first steps in environmentally compatible fly control and will provide long-term discouragement of additional pest invaders.

I recommend the following systematic approach: (1) prioritized leadership and hospital administrative commitment to sanitation; (2) empowering and educating food service workers in illness prevention; (3) emphasizing environmentally compatible sanitation methods coupled with strategic and specific applications of antipest agents when required; and (4) looking beyond surface cleanliness to follow food as an attractor of pests.

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# High minimum inhibitory concentrations among derepressed AmpC-beta-lactamase–producing *Enterobacter cloacae* complex isolates for ceftolozane with tazobactam

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*To the Editor*—*Enterobacterales*, such as *Enterobacter* spp, *Serratia marcescens*, *Citrobacter freundii*, *Providencia* spp and *Morganella morganii*, often referred to as the ESCPM group, may express high levels of chromosomal AmpC β-lactamases at high levels following exposure to β-lactams, mainly after third-generation cephalosporin therapy.[1] The induction or selection of derepressed isolates is a concern because they contribute to the isolation of organisms no longer susceptible to specific β-lactams and may lead to clinical failure, with scarce antimicrobial options.[2]

Ceftolozane with tazobactam (C/T) is a combination drug comprising a β-lactamase inhibitor (tazobactam) with a new cephalosporin (ceftolozane). Tazobactam inhibits class A extended-spectrum β-lactamases (EBSLs), and ceftolozane acts via a high affinity for some penicillin-binding-protein (PBPs). C/T is stable in the presence of AmpC β-lactamases and against OprD deficiency and efflux pumps. These characteristics make the C/T combination an important weapon in the treatment of infections due to extensively resistant *Pseudomonas aeruginosa* that are not carbapenemase producers.[3]

Despite the high efficacy described so far, emergence of resistance to C/T, mainly in *P. aeruginosa* isolates overexpressing AmpC-β-lactamase enzymes, have been reported.[4] Although derepressed AmpC may occur in *P. aeruginosa*, the main target for C/T use, this resistance mechanism is more robust in *Enterobacter cloacae* complex isolates, with a higher ability than others from the ESCPM group to derepress AmpC-β-lactamase production, which has important clinical and therapeutic implications.[5]

The main objective of this study was to determine the C/T minimum inhibitory concentration (MIC) among *E. cloacae* complex isolates, producing or not derepressed AmpC-β-lactamases. Additionally, meropenem and ceftazidime/avibactam MICs were also determined.

A set of 123 *E. cloacae* complex isolates recovered from inpatients between August 2016 and December 2017, in southern Brazil, were included in this study. Bacterial identification was made using the Vitek 2 automated system (bioMérieux, Marcy l'Etoile, France) and matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) for confirmation when necessary. Ertapenem susceptibility was determined using disc-diffusion testing.[6] The MICs of cefotolozane/tazobactam, meropenem, and ceftazidime/avibactam were determined using MIC test strips (MTS, Liofilchem, Italy) and were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) break points.[6] To attribute the resistance mechanism for the selected *E. cloacae* complex isolates, a synergistic test was applied using an enzymatic inhibition testing with clavulanic acid and cloxacillin and/or phenyl-boronic acid to detect ESBLs and AmpC enzymes, in that order, as reported elsewhere.[7] No isolate with carbapenemase production was included in this study, and for this study, all isolates were screened for a negative result using a blue-carba test to exclude class A and B carbapenemases and an OKN K-set immunochromatographic assay to exclude OXA-48-like production (ie, a carbapenemase with low hydrolysis activity for carbapenems and eventually resulting in a negative blue-carba test).[8]

Among the 123 isolates, 39 (31.7%) and 84 (68.3%) were characterized as derepressed and not-derepressed AmpC-β-lactamase

