

Rapid Phenotypic Detection of Gram-negative Bacilli Resistant to Oximinocephalosporins and Carbapenems in Positive Blood Cultures Using a Novel Protocol

Diego Josa

Fundacion Clinica Shaio

Ingrid Gisell Bustos

Universidad de La Sabana

Soad Amira Yusef

Universidad de La Sabana

Stephanie Crevoisier

Universidad de La Sabana

Edwin Silva-Monsalve

Fundacion Clinica Shaio

Natalia López-Moreno

Fundacion Clinica Shaio

Rafael Leal Avendaño

Fundacion Clinica Shaio

Isabel Torres Molina

Fundacion Clinica Shaio

Juan Pablo Osorio

Fundacion Clinica Shaio

Gerson Arias-Leon

Fundacion Clinica Shaio

Fabián Cortés

Fundacion Clinica Shaio

Carolina Sánchez

Fundacion Clinica Shaio

Luis Felipe Reyes (✉ luis.reyes5@unisabana.edu.co)

Universidad de La Sabana

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Abstract

Aim: To test a modified protocol designed to detect Gram-negative bacilli (GNB) resistant to oximinocephalosporins and carbapenems from positive blood cultures.

Design: This is a prospective, cohort study of consecutive patients.

Setting: A cardiovascular and University referral hospital.

Patients: Patients hospitalized in a third level hospital with bacteraemia.

Main variables of interest: We developed a modified protocol using HB&L® system to detect MDRP. We then attempted to determine accuracy, concordance and reduction of identification time of this novel method in a reference hospital. Descriptive statistics and logistical regressions were used.

Results: Ninety-six patients with BSI were included in the study. A total of 161 positive blood cultures were analysed. *Escherichia coli* (50%, 81/161) was the most frequently identified pathogen followed by *Klebsiella pneumoniae* (15%, 24/161) and *Pseudomonas aeruginosa* (8%, 13/161). Thirty-two percent of isolations had usual resistance patterns. However, in 34/161 (21%) of identified pathogens were producer of carbapenemases and 21/161 (13%) of extended-spectrum β -lactamases. Concordance among our HB&L® modified protocol and traditional method was 99% (159/161). Finally, identification times were significantly shorter using our HB&L® modified protocol than traditional methods (Median [IQR]; 19 hours [18, 22] Vs 61 hours [60, 64], $p < 0.001$).

Conclusions: Here we provided novel evidence that using our HB&L® modified protocol is an effective strategy to reduce the time to MDRP detection/identification; with a great concordance rate when compared to the gold standard. Further studies are needed to confirm these findings and to determine whether this method may improve clinical outcomes.

1. Background

Infections due to resistant gram-negative Bacilli (GNB) have emerged as a global public health problem [1, 2]. The impact on lives lost and costs for the health systems has been extensively studied in medical literature [3]. Up to 40% of patients diagnosed with sepsis and septic shock due to multi-drug resistant pathogens die as a result of these infections. Additionally, the survivors have prolonged hospital stays, with annual costs for the health system estimated at 16.7 billion dollars in the United States alone [4, 5].

The initiation of early antibiotic therapy improves the survival of patients with sepsis and blood stream infections caused by GNB [6, 12], but the emergence of antibiotic resistance poses a growing challenge in achieving this objective. Owing to its genetic plasticity, GNB rely on various resistance mechanisms that allow them to respond to a wide range of environmental threats; of these mechanisms, the main and most versatile is the production of extended spectrum β -lactamases (ESBL) [13]. The global expansion of ESBLs and carbapenemases is an unprecedented event, favored by the high mobility conditions of the population [14] and the non-prudent use of antibiotics both in the food and healthcare industries, among other factors [15, 16].

Latin America has been especially affected by this problem [17, 18]. Irrespective of the type of β -lactamase involved, epidemiological surveillance studies have shown a growing prevalence of Enterobacteriaceae resistance to oximino-cephalosporins in Latin American hospitals; which has led to a massive use of carbapenems, with the resulting emergence of isolates resistant to these agents [19]. In Colombia, GNB resistant to carbapenems are endemic. Colombia was the first Latin American country to identify isolate producers of *Klebsiella pneumoniae* carbapenemases (KPC) [20] and the first country in the world to deliver isolates of KPC-producing *Pseudomonas aeruginosa* [21]. On top of that, the Colombian national epidemiological surveillance has detected an endemic circulation of various types of carbapenemases (OXA, NDM, and VIM) [22, 23].

The use of conventional methods makes it possible to identify ESBL- and carbapenemase-producing GNB, but the total time required can be 96 hours after obtaining the isolate. However, rapid techniques have recently been evaluated to detect multi-drug resistant GNB in blood culture samples [24]. In addition to molecular methods [25, 26], biophysical techniques such as mass spectrometry, flow cytometry, laser nephelometry, immunochromatography, chemiluminescence, and bioluminescence are currently available [27]. The implementation of some of these techniques has shown a decrease in hospital length of stay, ICU length of stay, costs, and

mortality rates [28]. However, there are limitations such as their high cost, the use of physical space, and the need for trained staff, and therefore, these technologies are not available in every hospital around the world.

