that of mouthwash. Overall, our finding suggests that oral hygiene care using CHG gel is more practical and cost-effective than using CHG mouthwash in surgical ICUs.

This study has several limitations. It was conducted in a single institution in a short duration. The method of oral hygiene care and the cost of CHG may be different from other ICUs. Therefore, our finding may not be generalizable to other hospitals, and a further large-scale study is warranted to confirm our findings. In conclusion, CHG gel is a better choice than CHG mouthwash in oral hygiene care for preventing VAP.

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REFERENCES

1. Guillamet CV, Kollef MH. Update on ventilator-associated pneumonia. *Curr Opin Crit Care* 2015;21:430–438.
2. Guillamet CV, Kollef MH. Ventilator-associated pneumonia in the ICU: where has it gone? *Curr Opin Pulm Med* 2015;21:226–231.
3. O'Grady NP, Murray PR, Ames N. Preventing ventilator-associated pneumonia: does the evidence support the practice? *JAMA* 2012;307:2534–2539.
4. Chen Y, Mao EQ, Yang YJ, et al. Prospective observational study to compare oral topical metronidazole versus 0.2% chlorhexidine gluconate to prevent nosocomial pneumonia. *Am J Infect Control* 2016;44:1116–1122.
5. Zhang TT, Tang SS, Fu LJ. The effectiveness of different concentrations of chlorhexidine for prevention of ventilator-associated pneumonia: a meta-analysis. *J Clin Nurs* 2014;23:1461–1475.
6. Cocanour CS, Peninger M, Domonoske BD, et al. Decreasing ventilator-associated pneumonia in a trauma ICU. *J Trauma* 2006;61:122–129; discussion 129–130.
7. Hua F, Xie H, Worthington HV, et al. Oral hygiene care for critically ill patients to prevent ventilator-associated pneumonia. *Cochrane Database Syst Rev* 2016;10:Cd008367.
8. Fourrier F, Dubois D, Pronnier P, et al. Effect of gingival and dental plaque antiseptic decontamination on nosocomial infections acquired in the intensive care unit: a double-blind placebo-controlled multicenter study. *Crit Care Med* 2005;33:1728–1735.
9. Francis JR, Hunter B, Addy M. A comparison of three delivery methods of chlorhexidine in handicapped children. I. Effects on plaque, gingivitis, and toothstaining. *J Periodontol* 1987;58:451–455.

Does Second Place Count? Lessons from a Major Discrepancy Between Carbapenem-Resistant *Klebsiella pneumoniae* and Carbapenem-Resistant *Enterobacter cloacae* in a One-Year Follow-Up Study

To the Editor—Carbapenem-resistant Enterobacteriaceae (CREs) have become one of the most prevalent agents in nosocomial infections, and they are associated with poor outcomes.[1] In many Brazilian hospitals, *Klebsiella pneumoniae* carbapenemase (KPC)–producing *K. pneumoniae* (*Kp*), a main representative of the CRE group, has reached endemic levels and has been responsible for high morbidity and mortality rates.[2,3]

Since the emergence of KPC *Kp*, practically no other microorganism has managed to achieve prevalence levels as severe as those achieved by KPC *Kp*.[2,4,5] Some studies have shown the emergence of *Enterobacter* spp, especially *Enterobacter cloacae* and *Enterobacter aerogenes*, as a reflection of an increased prevalence rate, and they implicate CREs as one of the main bacteria with the ability to acquire and develop antimicrobial resistance, including carbapenem agents.[6,7]

In the past few years, the emergence of *Enterobacter* spp has been considered a second epidemic subsequent to the epidemic wave of KPC-producing microorganisms. However, in Brazil, few data are available to reveal how this microorganism has evolved over time, despite its recognized clinical and epidemiological status.

To verify the crude prevalence rate of CREs and to recognize a possible second potential CRE agent and assess its differences in relation to the most prevalent CRE, a retrospective survey from January 1 to December 26, 2016, was conducted at a tertiary hospital in Porto Alegre, Southern Brazil.

