Diagnostic Microbiology and Infectious Disease xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Prospective evaluation of rapid antimicrobial susceptibility testing by disk diffusion on Mueller-Hinton rapid-SIR directly on blood cultures

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ARTICLE INFO

Article history:
Received 10 May 2018
Received in revised form 24 July 2018
Accepted 25 July 2018
Available online xxxx

Keywords:
Rapid antimicrobial susceptibility test
Mueller-Hinton rapid-SIR
Disk diffusion
Blood cultures

ABSTRACT

Background: With the worldwide spread of antibiotic resistance, delivering antibiotic susceptibility test (AST) results in a timely manner represents a major challenge. In cases of sepsis, rapid AST may facilitate early optimization of empiric antibiotic therapy. Disc diffusion is a well-standardized AST method, however 16 to 24 h are required to achieve an overall AST profile according to antimicrobial societies.
Methods: In this prospective pilot study, we evaluated the performance of Mueller-Hinton-Rapid-SIR (MHR-SIR) agar after 6–8 h of incubation in comparison with standard MH agar after 16 h of incubation directly on positive blood cultures caused by Enterobacteriaceae and *Staphylococcus aureus* from routine clinical microbiology. A total of 133 positive blood samples including 110 Enterobacteriaceae (83%) and 23 *Staphylococcus aureus* (17%) were tested in parallel by two direct AST methods, each using EUCAST breakpoints. For each combination bacterium and antibiotic, we compared the categorical agreement and the correlation between the diameters obtained by MHR-SIR and by standard MH.
Results: Our results showed 97.7% categorical agreement for Enterobacteriaceae, with 1.4% minor errors, 0.4% major errors and 0.5% very major errors. For *S. aureus*, we observed 97.8% categorical agreement, 1.9% minor errors, 0.3% major errors and no very major errors.
Conclusion: Our results showed excellent categorical agreement and correlations between diameters for MHR-SIR and standard MH methods. MHRSIR can predict the result of overall AST profile within 6–8 h with reliable results. AST is obtained on the same day the blood culture becomes positive, with a very moderate cost.

1. Introduction

During bloodstream infections, antibiotic therapy is started early and empirically until the causative agents and their resistance profile is obtained. Although Gram stain and bacterial identification by Matrix-Assisted Laser Desorption Ionization–Time Of Flight Mass Spectrometry (MALDI-TOF MS) directly on positive blood cultures (Christner et al., 2010) can help physicians to focus empiric antibiotic therapy, antimicrobial susceptibility testing (AST) is crucial for management of bloodstream infections. In the context of global spread of bacterial resistance (Laxminarayan et al., 2013), the susceptibility of causative bacteria to clinically relevant antibiotics is uncertain and can lead to therapeutic failures. Rapid AST results allow optimization of treatment (Frickmann et al., 2014) and yield to reduce mortality, length of hospital stay and additional associated costs (Fraser et al., 2006; Kumar et al., 2009; MacArthur et al., 2004; Perez et al., 2014).

In clinical microbiology laboratories, the disk diffusion method described by Bauer et al. in 1966 (Bauer et al., 1966) is still one of the most frequently used methods for AST. The incubation should range between 16 and 24 h before reading and interpretation as recommended by EUCAST (2017). Faster AST systems are increasingly being developed and utilized in microbiology laboratories, especially automated systems of broth microdilution. New rapid and direct methods are also being developed, using microcalorimetry (Entenza et al., 2014), microfluidic platforms (Mohan et al., 2013), MALDI-TOF technologies (Burrer et al., 2015), DNA amplification (Timbrook et al., 2017), microarrays (Bogaerts et al., 2013), multiplex PCR systems (Saito et al., 2017; Straub et al., 2017) and rapid colorimetric tests (Dortet et al., 2014) to detect resistances in bacteria (Maurer et al., 2017). Although these techniques are rapid and efficient, they remain expensive and require special equipment. Moreover, they have certain limitations, for example PCR-based techniques identify resistance genes but the presence of these

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https://doi.org/10.1016/j.diagmicrobio.2018.07.016
0732-8893/

genes may not always correlate with phenotypic resistance, and broth microdilution systems can fail to correctly detect ESBL production, thereby requiring controls be performed on solid media (Bobenchik et al., 2015).

Recent studies have demonstrated that the incubation time of Mueller-Hinton agar could be reduced (Fröding et al., 2016; Hombach et al., 2017; Le Page et al., 2016; Van den Bijllaardt et al., 2017) to accelerate AST determination from isolated colony. Timely and reliable susceptibility results facilitate antimicrobial stewardship programs to change and focus empiric antibiotic therapy (Pulcini and Mainardi, 2014). An interesting alternative strategy would combine the robustness of classic disk diffusion methods on a Mueller-Hinton Rapid-SIR agar (MHR-SIR), in order to obtain the complete susceptibility profile of the bacteria up to 6–8 h directly from blood samples.

