

diseases perspective, the only thing that is standardized are the outpatients, and they are probably 50% of the volume.”

This was also true for patients undergoing cardiac surgery in the STOP SSI study (cited by Ariyo et al)<sup>2</sup> and similar subsequent unpublished studies. In the STOP SSI study, the significant reduction in complex *S. aureus* SSIs was only seen among orthopedic surgery patients but not cardiac surgery patients. The orthopedic surgery patients tended to be scheduled for elective surgery and had outpatient preoperative clinic visits in the 30 days prior to surgery. During this visit, the orthopedic patients were provided chlorhexidine body wash, were screened for *S. aureus* colonization and, if positive, were provided with a 5-day supply of mupirocin nasal ointment. In contrast, the cardiac surgery patients did not have a standardized outpatient preoperative clinic appointment and were often only seen in the inpatient setting.

Similarly, only 1.6% of patients undergoing urgent/emergent operations were fully adherent to the STOP SSI bundle, 40% of patients undergoing scheduled operations were fully adherent. This factor was reflected in the outcomes, in which there was only a statistically significant reduction in complex *S. aureus* SSIs among the scheduled operations and not the urgent/emergent operations. In a similar study performed in Veterans Affairs (VA) hospitals, a cardiac case manager from a small VA hospital stated:

“[The patient] could have a scheduled outpatient [cardiac catheterization] here and end up on a balloon pump, and then [be sent] via ambulance to the University for [urgent] open heart surgery. Those are not going to be ones we catch, obviously.”

Many infection prevention interventions, such as chlorhexidine washes and MRSA nasal screening and decolonization, require significant upfront investment of time and resources. Thus, providing these services to less stable patients and to add-on patients is difficult, if not impossible. Environmental barriers do not allow for showering and time restrictions do not allow for MRSA screening and multiple-day decolonization regimens.

These implementation challenges are critical areas of future research. First, as a field, we need better ways to implement

infection prevention interventions and to track which patients receive them and which do not. In addition to healthcare-associated infection outcomes, consideration of intermediate, implementation outcomes (eg, adherence to the intervention), should be included in assessments of efficacy and effectiveness, so that the potential for confounding by indication can be assessed and addressed. Second, we need to find better ways to triage infection prevention strategies. Under the current system, the most aggressive care is being targeted to the lowest-risk patients. Infection prevention interventions that can be rapidly implemented in the day-of-surgery area or the operating room could be more effective than interventions that need to be started multiple days before surgery. More research is needed to determine how we can improve implementation and overcome barriers for the most vulnerable patients, rather than targeting care where it is most convenient to do so.

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# Rapid and economical detection of eight carbapenem-resistance genes in *Enterobacteriaceae*, *Pseudomonas* spp, and *Acinetobacter* spp directly from positive blood cultures using an internally controlled multiplex-PCR assay

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*To the Editor*—The World Health Organization (WHO) has recognized carbapenem-resistant *Enterobacteriaceae* (CRE), *Pseudomonas aeruginosa* (CRPsA), and *Acinetobacter baumannii* (CRAB) as critical pathogens that cause significant morbidity and mortality in patients with bloodstream infections (BSIs),

**Table 1.** Distribution of Different Carbapenemases in *Enterobacteriaceae*, *Pseudomonas* spp, and *Acinetobacter* spp Isolated From Blood Samples

Organism (n)	Single Carbapenemase						Double Carbapenemase						
	NDM	OXA-48	KPC	VIM	OXA-23	OXA-58	NDM/OXA-48	NDM/OXA-58	KPC/VIM	NDM/OXA-23	NDM/VIM	OXA-48/OXA-58	
<i>E. coli</i> (32)	25	3	ND	ND	ND	ND	4	ND	ND	ND	ND	ND	
<i>K. pneumoniae</i> (82)	2	62	2	ND	ND	ND	16	ND	ND	ND	ND	ND	
<i>K. oxytoca</i> (1)	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
<i>E. cloacae</i> (7)	6	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
<i>Citrobacter freundii</i> (1)	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
<i>A. baumannii</i> (13)	2	ND	ND	ND	4	2	ND	3	ND	2	ND	ND	
<i>Acinetobacter</i> spp (2)	1	ND	ND	ND	ND	ND	ND	1	ND	ND	ND	ND	
<i>P. aeruginosa</i> (15)	11	ND	ND	1	ND	ND	ND	ND	2	ND	1	ND	
<i>Pseudomonas</i> spp (1)	ND	ND	ND	1	ND	ND	ND	ND	ND	ND	ND	ND	

Note: ND, not detected by polymerase chain reaction assay.

especially in healthcare settings.<sup>1,2</sup> Carbapenem resistance challenges empirical and targeted antibiotic treatment involving  $\beta$ -lactam antibiotics, including carbapenems, and it results in expensive and complicated alternative anti-infective strategies with polymyxin-B, colistin, tigecycline, and fosfomycin.<sup>3</sup> Despite their high cost (~US\$62 per test), commercially available PCR-based tests have been used to screen clinically important carbapenemases, known as the “Big 5” genes: metallo- $\beta$ -lactamases (MBLs) (ie, NDM, IMP, and VIM), KPC, and the OXA-48 family. These tests use rectal swab samples with short turnaround times (TATs), which contribute significantly to infection control efforts.<sup>2,4,5</sup> Few validated tests are available to meet the acute need in BSI cases.<sup>2,4</sup> A rapid, inexpensive, and accessible testing tool for carbapenemase producing-carbapenem resistant organisms (CP-CRO) as pathogens of BSI is of paramount importance for early institution of effective antimicrobial therapy and infection prevention and its control. In the present study, a pre-designed multiplex PCR assay was validated that targets the OXA carbapenemases (OXA-23, OXA-24/-143, and the OXA-58 family) commonly harbored by *Acinetobacter* spp, as well as with the “Big 5” genes, in DNA extracts of gram-negative bacilli positive blood-culture (BC) broths.

