Preliminary readings of antimicrobial susceptibility panels: A simple, fast and inexpensive way to detect bacterial resistance and enhance antibiotic treatment of bloodstream infections

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ABSTRACT

Increasing incidence of resistant bacteria needs faster identification (ID) and antibiotic susceptibility testing (AST) in order to improve antimicrobial treatment of severe infections. We propose a preliminary reading of the AST MicroScan® panels coupled with mass spectrometry ID. A total of 157 bacterial clinical isolates were processed for routine ID and AST (in 22 cases, ID and AST were performed directly from positive blood culture bottles). For gram-negatives, data from the initial and final readings were recorded and compared [89.9% category agreement (CA), 6.9% very major errors (VME)]. In adition all the 32 ESBL producers were detected at 5.3-8.6 hours. For Staphylococcus aureus, all the 16 MRSA isolates were detected at 4.5 to 7.5 hours. Thus, we find our preliminary readings approach as a simple, inexpensive and reliable way to detect and identify the most prevalent resistant bacteria in our institution on the same day that ID/AST is performed.

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1. Introduction

Early antimicrobial treatment has proven to have beneficial effects on the clinical evolution of the infected patient and this is even more so in the case of severe infections (Kumar et al., 2006). Thus, there is a consensus that speeding up bacterial identification and antibiogram availability decreases morbidity and mortality, improves antibiotic treatment and reduces the cost of patient care (Barenfanger et al., 1999; Doern et al., 1994; Kerremans et al., 2008; Perez et al., 2014), and this is particularly important in ICU patients (Russotto et al., 2015). Multidrug resistance among bacterial pathogens is an increasing global problem and the wise use of antimicrobial agents is required to prevent emerging resistance (Giammanco et al., 2017; Mammina et al., 2012). Accordingly, hospital antibiograms are commonly used to monitor local trends in antimicrobial resistance and to prepare antibiotic policies to target empiric therapy aimed at initiating prompt, appropriate antimicrobial treatment (Rodríguez-Baño et al., 2012; Rodriguez-Maresca et al., 2014; Wilson et al., 2010).

Moreover, early detection of resistant bacteria quickens the implementation of infection control measures and thus helps prevent further spreading of these bacteria. Unfortunately, traditional microbiological diagnosis, that is, isolation, identification (ID) and antimicrobial

susceptibility testing (AST) of a microorganism entails a delay of 48–72 h until a directed antimicrobial treatment can be started. Currently, AST is performed by manual phenotypic methods based on agar diffusion (using either disks or strips containing an antibiotic gradient – Etest-) or by growth-dependent automated systems, based on broth microdilution testing such as the Becton Dickinson Phoenix, the Beckman Coulter MicroScan WalkAway, or the BioMérieux Vitek 2. In order to shorten this process, different methods to identify microorganisms and perform rapid AST from positive blood cultures have been developed (Opota et al., 2015). Many of these methods are based on new technologies (March-Roselló and Bratos, 2016; Van Belkum and Dunne, 2013) and one is now commercially available (Marschal et al., 2017).

Alternatively, molecular techniques have been used directly in clinical samples, enabling the infectious agent to be identified and at the same time detecting genes encoding for resistance mechanisms of clinical importance (Ginn et al., 2017; Tziolos and Giamarellos-Bourboulis, 2016). Sometimes, these tests are just focused on the detection of specific resistance to an antibiotic group that is to be used as a broad-spectrum empiric treatment (Cortegiani et al., 2016). However, the volume of sample needed, the restricted number of resistance genes determined and the high cost incurred are serious drawbacks to this approach replacing conventional methods. Obviously, not only raw data of susceptibility to antibiotics are needed; in addition, the microbial pathogen must be identified to enable the correct interpretative reading of the antibiogram. This factor, in itself, provides important diagnostic,

