Long-term study of the frequency of *Escherichia coli* and *Klebsiella pneumoniae* isolates producing extended-spectrum β-lactamases

L. Romero1, L. López1, J. Rodríguez-Baño2, J. Ramón Hernández1, L. Martínez-Martínez1 and A. Pascual1

1Department of Microbiology and 2Section of Infectious Diseases, Service of Internal Medicine, Hospital Universitario Virgen Macarena, Seville, Spain

**ABSTRACT**

In total, 438 (1.7%) *Escherichia coli* and 125 (3.98%) *Klebsiella pneumoniae* isolates were found to be producers of extended-spectrum β-lactamase (ESBL) during 1995–2003 in southern Spain. There was a significant increase in the frequency of ESBL-producing *E. coli* isolates, from < 0.36% before 1999 to 4.8% in 2003, while the frequency of ESBL-producing *K. pneumoniae* isolates decreased during the same period. The most common ESBLs detected in *K. pneumoniae* were SHV type, whereas both CTX-M and SHV types were detected in *E. coli*. In addition, *E. coli* isolates showed greater clonal diversity (84 distinct REP-PCR patterns, compared with five in *K. pneumoniae*), fewer enzymes per isolate, and a higher number of isolates recovered from outpatients. These differences may have implications for the control measures that should be used for these two microorganisms.

**Keywords** Cephalosporin resistance, epidemiology, *Escherichia coli*, extended-spectrum β-lactamases, *Klebsiella pneumoniae*, nosocomial infection

**Original Submission:** 26 October 2004; **Revised Submission:** 14 February 2005; **Accepted:** 24 March 2005

*Clin Microbiol Infect* 2005; 11: 625–631

**INTRODUCTION**

Resistance to β-lactams in Enterobacteriaceae, associated with plasmid-mediated extended-spectrum β-lactamases (ESBLs), has become a worldwide problem [1,2]. ESBLs confer clinically significant resistance to broad-spectrum penicillins, monobactams and cephalosporins (except cephemycins), and are often associated with resistance to other non-related antibiotics in multiresistant pathogens [3]. The first ESBLs were reported in *Klebsiella pneumoniae* in 1983 [4], and ESBLs spread subsequently to *Escherichia coli* and, occasionally, other Enterobacteriaceae. However, *Klebsiella* spp. and *E. coli* continue to be the two most important organisms associated with ESBL-mediated resistance. ESBLs have been classified into types, based on their deduced amino-acid sequences (i.e., the TEM, SHV, CTX-M, PER, VEB, GES, TLA, BES and OXA types). TEM or SHV derivatives have been the most prevalent types of ESBL, but the prevalence of the CTX-M type has increased dramatically since 1995 in most parts of the world, including Europe, Asia, South America [1,5] and North America [6].

The epidemiology of ESBL-producing isolates of *K. pneumoniae* has been studied extensively. Such isolates are involved frequently in outbreaks of infection, particularly in high-risk areas, such as intensive care or neonatal units. Outbreaks involving spread between hospitals [7] and long-term care centres [8] have also been described. Although there are numerous reports describing the spread and epidemiology of ESBL-producing *K. pneumoniae*, the behaviour of ESBL-producing *E. coli* is less well-known. To date, few outbreaks caused by a single ESBL-producing *E. coli* strain [9–12], or involving nosocomial spread of plasmids between ESBL-producing *K. pneumoniae* and ESBL-producing *E. coli* strains [8,13], have been described. However, recent studies have suggested that ESBL-producing
isolates of *E. coli* have begun to spread in community settings [14–16].

Despite the emergence of the CTX-M type, the impact of these new enzymes on the local prevalence of ESBL-producing Enterobacteriaceae in a specific region has been neither analysed nor compared with that of other ESBLs. Longitudinal descriptions over a long period in a single area are limited and have dealt mostly with *K. pneumoniae* isolates from inpatients [17]. For *E. coli*, most reports describe only periods of 1–2 years [16], or are focused on the production of one particular ESBL [18]. Although there are several previous reports highlighting the increasing prevalence of ESBL [18], there are no comparative analyses of the two species have been described. Therefore, the aims of the present study were: (1) to describe the epidemiological evolution of ESBL-producing *E. coli* and *K. pneumoniae* isolates, in the context of the worldwide spread of CTX-M, during a 9-year period; (2) to determine the epidemiological relationship between isolates and the types of enzyme produced; and (3) to determine the differential characteristics of the two species involved.