Laser nephelometry (HB&L® Alifax, Italy), unlike molecular methods, offers an excellent diagnostic performance at a lower cost. They have a wide range of uses, including sifting strategies, quantitative cultures, and antimicrobial susceptibility determination. The most significant contribution in terms of antibiotic resistance has been the implementation of this technology as an active surveillance strategy for the early identification of multi-drug resistant GNB carriers in rectal swabs [29–31]. To date, its use in blood samples has been limited [32, 33]. Our hypothesis is that this technique is efficient for the rapid detection of multi-drug resistant GNB in patients with bacteremia. Therefore, the objective of this study is to evaluate the operational characteristics resulting from the combination of the Bact/Alert 3D®, HB&L®, and Vitek2® techniques to save time in the identification of infections due to oximino-cephalosporins- and carbapenemase-resistant GNB in positive blood cultures compared with the conventional techniques.

2. Methods

This is a prospective cohort observational study of diagnostic tests in which the operational characteristics are evaluated, in addition to the degree of concordance and the diagnostic time resulting from the combination of the Bact/Alert 3D®, HB&L®, and Vitek2® techniques compared with the reference standard in a high-complexity hospital in the city of Bogota, Colombia. The research protocol was approved by the Fundación Clínica Shaió's Ethics and Research Committee (Memorandum of Approval No. 273). Obtaining informed consent was not necessary because of the absence of direct intervention in patients and the observational characteristics of the study.

Sample processing

All blood cultures sent to the microbiology laboratory from July 1, 2017 to March 31, 2019 were collected in a consecutive and prospective manner. All positive blood cultures with GNB identified via direct Gram stain microscopy were included, and there were no exclusion criteria.

Setting up blood cultures for laser nephelometry using HM&L-ESBL/AmpC® and HB&L-Carbapenemase® kits

The extraction was performed directly from the sample in the positive blood culture bottles. Subsequently, two drops were released into a plastic tube with 2 ml of 0.9% saline solution and mixed using a vortexer. Based on this prepared suspension, 200 µl was released into the green-lidded vial from the HB&L-ESBL/AmpC® kit together with 200 µl of the reagent from the kit (containing a mix of antibiotics) and another 200 µl into the red-lidded vial from the HB&L® Carbapenemase kit with 200 µl of the specific reagent; the vials were then deposited in the automated HB&L® system in the pre-established programs ESBL and KPC, respectively. The results were subsequently read after 6 hours (Fig. 1).

Setting up the tests through conventional method

Culturing was performed through the conventional method in solid culture mediums. For this, a sample of positive blood culture was extracted, releasing a drop into blood agar and a drop into MacConkey agar. Subsequently, using a handle, culturing was performed by dropping, and cultures were incubated at 37 °C for 24 hours. The following day, the growth and morphology of the colonies present were observed in the culture media, and the biochemical identification and susceptibility profile test were performed using Vitek2® automated system (Biomerieux®) with GNB and AST-272 cards.

Phenotypic tests for confirmation of carbapenemase-producing GNB

The cultures processed via conventional methods, with carbapenemase-resistant susceptibility profile results, were subjected to the following confirmatory tests: Hodge Test and EDTA disk synergy and boronic acid disks tests. This methodology was considered the reference standard for the confirmation of carbapenemase production.

Test controls

The following control strains were used in setting up each of the tests: *K. pneumoniae* ATCC BAA 1705 (blaKPC+), *K. pneumoniae* ATCC BAA 2146 (blaNDM+), ESBL-producing *K. pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922, and *P. aeruginosa* ATCC 27853.

Clinical variables

Upon completion of the collection of blood culture bottles, the subjects' clinical chart was retrospectively reviewed, identifying GNB in the blood cultures to extract the relevant clinical variables. Demographic characteristics, comorbidities, origin of samples, source of infection, and severity of illness were evaluated.

Statistical analysis

A non-probabilistic convenience sampling was conducted, calculating a sample size of 134 blood culture samples to reach a 95% power with an alpha error of 5% and an accuracy of 3%. Central tendency and dispersion were used for the quantitative variables, whereas percentages and frequencies were implemented to measure the qualitative variables. Operational characteristics were separately analyzed for each one of the tests (HB&L-ESBL/AmpC® and HB&L-Carbapenemase® kits). Sensitivity (S), specificity (E), positive predictive value (VPP), and negative predictive value (VPN) were evaluated for each one of the tests. Additionally, the likelihood ratios (LR + and LR-) were calculated. To evaluate the degree of concordance among the diagnostic techniques, correlation coefficient (kappa coefficient) was estimated. Additionally, the time to positivity was evaluated (identification of multi-drug resistant GNB) using the proposed identification protocol, and the time to microbiological diagnosis was evaluated through conventional methods. The data were analyzed using SPSS 25 software for Mac.

3. Results

We included 161 positive blood culture bottles with GNB from 96 subjects hospitalized due to different diagnoses. The majority of samples were obtained from women (55.2%, 53/96), with a median (interquartile range, IQR) age of 73 years (59, 80). Arterial hypertension was the most frequently identified comorbidity (55.2%, 53/96). Most infections were community acquired (63.5%, 61/96); however, a remarkable percentage of nosocomial infections were observed (34.4%, 33/96). A vast majority of bacteremia was secondary (92.7%, 89/96), with urinary sepsis being the main etiology (46.9%, 45/96), followed by abdominal sepsis (12.5%, 12/96), biliary sepsis (10.4%, 10/96), and pulmonary-originated sepsis (8.3%, 8/96). The severity of illness was moderate, as calculated by APACHE II and SOFA (Table 1).