Here, we evaluated prospectively the performance of MHR-SIR agar (i2a, Montpellier, France) coupled with an automatic reading using SIRscan® 2000 Automatic system (i2a, France) after 6–8 h of incubation in comparison with standard MH agar after 16 h of incubation directly on routine positive blood cultures obtained in our laboratory.

2. Material and methods

2.1. Setting

The Groupe Hospitalier Paris-Saint Joseph is a 670-bed tertiary-care teaching hospital, located in Paris, France. The Department of Clinical Microbiology has an integrated clinical microbiology laboratory with microbiologists and infectious disease specialists. An antimicrobial stewardship team manages in routine every severe infection including bacteremia. The laboratory is open on weekdays from 7:00 until 6:30 pm and on Saturdays and Sundays from 8:30 until 5 pm.

2.2. Specimen collection

This prospective pilot study was carried out in a routine clinical microbiology laboratory between August 2016 and April 2017. Blood cultures were collected on BacT/ALERT® bottles and incubated in Virtuo (bioMérieux, La Balme-les-Grottes, France). Once the blood culture was flagged positive, Gram stain was performed, followed by identification by mass spectrometry with MALDI-TOF MS Andromas® (Beckman Coulter, Villepinte, France) directly on blood culture pellets using the A-MUST®, as recommended by the manufacturer's instructions (Mizrahi et al., 2018).

Positive monobacterial blood cultures from routine samples obtained on Monday to Friday morning and containing Enterobacteriaceae and *Staphylococcus aureus* after identification by MALDI-TOF MS were selected for inclusion in this study.

2.3. Disk diffusion testing

AST was performed on positive blood samples by direct inoculation as recommended by the British Society for Antimicrobial Chemotherapy (BSAC) (Wootton, 2013). The same inoculum of blood culture sample was tested in parallel by two methods: standard MH (Bio-Rad Laboratories, Marnes-la-coquette, France) incubated 16 h and MHR-SIR (i2a, France) incubated between 6 and 8 h. Inhibition zones were read from digital images with the SIRscan® 2000 Automatic system (i2a, France) and were interpreted using Comité de l'Antibiogramme de la Société Francaise de Microbiologie–European Committee on Antimicrobial Susceptibility Testing (CASFM-EUCAST) 2015 breakpoints (Bonnet et al., 2015; EUCAST, 2015). The reading of the MHR-SIR was performed after 6 h of incubation. MHR-SIR was re-incubated and read every 20 minutes until the reading was allowed by the SIRscan® with a sufficient visual growth or, failing, after 8 h of incubation. Seventeen antibiotic disks were tested for Enterobacteriaceae (AMX: amoxicillin; TIC: ticarcillin; C: cephalexin; ETP: ertapenem; CTX: cefotaxime; AMC: amoxicillin–clavulanic acid; CAZ: ceftazidime; IPM: imipenem; TZP: piperacillin–tazobactam; FEP: cefepime; SXT: trimethoprim–sulfamethoxazole; GM: gentamicin; AN: amikacin; NA: nalidixic acid; OFX: ofloxacin; CIP: ciprofloxacin; temocillin) and 16 antibiotic disks for *Staphylococcus aureus* (K: kanamycin; TM: tobramycin; GM: gentamicin; C: chloramphenicol; L: linezolid; E: erythromycin; CM: clindamycin; PT: pristinamycin; OFX: ofloxacin; TE: tetracycline; SXT: trimethoprim-sulfamethoxazole; RA: rifampicin; FA: fusidic acid; P: penicillin G; FOX: cefoxitin; MOX: moxalactam). Diameters for pristinamycin and moxalactam and were not interpreted because not recommended by the CASFM-EUCAST.

2.4. Quality control

The laboratory integrates its activity into a quality management system specified by the ISO1589 international standard. As such, the laboratory periodically checks the performance of the blood culture automated system and participates in periodic external quality controls. Similarly, a weekly quality control is carried out to verify the validity of the MHR and Mueller-Hinton agar and the conformity of the results of the inhibition diameters. *Escherichia coli* strain ATCC® 25,922 and *Staphylococcus aureus* strain ATCC® 25,923 were tested directly on MHR and MH agar once a week.

For comparison, the antimicrobial susceptibilities of reference strain isolates from recognized cultures were determined using a standardized method according to CA-SFM-EUCAST (Bonnet et al., 2015; EUCAST, 2015).

