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Prevalence of carbapenem-resistant *Enterobacteriaceae* in in- and out-of-hospital environments

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Abstract

Introduction: Healthcare is recognized as a condition of high selective pressure (SP) due to the use of antimicrobials. The emergence of community bacterial species resistant to the antimicrobials most extensively used in hospitals is of concern, as is the case with Gram-negative rods producing extended-spectrum beta-lactamases (ESBL). Hospital colonization by carbapenem-resistant Enterobacteria (CRE) can persist for many months after hospital discharge, and their encoding genes can be transferred among different species. Methodology: Because of the scarcity of options due to resistance to carbapenems, we sought to evaluate the occurrence of CRE in stool samples from outpatients and inpatients of a University Hospital, for whom clinical and epidemiological data were obtained from the analysis of medical records. After parasitological examination, stool samples from outpatients and inpatients were diluted in saline (1:20, 1:1000 and 1:2000) solution and 0.1 mL seeding was performed on MacConkey Agar (MA) containing gentamicin 8 µg/mL and MA containing cephalexin 32 µg/mL. Once isolated, strains were identified by biochemical tests and AST (with imipenem, ertapenem, and meropenem) was performed according to CLSI (2020). Results: We isolated carbapenem-resistant strains of enterobacteria in 13 (43.3%) of the 30 inpatients and in 13 (29%) of the 31 outpatients (p>0.05). In 7 (23.3%) of the 30 inpatients and in 5 (16.1%) of the 31 outpatients, we isolated CRE at 1:1000 or 1:5000 dilutions of stool samples, which correspond to "microbial loads". Ten bacterial species were isolated in the CRE related to the 13 inpatients and 13 outpatients, among whom we identified two or more species in 9 (69.2%) and 10 (76.9%), respectively. Even taking into account the limitations of the study due to the possibility of bias arising from the absence of data in the medical records, the detection of intestinal colonization with CRE in non-hospitalized individuals is of concern and may jeopardize the implementation of rational empirical therapy in patients of that community. Conclusion: It is possible that, as was the case of ESBL-producing strains decades ago, infections by carbapenem-resistant strains have become endemic in the community. In general, this development is a cause of concern, for plasmid-associated antimicrobial resistance strains, due to the fact that an often-recognized plasmid-associated fitness of bacterial cell favors persistence of the strains, even in the absence of antimicrobial (co-)selective pressures.

Keywords: Feacal enterobacteriaceae; Carbapenemases; Inpatients; Outpatients; Microbial loads.

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Introduction

Reports of the detection of community-acquired carbapenem-resistant *Enterobacteriaceae* (CREs) are of great concern.[1,2] Nordmann and colleagues, 2011[3] emphasize that enterobacteria are responsible for infections acquired in hospitals or in the community, since they are easily disseminated among humans because of the contamination of hands, food, and water.[3] These bacteria are recognized as especially competent in the horizontal transfer of genes carried by transposons and plasmids. Such carbapenemase-producing, multidrug-resistant strains can be considered to be endemic in various parts of the world.[4,5]

The circulation of these microorganisms and the resulting endemic condition of infections in the community is thought to be a consequence of several factors acting together: contaminated food; natural or artificial water reservoirs; poor sanitation and hygiene; and uncontrolled use of antimicrobials (including self-medication, etc.).[6,7] It is considered that, more generally, the wide global spread of antimicrobial resistance is due to the complex interactions between human health, animal breeding and veterinary science, with antimicrobial use, sanitation, hygiene, sewage and water treatment playing a significant role.

Currently, according to the World Health Organization (WHO), carbapenem-resistant CRE as well as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the main threats to human health.[8] In 1993, in France, the first carbapenemase-producing *Enterobacteriaceae* (NmcA) were detected in *Enterobacter cloacae*.[9]

The most frequently detected carbapenemases worldwide are *Klebsiella pneumoniae* carbapenemase (KPC), Ambler class A, Verona integron-encoded metallo-beta-lactamase, type B (VIM), New Delhi metallo-beta-lactamase, class B (NDM) and oxacylinase-48, class D (OXA-48). One of the most frequently detected genes is blaKPC-2.[10] Resistance to carbapenems, as occurs in the common production of "classical" ESBLs, is encoded in bacterial mobile genetic elements.[11] It is very relevant that hospital colonization by CRE can persist for many months after hospital discharge, and that their coding genes can be transferred between different Gram-negative bacterial species.[4]