Table 1
General characteristics of patients with bacteremia

Characteristic	Total (n = 96)
Sex, n (%)	
Men	43 (44.8)
Women	53 (55.2)
Age, median (IQR)	73 (59, 80)
Comorbidities, n (%)	
Arterial hypertension	53 (55.2)
Coronary disease	16 (16.7)
COPD	18 (18.8)
OSAHS	8 (8.3)
Diabetes Mellitus	33 (33.4)
Obesity	12 (12.5)
Chronic kidney disease	28 (29.2)
Severity, median (IQR)	
APACHE II	12.5 (8, 20.75)
SOFA	4 (2.25, 6.75)
Origin of samples, n (%)	
Hospitalization	48 (45.8)
Intensive Care Unit	40 (41.7)
Emergencies	11 (11.5)
Source of infection, n (%)	
Community acquired	61 (63.5)
Nosocomial infection	33 (34.4)
Bacteremia, n (%)	
Primary	7 (7.3)
Secondary	89 (92.7)
Origin, n (%)	
Urine	45 (46.9)
Abdomen	12 (12.5)
Bile duct	10 (10.4)
Lung	8 (8.3)
Time, hours (median [IQR])	
Bact/Alert 3D® positivity	13 (12, 16)
HB&L® positivity	19 (18, 22)
Conventional culture®	61 (60, 64)

Characteristic	Total (n = 96)
APACHE II: Acute Physiology and Chronic Health Evaluations II; COPD: Chronic Obstructive Pulmonary Disease; OSAHS: Obstructive Sleep Apnoea-Hypopnoea Syndrome; SOFA: Sequential Organ Failure Assessment, IQR: Interquartile Range	

The most commonly identified germ was *E. coli* (50.1%, 81/161), followed by *K. pneumoniae* (14.9%, 24/161) (Fig. 2). A certain degree of anti-microbial resistance was observed in 67% (109/161) of the samples, with carbapenemase resistance being the most frequently identified (21%, 34/161), followed by ESBL (13%, 21/161) and amplified-spectrum β -lactamase (11%, 18/161). It is important to highlight that in 33% (53/161) of the samples, a common sensitivity profile was observed (Table 2).

Table 2

Distribution of resistance profiles and concordance between the conventional identification method and antibiogram and the method proposed by HB&L

	Microorganism	(n)	Conventional method/ HB&L method						
			Sensitive	IRT	ASBL	ESBL	Repressed AmpC	Unrepressed AmpC	Resistance to carbapenemase
Enterobacteria	<i>E. coli</i>	81	34/34	13/13	15/15	17/17			2/2
	<i>K. pneumoniae</i>	24	6/6		1/1	2/2			15/15
	<i>S. marcescens</i>	9					4/4	5/5	
	<i>K. oxytoca</i>	7	4/4		2/2	1/1			
	<i>P. mirabilis</i>	5	5/5						
	<i>E. aerogenes</i>	4					1/1	1/1	2/2
	<i>E. cloacae</i>	2						2/2	
	<i>Salmonella</i> spp	2	1/1			1/1			
	<i>P. agglomerans</i>	2							2/2
	<i>M. morgani</i>	1						1/1	
<i>P. stuartii</i>	1					1/1			
NF GNB	<i>P. putida</i>	2							2/2
	<i>P. aeruginosa</i>	13					5/5	3/1**	5/5
	<i>B. cepacea</i>	3	2/2						1/1
	<i>S. maltophilia</i>	3							3/3
	<i>A. baumannii</i>	2							2/2

Operational characteristics of the HB&L-ESBL/AmpC® test

Sensitivity of the HB&L-ESBL/AmpC® test in blood cultures was 95.4% with 100% specificity. Predictive values VPP and VPN were 100% and 98.3%, respectively (Table 3). Compared with the conventional method, concordance, assessed through kappa correlation coefficient, was 0.92 with a 97.5% concordance rate.

Table 3. Operational characteristics of the *ESBL/AmpC* and *HB&L Carbapenemase* kit tests.

HB&L.

	ESBL/AmpC® Vial				Carbapenemase® Vial			
	Positive	Negative	Total		Positive	Negative	Total	
Positive conventional culture	61	2	63	S (95%)	34	0	34	S (100%)
Negative conventional culture	0	98	98	E (100%)	0	127	0	E (100%)
Total	61	100	161		34	127	161	
	VPP 100%	VPN 98%			VPP 100%	VPN 100%		

S: Sensitivity; E: Specificity; VPP: Positive predictive value; VPN: Negative predictive value

Operational characteristics of the HB&L-ESBL/Carbapenemase® test

All operational characteristics (S, E, VPN, and VPP) from the vial for carbapenemase were 100% (Table 3), with a correlation coefficient of 100%.

Time to identification of multi-drug resistant germs

The median (IQR) time to identification of GNB in the Bact/Alert 3D® system was 13 hours (12, 16). When the modified HB&L® method for positive blood culture samples was compared with the conventional identification method, the presumptive positivity for the identification of carbapenemase-producing GNB was much faster when the modified HB&L was used (19 hours [18, 22] vs. 61 hours [60, 64]; $p < 0.001$), including the positivity time from the Bact/Alert 3D® system. In other words, a 42-hour reduction was achieved in the early recognition of carbapenemase and/or oximino-cephalosporin resistance (Fig. 3). At the time of the identification of carbapenemase-producing GNB, their presence was confirmed through phenotypic testing: Hodge Test and EDTA disk synergy and boronic acid disks tests.