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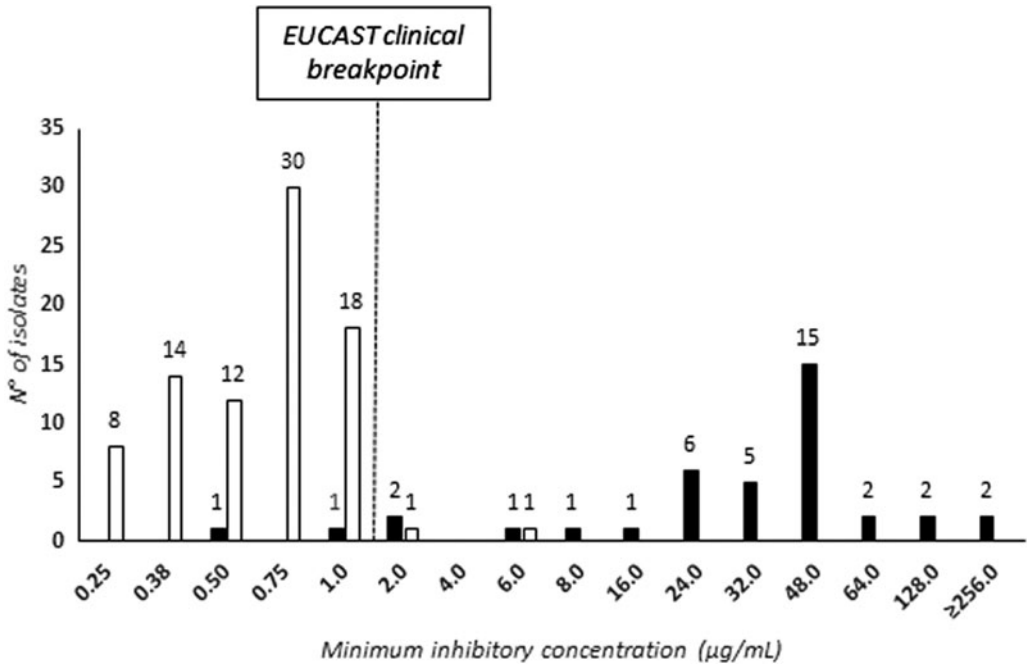


**Fig. 1.** Distribution of ceftolozane/tazobactam minimum inhibitory concentrations of 39 derepressed AmpC β-lactamase–producing organisms and 84 organisms not producing derepressed AmpC-β-lactamase. Black bars represent organisms expressing derepressed AmpC-β-lactamases based on positive results with cloxacillin synergy testing, and gray bars represent organisms not expressing derepressed AmpC-β-lactamases based on negative results with cloxacillin synergy testing.

producers, respectively, according to phenotypic testing. For derepressed AmpC-β-lactamase producers, 36 of 39 isolates (92.3%) were resistant to ertapenem according to disc-diffusion testing. For all isolates, meropenem and ceftazidime/avibactam showed excellent activity: $MIC_{90}$ of 0.75 and 2.0 mg/L, respectively. No ceftazidime/avibactam resistance was observed among the isolates. However, 39 of 123 isolates (31.7%) were resistant to C/T and 37 of these 39 (94.9%) were derepressed AmpC-β-lactamase producers. Only 2 derepressed AmpC isolates were susceptible to C/T when EUCAST break points (≤1.0 and >1.0 mg/L, for susceptible and resistant, respectively) were considered (Fig. 1).

*Enterobacter* spp, particularly *Enterobacter cloacae* complex, are the most problematic pathogens because of their potential for derepressing AmpC β-lactamase production, presenting an approximate rate that is 10-fold higher than that of other Enterobacterales.[9]

Ceftolozane/tazobactam can overcome inactivation by ESBL β-lactamases, usually allowing maintenance of its activity against Enterobacterales producing the globally important ESBLs CTX-M-14 and CTX-M-15. On the other hand, C/T may be influenced by AmpC β-lactamase activity, based on the results presented here, despite the fact that this drug combination is considered to have improved steric hindrance to prevent AmpC β-lactamase-mediated hydrolysis.

High-level AmpC expression appears to confer a fitness cost to an organism because of the high metabolic energy required to express *ampC* regulation. Nevertheless, in the face of a persistent stimulus (eg, β-lactam exposure), this phenotype may be sustained, and for this reason, C/T (like many expanded-spectrum cephalosporins) may be discouraged for the treatment of infections caused by *E. cloacae* complex.

Some other points should also be considered. First, C/T was primarily designed to treat *Pseudomonas* infections, and because this bacterium is highly susceptible to this drug,[10] it is reasonable to conclude that *Pseudomonas* usually does not derepress chromosomal AmpC enzymes, even those with an extensively drug-resistant phenotype. Second, C/T is an inappropriate drug for use in empirical therapeutic approaches; it should be preserved for the specific niche for *Pseudomonas* infection. Third, high inactivation of C/T by derepressed AmpC organisms justifies strict monitoring of its resistance level in both Enterobacterales and *Pseudomonas*, particularly those associated with poor outcomes when high MICs (>2 g/mL) are observed.[10]

In conclusion, in this study, we identified a mechanism that seems to be predictive of high C/T resistance levels. More prominent among bacteria that are capable of derepressing AmpC β-lactamases, mainly the *E. cloacae* complex (but also *Pseudomonas* on a minor scale), this resistance to C/T should lead to stricter use and monitoring of this drug.

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