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High incidence of extended-spectrum β-lactamases among outpatient clinical isolates of *Escherichia coli:* A phenotypic assessment of NCCLS guidelines and a commercial method

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Abstract

The production of extended-spectrum β-lactamases (ESBLs) among 357 clinical isolates of *Escherichia coli* and 175 of *Klebsiella* spp. was studied using both the National Committee for Clinical Laboratory Standards disk diffusion method and the semiautomated Wider system. We highlight the predominance of *E. coli* (50, 92.6%) among positive samples and the largely outpatient origin of these (40, 80%), including 39 samples of urine (97.5%) and one of urethral exudate. There were only four ESBL-producing isolates of *Klebsiella* spp. (7.4%), and three were in outpatient urine samples (75%, 2 *K. oxytoca* and 1 *K. pneumoniae*). The positive and negative predictive values for the Wider system were 81% and 98.5%, respectively. We stress the high incidence of ESBL in our setting, the predominance of cases in the outpatient setting, and the acceptable detection of ESBL by means of the Wider system in *E. coli* and *Klebsiella* spp.

Keywords: Escherichia coli; *Klebsiella* spp.; Extended-spectrum β-lactamases; Urine

1. Introduction

Extended-spectrum β-lactamases (ESBLs) represent an important mechanism of resistance in *Enterobacteriaceae*, because they inactivate third-generation cephalosporins and aztreonam. In vitro, among β-lactams, only cephamycins, betalactam/beta-lactamase inhibitor combinations, and carbapenems retain activity. A study by Knothe et al. (1983) in Germany demonstrated the transmission of resistances in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*, which were subsequently related to the presence of ESBLs. In Spain, the first outbreak was reported in 1988, although study of archived clinical isolates has revealed their earlier presence (Baquero et al., 1988). Recent studies have reported a larger number of ESBL-producing isolates of *E. coli* than of *K. pneumoniae* in absolute terms, although the latter species has shown a greater proportion of ESBLs (Hernández et al., 2003). The largest nosocomial outbreak to date occurred between 1993 and 1995 at Bellvitge Hospital, Barcelona and was due to clonal dissemination of a strain of *K. pneumoniae* (Peña et al., 1998). A study of clinical isolates of *Klebsiella* spp. at Ramón y Cajal Hospital in Madrid between 1989 and 2000 showed that 4.8% of them produced ESBLs. Most of these came from the intensive care unit and surgical units and showed a wide genetic variability, although one clone had been responsible for an outbreak in the neonatal intensive care unit between 1997 and 1998 (Asensio et al., 2000; Coque et al., 2002).

Nevertheless, information on the presence of ESBL-producing clinical isolates of *E. coli* and *Klebsiella* spp. in Spain may be distorted by the frequent use of automated detection systems (MicroScan, VITEK, Wider systems) that differ from the method recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002).

The aim of the present work was to study the presence in our setting of ESBL-producing *E. coli*, *K. pneumoniae*, and *K. oxytoca* clinical isolates using the NCCLS reference method and to assess the capacity of the Wider automated system (Francisco Soria Melguizo, Spain) to detect these clinical isolates.

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2. Materials and methods

During October and November 2002, 744 *Enterobacteriaceae* (357 *E. coli*, 132 *Klebsiella pneumoniae*, 43 *Klebsiella oxytoca*, and 212 of other species) were isolated consecutively from single patients treated at the San Cecilio University Hospital, Granada. This tertiary-level hospital serves the west central hospital area of Granada province (Andalusia region), which includes a population of 320,000 inhabitants and two primary care districts. Demographic characteristics of the patients were gathered from their hospital records. All species were identified using the Wider automated system (Cantón et al., 2000). Seventy-seven percent of the isolates were of outpatient origin, and 67% came from females.

The production of ESBLs in the clinical isolates of *E. coli*, *K. pneumoniae*, and *K. oxytoca* was studied by the NCCLS diffusion method (NCCLS, 2002), as reference test, and by the Wider automated system (Cantón et al., 2000) (Panel MIC/ID, GRAM NEGATIVOS REV. 1).

K. pneumoniae American Type Culture Collection (ATCC) 700603 and *E. coli* ATCC 25922 were used as reference strains in each run of 20 clinical isolates. The inhibition zone diameters of *K. pneumoniae* ATCC 700603 were (in mm) 10/22 for ceftazidime/ceftazidime plus clavulanic acid, 20/28 for cefotaxime/cefotaxime plus clavulanic acid, 11/21 for cefpodoxime/cefpodoxime plus clavulanic acid in all assays; the zone diameters of *E. coli* ATCC 25922 were 28/28 for ceftazidime/ceftazidime plus clavulanic acid, 30/30 for cefotaxime/cefotaxime plus clavulanic acid, and 24/24 for cefpodoxime/cefpodoxime plus clavulanic acid in all assays. All in vitro assays with the Wider system showed the control strain of *K. pneumoniae* (ATCC 700603) to be resistant to amoxicillin, cefoxitin, and ceftazidime and sensitive to amoxicillin–clavulanic acid, piperacillin-tazobactam, cefotaxime, cefepime, and carbapenems, whereas *E. coli* ATCCC 25922 was found to be sensitive to all of the antibiotics assayed.

