Relationship between ceftriaxone use and resistance to third-generation cephalosporins among clinical strains of Enterobacter cloacae

A. Muller¹, J. M. Lopez-Lozano², X. Bertrand¹ and D. Talon¹*

¹Service d’Hygiène Hospitalière et d’Épidémiologie Moléculaire, Centre Hospitalier Universitaire Jean Minjoz, Besançon, France; ²Preventive Medicine Unit, Hospital Vega Baja, Orihuela, Spain

Received 17 October 2003; returned 10 February 2004; revised 3 March 2004; accepted 10 April 2004

Objective: To investigate the potential correlation between the use of extended-spectrum cephalosporins (ESCs) and resistance to this antibiotic class among clinical isolates of Enterobacter cloacae in a university-affiliated hospital.

Materials and methods: Data on antimicrobial resistance and antimicrobial use concerning E. cloacae and ESCs were collected over a 4 year period. Various statistical tools were used to explore the potential relationship.

Results: From 1999 to 2002, the proportion of E. cloacae isolates resistant to ESCs increased from 24.3% to 29.6%. \((P = 0.04)\), and the quantity of ESCs prescribed and given did not change. Within the subclass constituted by first-line ESCs, the proportion of ceftriaxone increased from 64.3% to 77.6% and the proportion of cefotaxime decreased accordingly, from 35.7% to 22.4%. Statistical analyses showed that E. cloacae resistance to ESCs correlated with ceftriaxone use regardless of the other ESCs. For every defined daily dose of ceftriaxone per 1000 patient days used in our hospital, resistance of E. cloacae isolates to ESCs increased by 1.36%.

Conclusion: This study demonstrates a specific correlation between ceftriaxone use and the development of resistance in E. cloacae clinical isolates. The high biliary elimination of ceftriaxone compared with other ESCs may be responsible for a greater impact of this antibiotic on the digestive flora.

Keywords: E. cloacae, antibiotic resistance, ceftriaxone, cefotaxime, statistical analysis

Introduction

In recent years, Enterobacter cloacae has emerged as an important nosocomial pathogen.¹ Moreover, resistance to antimicrobial agents is increasing within this species, and particularly resistance to third-generation cephalosporins.²,³ Monitoring this antimicrobial resistance is important, because resistance has been reported to be associated with increased patient morbidity and mortality, prolonged hospitalization and increased hospital expenditure, particularly for bacteraemia and ventilator-associated pneumonia.⁴ There are two basic modes of spread of antimicrobial resistance: (i) dissemination of epidemic multiresistant strains by cross-transmission; and (ii) acquisition of resistance by susceptible strains. For E. cloacae in our hospital, the second mode is mostly responsible: resistance to extended-spectrum cephalosporins (ESCs) in this species is practically exclusively mediated by high-level production of the chromosome-encoded Bush group 1 \(\beta\)-lactamase.⁵,⁶ The proportion of E. cloacae isolates resistant to third-generation cephalosporins increased between 1999 and 2002. Concomitantly, the relative part of our two first-line ESCs, cefotaxime and ceftriaxone, had changed: cefotaxime was progressively replaced with ceftriaxone. The objective of this study was to determine whether there was a correlation between these two events at a collective level.

Materials and methods

Setting and study period

Besançon Hospital is a university-affiliated hospital with 1219 acute-care beds divided into 59 units (35 medical units, 21 surgical...
units and three intensive care units). Specialty services include cardiothoracic surgery, and organ and bone marrow transplantation. Approximately 50,000 inpatients are admitted per year, for a total of 350,000 patient days. Data were collected from 1 January 1999 to 31 December 2002.

**Bacteriological culture and antibiotic susceptibility testing**

The organisms included in this study were isolated for diagnostic purposes. They came from blood cultures (7.6%), urine samples (32.8%), superficial swabs (28.6%), broncho-pulmonary tract (14.8%) and other sites (16.2%). We did not collect clinical information to distinguish between infections and simple colonization; hence patients with positive clinical specimens should be considered as colonized/infected. Isolates were not collected as a part of a surveillance programme. All Enterobacteriaceae isolated were identified to the species level by biochemical characteristic analysis (API 20E and ID 32 E Strips; bioMérieux, Marcy l’Etoile, France). Susceptibilities to common antibiotics were determined by the disc diffusion method. Isolates were classified as being susceptible, intermediate or resistant according to the criteria recommended by the Antibiogram Committee of the French Microbiology Society5 (Table 1). All E. cloacae isolates showing reduced susceptibility or resistance to ceftazidime (<21 mm for a 30 µg disc) and/or cefotaxime (<21 mm for a 30 µg disc) were considered to be resistant (intermediate + resistant) to ESCs. They were tested for extended-spectrum β-lactamase (ESBL) production by standard double-disc diffusion testing.2 Isolates were classified as high-level-expressing β-lactamase producers (HLBL) or as ESBL producers according to the production of ESBL. These data were extracted from the clinical microbiology laboratory information system for all E. cloacae isolated during the study period. Duplicate isolates were defined on the basis of patient identity and the antibiotic susceptibility profile to ESCs (susceptible or resistant).

