Foodborne Nosocomial Outbreak of SHV1 and CTX-M-15–producing Klebsiella pneumoniae: Epidemiology and Control

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**Background.** We describe a foodborne nosocomial outbreak due to extended-spectrum β-lactamase (ESBL)–producing Klebsiella pneumoniae.

**Methods.** An outbreak of ESBL K. pneumoniae was detected in March 2008. Initial control measures included contact isolation and a protocol for routine detection and reinforcement in hand hygiene practices. ESBL producers were screened for the blaTEM, blaSHV, and blaCTX-M genes. Pulsed-field gel electrophoresis analysis was performed using XbaI as a restriction endonuclease.

**Results.** One hundred fifty-six colonized and/or infected patients were identified, 35 (22.4%) of whom had infection. The outbreak affected all hospital wards. Fecal carriage was up to 38% of patients in some wards. Of note, investigation revealed a very short delay between admission and colonization. None of the health care workers or environmental surfaces in the wards was found to be colonized. This prompted an epidemiological investigation of a possible foodborne transmission. We found that up to 35% of the hospital kitchen–screened surfaces or foodstuff were colonized and that 6 (14%) of 44 food handlers were found to be fecal carriers. Phenotypic and genotypic analysis of all clinical, environmental, and fecal carrier isolates showed the dissemination of a single strain of SHV-1 and CTX-M-15–producing K. pneumoniae. At that time, structural and functional reforms in the kitchen were performed. These were followed by a progressive reduction in colonization and infection rates among inpatients until complete control was obtained in December 2008. No restrictions in the use of antibiotics were needed.

**Conclusions.** To our knowledge, this is the first reported hospital outbreak that provides evidence that food can be a transmission vector for ESBL K. pneumoniae.

*Klebsiella pneumoniae* has been associated with 2%–5% of nosocomial infections, particularly those involving the urinary and respiratory tracts [1, 2]. Resistance of this species to third-generation cephalosporins was first described in 1983 [3]. Since that time, the prevalence of extended-spectrum β-lactamase (ESBL)–producing *K. pneumoniae* strains in hospitals has progressively increased. Especially feared are epidemic hospital infections caused by multidrug-resistant strains. In a recent international surveillance survey, 31% of episodes of nosocomial *K. pneumoniae* bacteraemia were caused by ESBL-producing strains [4]. In intensive care units, where antibiotic consumption is heaviest and the potential for patient to patient transmission of organisms is greatest, 43.5% of episodes of bacteraemia due to *K. pneumoniae* were caused by ESBL-producing strains [4]. Highest frequencies have been reported in some areas, such as Brazil, where up to >50% of *K. pneumoniae* isolates in bloodstream infections were
ESBL producers [5]. To date, >30 hospital outbreaks due to ESBL-producing K. pneumoniae (ESBL-KP) have been reported [6].

Classically, the lower gastrointestinal tract of colonized patients has been thought to be the main reservoir of these microorganisms, and cross-contamination has been documented through the hands of health care workers in the hospital setting [7]. However, increasing evidence suggests that food can contribute to the dissemination of ESBL-producing Enterobacteriaceae in the community [8, 9].

Nosocomial outbreaks of foodborne ESBL-KP have not been reported. The present study describes the epidemiology and measures taken for control of a foodborne nosocomial outbreak of ESBL-KP in an acute care hospital.

MATERIALS AND METHODS

Setting
The Hospital Universitari Mútua Terrassa is a 500-bed acute care institution in Barcelona, Spain, with ∼26,000 hospital admissions/year, for an area of with 350,000 inhabitants.

Definition and Extent of the Outbreak
The outbreak started in March and ended in December 2008. During the study period, 22,484 admissions were registered.

The outbreak investigation started on 2 June with the identification during routine infection control surveillance of 2 simultaneous infected patients with a multidrug-resistant ESBL-KP strain. Both patients had shared a room in a medical ward for 13 days. Records of the Microbiology Laboratory were reviewed, and 4 other cases were identified since March. Then, a prospective epidemiological study was undertaken. A case was defined in any inpatient by culture demonstration of colonization and/or infection due to ESBL-KP. Nosocomial infections were diagnosed and classified according to standard definitions of the Centers for Disease Control and Prevention [10].

After the first 2 cases were identified, a protocol for routine detection of colonization was implemented. On 6 June, a rectal swab from all inpatients allocated in the affected ward was obtained. Because of the high prevalence of colonization, the protocol was extended progressively to the other hospital wards. Nine of 14 wards were screened ≥1 times from 6 June through 18 November. Cohorting isolation measures were applied to colonized or infected patients until discharge.

