Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae in Yaoundé, Cameroon

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Organisms producing extended-spectrum β-lactamases (ESBLs) have been reported in many countries, but there is no information on the prevalence of ESBL-producing members of the family Enterobacteriaceae in Cameroon. A total of 259 Enterobacteriaceae strains were isolated between 1995 and 1998 from patients at the Yaoundé Central Hospital in Cameroon. Enterobacterial isolates resistant to extended-spectrum cephalosporin and monobactam were screened for ESBL production by the double-disk (DD) synergy test. Thirty-one (12%) of these Enterobacteriaceae strains were shown to be positive by the DD synergy test, suggesting the presence of ESBLs. Resistance to oxyimino-cephalosporins and monobactams of 12 (38.7%) of the 31 strains—i.e., 6 Klebsiella pneumoniae, 4 Escherichia coli, 1 Citrobacter freundii, and 1 Enterobacter cloacae strain—was transferred to E. coli HK-225 by conjugation. Resistance to gentamicin, gentamicin plus trimethoprim-sulfamethoxazole, or trimethoprim-sulfamethoxazole was cotransferred into 6, 2, and 1 of these transconjugants, respectively. All 12 transconjugants were resistant to amoxicillin, piperacillin, all of the cephalosporins, and aztreonam but remained susceptible to cefoxitin and imipenem. Crude extracts of β-lactamase-producing transconjugants were able to reduce the diameters of inhibition zones around disks containing penicillins, narrow- to expanded-spectrum cephalosporins or monobactams when tested against a fully susceptible E. coli strain but had no effect on such zones around cefoxitin, imipenem, and amoxicillin-clavulanate disks. The β-lactamases produced by the 12 transconjugants turned out to be SHV-12 by DNA sequencing. Therefore, the ESBL SHV-12 is described for the first time in Cameroon.

Many extended-spectrum β-lactamases (ESBLs) are plasmid-mediated derivatives from TEM- and SHV-type enzymes and cause resistance to expanded-spectrum cephalosporins. They belong to Bush group 2be (6). Since their initial description in Germany in 1983 (13), ESBLs have diversified and spread worldwide. Several ESBLs appear to be particularly widely disseminated, being found in many countries, whereas others seem to occur more commonly in one or few countries (4). The various national patterns of antibiotic consumption in hospitals probably account for the differences in distribution of these enzymes. In an attempt to detect and study the dissemination of ESBLs in a central African country (Cameroon), we collected and characterized producers of such enzymes among clinical isolates of Enterobacteriaceae at Yaoundé Central Hospital between 1995 and 1998. The ESBL SHV-12 was found in several species of Enterobacteriaceae for the first time in Cameroon.

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MATERIALS AND METHODS

Bacterial strains. A total of 259 isolates, members of the Enterobacteriaceae family were collected from patients in Yaoundé Central Hospital (Table 1). Isolates were collected over 3-year period (April 1995 to March 1998) from urine, pus, and blood. The isolates were identified by conventional techniques (9) and were confirmed by the API 20 E (bioMérieux, France). There were no replicate strains isolated from any patient in the present study. Escherichia coli HK 225 (12), which is resistant to rifampin and streptomycin, was used as a recipient strain for transfer experiments by conjugation. E. coli ATCC 25922 was used as a control strain for antimicrobial susceptibility testing and as a negative control for PCR experiments. E. coli K12R111 (provided by Danièle Sirot) encoding TEM-1 and plasmid pMPA encoding SHV-2A were used as positive controls for blaTEM and blaSHV genes, respectively.

Antimicrobial susceptibility testing and detection of ESBL producers. Antimicrobial susceptibility was determined by disk diffusion tests according to the methods of the Clinical and Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards) (16) by using disks from BBL Microbiology Systems (Cockeysville, Md.). The tested antibiotics were amoxicillin, amoxicillin-clavulanate, piperacillin, cefazolin, cefoxitin, cefotaxime, ceftazidime, aztreonam, imipenem, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole.