2.5. Discrepancies

For each bacterium and each antibiotic, we compared the concordance of interpretation between both methods: Susceptible (S), Intermediate (I) or Resistant (R). The discrepancies were classified as follows: minor error, major error and very major error. Strains interpreted "S" or "I" with a method and respectively "I" or "R" with the other method were classified as minor errors. The major error (ME) represented the strains interpreted "R" with MHR-SIR method and "S" with the standard method. The very major errors (VME) represented the strains interpreted "S" with MHR-SIR method and "R" with the standard method.

In case of discrepancies (ME and VME) between results of inhibition zones with MH and MHR-SIR agar, minimum inhibitory concentration (MIC) was determined retrospectively from −80 °C conserved frozen strains using an isolated colony by ETEST® method (bioMérieux, La Balme-les-Grottes, France), interpreted with CASFM-EUCAST 2015 criteria. These results were verified by broth microdilution method (Sensititre; Thermo Fisher Scientific, Dardilly, France).

3. Results

During this study period, 141 clinical isolates from positive blood culture samples were tested; 133 samples were included, consisting of 110 *Enterobacteriacae* (83%) and 23 *Staphylococcus aureus* (17%). Five positive blood bottles with Enterobacteriaceae and 3 with *S. aureus* were excluded because of an insufficient visual growth or polymicrobial samples. The *Enterobacteriacae* were distributed as follow 68 (62%) *Escherichia coli*, 18 (16%) *Klebsiella pneumoniae*, 10 (9%) *Enterobacter cloacae*, 3 (3%) *Enterobacter aerogenes*, 3 (3%) *Klebsiella oxytoca*, 2 (2%) *Proteus mirabilis*, 2 (2%) *Citrobacter freundii*, 2 (2%) *Serratia marcescens*, 1 (1%) *Citrobacter koseri* and 1 (1%) *Salmonella enterica*.

3.1. Enterobacteriaceae results

Among Enterobacteriaceae studied, different mechanisms of beta-lactam resistance analyzed by phenotypic approach were observed. The population comprised of 49% wild type strains for beta-lactams

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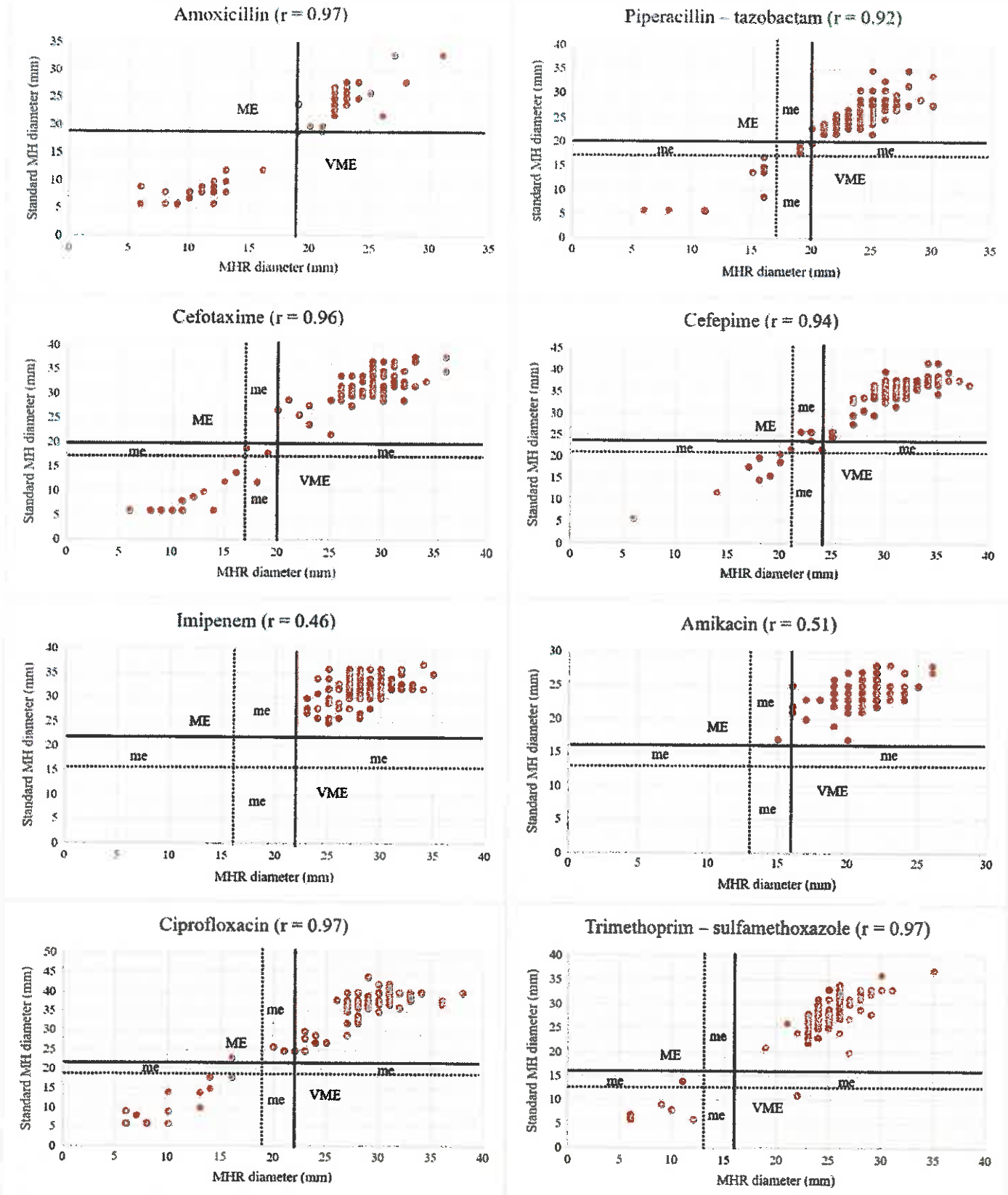

