% and 51% of the isolates, respectively. All 116 strains were classified as ESBL-producing MDR *P. stuartii*, since 102 (88%) isolates were cross-resistant to ciprofloxacin and amikacin, and the other 14 (12%) strains were cross-resistant to ciprofloxacin and gentamicin.

The average age of the patients was 46 ± 18 years (range 28–92); 128 patients (57%) were men. None of the patients was an active intravenous drug abuser. The mean APACHE III score was 31 ± 14 at the diagnosis of *P. stuartii* infection.

Analysis of risk factors for the development of ESBL-producing infections is shown in Table 2. Advanced age (OR = 1.06, 95% CI = 1.03–1.10, *P* < 0.001), previous hospitalization (OR = 18.10, 95% CI = 4.43–28.45, *P* < 0.01), neoplastic diseases (OR = 15.23, 95% CI = 3.09–74.99, *P* < 0.001) and previous antibiotic therapy (OR = 20.22, 95% CI = 5.68–71.86, *P* < 0.001) were independent risk factors at logistic regression analysis.

The mean length of total hospitalization was 49 days for patients with infections caused by ESBL-producing strains and 27 days for the others (*P* = 0.001). No statistically significant difference was observed in the outcome of patients with urinary tract infections due to ESBL-producing or non-producing *P. stuartii* strains. In bacteremic patients, the overall mortality rate was 37%. The mortality rate among patients with *Providencia* bacteraemia presenting higher APACHE III scores (mean 42 ± 26; *P* = 0.01) and ESBL-producing *P. stuartii* infections (69% versus 21%; *OR* = 15.13; 95% CI = 1.90–323.13; *P* = 0.001) was significantly higher than that observed in patients without these variables.

In the entire hospital, mean DDDs per year of parenteral ciprofloxacin (3199), cefazidime (14 299) and amikacin (7315) were stable over the study period. The mean DDD of oral ciprofloxacin was 23.180 per year, increasing from 18.895 to 26.935 (*P* > 0.05 χ² for trend). The mean DDDs for the above mentioned antibiotics were higher in ICUs, but we did not find a statistically significant correlation between the previous use of these three antibiotics and the occurrence of ESBL-producing MDR *Providencia* infections.

**Discussion**

In the last two decades there has been a sharp increase in infections caused by Enterobacteriaceae resistant to many antibiotics. Among members of the family Enterobacteriaceae, the production of plasmid-mediated ESBLs has emerged as an important mechanism of transferable resistance to β-lactams, which account for ~50% of antibiotic consumption. ESBLs are widespread the world over, but the prevalence and phenotypic characteristics among clinical isolates may vary between geographical areas. The majority of ESBLs are derivatives of TEM-1 and TEM-2 (common plasmid-mediated β-lactamases of *E. coli*) or SHV-1 (the chromosomal encoded enzyme of *K. pneumoniae*).

Several data have indicated that the production of ESBLs has an important clinical impact. Studies on the effect of inocula, animal experimental data and limited clinical data have raised the possibility that using cephalosporins and penicillins to treat infections caused by ESBL-producing strains may result in adverse treatment outcomes. Recent surveys, both in Europe and the USA, have indicated that the emergence of infections due to ESBL-producing strains of Enterobacteriaceae is increasing with a consequent significant impact on mortality and hospital costs. A low number of cases of ESBL-producing *P. stuartii* infections have been reported.

This 4 year surveillance of *Providencia* infections clearly indicates that ESBL-producing *Providencia* infections are an emerging problem. Before 1999 no patient with ESBL-producing *P. stuartii* infection was observed in our hospital; after that date we observed
ESBL-producing *P. stuartii* infections

a statistically significant increase, up to 62.5% of *P. stuartii* isolates in 2002.

The results of the molecular analysis suggest that dissemination of ESBL-producing *P. stuartii* strains in our hospital has probably occurred through different pathways, namely, the dissemination of one clone (e.g. strains producing the TEM-72 ESBL enzyme) has occurred through a limited outbreak in an ICU in the year 2000, only. The remaining two clones, both producing the TEM-52 ESBL enzyme, were endemic as they were isolated throughout the entire period of the study in several wards.

It should be noted that a precise association has not always been found between the ESBL type produced and the susceptibility to different β-lactams, since this last variable is multifactorial, depending on ESBL substrate specificity, production of additional β-lactamases and changes in outer membrane permeability. Nonetheless in our study, we observed the same resistance pattern both for ESBL-TEM-52- and ESBL-TEM-72-producing organisms. All ESBL-producing strains maintained susceptibility to imipenem, whereas 50% of the strains were susceptible to cefotixin.

As noted above, treatment of infections caused by organisms that produce ESBL enzymes is difficult, because strains are often resistant to several antimicrobial agents. Our survey confirms the above-cited reports and, in addition, indicates a marked association between ESBL production and multidrug resistance.

Our data indicate that a valuable alternative to imipenem for treatment is represented by gentamicin, a bactericidal drug effective against 88% of strains. In addition, among β-lactam/β-lactamase inhibitor combinations only piperacillin/tazobactam was active in vitro against all isolates.

As regards the analysis of risk factors, several reports have described the clinical outcomes of hospitalized patients with infections caused by ESBL-producing Enterobacteriaceae other than *Providencia*. Our prospective surveillance on 116 subjects clearly indicates, at multivariate analysis, a significant correlation between the presence of ESBL-producing *P. stuartii* infections and advanced age, previous hospitalization and previous antibiotic use by the patients. Indeed, advanced age is a well-known risk factor for bacterial infection, whereas previous hospitalization and previous antibiotic therapy are often implicated in infections due to antibiotic-resistant strains of Enterobacteriaceae.

In addition, we observed a statistically significant increased risk of infections caused by ESBL-producing strains of *Providencia* in patients with neoplastic diseases. Most studies have not evaluated the association between malignancy and ESBL-producing strains of Enterobacteriaceae. However, one prospective survey noted malignancy to be a risk factor for infections caused by ESBL-producing strains, but another indicated a lower incidence of colonization, a well known risk factor for infection, by ESBL-producing strains in patients with neoplastic diseases.

An interesting point is that the rates of ciprofloxacin, ceftazidime and amikacin use were similar, according to DDD analysis, throughout the different medical and surgical wards, although the level of resistance was higher in subjects admitted to surgical wards. Similar observations have been reported for ICUs in a different surveillance among antibiotic-resistant infections in the USA. The lack of absolute correlation between hospital antibiotic use and development of resistance suggests that other factors, such as underlying diseases, case mix of the ward or use of other drugs, may play an important role in the propagation of antibiotic-resistant organisms.

This prospective surveillance underscores the important role of *P. stuartii* infections in the hospital setting and, for the first time, provides data on the emerging trends in infections due to multidrug-resistant ESBL-producing strains of *Providencia* and timely data on their epidemiology.

The main conclusion of our study is that during the past 4 years we have observed an increase in infections caused by ESBL-producing multidrug-resistant *P. stuartii*.

This observation might not be limited to our hospital, or even our nation, as the lack of NCCLS standardized procedures to detect ESBL production of *Providencia* strains could underestimate the real scale of this problem in other geographical areas.

References

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