4. Discussion

The rapid detection of antibiotic resistance is one of the most important objectives in the treatment approach for patients with blood stream infections. Thus, in our study a 42-hours reduction was noted in the identification of carbapenemase and oximino-cephalosporin resistance in GNB from blood cultures. Additionally, it was found that compared with the conventional method, the combination of the Bact/Alert 3D®, HB&L®, and Vitek2® techniques resulted in a concordance of 100% for the early detection of carbapenemase and a concordance of 95% in the detection of phenotypic resistance to oximino-cephalosporins. This time saved might have a positive impact on the clinical outcomes in patients with infections due to multidrug resistant GNB; however, this should be evaluated in further studies.

The early initiation of effective antibiotic therapy is a predictor of the outcomes of bacteremia due to GNB [6, 8–12]. Tumbarello et al. [8] showed that the initiation of inappropriate antibiotic treatments was a strong predictor of mortality in patients with bacteremia due to ESBL-producing GNB (59.5% vs. 18.5%; OR:2.28; 95% CI:1.76–3.22; $p < 0.001$). A meta-analysis conducted by Kohler et al. reported a mortality of 46% in patients with infections caused by carbapenemase-producing GNB, which was even higher in those receiving inappropriate treatment (10% higher, OR:1.28; 95% CI: 1.04–1.58; $p = 0.02$) [12]. Likewise, the INCREMENT cohort reported a 22% increase in mortality resulting from the initiation of inappropriate empiric therapy [11]. Therefore, it is evident that tests are necessary for the rapid detection of multi-drug resistant germs to ensure adequate antibiotic treatment.

Rapid detection tests showed a reduction in the hospital and ICU length of stays, mortality rate, and costs in various studies [25–27]. Perez et al. incorporated the rapid identification of pathogens through Matrix-Assisted Laser Desorption/ionization Time (MALDI-TOF) and susceptibility through BD Phoenix™, with a decrease of more than 50% in the microbiological identification times (40.6 vs. 14.5 hours; $p < 0.001$), adjustment to effective treatment (89.7 vs. 32 hours; $p < 0.001$), reduced hospital stay (23.3 vs. 15.3 days; $p = 0.001$) and ICU stay (16 vs. 10.7 days; $p = 0.008$), and 30-day mortality (21% vs. 8.9%; $p = 0.01$) [28]. Sakarikou et al. also used MALDI-TOF for identification purposes as well as VITEK-2® for susceptibility test in blood culture samples with GNB. In this case, the sample was taken directly from the blood culture without moving it to a solid medium, saving 8 hours compared with the conventional method (5 vs. 11 hours; $p \leq 0.001$, without taking time for blood culture positivity into account) and with a concordance of 98.5%

[34]. This data confirms that by performing an early identification of multi-drug resistant bacteria, it is possible to provide a rapid targeted therapy and therefore improved clinical outcomes. Unfortunately, the MALDI-TOF availability is limited to a few hospitals owing to the high initial installation cost.

PCR-based techniques conducted microbiological identification in 1–2 hours. However, antibiotic susceptibility is limited to the genes included in each panel, such as Film Array® platform (Biofire®), which only detected the presence of the KPC genes. Additionally, in some of these techniques, a decreased efficiency is observed in polymicrobial infections, thereby making the combination of additional techniques necessary [26–28]. On the other hand, the Bact/Alert 3D®, VITEK®, and HB&L® techniques are less costly and do not require specialized training, resulting in easy implementation in hospitals. Studies such as those conducted by Hogan and Höring (30, 31) on GNB-positive blood culture involved direct inoculation to evaluate susceptibility through VITEK-2® [35, 36]; the concordance with the conventional method was over 95% in both studies. However, false susceptibilities to carbapenemases were documented, which constitutes a major error. Recently, Athamna et al. used Uro4 HB<M laser nephelometry, compared with VITEK-2®, in their study on ESBL/AmpC susceptibility in Enterobacteria, with 91.3% concordance. However, carbapenemase identification was not included, and for *P. mirabilis*, the concordance was only 58.3% with zero sensitivity [32]. In contrast, our study integrated the Bact/Alert 3D®, VITEK®, and HB&L® techniques in GNB-positive blood cultures, with direct inoculation using both kits (ESBL/AmpC® and Carbapenemase®). Identification time and susceptibility decreased 42 hours compared with the conventional methodology, which involves a significant decrease in the identification times that could potentially improve the clinical outcomes of patients with bacteremia due to GNB. Importantly, the efficiency was 100% in all operational characteristics using the HB&L Carbapenemase® kit, including the identification of serine carbapenemases and metallo-β-lactamases. Thus, this study opens the way for new low-cost strategies aimed at the rapid detection of these pathogens; however, it must be assessed whether the routine use of this protocol has an impact on the clinical outcomes of patients with bacteremia.

