AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

The Brief Case: IMP, the Uncommonly Common Carbapenemase

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KEYWORDS IMP, IMP-27, carbapenemase

CASE

A 51-year-old male on hemodialysis with diabetes mellitus, end-stage renal disease, dyslipidemia, hypertension, and peripheral vascular disease was admitted to the hospital for bilateral lower leg diabetic foot infection and osteomyelitis. A wound swab was collected from a chronic ulcer on his left foot. The Gram stain was reported as +3 polymorphonuclear cells, +1 squamous cells, and occasional Gram-positive cocci in chains. Culture on a Columbia colistin nalidixic acid (CNA) agar plate revealed light growth of *Enterococcus faecalis*, while culture on a MacConkey agar plate produced light growth of *Proteus mirabilis*.

Automated susceptibility testing for *P. mirabilis* was performed on the Vitek 2 AST-N208 system (bioMérieux, Marcy l'Étoile, France). The isolate was found to be resistant to cefazolin, cefixime, cefoxitin, ceftriaxone, cephalothin-cephalexin, ertapenem, and meropenem while remaining susceptible to ceftazidime, piperacillin-tazobactam, amikacin, gentamicin, tobramycin, ciprofloxacin, and trimethoprim-sulfamethoxazole. The isolate was then tested by the NG-Test CARBA 5 immunochromatographic assay (NG Biotech, Guipry, France) and was clearly negative for NDM, KPC, OXA, VIM, and IMP carbapenemases. Due to the suspicion of a possible carbapenemase, the isolate was further tested using a laboratory-developed PCR for bla_{NDM}, bla_{KPC}, bla_{OXA-48}, bla_{IMP}, and bla_{VIM}. The multiplex PCR was negative for bla_{NDM}, bla_{KPC}, bla_{OXA-48}, and bla_{VIM} but positive for bla_{IMP} (threshold cycle [C_T] value, 32). The PCR was repeated twice, with reproducible results (C_T values, 28 and 30). Consequently, the detection of bla_{IMP} was reported, and the Infection Prevention and Control (IPAC) team was alerted to a carbapenemase-producing organism recovered in a patient not previously known to be colonized or infected.

The isolate was subsequently forwarded to the hospital's reference laboratory, which performed a different laboratory-developed multiplex assay for carbapenemase genes, and the isolate was reported as negative. To investigate the discrepancy further, the isolate was tested by the Xpert Carba R assay (Cepheid, Sunnyvale, CA) and was again negative for all carbapenemase genes. A phenotypic method (the modified carbapenem inactivation method [mCIM]) was positive (zone of inhibition, 15 mm). Further confirmation of metallo-beta-lactamase phenotypic activity with an EDTA-modified CIM (eCIM) was not performed, as the multiplex PCR detected bla_{IMP}. Due to the discrepancy in results, we performed amplicon-based sequencing of the bla_{IMP} gene using a MiSeq sequencer (Illumina, San Diego, CA). The next-generation sequencing reaction covered bp 220 to 660, and the sequence was compared to those of known IMP genes through a BLAST search. Analysis of the sequencing results determined that the bla_{IMP} sequence had 100% correlation with bla_{IMP-27}, bla_{IMP-64}, and bla_{IMP-67} (based on the bla_{IMP-27} sequence, GenBank accession no. JF894248, bla_{IMP-64} sequence, accession no. NG_054710, and bla_{IMP-67} sequence, accession no. NG_055271). Further dis-

Citation Lowe CF, Matic N, Champagne S, Romney MG, Leung V, Ritchie G. 2020. The Brief Case: IMP, the uncommonly common carbapenemase. J Clin Microbiol 58:e01094-19. https://doi.org/10.1128/JCM.01094-19.

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For answers to the self-assessment questions and take-home points, see https://doi.org/10.1128/JCM.01126-19 in this issue.

Published 25 March 2020

crimination between the three IMP variants could not be performed based on the coverage of the amplicon.

DISCUSSION

IMP (active against imipenem; imipenemase) is an Ambler class B metallo-beta-lactamase group that is frequently identified from *Pseudomonas aeruginosa* but can also be detected in *Enterobacterales* (*Enterobacteriaceae*). It is a diverse group of metallo-beta-lactamases comprising at least 52 different variants (1). Two case reports describing the identification of IMP-27 variants in *P. mirabilis* and *Morganella morganii* have been published (2, 3). These isolates have been reported to have unique susceptibility profiles, notably that they are susceptible to ceftazidime and piperacillin-tazobactam (2). The Centers for Disease Control and Prevention have reported that IMP was identified in 0.4% of Gram-negative bacteria tested in 2017 (4). In Canada, IMP-producing carbapenemase producers are also rare, with only one isolate reported at Canadian surveillance sites from 2013 to 2017 (https://www.canada.ca/en/public-health/services/publications/science-research-data/summary-report-healthcare-associated-infection-antimicrobial-resistance-antimicrobial-use-surveillance-data-2013-2017.html). Unlike other, less commonly detected carbapenemases (e.g., GES and IMI), imipenemases have been included on commercial assays for carbapenemases (including KPC, NDM, OXA-48, and VIM). Due to the relative rarity of isolating bacteria harboring IMP, detection in a clinical microbiology laboratory still represents a significant challenge and may potentially contribute to the underestimation of the true burden of IMP carbapenemases.