Fig. 1. Correlation between MHR-SIR and MH methods for amoxicillin, piperacillin-tazobactam, cefotaxime, cefepime, imipenem, amikacin, ciprofloxacin and trimethoprim-sulfamethoxazole for Enterobacteriaceae. Full lines represent the R/I breakpoints, dotted lines represent the I/S breakpoints (CASFM-EUCAST 2015). r = correlation coefficient; me = minor error; ME = Major Error; VME = Very Major Error; S = Susceptible; I = Intermediate; R = Resistant.

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(n = 54), 27% acquired penicillinase producers (n = 30), 12% extended-spectrum beta-lactamase (ESBL) producers (n = 13) and 12% other mechanisms (e.g. AmpC, inhibitor-resistant TEM [IRT]) (n = 13). No carbapenemase production was observed among the strains tested.

With regards diameter correlation between both methods (Fig. 1), correlation coefficients r > 0.90 were observed for amoxicillin, piperacillin-tazobactam, cefotaxime, cefepime, ciprofloxacin and trimethoprim-sulfamethoxazole for Enterobacteriaceae, with the same distribution of the diameters (Supplementary data 1). Correlation coefficients for imipenem and amikacin were less satisfactory (r = 0.46 and r = 0.51 respectively). However, all strains included in this study were susceptible to these two antibiotics and neither ME nor VME were observed. Only one minor error was observed for amikacin.

Over the 1847 tested combinations, there were 1804 (97.7%) concordances, 25 (1.4%) minor errors, 8 (0.4%) ME and 10 (0.5%) VME (Supplementary data 1). Antibiotics as amoxicillin, ticarcillin, imipenem and gentamicin showed 100% concordance. More than 98% categorical agreement was observed for cefotaxime, trimethoprim-sulfamethoxazole, amikacin and nalidixic acid.

The minor errors were observed mainly with ofloxacin (5.4%) and cefepime (4.6%) (Fig. 2B).

Four ME were observed for temocillin (4.4%) and 1 ME (1%) was observed respectively for amoxicillin–clavulanate, ceftazidime, ofloxacin and ciprofloxacin.

Cephalexin was the cause of most VME (5 VME, 4.5%) corresponding to 50% of all VME. Of the 5 VME for cephalexin, 4 involved group 3 Enterobacteriaceae (*Enterobacter sp*) (Fig. 2A). Three VME were observed for amoxicillin-clavulanic acid (2.8%). These VME concerned diameters close to the category breakpoint diameter.

Focusing on the 26 beta-lactam resistant strains composed of ESBL, IRT, AmpC and derepressed AmpC, corresponding to 440 combinations of antibiotic-bacteria, we observed 414 (94.1%) categorical agreement, 20 (4.5%) minor errors, 3 (0.7%) ME and 3 (0.7%) VME.

