Faecal carriage of multidrug-resistant Gram-negative bacilli during a non-outbreak situation in a French university hospital

Laure Vidal-Navarro, Caroline Pfeiffer, Nicole Bouziges, Albert Sotto and Jean-Philippe Lavigne*

1Institut National de la Santé et de la Recherche Médicale, Espri 26, Université Montpellier 1, UFR de Médecine, 30908 Nîmes cedex 08, France; Laboratoire de Bactériologie, Virologie, CHU Caremeau, 30029 Nîmes cedex 09, France; Service des Maladies Infectieuses et Tropicales, CHU Caremeau, 30029 Nîmes cedex 09, France

*Corresponding author. Laboratoire de Bactériologie, Virologie, CHU Caremeau, Place du Professeur Robert Debre, 30029 Nîmes cedex 09, France. Tel: +33-4-666-832-02; Fax: +33-4-666-842-54; E-mail: jean.philippe.lavigne@chu-nimes.fr

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Objectives: To determine the prevalence of multidrug-resistant (MDR) Gram-negative bacilli and extended spectrum β-lactamase (ESBL)-producing isolates in stool specimens obtained from patients hospitalized for acute diarrhoea in a French university hospital.

Methods: Bacteria in stool specimens were screened for ESBL production on Drigalski agar supplemented with ceftazidime, ESBL CHROMagar and CTX CHROMagar media and confirmed by the double-disc synergy test. Genetic detection was performed by PCR and sequencing with bacterial DNA extracted from isolates.

Results: The presence of MDR bacteria was markedly high (96 of 303 patients, 31.7%). The majority of MDR bacteria were Enterobacter cloacae (44, 38%) and Escherichia coli (32, 28%). Moreover, the prevalence of ESBL and CTX-M producers among all included patients was 15.8% and 5.9%, respectively. The clone E. coli O25b:H4-ST131 was detected in 63% of CTX-M strains. Surprisingly, 16 carbapenemases (5.3% of patients) were isolated.

Conclusions: The study revealed the widespread dissemination of MDR bacteria, including carbapenemase producers, in a French hospital during a non-outbreak situation. Public health efforts to combat emergence and dissemination of MDR organisms need to be developed.

Keywords: cephalosporinases, extended-spectrum β-lactamases, metallo-β-lactamases, multidrug-resistant bacteria, prevalence, stool

Introduction

The potential of different antimicrobial agents to cause ecological disturbances in the normal digestive microflora is of importance. A major consequence is the emergence of multidrug-resistant (MDR) bacteria. Production of β-lactamases, such as extended-spectrum β-lactamases (ESBLs), is a common resistance mechanism in Gram-negative bacilli and the rapid dissemination of genes encoding these enzymes reflects the impact of the selective pressure of antibiotic usage. To date, more than 100 different ESBL types have been described and CTX-M β-lactamases are currently the most widespread. Although the spread of ESBLs is frequently due to the dissemination of mobile genetic elements, clonal dissemination of CTX-M-15-producing Escherichia coli belonging to phylogenetic groups B2 and ST131 has been described around the world. However, the misuse of antibiotics has led to the emergence of other MDR bacteria, notably carbapenem-resistant Enterobacteriaceae. Targeted surveillance of high-risk patients and screening is essential to prevent outbreaks of nosocomial infections by these organisms. Different studies worldwide have reported high levels of prevalence of MDR bacteria, which represents a growing concern among nosocomial and community-acquired infections. This prevalence can be related to the increase in faecal carriers over time. In France, investigations of faecal carriage are rare. The aim of this study was to evaluate the prevalence of MDR Gram-negative bacilli and of ESBL producers in patients with acute diarrhoea in a university hospital.

Materials and methods

Data collection and bacterial isolates

From September 2009 to November 2009, a total of 303 faecal samples from 303 patients hospitalized for acute diarrhoea were prospectively...
and consecutively collected and screened for the presence of MDR bacteria and ESBL-producing Enterobacteriaceae. These patients either lived in their own homes or were residents in a nursing home or a healthcare centre. Sampling was carried out at admission to the hospital from patients with acute diarrhoea during a non-outbreak period that was at least 1 year from the time of a recorded epidemic. The following clinical data were collected prospectively: demographic data; clinical ward; hospitalization or surgical treatment in the last 12 months; transfer from another hospital, intensive care unit (ICU) or nursing home; and antimicrobial treatment in the previous month.

