Official journal of the Spanish Society of Chemotherapy

Original

Revista Española de Quimioterapia
doi:10.37201/req/043.2023

Jorge Ligero-López[1]
Iker Falces-Romero[1,2]
Alfonso Aranda-Díaz[1]
Diana García-Ballesteros[1]
Julio García-Rodríguez[1,2]
Emilio Cendejas-Bueno[1,2]

Rapid AST: Possibility of inferring resistance mechanisms with complex phenotypes

[1]Clinical Microbiology Department, Hospital Universitario La Paz, IdiPaz, Madrid, Spain.
[2]CIBERINFEC, Instituto de Salud Carlos III, Madrid, Spain

Article history
Received: 18 April 2023; Revision Requested: 6 June 2023; Revision Received: 5 September 2023; Accepted: 13 September 2023; Published: 8 November 2023

ABSTRACT

The new automated systems designed for rapid performance of AST have significantly reduced the response time for susceptibility testing of microorganisms causing bacteremia and sepsis. The Accelerate Pheno® system (AAC) is one such system. Our objective for this study was to determine whether the AAC system is capable of providing an accurate susceptibility profile to infer resistance mechanisms in different carbapenemase-producing isolates when compared to the MicroScan WalkAway System (MWS). Disk diffusion method was also performed on all isolates as a reference method. Additionally, we compared the results obtained with the routine AST production system. We selected 19 isolates from the cryobank of the Microbiology department, all of which were carbapenemase-producing gram-negative bacilli. AAC was able to identify and infer the resistance of a total of 10 isolates, with an EA and CA of 84.2% for meropenem and 88.2% and 64.7% for ertapenem EA and CA, respectively. If we consider the disk diffusion technique, the CA was 57.9% and 76.5% for meropenem and ertapenem. However, in the presence of carbapenemases, AAC was not able to provide adequate MICs or infer the resistance mechanisms of the isolates accurately. Further studies with a larger number of isolates, including the new antibiotics ceftolozane/tazobactam and ceftazidime/avibactam, are needed for a more comprehensive comparison.

Keywords: Accelerate Pheno® system, carbapenemases, Gram-negative bacilli

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 antibiogramas 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 en diferentes aislados productores de carbapenemasas en comparación con el sistema MicroScan WalkAway (MWS). El método de disco difusión fue incluido también en todos los aislados como método de referencia. Además, comparamos los resultados obtenidos con el sistema rutinario de producción de antibiogramas rápidos. Seleccionamos 19 aislados del criobanco del departamento de 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 el meropenem y del 88,2% y 64,7% para la EA y CA del ertapenem, respectivamente. Si consideramos la técnica de disco difusión, la CA fue de un 57.9% y de un 76.5% para meropenem y ertapenem. Sin embargo, en 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 incluyendo también los nuevos antibióticos ceftolozano/tazobactam y ceftazidima/avibactam para una comparación más exhaustiva.

Palabras clave: sistema Accelerate Pheno®, carbapenemasas, bacilos gramnegativos

Correspondence:
Emilio Cendejas-Bueno
Clinical Microbiology Department, Hospital Universitario La Paz, Madrid, Spain, Paseo de La Castellana 261, 28046 Madrid, Spain.
E-mail: ecendejas77@gmail.com

Table 1 Carbapenemase producing gram negative rods and carbapenemase types evaluated.

Species	Number of isolates	Carbapenemase resistance genes and number of isolates with this resistance
E. coli	2	OXA-48 (1 isolate) VIM (1 isolate)
K. pneumoniae	9	VIM (3 isolates) OXA-48 (5 isolates) KPC (1 isolate)
C. freundii	2	KPC + VIM (2 isolates)
K. oxytoca	1	VIM
P. aeruginosa	2	VIM (2 isolates)
E. cloacae	3	VIM (2 isolates) OXA-48 (1 isolate)

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.

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-

Table 2 **Carbapenamase producers gram negative rods evaluated. MIC by the three methods tested for meropenem and ertapenem are showed.**