We subsequently performed MIC on the strains which produced ME and VME (Table 1). MICs were not performed for cefalexin because the VME observed related to AmpC cephalosporinases, natural resistances, not detected by MHR-SIR method. Of the 14 errors analyzed, MHR-SIR interpretations matched with MIC results for amoxicillin–clavulanic

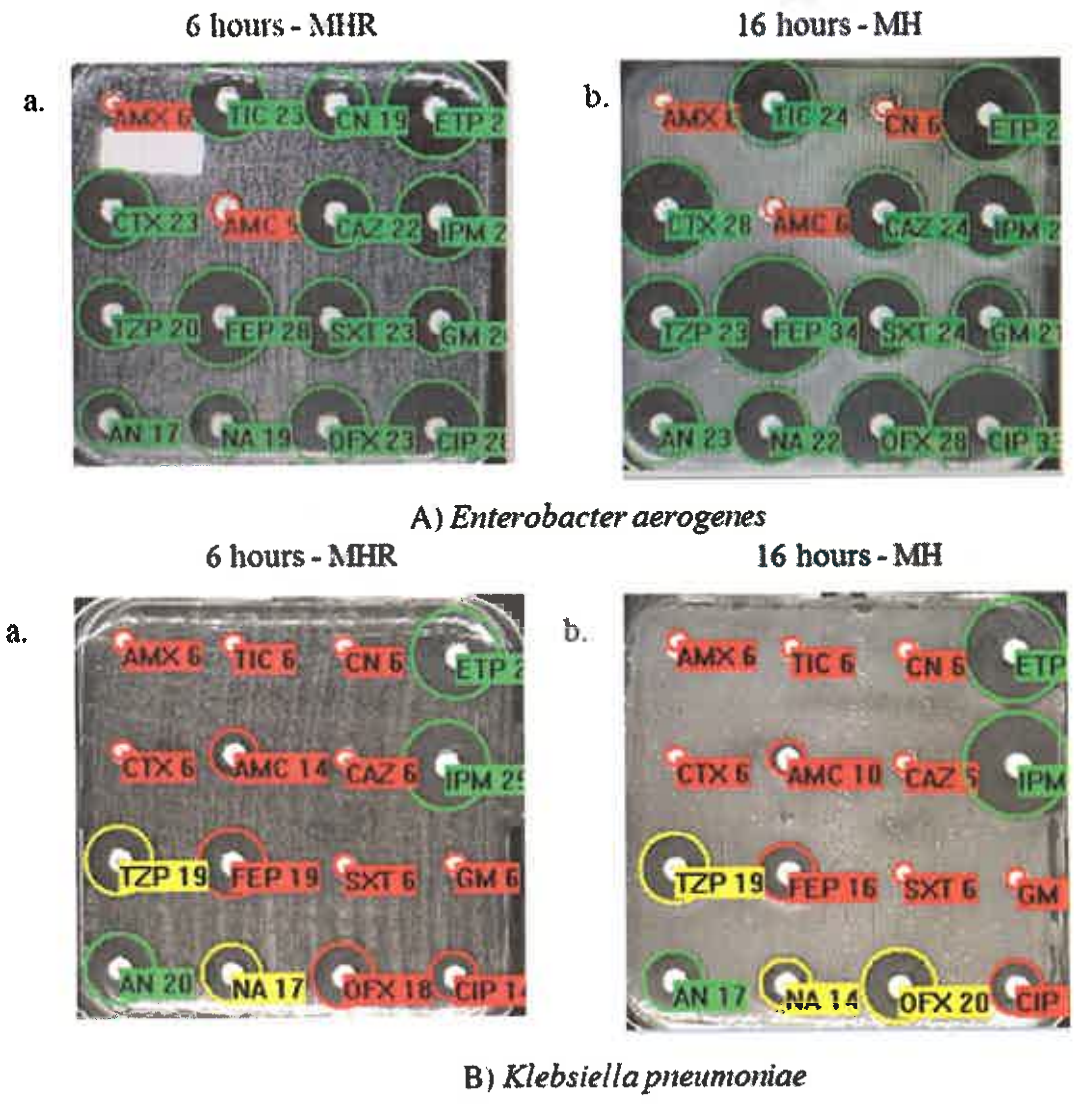

Fig. 2. SIRscan® 2000 Automatic photos of a wild type *Enterobacter aerogenes* strain (A) and ESBL *Klebsiella pneumoniae* (B), on MHR-SIR (a) and standard MH (b) after 6 and 16 h incubation, respectively. A. The pictures illustrate the AST profile of a wild type *E. aerogenes* strain. Resistance to cephalexin is not detected after 6 h of incubation on MHR-SIR media (a) but is detected on standard MH after 16 h of incubation (b). B. The pictures illustrate the AST profile of a *K. pneumoniae* producing ESBL. AST results are comparable between the two methods MHR-SIR (a.) and MH (b.), except a minor error observed with ofloxacin. MH = Mueller-Hinton, MHR = Mueller-Hinton Rapid-SIR; AMX = amoxicillin; TIC = ticarcillin; C = cephalexin; ETP = ertapenem; CTX = cefotaxime; AMC = amoxicillin–clavulanic acid; CAZ = ceftazidime; IPM = imipenem; TZP = piperacillin–tazobactam; FEP = cefepime; SXT = trimethoprim–sulfamethoxazole; GM = gentamicin; AN = amikacin; NA = nalidixic acid; OFX = ofloxacin; CIP = ciprofloxacin.