2.1. Disk diffusion method (NCCLS)

The disk diffusion method was used on Mueller-Hinton agar plates (bioMérieux, France) inoculated with isolates in a 0.5 McFarland bacterial suspension of cefpodoxime (CPD) (10 μg) (Oxoid, England), cefpodoxime plus clavulanic acid (CD01) (10/1 μg) (Oxoid), ceftazidime (CAZ) (30 μg) (Oxoid), ceftazidime plus clavulanic acid (CD02) (30/10 μg) (Oxoid), cefotaxime (CTX) (30 μg) (Oxoid), and cefotaxime plus clavulanic acid (CD03) (30/10 μg) (Oxoid). The presence of ESBL was suspected when inhibition halos of $\leq$17, 22, and 27 mm were obtained for CPD, CAZ, or CTX disks, respectively. Their presence was confirmed by the demonstration of synergy between cephalosporin with reduced inhibition zone and clavulanic acid (Synergy was defined when the inhibition zone of the disk of cephalosporin plus clavulanic acid was $\geq$5 mm greater than that of the disk of cephalosporin alone).

Although it is not an NCCLS criterion, a cefoxitin susceptibility test was also carried out using the disk diffusion method (30 μg) (Oxoid).

2.2. Wider system

According to the manufacturer, the Wider system permits the identification and susceptibility study of Gram-negative bacilli through microdilution at critical concentrations. For all *Enterobacteriaceae*, ESBL detection was based on data from wells containing ceftazidime (TAZ) (1–16 μg/mL), ceftazidime plus clavulanic acid (TZ/C) (8/4 and 1/4 μg/mL), amoxicillin (AMX) (4–16 μg/mL), amoxicillin plus clavulanic acid (A/C) (4/2 and 16/8 μg/mL), and cefotaxime (CTX) (1–8 μg/mL). According to the manufacturer, presence of ESBLs is indicated when the antibiogram is interpreted as AMX resistant, A/C nonresistant, and TAZ nonsusceptible and when CTX is also nonsusceptible, or the minimal inhibitory concentration for TZ/C is $\leq$ 1/4 μg/mL.

Disk diffusion and Wider system tests were repeated for strains showing discrepant results.

3. Results

According to the reference method, ESBLs were produced by 50 clinical isolates of *E. coli* (14% of this species), two of *K. pneumoniae* (1.5%), and two of *K. oxytoca* (4.6%). All presented around diameters of >18 mm around the cefoxitin disk.

Overall, 66.7% of the isolates came from females.

Among these strains, *E. coli* predominated (50, 92.6%). The ESBL-positive samples were largely of outpatient origin (40, 80%), including 39 samples of urine (97.5%) and one of urethral exudate. Among the inpatient samples (10, 20%), there was a lower percentage of *E. coli* isolates in urine (6, 60%) and a higher percentage in wound secretions (3, 30%) and vulva (1, 10%).

There were only four isolates of *Klebsiella* spp. (7.4%), three in outpatient urine samples (75%, 2 *K. oxytoca*, and 1 *K. pneumoniae*) and one of *K. pneumoniae* (25%) in an inpatient urine sample.

Both methods demonstrated the presence of ESBLs in 47 of the isolates and their absence in 467. The Wider system failed to detect 7 of 54 (13%) ESBLs: five of *E. coli* (80% from urine samples and 20% from wound secretions), one of *K. pneumoniae* (from inpatient urine sample), and one of *K. oxytoca* (from outpatient urine). The results obtained for these strains with the reference test are displayed in Table 1 Only 47 of 58 (81%) isolates identified as ESBL by the Wider system were true ESBLs; nine isolates of *E. coli* (81.9%) and two of *K. pneumoniae* (18.1%) were false positives. Table 2 exhibits the results obtained for these

Table 1
Results obtained with disk diffusion method (diameters in mm) in extended-spectrum β-lactamase–producing clinical isolates undetected with the Wider system

Microorganism	CAZ	CD02	CTX	CD03	CPD	CD01
E. coli	21	32	14	32	6	22
E. coli	28	30	20	30	6	21
E. coli	20	35	12	35	6	24
E. coli	29	30	22	30	6	20
E. coli	26	29	14	30	6	21
K. pneumoniae	20	30	19	36	6	26
K. oxytoca	18	30	19	32	6	23

CPD, cefpodoxime (10 μg); CD01, cefpodoxime plus clavulanic acid (10/10 μg); CTX: cefotaxime (30 μg); CD03, cefotaxime plus clavulanic acid (30/10 μg); CAZ, ceftazidime (30 μg); CD02, ceftazidime plus clavulanic acid (30/10 μg).

strains with the disk diffusion method and the Wider system. In detection of ESBLs among the *E. coli* and *Klebsiella* spp. isolates, the Wider system showed a positive predictive value of 81% and a negative predictive value of 98.5%.