Identification of bacterial species as well as an antimicrobial susceptibility profile were initially performed using an automated broth microdilution system (MicroScan, Beckman Coulter, Brea, CA); the results were confirmed using the disk diffusion method. Determination of the resistance mechanism attributable to carbapenem agents was performed by applying a synergistic test with phenyl-boronic acid and ethylenediamine tetra-acetic acid for detecting KPC and metallo-betalactamase enzymes, respectively, and by enzymatic inhibition using clavulanic acid and cloxacillin for detecting extended-spectrum β-lactamases (ESBLs) and *Amp*C enzymes, in that order, as previously described.[2]

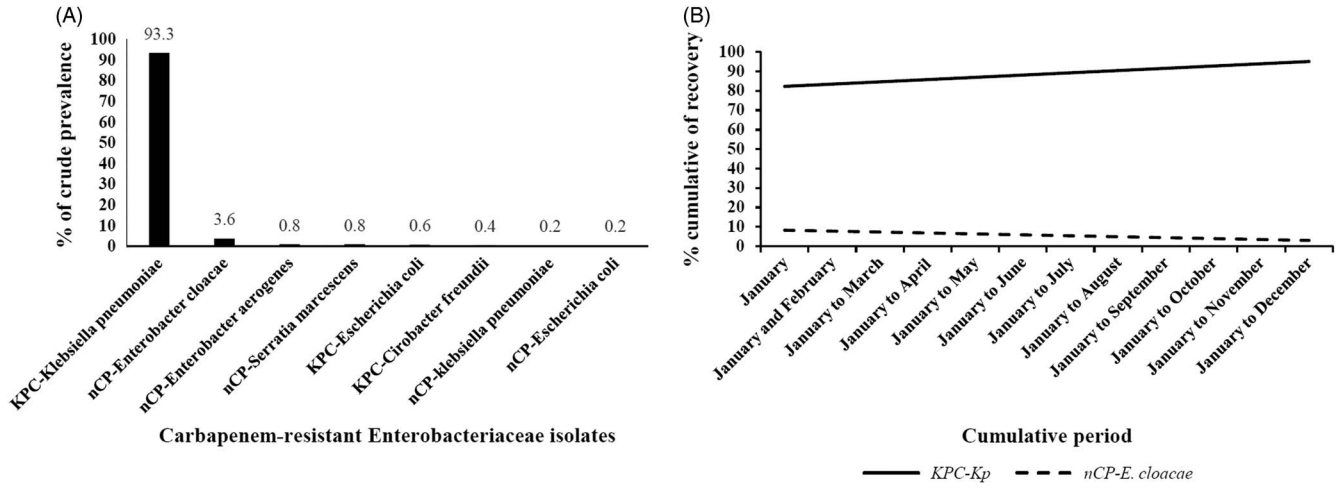

FIGURE 1. (A) Crude prevalence of all CRE isolates found in this study and (B) cumulative prevalence of KPC-*Kp* and nCP-*E. cloacae* (the 2 most prevalent agents) recovered from some clinical specimens during the study period.

During the study period, 472 CRE isolates were recovered from clinical sites. Among them, 445 (94.3%) were KPC producers and the remaining 27 isolates (5.7%) were noncarbapenemase producers (nCPs).

Among the KPC-producing isolates, *K. pneumoniae* (98.9%; 440 of 445) was by far the most prevalent microorganism, followed by *Escherichia coli* (0.7%; 3 of 445) and *Citrobacter freundii* (0.4%; 2 of 445). Among the nCP isolates, *Enterobacter cloacae* (63%; 17 of 27) was the most common agent, and the remaining isolates were *E. aerogenes* and *Serratia marcescens* (14.8%; 4 isolates each), and *K. pneumoniae* and *Escherichia coli* (3.7%; 1 isolate each).

