Pruebas rápidas de sensibilidad a los antimicrobianos: ¿es posible inferir mecanismos de resistencia con fenotipos complicados?

RESUMEN

Los nuevos sistemas automatizados diseñados para la realización rápida de antibiógramas han reducido significativamente el tiempo de respuesta para las pruebas de susceptibilidad de los microorganismos causantes de bacteriemia y sepsis. El sistema Accelerate Pheno® (AAC) es uno de ellos. Nuestro objetivo para este estudio era determinar si el sistema AAC es capaz de proporcionar un perfil de sensibilidad preciso para inferir mecanismos de resistencia de los aislados productores de carbapenemasas en comparación con el sistema MicroScan WalkAway (MWS). Además, comparamos los resultados obtenidos con el sistema rutinario de producción de antibiógramas rápidos. Se seleccionó un total de 19 aislados del criobanco de la Microbiología, todos ellos bacilos gramnegativos productores de carbapenemasas. AAC fue capaz de identificar e inferir la resistencia de un total de 10 aislados, con una EA y CA del 84.2% para meropenem y 88.2% y 64.7% para ertapenem, respectivamente. Sin embargo, en la presencia de carbapenemasas, AAC no fue capaz de proporcionar CMIs adecuadas ni de inferir con precisión los mecanismos de resistencia de los aislados. Se necesitan más estudios con un mayor número de aislados para una comparación más exhaustiva.

Palabras clave: sistema Accelerate Pheno®, carbapenemasas, bacilos gramnegativos
Department’s cryobank. The distribution of species and carbapenemase types is shown in Table 1. All strains were chosen based on their antimicrobial phenotype and carbapenemase detection using commercial molecular methods (OXVIKP (Progenie Molecular) and Xpert® Carba-R (Cepheid)), with varying MIC values determined by the microdilution broth method for different carbapenems available in the susceptibility panels.

### INTRODUCTION

Various approaches are utilized to expedite laboratory results in the management of blood cultures (BC). One such approach involves incorporating Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) directly from positive BC bottles, along with preparing the Antimicrobial Susceptibility Testing (AST) from the same positive blood culture. This process can provide rapid identification and a susceptibility profile within 16 to 24 hours of the MALDI-TOF MS identification [1].

Molecular methods can detect carbapenemase genes in positive BC in less than two hours. However, the Minimal Inhibitory Concentrations (MIC) of carbapenems are not provided until the phenotypic AST is completed [2]. MIC of antimicrobials is still essential for personalized and targeted therapy [3]. Recent IDSA guidelines recommend long-term infusion of meropenem as the preferred treatment agent in certain situations for resistant gram-negative bacteria [4].

The Accelerate Pheno® system (AAC) (Accelerate Diagnostics, Tucson, AZ) is a diagnostic tool that can quickly identify bacterial strains and provide AST results. The AAC has been proven to significantly reduce turnaround time in the diagnosis of bloodstream infections [5]. The reliable determination of MIC in all types of strains has been published [6].

The objective of this study was to determine whether the AAC is capable of providing a reliable susceptibility profile to detect and infer the resistance mechanisms in different strains of carbapenemase-producing gram-negative bacteria. The secondary objective of this research is to compare our routine AST method with the AAC in terms of these antimicrobial phenotypes.

### MATERIAL AND METHODS

#### Selection of bacterial isolates.

Nineteen carbapenemase-producing strains were selected from our Microbiology Department’s cryobank. The distribution of species and carbapenemase types is shown in Table 1. All strains were chosen based on their antimicrobial phenotype and carbapenemase detection using commercial molecular methods (OXVIKP (Progenie Molecular) and Xpert® Carba-R (Cepheid)), with varying MIC values determined by the microdilution broth method for different carbapenems available in the susceptibility panels.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of isolates</th>
<th>Carbapenem resistance genes and number of isolates with this resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>2</td>
<td>OXA-48 (1 isolate) VM (1 isolate)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>9</td>
<td>VM (3 isolates) OXA-48 (5 isolates) KPC (1 isolate)</td>
</tr>
<tr>
<td><em>C. freundii</em></td>
<td>2</td>
<td>KPC + VM (2 isolates)</td>
</tr>
<tr>
<td><em>K. oxytoca</em></td>
<td>1</td>
<td>VM</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>2</td>
<td>VM (2 isolates)</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>3</td>
<td>VM (2 isolates) OXA-48 (1 isolate)</td>
</tr>
</tbody>
</table>

#### Accelerate Pheno system test using spiked blood cultures.

Spiked blood cultures were prepared as follows: BD BACTEC™ Plus aerobic and anaerobic Culture Vials (Becton Dickinson, Madrid, Spain) were inoculated with 10 ml of blood from healthy volunteers. Each bottle was then inoculated with 500 μl of a suspension adjusted to $10^3$ bacteria/ml in 0.9% sodium chloride and incubated at 35 °C with agitation in a BACTEC FX automated blood culture system until bottles flagged positive. For control tests, each bottle was inoculated with 10 ml of blood from healthy volunteers and 100 μl of saline serum.

#### Accelerate PhenoTest™ BC kit testing.

The Accelerate PhenoTest™ BC kits were run on a two-module AAC. The positive blood culture bottles were immediately processed using the AAC. Five hundred μl of positive blood culture was introduced into the sample vial and loaded into the AAC following the manufacturer’s instructions. AAC infers resistance mechanisms involving carbapenem resistance through the presence of higher MIC values (without changing the clinical category to intermediate or resistant) in meropenem and ertapenem antibiotics, as compared to isolates that do not possess these resistance mechanisms.