Human gut microbiota, especially, are known to be important reservoirs of CRE and other relevant pathogens. Surveillance cultures have become a practice of great relevance for the detection of multidrug-resistant bacteria that require contact prevention measures, among others, to control the spread of these potential agents of infection.[10]

Chika and colleagues, 2017[12] isolated metallo-beta-lactamase-producing GNR in rectal swabs from cattle and poultry. The authors highlight the importance of food as a transmission vehicle of CRE in the environment and emphasize the need for continuous monitoring. They highlight the importance of continuous monitoring of multidrug-resistant strains, including MBL-producing enterobacteria, to detect the occurrence of transmission, between hospital and community, in both directions. Reports of CRE infection in the community are unable to fully confirm the origin (whether hospital or community) of the strains.[13-17]

We can consider monitoring to include more than hospital colonization surveillance, despite recognizing the great importance of the latter. Thus, the aim of this study is to evaluate the occurrence of CRE in stools of patients treated at a University Hospital Outpatient Clinic.

Methodology

We sought to evaluate the occurrence of CRE in stool samples from outpatients and inpatients of a University Hospital, whose clinical and epidemiological data were obtained from the analysis of clinical records. The use of antimicrobials prior to hospitalization, prior hospitalization or treatment in out-of-hospital healthcare environments were considered to be exclusion criteria. Stool samples obtained for parasitological examination from outpatients and inpatients were diluted in saline (1:20, 1:1000 and 1:2000) solution and 0.1 mL seeding was performed on MacConkey Agar (MA) containing gentamicin 8 µg/mL and MA containing cephalexin 32 µg/mL. Isolated strains were stained using the Gram method and observed by microscopy. The strains were identified by biochemical tests and AST for carbapenems (with imipenem, ertapenem, and meropenem) and for several other antimicrobials, performed according to the stipulations of the Clinical and Laboratory Standards Institute (CLSI).[18] The data obtained were analyzed using the Statistical Package Epi InfoTM - CDC/USA. The study was approved by the Ethics Committee of the Pedro Ernesto University Hospital (CEP/HUPE), under the number CAAE 44685015.9.0000.5259/2015.

Results

We isolated strains of carbapenem-resistant *Enterobacteriaceae* in 13 (43.3%) of the 30 inpatients and in 9 (29.0%) of the 31 outpatients ($p>0.05$), which is not statistically significant. In 7 (23.3%) of the 30 inpatients and in 5 (16.1%) of the 31 outpatients, we isolated CRE at 1:1000 or 1:5000 dilutions of stool samples, which correspond to "microbial loads" (Table 1). Ten bacterial species were isolated both among the CRE originating from the 13 inpatients and those isolated from the 13 outpatients, in which we identified two or more species in 9 (69.2%) and 10 (76.9%), respectively (Table 2).

It seems important, here, to report the diversity of species isolated in a large proportion of the isolated cases (individual stool samples) from the stool of both inpatients and outpatients (Table 2). We found multiple CRE resistance profiles in individual stool samples (from the same patient) for both types of patients. The association of several determinants associated with carbapenem resistance for the different isolated strains for both inpatients and outpatients is, therefore, evident.

Discussion

Even taking into account the limitations of the study due to the possibility of bias arising from the absence of data in the medical records, the detection of and intestinal colonization with CRE in non-hospitalized individuals is cause for concern. Furthermore, our results may possibly be subject to some bias, since the fecal samples were obtained for parasitological examination and may include patients presenting dysbiosis, which favors bowel colonization by antimicrobial resistant bacteria. Analyses involving stool dilutions allowed the detection of CREs in some cases in dilutions of up to 1/5,000.

Nordmann and colleagues, 2011[3] found few reports of community-acquired CRE infections; however, considering the numbers of cases in endemic areas worldwide, the true number may, in fact, be higher. In fact, the authors assert that selective pressure processes may occur due to structurally unrelated antimicrobials for which carbapenem-producing strains express resistance genes (co-selection), promoting the spread of these strains. Case reports exist of

Table 1. Frequencies of patients with CRE isolation at different dilutions of stool samples. For each patient, we considered the highest dilution at which a strain of CRE was isolated

Highest dilution where CRE were found	Number of outpatients	Number of inpatients
1:20	8 (62%)	6 (46%)
1:1000	3 (23%)	2 (15%)
1:5000	2 (15%)	5 (39%)
Total	13 (100%)	13 (100%)

Source: The authors (2022).