The strengths and weaknesses of our study are noteworthy. Despite being a unicentric study, the incidence of antibiotic resistance resembles that at the international level; thus, similar endemic populations with multi-drug resistance could benefit from the use of this methodology and from the results presented in this manuscript. With respect to cost, the systematic use of HB&L® for all GNB-positive blood cultures could initially increase costs; however, it could be considered cost-effective when comparing it with alternative quick tests and evaluating the impact of the optimal initiation of therapy in terms of other cost-effective scenarios such as hospital length of stay, ICU length of stay, antibiotic days savings, and mortality rate. However, the assessment of the economic and clinical impacts of this protocol was out of the scope of this study and needs to be further studied. The phenotypic diagnosis led to limitations owing to the presence of combinations in increasing resistance mechanisms; however, in these limited resource scenarios, we believe that this methodology is one of the best diagnostic options. Moreover, it provides another fast-diagnostic opportunity using concentrated biomass in the HB&L® equipment for the application of other methods such as direct immunochromatography or lateral flow for carbapenemases detection, a methodology that has been experimentally developed so far.

5. Conclusion

The combination of the Bact/Alert 3D®, HB&L®, and Vitek2® techniques was comparable to the reference standard and allowed the identification of oximino-cephalosporins- and carbapenemases-resistant GNB 42 hours earlier compared with the conventional techniques. Additionally, this rapid detection of resistant GNB could be compared with other similar techniques, but its cost is even lower. Whether these results are reflected in the clinical outcomes is yet to be evaluated in prospective trials. However, we believe that this entails remarkable progress in the early detection of antimicrobial resistance.

Declarations

Ethics approval and consent to participate:

This study was approved by the Committee of the Research Ethics of the Fundacion Clinica Shaio and Universidad de La Sabana. This study was declared except and does not required informed consent. The patient's privacy and the availability of services were guaranteed by the investigator team even if the patients decided to leave the study. This study did not involve any potentially harmful intervention for the patient. The confidentiality of the data was preserved until the writing of the manuscript for publication.

Consent for publication:

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

Availability of data and materials:

Not applicable

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Author contribution:

DJM, IGB, SAY, SC, ESM, NLM, RLA, ITM, JPO, GAL, FC, CS and LFR contributed to the planning, writing and, correction of each section of the manuscript.

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References

1. Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance—the need for global solutions. *Lancet Infect Dis* 2013; 13:1057-98.
2. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018; 18:318 – 27.
3. Giske CG, Monnet DL, Cars O, Carmeli Y, Resistance R-AoA. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. *Antimicrob Agents Chemother* 2008; 52:813 – 21.
4. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001; 29:1303-10.
5. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; 348:1546-54.
6. Kang CI, Kim SH, Park WB, et al. Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. *Antimicrob Agents Chemother* 2005; 49:760-6.
7. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006; 34:1589-96.
8. Tumbarello M, Sanguinetti M, Montuori E, et al. Predictors of mortality in patients with bloodstream infections caused by extended-spectrum-beta-lactamase-producing Enterobacteriaceae: importance of inadequate initial antimicrobial treatment. *Antimicrob Agents Chemother* 2007; 51:1987-94.
9. Paul M, Shani V, Muchtar E, Kariv G, Robenshtok E, Leibovici L. Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. *Antimicrob Agents Chemother* 2010; 54:4851-63.
10. Zarkotou O, Pournaras S, Tselioti P, et al. Predictors of mortality in patients with bloodstream infections caused by KPC-producing *Klebsiella pneumoniae* and impact of appropriate antimicrobial treatment. *Clin Microbiol Infect* 2011; 17:1798 – 803.
11. Gutiérrez-Gutiérrez B, Salamanca E, de Cueto M, et al. Effect of appropriate combination therapy on mortality of patients with bloodstream infections due to carbapenemase-producing Enterobacteriaceae (INCREMENT): a retrospective cohort study. *Lancet Infect Dis* 2017; 17:726 – 34.
12. Kohler PP, Volling C, Green K, Uleryk EM, Shah PS, McGeer A. Carbapenem Resistance, Initial Antibiotic Therapy, and Mortality in *Klebsiella pneumoniae* Bacteremia: A Systematic Review and Meta-Analysis. *Infect Control Hosp Epidemiol* 2017; 38:1319-28.
13. Bush K, Fisher JF. Epidemiological expansion, structural studies, and clinical challenges of new β -lactamases from gram-negative bacteria. *Annu Rev Microbiol* 2011; 65:455 – 78.
14. Hawkey PM. Multidrug-resistant Gram-negative bacteria: a product of globalization. *J Hosp Infect* 2015; 89:241-7.
15. Berendonk TU, Manaia CM, Merlin C, et al. Tackling antibiotic resistance: the environmental framework. *Nat Rev Microbiol* 2015; 13:310-7.
16. Wellington EM, Boxall AB, Cross P, et al. The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. *Lancet Infect Dis* 2013; 13:155 – 65.
17. Munoz-Price LS, Poirel L, Bonomo RA, et al. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect Dis* 2013; 13:785 – 96.