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prognostic and epidemiologic information. Since most clinical microbiology laboratories include mass spectrometry as a routine bacterial identification method, we have used it in conjunction with a preliminary reading of MicroScan® panels to obtain crucial resistance results on the same day that a microorganism is isolated or when growth is detected in a blood culture. Although previous studies have combined MALDI-TOF identification with the application of rapid antibiograms (Hrabák et al., 2013; Maelegheer and Nulens, 2017), the scheme we propose only needs the identification step, because the susceptibility data are derived from an initial reading of the routine AST panels. Thus, no additional procedures are required, which is an important consideration in a setting of limited human resources, as is our case. To enhance the value of the results presented, our analysis is focused on pivotal bacterial resistance traits which pose a challenge for empiric antimicrobial treatment at our hospital: namely, extended spectrum β-lactamases (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae* and methicillin resistance in *Staphylococcus aureus*. The provision of same-day information about these two mechanisms of bacterial resistance will make antimicrobial treatment less iatrogenic, more rapid, appropriate and with less impact on the normal flora.

2. Material and methods

2.1. Bacterial isolates

During the study period (January–March 2017) a total of 135 bacterial clinical isolates were processed for routine ID and AST in our laboratory. In a further 22 cases, routine ID and AST were performed directly from positive blood culture bottles (BACTEC™, Becton Dickinson) using routine Gram stain as a guide to select appropriate ID and AST panels (Ginn et al., 2017). Since 5–10% of blood cultures are polymicrobial (Opota et al., 2015), careful microscopic examination is required to avoid unnecessary ID/AST processing. In our series there were no polymicrobial bacteraemias as the Gram stain correctly predicted. In this analysis, we focused on species of *Enterobacteriaceae*, *Staphylococcus* and *Enterococcus*, which represent about 80% of the bacteria recovered in blood cultures (Wilson et al., 2010). To ensure data reliability, some bacterial species with traits of resistance such as *Enterobacteriaceae*-producing ESBL and methicillin-resistant *Staphylococcus aureus* (MRSA) were overrepresented in this sample.

2.2. Routine bacterial identification (ID) and antibiotic susceptibility testing (AST)

The MicroScan WalkAway plus System (Beckman Coulter) was used for routine ID and AST. Interpretation of AST was performed taking into account the breakpoints provided by the Clinical and Laboratory Standards Institute (CLSI), 2016 edition. Combo panels (ID/AST) or only AST panels for Gram-negative and Gram-positive bacteria were used in accordance with the manufacturer's instructions. Briefly, during the morning shift, panels were inoculated with the bacterial isolates selected for ID and AST and then placed in the WalkAway system. For positive blood culture bottles, panels were directly inoculated with 50 μl of broth (González et al., 2009). After 18 h of incubation, ID/AST results were validated by a microbiologist and transmitted to the laboratory information system. A manual MicroScan autoSCAN-4 System was used for back-up and to obtain preliminary readings. In all cases, and according to the EUCAST guidelines, ESBL production were confirmed by double-disk synergy test (The European committee on antimicrobial susceptibility testing, 2017), AmpC production by synergy with cloxacillin (The European committee on antimicrobial susceptibility testing, 2017) and methicillin-resistance by disk diffusion with cefoxitin (The European committee on antimicrobial susceptibility testing, 2017).

2.3. Identification by mass spectrometry

Prior to the initial readings, bacterial isolates were identified by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF, Microflex III MS, Bruker Daltonics GmbH, Leipzig, Germany). Panels inoculated directly from positive blood culture bottles were identified from the incipient growth on the blood agar plates.

2.4. Preliminary readings

During the afternoon shift, routine panels for the same bacterial species were extracted from the WalkAway system and read manually with the MicroScan autoSCAN-4, using the identification provided by MALDI-TOF. Because the Id/AST panels were processed all along the morning shift, preliminary readings were set out at 7:00 p.m. to make sure that most of them showed enough growth to be read and allow sufficient time for reporting critical results before the end of the afternoon shift. They were then returned to the WalkAway system to continue the ID/AST process. Panel loading and reading times were recorded. The next day, initial and final readings were compiled in a database for comparison and analysis (Fig. 1).