Table 2. Distribution of *Enterobacteriaceae* species isolated from patient stools by origin and by dilution of clinical specimen

Numbers of carbapenem-resistant *Enterobacteriaceae* strains									
Bacterial species	Inpatients				Outpatients				Total
	Dilutions of stool			Subtotal	Dilutions of stool			Subtotal	
	1:20	1:1000	1:5000		1:20	1:1000	1:5000		
Escherichia coli	6	6	0	12 (27%)	5	1	0	6 (21%)	18 (25%)
Klebsiella pneumoniae	5	2	2	9 (21%)	0	0	0	0	9 (13%)
Morganella morganii	5	1	1	7 (16%)	2	0	0	2 (7%)	9 (13%)
Enterobacter cloacae	1	1	1	3 (7%)	6	0	0	6 (21%)	9 (13%)
Citrobacter freundii	3	1	0	4 (9%)	2	1	1	4 (14%)	8 (11%)
Klebsiella oxytoca	0	1	1	2 (5%)	1	1	1	3 (11%)	5 (7%)
Citrobacter diversus	0	0	0	0	4	0	0	4 (14%)	4 (5%)
Pantoea agglomerans	2	0	2	4 (9%)	0	0	0	0	4 (5%)
Enterobacter aerogenes	1	0	0	1 (2%)	1	0	0	1 (4%)	2 (3%)
Providencia stuartii	1	0	0	1 (2%)	0	0	0	1 (4%)	2 (3%)
Enterobacter asburiae	0	0	0	0	0	1	0	1 (4%)	1 (1%)
Enterobacter sakasakii	1	0	0	1 (2%)	0	0	0	0	1 (1%)

Source: The authors (2022).

CRE infections with a presumed community origin, but it is difficult, from these sources, to exclude the occurrence of exposure to hospital or other healthcare environments – a possible source of colonization with CRE and/or other antimicrobial multidrug-resistant bacteria.

Okazaki and colleagues, 2015[13] report on community pyelonephritis, with no history of hospitalization. However, the patient resided in a "nursing home". Such environments are consid-

ered a risk factor for colonization by multidrug-resistant strains.[11] In fact, the authors draw attention to the importance of emergency services professionals considering the occurrence of multidrug-resistant microorganism infections, even in non-hospital institutions. Song and colleagues, 2019[16] report a hospital outbreak of KPC2-Kp infections in Seoul, South Korea. This outbreak was well-characterized, by phenotypic and molecular analysis, as originating from hospitalization of a patient with community-acquired infection (*index* case), where conjugative plasmid carrying the gene *kpc* was detected. From the information provided in the study, we cannot totally exclude the occurrence of healthcare-associated infection (HAI) in this patient index. Khatri and colleagues, 2015[14] report the successful treatment of a pregnant patient with pyelonephritis caused by KPC-producing *Klebsiella pneumoniae*. The patient did not report previous hospitalization, but reportedly lived with her mother, who was undergoing cancer treatment in out-of-hospital healthcare units. It seems that the importance of this contact in the eventual acquisition of CRE cannot be dismissed. In the Brazilian context, Azevedo and colleagues, 2019[15] detected, as CRE, an expressive proportion of *K. pneumoniae* isolated from urinary tract infections considered to be community-based. From 48 strains of *K. pneumoniae* isolated from "community" patients with urinary tract infection, 29 (60.4%) were found to be multidrug-resistant strains. The authors detected 46 genes specific for beta-lactamases in 27 (56.3%) samples. They found 73 genes encoding virulence factors in 30 strains (62.5%). The authors, however, do not report on the possible occurrence of prior hospitalization of patients. They draw attention to the need for epidemiological surveillance to prevent patients being discharged from hospital, thus preventing colonization that leads to the spread of resistant strains and the occurrence of infections in the community.

