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Jason P. Burnham

Mario F. Feldman

Juan J. Calix

# Seasonal Changes in the Prevalence of Antibiotic-Susceptible *Acinetobacter calcoaceticus-baumannii* Complex Isolates Result in Increased Multidrug Resistance Rates During Winter Months

Jason P. Burnham,<sup>1</sup> Mario F. Feldman,<sup>2</sup> and Juan J. Calix<sup>1</sup> 

<sup>1</sup>Division of Infectious Diseases, Department of Medicine; <sup>2</sup>Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri

Temporal analysis of *Acinetobacter calcoaceticus-baumannii* complex isolates in a large, US healthcare system demonstrated decreased occurrence of antibiotic-susceptible isolates between November and May, while resistant isolate occurrence was temporally stable. This resulted in 50%–100% seasonal increases of resistance rates. This work offers insight into the phenomenon of Gram-negative pathogen seasonality.

**Key words.** *Acinetobacter baumannii*; multidrug resistance; seasonality.

## INTRODUCTION

*Acinetobacter calcoaceticus-baumannii* complex (Abc) is a major global health threat due to its ability to survive in multiple environments and its association with high levels of antibiotic resistance. Though most notorious for their role in hospital-acquired infections, multiple studies report that 25%–65% of Abc clinical isolates are obtained in ambulatory settings or within 48–72 hours of hospital admission [1–3]. Further understanding Abc epidemiology is essential for developing interventions to curb the growing impact of multidrug-resistant (MDR) Abc infections.

Many Gram-negative pathogens, including Abc, increase in incidence during warmer, summer months, a phenomenon commonly referred as seasonality [4]. Accurate characterization of these seasonal trends is important for effective surveillance and infection control efforts. However, reports from single center studies conflict on Abc seasonality. For example, a 9-year Korean study of 3520 unique Abc isolates demonstrated

that community-acquired isolates (n = 922), but not hospital-acquired isolates (n = 2598), had higher rates of incidence during warmer months [2]. An 11-year Pennsylvania study of 1476 isolates reported that non-MDR isolates (n = 692) exhibited seasonality, but contemporaneous MDR Abc isolates (n = 784) lacked seasonality [5]. In contrast, a 7-year study performed in Baltimore reported that all Abc isolates (n = 1444) exhibited seasonality independent of duration of hospital admission and antibiotic resistance [1]. We performed a retrospective analysis of Abc clinical isolates identified in and around St. Louis, Missouri, over a decade. Our aim was to analyze this cohort, of which over 45% of isolates were MDR, to investigate Abc seasonality in different epidemiological subgroups.

## METHODS

Our retrospective analysis will be described elsewhere (unpublished data). Briefly, we compiled clinical and microbiology data on 1948 Abc isolation events from January 1, 2007, through December 31, 2016, in 11 BJC HealthCare System (BJC) hospitals located in and around Saint Louis. Only the first isolation event (index culture) per patient age >18 years was included. Similar to prior studies [1, 2], isolates were labelled as hospital-acquired (HA) if the index culture was obtained ≥48 hours after hospital admission, and all other isolates were labelled as nonhospital-acquired (nHA). Isolates were also grouped according to specimen source as respiratory, skin and soft tissue or musculoskeletal (SST/MSK), urinary, endovascular, or other. For antibiotic susceptibility analysis, isolates were classified as resistant if they were reported as resistant or intermediate per the Clinical and Laboratory Standards Institute's guidelines [6]. The following antibiotics were grouped into classes: meropenem and imipenem (MEM/IPM) as carbapenems; ciprofloxacin and levofloxacin (CIP/LVX) as fluoroquinolones; piperacillin-tazobactam and ticarcillin-clavulanic acid (TZP/TIM) as antipseudomonal penicillins plus β-lactamase inhibitor; and tetracycline and doxycycline (TET/DOX) as tetracyclines. If an isolate was nonsusceptible to any antibiotic in a class, it was labelled resistant for that class.