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t1.1 **Table 1**
t1.2 Determination of MIC for strains showing major and very major differences, except cefalexin. For cefalexin, the very major errors observed concerned natural resistances, not detected by
t1.3 MHR-SIR method. MIC was interpreted according to CASFM-EUCAST 2015 guidelines.

t1.4	Bacteria	Antibiotic	MHR-SIR result	MH result	Difference	MIC measure Micro-dilution broth (mg/L)	MIC cut off* (mg/L) S ≤ R>	Interpretation MIC result	Correct method
t1.5	*K. pneumoniae*	Temocillin	R	S	ME	<4	8	S	MH
t1.6	*E. aerogenes*	Temocillin	R	S	ME	<4	8	S	MH
t1.7	*E. coli*	Temocillin	S	R	VME	16	8	R	MH
t1.8	*E. coli*	Temocillin	R	S	ME	8	8	S	MH
t1.9	*E. coli*	Temocillin	S	R	ME	8	8	S	MHR-SIR
t1.10	*E. coli*	Amoxicillin-clavulanate	S	R	VME	8	8	S	MHR-SIR
t1.11	*E. coli*	Amoxicillin-clavulanate	S	R	VME	16	8	R	MH
t1.12	*E. coli*	Amoxicillin-clavulanate	S	R	VME	16	8	R	MH
t1.13	*K. pneumoniae*	Ceftazidime	R	S	ME	1	1–4	S	MH
t1.14	*E. coli*	Trimethoprim-Sulfamethoxazole	S	R	VME	>8	2–4	R	MH
t1.15	*E. coli*	Ofloxacin	R	S	ME	1	0,5–1	I	MH/MHR-SIR
t1.16	*E. coli*	Ciprofloxacin	R	S	ME	1	0,5–1	I	MH/MHR-SIR
t1.17	*S. aureus*	Clindamycin	S	R	VME	1	0.25–0.5	R	MH

t1.18 S = Susceptible; I = Intermediate; R = Resistant.
t1.19 me = minor error; ME = Major Error; VME = Very Major Error.
t1.20 MH = Mueller-Hinton; MHR = Mueller-Hinton Rapid-SIR.
t1.21 MIC = Minimum Inhibitory Concentration.
t1.22 * CASFM-EUCAST 2015.

acid despite the VME observed. For the ME and VME observed with temocillin, MIC results matched with MH interpretations. For the other antibiotics which resulted in ME and VME, MIC matched alternatively with MH or MHR-SIR results.

3.2. *Staphylococcus aureus* results

Among the 23 strains of *S. aureus* tested (19 SMSA and 4 MRSA), the results showed 315 (97.8%) categorical agreements, 6 (1.9%) minor errors, 1 (0.3%) VME and no ME (Supplementary data 2). The correlation coefficients were high ($r > 0.95$) for penicillin G, gentamicin, erythromycin and lower for clindamycin ($r = 0.74$) (Fig. 3). Among the minor errors, 5 out of 6 were due to cefoxitin, and the other minor error due to erythromycin. The only VME was observed for clindamycin. Antibiotics tested showed 100% of concordance for penicillin G, kanamycin, tobramycin, gentamicin, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, linezolid, ofloxacin, rifampicin and fusidic acid (Supplementary data 2).

Regarding the minor errors for cefoxitin (21.7%), we interpreted the diameters according to CA-SFM-EUCAST 2015 breakpoints (EUCAST, 2015). An "uncertain zone" was defined between 22 and 25 mm, where resistance to methicillin must be determined by identifying presence of the *mecA* gene or PBP 2a production. Analyzing our MHR-SIR results, the 5 minor errors described concerned strains which were classified as "uncertain" with MHR-SIR results and "susceptible" with MH (SMSA). According to CA-SFM-EUCAST 2015 guidelines, methicillin resistance of *S. aureus* was not always predictable with MHR-SIR method (Fig. 4).

