

Sensitive and Specific Modified Hodge Test for KPC and Metallo-Beta-Lactamase Detection in *Pseudomonas aeruginosa* by Use of a Novel Indicator Strain, *Klebsiella pneumoniae* ATCC 700603[▽]

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We evaluated the ability of the modified Hodge test to discriminate between KPC- and metallo-beta-lactamase (MBL)-producing *Pseudomonas aeruginosa* isolates and carbapenemase nonproducers. With *Escherichia coli* ATCC 25922 as the indicator strain, the MHT resulted in low sensitivity, specificity, and repeatability. Replacing the indicator strain with *Klebsiella pneumoniae* ATCC 700603 led to an improved performance (100%, 97%, 0%, and 100% sensitivity, specificity, indeterminate results and repeatability, respectively).

Detection of carbapenemase producers in the clinical laboratory is of major importance for the determination of appropriate therapeutic schemes and the implementation of infection control measures (1, 5). The modified Hodge test (MHT) has been widely used for carbapenemase screening by routine labs because it directly analyzes the carbapenemase activity of a tested strain. Because of its simplicity, the CLSI published a recommendation that *Enterobacteriaceae* with elevated carbapenem MICs or reduced disk diffusion inhibition zones be tested for the production of carbapenemases by means of the MHT (2). However, this recommendation does not include *Pseudomonas aeruginosa*, in which acquired carbapenemases are emerging with increasing prevalence. Only two reports addressed the usefulness of the MHT using *P. aeruginosa* isolates of known genotype as the gold standard (4, 6). Using the methodological standardization for *Enterobacteriaceae*, we reported that the background lawn formation by *E. coli* ATCC 25922 was inhibited by a large proportion of the tested strains, defined as an equivocal or indeterminate result (6). Similar results were described in the report of Lee et al. (4). It is clear then, that the traditional MHT needs to be redefined for use in *Pseudomonas aeruginosa*, with the optimum test conditions defined to ensure a higher performance.

On the other hand, several inhibitor-based tests have been developed for the detection of carbapenemases in *P. aeruginosa* (5, 6). However, misdetection of newly emerging isolates with a combination of carbapenemases (3) could occur with these methods. Thus, other phenotypic methods such as the MHT are needed to complement these inhibitor-based tests.

Here we optimized the MHT for a more accurate and reliable detection of carbapenemase production in *P. aeruginosa* by using a novel indicator strain, *K. pneumoniae* ATCC 700603, and named this test the *P. aeruginosa* MHT (PAE-MHT).

Selection of the optimal indicator strain. The main limitation of the MHT for carbapenemase screening in *P. aeruginosa* was the inhibition of growth of the indicator strain by the tested clinical isolate. Therefore, we first evaluated the performance of five putative indicator strains: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* ATCC 700603. For this purpose, the MHT was challenged with a panel of 64 *P. aeruginosa* isolates: 42 carbapenemase producers [KPC ($n = 20$), VIM-like ($n = 6$), IMP-13 ($n = 3$), VIM-11 ($n = 3$), SPM-1 ($n = 3$), VIM-2 ($n = 3$), IMP-16 ($n = 2$), and IMP-like ($n = 2$)] and 22 carbapenemase nonproducers. The strains had been characterized as part of a previous work (6). The isolates were from clinical sources, and there was a single isolate from each patient. The MHT was performed as previously described (2, 4). Briefly, a 1/10 dilution of an inoculum of the indicator organisms, adjusted to a 0.5 McFarland turbidity standard, was used to inoculate the surfaces of Mueller-Hinton agar (Difco, Becton Dickinson) plates (diameter, 100 mm) by swabbing. After the plates had been allowed to stand for 10 min at room temperature, one disk with meropenem (10 µg; Difco, Becton Dickinson) was placed on each plate. Subsequently, by use of a 10-µl loop, three to five colonies of the test organisms, grown overnight on an agar plate, were inoculated onto the plate in a straight line from the edge of the disk to the periphery of the plate. The presence of growth of the indicator strain toward a meropenem disk was interpreted as a positive result for carbapenem hydrolysis (carbapenemase pattern).

Carbapenemase producers were not detected with *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 indicator strains (Table 1). Both the indicators *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 produced indeterminate results in 32% and 35% of the strains, respectively, leading to an unacceptable performance (Table 1). Indeterminate results were not obtained for KPC producers. Conversely, indeterminate results were observed for metallo-beta-lactamase (MBL) producers (12 and 14% with *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, respectively) and carbapenemase nonproducers (45% and 80% with *E. coli* ATCC 25922 and *P. aeruginosa*

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TABLE 1. Sensitivity, specificity, and indeterminate results of the modified Hodge test for detection of carbapenemase production in *P. aeruginosa* with different indicator strains

Stage and organism	No. of tested strains	Percent		
		Sensitivity	Specificity	Indeterminate
Selection of the optimal indicator strain				
<i>S. aureus</i> ATCC 25923	64	0	100	0
<i>E. faecalis</i> ATCC 29212	64	0	100	0
<i>E. coli</i> ATCC 25922	64	78	57	32
<i>P. aeruginosa</i> ATCC 27853	64	88	15	35
<i>K. pneumoniae</i> ATCC 700603	64	100	98	0
Challenge of the selected indicator strain				
<i>K. pneumoniae</i> ATCC 700603	173	100	97	0

ATCC 27853, respectively). The PAE-MHT demonstrated 100% sensitivity and 98% specificity for detection of carbapenemase activity without indeterminate results (Table 1). Figure 1 shows indeterminate results for a VIM-producing isolate with *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 indicator strains, but these inconveniences were solved with the PAE-MHT.