18. Escandón-Vargas K, Reyes S, Gutiérrez S, Villegas MV. The epidemiology of carbapenemases in Latin America and the Caribbean. *Expert Rev Anti Infect Ther* 2017; 15:277 – 97.
19. Bonelli RR, Moreira BM, Picão RC. Antimicrobial resistance among Enterobacteriaceae in South America: history, current dissemination status and associated socioeconomic factors. *Drug Resist Updat* 2014; 17:24–36.
20. Villegas MV, Lolans K, Correa A, et al. First detection of the plasmid-mediated class A carbapenemase KPC-2 in clinical isolates of *Klebsiella pneumoniae* from South America. *Antimicrob Agents Chemother* 2006; 50:2880-2.
21. Villegas MV, Lolans K, Correa A, et al. First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing beta-lactamase. *Antimicrob Agents Chemother* 2007; 51:1553-5.
22. Ovalle MV, Saavedra SY, González MN, Hidalgo AM, Duarte C, Beltrán M. Results of the national surveillance of antimicrobial resistance of Enterobacteriaceae and Gram negative bacilli in health care-associated infections in Colombia, 2012–2014. *Biomedica* 2017; 37:473 – 85.
23. Hernández-Gómez C, Blanco VM, Motoa G, et al. [Evolution of antimicrobial resistance in Gram negative bacilli from intensive care units in Colombia]. *Biomedica* 2014; 34 Suppl 1:91–100.
24. Banerjee R, Humphries R. Clinical and laboratory considerations for the rapid detection of carbapenem-resistant Enterobacteriaceae. *Virulence* 2017; 8:427 – 39.
25. Findlay J, Hopkins KL, Meunier D, Woodford N. Evaluation of three commercial assays for rapid detection of genes encoding clinically relevant carbapenemases in cultured bacteria. *J Antimicrob Chemother* 2015; 70:1338-42.
26. Rodríguez-Lucas C, Rodicio MR, Costales I, Boga JA, Vazquez F, Fernández J. Evaluation of Sepsis Flow Chip for identification of Gram-negative bacilli and detection of antimicrobial resistance genes directly from positive blood cultures. *Diagn Microbiol Infect Dis* 2018; 91:205-9.
27. March-Rosselló GA. Rapid methods for detection of bacterial resistance to antibiotics. *Enferm Infecc Microbiol Clin* 2017; 35:182-8.
28. Perez KK, Olsen RJ, Musick WL, et al. Integrating rapid diagnostics and antimicrobial stewardship improves outcomes in patients with antibiotic-resistant Gram-negative bacteremia. *J Infect* 2014; 69:216 – 25.
29. Josa DF, Bustos G, Torres IC, Esparza S G. [Evaluation of three screening methods for detection of carbapenemase-producing Enterobacteriaceae in rectal swabs]. *Rev Chilena Infectol* 2018; 35:253 – 61.
30. Arena F, Giani T, Antonelli A, et al. A new selective broth enrichment automated method for detection of carbapenem-resistant Enterobacteriaceae from rectal swabs. *J Microbiol Methods* 2018; 147:66 – 8.
31. Marani S, Pedna MF. Evaluation of the HB&L carbapenemase and extended spectrum beta lactamase-AmpC automated screening kits for the rapid detection of resistant Enterobacteriaceae in rectal swabs. *Microbiologia Medica* 2017; 32:6709.
32. Athamna A, Freimann S. A new rapid method for detecting extended-spectrum beta-lactamase/AmpC-producing Enterobacteriaceae directly from positive blood cultures using the Uro4 HB&L™ system. *Braz J Microbiol* 2019.
33. Barnini S, Brucculeri V, Morici P, Ghelardi E, Florio W, Lupetti A. A new rapid method for direct antimicrobial susceptibility testing of bacteria from positive blood cultures. *BMC Microbiol* 2016; 16:185.
34. Sakarikou C, Altieri A, Bossa MC, et al. Rapid and cost-effective identification and antimicrobial susceptibility testing in patients with Gram-negative bacteremia directly from blood-culture fluid. *J Microbiol Methods* 2018; 146:7–12.
35. Hogan CA, Watz N, Budvytiene I, Banaei N. Rapid antimicrobial susceptibility testing by VITEK®2 directly from blood cultures in patients with Gram-negative rod bacteremia. *Diagn Microbiol Infect Dis* 2019; 94:116 – 21.
36. Höring S, Massarani AS, Löffler B, Rödel J. Rapid antibiotic susceptibility testing in blood culture diagnostics performed by direct inoculation using the VITEK®-2 and BD Phoenix™ platforms. *Eur J Clin Microbiol Infect Dis* 2019; 38:471-8.

