AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

COMMENTARY

Success and Challenges Associated with Large-Scale Collaborative Surveillance for Carbapenemase Genes in Gram-Negative Bacteria

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ABSTRACT The emergence and spread of antimicrobial resistance, especially in Gram-negative bacteria, has led to significant morbidity and increased cost of health care. Large surveillance studies such as the one performed by the Antibiotic Resistance Laboratory Network are immensely valuable in understanding the scope of resistance mechanisms, especially among carbapenemase-producing Gram-negative bacteria. However, the routine laboratory detection of carbapenemases in these bacteria remains challenging and requires further optimization.

KEYWORDS Gram negative, carbapenemase detection, surveillance

The emergence and spread of antimicrobial resistance, especially in Gram-negative bacteria is a global threat of major concern (1). The presence of plasmids conferring resistance to multiple first-line antibiotics leads to a significant increase in morbidity and treatment failures and subsequent use of multiple broader spectrum antibiotics which are often less effective, thus increasing the cost of health care (2, 3).

While several new antimicrobial agents are in development and several have received approval in recent years, the current antimicrobial development pipeline is not robust, with the additional liability of requiring an expensive and lengthy process before a new drug can be approved. Most of the new agents lack innovation as they are modifications of existing ones. Thus, resistance to new agents is often quick to emerge. Appropriate use of antimicrobial agents together with antimicrobial stewardship is a practical and sustainable approach to reducing antibiotic resistance; surveillance of resistance mechanisms plays an integral part in this approach.

One of the most important sets of information disseminated by clinical and public health laboratories is the prevalence of antibiotic-resistant Gram-negative organisms and the mechanism of resistance circulating in a particular region. The *a priori* knowledge of the resistance mechanism(s) in a particular pathogen is crucial in the choice of treatment and, in turn, influences the selection pressure exerted during therapy. For example, the selection of antimicrobial therapy would be different for a resistant *Enterobacter cloacae* isolate if resistance were known to be due to carbapenemase production instead of the presence of a permeability barrier in the outer or inner membrane.

The recent study by Sarah Sabour and coauthors (4) describes the results for surveillance through the Antibiotic Resistance Laboratory Network (AR Lab Network) established by the Centers for Disease Control (CDC). It reports findings from a robust study that describes the epidemiology of carbapenemase-producing carbapenem-resistant Enterobacterales (CP-CRE), *Pseudomonas aeruginosa* (CR-PA), and *Acinetobacter baumannii* (CRAB) isolates collected from clinical laboratories throughout United States (US) sites during the first 3 years of the AR Lab Network. Both infecting pathogens and colonizing bacteria were examined in an approach not usually reported in carbapenemase surveillance studies. The CDC study

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For the article discussed, see https://doi.org/10.1128/AAC.01105-21.

The authors declare no conflict of interest.

The views expressed in this article do not necessarily reflect the views of the journal or of ASM.

Accepted manuscript posted online 20 December 2021

Published 15 February 2022

further defines the distribution of carbapenemase genes in the different species of bacteria identified and correlates the presence of these genes with the resistance profile of the isolates to carbapenems. This is exceedingly beneficial in understanding the mechanisms associated with these genes and how they interact with the commonly used β-lactam agents.

A major strength of this study is the inclusion of a very large database of isolates (n = 76,887) from different regions of the US, with the detailed methodology of the surveillance network provided. In addition, the inclusion of colonization and screening specimens as well as clinical isolates provides a more comprehensive representation of the circulating resistance plasmids. The study also demonstrated a robust surveillance methodology for antimicrobial resistance and confirmed the validity of current laboratory methods used for the detection of carbapenemase genes. The inclusion of CRE, CR-PA, and CRAB isolates provided a broader evaluation of the spread of carbapenemase production among US clinical isolates, not just the CRE pathogens that often are the focus of carbapenem resistance studies. This is the kind of national study expected from the collaborative efforts of the AR Laboratory Network, and it does not disappoint.