The detection of carbapenemases continues to evolve, with the goal of enabling all clinical microbiology laboratories to have the capacity to detect carbapenemase-producing organisms due to their clinical significance, lack of active antimicrobials, and transmissibility within health care facilities. Because carbapenemase producers are being reported in the literature with increasing frequency, various phenotypic assays which can potentially be performed by local microbiology laboratories rather than reference laboratories have been described; examples are commercial colorimetric assays such as CarbaNP (bioMérieux) and β-Carba (Bio-Rad), disk diffusion confirmation assays such as the Rosco KPC/MBL/OXA-48 Confirm kit (Rosco Diagnostica), and manual laboratory methods such as the modified or EDTA carbapenem inactivation method (mCIM or eCIM, respectively) (5). Although mCIM and eCIM require no additional equipment and are relatively inexpensive, these tests have a long turnaround time for reporting and may result in false-positive (AmpC with porin loss) or false-negative (weak carbapenemases or low carbapenem MICs) reactions (6). There are other emerging modalities for carbapenemase detection, such as the use of matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry to assess carbapenem hydrolysis. These methods can detect carbapenemase activity but are unable to differentiate between the different carbapenemase genes. The CLSI M100 recommendations advise that laboratories utilizing the updated *Enterobacterales* MIC breakpoints for carbapenems are not routinely required to confirm carbapenemase activity (5), but identification of carbapenemase genes is essential for IPAC and public health interventions to prevent the spread of carbapenemases in health care facilities (4). Specific identification of the carbapenemase gene is also critical for treatment considerations, as novel beta-lactam combination antimicrobials (e.g., ceftazidime-avibactam and meropenem-vaborbactam) are not active against metallo-beta-lactamases, including IMP.

There are limited assays available for identification of specific carbapenemase genes. The NG-Test CARBA 5 is a simple and rapid immunochromatographic assay that can easily be implemented in frontline laboratories to enable the detection of specific carbapenemases. It has been reported to have an overall sensitivity of 88.2 to 97.3% (7, 8) However, with specific regard to its performance for IMP, the package insert indicates reliable detection of IMP-1, IMP-8, and IMP-11. Evaluation of NG-Test CARBA 5 noted that only 12/17 (70.6%) IMPs were detected (IMP-13 and IMP-14 were falsely negative) (8). Other IMP variants (IMP-28, IMP-50) have also been reported to be falsely negative by NG-Test CARBA 5 (7).

Molecular testing can serve as a confirmatory test for carbapenemases but has its own potential pitfalls. While our multiplex assay was reproducible at a C_T of 32, late C_T values may contribute to false-negative results of other molecular assays. In addition to the variety of bla_{IMP} variants described, the mutations associated with bla_{IMP} variants have been reported to span the sequence of the bla_{IMP} gene, making the design of a comprehensive bla_{IMP} PCR difficult (8). The only currently FDA-approved commercial PCR which includes bla_{IMP} as a target is the Xpert Carba R assay. The package insert specifies and describes the test characteristics for the detection of bla_{IMP-1} and cautions laboratories regarding the potential for false-negative results due to variants. Xpert Carba R has been reported to have a sensitivity of 44.4% for IMP-producing isolates (it detected $bla_{IMP-1/-4/-28}$ but not $bla_{IMP-8/-13/-14/-22/-50}$) (7). In addition, variants, including $bla_{IMP-1/-2/-4/-6/-10/-11}$ have been reported to be detected by Xpert Carba R, while $bla_{IMP-7/-13/-14}$ variants have not (9). Depending on the targets utilized for a laboratory-developed PCR, it may also be a challenge to implement a PCR to include all variants of bla_{IMP}. Primer and probe designs are critical, both in initial design and for ongoing evaluation of their ability to detect newly described variants. As an example, we aligned the reference sequences for bla_{IMP-1} (GenBank accession number NG_049172.1) and bla_{IMP-27} (GenBank accession number KY947875.1) and identified approximately 130-bp differences. At our laboratory, to mitigate the risk of a false-negative result and to minimize primer-binding mismatches due to point mutations in the bla_{IMP} gene, we have incorporated two targets for bla_{IMP} into our multiplex PCR for carbapenemases, modified from previously published assays (10, 11). A similar case of a false-negative PCR for carbapenemase genes, which was identified as *Morganella morganii* carrying the bla_{IMP-27} gene, was recently described. The false negative was attributed to four (1-bp) mismatches located in the forward-primer binding site. Sequencing was subsequently required to resolve the discordant carbapenemase investigation (3). While sequencing may provide definitive confirmation of the presence of bla_{IMP} (or the presence of another carbapenemase gene), this technology has traditionally been available only at reference laboratories, and turnaround times for results are typically not timely enough for treatment decisions, infection control purposes, and the prevention of transmission.

Commercial assays to detect IMP carbapenemases or bla_{IMP} variants, unlike those to detect the most common carbapenemase genes detected (bla_{NDM}, bla_{KPC}, bla_{OXA-48}), are currently suboptimal. IMP is not the most commonly identified carbapenemase but presents frequently enough that microbiology laboratories should be proficient in the detection of bla_{IMP}. Revisions to the algorithm for a workup of carbapenem resistance has recently been proposed; it utilizes an algorithm of a rapid nonmolecular assay, followed by CIM and/or molecular testing for the specimens that are negative by rapid testing (7). Microbiology laboratories need to continue to develop and refine their own algorithms to be able to rapidly identify rarely encountered carbapenemases, with recognition that there are gaps in the detection of IMPs in currently available commercial assays targeting specific carbapenemases.

SELF-ASSESSMENT QUESTIONS

1. How prevalent is the IMP carbapenemase in Gram-negative organisms in North America?
    a. <1%
    b. 5%
    c. 10%
    d. >10%
2. What type of carbapenemase is IMP?
    a. Ambler class A
    b. Ambler class B
    c. Ambler class C
    d. Ambler class D

3. What is the estimated reported sensitivity of nonphenotypic commercially available assays which target IMP carbapenemase or bla_{IMP-1}?
    a. >95%
    b. >85%
    c. >75%
    d. <75%

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