Figures

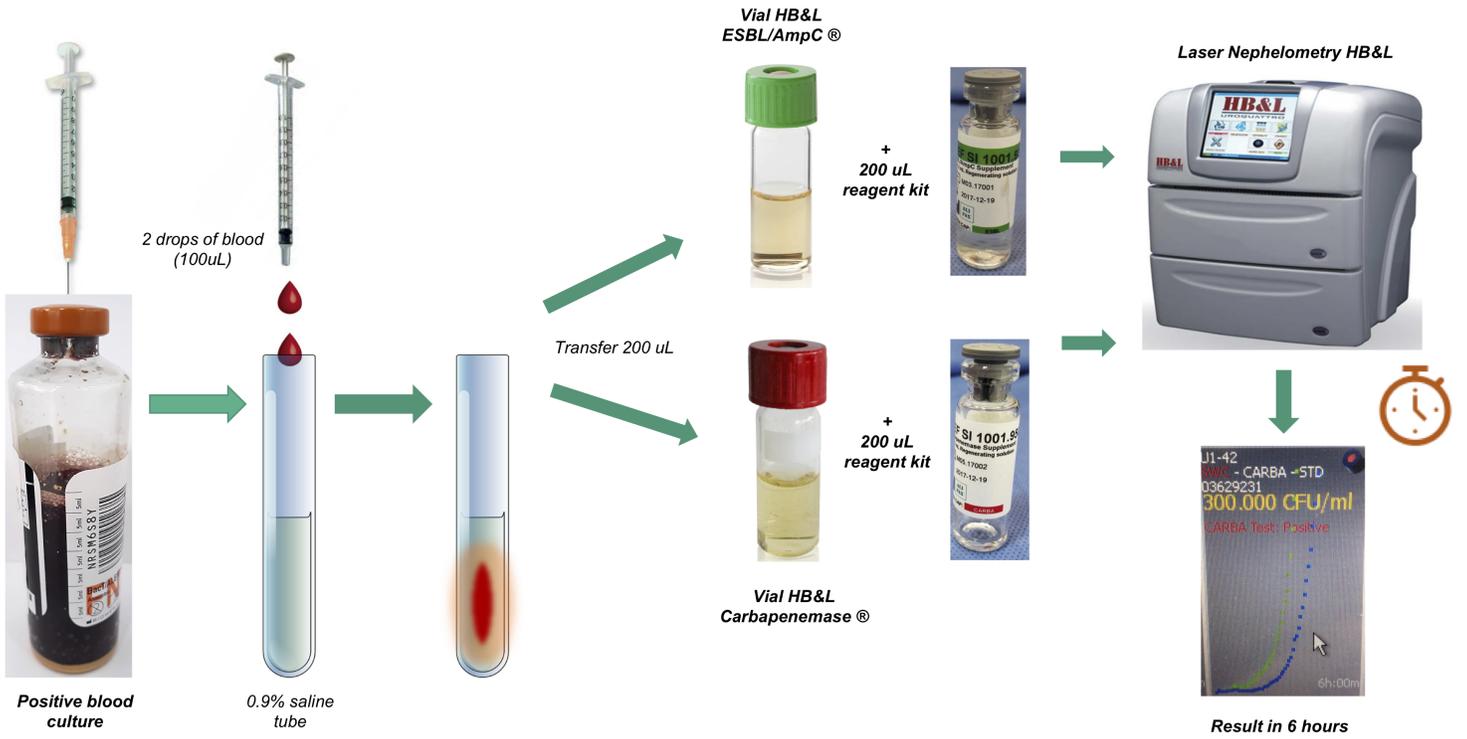


Figure 1

Processing of positive blood culture samples by ESBL/AMPC and HB&L Carbapenemase kit tests.