The double-disk (DD) synergy test (11) was used for detection of ESBLs in clinical and transconjugant strains. A central disk of amoxicillin-clavulanate was surrounded by disks with cefotaxime, ceftriaxone, ceftazidime, and aztreonam at a distance of ca. 19 mm (center to center) on a Mueller-Hinton agar plate (Difco Laboratories, Detroit, MI) inoculated according to the standard procedures (16). Distortion of the peripheral inhibition zones of surrounding antibiotics toward the central disk with clavulanate was indicative for an ESBL. The tests were repeated with a disk spacing of 15 mm (center to center).
from blood) belonging to the family *Enterobacteriaceae* were studied, among which 31 (12%) potential ESBL producers were identified by the DD synergy test. Characteristic clavulanate-induced distortions of inhibition zones that were indicative for ESBL production were found in 86, 90, 62, and 97% of the strains around the disks containing cefotaxime, ceftriaxone, ceftazidime, and aztreonam, respectively. The ESBL producer’s isolates were investigated, and the ESBL-producing phenotype was found most frequently among *Klebsiella* species (18.8%; *K. pneumoniae* [n = 11] and *K. oxytoca* [n = 1]), followed by *Citrobacter* species (17.6%; *C. freundii* [n = 3]) and *E. coli* (14.3%) (Table 1). Of the ESBL-producing strains, 15 were from urine, 11 were from pus, and 5 were from blood; isolates were mainly from patients in the intensive care unit (ICU) (38.7%) and the surgical (45.1%) ward (Table 2).

MICs of extended-spectrum cephalosporins exhibited a notable variability among different isolates, but MICs of CAZ were always ≥2 μg/ml (Table 2). All of the isolates were resistant to amoxicillin, piperacillin, and cephalothin, and most of them were also resistant to amoxicillin-clavulanate, gentamicin, and trimethoprim-sulfamethoxazole. All of the isolates were susceptible to imipenem, and most of them were also susceptible to cefoxitin, ciprofloxacin, and amikacin (Table 2).

**Transferability of ESBL genes.** Among potential ESBL producers, 12 (38.7%) were transferred oxyimino-cephalosporin resistance to *E. coli* HK 225. The transfer frequencies were between 9 × 10⁻⁸ and 6.7 × 10⁻⁴ per input donor. The isolates included six of *K. pneumoniae*, four of *E. coli*, one of *E. cloacae*, and one of *C. freundii*. Resistance determinants against the following non-beta-lactam antibiotics were cotransferred to *E. coli* HK 225: gentamicin (six cases), gentamicin and trimethoprim-sulfamethoxazole (two cases), and trimethoprim-sulfamethoxazole (one case). In contrast, resistance to cefoxitin or to other antibiotic classes was not transferred (Table 3).

**Beta-lactamase type.** Supernatants containing crude beta-lactamase extracts from transconjugants did not affect the inhibition zones of *E. coli* HK 225 around cefoxitin, imipenem, and amoxicillin-clavulanate disks. The supernatants did, however, significantly reduce the inhibition zones around disks containing amoxicillin, piperacillin, cefazolin, cefotaxime, ceftaxime, ceftazidime, and aztreonam (data not shown). These results were consistent with the presence of the detected ESBLs.

All 12 transconjugants were subjected to molecular detection and characterization procedures aimed at *bla* TEM and *bla* SHV genes. From all 12 strains, no PCR product was obtained with TEM specific primers, whereas a characteristic 1,017-bp amplifier was synthesized, which proved to be degradable by the restriction enzyme NheI into two fragments of 770 and 247 bp in length. This positive PCR/NheI test indicated that an SHV-ESBL was produced by all transconjugants and hence by the 12 corresponding donor clinical isolates (Table 3).

