Faecal carriage of ESBL-producing Enterobacteriaceae and carbapenem-resistant Gram-negative bacilli in community settings

Hugo Edgardo Villar, Marisa Noemí Baserni, Monica Beatriz Jugo

Department of Clinical Bacteriology, Laboratorio Hidalgo, Buenos Aires, Argentina

Abstract
Introduction: The aim of this study was to determine the prevalence of intestinal carriage of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae and carbapenem-resistant Gram-negative bacilli in the community in Buenos Aires, Argentina.

Methodology: Faecal samples from 164 non-hospitalized patients were cultured on CHROMagar KPC and CHROMagar ESBL plates. Isolates resistant to third-generation cephalosporins or carbapenems were selected for further study. The minimal inhibitory concentration (MIC) of the isolates was determined using the E-test method. The phenotypic detection of ESBLs and carbapenemases was performed using the double-disc synergy test.

Results: The rate of faecal carriage of Enterobacteriaceae resistant to third-generation cephalosporins was 26.8%. Escherichia coli represented a large majority (75%) of the isolates recovered. Thirty-three ESBL-producing isolates were detected from 31 faecal samples (18.9% of the collected specimens). Eight carbapenem-resistant Gram-negative bacilli were recovered from eight specimens (4.9%).

Conclusions: This study revealed a high prevalence of faecal carriage of multidrug-resistant Gram-negative bacteria, including ESBLs, in Buenos Aires. Therefore, the use of surveillance cultures will be helpful for tracking and monitoring the spread of ESBL-producing Enterobacteriaceae within community settings.

Key words: extended-spectrumβ-lactamase; multidrug-resistant bacteria; carbapenemase; faecal carriage


(Received 26 July 2012 – Accepted 28 October 2012)

Copyright © 2013 Villar et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Multidrug-resistant Gram-negative bacteria are a major public health threat. However, despite intense efforts to limit their spread, the number of multidrug resistant Gram-negative bacteria continues to increase globally.

Since 2000, the incidence of community-acquired enterobacteria (Escherichia coli) that produce extended-spectrum beta-lactamases (ESBLs) capable of hydrolysing almost all cephalosporins (except carbapenems) has increased worldwide [1]. The prevalence of Pseudomonas aeruginosa, which produces metallo-beta-lactamases (MBLs), is increasing, and transferrable multidrug resistance associated with IMP- and VIM-type MBLs has emerged in other clinically relevant species, including E. coli, Klebsiella pneumoniae and Acinetobacter baumannii [2].

A novel type of carbapenemase, New Delhi metallo beta-lactamase-1 (NDM-1), was first identified in two Enterobacteriaceae isolates in 2008, both from a Swedish patient transferred from India [3].

The emergence of NDM-1 on all continents and the appearance and rapid spread of organisms that produce Klebsiella pneumoniae carbapenemase (KPC)-type β-lactamases are two of the most recent developments in the epidemiology of carbapenem resistance [4].

High resistance rates to third-generation cephalosporins and carbapenems were detected among Gram-negative bacilli isolated from Latin American medical centres enrolled in the SENTRY Antimicrobial Surveillance Programs. ESBL rates were 18.1% among Escherichia coli and 60.4%, among Klebsiella spp. from Argentina. Meropenem-nonsusceptible P. aeruginosa was observed in 53.8% of strains from Argentina [5].

Gastrointestinal colonization by multidrug resistant bacteria is associated with subsequent clinical infection. A recent study emphasized the importance of identifying individuals carrying antimicrobial-resistant bacteria in both patient and healthy populations [6]. An increase in the proportion of carriers in the community increases the risk that other
individuals will also become carriers via human-to-
human transmission [7]. In addition, the admission
into hospital of patients harbouring resistant bacteria
increases the risk of other hospitalized patient
contracting an infection [8].

To date, no studies have investigated the
prevalence of faecal carriage of multidrug-resistant
bacteria in the community setting in Argentina.
Therefore, the aim of this study was to examine the
prevalence of intestinal carriage of ESBL-producing
Enterobacteriaceae (ESBL-E) and carbapenem-
resistant gram-negative bacilli (CRGNB) in the
community.

Methodology
Setting, patient selection, and collection of
surveillance specimens
The study was conducted at the Laboratorio
Hidalgo, Buenos Aires, Argentina. From March 2012
through July 2012, the laboratory reception area
received faecal samples from 164 patients (52.4%-
were women aged 1 to 83 years; median age of 58
years) with gastrointestinal complaints, none of whom
had been hospitalized within the previous two months.
The samples were then screened for the presence of
ESBL-E and CRGNB within 8 hours of collection.