When discordant results between the reference method and Wider system were repeated, the discrepancy was maintained in all 11 isolates (Table 2); according to the reference method, they were sensitive isolates, whereas the Wider system determined them to be ESBL producers.

4. Discussion

In 2000, a Spanish multicenter study reported a much lower frequency of ESBLs than that detected in the present study (Hernández et al., 2003). Besides the increment due to the passage of years, the precise reasons for this increase are not clear. The increase may be due to: (1) the different methodology used for the isolate selection, because the clinical isolates were not consecutively obtained (2) a predominance of inpatient isolates in the previous study; or (3) the possible lack of a systematic search for the presence of ESBLs in clinical isolates at some centers. The much higher frequency in our outpatients' samples suggests that studies of ESBLs in *E. coli* and *Klebsiella* spp. often include only inpatient samples and that their presence in outpatient samples is at least underreported. This occurs because third-generation cephalosporins are not indicated as first-line drugs for infections in the outpatient setting and are not, therefore, subject to regular laboratory testing. Larger studies in neighboring countries (De Champs et al., 2000; Spanu et al., 2002) were performed on inpatient samples, and their findings were very similar to those of the Spanish multicenter study (Hernández et al., 2003).

In the latter study (Hernández et al., 2003), 51% of ESBL-producing *E. coli* came from outpatient samples, compared with 80% in the present study. Likewise, 93% of ESBL-producing *Klebsiella* spp. came from inpatient samples in the multicenter study (Hernández et al., 2003), whereas most of the few cases in the present series came from outpatient samples. Nevertheless, ESBL-producing *E. coli* was the most frequent species in both studies, especially in urine samples, and females provided most of the ESBL-producing isolates of *E. coli* (54% and 66.7% in multicenter and present study, respectively).

In October 2002, a 7.5% prevalence of ESBL-producing

Table 2
Disk diffusion method results in clinical isolates that were false positives with the Wider system

Species	Disk diffusion diameters (mm)						Wider MIC (μg/mL)				
	CAZ	CD02	CTX	CD03	CPD	CD01	AMX	A/C	TAZ	TZ/C	CTX
E. coli	30	32	34	34	27	27	<4	<4/2	16	<1/4	<1
E. coli	25	25	28	28	24	24	<4	<4/2	16	<1/4	<1
E. coli	26	26	30	30	22	22	<4	<4/2	16	<1/4	<1
E. coli	26	27	30	30	23	24	>16	<4/2	>16	<1/4	<1
E. coli	30	34	30	30	23	27	>16	<4/2	<1	<1/4	>8
E. coli	30	30	30	30	22	24	>16	<4/2	16	<1/4	>8
E. coli	26	26	28	28	23	23	>16	8/4	>16	<1/4	<1
E. coli	27	27	30	30	25	25	>16	16/8	<1	<1/4	<1
E. coli	32	32	36	36	25	25	>16	8/4	16	<1/4	<1
K. pneumoniae	25	26	32	32	26	26	>16	<4/2	>16	<1/4	>8
K. pneumoniae	29	31	36	38	30	30	>16	<4/2	<1	<1/4	>8

CPD, cefpodoxime (10 μg); CD01, cefpodoxime plus clavulanic acid (10/10 μg); CTX, cefotaxime (30 μg); CD03, cefotaxime plus clavulanic acid (30/10 μg); CAZ and TAZ, ceftazidime (30 μg); CD02 and TZ/C, ceftazidime plus clavulanic acid (30/10 μg); AMX, amoxicillin; A/C, amoxicillin plus clavulanic acid; MIC, minimal inhibitory concentration.

E. coli was observed in outpatient samples at the Santa Creu i Sant Pau Hospital in Barcelona, representing a considerable increase over the 2.1% prevalence reported in a study between February and May 2001 (Mirelis et al., 2003). Higher percentages were recorded in the present study, with ESBL production detected in 50 out of 357 *E. coli* isolates (14%).

β-lactam therapy can be applied to infections with these strains by the use of carbapenems (severe cases) or combinations of penicillins and β-lactamase inhibitors, such as amoxicillin plus clavulanic acid or piperacillin plus tazobactam, with the latter being the treatment of choice in severe infections. Nevertheless, clinical failures have been reported after treatments with these combinations, because of the hyperproduction of ESBLs (Pujol and Peña, 2003).

The performance of the Wider system to detect extended-spectrum beta-lactamases was assessed in a previous study (Cantón et al., 2000), obtaining similar results. Given the ESBL detection results with the Wider system in our series and the potential clinical repercussions of a false result, all positive results obtained with this system should be confirmed with an NCCLS reference test, at least for clinical isolates from inpatients. The difference between the Wider system and disk diffusion outcomes may be due to the hyperproduction of AmpC or of other plasmid β-lactamases such as SHV-1, which produces elevation of minimal inhibitory concentration to third-generation cephalosporins (Miró et al., 1998).

In conclusion, there is a high incidence of ESBLs in our setting, predominantly among outpatients, and the Wider system has an acceptable ability to detect the presence of ESBLs in *E. coli* and *Klebsiella* spp.

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