**Antibiotic use**

The monthly quantities of each antimicrobial agent delivered to each unit of the hospital were obtained from the pharmacy information system. Grams and international units of antimicrobials were further converted into defined daily doses (DDD) following the recommendations of the WHO.6 The use of various antibiotics is expressed in DDD/1000 days of hospitalization.

**Statistical analysis**

The study approach was ecological. Statistical analysis was conducted to explore the relationships between total resistance of E. cloacae isolates to ESCs (we did not differentiate between HLBL and ESBL producers) and ESC use. First, this association was tested for the year 2002, with the individual hospital units that patients were admitted to as the statistical unit. Association between each ESC and resistance was tested in univariate analysis using the Spearman rank correlation coefficient. We retained the ESCs for which use seemed to be significantly associated with ESC resistance with a threshold value of $P \leq 0.20$. Then, logistic regression was used in multivariate analysis. A $P$ value of $\leq 0.05$ was considered to be statistically significant. Statistical analysis was performed with R software (The R Project for Statistical Computing; http://www.r-project.org).

In a second step, we conducted time-series analysis of monthly ESC resistance values and the monthly data concerning use of different ESCs (ceftriaxone, cefotaxime, ceftazidime, cefpodoxime and cefepime) to investigate the relationship between antimicrobial resistance and use. This technique, developed by Lopez-Lozano et al.7 and Monnet et al.8 was applied to our data according to methodology previously described.3 It is based on autoregressive integrated moving average models, which are used to analyse the temporal behaviour of a variable as a function of its previous values, its trends and any abrupt changes in the recent past. Once the basic characteristics of the series were established, the relationships between antimicrobial use and resistance were quantified through the use of dynamic time-series modelling techniques. Specifically, polynomial distributed lag (PDL) models were utilized for detection and quantitation of lagged effects of antimicrobial use on resistance. In a PDL model, the relationship between the independent variables (past resistance and antimicrobial use) and the dependent variable (resistance) should evolve smoothly over time, through the use of ‘polynomial lags’. The optimum PDL model for the datasets emerged via the ‘general-to-specific’ econometric methodology. This meant that initially, many possible independent variables were included in the model, some of which were ultimately found to be irrelevant. The model was then progressively simplified by eliminating these irrelevant independent variables. The validity of the simplified model was then checked by a battery of specification and diagnostic tests to ensure that the simplification from the initial model was appropriate. Using the approach proposed by Pankratz,9 we adjusted a linear transfer function model. Data

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>NCCLS</th>
<th>BSAC</th>
<th>CA-SFM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
<td>R</td>
</tr>
</tbody>
</table>

S, susceptible; I, intermediate; R, resistant.
*Expressed in mm.
*Expressed in mg/L.
were collected at the hospital level and analysed using the SCA system (Scientific Computer Associates; Chicago, IL, USA; www.scausa.com).

Results

From 1999 to 2002, the proportion of *E. cloacae* isolates resistant to ESCs had increased from 24.3% to 29.6% (*P* = 0.03). During the study period, 770 patients were colonized and/or infected with *E. cloacae*. We included in the analysis 806 isolates of *E. cloacae*, of which 211 were resistant to ESCs (Figure 1). Considering only the first isolate from each patient, the level of resistance was not significantly different from that of all isolates (data not shown). Acquisition of resistance during the course of a colonization/infection (i.e. patients with clinical specimens testing positive for a susceptible isolate and subsequently positive for a resistant one) occurred for 29 patients (3.8%), who had stayed in hospital for >40 days, and seven patients (0.9%) had clinical specimens testing positive for a resistant isolate and subsequently a susceptible one. More than 95% of the *E. cloacae* isolates resistant to ESCs were HLB; only five patients had specimens testing positive for ESBL-producing *E. cloacae*, giving a total of five isolates included in the statistical models. Broadly, 20 000 DDD of ESCs are prescribed and given each year in our hospital. This quantity did not change significantly between 1999 and 2002. Table 2 reports the changes in use of each molecule of this class of antibiotics. Only two first-line ESCs were distributed in our institution: cefotaxime and ceftriaxone. During the study period, the proportion of ceftriaxone in this subclass increased from 64.3% to 77.6%, and the proportion of cefotaxime decreased conversely from 35.7% to 22.4%. The only available oral third-generation cephalosporin (cefepodoxime) accounted for ~5% of the total ESC use (Table 2).

