


Equal, but different: Fluctuant biofilm formation and its impact on polymyxin B susceptibility among a clonal spreading of KPC-2-producing *Klebsiella pneumoniae* isolates

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To the Editor—*Klebsiella pneumoniae* often causes severe nosocomial infections against which carbapenem agents are frequently used when isolates produce broad-spectrum β -lactamase enzymes (ie, extended-spectrum β -lactamase [ESBL] or *ampC* type). In the last decade, carbapenem-resistant *K. pneumoniae* has been increasing worldwide, mainly due to *K. pneumoniae* carbapenemase (KPC) enzymes. *Bla*_{KPC-2} is especially common in Brazil, conferring resistance to almost all available antibiotics in association with high morbidity and mortality rates.^{1,2}

Biofilm formation is a well-recognized pathway by which pathogenic bacteria evade adverse conditions (oxygen and nutrient deprivation) as well as bactericidal antimicrobial agents. Matrix-enclosed biofilms are formed preferentially on a variety of surfaces inside the body and on invasive devices.³

Recently, we evaluated the ability to produce biofilms among *Pseudomonas aeruginosa* clinical isolates and their impact on antimicrobial susceptibility profiles; there was an alarming impact when overlapping resistance mechanisms (biofilm plus enzymatic production) were present.⁴ These results prompted a study evaluating these overlapping mechanisms because few reports are available regarding biofilm production among KPC-2-producing *K. pneumoniae* (KPC-2-*Kp*) isolates.^{5,6}

In the present report, we describe for the first time the ability to produce biofilms among KPC-2-*Kp* clinical isolates from extensive clonal spread in patients in an intensive care unit. This is a sub-study of the evaluation of the impact of biofilms on the potential development of antimicrobial resistance.⁷

We analyzed 10 pairs of KPC-2-*Kp* isolates from rectal swabs and clinical isolates obtained from 10 unique patients as previously described.² These isolates were subjected to microtiter plate assays performed in triplicate, biofilm status characterization, and determination of both polymyxin B minimum inhibitory concentrations (MIC) and minimum biofilm eradication concentrations (MBEC) as described elsewhere.⁴

All KPC-2-*Kp* isolates were identical by molecular typing,² but most of them had different biofilm profiles (Table 1). Four pairs of KPC-2-*Kp* had the same biofilm formation status: 3 of these isolates were recovered from blood and 1 was recovered from cerebrospinal fluid. However, for the remaining 6 pairs, an increase in biofilm production was observed in the clinical isolates compared

to their colonizing counterparts (Table 1). A worrisome consequence of these results was the association with increasing resistance levels or bacterial tolerance in the face of higher polymyxin B concentrations. Moreover, polymyxin B MBEC results revealed that 90% of clinical isolates showed a 2–16-fold increase in resistance in biofilms (Table 1). Similar to our results, Naparstek et al.⁵ found a dramatic increase in polymyxin resistance with *K. pneumoniae* isolates in biofilms. Although *K. pneumoniae* are not as notorious for biofilm formation as *Pseudomonas aeruginosa*, we did find that *K. pneumoniae* clinical isolates indeed produce robust biofilms compared with the same bacterial clone previously recovered from surveillance cultures reflecting gut colonization.

Many bacteria grow on surfaces forming biofilms, but high dosages of antimicrobial often cannot clear infectious biofilms. Most of our isolates were recovered from patients with some invasive medical device (eg, bladder catheter, central venous catheter, or cerebrospinal fluid diversion), which is a well-characterized risk factor for both an infection process and biofilm production.⁸

A potential limitation of this study was the lack of information about the genetic lineage of our KPC-2-*Kp* isolates. Some evidence shows that ST258 are widely disseminated in many Brazilian hospitals⁹ and that this ST258 lineage has a lower biofilm biomass than other lineages.⁵ Little is known about the ability to produce biofilms in sets of the same KPC-2-*Kp* clonal isolates when recovered from distinct clinical specimens in the same patient.

In conclusion, a KPC-2-*Kp* predominant clone exhibits exuberant biofilm formation and high polymyxin B resistance levels, as noted with other species.^{4,6} Although our results should be validated by further studies involving other clonal lineages, the ability to produce biofilm may be responsible for maintaining refractory infections due to KPC-2-*Kp* isolates, with an impact on polymyxin B resistance development.

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Table 1. Biofilm Status for KPC-2-*Klebsiella pneumoniae* Colonizing and Infecting Isolates and Polymyxin B MBEC/MIC Determination for Infecting Isolates

Patient	Clinical Site	Biofilm Status		Polymyxin B MBEC/MIC (Ratio)
		Rectal	Clinical	
Patient 1	Blood	Nonproducing	Nonproducing	2.0/2.0 (1)
Patient 2	Endotracheal aspirate	Nonproducing	Moderate	32.0/16.0 (2)
Patient 3	Urine	Weak	Moderate	8.0/2.0 (4)
Patient 4	Cerebrospinal fluid	Moderate	Moderate	8.0/2.0 (4)
Patient 5	Urine	Weak	Moderate	32.0/8.0 (4)
Patient 6	Blood	Moderate	Strong	16.0/4.0 (4)
Patient 7	Endotracheal aspirate	Weak	Strong	128.0/8.0 (16)
Patient 8	Blood	Weak	Weak	64.0/32.0 (2)
Patient 9	Blood	Weak	Weak	1.0/0.5 (2)
Patient 10	Urine	Nonproducing	Moderate	2.0/ 1.0 (2)

Note. MBEC, minimum biofilm eradication concentration; MIC, minimum inhibitory concentration.

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Prevalence of bloodstream infections in neutropenic patients with bacteriuria

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To the Editor—Patients with hematologic malignancies and neutropenia are at increased risk for several infectious complications. They are predisposed to higher rates of bloodstream infections (BSIs) than the general population. Although BSI is caused by bacteriuria in ~3% of cases within the general population,¹ malignancy (odds ratio [OR], 1.94) and receiving immunosuppression within 14 days of bacteriuria (OR, 8.13) are risk factors for urinary-tract-

related BSIs.² However, few studies have evaluated the occurrence of secondary BSIs from bacteriuria in neutropenic patients. This study was performed to define the incidence of secondary BSIs in patients with acute myeloid leukemia (AML) with neutropenia and bacteriuria and to determine whether neutropenic patients with asymptomatic bacteriuria have a higher rate of BSI than the general population.

Our study took place in a 900-bed Midwestern tertiary-care hospital with ~2,000 annual hematology and oncology admissions. We performed a cohort study of all adult patients with AML who were admitted to the hematology service between February 2010 and November 2013. A case was defined as any patient with neutropenia and bacteriuria during the study period.

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