2.5. Data analysis

Categorical agreement (CA), minor errors (ME) and very major errors (VME) were calculated for ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, cefuroxime, cefotaxime, ceftazidime, cefepime, ciprofloxacin, levofloxacin, gentamicin, tobramycin and amikacin for Gram-negative bacteria, and for oxacillin and cefoxitin for Gram-positive bacteria.

Due to the nature of this study, errors that imply a change towards an increase in susceptibility from the preliminary to the final readings (R- > S, R- > I or I- > S) were unlikely and, in fact, were nor detected in our study. So, we did not find any degree of false resistance.

3. Results

3.1. Gram-negative bacteria

A total of 97 isolates were tested using MicroScan panels. Preliminary readings were obtained for all except six, in which no growth was detected by the MicroScan autoSCAN-4. As mentioned above, microbial identification was provided by MALDI-TOF. The time elapsed from inoculation of the panels until the initial reading was also noted. The final reading was taken at 18 h in all cases. The following isolates provided initial readings: *Escherichia coli* (45), *Klebsiella pneumoniae* (28) and the *Enterobacteriaceae Citrobacter diversus* (Kumar et al., 2006), *Enterobacter aerogenes* (Kumar et al., 2006), *Enterobacter cloacae* (Giammanco et al., 2017), *Morganella morganii* (Kumar et al., 2006) and *Proteus mirabilis* (Mammina et al., 2012).

In this analysis, we focused on antibiotics considered to be useful both for bloodstream infection treatment and for detecting resistance phenotypes. Data for these antibiotics are shown in Table 1, except for cefotaxime and ceftazidime, which were used with their clavulanate combinations to detect the presence of extended spectrum β-lactamases. Analysis of carbapenems was omitted because none of the isolates tested showed resistance to these agents.