In univariate analyses, the use of both ceftriaxone and ceftazidime was significantly associated with resistance (Table 3). In multivariate analysis, only ceftriaxone use correlated with resistance (*P* = 0.016), and the model indicated that the use of 1 DDD/1000 patient days of ceftriaxone led to an increase of 0.91% in the resistance.

The adjusted linear transfer function model showed a significant relationship between the ceftriaxone use time-series and the ESC resistance series. This was observed for contemporaneous data and with a lag of 1 month (Table 4). By cumulating the calculated coefficient, the model shows that the use of 1 DDD/1000 patient days of ceftriaxone led to an increase of 1.36% in the resistance, 1 month later. Another relationship was found between the cefotaxime use time-series and the ESC resistance series: with a lag of 3 months (Table 4), the effect of cefotaxime on ESC resistance was 1.26%. Two stochastic terms were introduced in order to assess a white noise model for residuals: a 4 month autoregressive term and an 8 month moving average term (Table 4). The model explained 60% of the variability of the resistance series (*r*² = 0.601). No relationship was observed between the hospital use of ceftazidime and resistance.

Discussion

In our hospital, the use of ESCs overall did not vary during the study period. The only observed change was the increasing

Table 2. ESC use in Besançon Hospital (1999–2002)

<table>
<thead>
<tr>
<th>Year</th>
<th>Ceftriaxone</th>
<th>Cefotaxime</th>
<th>Ceftazidime</th>
<th>Cefepime</th>
<th>Cefpodoxime</th>
<th>Total ESC use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>22.02</td>
<td>12.23</td>
<td>11.91</td>
<td>6.66</td>
<td>3.67</td>
<td>56.61</td>
</tr>
<tr>
<td>2000</td>
<td>23.6</td>
<td>8.73</td>
<td>13.26</td>
<td>6.92</td>
<td>2.79</td>
<td>55.3</td>
</tr>
<tr>
<td>2001</td>
<td>29.16</td>
<td>9.72</td>
<td>12.13</td>
<td>6.56</td>
<td>3.01</td>
<td>60.58</td>
</tr>
<tr>
<td>2002</td>
<td>32.03</td>
<td>8.99</td>
<td>12.91</td>
<td>4.91</td>
<td>3.15</td>
<td>61.99</td>
</tr>
</tbody>
</table>

Extended-spectrum cephalosporin use is expressed as DDD/1000 patient days.
A. Muller et al.

Table 3. Correlation coefficients between ESC use and resistance of E. cloacae isolates in Besançon Hospital (2002)

<table>
<thead>
<tr>
<th>Various ESCs</th>
<th>Ceftriaxone</th>
<th>Cefotaxime</th>
<th>Ceftazidime</th>
<th>Cefepime</th>
<th>Cefpodoxime</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )</td>
<td>( P )</td>
<td>( r )</td>
<td>( P )</td>
<td>( r )</td>
</tr>
<tr>
<td>Frequency of resistance</td>
<td>0.46</td>
<td>0.002</td>
<td>-0.08</td>
<td>0.61</td>
<td>0.39</td>
</tr>
</tbody>
</table>

\( r \), correlation coefficient using Spearman coefficient.

Table 4. Transfer function model for percentage of ESC-resistant E. cloacae taking into account hospital third-generation cephalosporin use (CHU Jean Minjoz, Besançon, France, 1999–2002)

<table>
<thead>
<tr>
<th>Term</th>
<th>Order( ^a )</th>
<th>Parameter (S.E.)( ^b )</th>
<th>T-ratio</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0</td>
<td>-24.526(8.993)</td>
<td>-2.727</td>
<td>0.010</td>
</tr>
<tr>
<td>Ceftriaxone use</td>
<td>0</td>
<td>0.563(0.188)</td>
<td>2.998</td>
<td>0.005</td>
</tr>
<tr>
<td>Ceftriaxone use</td>
<td>1</td>
<td>0.850(0.176)</td>
<td>4.836</td>
<td>0.000</td>
</tr>
<tr>
<td>Ceftriaxone use</td>
<td>3</td>
<td>1.257(0.543)</td>
<td>2.314</td>
<td>0.026</td>
</tr>
<tr>
<td>AR</td>
<td>4</td>
<td>-0.472(0.148)</td>
<td>-3.192</td>
<td>0.003</td>
</tr>
<tr>
<td>MA</td>
<td>8</td>
<td>-0.865(0.031)</td>
<td>-27.511</td>
<td>0.000</td>
</tr>
</tbody>
</table>

\( ^a \)Delay necessary to observe the effect (in months).