A survey of environmental colonization of ESBL-KP was performed in selected wards. Samples were obtained for culture by rubbing moistened gauzes repeatedly over designated sites in the immediate vicinity of the patient environment, over equipment used in patient care, and in the general areas in all the compartments of the studied wards. Gauzes were immersed, using sterile gloves, in a screw-cap sterile container with 10 mL of thioglycolate broth. Then, gauzes were rubbed over designated sites, and they were returned to the container. Gloves were removed, and hands were washed. The containers were incubated overnight to 37°C and then sampled on ChromID ESBL (bioMerieux).

Simultaneously, a global proactive campaign to reinforce hand hygiene practices and glove use was started.

On 22 July, a number of events seemed to justify a modification of the surveillance and control strategy. First, the number of colonized patients was so high that the isolation measures were impossible to perform, and second, the investigations conducted suggested that the possible outbreak source was related to the food chain. Consequently, isolation practices for colonized patients were abandoned.

Clinicians were informed about the ongoing outbreak and advised to pay special attention to empirical therapy of nosocomial infections. No antimicrobial use restrictions were applied.

A case-control study to compare the frequency of exposure and the clinical features of case patients with those of control patients was performed to identify and quantify potential independent risk factors associated with ESBL-KP colonization and/or infection. Control patients were any culture-negative inpatients admitted during the outbreak period in any of the screened wards. Control patients were matched 1:1 by sex, age, ward, and date of culture. Demographic characteristics, comorbidities, exposure to different antibiotics, length of stay before colonization and/or infection, diet, menu, and allocation were analyzed.

Verbal consent was obtained from all patients or by a first-degree relative and from all hospital employees screened.

Bacterial Isolates
A representative sample of the isolates (1 sample per patient [94 strains]) and from environmental sources (21 strains) was submitted for further study.

Determination of ESBL Production
All K. pneumoniae isolates that exhibited ESBL production and were obtained during the study period from any clinical specimen by conventional laboratory procedures were included. Screening samples were plated on ChromID ESBL (bio-Mérieux). K. pneumoniae identification and susceptibility testing were performed using Vitek Two (bioMérieux). ESBL production was detected using the double-disk synergy test (CLSI 2009, M100–S19) [11].

Characterisation of the bla Genes by Polymerase Chain Reaction (PCR) and DNA Sequencing
ESBL producers were screened for the blaTEM, blashv, and blaCTX-M genes. Individual PCR was performed in the first 2 cases, whereas a multiplex PCR was used to characterize the
specific CTX-M group (1, 2, 8, 9, and 25). After CTX-M group identification, specific primers for each group were used to determine the allelic variant. In the present study, only primers CtxM-1F and CtxM-1R, used to identify members of group 1, were required. Furthermore, discrimination between CTX-M15 and CTX-M28, both group 1 members, was performed using primers Ctx1-15/28F and Ctx1-15/28R to amplify the 3’ end of the gene. DNA sequencing was then performed using the BigDye Terminator, version 3.1, cycle sequencing kit and an ABI prism 3700 sequencer (Applied Biosystems). All primers are listed in Table 1 [11–15].

Molecular Typing
To determine the genetic relationship of the isolates in our study, pulsed-field gel electrophoresis analysis was performed using XbaI as a restriction endonuclease and electrophoresing the genome in a CHEF DR III system (Bio-Rad) at 6 volts with pulse times of 5–35 s and linear ramping at a temperature of 14°C for 20 h. Clonality was accepted in accordance with Tenover’s criteria [16].

Statistical Analysis
To investigate potential independent risk factors associated with ESBL-KP colonization and/or infection, case patients were compared with control patients in terms of exposure to the different variables studied. For independent samples, χ² test or Fisher’s exact test were used for categorical variables; for continuous variables, Student’s t test and the Mann-Whitney U test were used. The accepted statistical significance level was P < .05. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. The OR was calculated using conditional logistic regression. SPSS, version 11.5 was used for analysis.

RESULTS
The outbreak lasted for 9 months. During the outbreak, 1100 screening samples were obtained. One hundred fifty-six colonized and/or infected patients were identified (Figure 1). The mean age of the patients was 69 years, and 57% were men. Thirty-five patients (22.4%) were infected (24 urinary tract infections, all in patients with indwelling urinary catheter; 5 surgical site infections; 5 cases of primary bacteremia; 1 pneumonia). Twelve colonized and 2 infected patients were in the intensive care unit. Five patients died. No death was related to infection.