**MATERIALS AND METHODS**

**Setting**

The study was carried out in the area served by the University Hospital Virgen Macarena, located in the north of Seville (Spain). The Clinical Microbiology Laboratory receives samples from the 1000-bed hospital, the outpatient clinic, a nearby chronic-care hospital, and the primary-care services of an area with 500 000 inhabitants.

**Isolates**

During the period January 1995 to December 2003, all *E. coli* and *Klebsiella* spp. isolates from significant clinical samples were analysed for ESBL production. From 1995 until 2000, identification and preliminary antimicrobial susceptibilities were determined by the MicroScan system (Dade Behring, Sacramento, CA, USA), and from 2001 until 2003, by the VITEK2 system (bioMérieux; Hazelwood, MO, USA). Samples referred from primary-care services, the outpatient clinic and the emergency department were considered to come from non-hospitalised patients, while those referred from hospital wards were considered to come from hospitalised patients. Differences in proportions among categorical data were calculated by the chi-square test, with *p* < 0.05 considered to be significant.

**Screening for ESBL producers**

All isolates with a cefpodoxime MIC ≥8 mg/L and/or a cefotaxime/cefazidime MIC ≥2 mg/L with the automated methods were tested for ESBL production according to NCCLS criteria [19]. ESBL production was tested by the disk-diffusion method using Mueller–Hinton agar plates (Becton Dickinson, Le Point de Claix, France) and disks containing 30 μg of cefotaxime or cefazidime, with or without 10 μg of clavulanic acid, and 10 μg of cefpodoxime, with or without 1 μg of clavulanic acid, according to the manufacturer’s instructions. ESBL production was confirmed by the NCCLS microdilution technique [19]. A two-fold decrease in MIC of ≥3 mg/L for either cefazidime, cefotaxime or cefpodoxime when tested in combination with 4 mg/L of clavulanic acid, compared with the MIC when tested alone, was considered indicative of ESBL production [19].

**Antimicrobial agent susceptibility assays**

The activities of cefazidime, cefotaxime, cefoxitin, ciprofloxacin, gentamicin, amikacin, amoxycillin–clavulanate, piperacillin–tazobactam, imipenem and trimethoprim–sulphamethoxazole were determined by microdilution assay according to NCCLS guidelines [20,21]. *E. coli* ATCC 25922, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 700603 were used as control strains.

**Molecular typing**

Clonal relationships were determined by REP-PCR using primers REP-1 (5′-IIIGCCGGCCICATCAGGC-3′) and REP-2 (5′-ACGTCTATCACGGCCTAC-3′) [22] for isolates collected between 1995 and 2001. DNA was extracted by boiling a suspension of a colony at 100°C for 10 min. The supernatant from this suspension (24.5 μL) was used as the PCR template. All reactions were performed in a 50 μL-volume, using 2 U of *Taq* DNA polymerase (Invitrogen, Barcelona, Spain). Cycling conditions comprised 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 40°C for 1 min, and 65°C for 8 min, with a final extension at 65°C for 8 min. DNA band patterns were analysed visually following electrophoresis on agarose 1.5% gels. Two isolates were considered to be clonally related when the number of DNA bands and their positions matched.

**β-Lactamase characterisation**

Isoelectric focusing and PCR were used for preliminary characterisation of the β-lactamases produced and their respective *bla* genes. Isoelectric focusing (PhastGel IEF 3-9; Pharmacia, Barcelona, Spain) was performed to identify the numbers and isoelectric points (pI) of the β-lactamases present. The focused β-lactamases were detected by overlaying the gel with nitrocefin (0.5 mg/mL) [23–25]. Known producers of TEM-1, TEM-24, SHV-2 and CTX-M-9 β-lactamases were included as controls.