Briefly, a total of 0.5 g of each faecal sample was
placed in 1 mL of sterile 0.9% saline and then
vortexed. A small amount of the resulting suspension
(50 µl) was cultured on CHROMagar KPC and
CHROMagar ESBL (CHROMagar Company, Paris,
France) plates. For both commercial media, the colour
of the colonies was recorded according to the colour
chart provided by the manufacturer. Plates were
incubated at 35°C under aerobic conditions and
assessed after 24 and 48 hours of incubation. A
distorted inhibition zone was interpreted as a positive result for carbapenem
hydrolysis screening.

Strains hyperproducing AmpC β-lactamases were
suspected when an MIC ≥ 32 µg/ml for ceftaxime, an
MIC ≥ 32 µg/ml for cefotaxime, and a negative ESBL
test were found.

Results
Of the 164 samples analysed, 111 (67.7%) did not
grow on CHROMagar ESBL medium; however, 53
(32.3%) grew after 48 hours of incubation. In total, 59
isolates were recovered, 44 (26.8%) of which were
Enterobacteriaceae strains that were resistant to third-
generation cephalosporins. Of these, 11 overexpressed
cephalosporinase and 33 produced ESBL. These 33
ESBL-producing isolates were recovered from 31
faecal samples (18.9% of the collected specimens). E.
coli represented the majority (75%) of the isolates
recovered. The species distribution and phenotypic

Identification and antimicrobial susceptibility testing
The API 20 E system (bio-Merieux, Marcy
L’Étoile, France) was used for the biochemical
identification of all Enterobacteriaceae. Nonlactose-
fermenting organisms were identified at the species
level using the API 20 NE system (bio-Merieux,
Marcy L’Étoile, France).

Antimicrobial susceptibility testing of all isolates
was performed using the Kirby-Bauer disk diffusion
method. The minimal inhibitory concentration (MIC)
of each isolate was determined using the Epsilometer
test (E-test; AB Biodisk, Solna, Sweden). MIC breakpoints were defined according to Clinical and
Laboratory Standards Institute (CLSI) criteria [9].

Screening for ESBL production was performed according to CLSI guidelines [9] using 30 µg
cefotaxime and ceftazidime discs (Oxoid Ltd,
Basingstoke, United Kingdom) in the presence or
absence of 10 µg clavulanic acid. Carbapenemase
production was phenotypically confirmed using
carbapenemase inhibition tests. An inhibitor (3-
aminophenylboronic acid; BAC) was used to detect
class A carbapenemases, while ethylenediaminetetraacetic acid (EDTA) was used to
detect class B metallo-carbapenemases. Double-disk
synergy tests (disk approximation methods) were
performed at different distances (range 0.5–2 cm).
Discs of BAC (300 µg) and EDTA (372 µg) plus
sodium mercaptoacetic acid (SMA 900 µg) were
purchased from Laboratorios Britania (Britania,
Buenos Aires, Argentina).

The modified Hodge test (MHT) was performed as
described previously [9]. Briefly, the indicator
organism, E. coli ATCC 25922, at a turbidity
equivalent to that of a 0.5 McFarland standard, was
used to inoculate, with a swab, the surface of a
Mueller-Hinton agar plate (bio-Merieux, Marcy
L’Étoile, France), and the test strain was heavily
streaked from the centre to the plate periphery. After
brief drying, a 10 µg imipenem disc (Oxoid Ltd,
Basingstoke, United Kingdom) was placed at the
centre, and the plate was incubated overnight at 35°C.
The presence of a distorted inhibition zone was
interpreted as a positive result for carbapenem
hydrolysis screening.

Strains hyperproducing AmpC β-lactamases were
suspected when an MIC ≥ 32 µg/ml for ceftaxime, an
MIC ≥ 32 µg/ml for cefotaxime, and a negative ESBL
test were found.
characteristics of the *Enterobacteriaceae* isolates resistant to third-generation cephalosporins are shown in Table 1. Of the 33 ESBL-producers, 15 (45.4%) had MIC values for cefotaxime that were at least eight times higher than those for ceftazidime, suggesting that they expressed a cefotaximase-type enzyme. The remaining ESBL-producing strains had MIC values ≥ 32 μg/ml for cefotaxime and ceftazidime. The frequency of co-resistance among the ESBL-producing isolates was as follows: 63.6% were resistant to nalidixic acid; 57.6% to ciprofloxacin; 48.5% to trimethoprim sulfamethoxazole; 42.4% to gentamicin; 3.0% to amikacin; 3.0% to nitrofurantoin; and 0% to imipenem. Faecal carriage of ESBL-producing isolates was not related to age [median age 51 years (1 to 77)] or sex (54.8% women).