Of the 32 isolates categorized as ESBL producers, 15 were *Escherichia coli* and 17 were *Klebsiella pneumoniae*. Among the *Escherichia coli* and according to the final readings, 11 isolates showed synergy between clavulanic acid and both cefotaxime and ceftazidime, while four showed synergy only with cefotaxime. The preliminary readings correctly categorized the four isolates with synergy only with cefotaxime, and nine of the eleven that presented synergy with both cefotaxime and ceftazidime. The remaining two isolates were categorized as having synergy only with ceftazidime. Overall, the preliminary readings categorized all of them as ESBL producers. In the case of *Klebsiella pneumoniae* and
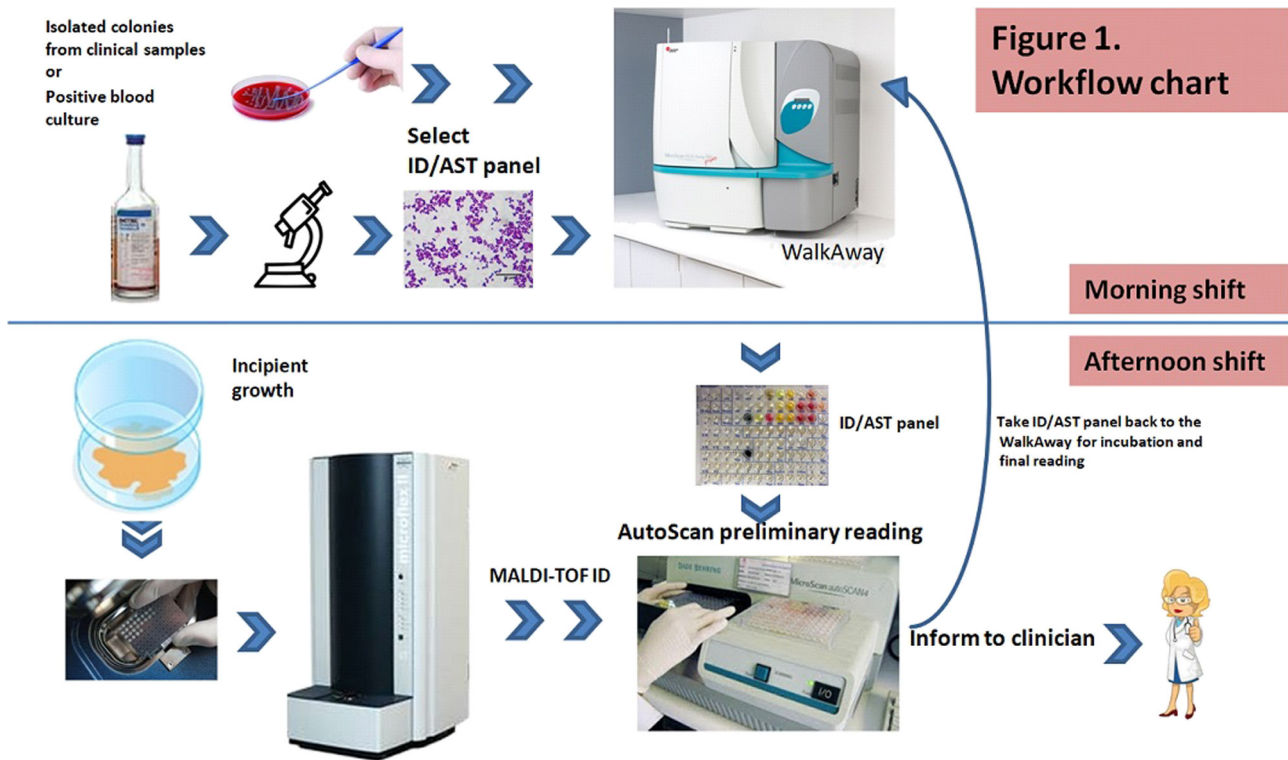
Fig. 1. Workflow chart.

according to the final readings, all 17 isolates showed synergy between clavulanic acid and both cefotaxime and ceftazidime, while the preliminary readings detected synergy between clavulanic acid and both cefotaxime and ceftazidime in 15 isolates and synergy only with cefotaxime in the remaining two isolates. Thus, once again all the *Klebsiella pneumoniae* ESBL producers were correctly categorized by the preliminary readings (Table 2). Three *Escherichia coli* isolates were resistant to third-generation cephalosporins by AmpC cephalosporinase production. For those isolates, the preliminary readings for cefotaxime and ceftazidime were 2, 2 and 16 μg/mL and 8, 4 and >8 μg/mL respectively.

3.2. Gram-positive bacteria

A total of 60 isolates were tested in the same way as the Gram-negative ones (in three of them no growth was detected by the

MicroScan autoSCAN-4). These isolates were *S. aureus* (29), coagulase-negative *Staphylococci* (CoNS) (Maelegheer and Nulens, 2017) and *Enterococcus species* (Wilson et al., 2010). Among the *S. aureus* isolates, 16 were methicillin resistant and 13 were susceptible to methicillin. Preliminary readings detected resistance to both oxacillin and cefoxitin in 15 of the 16 methicillin-resistant isolates. Only one isolate showed discrepant preliminary reading results, being resistant to oxacillin and susceptible to cefoxitin (Table 3). The species of CoNS tested were *Staphylococcus epidermidis* (Rodriguez-Maresca et al., 2014), *Staphylococcus hominis* (Perez et al., 2014), *Staphylococcus haemolyticus* (Barenfanger et al., 1999) and *Staphylococcus cohnii* (Kumar et al., 2006). Of these, 16 were categorized as methicillin-resistant. Three cases were cefoxitin-susceptible in the preliminary readings, although two of these isolates showed resistance to oxacillin (data not shown). Finally, nine *Enterococcus species* were tested, composed of *Enterococcus*

Table 1
Analysis of antibiotic readings.