The presence of *bla* genes was detected with oligonucleotide primers designed to amplify the most common subgroups within the ESBL family: *bla*TEM [26], *bla*SHV [26], *bla*CTX-M-10 [27] and *bla*CTX-M-9 [28]. The primers used for each individual isolate were selected according to the isoelectric focusing results, namely pI 4.5–6.5 for *bla*TEM, and pI 7.0–8.2 for *bla*SHV and *bla*CTX. All reactions were performed in 25-μL volumes with 2 U of FastStart Polymerase (Roche Diagnostics, Mannheim, Germany). Cycling parameters comprised 95°C for 4 min, followed by 35 cycles of 95°C for 30 s, 58°C for 30 s for TEM and SHV, 62°C for 30 s for CTX-M-9 or 60°C for 30 s for CTX-M-10, and 72°C for 1 min, with a final extension at...
72°C for 7 min. Positive controls were strains of *E. coli* containing either the *bla*<sub>TEM-1</sub>, *bla*<sub>TEM-3</sub>, *bla*<sub>SHV-1</sub>, *bla*<sub>SHV-5</sub>, *bla*<sub>CTX-M-9</sub> or *bla*<sub>CTX-M-10</sub> genes, with *E. coli* J53 Rif-R as a negative control.

Amplicons were sequenced by CSIC (Madrid, Spain), equipped with an ABI PRISM 377 sequencer (Applied Biosystems, Foster City, USA). Sequences were analysed with the Chromas application and the BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and Traduction Multiple (Infobiogen; http://www.Infobiogen.fr/services/analyzeq/cgi-bin/traduc-in.pl) algorithms.

**RESULTS**

In total, 25 447 *E. coli* and 2790 *K. pneumoniae* isolates were recovered during 1995–2003. ESBL production was detected in 438 (1.7%)*E. coli* isolates recovered from 438 patients, and in 125 (3.98%) *K. pneumoniae* isolates from 122 patients.

The median age of patients infected with an ESBL-producing isolate was 67 years (range 0–89 years), and a higher proportion of female patients was infected with ESBL-producing *E. coli* than with ESBL-producing *K. pneumoniae* (69% vs. 44%; p 0.0003). The ESBL-producing *E. coli* and *K. pneumoniae* isolates were recovered from the following specimens: urine, 70% and 54% (p 0.004); blood, 9% and 14% (p 0.2); wounds, 13% and 11% (p 0.6); abscesses, 4% and 6% (p 0.5); respiratory tract, 1% and 9% (p 0.009); and others (catheter, faeces and biopsies), 3% and 6% (p 0.3), respectively.

By the microdilution method, all ESBL-producing *E. coli* and *K. pneumoniae* isolates were susceptible to carbapenems, and showed similar rates of susceptibility to amikacin (91% and 94%; p 0.4), gentamicin (60% and 64%; p 0.5), amoxycillin-clavulanate (69% and 61%; p 0.2) and trimethoprim–sulphamethoxazole (38% and 40%; p 0.7). However, susceptibility rates differed between these microorganisms for piperacillin–tazobactam (75% of ESBL-producing *E. coli* and 52% of ESBL-producing *K. pneumoniae*; p 0.0001) and ciprofloxacin (17% of ESBL-producing *E. coli* and 50% of ESBL-producing *K. pneumoniae*; p < 0.0001).

During the study period, the annual distribution of ESBL-producing microorganisms was different for *E. coli* and *K. pneumoniae*. From 1995 to 1998, the absolute number of ESBL-producing *E. coli* isolates was less than seven per annum. After 1999, a steady increase was observed, reaching 139 isolates in 2003. In contrast, the annual number of ESBL-producing *K. pneumoniae* isolates decreased gradually from 34 in 1995 to nine in 2003. Similarly, the frequency of ESBL-producing *E. coli* isolates also increased gradually, from 0.31% in 1995 to 4.8% in 2003, while the frequency of ESBL-producing *K. pneumoniae* isolates decreased. The median frequency of ESBL-producing *K. pneumoniae* isolates in the period 1995–1997 was 7.2% (range, 5.2–9.7%), compared with 2.5% (range, 1.7–3.2%) in the period 1998–2003.

The ESBL-producing isolates of *E. coli* and *K. pneumoniae* were from non-hospitalised patients in 64% and 36% of the cases, respectively (p < 0.0001). The annual distributions for both microorganisms according to origin are shown in Fig. 1. The increase in ESBL-producing *E. coli* occurred almost simultaneously in non-hospitalised and hospitalised patients, although the increase was observed in inpatients a year earlier.