4. Discussion

Given that microbial acquired resistance is unpredictable, rapid AST result is a real challenge for microbiologists and infectious diseases specialists. The objective of our study was to evaluate in a routine use, a rapid disc diffusion method using MHR-SIR. Blood samples were cultured on MHR-SIR for direct AST and read after 6–8 h of incubation, comparing to the standard method MH incubated for 16 h. When this study was undertaken, to the best of our knowledge, only BSAC suggested a protocol to perform AST directly from positive blood cultures (Wootton, 2013). Thus, we followed these British recommendations in the absence of French guidelines, as has been already done with similar studies (Coorevits et al., 2015). Since February 2018, CA-SFM proposed guidelines to perform directly AST from positive blood cultures (Bonnet, 2018). Q4 Q

Our results show an excellent correlation between diameters obtained with standard MH and MHR-SIR. According to the FDA and Jorgensen (Jorgensen and Ferraro, 2009), (Jorgensen, 1993), the concordance is acceptable. Indeed, the results show 97.7% categorical agreement, 1.4% minor errors, 0.4% ME and 0.5% VME for Enterobacteriaceae. Regarding Enterobacteriaceae, cefalexin yielded 4.5% VME. This antibiotic does not well correlate early reading on MHR-SIR and is essentially used for cephalosporinase detection. This has no impact in clinical practice. Moreover, in our study amoxicillin–clavulanic acid shows 2.8% VME in the absence of a defined intermediate zone. Interestingly, the MIC performed on these strains showed that MHR-SIR results were concordant with MIC, suggesting that standard MH read at 16 h of incubation overestimated resistance to amoxicillin–clavulanate. For *S. aureus*, we observed 97.8% of categorical agreement, 1.9% of minor errors, 0.3% of VME and no ME. We observed 100% of concordance for the majority of antibiotics tested (penicillin G, kanamycin, tobramycin, gentamicin, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, linezolid, ofloxacin, rifampicin and fusidic acid). For cefoxitin, 21.7% of minor errors were described with diameter interpretation using CA-SFM/EUCAST 2015 (Bonnet et al., 2015). The minor errors observed concerned susceptible strains which are categorized as "uncertain zone" in MHR-SIR. According to these recommendations, methicillin resistance of *S. aureus* is not always predictable with MHR-SIR. However, reinterpretation of these diameters using CA-SFM/EUCAST 2017 (Bonnet et al., 2017; EUCAST, 2017), with the specific breakpoint of 22 mm, results in 100% categorical agreement for cefoxitin. Q6 The very good agreement between diameters interpreted at 6–8 h with MHR-SIR and 16 h with standard MH allows us to rely on MHR-SIR for early interpretation of bacterial susceptibilities and resistances. These conclusions had already been mentioned by Bauer et al. (1966) and recently by Fröding et al. (2016) and Van den Bijllaardt et al. (2017) discussed that early diameters could appear smaller than diameters after 16 h of incubation. We also noticed this observation

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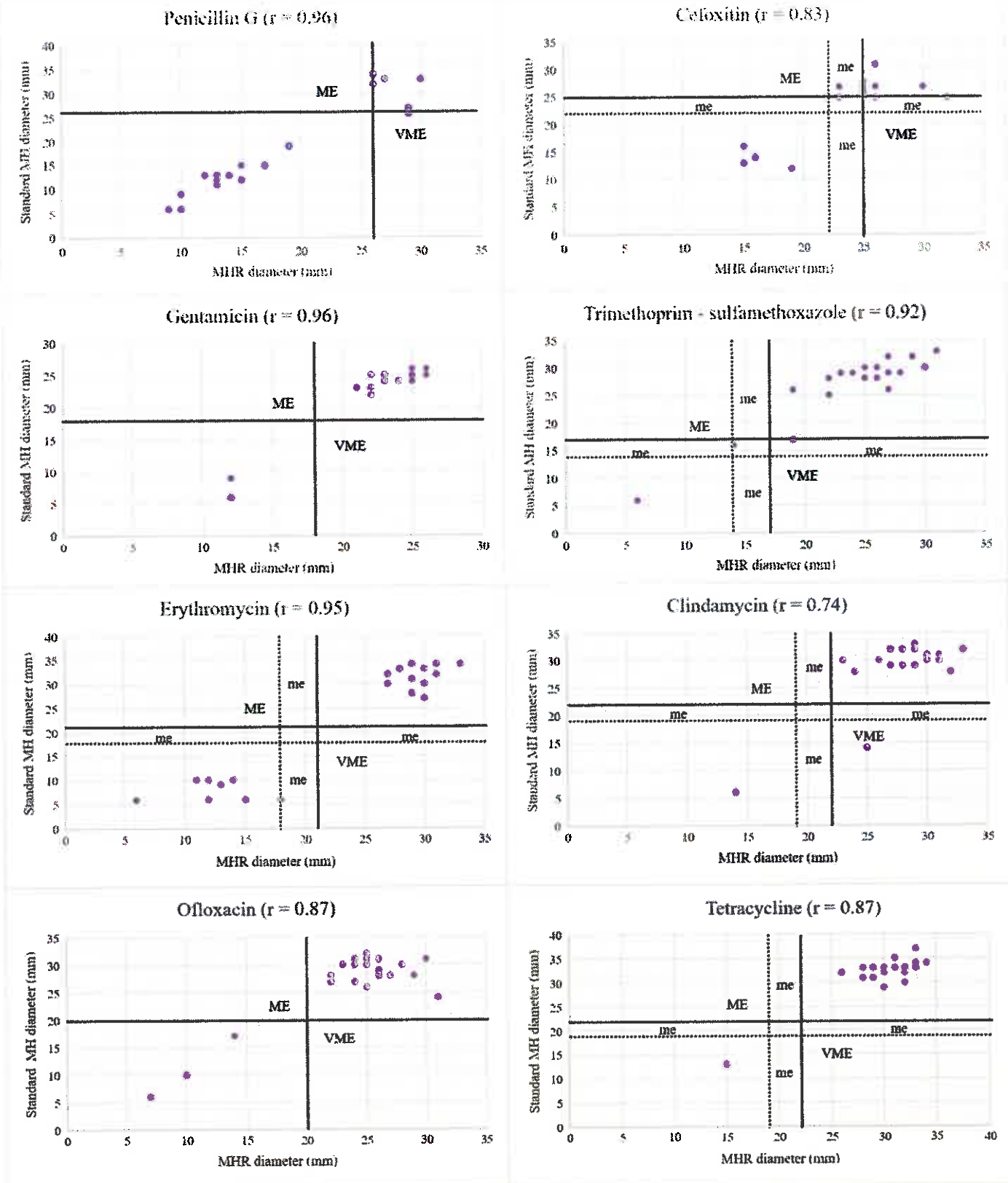