Repeatability. To investigate whether the PAE-MHT could provide consistent results, we assessed the repeatability (i.e., the variation in measurement obtained by a single person under the same conditions) by performing the test for five consecutive days. For this purpose, we tested 15 strains (10 carbapenemase producers and 5 nonproducers). The performance of PAE-MHT was compared to that of the traditional MHT. Surprisingly, we observed that when *E. coli* ATCC 25922 was used as the indicator strain, 9 of 15 test strains showed variable results over the test period (repeatability, 40%) as follows: (i) 3 carbapenemase producers (an SPM-1 producer, an IMP-16 producer, and one carbapenem-susceptible IMP-13 producer) gave positive and indeterminate results; (ii) 3 carbapenemase nonproducers (a CTX-M-2 producer, an efflux overproducer, and an efflux plus AmpC overproducer) alternated between indeterminate and negative results; and (iii) 3 carbapenemase producers (an IMP-13 producer, a GES-5 producer, and one carbapenem-susceptible VIM-11 producer) alternately gave positive and indeterminate but also false-negative results. In contrast, with the PAE-MHT, all tested strains showed the same expected results with day-to-day repetitions (repeatability, 100%).

Challenge of the PAE-MHT. Finally, we challenged the PAE-MHT with an extended panel of 173 characterized *P.*

aeruginosa clinical isolates: carbapenemase producers ($n = 99$) [IMP-like ($n = 41$), KPC ($n = 25$), VIM-like ($n = 14$), SPM-1 ($n = 7$), IMP-13 ($n = 6$), VIM-2 ($n = 4$), VIM-11 ($n = 4$), IMP-16 ($n = 2$), and GES-5 ($n = 2$)] and carbapenemase nonproducers ($n = 74$). The panel of strains was chosen to include major carbapenem resistance mechanisms and, in particular, carbapenemase-producing strains categorized as susceptible according to CLSI standards by both the disk diffusion and agar dilution methods (2). About 33% and 15% of the IMP and VIM producers in this collection, respectively, were carbapenem susceptible. In this large-scale trial we corroborated the absence of indeterminate results and obtained sensitivity and specificity values similar to those observed in the preliminary study conducted for the optimal selection of the indicator strain. Only two false-positive results (two cephalosporinase overproducer isolates) were obtained. However, these strains were correctly categorized as cephalosporinase overproducers by using an inhibitor-based test (6).

Conclusion. The difficulty of detecting carbapenemase-producing *P. aeruginosa* isolates, together with their potential to disseminate, highlights the need for an easy, sensitive, and accurate screening method. The MHT displayed a high rate of indeterminate results, even when standardized conditions for *Enterobacteriaceae* were used (4). In addition, we obtained a low consistency of results with *E. coli* ATCC 25922, and other studies did not assess repeatability (4, 6). In contrast, the PAE-MHT using *K. pneumoniae* ATCC 700603 as the indicator strain eliminated the incidence of indeterminate results. In addition, the PAE-MHT exhibited high repeatability and optimal sensitivity and specificity.

Our study demonstrates that the PAE-MHT is able to detect currently circulating carbapenemase-producing strains, including those susceptible to carbapenems, which makes it a good candidate as a key diagnostic tool for routine laboratories, especially for those where inhibitor-based tests are not available. In this scenario, we recommend using the PAE-MHT to screen all ceftazidime-resistant isolates of *P. aeruginosa* with meropenem halos of ≤ 23 mm or MICs of ≥ 1 $\mu\text{g/ml}$, an epidemiological breakpoint that suggests carbapenemase production (6). For labs where inhibitor-based tests are available, the PAE-MHT can be an ideal complementary method to overcome potential false-negative results due to the emergence of new combinations of carbapenemases. Thus, we suggest using the PAE-MHT to test strains with a high level of resistance to meropenem (halo, 6 mm; MIC, ≥ 128 $\mu\text{g/ml}$) and negative



FIG. 1. Results of the modified Hodge test for a representative VIM-producing *P. aeruginosa* isolate. Comparative performance was assessed with *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *K. pneumoniae* ATCC 700603 as indicator strains. The final interpretation of the MHT is shown below each strain.

results with class A (3-aminophenyl-boronic acid) and class B (EDTA, dipicolinic acid, etc.) inhibitors.

In conclusion, the PAE-MHT with *K. pneumoniae* ATCC 700603 as the indicator strain will enable routine labs to identify *P. aeruginosa* isolates suspected of producing carbapenemases, including some of the most important epidemiological challenges of recent times, such as KPCs and carbapenem-susceptible MBLs.

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