Antibiotic (number)	Category agreement P. reading/F. reading				Minor errors P. reading/F. reading			VME	%VME
	S/S	R/R	I/I	Total	S/I	I/R	Total (%)	(S/R)	
Ampicillin (91)*	11	70	-	81 (89.0%)	2	6	8 (8.8%)	2[1]	2.56 (2/78)
Amoxicillin-clavulanate (91)*	50	17	4	71 (78.0%)	14	5	19 (20.9%)	1[2]	4.34 (1/23)
Cefuroxime (91)*	45	38	1	84 (92.3%)	2	1	3 (3.3%)	4[3]	9.3 (4/43)
Piperacillin/Tazobactam (91)*	78	2	2	82 (90.1%)	5	2	7 (7.7%)	2[4]	33.3 (2/6)
Cefotaxime (91)*	48	34	-	82 (90.1%)	2	3	5 (5.5%)	4[5]	9.7 (4/41)
Ceftazidime (91)*	56	21	-	77 (84.6%)	5	5	10 (11.0)	4[6]	13.3 (4/30)
Cefepime (74)**	41	17	2	60 (81.1%)	1	12	13 (15.5)	1[7]	3.3 (1/30)
Ciprofloxacin (91)*	38	46	1	85 (93.4%)	3	1	4 (4.4)	2[8]	4.08 (2/49)
Levofloxacin (63)***	28	30	2	60 (95.2%)	1	2	3 (4.7%)	0	0
Gentamicin (91)*	59	27	0	86 (94.5%)	0	2	2 (2.2%)	3[9]	9.37 (3/32)
Tobramycin (91)*	56	14	3	73 (80.2%)	4	11	15 (16.5%)	3[10]	10.7 (3/28)

[1]Two *K. pneumoniae* isolates; [2]*E. cloacae;* [3]*P. mirabilis, K. pneumoniae* (ESBL), *E. cloacae* and *E.coli* (ESBL); [4]*K. pneumoniae* (ESBL) and *E. coli* (ESBL); [5] 2 *E.coli* (ESBL), *M. morganii* and *E. aerogenes*; [6] 2 *K. pneumoniae* (ESBL), *E. coli* (ESBL) and *M. morganii*; [7] *K. pneumoniae* (ESBL); [8] *E. coli* (AmpC) and *E. cloacae.* [9]*E. coli* (ESBL), *K. pneumoniae* (ESBL) and *P. mirabilis;* [10] *E. coli* (ESBL); *P. mirabilis* and *E. coli.*
*Fifteen directly inoculated from positive blood bottles and 76 from isolated colonies; **Fifteen directly inoculated from positive blood bottles and 59 from isolated colonies; ***Seven directly inoculated from positive blood bottles and 56 from isolated colonies.

Table 2
Preliminary readings of extended spectrum β-lactamases.

	Escherichia coli ESBL+		*Klebsiella pneumoniae* ESBL+	
	Final reading (18 h)	Initial reading	Final reading (18 h)	Initial reading
Ceftazidime and cefotaxime synergy	11	9(1)[*]	17	15(2)
Ceftazidime synergy only	-	2(1)	-	-
Cefotaxime synergy only	4	4	-	2
No synergy	27	27(8)	11	11
Total	42 (15 ESBL)		28 (17 ESBL)	

[*] In brackets ID/AST panels directly inoculated from blood bottles.

faecalis (Russotto et al., 2015), all of which were susceptible to ampicillin and vancomycin, together with *Enterococcus faecium* (Doern et al., 1994), both of which were resistant to ampicillin and susceptible to vancomycin and the only discrepancy, an *Enterococcus faecium* isolate that was susceptible to ampicillin (MIC = 8 g/ml) in the preliminary reading (data not shown).

