more noticeable for MDR (17.6% ± 10.8%). The cumulative prevalence of these enterobacterial isolates regarding the PMB resistance mechanisms are shown in Figure 1.

The development of PMB resistance is of utmost concern. Although the resistance rate was lower among MDRs than among KPC producers (17.6% vs 33.2%, respectively) during the same period of evaluation, the microbiological outcome reported here may illustrate a crucial impact of PMB use on the resistance development in bacteria whose infectious processes need not be treated with it (eg, ESBLs).[7]

Notably, a remarkable increase in adaptive PMB resistance rates was observed during the study period despite a stable cumulative prevalence of PMB-IR organisms. This fact may suggest a major predilection for the development of resistance among bacteria previously susceptible to this class of drug. Also, it is reasonable to speculate that such organisms might not have any fitness advantage (eg, virulence factors) other than resistance to PMB when compared to organisms more able to adapt and survive, such as *K. pneumoniae* and *Enterobacter* spp.[8]

A limitation of this study was that no evaluation of the genetic background of the isolates was performed. Thus, an increased PMB resistance, especially among KPC producers, where *K. pneumoniae* emerges from other species, may be due to the selection of a PMB-resistant clone. However, increased resistance was also observed in the MDR group, where *Enterobacter* spp were expressive. This finding indicates a trend of PMB resistance development among other enterobacterial species.[7]

In conclusion, an increase in the prevalence of an adaptive resistance mechanism, inferred by the increased prevalence of PMB resistance rates in KPC and MDR groups, was identified. In addition, the prevalence rate of those PMB-IR organisms remained stable over the same survey period. Exposure to PMB does not seem to protect against an increase in adaptive resistance, and this finding emphasizes the need for a constant monitoring program to prevent the emergence of PMB resistance and for a better therapeutic approach ensuring its safe use.

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Characteristics of *Enterobacteriaceae* Isolates Coharboring Distinct Carbapenemase Genes

To the Editor—The emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) isolates is an important public health problem; the treatment of carbapenem-resistant isolates is extremely difficult because few options remain available for clinical use.[1] Usually, CPE harbors only 1 carbapenemase gene, although other resistance mechanisms (ESBL, porin loss, eflux pumps) may also be present. However, relatively few studies have reported *Enterobacteriaceae* isolates producing more than 1 carbapenemase.[2] In the present study, we describe the characteristics of 10 *Enterobacteriaceae* coharboring carbapenemase genes.

The isolates were selected from an epidemiologic study evaluating *Enterobacteriaceae* with reduced susceptibility to carbapenems in several hospitals in the southernmost state of Brazil. The methods of this epidemiologic study are detailed