Nucleotide sequencing revealed that all 12 amplifiers were 100% homologous to each other along their entire length. This also confirmed the PCR/NheI results and showed that the deduced amino acid sequences differed in the following positions (Ambler numbering [1]) from the SHV-1 standard (4): leucine 35—glutamine, glycine 238—serine, and glutamic acid 240—lysine. Thus, SHV-12 (http://www.lahey.org/studies/webt.htm) was identified in all of the 12 strains (Table 3). More-
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<th>Organism</th>
<th>Gene(s)</th>
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<td>Proteus mirabilis</td>
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<tr>
<td>Citrobacter freundii</td>
<td>Amx</td>
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<td>Klebsiella oxytoca</td>
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ESBLs were present in 31 (12%) isolates that expressed an ESBL producer phenotype among Enterobacteriaceae. Table 3 presents the characteristics of transconjugant strains. The susceptibility test data showed that the ESBL producers which were resistant to most β-lactams and non-β-lactams such as gentamicin and trimethoprim-sulfamethoxazole were multidrug-resistant strains. The ESBL producers usually carry a multiresistant plasmid, the genes conferring resistance to β-lactam and non-β-lactam antibiotics (10, 23). All of the ESBL producers were susceptible to imipenem, and most were also susceptible to cefoxitin, amikacin, and ciprofloxacin. If the patients are infected by ESBL producers, carbapenem may be used (5, 14).

Table 4 shows the distribution of SHV-12-producing clinical isolates of Enterobacteriaceae. The prevalence among genera is varied, with rates of 18.8% for Klebsiella spp. and 1.8 to 17.6% for all other genera. Of 31 ESBL producer isolates, 12 (38.7%) transferred the ESBL gene to E. coli HK 225 and were also found to produce SHV-12 ESBL. These data represent the first report of the prevalence of ESBL producers among Enterobacteriaceae in Cameroon.

In the present study, most ESBL producers were collected from patients in the surgical ward and the ICU. In these wards, isolates are exposed to great antibiotic pressure. Furthermore, many of these patients are particularly vulnerable to infection because they are immunocompromised or have an easy avenue of access for bacteria (23).

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The results of the present study are evidence of an ongoing outbreak during the sampling period from 1996 to 1998 at Yaoundé Central Hospital of ESBL-producing organisms attributable to at least four species of Enterobacteriaceae: K. pneumoniae, E. coli, C. freundii, and E. cloacae. Although the \( \text{bla}_{\text{SHV}-12} \) gene was cotransferable along with other resistance determinants upon conjugation and was found in several species, it seemed possible that the determinant is located on a large low-copy broad-host-range plasmid, as is usually the case (10, 21). In the present study, we focused on determinants that were easily transferable by conjugation in vitro (more than a third of the ESBL isolates from Yaoundé Central Hospital) because these are the most clinically relevant factors with regard to the speed of dissemination.

Within the collection of 31 strains that yielded positive DD results, ceftazidime and aztreonam showed the lowest (62%) and highest (96%) rates of detection, respectively. These results indicate that at maximum sensitivity, when looking for ESBLs, several oximino-cephalosporins and aztreonam should be used simultaneously for DD testing. This is in agreement with the recommendations by Coudron et al. (7). Moreover, this finding underlines the fact that synergy tests and other physiological tests in general are of limited sensitivity in detecting ESBLs, a fact that has been stated already in 1995 (14) and confirmed by careful studies involving site-directed mutagenesis, as well as different copy number cloning vectors in isogenic systems (20).

ESBLs are now a problem for hospitalized patients worldwide. The rates of ESBL producers among Klebsiella sp. and E. coli at our center are 18.8 and 14.3%, respectively. This prevalence is lower for Klebsiella sp. and higher for E. coli than that reported by the SENTRY worldwide surveillance program, in which the ESBL prevalences in K. pneumoniae and E. coli were 45 and 8.5% (Latin America), 25 and 7.9% (Western Pacific), or 23 and 5.3% (Europe), respectively (22).

In conclusion, the present study emphasizes the importance of screening for ESBLs even in countries where such enzymes...
have not been reported previously. The plasmid-mediated ESBLs have been already disseminated to four different species of Enterobacteriaceae and escalated into a multiclinic outbreak at Yaounde Central Hospital by the time they were discovered.

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