3.3. Preliminary readings

Overall, the mean incubation time for the preliminary readings was 6.80 h (range: 4.45–10.7 h; SD: 1.20). For Gram-negative bacteria, it was 6.84 h (range: 4.93–10.07 h; SD: 1.22) and for Gram-positive bacteria it was 6.75 h (range: 4.45–10.7 h; SD: 1.19). For the antibiotics included in this study, a total of 1582 paired readings, preliminary and final, were recorded. In 1296 cases, the final and preliminary values for the minimum inhibitory concentration (MIC) were coincident (mean reading time 6.89 h) while in 286 cases, the MIC values were different (mean reading time 6.69 h, $P < 0.001$).

3.4. ID/AST panels rejected due to insufficient growth

For nine (5.7%) bacterial isolates, six of which were Gram-positive and three, Gram-negative, no growth was detected when the preliminary reading was performed.

4. Discussion

We describe a simple and inexpensive method to obtain a rapid profile of antibiotic resistance based on a preliminary reading of routine MicroScan® ID/AST panels. For Gram-negative bacilli, data from the initial and final readings of ampicillin, amoxicillin-clavulanate, cefuroxime, piperacillin/tazobactam, ciprofloxacin, levofloxacin, gentamicin, tobramycin and amikacin were recorded and compared. The CA and VME rates obtained were 89.9% and 6.9%, respectively. The CA was on the limit of acceptability, but the VME rate was higher than is admitted for validating an AST procedure (Clark et al., 2009). However, rather than validating a rapid antibiogram, our goal was to accelerate the detection of bacterial resistant isolates prevalent at our institution, thus enhancing antibiotic treatment for severe infections, especially bacteraemia. Thus, for all the 32 isolates that were producers of ESBL (15 *Escherichia coli* and 17 *Klebsiella pneumoniae*), at least one test of

synergy with cefotaxime or ceftazidime was found to be positive at 5.3–8.6 h from the inoculation of the ID/AST panels. In consequence, this crucial information could be delivered on the same day that AST was performed or when the blood culture was found to be positive, as in the four cases in which ID/AST panels were directly inoculated from blood positive bottles. Moreover, the three *Escherichia coli* isolates resistant to third-generation cephalosporins by AmpC production were detected in the preliminary readings.

Although VME rates were high, many had little clinical significance. Thus, when testing ampicillin the two VME found were in two *Klebsiella pneumoniae* isolates, and these should be considered resistant, irrespective of the preliminary readings. The same is true for one of the VME when testing for amoxicillin-clavulanate, because *Enterobacter cloacae* is intrinsically resistant to this antibiotic combination. This is also the case for cefuroxime, because three of the four VME were in *Enterobacter cloacae*, *Klebsiella pneumoniae* ESBL and *Escherichia coli* ESBL isolates, where again, there is no indication for cefuroxime treatment. For piperacillin/tazobactam, the two VME were in two isolates of *Klebsiella pneumoniae* and *Escherichia coli*, both of which are ESBL producers. In fact, the use of this antibiotic combination is controversial in such cases (Ng et al., 2016; Paterson et al., 2004; Rodríguez-Baño et al., 2012).

On the whole, the quinolones performed well. Thus, no VME were found for levofloxacin and only two for ciprofloxacin, although one of these was resistant to levofloxacin in the preliminary reading and so quinolone treatment could have been ruled out. Conversely, aminoglycosides showed VME rates of nearly 10% in gentamicin and tobramycin. In addition, the only isolate resistant to amikacin was susceptible, according to the preliminary reading (date not shown). Nevertheless, 27 and 14 isolates resistant to gentamicin and tobramycin, respectively, were detected in the preliminary readings and five of the seven VME for aminoglycosides showed resistance to gentamicin, tobramycin or both, and so a conservative analysis could have precluded the use of aminoglycosides in these cases if indicated.

