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Short Communication

β-Lactam and chloramphenicol-resistant enterobacteria in hospital surfaces

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The following study aimed to research the Enterobacteriaceae present on the material surfaces of a hospital environment in a Community Health Unit in Ceara-Brazil. Data was collected in 10 different rooms and facilities by rubbing sterile swabs in an enclosed area of 10 cm² for a minute. Bacterial growth was observed in all surveyed areas. However, Enterobacteriaceae were only found in surfaces from the kitchen and the reception. From the isolated strains (n = 10), the vast majority were identified as *Enterobacter* (n = 7). Four of those *Enterobacter* strains were found to be resistant, with the following resistance profiles: monoresistance to ampicillin (n = 2) and chloramphenicol (n = 1) and cross-resistance to beta-lactam (n = 1). The results serve as an alert to public health authorities, for enteric bacteria resistant to drugs were found in two environments in the facility.

Key words: Enteric bacteria, antimicrobial resistance, hospital environment.

INTRODUCTION

Hospital-associated bacteria have been commonly related to outbreaks (Brust et al., 2013) and environmental surfaces may contribute to transmission of nosocomial pathogens (Livshiz-Riven et al., 2015). Among contaminant bacteria found in those surfaces, enterobacteria are worth mentioning, since these microorganisms are associated with infections (Ito et al., 2015). For Loftus et al. (2015), Gram-negative bacteria (GNB) are an important health care concern due to increasing prevalence of infection and community spread. In this context, the isolation of Gram-negative enteric bacilli on surfaces from hospital environments (Thurlow et al., 2013; Freeman et al., 2014) has been previously reported, suggesting that these surfaces may contribute to the transmission of pathogens implicated in nosocomial infections. The alert is also extended to the spread of antibiotic-resistant strains, as these microorganisms may become part of the allochthonous microbiota

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Abbreviations: GNB, Gram-negative bacteria; **CRE,** carbapenem-resistant enterobacteriaceae.

of a hospital setting (Di Conza et al., 2014) if the disinfection procedures are not properly conducted. For Pelege and Hooper (2010), GNB are highly efficient at up-regulating or acquiring genes that code for mechanisms of antibiotic resistance.

In hospital environmental, the emergence of drug-resistant enterobacteria is currently a worldwide problem. Carbapenem-resistant Enterobacteriaceae (CRE) has been detected as contaminant of the environmental surfaces of hospital room (Weber et al., 2015). In a tertiary care hospital in Mexico City, Torres-Gonzalez et al. (2015) report an outbreak caused by a New Delhi Metallo- β-lactamase 1 (NDM-1) harboring plasmid spread by *Escherichia coli* (ST617) and *Enterobacter cloacae* (ST182). Thus, considering that understanding microbial populations in hospital environments is crucial for improving human health (Poza et al., 2012), the present study aimed to verify the occurrence of enterobacteria in hospital surfaces from a Community Health Unit in Ceará-Brazil, by isolating and identifying enteric bacteria, as well as proceeding with the antimicrobial susceptibility profile of the isolates.

MATERIALS AND METHODS

Samples for microbiological analysis were collected from a Health Unit located in Ceara-Brazil. They were collected from surfaces in ten different rooms: dining room table, kitchen counter, reception counter, reception chair, external knob of the bathroom door, internal knob of the bathroom door, seat in the nursing room, workbench in the nursing room, table in the doctor's office and workbench in the doctor's office. Collection procedure corresponded to rubbing sterile swabs for about a minute in an enclosed area (10 cm^2) from each surface. After the sampling, swabs were placed in tubes containing Brain Heart Infusion Broth (BHI - Difco) medium, incubated at 35 ± 1.0°C for 24 h. Then, aliquots were removed, plated on McConkey agar and incubated at 35°C ± 1.0/24 h. Colonies with phenotypic characteristics consistent with those of enterobacteria were isolated on tryptone soy agar (TSA), followed by incubation at 35°C ± 1.0 / 24 h. Gram stain analysis and oxidase test were subsequently performed. The Gram-negative oxidase-negative bacilli cultures were selected for the identification process via EPM – MiLi – Simmons Citrate kit enterobacteriaceae identification (Indol, L-triptofano, sacarose, H_2S, gas glicose, l-lisina, motilidade), with incubation at 35°C ±1.0/ 24 h. Strains confirmed as enterobacteria were kept in TSA until the antimicrobial susceptibility testing.

The determination of antimicrobial susceptibility profiles were performed by the agar diffusion disk technique using Agar Mueller-Hinton (MH) medium, as detailed in the Clinical and Laboratory Standards Institute (CLSI, 2012). The following antimicrobials were tested: Amikacin (30 µg), Ampicillin (10 µg), Cefotaxime (30 µg), Cefepime (30 µg), Ceftriaxone (30 µg), Cefuroxime (30 µg), Chloramphenicol (30 µg), Streptomycin (10 µg), Gentamicin (10 µg), Imipenem (10 µg), Meropenem (10 µg), and Tetracycline (30 µg). All enterobacteria strains were diluted in 0.85% saline in order to match a turbidity of a McFarland 0.5 scale. Aliquots were removed from the diluted cultures and plated onto MH medium, followed by the application of antibiotic disks. Plates were incubated at 35°C ± 1.0 and the reading and interpretation of inhibition zones were in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2012).