The crude prevalence rates for all CRE isolates found in this study, not including the carbapenem-resistance mechanism involved (as described above), are illustrated in Figure 1(A). Although the nCP *E. cloacae* was the second most prevalent CRE, its recovery rate was extremely low compared to KPC *Kp* (3.6% vs 93.3%, respectively). In addition, the prevalence of nCP *E. cloacae* decreased over the study period, contrary to the trend observed in other countries.[6,8]

In past few years, *K. pneumoniae* and *E. cloacae* have proven to be challenging microorganisms regarding their pathogenesis, transmission, and ability to acquire antimicrobial resistance. Therefore, these organisms are highlighted in a faction of agents termed by the Infectious Diseases Society of America as the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp),[9] and their prevalence rates are not constantly monitored. Likewise, it is important that the differences among them be evaluated to produce a better strategy to combat their spread.

This study produced several conclusions: (1) nCP *E. cloacae* is the second most prevalent CRE in our institution, and it is far less prevalent than KPC *Kp*. (2) Different gender and/or species may reflect a better ability to become established in a nosocomial environment. Moreover, 440 of 1,099 of all *K. pneumoniae* isolates (40%) recovered during the study period were KPC producers, whereas only 17 of 144 of all *E. cloacae* (11.8%) recovered in the same period were carbapenem-resistant isolates. (3) The type of resistance mechanism (ie, those that confer resistance to broad-spectrum activity agents, such as carbapenems) is as important as gender and/or species differences. Furthermore, the KPC resistance mechanism seems to be more dangerous than any other, such as ESBL or *Amp*C β-lactamases, efflux pumps, outer membrane impermeability or even a sum of them.

Why does a microorganism with relatively minor virulence and resistance remain a second potential CRE agent? Although this question is still under investigation, a reasonable explanation would be the fact that *E. cloacae*, like *K. pneumoniae*, has a greater ability to become resistant to polymyxins, which are currently widely used.[6,8]

In conclusion, the results of this study confirm KPC *Kp* as the most prevalent CRE, far more prevalent than an nCP *E. cloacae* isolate, which was the second most common CRE pathogen identified in this survey. The presence of the bla_{KPC} gene seems to be mandatory for this major difference between species. However, a well-structured and well-coordinated surveillance plan must be implemented together with a strict program of antimicrobial use to prevent the spread of the most prevalent CRE as well as the less prevalent CREs.

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REFERENCES

1. Tamma PD, Goodman KE, Harris AD, Tekle T, Roberts A, Taiwo A, Simner PJ. Comparing the outcomes of patients with carbapenemase-producing and non-carbapenemase-producing carbapenem-resistant Enterobacteriaceae bacteremia. *Clin Infect Dis* 2016;64:257–264.
2. Perez LR. Carbapenem-resistant Enterobacteriaceae: a major prevalence difference due to the high performance of carbapenemase producers when compared to the nonproducers. *Infect Control Hosp Epidemiol* 2015;36:1480–1482.
3. Rodrigues Perez. Emergence of infections due to a polymyxin B-resistant KPC-2-producing Klebsiella pneumoniae in critically ill patients: What is the role of a previous colonization? *Infect Control Hosp Epidemiol* 2015;37:240–241.
4. Perez LR. Increase in prevalence of KPC-2-producing *Klebsiella pneumoniae* recovered from respiratory secretions of intensive care patients—getting a free ride on a menacing colistin resistance. *Infect Control Hosp Epidemiol* 2016;37:1521–1522.
5. Perez LR. The impact of carbapenem-resistant Enterobacteriaceae type on clinical outcomes after the recovery of this organism from urine of critically ill patients. *Infect Control Hosp Epidemiol* 2016;37:1257–1258.
6. Ronveaux O, Gheldre Y, Glupczynski Y, Struelens M, Mol P. Emergence of *Enterobacter aerogenes* as a major antibiotic-resistant nosocomial pathogen in Belgian hospitals. *Clin Microbiol Infect* 1999;5:622–627.
7. Gales AC, Jones RN, Sader HS. Global assessment of the antimicrobial activity of polymyxin B against 54731 clinical isolates of gram-negative bacilli: report from the SENTRY antimicrobial surveillance programme (2001–2004). *Clin Microbiol Infect* 2006;12:315–321.
8. Kanamori1 H, Parobek CM, Juliano JJ, et al. A prolonged outbreak of KPC-3-producing *Enterobacter cloacae* and *Klebsiella pneumoniae* driven by multiple mechanisms of resistance transmission at a large academic burn center. *Antimicrob Agents Chemother* 2016. doi: 10.1128/AAC.01516-16.
9. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J Infect Dis* 2008;197:1079–1081.