Screening for MDR isolates
To screen Gram-negative bacilli for MDR production, swab samples were placed in 1 mL of sterile 0.9% saline and then vortexed. From this suspension, 100 μL was inoculated on three culture media: Drigalski agar supplemented with ceftazidime (2 mg/L); ESBL CHROMagar® (CHROMagar, Paris, France); and CTX CHROMagar® (CHROMagar). Plates were incubated at 37°C under aerobic conditions and assessed after 24 and 48 h of incubation. For both commercial media, the colour and intensity of the colonies were recorded according to the colour chart provided by the manufacturer.

Susceptibility testing and MDR confirmation
The Vitrek 2 automated system (bioMérieux, Marcy l’Étoile, France) was used for biochemical identification of all isolates growing on the three media and for antibiotic susceptibility testing. The following antibiotics were tested: β-lactams (amoxicillin, amoxicillin–clavulanic acid, piperacillin–tazobactam, cefepime, cefazolin, cefotaxime, ceftazidime, cefoxitin, imipenem and aztreonam); aminoglycosides (kanamycin, gentamicin, netilmicin, tabramycin and amikacin); quinolones (nalidixic acid and ciprofloxacin); chloramphenicol; nitrofurans; and co-trimoxazole. Strains were classified as susceptible, intermediate-resistant or resistant to antibiotics according to the recommendations of the Antibiotic Committee of the French Society for Microbiology (http://www.sfm.asso.fr/nouv/general.php?pa=2).

To be classed as MDR, Gram-negative bacilli should be resistant to at least three antibiotic families. These isolates included extended-spectrum cephalosporin (ESC)-resistant Enterobacteriaceae, Pseudomonas aeruginosa resistant to ceftazidime, Acinetobacter baumannii resistant to ceftazidime and Stenotrophomonas maltophilia. ESBL production was confirmed by the double-disc synergy test using ceftazidime, cefepime, cefotaxime, cefpodoxime, ceftriaxone, aztreonam and amoxicillin/clavulanic acid. Strains hyperproducing AmpC β-lactamases were suspected when an MIC of ≥32 mg/L for cefoxitin and an MIC of ≥32 mg/L for cefotaxime were found. Chromosomally encoded AmpC hyperproduction was confirmed by determining MICs of ceftazidime, cefepime and imipenem by the Etest method using Mueller–Hinton agar without and with 250 mg/L cloxacillin (http://www.sfm.asso.fr/nouv/general.php?pa=2).

Characterization of genes encoding β-lactamases
Plasmid DNA was extracted from the isolates by the use of a Qiagen Plasmid DNA Midi Kit (Qiagen, Courtaboeuf, France). The genotypic characterization of ESBL resistance mechanisms was determined by PCR assays targeting the blaTEM, blaSHV, blaCTX-M, blaOXA-23, blaIMP-1, and blaVIM genes and the PCR products were identified by sequencing.11–13 Triplex PCR specific for clone E. coli O25b:H4-ST131 producing CTX-M-15 was used.12 Detection of plasmid-mediated AmpC β-lactamase genes in different suspected strains was performed by using a multiplex PCR.13

Results
Main characteristics of patients
A total of 303 stool specimens—one specimen for each patient [50.5% of them women, median age 64 years (0–97)] hospitalized for acute diarrhoea—were examined in this study. Of the 303 patients, 96 (31.7%) harboured MDR bacteria. Among them, 86 (89.6%) had a previous hospitalization or lived in a nursing home or a healthcare centre in the last year and 57 (59.4%) had been prescribed antibiotics in the previous month, 34 of whom (59.7%) had received β-lactams. The patients belonged to the following units: internal medicine (38/106, 35.8%); geriatrics (20/58, 34.5%); surgical (13/27, 48.1%); emergency (10/76, 13.2%); recovery units (9/15, 60.0%); and ICU (6/21, 28.6%). Ten patients (six from ICU and four from emergency) who were carriers of MDR bacteria had no previous hospitalization. Single isolates were identified in 74 patients, two isolates in 16 patients and three isolates in 3 patients.

Distribution of MDR strains
MDR strains were mainly isolated in Enterobacter cloacae strains (44, 38% of the MDR strains) and E. coli (32, 28% of the MDR strains). Among the studied population, the prevalence of ESBLs was 15.8%. The prevalences of TEM, CTX-M and SHV producers were 9.2%, 5.9% and 0.7%, respectively. The distribution of the different types of ESBLs in the different species recovered during this study is shown in Table 1. Among the CTX-M producers, 16 (89%) strains produced CTX-M-15 β-lactamases. Sixty-three percent of the patients of the CTX-M-15-producing strains (10/16) belonged to the E. coli O25b:H4-ST131 clone. These strains were mainly isolated from patients with no previous hospitalization (5/10). TEM-producing strains were particularly prevalent in Enterobacter aerogenes (50% of the strains) and P. aeruginosa (43% of the strains). Finally, 48 patients (15.8% of the included patients) had strains hyperproducing chromosomally encoded AmpC. No plasmid-encoded AmpC isolate was detected. Sixteen (5.3%) patients carried strains resistant to carbapenems [OXA-23 (eight strains), IMP-1 (seven strains) and VIM-1 (one strain)]. These carbapenemases were produced by A. baumannii and E. cloacae, and were detected in patients hospitalized in different wards with no link. All these patients had had a previous hospitalization in the last year and had received antibiotic therapy in the last month.

