Sunnyvale, CA), the cost of the current assay was significantly less with a convenient TAT (same-day result) using this noncommercial DNA extraction method and end-point multiplex PCR format.[4,5,9] In this study, we validated the diagnostic performance of internally controlled multiplex-PCRs targeting 8 carbapenemases in BC-positive samples. In the wake of increased carbapenem-based antimicrobial therapies, multiplex PCR may prove to be a useful tool for detecting carbapenem resistance and thus facilitating early infection control actions in clinical settings. The strengths of the current study include (1) demonstration of the molecular epidemiology of carbapenem resistance genes among a group of oncology patients in eastern India, (2) development of a rapid and cost effect test suitable for implementation in resource constrained settings; (3) application of molecular tests for gram negative bacteremia to optimize antibiotic therapy; (4) identification of potential targets for developing new drugs to treat NDM and OXA positive GNB infections, and (5) use of OXA-23/-24/-58 PCRs, which has been rarely investigated for diagnostic purposes apart from its importance as a marker in CRAB associated with outbreaks (OXA-23).[5] The implementation of this new, low-cost tool in resource-limited settings may enable better management of gram-negative sepsis.[10]

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Impact of biofilm production on polymyxin B susceptibility among *Pseudomonas aeruginosa* clinical isolates

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To the Editor—Polymyxin B is an old class of nonribosomal cyclic lipopeptide antibiotics that has been used for the treatment of gram-negative bacterial infection such as multidrug-resistant *Pseudomonas aeruginosa*, especially carbapenem-resistant isolates.[1]

Bacteria are usually able to evolve different strategies to sense, respond, and adapt to bactericidal agents including polymyxins. Although biofilm is a well-established response strategy to an antimicrobial agent, little is known about the impact of biofilms produced by *P. aeruginosa* regarding polymyxin susceptibility.[2]

Biofilms—designated as an aggregation of bacterial cells—are crucially important due to a high adhesion ability on surfaces and a worrying ability to withstand high concentrations of different classes of antimicrobial agents. This ability to resist may be due to some factors such as a lower metabolic rate of the bacterial cell, resistance gene transfer, or even the inability of the antimicrobial agent for permeating into the biofilm.[3]

Polymyxin B has been used on a massive scale in Brazilian hospitals due to the high rate of *Klebsiella pneumoniae* carbapenemase dissemination.[4] On the other hand, little is known about what impact on the polymyxin resistance development would have when different forms of bacterial presentation, such as biofilms (which often present in device-related infections), are present.

The aim of this study was to evaluate the impact of biofilm production by *P. aeruginosa* isolates on polymyxin B susceptibility by comparing the minimum biofilm eradication concentration (MBEC) with the minimum inhibitory concentration (MIC). In this

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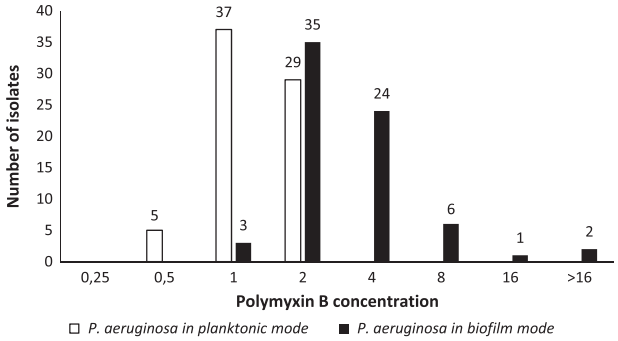

Fig. 1. Distribution of 71 *P. aeruginosa* isolates among a polymyxin B concentration gradient considering planktonic and biofilm mode of growth.

substudy, we evaluated the impact of biofilms produced by *P. aeruginosa* on the potential development of antimicrobial resistance.[5]

We selected 82 *P. aeruginosa* clinical isolates, recovered from hospitalized patients, as previously described.[5] All isolates were submitted to microtiter plate assay for biofilm status characterization and MBEC determination.[6] All biofilm experiments were performed in triplicate for each isolate. Polymyxin B MICs were evaluated using broth microdilution and the results were interpreted according to European Committee on Antimicrobial Susceptibility Testing guidelines.[7] Polymyxin B at different concentrations (ie, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 μg/mL) were used for MIC and MBEC determinations.

Figure 1 illustrates the distribution of cells in planktonic form and biofilm mode of growth (ie, weak, moderate, or strong producers) along a polymyxin B concentration gradient. Non–biofilm-producing *P. aeruginosa* isolates presented the same MIC and MBEC values and are not shown in Figure 1. Among the biofilm-producing isolates, most were moderate or strong producers (71 isolates, 86.5%). The modal polymyxin B MIC obtained for the isolates tested in our study was lower than that obtained for the modal polymyxin B MBEC (2.0 μg/mL and 4.0 μg/mL, respectively). Notably, a significant reduction in susceptibility to polymyxin B was observed when the isolates were in biofilm mode of growth compared to their planktonic counterparts ($MBEC_{90} = 8.0$ μg/mL versus $MIC_{90} = 2.0$ μg/mL) (Fig. 1).

Biofilm production appears to be a strategy to evade antimicrobial treatment and is crucially important to determine the persistence in environments with adverse conditions to the bacterial cell.[5] In fact, the biofilm mode of growth of *P. aeruginosa* may require up to 1,000 times the concentration of a determined antibiotic to be effective compared to its planktonic form, and antimicrobial exposure can stimulate biofilm formation when the antimicrobial agent reaches doses only at sub-MIC levels.

Among our isolates, 3 (ie, 2 moderate and 1 strong biofilm producer) harbored the bla_{SPM-1} enzyme, a metallo-β-lactamase that confers a high resistance level to all β-lactams agents, independently of the ability to produce biofilm (data not shown). Importantly, these results are alarming because these overlapping features (eg, carbapenemase production plus biofilm formation) can make the bacteria more refractory to the antimicrobial therapy.[8] Thus, biofilm production and its influence on resistance mechanisms must be constantly monitored to prevent the development and spread of resistance.

In conclusion, our results show that polymyxin B susceptibility is highly affected when *Pseudomonas* biofilm is involved. Antimicrobial susceptibility testing based on MIC values alone cannot accurately determine the exact susceptibility of *P. aeruginosa* biofilm. For those isolates harboring a specific resistance mechanism (eg, carbapenemases), the ability to produce biofilms enhances the capacity to acquire antimicrobial resistance. Knowledge about *Pseudomonas* biofilms are needed to select the best therapeutic strategy.

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