	Meropenem AxDxcall (MIC mg/L)	Meropenem VITEK2 Call (MIC mg/L)	Meropenem MWS Call (MIC mg/L)	Disk diffusion Meropenem (mm)	Ertapenem AxDxcall (MIC mg/L)	Ertapenem VITEK2 Call (MIC mg/L)	Ertapenem MWS Call (MIC mg/L)	Disk diffusion Ertapenem (mm)	Carbapenemase type	C/A E-test (MIC mg/L)	Microorganism
(EBCBP1)	S (≤0.25)	S (1)	S (0.5)	18 (I)	R (4)	R (>1)	I (1)	15 (R)	OXA-48	0.25 (S)	*K. pneumoniae*
(EBCBP2)	S (≤0.25)	S (1)	I (4)	20 (I)	R (4)	R (>1)	R (4)	17 (R)	KPC+VIM	-	*C. freundii*
(EBCBP3)	S (≤0.25)	S (1)	S (0.5)	19 (I)	R (2)	R (>1)	R (2)	14 (R)	OXA-48	0.25 (S)	*K. pneumoniae*
(EBCBP4)	S (≤0.25)	S (≤0.25)	S (0.03)	31 (S)	S (0.25)	S (≤0.5)	S (0.06)	28 (S)	VIM	-	*E.cloacae*
(EBCBP5)	S (≤0.25)	R (≥16)	S (2)	30 (S)	S (0.25)	S (≤0.5)	I (1)	29 (S)	VIM	-	*K. pneumoniae*
(EBCBP6)	R (≥16)	R (≥16)	R (128)	10 (R)	NR	NR	NR	NR	VIM	-	*P. aeruginosa*
(EBCBP7)	S (≤0.25)	S (≤0.25)	S (1)	29 (S)	S (0.25)	S (≤0.5)	S (0.25)	29 (S)	VIM	-	*E. coli*
(EBCBP8)	I/R (≥8)	R (≥16)	R (64)	12 (R)	R (2)	R (>1)	R (2)	9 (R)	KPC	6 (S)	*K. pneumoniae*
(EBCBP9)	S (≤0.25)	S (1)	S (0.125)	13 (R)	S (0.25)	S (≤0.5)	S (0.125)	9 (R)	VIM	-	*K. oxytoca*
(EBCBP10)	S (≤0.25)	S (≤0.25)	S (0.03)	13 (R)	S (0.25)	S (≤0.5)	S (0.06)	13 (R)	OXA-48	0.5 (S)	*K. pneumoniae*
(EBCBP11)	S (≤0.25)	I (4)	S (1)	23 (S)	S (0.25)	S (≤0.5)	S (0.5)	23 (S)	VIM	-	*K. pneumoniae*
(EBCBP12)	I/R (≥8)	R (≥16)	R (16)	14 (R)	NR	NR	NR	NR	VIM	-	*P. aeruginosa*
(EBCBP13)	S (≤0.25)	S (2)	S (1)	16 (I)	R (2)	R (>1)	R (4)	12 (R)	OXA-48	0.5 (S)	*K. pneumoniae*
(EBCBP14)	S (≤0.25)	I (4)	S (0.5)	17 (I)	R (2)	R (>1)	I (1)	14 (R)	OXA-48	0.25 (S)	*E. coli*
(EBCBP15)	S (≤0.25)	I (4)	I (4)	24 (S)	S (0.25)	R (>1)	R (4)	27 (S)	KPC+VIM	-	*C. freundii*
(EBCBP16)	S (≤0.25)	I (4)	S (1)	25 (S)	S (0.25)	R (>1)	I (1)	24 (I)	VIM	-	*K. pneumoniae*
(EBCBP17)	S (≤0.25)	I (4)	S (0.5)	24 (S)	S (0.25)	I (1)	S (0.5)	25 (S)	VIM	-	*E. cloacae*
(EBCBP18)	I/R (≥8)	I (4)	S (2)	13 (R)	I (1)	R (>1)	R (8)	10 (R)	OXA-48	0.5 (S)	*E. cloacae*
(EBCBP19)	S (0.5)	S (2)	S (1)	19 (I)	R (2)	R (>1)	R (2)	16 (R)	OXA-48	0.5 (S)	*K. pneumoniae*

S (susceptible) I (intermediate), R (Resistant) C/A (ceftazidime/avibactam). MIC breakpoints were interpreted with the EUCAST 2018 rules.

tionally, AST was performed on all isolates using the MicroScan WalkAway System (MWS) (Beckman Coulter, Madrid, Spain) as a comparative method. Disk diffusion method was also performed on all isolates as a reference method. Although the kit did not include ceftazidime/avibactam, ceftazidime/avibactam E-tests were performed on all isolates except those with VIM-type carbapenemases. This study was conducted in 2019, and therefore, the breakpoints used were those specified in version 8.0 published by EUCAST in 2018. The isolates, MICs obtained by the three methods, MICs obtained with Ceftazidime/Avibactam and resistance genes are described in Table 2.

Data Analysis. To assess the accuracy of the AST, we calculated the values of essential agreement (EA), categorical agreement (CA), minor error (MiE), major error (ME), and very major error (VME) by comparing the results of AAC with the comparative method. Essential agreement is defined as MIC result with the susceptibility testing system that is within plus or minus one doubling dilution step from the MIC value established with the comparative method. CA is the percentage of isolates whose clinical category matches that of the comparative technique. MiE was defined as an intermediate result by one method and a susceptible or resistant result by the other method or vice versa. ME is the percentage of isolates that are resistant by AAC and susceptible by the comparative technique. VME is the percentage of isolates that are susceptible by AAC and resistant by the comparative technique.

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 agreement 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 carbapenemase-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.

FUNDING

None to declare

CONFLICT OF INTEREST

Authors declare no conflict of interest

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