Characterization of Transformants Obtained From NDM-1–Producing *Enterobacteriaceae* in Brazil

To the Editor—The emergence of carbapenemase-producing Enterobacteriaceae (CPE) is an important public health problem because the treatment of CPE is difficult; few options remain available for clinical use.[1] The New Delhi metallo-β-lactamase (NDM-1) is the most common class B carbapenemase among Enterobacteriaceae, and it has been detected increasingly frequently in several countries,[2] including Brazil.[3–8] The aim of this study was to evaluate the characteristics of transformants obtained from NDM-1–production in different bacterial species of *Enterobacteriaceae* identified in southern Brazil.

Isolates were selected from a surveillance study that evaluated *Enterobacteriaceae* with reduced susceptibility to carbapenems in Rio Grande do Sul State, southern Brazil. A total of 9 clinical NDM-producing isolates from 4 hospitals were selected for this study: 3 *Klebsiella oxytoca*, 2 *Enterobacter clocae* complex, 1 *Klebsiella pneumoniae*, 1 *Morganella morganii*, 1 *Escherichia coli*, and 1 *Citrobacter freundii*. These isolates were initially identified by the VITEK2 system (bioMèrieux, Marcy-l'Étoile, France) and confirmed by 16S rRNA sequencing. The bla_{NDM} gene was detected by a multiplex real-time polymerase chain reaction (PCR), which also included primers for the bla_{KPC}, bla_{VIM}, bla_{GES}, bla_{NDM}, bla_{OXA-48}, and bla_{IMP} genes.[9] The presence of bla_{NDM} was further confirmed by conventional PCR, and the amplicons were purified and sequenced using a BigDye Terminator Kit (version 3.1, Thermo Fisher Scientific, Waltham, MA) and an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). GenBank was used to access the bla_{NDM} sequences deposited to date, and the BioEdit program was used to compare similarities between sequences. The plasmids were extracted by alkaline lysis and were transformed into *E. coli* TOP10 electrocompetent cells by electroporation. Transformants were selected on Luria-Bertani agar containing 2 μg/mL ceftazidime. The transformants were evaluated for the bla_{NDM} gene by conventional PCR with specific primers. The minimum inhibitory concentrations (MICs) of imipenem, meropenem, doripenem, piperacillin/tazobactam, ceftriaxone, cefepime, aztreonam, gentamicin, amikacin, polymyxin, and tigecycline were assessed by Etest (bioMèrieux, Marcy-l'Étoile, France). The modified Hodge test (MHT) and the combination-disc test (ie, meropenem and imipenem with and without ethylenediaminetetraacetic acid [EDTA]) were used as phenotypic methods for carbapenemase and metallo-β-lactamase detection, respectively.

It was possible to obtain transformants from all 9 clinical isolates. The transformants obtained from each isolate presented higher MICs than the original *E. coli* TOP10 for β-lactams. In fact, the MICs of transformants were similar to those of the donor NDM-positive clinical isolates, which showed high levels of resistance (Table 1).

Notably, the combined-disc assay with EDTA proved to be positive (ie, EDTA inhibited the carbapenem activity) for all NDM-1–producing clinical isolates and transformants.

Plasmid analysis indicated that most transformants contained a 110-kb plasmid: 2 from the *E. clocae* complex and 1 each from *K. oxytoca*, *M. morganii*, *E. coli*, and *C. freundii*. However, it was also possible to identify the presence of a 52-kb plasmid in a transformant from *K. oxytoca*, a 154-kb plasmid from a *K. oxytoca*, and a *K. pneumoniae* (Table 1).