It is not surprising that the study reports the predominance of bla_{KPC} and identifies it as the most common carbapenemase gene (86%) among CRE in the United States; similar results have been reported previously (5, 6). It is interesting to note, however, that in a comparable recent global surveillance study that examined isolates collected between 2012 and 2017, bla_{KPC} was also noted as the most common carbapenemase gene in CRE but was found in a much lower number of isolates (47.4%). Metallo-β-lactamases (MBLs) were found in a much larger proportion of isolates (20.6% of CRE), contrasting with that reported by Sabour and colleagues (11% MBL genes) (4, 7). The increased prevalence of bla_{KPC} in CRE compared to MBL-encoding genes contrasts with what is being reported from Asia and Africa but mirrors the picture in Latin America and Europe (7, 8). The regional difference in the relative distribution of carbapenemase genes is also exemplified both locally (noted by Sabour et al. (4) in the lower prevalence of CP-CRE in the Central region and higher levels of bla_{VIM} and bla_{IMP} in the Midwest and Central regions of the US, respectively) and globally (noted by Kazmierczak et al. (7) highlighting the predominance of OXA-48 like β-lactamases in Europe while KPC-producing isolates were more common in Latin America and North America). Among CR-PA and CRAB, MBL-encoding genes were more common than $bla_{KPC,}$ in contrast to what was observed in CRE. No OXA-48-like genes were seen in either set of these nonfermentative organisms. Notably, the CDC study confirms the low prevalence of OXA-48-like enzymes throughout the US, although these pathogens deserve to be watched closely to prevent their dissemination as seen in some areas in Europe (7). This highlights the need for continued surveillance studies that should have a comprehensive repository of isolates inclusive of regions with different antimicrobial prescribing practices.

One interesting methodological detail brought up in the study by Sabour et al. (4) is the difference in techniques used by the different labs to screen for and detect carbapenemase production. This single study reported four different molecular methods to detect carbapenemase genes. In addition, whole-genome sequencing was used to adjudicate all phenotypic/genotypic discrepancies. This highlights a problem faced by the clinical laboratories in determining the isolates that need to be further characterized for resistance genes. Only one of the molecular methods described by Sabour and colleagues (gene Xpert Carba-R) is approved by FDA for use in clinical laboratories to detect carbapenemase genes in bacterial isolates and surveillance specimens, such as perirectal swabs. This makes it difficult for clinical microbiology laboratories with limited resources to detect and differentiate carbapenemase producers among clinical and surveillance isolates. Laboratories often depend on phenotypic markers or antibiotic resistance profiles as indicators of carbapenemase production. We, therefore, need to institute better guidelines regarding the resistance mechanisms that need investigation and define simple methods and algorithms to identify them at a laboratory bench. While this is easy to recommend, it may not be as easy to implement as we continue

to introduce newer β-lactams and β-lactam/β-lactamase inhibitor combinations, while we establish new breakpoints and consider revision of old ones.

Carbapenem resistance among *P. aeruginosa* isolates is a cause of concern in the laboratory. However, the prevalence of carbapenemase genes was only 2% in CR-PA, in the CDC study that included a very large number of isolates (4). This is a significant finding that requires further investigation. This and other surveillance studies fail to recognize and address the nonspecificity of screening algorithms for the investigation of CR-PA, a major limitation of current laboratory procedures. The threshold set for screening for carbapenemase producers is nonsusceptibility to carbapenems. However, there are instances where susceptibility profiles may also be consistent with an AmpC hyperproducer, or a membrane permeability defect, or production of an efflux pump. This is especially true of *P. aeruginosa* isolates where the screening of carbapenemase producers based on carbapenem resistance has been challenged and an alternate algorithm, including nonsusceptibility to cefepime, ceftazidime, and a β-lactam/β-lactamase inhibitor combinations (piperacillin-tazobactam and ceftolozane-tazobactam), has been recommended for increased specificity (9). Conversely, although less commonly, CRE isolates harboring carbapenemases $bla_{\text{OXA-48-like}}$ or bla_{IMP} could be less resistant to some carbapenems leading to a genotype-phenotype discrepancy. This highlights the importance of collaboration with infection control and antimicrobial stewardship programs to institute surveillance algorithms that capture all resistance mechanisms of significance in Gram-negatives. These surveillance endeavors not only provide guidance for empirical therapy but also help identify opportunities for the development of novel antimicrobial agents or innovative treatment algorithms.

Finally, the need for surveillance studies cannot be denied but there is also a need for these studies to be more equitably dispersed among human, agricultural and veterinary sources. Antimicrobial resistance should be screened, not only from human clinical specimens but also from food and environmental sources as a part of the One Health Perspective. This approach will provide an even broader perspective regarding the dissemination of resistance genes that will influence the selection of appropriate therapeutic interventions and incentivize antibiotic research in the future.

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