Discussion
This study demonstrates for the first time the high faecal carriage of MDR bacteria in patients hospitalized for acute diarrhoea in a French university hospital, the presence of carbapenemases being notable. The types of species isolated in this study are very diverse. The high level of faecal carriage of MDR bacteria is not a surprising finding, considering the elderly population with previous hospitalization encountered in our hospital. However, 14 (15% of the patients carrying MDR bacteria) were under 25 years of age and 10 had never been hospitalized. This young population, the worldwide E. coli clone was detected in three cases, confirming the spread of this worrisome strain in the community. Different studies have previously reported the
prevalence of faecal carriage of ESBL-producing isolates. \textsuperscript{5,6,14,15}
Marked variations were observed in the incidence and genotype of these strains in hospitals, nursing homes or communities among countries. Infections due to MDR strains are classically associated with prolonged hospital stays, increased healthcare costs and increased mortality. \textsuperscript{15} Previous exposure to antibiotics seems to be an important factor, as this study also suggests. Moreover, person-to-person transmission and acquisition from a common source may contribute to ESBL dissemination and represent a risk of being a carrier compared with unrelated persons.\textsuperscript{15}

Certain points must be highlighted. The prevalence of ESBL-producing strains in stool samples (48/303, 15.8\%) was higher than that observed in clinical samples from the same period. During 2009, 618 clinically relevant MDR strains out of 5764 Gram-negative bacteria (10.7\%) were isolated in our hospital. Moreover, the prevalence of infection with ESC-resistant Enterobacteriaceae and \textit{E. coli} strains was 8.2\% (420 out of 5093 Enterobacteriaceae isolated in 2009) and 5.5\% (174 out of 3181 \textit{E. coli} isolated), respectively. Over recent years, we, and many other hospitals, have observed the rapid emergence of the CTX-M-type enzyme as the predominant ESBL in infected patients. However, in our study the proportions of the different ESBL types found in faecal carriers were totally different. TEM-family $\beta$-lactamases were the most predominant enzymes in ESBL-producing strains (58.3\% of ESBLs found) found in faecal carriage. This trend is different from previous studies.\textsuperscript{5,16,17} TEM producers are classically detected in our hospital, but their involvement in infections has recently declined in France.\textsuperscript{18} Noticeably, TEM-21-producing \textit{P. aeruginosa} is commonly isolated in France, but we have never previously isolated it in our hospital.\textsuperscript{19} Finally, we have detected carbapenemases in our hospital for the first time. The carbapenemases we found were either metallo-$\beta$-lactamases or OXA-type carbapenemases. The metallo-$\beta$-lactamases that belong to Ambler class B (VIM and IMP) have been identified in different countries as a source of several nosocomial outbreaks.\textsuperscript{20} MDR \textit{E. cloacae} strains represent an emerging problem in France.\textsuperscript{18} The ESC-resistant strains have progressively replaced the epidemic clone of \textit{E. aerogenes} carrying TEM-24.\textsuperscript{21} We also emphasize the first description of IMP-1 and VIM-1 enzymes in our hospital, not identified in clinical samples to date, but reported in Turkey and Spain.\textsuperscript{22,23} Concerning OXA-type carbapenemases, two cases of urinary colonization with OXA-23-producing \textit{A. baumannii} have been previously observed in our hospital (J.-P. Lavigne, unpublished data). This type of $\beta$-lactamase was previously reported in France.\textsuperscript{24} This emergence is worrisome since the antimicrobial treatment options are very restricted.

In conclusion, in a French university hospital, hospitalized patients have a high prevalence of gut carriage of ESBL- and carbapenemase-producing Gram-negative bacilli. As the epidemiology of faecal carriage is different compared with infections currently found, routine screening for MDR Gram-negative bacilli at admission seems unnecessary, but implementation of periodic active surveillance will be useful to monitor the changing epidemiology of these resistant strains. Moreover, basal hygiene (strict compliance to hand washing to prevent transmission via carriage on hands) must be reinforced, risk factors for acquisition of these strains must be detected and a decrease in the pressure of excessive antibiotic use should be an obligation.

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