## Seasonality Analysis

According to the month an index culture was obtained, isolates were grouped into quarters as follows: December (from prior year) through February as Quarter 1 (Q1); March through May as Quarter 2 (Q2); June through August as Quarter 3 (Q3); and September through November as Quarter 4 (Q4). The number of isolates per quarter was plotted, starting with Q2 in 2007 ("07Q2"). To normalize isolate occurrence across multiple years for comparative analysis, we converted quarterly occurrence to a percentage of annual isolates (ie, [# of isolates

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Correspondence: J. Calix, MD, PhD, Washington University School of Medicine, Division of Infectious Diseases, 4523 Clayton Avenue, Campus Box 8051, St. Louis, MO 63110 (jjcalix@wustl.edu).

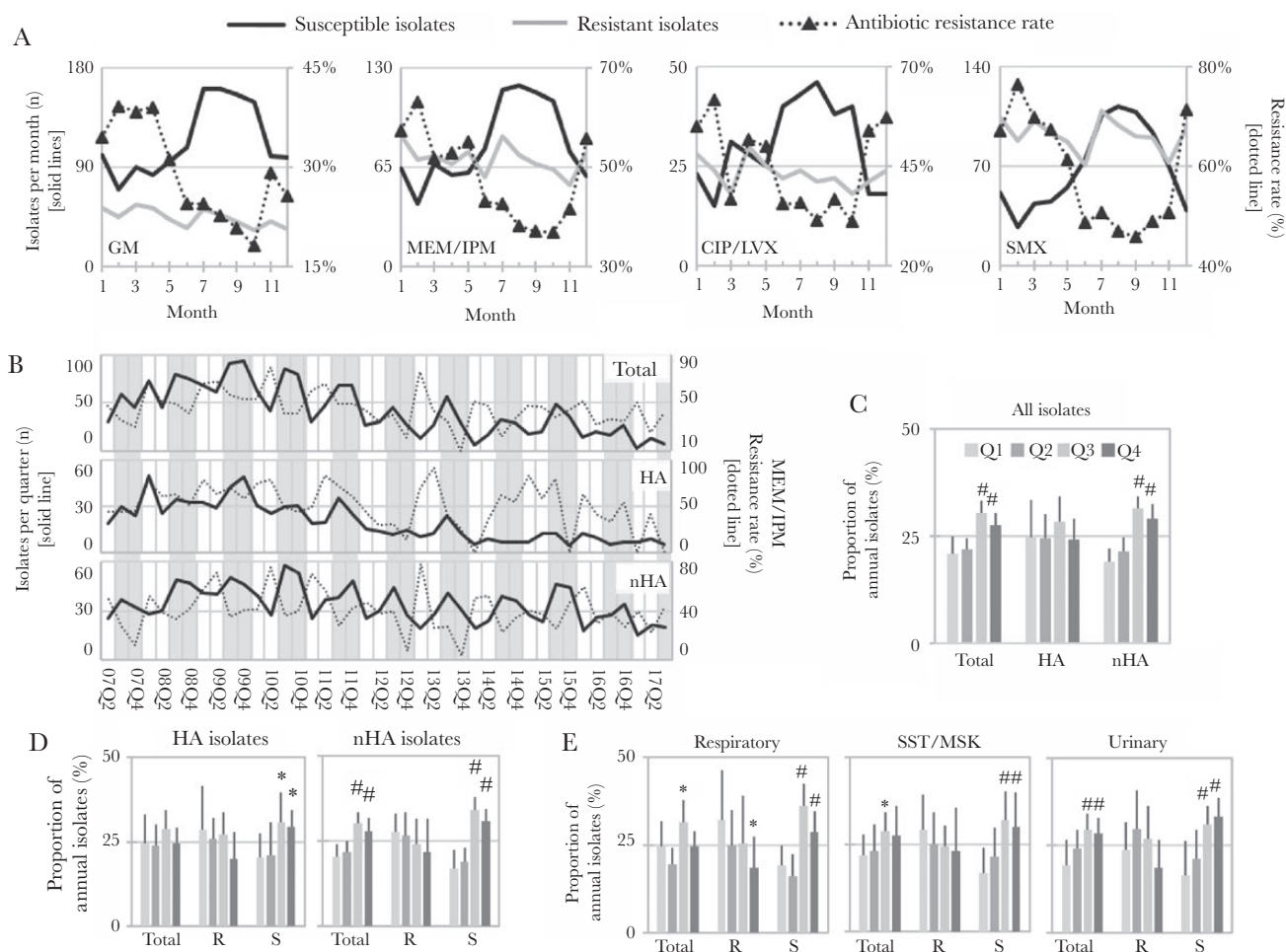
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in a quarter]/[# of isolates in all 4 quarters in the corresponding year]  $\times 100 =$  “normalized occurrence”). Using this method we expect 25% of annual isolates, on average, to occur in each quarter, in the absence of seasonal variation. We also determined the resistance rates exhibited by isolates in each quarter. Normalized occurrence and resistance rates were averaged for all Q1–Q4 in the study period. Pairwise comparisons between quarters (eg, Q1 vs Q2, Q1 vs Q3, etc.) were performed using 2-sample independent *t* test analysis with SPSS v25 (IBM, USA). To compare our cohort to those from prior analyses [1, 2, 5], we performed subgroup seasonality analysis according to whether isolates were HA or nHA, whether they were susceptible or resistant to an antibiotic, and according to isolate tissue source.