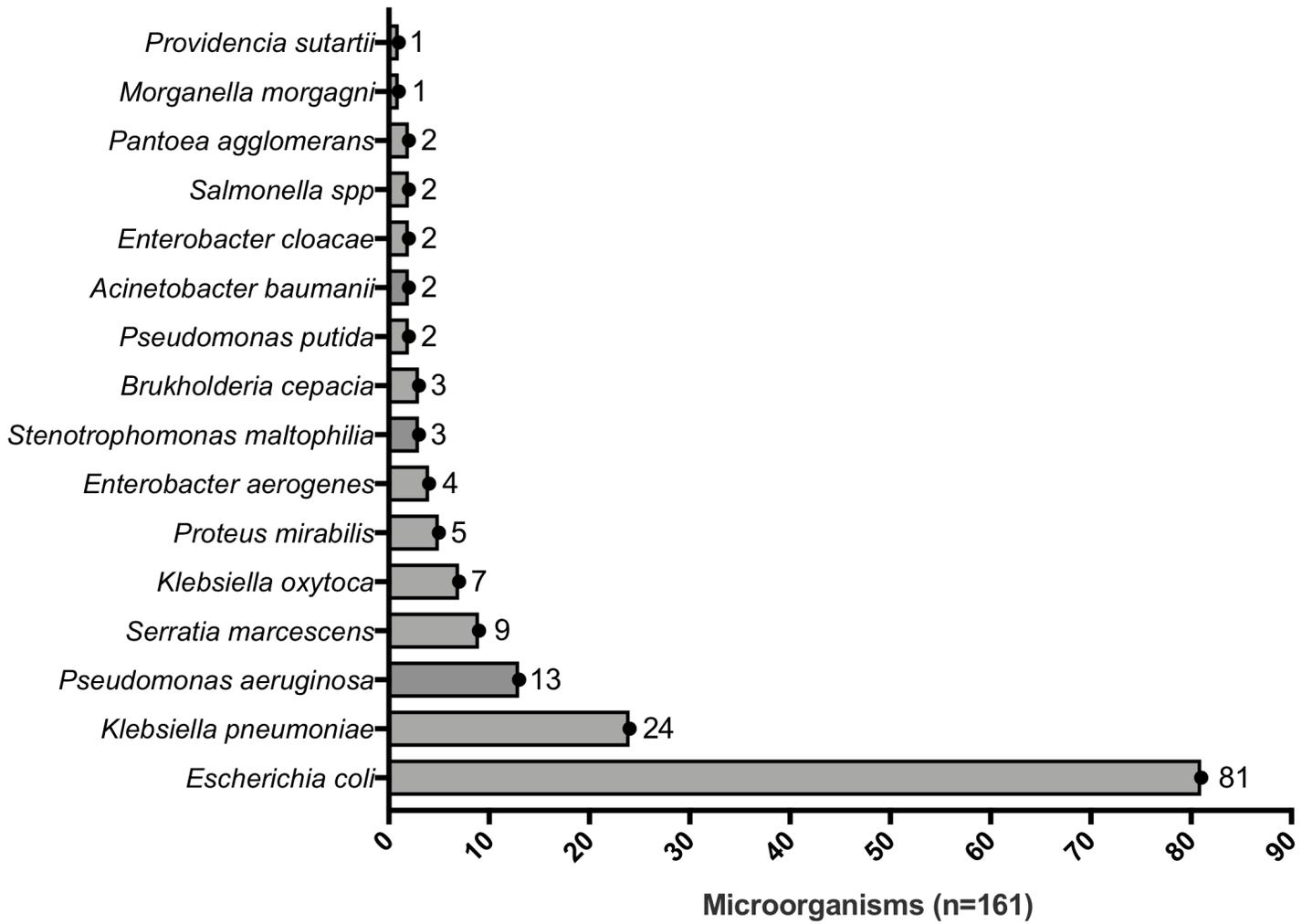


Figure 2

Distribution of microorganisms retrieved from patients with bacteremia included in the study.

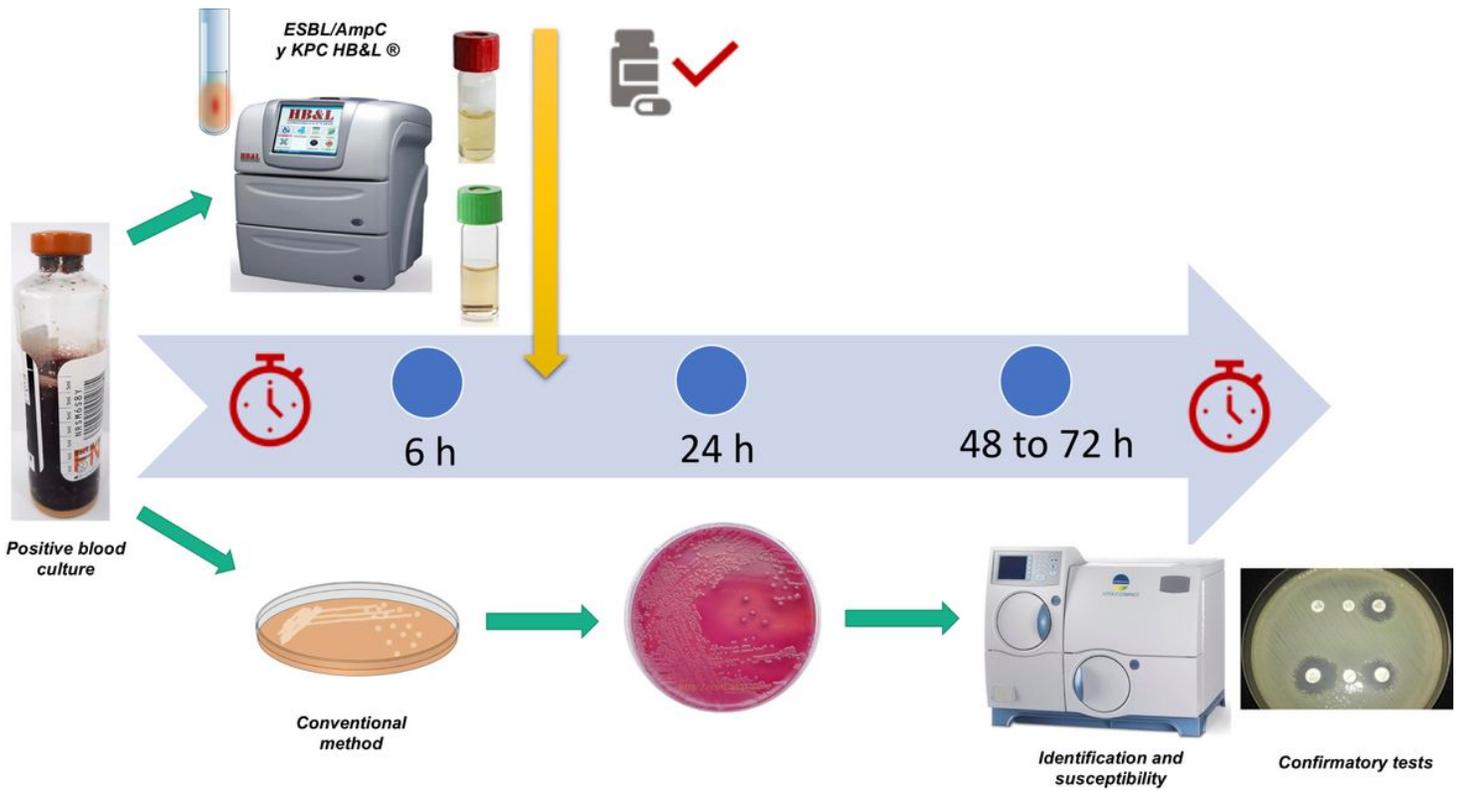


Figure 3

Comparison of identification times for each technique (turnaround time).