From June through November 2008, patients in medical and surgical wards and in the intensive care units were periodically screened. In Figure 2, we summarize the percentage of fecal carriers out of the total number of cultured cases on a monthly basis. Only the ward with the highest proportion of colonized patients is shown. In June and July, 26% and 38% of cultured patients were colonized, respectively.

Because of the rapid and massive spread of the outbreak, we decided to exclude a community origin. Rectal swab specimens were obtained from 30 outpatients with no previous hospital admission who visited the emergency department in June 2007. Considering the high incidence found among inpatients, we

Table 1. Primers Used in the Characterization of the Extended-Spectrum β-Lactamase –Producing Klebsiella pneumoniae

<table>
<thead>
<tr>
<th>PCR target</th>
<th>Primer name (sequence)</th>
<th>Temperature, °C</th>
<th>Fragment size, bp</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single PCR</td>
<td>blaTEM</td>
<td>Tem-F GACAGTTACCAATGCTTAATC Tem-R ATAAAAATCTTGAAGACGAAA</td>
<td>55</td>
<td>1079</td>
</tr>
<tr>
<td></td>
<td>blaSHV</td>
<td>SHV_F CGCCGGTGTATTCATTTTGTGC SHV_R TCTTCTCGATGCAGCGAGCGAGTCGA</td>
<td>67</td>
<td>1017</td>
</tr>
<tr>
<td></td>
<td>blaCTX-M</td>
<td>CtxM-1F ATGGTTAAAAATCACTG CtxM-1R TTACAAACCGTYGGTGAC</td>
<td>50</td>
<td>876</td>
</tr>
<tr>
<td></td>
<td>blaCTX-M_15/28</td>
<td>Ctx1-15/28F ATGGTTAAAAATCACTG Ctx1-15/28R GAATTTTGACGATCGGGG</td>
<td>54</td>
<td>483</td>
</tr>
<tr>
<td>Multiplex PCR</td>
<td>Group 1 blaCTX-M</td>
<td>Ctxm1-F AAAAATCACTGGCCAGTCGAGTC</td>
<td>52</td>
<td>415</td>
</tr>
<tr>
<td></td>
<td>Group 2 blaCTX-M</td>
<td>Ctxm2-F CGACGGCTAATCCGCTTAGT Ctxm2-R CGACGGCTAATCCGCTTAGT</td>
<td>52</td>
<td>552</td>
</tr>
<tr>
<td></td>
<td>Group 9 blaCTX-M</td>
<td>Ctxm9-F CAAAGAGAGTGCAACGGATG Ctxm9-R ATTGGAAAGCTTATCACC</td>
<td>52</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>Group 8 blaCTX-M</td>
<td>Ctxm8-F TGGCCATGGGCAAGGATG Ctxm8-R AACCCACGATGTTGGTAG</td>
<td>52</td>
<td>666</td>
</tr>
<tr>
<td></td>
<td>Group 25 blaCTX-M</td>
<td>Ctxm25-F GCAACGATGACATCGGGG Ctxm25-R AACCCACGATGTTGGTAG</td>
<td>52</td>
<td>327</td>
</tr>
</tbody>
</table>

Abbreviation: PCR, polymerase chain reaction
thought that the 30 negative consecutive fecal samples from 30 outpatients were enough. We realize that this sample size is too small to absolutely exclude that possibility.

Thereafter, a foodborne outbreak was contemplated. Food catering for inpatients was provided by an external company and had 2 differentiated lines. One line was exclusive for hospital employees (health care workers and administrative personnel), and the other was for inpatients. To explore this hypothesis, on 11 July, stool cultures of food handlers were performed. In addition, on 22 July, rectal swab specimens were obtained from 54 hospital employees. The cultured group included 30 administrative personnel (with no patient contact) and 24 health care workers. These cohorts did not share the catering service with the inpatients.

During the outbreak, many factors led the investigation to the hospital kitchen and food processing chain: (1) the high prevalence of fecal colonization in many different hospital units (up to 38% in some wards), (2) the rapidity of its spread (all medical and surgical wards were simultaneously affected), (3) the early colonization soon after admission (8 patients were colonized < 48 h after admission), (4) the all results of environmental cultures (n = 31) from hospital surfaces were negative, (5) no colonization among outpatients was detected, and (6) none of the 54 hospital employees from who samples were obtained were fecal carriers. Moreover, in the kitchen, 6 (14%) of 44 workers were found to be transient fecal carriers. One of them was directly a food handler. The remaining 5 were cleaning staff. All of them worked during the evening shift, and all previously ate dinner in the hospital and shared exactly the same daily menu of inpatients. None of the food handlers employed in the morning shift was colonized. Some of them previously ate lunch in the hospital, but they received the same menu than the rest of hospital employees that was different than the inpatients menu.