## RESULTS

We plotted the 10-year cumulative total of isolates obtained in each month, grouped according to susceptibility to gentamicin (GM), carbapenems (MEM/IPM), fluoroquinolones (CIP/LVX), or trimethoprim-sulfamethoxazole (SMX). As seen in Figure 1A, there was a peak in susceptible isolates between June and November without a corresponding peak for resistant isolates. These diverging epidemiological behaviors resulted in net antibiotic resistance rates being lowest in September or October and highest in February. Similar observations were made when examining isolate occurrence and resistance rates by quarter: there were annual peaks of Abc in Q3 and Q4, while resistance rates peaked during Q1 and Q2 (Supplementary Figure S1).



**Figure 1.** Seasonality among antibiotic-susceptible, but not resistant, Abc isolates. A, The cumulative total (solid lines, left axis) and antibiotic resistance rates (dotted lines, right axis) of isolates obtained in each month (1–12, January through December) over the study period. Graphs depict numbers of isolates that are susceptible (gray line) or resistant (black line) to each antibiotic or antibiotic class (GM, gentamicin; MEM/IPM, meropenem/imipenem; CIP/LVX, ciprofloxacin/levofloxacin; SMX, trimethoprim/sulfamethoxazole); B, Total, hospital-acquired (HA), and nonhospital-acquired (nHA) Abc cases per quarter (YY–QQ) are plotted with solid lines. Corresponding quarterly MEM/IPM resistance rates are plotted with dotted lines. Shaded areas highlight peaks in seasonal occurrence in Q3 and Q4. C, D, E, Average proportion of annual isolates occurring in each quarter, according to Abc subgroups. In panel C, isolates were grouped into total, HA, and nHA cases. In panels D and E, HA and nHA isolates (panel D) and isolates from each anatomical source (panel E), were grouped into total (all isolates with susceptibility data), MEM/IPM resistant (R) and susceptible (S) isolates. Color key for panels C–E is located in panel C. Error bars represent standard deviations. Multi-year averages for each quarter were compared to corresponding Q1 average by independent *t* test. \*,  $P < 0.05$ ; #,  $P \leq 0.001$ .

We next tested whether Abc seasonality was exhibited in different populations. Both total isolates ( $n = 1948$ ) and nHA isolates ( $n = 1202$ , 61.7%) exhibited annual peaks during Q3 and Q4 (Figure 1B), resulting in a higher proportion of isolates during Q3 and Q4 compared to Q1 and Q2 (Figure 1C). Though the occurrence of HA isolates ( $n = 746$ , 38.3%) was indistinguishable between quarters (Figure 1C), MEM/IPM resistance rates among both nHA and HA isolates repeatedly peaked during Q1 and Q2 (Figure 1B). When HA and nHA isolates were grouped according to carbapenem susceptibility, a higher proportion of susceptible HA and nHA isolates occurred in Q3 and Q4, while carbapenem-resistant HA and nHA isolates lacked seasonal variation (Figure 1D). Similarly, when Abc cases were grouped according to anatomic site of isolation, only susceptible isolates exhibited increased occurrence during Q3 and Q4 (Figure 1E). The association between antibiotic susceptibility and increased Q3 and Q4 occurrence was observed regardless of antibiotic class (Supplementary Figure S2). The only exception was ceftriaxone (CRO), where both CRO-resistant and CRO-susceptible isolates displayed higher incidence during Q3 and Q4 compared to Q1 and Q2, though seasonal changes were of lower magnitude among CRO-resistant isolates (Supplementary Figure S2). Lastly, antibiotic resistance rates were lower during Q3 and Q4 compared to Q1 and Q2, regardless of tested antibiotic (Supplementary Figure S3A), classification as HA or nHA, and tissue source (Supplementary Figure S3B).