![Fig. 1. Comparison of the frequency of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* according to origin. (a) Frequency of ESBL-producing *E. coli* from inpatients and outpatients. (b) Frequency of ESBL-producing *K. pneumoniae* from inpatients and outpatients.](image-url)
All ESBL producers (n = 415) isolated during 1995–2002 were examined by isoelectric focusing and PCR for the presence of the blaCTX-M, blTEM and blaSHV genes. All β-lactamase bands with a pI that might correspond to TEM-1, TEM-2 or SHV-1 were excluded from the analysis. Multiple β-lactamase genes were identified in 53 (17%) ESBL-producing E. coli isolates (48 with two genes; five with three genes) and in 59 (51%) ESBL-producing K. pneumoniae isolates (Tables 1 and 2). SHV enzymes were the most common ESBLs detected in K. pneumoniae (97% of the isolates) (Table 2), whereas CTX-M and SHV enzymes were the most common types in E. coli, and were observed at similar frequencies (46% and 44%, respectively; Table 1). During 1995–1998, the rare ESBL-producing E. coli isolates were predominantly SHV producers, while the increase in the total percentage of ESBL-producing E. coli isolates from 1999 was associated with isolates producing any of the three types of ESBL. However, the relative frequency of isolates producing CTX-M-type enzymes tended to increase, while that of those producing SHV tended to decrease (Table 1). In contrast, all K. pneumoniae isolates produced SHV-type enzymes during 1995–2000, often in combination with TEM-type enzymes, but a few CTX-M-producing K. pneumoniae isolates began to appear during 2001 and 2002 (Table 2).

In total, 262 (48%) bla genes were sequenced, of which 68% encoded CTX-M enzymes (100 (97%) CTX-M-14 and three (3%) CTX-M-9), 56% encoded SHV enzymes (112 (80.5%) SHV-12, 25 (18%) SHV-4, and two (1.5%) SHV-2a), and 14% encoded TEM enzymes (ten (50%) TEM-4, eight (40%) TEM-20, and two (10%) TEM-53).

The clonal relationships of all ESBL-producing isolates detected from January 1995 to June 2001 (119 E. coli and 103 K. pneumoniae) were evaluated by REP-PCR. For the 119 ESBL-producing E. coli isolates, 84 distinct REP-PCR patterns were obtained, distributed in 69 non-related patterns and 15 clonally related groups. There was great variability in the different genotypes of E. coli, with a median of 2–5 different bands among the most related isolates. During the total period, 13 clonal groups of 2–4 isolates were observed, with two larger groups (seven and nine isolates, respectively) during the 2000–2001 period. The largest group comprised isolates from inpatients in the long-term care hospital, and the other clonally related ESBL-producing E. coli groups also comprised isolates mainly (63.4%) from hospitalised patients. In contrast, ESBL-producing K. pneumoniae isolates were more related, with the 103 isolates belonging to only five clonally related groups, of which one predominated (containing 92 isolates from different wards throughout the total study period).

## DISCUSSION

ESBL-producing Enterobacteriaceae are among the most problematic multiresistant bacteria worldwide and are being isolated with increased frequency [2,29–31]. Most isolates are recovered from hospitalised patients, but recent data indicate an increasing problem in nursing homes and in the community [16,31]. Such surveys usually describe a transient situation for an institution or region at a certain time-point, and it is difficult to gain an overview of the general situation regarding the main ESBL-producing organisms.

### Table 1. Annual distribution of extended-spectrum β-lactamase types in Escherichia coli

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of isolates</th>
<th>TEM n %</th>
<th>SHV n %</th>
<th>CTX-M n %</th>
<th>No. of enzymes per isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>34</td>
<td>2 6</td>
<td>34 100</td>
<td>0 0</td>
<td>32 2 0</td>
</tr>
<tr>
<td>1996</td>
<td>18</td>
<td>14 78</td>
<td>18 100</td>
<td>0 0</td>
<td>4 14 0</td>
</tr>
<tr>
<td>1997</td>
<td>21</td>
<td>15 71</td>
<td>21 100</td>
<td>0 0</td>
<td>6 15 0</td>
</tr>
<tr>
<td>1998</td>
<td>12</td>
<td>10 83</td>
<td>12 100</td>
<td>1 8</td>
<td>1 11 0</td>
</tr>
<tr>
<td>1999</td>
<td>9</td>
<td>7 78</td>
<td>9 100</td>
<td>0 0</td>
<td>2 7 0</td>
</tr>
<tr>
<td>2000</td>
<td>5</td>
<td>4 80</td>
<td>5 100</td>
<td>0 0</td>
<td>1 4 0</td>
</tr>
<tr>
<td>2001</td>
<td>8</td>
<td>2 25</td>
<td>8 100</td>
<td>1 12</td>
<td>5 3 0</td>
</tr>
<tr>
<td>2002</td>
<td>9</td>
<td>3 33</td>
<td>6 66</td>
<td>3 33</td>
<td>6 3 0</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>57 49</td>
<td>113 97</td>
<td>5 4</td>
<td>57 59 0</td>
</tr>
</tbody>
</table>