RESULTS AND DISCUSSION

Microbial growth was observed on all analyzed surfaces. However, enterobacteria were only confirmed on surfaces 2 (kitchen counter) and 3 (reception counter). Out of the Enterobacteriaceae identification, seven out of 10 isolated strains were identified as *Enterobacter* (areas 2 and 3), two were of indeterminate profile and one was confirmed as *Citrobacter.*

The bacterial genus which represented 70% of total isolates - *Enterobacter* - is a common nosocomial microorganism (Tuon et al., 2015). Its isolation from samples taken on hospital surfaces is not an unusual fact: Matoušková and Holy (2014) conducted a monitoring of a transplant unit, hemato/oncology clinic in Czech Republic and detected, from samples taken from surfaces, that the second most frequently isolated Gram-negative strains were from the *Enterobacter* (28%) genus.

The occurrence of bacteria in hospitals has been commonly related to some possible sources of dissemination: bottle soap (Buffet-Bataillon et al., 2009), hands of healthcare professionals (Tan et al., 2013), gloves and gowns (Rock et al., 2014), mobile phones (Ustun and Cihangiroglu, 2012) paper money and coins (Angelakis et al., 2014). Besides those vectors, the occurrence of hospital pests must also be highlighted. Menasria et al. (2014) researched the bacterial load of a cockroach species (*Blattella germanica*) found in hospital environment, and isolated *Enterobacter* and *Citrobacter* from both external surface and digestive tract of the insect.

In the present study, enteric bacteria were isolated from the bench designed for processing the hospital's food. This is a fact of major concern, and it serves to alert the risk of food contamination through inadequate hygiene practices, since these microorganisms can be indicators of fecal contamination (Van Hoek et al., 2015). In a similar study, Staskel et al. (2007) evaluated the microbiota of foodservice surfaces in several Texas child-care centers, and isolated Enterobacteria (*Klebsiella pneumonia* and *Salmonella* Paratyphi A) that are considered nonopportunistic and can infect healthy individuals. The authors stated that it is vital that the staff wash their hands often and disinfect every surface, for even those that appear to be clean may harbor microorganisms.

The reception counter was another place where enterobacteria were detected. This may be related to the continuous flow of patients and professionals, which could cause not only the contamination per se, but its spread to other sectors in the facility.

All isolates confirmed as enterobacteria (n = 10) were submitted to an antibiogram. Four strains resistant to the following profiles were detected: monoresistance to ampicillin (n = 2), monoresistance to chloramphenicol (n = 1), and cross-resistance to the β–lactam Amp, Cro, Ipm e Mer (n = 1). No strain presented multi-resistance (Table

Table 1. Antimicrobial resistance profile of *Enterobacter* strains isolated from hospital surfaces.

Enterobacteria	n	Source	Antimicrobial resistance
Enterobacter	1	Kitchen	Amp, Cro, Crx, Ipm, Mer
Enterobacter	1	Kitchen	Clo
Enterobacter	1	Reception	Amp
Enterobacter	1	Reception	Amp

*Amp, Ampicillin; Cro, ceftriaxone; Crx, cefuroxime; Imp, imipenem; Mer, meropenem; Clo, chloramphenicol.

1). Among the group of β-lactam antibiotics, ampicillin was the antibiotic for which the resistance index was the highest (n = 3). Besides, resistance to second (cefuroxime) and third (ceftriaxone) generation of cephalosporin was observed (Table 1). In accordance to our findings, Vasques et al. (2011) isolated *Enterobacter* spp. in hospitals and detected resistance to ampicillin and ceftriaxone. To the authors, there is a high incidence of infections caused by betalactam-producing Gram-negative microorganisms in Brazil. These organisms are of clinical and epidemiological importance, since their mobile genetic elements helps in the cross-infection process.

Hawkey (2015) alerted to the fact that in Asian countries a consequence of high rates of ESBL production among Enterobacteriaceae is that there is a substantial use of carbapenem antibiotics, resulting in the emergence of plasmid-mediated resistance to this class of drugs. In the present study, one strain resistant to imipenem and meropenem (Table 1) was found, which characterizes the health unit studied as a potential source of a carbapenem-producing bacteria spread. The low isolation rate of Carbapenem-resistant Enterobacteriaceae (CRE) is in accordance to the findings of Werber et al. (2015), who researched the survival of CRE in inoculated surfaces of hospital rooms. The authors verified that three species of CRE (*Klebsiella*, *Enterobacter*, and *Escherichia)* survived poorly (>85% die-off in 24 h) when ~2 $\log_{10}$ CFU were inoculated onto 5 different environmental surfaces.

Increasing on the incidence of antibiotic-resistant Gram-negative infections has become the most pressing issue in bacterial resistance. Indiscriminate antimicrobial use in humans and animals, combined with an increased global connectivity fostered the transmission of Gram-negative infections harboring extended-spectrum β-lactam in the 1990s. Carbapenem-producing Enterobacteriaceae have been the latest affliction since late 1990s and early 2000s (Vasoo et al., 2015).

One strain of a mono-chloramphenicol-resistant *Enterobacter* was found (Table 1). Čivljak et al. (2014) stated that chloramphenicol is a broad spectrum antibiotic that was abandoned in developed countries due to its association with fatal aplastic anemia. However, it is still widely used in 3rd world countries. In the light of the emerging problem of multi-drug resistant pathogens, its role should be reassessed. Susceptibility patterns for Gram-positives were good, although less favorable for Gram-negatives.

The findings in this study serve as a warning to political and social authorities in Public Health as an evidence that hospital surfaces can be antibiotic-resistant enteric bacteria reservoirs. Thus, it is important to be extremely cautious about the quality of the cleaning and disinfecting in hospital environments, providing orientation to both patients and visitors, as well as continuing education measures to all the health professionals in different units.

Conflict of interest

The authors did not declare any conflict of interest.

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