For the Gram-positive isolates, combined MICs to oxacillin and cefoxitin detected the 16 MRSA isolates at 4.5–7.5 h from the ID/AST panel inoculation and in two cases the ID/AST panels were directly inoculated from blood-positive bottles. Likewise, oxacillin and cefoxitin jointly detected 15 of 16 CoNS as resistant to methicillin (data not shown), and oxacillin/cefoxitin resistance identified 15 of 16 CoNS as resistant to methicillin. Of the nine isolates of *Enterococcus species* included in this study, only two, both *Enterococcus faecium,* were resistant to ampicillin. One was susceptible to ampicillin in the preliminary reading, although the MIC recorded was high (4 mcg/ml) and therefore resistance could be suspected, but due to the low number of isolates tested no firm conclusions can be drawn.

Obviously, the time of the preliminary reading can influence the accuracy of the MIC readings, but its clinical importance is uncertain and further studies should be conducted to clarify this question. In addition, variability in the reading time is a limitation to the standardization of the procedure. In our opinion, the time interval selected fits best with the laboratory routine and, at the same time, produces useful results. Other limitations, as mentioned previously, are that some resistance mechanisms, such as AmpC cephalosporinase production in Gram-negative isolates or ampicillin resistance in *Enterococcus species*, were largely absent from our sample, and others such as resistance to carbapenems, vancomycin, daptomycin or linezolid were completely absent,

Table 3
Preliminary readings of methicilin resistance.

	Methicillin-resistant *Staphylococcus aureus* (MRSA)		Methicillin-susceptible *Staphylococcus aureus* (MSSA)	
	Final Reading (18 h)	Initial Reading	Final Reading (18 h)	Initial Reading
Oxacillin R/Cefoxitin R	16	15(1)[*]	-	-
Oxacillin R/Cefoxitin S	-	1	-	-
Oxacillin S/Cefoxitin S	-	-	13	13(1)[*]

[*] In brackets ID/AST panels directly inoculated from blood bottles.

so further studies including bacteria with these resistance mechanisms are required. Furthermore, in our institution, *Klebsiella pneumoniae* resistance to third-generation cephalosporins by ESBL production is almost entirely due to CTX-M-15 enzymes (data not published). Accordingly, it would be useful in the future to test bacterial populations with greater enzymatic diversity. Finally, not all bacterial species causing serious infections have been included in this study, especially *Pseudomonas aeruginosa*, which is less prevalent as an agent of bacteraemia and probably requires prolonged incubation times to obtain reliable results.

The relatively low clinical significance of some of the VME detected justifies antibiotic treatment de-escalation, especially if an interpretative reading of the antibiogram times reasonably allows resistant phenotypes to be ruled out (Livermore et al., 2001). Thus, 38 Klebsiella/ Escherichia isolates could have been classed as "non-ESBL producers" and 13 *Staphylococcus aureus* isolates could have been classed, on the same day, as susceptible to methicillin.

In conclusion, our preliminary readings approach is simple, inexpensive and reliable, enabling us to detect and identify the most prevalent resistant bacteria in our institution, and what is most significant, if the ID/AST panels are directly inoculated from the positive blood bottles, this means that directed antibiotic treatment could be started on the same day that a blood culture becomes positive. We consider it would be a powerful tool for the clinicians who are in charge of the patient and that critical decisions about treatment could be made at the reporting moment. The knowledge of the species and resistance phenotype of the microorganism causing infection not only guarantee an early and appropriate antibiotic treatment but also allows antibiotic de-escalation. Moreover, in settings of limited resources, such as developing countries, MicroScan autoSCAN-4 readings could be coupled with chromogenic media or rapid phenotypic identification methods to advance antibiogram results in critical samples. Antibiotic de-escalation is possible if it is based on an interpretative reading of the antibiogram by clinicians or microbiologists acquainted with the local profile of bacterial resistance.

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