### Table 2. Annual distribution of extended-spectrum β-lactamase types in Klebsiella pneumoniae

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of isolates</th>
<th>TEM n %</th>
<th>SHV n %</th>
<th>CTX-M n %</th>
<th>No. of enzymes per isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>34</td>
<td>2 6</td>
<td>34 100</td>
<td>0 0</td>
<td>32 2 0</td>
</tr>
<tr>
<td>1996</td>
<td>18</td>
<td>14 78</td>
<td>18 100</td>
<td>0 0</td>
<td>4 14 0</td>
</tr>
<tr>
<td>1997</td>
<td>21</td>
<td>15 71</td>
<td>21 100</td>
<td>0 0</td>
<td>6 15 0</td>
</tr>
<tr>
<td>1998</td>
<td>12</td>
<td>10 83</td>
<td>12 100</td>
<td>1 8</td>
<td>1 11 0</td>
</tr>
<tr>
<td>1999</td>
<td>9</td>
<td>7 78</td>
<td>9 100</td>
<td>0 0</td>
<td>2 7 0</td>
</tr>
<tr>
<td>2000</td>
<td>5</td>
<td>4 80</td>
<td>5 100</td>
<td>0 0</td>
<td>1 4 0</td>
</tr>
<tr>
<td>2001</td>
<td>8</td>
<td>2 25</td>
<td>8 100</td>
<td>1 12</td>
<td>5 3 0</td>
</tr>
<tr>
<td>2002</td>
<td>9</td>
<td>3 33</td>
<td>6 66</td>
<td>3 33</td>
<td>6 3 0</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>57 49</td>
<td>113 97</td>
<td>5 4</td>
<td>57 59 0</td>
</tr>
</tbody>
</table>
The present study offers a view of the dynamic evolution of the two most significant ESBL-producing organisms, with isolates from both hospitalised and non-hospitalised patients. The results show an important increase in the frequency of ESBL-producing E. coli isolates in this area of Spain, beginning in 1999, confirming the impression that this organism is an emerging multiresistant pathogen. During 1999, the isolation of ESBL-producing E. coli strains found in the SENTRY project was 3.3–8.5% in Latin America [29], with an even higher frequency of 23.6% reported from China [32], compared with a very low frequency of 0.25% reported from Canada in 1999–2000 [12]. The final 3 years of the present study found a median incidence of 24 cases/100 000 population/year, which is significantly higher than that recorded in the Calgary region (5.5/100 000 inhabitants/year) during the same period, including both inpatients and outpatients [16]. In contrast, the overall ESBL-producing K. pneumoniae prevalence (3.8%) was lower than in previous studies [2,29]. The decreased isolation frequency of ESBL-producing K. pneumoniae observed during the last 6 years could be related to the strict and successful infection control measures introduced in this institution to control methicillin-resistant Staphylococcus aureus and multiresistant Acinetobacter baumannii [33].