A total of 147 (89.6%) specimens failed to grow on CHROMagar KPC plates; however, 17 (10.4%) did grow. Overall, eight CRGNB isolates (excluding *Pseudomonas aeruginosa* and *Acinetobacter* spp strains, which were resistant to ertapenem only) were recovered from eight specimens (4.9%). The characteristics of each of these species are shown in Table 2.

The most frequently identified species of non-fermenting Gram-negative rod was *Pseudomonas aeruginosa*. An enhancement of the inhibition zone between IPM and EDTA-SMA and a positive MHT were detected in 1/4 *P. aeruginosa* isolates, suggesting the production of a class B metallo-beta-lactamase. An imipenem-resistant *Enterobacter cloacae* strain was also recovered and was found to be susceptible to aminoglycosides, quinolones, trimethoprim-sulfamethoxazole, cefotaxime (0.5 μg/ml), and ceftazidime (0.5 μg/ml). The MHT was positive and imipenem susceptibility was restored after treatment with BAC, which suggests that the strain contained produced a class A carbapenemase.

### Table 1. Characteristics of the 44 *Enterobacteriaceae* strains recovered from CHROMagar ESBL plates, which were resistant to third generation cephalosporins

<table>
<thead>
<tr>
<th>Strains</th>
<th>Number</th>
<th>Hyper-production of AmpC</th>
<th>ESBLs</th>
<th>ESBLs+E with MIC CTX/CAZ ≥ 8</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>33 (75.0%)</td>
<td>5</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>3 (6.8%)</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>3 (6.8%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>2 (4.5%)</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>2 (4.5%)</td>
<td>-</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>Morganella morganii</em></td>
<td>1 (2.3%)</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CTX, cefotaxime; CAZ, ceftazidime; ESBLs, extended-spectrum β-lactamases; ESBL-E, ESBL-producing *Enterobacteriaceae*

### Discussion

The present study revealed that faecal carriage of ESBL-E (18.9%) is common in community-dwelling patients with gastrointestinal complaints. The prevalence of ESBL-E in the faecal samples of community-dwelling individuals is reported to be between 1.7% and 15.4% [10-15]. Notably, ESBL-E was isolated from the faeces of 58.2% of healthy individuals in Thailand [16]. Also, *E. coli* species harbouring CTX-M type ESBLs, particularly CTX-M-14 and CTX-M-15, have been identified worldwide [17,18].

The present study has some limitations. First, it was not possible to perform molecular characterization of the enzymes expressed by the bacteria or to detect isolates expressing multiple enzymes. However, the MIC values for cefotaxime and ceftazidime against several of the *E. coli* isolates suggest that these strains did produce a cefotaximase-type enzyme. In addition, although there was no history of recent hospitalization or antibiotic use among the community-dwelling study subjects, many were likely to have been exposed to multiple courses of antibiotics due to the unrestricted over-the-counter availability of these drugs in developing countries.

The emergence and spread of CRGNB is a great concern; therefore, we must constantly monitor the development of carbapenem resistance, particularly in organisms that are naturally susceptible to this antibiotic. With the exception of *Stenotrophomonas maltophilia* and *Burkholderia cepacia*, carriage of carbapenem-resistant organisms was not a common finding in the present study (1.2% of the collected specimens). However, a *P. aeruginosa* strain producing metallo-beta-lactamases (MBLs) was recovered from a 72-year-old patient frequently exposed to health-care settings, and an *E. cloacae*
isolate that was resistant to imipenem but susceptible to cefotaxime and ceftazidime was isolated from a patient with celiac disease who had not used antibiotics within the last three months. The phenotypic characteristics of this strain (probably NMC-A) were similar to those reported previously by Radicce et al. [19]. The screening strategy using the APB test for class A carbapenemase in P. aeruginosa is not recommended. In spite of this, the MHT was negative for strains LH5, LH21, and LH138.

Conclusion

Faecal carriage of ESBL-E is prevalent in the urban communities of Buenos Aires; however, carbapenem-resistance, particularly in naturally carbapenem-susceptible organisms, was not common. We believe that collecting local epidemiological data is important if we aim to track and monitor the spread of ESBL-E in community settings.

References


**Corresponding author**
Hugo Edgardo Villar
Department of Clinical Bacteriology
Ladislao Martinez 43
Martinez (B1640EYA) Prov. de Buenos Aires
Argentina
Email: hugo.villar@laboratoriohidalgo.com

**Conflict of interests:** No conflict of interests is declared.