The first environmental and food samples in the kitchen were obtained for culture on 25 July. Eighteen (37.5%) of 48 cultured surfaces and 1 of 12 foodstuff samples were contaminated. Among foodstuff tested, only 1 handmade fruit puree was found to be contaminated. When these findings became known, structural and functional changes and cleaning measures in the kitchen area were performed. No more fruit purees were served in the hospital. Since then, no other sample was found to be contaminated. Three (10%) of 30 environmental kitchen samples on 7 August and 1 (2.13%) of 47 samples on 3 October were still positive. These findings could explain at least in part the persistence of the outbreak over time. New special cleaning measures were applied on 17 August. The last kitchen environmental cultures were done on 2 December. All 48 samples taken were negative.

During the outbreak, we identified the only 2 populations at risk of infection: inpatients with indwelling urinary catheter and patients with solid cancer or hematologic malignancies. Therefore, from 22 July through 28 October, we decided to restrict the surveillance to weekly urine cultures for all inpatients with indwelling urinary catheters and rectal swabs for immunocompromised patients and for patients admitted to the intensive care unit. Figure 3 shows the prevalence of urinary colonization.

After the intervention in the hospital kitchen, the percentage of colonized and/or infected patients experienced a progressive reduction. None of the 35 patients in November 2008 was positive. A new screening surveillance was conducted in May 2009. Fecal colonization was screened in 39 inpatients allocated in the wards with the previous highest colonization rates. No new cases of nosocomial colonization or infection were identified.

In bivariate analysis, no statistically significant differences were found. Therefore, we included in the multivariate analysis the variables with the highest ORs in bivariate analysis and that were considered to be clinically relevant: sex, age, use of previous surfaces and a...
antibiotic, length of stay, and consumption of pasta, soup, or meat. Multivariate analysis identified previous exposure to any antimicrobial (OR, 4.87; 95% CI, 1.65–14.37) and having eaten soup (OR, 6.34; 95% CI, 1.26–31.76) as risk factors for colonization or infection.

The antimicrobial susceptibility pattern of the ESBL-KP showed resistance to penicillins and third-generations cephalosporins. In addition, 85.2% of the strains were resistant to amoxicillin-clavulanate, and 11.1% were resistant to piperacillin-tazobactam. All strains were resistant to ciprofloxacin and susceptible to carbapenems and amikacin (table 2).

Phenotypic and genotypic analysis of the isolates showed that there was a predominant clone (clone A; 87 of 94 isolates studied); the remaining isolates belong to different clones (B–F) and clone G, which included 2 isolates (Figure 4). The strain isolated in the initially infected patients was clone A. All 21 strains from environmental sources also belonged to clone A. Further characterization of the underlying mechanisms of resistance to cephalosporines showed that clone A carried 2 different enzymes: SHV-1 and CTX-M-15.

**DISCUSSION**

We report the rapid and widespread dissemination of an epidemic clone of CTX-M-15 and SHV-1–producing *K. pneumoniae* through the food chain in an acute care hospital. Many findings support the hypothesis of (1) the early and massive fecal colonization among inpatients allocated in all hospital wards, (2) the lack of environmental contamination in the wards affected or among health care workers, and (3) the identification of fecal carriers among food handlers and persistent contamination on surfaces of tables, sinks, and washing machines in the kitchen.

There is evidence that food can be a transmission vector for ESBL-producing *Enterobacteriaceae* in community-acquired outbreaks of gastroenteritis [9]. The prevalence of ESBL-producing enterobacteria in outbreaks of foodborne infections (up to 31%) and the high percentage of carriers in each outbreak (range, 4.4%–66.6%) reinforce the hypothesis that ESBL-producing *Enterobacteriaceae* could be transmitted via the food supply [17]. In the same vein, several studies have shown that these strains can be recovered from food animals and pets [8, 18]. A recent paper has evaluated ESBL-producing *Escherichia coli* isolates obtained from infected patients and from retail meat. The authors reported that the ESBL gene content of the *E. coli* isolates of food origin correlated well with the genes that were locally prevalent in the clinical isolates [19].

It was unclear how the first ESBL-KP strain was imported to the kitchen and, consequently, to the food chain and food handlers. In fact, food and food handlers have been recognized as possible reservoirs for ESBL-producing *Enterobacteriaceae* [9].