Based on the origin of the isolates, ESBL-producing E. coli and K. pneumoniae seem to show different kinds of epidemiological behaviour. While ESBL-producing K. pneumoniae has been, and still is, mainly a nosocomial pathogen, ESBL-producing E. coli is also emerging as an important pathogen in outpatients [16]. The ESBL-producing E. coli isolates from non-hospitalised patients emerged a year after the observed increase in the number of isolates from hospital wards, but there was subsequent parallel development. Previous hospitalisation was identified as one of the main risk-factors for ESBL-producing E. coli infections in non-hospitalised patients [15], and this could partly explain the emergence of these strains in non-hospitalised patients after a delay of 1 year. However, these data should not lead to an assumption that the emergence of ESBL-producing E. coli in the community proceeds mainly from the dissemination of hospital strains. Some patients might have acquired ESBL-producing E. coli in the community, but then developed infections caused by these organisms following hospital admission, since hospitalised patients more frequently have risk-factors (antimicrobial use, debilitating underlying diseases, invasive procedures) for infection with multiresistant organisms. Of concern was the fact that E. coli isolates were more frequently ciprofloxacin-resistant than were K. pneumoniae isolates. This association, which has also been observed previously by Tolun et al. [34], has not been explained, although previous fluoroquinolone treatment has been identified as a risk-factor for ESBL-producing E. coli infection in non-hospitalised patients [15].

Another characteristic differentiating between ESBL-producing E. coli and K. pneumoniae isolates was the number of clonally related groups found during the study period. The emergence in our area of ESBL-producing E. coli since 1999 was caused mainly by genetically unrelated isolates. This phenomenon has been observed previously in isolates from an intensive care unit [32] and from the community [14]. Although E. coli is associated less frequently with clonal dissemination, the small group of nine strains isolated in the long-term care hospital was an interesting observation, reflecting reports of small groups of ESBL-producing E. coli in different Russian hospitals [18], and the transmission of an epidemic strain in three geriatric hospitals [9]. In contrast, ESBL-producing K. pneumoniae isolates were more genetically related, with the endemic persistence of one of the five REP-PCR genotypes. Similar results, with a large number of isolates in each cluster, were obtained in a previous 1-year analysis of strains from an intensive care unit [32].

The number of isolates producing multiple β-lactamases was much higher among K. pneumonia than among E. coli, but was similar to that found in a previous survey [32]. The most frequent type of ESBL in K. pneumoniae in the present study was SHV, compared with SHV and CTX-M in E. coli. TEM has been reported to be more prevalent in E. coli in some surveys [32], and CTX-M in others [35], while a clear predominance has not been found in K. pneumoniae [17,32,36]. In our area, the CTX-M-producing E. coli isolates emerged in 1999, and have gradually become more prevalent. This agrees with the increased frequencies of CTX-M-producing bacteria in reports from Europe, North and South America, Africa and Asia [1,5].

In summary, these results demonstrate the emergence of ESBL-producing E. coli in the
context of increasing control over ESBL-producing \textit{K. pneumoniae}. The present data also indicate that the epidemiologies of these two species show substantial differences, in that ESBL-producing \textit{E. coli} strains were isolated from hospitalised and non-hospitalised patients, produced different types of ESBLs and were clonally diverse, while ESBL-producing \textit{K. pneumoniae} strains were isolated mainly from hospitalised patients, produced predominantly SHV-type enzymes and tended to be clonally related. These epidemiological characteristics are shared with other ESBL-producing enterobacteria, such as \textit{Enterobacter aerogenes} and \textit{Proteus mirabilis}, which are detected increasingly in France [37], Belgium [38] and Italy [39,40]. These differences may have implications for the underlying rationale of the control measures used for these microorganisms. In the period of analysis, except for well-defined cases, there was no real epidemic or endemic problem, but, rather, an ‘allodemic situation’, to use the term proposed by Baquero \textit{et al.} [41].

The present study had some limitations, in that the clinical aspects were not considered, the colonisation rate in the community was not known, and plasmid analysis was not performed. Several issues remain unresolved, including the question as to whether dissemination of a plasmid or transposable element was involved, or whether these observations result from the evolutionary success of a particular enzyme (CTX-M type vs. SHV type). Further investigations are required to evaluate the impact of antibiotic selection, the dynamic flow of organisms and genes between hospital and community, community colonisation, and the multiple origins of isolates. On the basis of these data, different interventions to control host-to-host spread should be designed which take into account the fact that the hospital is not a microbiologically separate entity from the rest of the care system.

\section*{Acknowledgements}
This study was supported, in part, by REIPI (Red Española de Investigación en Patología Infecciosa) grant CO3/14, Spain.

\section*{References}


16. Pitout J, Hanson N, Church D, Laupland K. Population-based laboratory surveillance for \textit{Escherichia coli} producing extended-spectrum beta-lactamases: importance of com-