## DISCUSSION

Consistent with prior observations [5], we found that Abc seasonality was restricted to antibiotic-susceptible isolates. Seasonal cycling of antibiotic-susceptible, but not antibiotic-resistant, Abc resulted in seasonal fluctuations in antibiotic resistance rates. This phenomenon held true for all tested antibiotics and among isolates from different epidemiologic compartments. As reported by others [2], no seasonality was detected among HA isolates. Subgroup analysis, however, revealed higher Q3 and Q4 occurrence of antibiotic-susceptible, but not resistant, HA isolates. It is possible that the high proportion of HA resistant isolates masked the seasonality of susceptible HA isolates in this and prior studies.

Seasonal variation was first described in *Acinetobacter* in the 1970s, and since then has been described in many other Gram-negative and nosocomial infections [4]. The mechanisms mediating seasonality remains unclear. It has been argued that warmer, humid conditions or summer-associated host behaviors lead to a bloom of pathogenic bacteria [1, 2]. However, seasonality has been observed in tropical environments where temperature remains relatively stable throughout the year [7]. Furthermore, climate-dependent models do not explain the lack of seasonality among resistant Abc isolates. Notably, seasonality analyses typically have not controlled for seasonal antibiotic prescribing behaviors. A winter-associated increase in community

antibiotic prescriptions has been repeatedly demonstrated [8, 9] and temporally-linked to seasonal increases in resistance rates among common human pathogens [8, 10]. It is possible that the higher use of community antibiotics (eg, tetracyclines, macrolides, sulfonamides, etc.) during winter months indirectly suppresses drug-susceptible organisms, mediating Gram-negative seasonality. This would explain the observation of decreased overall Gram-negative pathogen incidence and increased resistance rates during colder months (and vice versa). Furthermore, in Abc, where resistance to various antibiotics is frequently genetically linked through chromosomal and, to a lesser extent, plasmid-encoded resistance islands [11], pressure from antibiotic use in the community has the potential to select for resistance to multiple other drugs (eg, aminoglycosides, carbapenems, etc.).

Our findings have potentially large clinical and research implications. First, predictable seasonal changes in antibiotic resistance rates can help inform antibiotic choice for Abc infections while awaiting susceptibility testing results. Furthermore, our findings highlight why surveillance studies must account for annual fluctuations in resistance rates. For example, a study on Abc resistance rates analyzing isolates only obtained from June to October may mistakenly underestimate annual resistance rates. Finally, the potential link between community antibiotic practices and multidrug resistance must be further investigated, as establishing this association would emphasize the urgency to restrict unnecessary antibiotic usage, especially during late fall and winter months.

Our study is limited by its retrospective, single-regional healthcare system design and by examination of a single group of Gram-negative pathogens. However, the putative link between antibiotic resistance and seasonality is consistent with prior observations of Abc [2, 5] and other Gram-negative pathogens [8, 10]. In this unadjusted analysis, we cannot exclude unmeasured confounders that could influence the seasonality of drug-resistant phenotypes. Future studies should directly address whether seasonality in other Gram-negative pathogens also is correlated to drug susceptibility and whether selective pressure arising from seasonal antibiotic usage is the principle factor contributing to seasonal trends in incidence and resistance rate. Regardless, our findings suggest that future investigations on pathogen seasonality should control for fluctuations in local antibiotic usage and susceptibility rates.

## Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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