Horizontal spread has been repeatedly recognized as the major mechanism for the emergence and maintenance of

**Table 2. Antimicrobial Susceptibility of 72 Isolates of the Epidemic Extended-Spectrum β-Lactamase–Producing *Klebsiella pneumoniae* Strain**

<table>
<thead>
<tr>
<th>Susceptibility</th>
<th>AMP</th>
<th>AMC</th>
<th>CXM</th>
<th>FOX</th>
<th>CTX</th>
<th>CAZ</th>
<th>CEF</th>
<th>AZT</th>
<th>GM</th>
<th>TO</th>
<th>AK</th>
<th>SXT</th>
<th>CIP</th>
<th>IMI</th>
<th>PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range, μg/mL</td>
<td>≥32</td>
<td>&gt;64</td>
<td>&lt;4</td>
<td>&gt;64</td>
<td>16</td>
<td>2</td>
<td>R</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&gt;1</td>
<td>&gt;4</td>
<td>&lt;1</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>128</td>
</tr>
<tr>
<td>MIC90, μg/mL</td>
<td>≥32</td>
<td>&gt;64</td>
<td>&lt;4</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>R</td>
<td>4</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td>16</td>
<td>4</td>
<td>16</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Resistance, %</td>
<td>100</td>
<td>85.2</td>
<td>100</td>
<td>11.1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>7.4</td>
<td>95.1</td>
<td>60</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>11.1</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AK, amikacin; AMC, amoxicillin-clavulanic; AMP, ampicilin; AZT, aztreonam; CAZ,cefazidime; CEF, cefepime; CIP, ciprofloxacin; CTX, cefotaxime; CXM, cefuroxime; GM, gentamicin; IMP, imipenem; MIC90, 90% minimum inhibitory concentration; PT, piperacillin-tazobactam; STX, trimethoprim-sulfamethoxazole; TO, tobramycin.
ESBL-producing K. pneumoniae outbreaks. Traditionally, ESBL-producing K. pneumoniae outbreaks have been related to cross transmission, especially in areas where antibiotic use is heaviest and the potential for patient to patient transmission of organisms is greatest, such as intensive care units, neonatal units, and surgical units. Several recent reports confirm this assumption [4].

In previously reported outbreaks of ESBL-producing K. pneumoniae, removable environmental sources were rarely identified. Only in a few cases, a common source causing the dissemination of the ESBL has been reported [20, 21]. Rather, as it has been explicitly documented by several investigations [22–25], ESBL-producing K. pneumoniae transiently colonizes the hands of hospital staff members, thereby facilitating patient to patient transmission.

Because of the unusual mode of transmission of the present outbreak, infection control measures, such as isolation of colonized patients, use of gloves, and hand washing, did not halt the outbreak, although they were probably effective in preventing horizontal transmission and a wider dissemination of the multidrug-resistant strain. The outbreak was stopped only after control measures were applied in the kitchen. The absence of new cases during the 14-month follow-up period suggests the effectiveness of these measures.

Studies of outbreaks of ESBL-producing Klebsiella species have shown that their emergence is usually associated with the use of third-generation cephalosporins [28–30] or all cephalosporins [28–30] is followed by a decrease in the occurrence of ESBL-producing organisms. We also identified previous exposure to antibiotics as a risk factor for colonization or infection due to the ESBL-KP epidemic strain. Nevertheless, the present outbreak differs from those previously described in an important aspect: no antibiotic restrictions were applied. The identification and control of the source were enough to achieve a rapid control of the outbreak.

The strain causing the outbreak was a CTX-M-15 producer. During the past decade, the CTX-M type has represented the most rapidly growing group of ESBLs. Specifically, CTX-M-15 has emerged as the dominant type of ESBL in gram-negative pathogens causing outbreaks in nosocomial and community settings [31]. The clonal dissemination of CTX-M-15–producing E. coli has been recognized in many areas [32–34]. This has generated an important genetic reservoir from which other species that share the same ecological environment, such as K. pneumoniae, could easily acquire this resistance gene. In fact, it was recently reported that CTX-M-15 has successfully spread into several clones of K. pneumoniae, both at the community and at the nosocomial level [35–37]. Outbreaks of CTX-M-15–producing K. pneumoniae isolates have been previously reported in some European countries [38, 39]; none of them reported any association with food as a source of the strain.

In conclusion, the present report describes the rapid and widespread dissemination of an epidemic clone of CTX-M-15 and SHV-1–producing K. pneumoniae causing a large nosocomial outbreak through the food chain. To our knowledge, this is the first report that provides insight on how transmission of ESBL-KP can occur through the food as the vehicle in the hospital setting. Infection control teams must take into account that food can be a transmission vector for multidrug-resistant strains in the hospital setting, and they should consider extending their surveillance to kitchen facilities and foodstuff.

Acknowledgments

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References