#### Confirmation, carbapenemase PCR Testing, and AST of the cryobank isolates.

Confirmation of identification and AST of the isolates were performed as previously described [1]. AST was conducted using the VITEK2 Compact system (VCS) (Biomerieux, France) directly from the blood cultures. Addi-
Rapid AST: Possibility of inferring resistance mechanisms with complex phenotypes

RESULTS AND CONCLUSIONS

For meropenem MICs, the EA between AAC and MWS was 84.2% (16/19 isolates), with a CA of 84.2% (16/19 isolates), two MiE (10.5%), and one ME (7.1%). The two MiE occurred in both C. freundii double carbapenemase producers (KPC and VIM), and the ME occurred in one Enterobacter cloacae OXA-48. The concordance in the clinical category (susceptible, intermediate, resistant) between AAC and VCS was 68.4% (13/19 isolates). Among the six discrepancies, AAC showed concordance with MWS in five out of six isolates. If we consider the diffusion disk technique, the CA was 57.9% (11/19 isolates) with six MiE (31.6%), and two VME (33.3%).

For ertapenem MICs, the EA between AAC and MWS was 88.2% (15/17 isolates), with a CA of 64.7% (11/17 isolates), five MiE (29.4%), and one very major error (VME) (14.3%). The five MiE occurred in three OXA-48 carbapenemases and two VIM carbapenemases, and the one VME occurred in a C. freundii double carbapenemase producer (KPC and VIM). The agreement in the clinical category between AAC and VCS was 76.5% (13/17 isolates). Among the four discrepancies, AAC showed concordance with MWS in one isolate. If we consider the diffusion disk technique, the CA was 76.5% (13/17 isolates) with two MiE (11.7%) and two VME (20%).

All isolates tested with ceftazidime/avibactam (isolates 1, 3, 8, 10, 13, 14, 18, and 19) were found to be susceptible. The isolate with the highest MIC was K. pneumoniae with carbapenemase type KPC (MIC = 6).
Our results for both EA and CA for carbapenems are lower than those published by some authors [5, 6]. For example, Patel YA et al. [7] found a CA of more than 90% in Enterobacterales for meropenem and ertapenem, although these authors tested all types of resistance, not just carbapenemase-producing gram-negative bacteria. Considering only carbapenemase-producing bacteria, Pantel A et al. [8] obtained a CA for ertapenem of 81.8% and for meropenem of 84.8% from a total of 33 isolates. These results are comparable for meropenem but lower for ertapenem in our evaluation.

A total of 10 carbapenem-producing isolates were identified as having carbapenem resistance by AAC, with a CA of 70% (7/10 isolates) compared to MWS. However, in three isolates (isolates 5, 15, and 16), AAC was not able to detect carbapenem resistance, unlike the other two methods. In four isolates, none of the three methods were able to detect carbapenem resistance. Interestingly, the VCS system detected two VIM-type carbapenemase-producing isolates (isolates 11 and 17), while the other two methods did not. Excluding the four isolates with carbapenemases that were not detected by any of the three methods, AAC was able to provide MICs of ertapenem and/or meropenem that allowed for the inference of the carbapenemase in 66.6% of the isolates (10/15 isolates), which is a lower percentage than that reported by Pantel A et al. [8]. In their report, AAC was able to detect 35/38 carbapenemase-producing bacteria. In the study by Marschal M et al. [9], which tested 3 isolates of multidrug-resistant *P. aeruginosa* with resistance to carbapenems, AAC was able to detect all 3 isolates. In the study by Sze DTT et al. [10], which tested 8 isolates with resistance to carbapenems (5 without carbapenem resistance genes and 3 with KPC), AAC was able to detect all 8 isolates, demonstrating good sensitivity.

Three out of the four isolates that were not detected by any method were VIM, and one was OXA-48. In these cases, several authors have demonstrated that metallo-beta-lactamases pose numerous practical difficulties for detection. Low-level expression of the enzyme is not always well detected by automated systems [11], and several studies have shown hetero resistance to carbapenems [12].

In conclusion, AAC demonstrated low EA and CA values for meropenem, with EA being very close to 90% for ertapenem. However, the high number of errors suggests that it cannot be relied upon as a good predictor of MICs, nor can it be used to infer the presence of carbapenemases since a change of category may impact the choice and timing of treatment for carbapenemase-producing bacteria. In cases where AAC shows low MICs for meropenem and ertapenem, which do not allow for inferring resistance, additional rapid tests such as NG-Test® CARBA-5 may be useful in identifying the carbapenemases, especially in patients with a high suspicion of bacteremia due to carbapenemase-producing bacteria. Saito K et al. [13] have reported a high sensitivity (99.1%) and specificity (100%) for NG-Test® CARBA-5, making it a valuable tool in the detection of carbapenemases.

The new AAC Kit, which includes ceftolozane-tazobactam and ceftazidime/avibactam, may offer better inference of carbapenemase production produced by gram-negative bacilli.

Our study has limitations; we only analyzed a small number of isolates, including only meropenem and ertapenem in the evaluation. Further studies are needed to evaluate and investigate the ability of these systems to detect and infer resistance produced by carbapenemase-producing bacteria.

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None to declare

**CONFLICT OF INTEREST**

Authors declare no conflict of interest

**REFERENCES**