TABLE 1. Phenotypic Characteristics of Coproducing *Enterobacteriaceae* Isolates

		Minimum Inhibitory Concentration (mg/L)										
Isolate	Bacteria	IMP	MEM	ERT	CAZ	AZT	CIP	AMK	GC	POL	Gene	Estimated Plasmid Size (kb)
828F	*Klebsiella pneumoniae*	64	32	64	≥256	≥32	64	128	4	0.25	bla_{KPC} + **$bla_{OXA-370}$**	**92**–128–154
T 828F[a]		4	2	16	64	32	16	1	0.25	0.25	**$bla_{OXA-370}$**	**92**
1233F	*Enterobacter cloacae* complex	16	16	64	≥256	≥32	64	128	4	0.5	bla_{NDM-1} + **$bla_{oxa-370}$**	**110**–128
T 1233F[a]		16	16	64	≥256	≥32	16	≥256	2	0.5	**$bla_{OXA-370}$**	**110**
3320F	*Enterobacter cloacae* complex	64	64	64	≥256	≥32	64	64	1	2	bla_{NDM-1} + **$bla_{oxa-370}$**	92–**128**–154
T 3320F[a]		64	1	4	16	4	16	≤0.5	0.25	1	**$bla_{OXA-370}$**	**128**
3323F	*Enterobacter cloacae* complex	64	32	64	≥256	≥32	64	128	2	0.5	bla_{NDM-1} + **$bla_{oxa-370}$**	110–**128**
T 3323F[a]		8	≤0.5	4	128	4	16	32	0.5	0.5	**$bla_{OXA-370}$**	**128**
3885F	*Enterobacter cloacae* complex	32	32	64	≥256	≥32	64	64	1	≤0.125	bla_{NDM-1} + **$bla_{oxa-370}$**	**92**–128–154
T 3885F[a]		16	≤0.5	4	128	32	16	≤0.5	0.13	≤0.125	**$bla_{OXA-370}$**	**92**
3888F	*Enterobacter cloacae* complex	32	32	128	≥256	≥32	64	128	1	0.5	bla_{NDM-1} + **$bla_{oxa-370}$**	92–**128**–154
T 3888F[a]		8	≤0.5	≤0.5	8	4	16	32	0.5	0.5	**$bla_{OXA-370}$**	**128**
4077F	*Klebsiella pneumoniae*	256	128	≥256	≥256	≥32	16	≥256	1	32	bla_{NDM-1} + **bla_{KPC}**	92–**128**–154
T 4077F[a]		64	2	16	16	1	32	32	0.25	0.5	**bla_{KPC}**	**128**
4517F	*Enterobacter cloacae* complex	64	64	128	≥256	≥32	128	≥256	2	0.25	**bla_{NDM-1}** + **bla_{KPC}**	***128*–154**
T 4517F a[a]		128	32	16	≥256	1	128	≤0.5	0.5	0.25	**bla_{NDM-1}**	**154**
T 4517F b[a]		8	2	16	128	≥32	16	1	0.5	≤0.125	**bla_{KPC}**	***128***
4521F	*Klebsiella pneumoniae*	32	128	256	≥256	≥32	32	≥256	2	0.25	bla_{NDM-1} + **bla_{KPC}**	**92**
T 4521F[a]		32	4	4	128	≥32	16	2	0.5	≤0.125	**bla_{KPC}**	**92**
4815F	*Klebsiella pneumoniae*	64	256	≥256	≥256	≥32	32	≥256	0.5	1	bla_{NDM-1} + **bla_{KPC}**	**130**
T 4815F[a]		4	1	8	128	32	16	2	0.5	1	**bla_{KPC}**	**130**
...	*Escherichia coli* TOP10	0.25	0.032	0.008	...	0.0125	...	2	0.06	≤0.125	...	...

NOTE. IMP, imipenem; MEM, meropenem; ERT, ertapenem; CAZ, ceftazidime; AZT, aztreonam; CIP, ciprofloxacin; AMK, amikacin; TGC, tigeciclyne; POL, polymyxin.
[a]Transformant.
Bold: Transferred gene and size of the transferred plasmid.

elsewhere.[3] Briefly, those isolates harboring more than 1 of the following genes were selected for further evaluation in this report: bla_{KPC}, bla_{VIM}, bla_{GES}, bla_{NDM}, bla_{OXA-48}, and bla_{IMP} (detected by a multiplex real-time polymerase chain reaction [PCR]).

These isolates were initially identified in the original institution by the VITEK2 system (bioMeriéux, France), and isolates coharboring more than 1 carbapenemase gene were confirmed by 16S rRNA sequencing. The presence of the carbapenemase genes was confirmed by conventional PCR, and the amplicons were purified and sequenced using a BigDie Terminator kit (version 3.1) and an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). GenBank was used to access the sequences deposited to date, and the BioEdit program was used to compare similarities among sequences.

Plasmids were extracted by alkaline lysis and were transformed into cells of an *Escherichia coli* TOP10 eletrocompetent by electroporation.[4] Transformants were selected on Luria-Bertani agar containing 2 mg/L ceftazidime. Estimation of plasmid size was performed after 0.7% agarose gel electrophoresis, using a curve obtained by plotting the distance (mm), compared to *E. coli* 39R861.[5]

Minimum inhibitory concentrations (MIC) of carbapenems were evaluated using the broth microdilution method and were interpreted according to the Clinical and Laboratory Standards Institute (CLSI).

Overall, 10 isolates coproducing carbapenemases were identified: 5 *Enterobacter cloacae* complexes with bla_{NDM-1} and $bla_{OXA-370}$ genes, 3 *Klebsiella pneumoniae,* 1 *E. cloacae* complex with bla_{NDM-1} and bla_{KPC-2} genes, and 1 *K. pneumoniae* with bla_{KPC-2} and $bla_{OXA-370}$ genes. We detected multiple plasmids in 8 clinical isolates (Table 1); 2 isolates presented only one plasmid.

One *Providencia rettgeri* presented bla_{GES} and bla_{IMP} genes in the multiplex PCR but the sequencing of the amplicon did not yield the specific variant of these genes. Notably, the *P. rettgeri* presented a peculiar result: it was positive for both bla_{IMP} and bla_{GES}, which are supposed to be in a plasmid, but the plasmid was not identified in either the clinical isolate or the transformant (data not show).