Reports of CRE infection in a community invariably raise some degree of doubt about the origin of the strains. We can consider a genuine community infection to be true if it occurs thanks to "circulating" CRE, relatively independent of institutional environments under high selective antimicrobial pressure. According to Nordmann and colleagues, 2011,[3] it is possible that, and of great concern for carbapenem resistance, a process similar to that for ESBL-producing strains decades ago will occur, causing endemic infections in the community. Furthermore, it is important to consider that, unlike what may be considered the selective pressure capable of favoring ESBL-producing strains in the community in general, the restrictive use of carbapenems "out-of-hospital" can delay the establishment of CRE. However, plasmids containing both the gene for carbapenem resistance and "classic" ESBLs have been described in CRE strains.[1]

The combination of several determinants associated with carbapenem resistance, particularly the resistance markers for aminoglycosides and quinolones for the different stool strains isolated, in patients from both origins, is very likely a sign of plasmid-encoding.[10] Plasmids that carry gene coding for resistance to carbapenems often also contain gene coding for ESBLs, in addition to other determinants of resistance. Also, plasmids containing two different genes codifying for carbapenemases have been reported.[19] Furthermore, many of the different plasmids already characterized in several plasmids of different incompability (Inc) groups are transferable by bacterial conjugation.[20]

It is likely that poor hygiene and sanitary conditions, environmental contamination, and/or contamination of the food and water used, as well as uncontrolled use of antimicrobials in India (as occurs in the Asian continent in general) may act as risk factors for the acquisition of CRE and ESBL-producing *Enterobacteriaceae* (ESBLPE). Such strains can be acquired by travelers. The authors recommend that patients receiving healthcare while traveling abroad should undergo surveillance cultures for colonization by these microorganisms upon hospitalization.[6]

Woerther and colleagues, 2017[21] concluded from an extensive review of international travel, particularly to sub-tropical regions, that these are important risk factors for ESBLPE colonization. We can consider that – even while acknowledging some differences – Brazilian sanitary and hygienic conditions are very often precarious, and may identify settings recognized as favorable for contamination, persistence, and circulation of CRE in the community.

CRE have been detected in the community environment in Guanabara Bay, Rio de Janeiro.[22] De Araújo and colleagues, 2016[23] detected different gene coding for the production of carbapenemases (bla_{KPC}, bla_{NDM}, bla_{GES}, and $bla_{OXA48\text{-}like}$ genes) in *Enterobacteriaceae* in two water bodies (matrices) in Rio de Janeiro, Brazil. The authors warn of the possibility of transmission of these strains and genes to the community.

We found multiple CRE resistance profiles in individual stool samples of both inpatients and outpatients. These are strains of different *Enterobacteriaceae* species isolated from the stool of the same patient. It seems valid to consider that the diversity of species may caused by the very diversity of contaminating strains in the environment, whether hospital or otherwise, or by successive contamination events. Hospital environments, especially some departments (e.g. ICUs), are recognized as being intensely and widely potentially contaminated (as to the diversity of multidrug-resistant strains, in general). But it should be considered that, very often, gene coding for carbapenem resistance is located in plasmids transferable by bacterial conjugation. We cannot rule out the transfer of plasmids among strains (possible even from different species) in the context of the microbiota of individual patients.

Considering what has been reported and discussed above, the stool colonization of patients with community-acquired infections and their domestic contacts may be considered CRE reservoirs, and can determine the risk of dissemination of resistant strains to healthy individuals in the community.

Cano and colleagues, 2018,[24] in a prospective study during an outbreak of KPC-Kp infections in a Spanish hospital, validated both models determining scores indicative of the occurrence of infection (not just sepsis) in patients with intestinal colonization, and a mortality prediction model for established infections. This result allowed the establishment of the most appropriate empirical therapy according to the clinical situation.

Nordmann and colleagues, 2011[3] alert that high rates of carbapenemase-producing *E. coli* infections could be reached worldwide. According to the authors, unlike viral epidemics, such as the H1N1 pandemic, the CRE epidemic would not spontaneously cease.

Conclusion

Our study has limitations because it is based on analyses of clinical material not obtained specifically for the proposed objective, but this material allowed the detection of intestinal colonization of inpatients and outpatients by different species of enterobacteria showing phenotypes of resistance to carbapenems. Analyses involving stool dilutions allowed the detection of CREs, in some cases in dilutions of up to 1:5,000. It is possible that, as occurred with ESBL-producing strains decades ago, infections by carbapenem-resistant strains will become endemic in the community.

Potential conflict of interest

The authors declare no conflicts of interest.

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