\( ^b \)Size and direction of the effect.

The causes of the emergence and spread of antimicrobial-resistant pathogens are multifactorial, but the excessive and inappropriate use of antimicrobials is clearly the principal determinant. In our hospital, as in other settings, the spread of resistance applied to the combination E. cloacae/ESCs was mainly the consequence of acquisition of resistance by susceptible strains. Although we have not demonstrated that there was no major outbreak during the study period, it was very likely that the spread of resistance to ESCs in E. cloacae was not the result of cross-transmission. So, for the combination ESCs/ E. cloacae, the role of antibiotic selective pressure was predominant in the emergence of resistance. The plausibility of this biological model was confirmed by time-series analysis, which demonstrated that resistance to ESCs in E. cloacae correlated to ceftriaxone use in the same month and in the preceding month. However, the biological model is not plausible for the association between resistance and cefotaxime use 3 months previously. Note that classical statistical methods did not indicate a correlation between cefotaxime use and resistance to ESCs.

By using time-series analysis, the power of the model is high. More than 60% of the variations in monthly hospital ESC-resistant E. cloacae over the period January 1999 to December 2002 were explained by the model. This evidence was obtained through the use of time-series analysis and dynamic modelling techniques, the advantages of which lie in their ability to detect and quantify the lagged effects of antibiotic consumption, as well as past values of hospital resistance series on bacterial resistance. Past values of hospital resistance are represented in the model by the two stochastic terms, which might not have any possible biological interpretation except the inertial influence explained by a setting exposed to a concrete bacterial contamination level. The model does not explain 40% of the variability because it was impossible to include other unknown contributing factors into the analysis.

Further analysis of the data showed that the level of resistance of the group of first isolates from each patient was not different from that of the total isolates. Acquisition of resistance during the treatment of an infection caused by E. cloacae was thus not a major factor; it was observed in only 3.8% of the patients, all hospitalized for a long period of time (>40 days). Administration of ESCs probably selects spontaneous mutants of E. cloacae producing HLBL among the digestive bacterial flora. This selected population may then be further involved in infectious processes. Our results suggest that ceftriaxone is more frequently associated with the hyperproduction of chromosomal β-lactamase in E. cloacae clinical isolates than other ESCs used in our hospital, and particularly cefotaxime. The pharmacokinetic properties of
Ceftriaxone and *E. cloacae*

Ceftriaxone may partially explain this observation. Indeed, although the residual concentration of ceftriaxone before a new administration, and the diffusion of this antibiotic into various tissues are excellent, the high biliary elimination of ceftriaxone in comparison with other ESCs may be responsible for a higher impact of this antibiotic on the digestive flora.\textsuperscript{11,15} The digestive tract is the main reservoir for Enterobacteriaceae involved in infections. Other ESCs for which the major route of elimination is via the kidney may therefore have less effect on promoting the development of resistance to ESCs. It is also possible that, intrinsically, ceftriaxone could, more frequently than other ESCs, lead to the derepression of the cephalosporinase gene. This possibility is consistent with the report by Fung-Tomc et al.\textsuperscript{16} who reported that *in vitro*, the development of resistance to ESCs was more rapid after exposure to ceftriaxone than to other ESCs.

Our study demonstrates a specific correlation between ceftriaxone use and development of resistance among *E. cloacae* clinical isolates. This observation needs to be confirmed in other settings and to be evaluated by patient-level studies. Additional research is needed to understand the relationship between *E. cloacae* and ESCs. Nevertheless, there is evidence supporting the implementation of programs to evaluate and improve prescriptions of first-line ESCs so as to minimize the spread of ESC resistance among *E. cloacae*. In our hospital, all the physicians can prescribe any antimicrobial agent available in the pharmacy. Our results emphasize the need for antibiotic policies and surveillance of emergence of antibiotic resistance in order to prevent scenarios as described in this paper.

Acknowledgements

This work was supported by the Programme Hospitalier de Recherche Clinique 2002.

References