The MICs of transformants were much higher than that of *E. coli* TOP10, which indicates that the plasmids are enough to confer resistance to antibiotics. The analysis of the antimicrobial susceptibility profile of the transformants compared to the wild-type isolates showed that most isolates present lower MICs for carbapenems. The transformants that received only the OXA-370 gene present very low MICs to both meropenem and imipenem, which may indicate that this OXA-48 variant lacks carbapenemase activity.

Plasmid analysis demonstrated a heterogeneous pattern of plasmid sizes: 92, 110, 128, 130, and 154 kbp. Moreover, we observed that carbapenemases were inserted in different plasmids, which was also observed in other studies. Balm et al[6] reported a *K. pneumoniae* isolate coharboring bla_{NDM} and $bla_{OXA-181}$ genes on ~160 kb and ~280 kb plasmids, respectively. Another study demonstrated coproduction of bla_{NDM-1} and $bla_{OXA-232}$ in *E. coli*: the bla_{NDM-1} gene was located on a plasmid of 129,085 bp and the $bla_{OXA-232}$ gene was located on a small plasmid of 6,141 bp.[7] A study of *K. pneumoniae* coharboring bla_{VIM} and bla_{KPC} revealed 2 plasmids of 70 and 150 kb, while the bla_{VIM} transconjugants had a single plasmid of 150 kb and the bla_{KPC}-bearing transconjugant had a single plasmid of 70 kb.[8] We were able to transfer at least 1 carbapenemase gene to the *E. coli* TOP10 receptor, with the exception of 1 (4517F) *E. cloacae* that transferred both carbapenemase genes (bla_{NDM-1} and bla_{KPC-2}).

The most frequently reported *Enterobacteriaceae* species carrying 2 or more carbapenemases is *Klebsiella pneumoniae*, although other species with this property have also been reported sporadically.[2] Moreover, a few studies have indicated that the number of bla_{NDM-1} and bla_{OXA-48} is constantly increasing, and this combination has been the most frequently described.[2,9,10] Relatively few reports of *Enterobacteriaceae* coproducing carbapenemases are available; here, we describe 2 additional species harboring 2 carbapenemases, and we observed other combinations such as a New Delhi metallo (NDM)-codifying gene with an OXA-48 variant carbapenemase.

In the present study, we observed the occurrence of 10 clinical isolates coproducing different carbapenemases located in a variety of plasmids, demonstrating the plasticity of these mobile genetic elements. The dissemination of double-carbapenemase–producing *Enterobacteriaceae* is worrisome because it potentially further narrows the therapeutic options. Furthermore, isolates producing more than 1 carbapenemase may impair the detection of carbapenemase production using some phenotypic methods, which reinforces the need for further investigation of these isolates.

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Pseudomonas aeruginosa Outbreak in a Neonatal Intensive Care Unit Attributed to Hospital Tap Water: Methodological and Statistical Issues to Avoid Misinterpretation

To the Editor—We were interested to read the May 2017 article by Bicking Kinsey et al.[1] The authors investigated an outbreak of *Pseudomonas aeruginosa* infections. They found that compared with controls, case patients had higher odds of being in a room without a point-of-use filter (odds ratio [OR], 37.55; 95% confidence interval [CI], 7.16–∞).[1]

Although these results are interesting, some methodological and statistical issues should be considered. The estimated effect size for some risk factors such as unfiltered water is biased due to sparse data bias. In other words, the data are inadequate to estimate a valid and precise OR. The main indicators of sparse data bias are a huge effect-size estimate and a remarkably wide and even infinite confidence interval limit.[2] The most common strategy to adjust sparse data bias is a correction of one-half, a conventional method in which one-half is added to each level of exposure–outcome combination prior to statistical analysis.[2] The problem with the conventional method is that it can lead to implausible ratio estimates.[2] Greenland and Mansournia proposed an advanced method, namely, penalization via data augmentation to adjust and minimize sparse data bias.[2,3] In this method, the effect-size estimate is assumed to falls in an acceptable and possible range, such 1/40 to 40. Using penalization, the effect-size estimates are reduced to the range specified.[2] We analyzed the presented data in the study conducted by Bicking Kinsey using the penalization method to test how the results can be influenced by sparse data bias. We found that the unfiltered water in univariable model had an estimated OR of 17.23 (95% CI, 3.56–83.19). Thus, we think the true and valid estimated OR for unfiltered water is different than 37.55 (95% CI, 7.16, ∞) as reported in the article.

The take-home message for readers is that sparse data bias is a common bias in biomedical research[4–7]; however, it is rarely addressed in analyses. Furthermore, sparse data bias can be minimized using efficient statistical methods.

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