In this prospective study, consecutive BC broth samples (n = 415) were collected from patients (n = 278) with laboratory-confirmed BSIs at Tata Medical Center, Kolkata, India, from July 2017 to September 2018. Blood culturing was performed using the BacT/Alert 3D system (bioMérieux, Marcy-l'Étoile, France), and identification and susceptibility testing of BC isolates were performed using the Vitek2 system (bioMérieux). Carbapenem susceptibility results were interpreted following Clinical Laboratory Standard Institute (CLSI) guidelines.<sup>6</sup>

DNA was extracted from positive BC broths using the alkali wash and heat lysis method.<sup>7</sup> Additionally, 20  $\mu$ L McFarland 3 turbidity standard of *A. baumannii* ATCC19606 was added to 500  $\mu$ L BC broth before extraction as an internal extraction control (IEC). Two multiplex PCRs were then performed to detect 8 carbapenemase genes (as described earlier) with a modification: CARBA (KPC, 353 base pairs [bp]; NDM, 603 bp; VIM, 437 bp; IMP, 387 bp; OXA-48 family, 265 bp) and OXA (OXA-23 family, 330 bp; OXA-24/143 family, 271 bp; OXA-58 family, 688 bp; with *Acinetobacter*-derived AmpC [ADC] as the IEC and PCR control, 1,059 bp).<sup>8</sup> The PCR amplicons were visualized on 3% prestained agarose gels. Bidirectional sequencing was performed using a 3500 DNA analyzer

(Applied Biosystems, Foster City, CA) for confirmation. Analytical specificities of multiplex PCRs were validated using bacterial and fungal reference strains and clinical isolates. For assay standardization, cultured reference strains were suspended in normal saline to a density of 0.5 McFarland turbidity standard ( $\sim 1.5 \times 10^8$  CFU/mL) using a Densimat (bioMérieux) followed by preparation of 10-fold serial dilutions ranging from  $10^7$  to  $10^2$  CFU/mL. Known negative BC samples (450  $\mu$ L) incubated for 5 days were spiked with bacterial dilutions (50  $\mu$ L), and DNA extraction followed by PCR was performed to check analytical sensitivity.

Statistical performance indicators of the multiplex PCRs were determined using MedCalc (MedCalc Software, Mariakerke, Belgium) using carbapenem susceptibilities of *Enterobacteriaceae*, *Pseudomonas* spp, and *Acinetobacter* spp (EPA) as references. Non-EPA, intrinsically CRO, and uncultivable organisms were considered negative controls. Interrater agreement using Cohen's  $\kappa$  was used to measure the concordance between culture and multiplex PCRs.

Of 153 CARBA-OXA multiplex PCR-positive samples, 122 had a single target (NDM, 48; OXA-48 family, 66; KPC, 2; VIM, 2; and OXA-23 family, 4) and 31 had double targets (NDM/OXA-48 family, 20; NDM/OXA-58 family, 5; KPC/VIM, 2; NDM/OXA-23 family, 2; NDM/VIM, 1; and OXA-48/OXA-58 family, 1) (Table 1). The analytical specificities of the PCRs were 100% for detecting the 8 carbapenemases; the sensitivities ranged between 500 and 5000 CFU/mL for different carbapenemases using spiked BC samples. Among 415 BC samples, accuracy of the assay was marginally improved (95.9% vs 96.9%) using both the “Big 5” CARBA and extended OXA panels (Supplementary Table 1 online). Discrepant results between the culture method and multiplex-PCRs were observed in 13 samples. The TAT for the detection of carbapenemases was <4 hours using BC positive broth, including DNA extraction ( $\sim 50$  minutes), PCR amplification ( $\sim 120$  minutes), and agarose-gel electrophoresis and documentation ( $\sim 30$  minutes). The cost of consumables and reagents for running the assays was only  $\sim$ US\$10 per sample.

Early and rapid detection of carbapenemases in common gram-negative bacilli is of paramount importance for optimizing antimicrobial therapy, as a tool for antimicrobial stewardship programs, for hospital infection control, and for improving patient outcomes with regard to morbidity, mortality, reducing length of hospitalization, and minimizing the cost of health care. Compared with commercial assays (eg, Carba R GeneXpert from Cepheid,

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.<sup>4,5,9</sup> 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).<sup>5</sup> The implementation of this new, low-cost tool in resource-limited settings may enable better management of gram-negative sepsis.<sup>10</sup>

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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.<sup>1</sup>

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.<sup>2</sup>

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.<sup>3</sup>

Polymyxin B has been used on a massive scale in Brazilian hospitals due to the high rate of *Klebsiella pneumoniae* carbapenemase dissemination.<sup>4</sup> 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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