Fig. 3. Correlation between MHR-SIR and MH methods for penicillin G, cefoxitin, gentamicin, trimethoprim-sulfamethoxazole, erythromycin, clindamycin, ofloxacin and tetracycline for *S. aureus*. Full lines represent the R/I breakpoints, dotted lines represent the I/S breakpoints. r = correlation coefficient; me = minor error; ME = major error; VME = very major error. S = Susceptible; I = Intermediate; R = Resistant.

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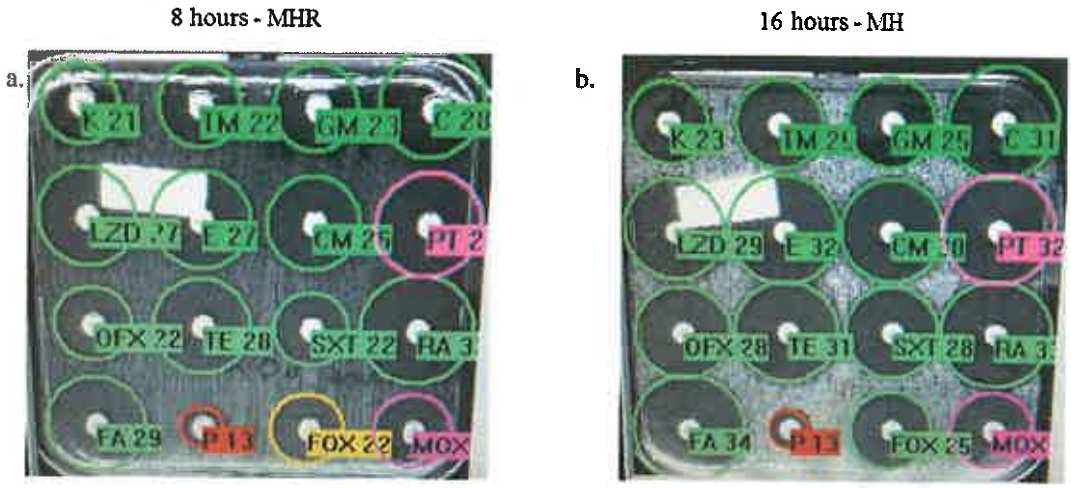

Fig. 4. SIRscan® 2000 Automatic photos of a penicillinase producing *Staphylococcus aureus* strain on MHR-SIR (a) and standard MH (b) after 8 and 16 h, respectively. MH = Mueller-Hinton; MHR = Mueller-Hinton Rapid-SIR; K = kanamycin; TM = tobramycin; GM = gentamicin; C = chloramphenicol; L = linezolid; E = erythromycin; CM = clindamycin; PT = pristinamycin; OFX = ofloxacin; TE = tetracycline; SXT = trimethoprim-sulfamethoxazole; RA = rifampicin; FA = fusidic acid, P = penicillin G; FOX = cefoxitin; MOX = moxalactam.

during our study. This could be due to the fact that microcolonies are not included in the diameter reading at 16 h, compared to 6–8 h. Furthermore, van den Bijllaardt et al. noted both increases and decreases were observed, mainly for susceptible and resistant strains, respectively.

Our study has certain limitations. A limitation concerns the mechanisms of beta-lactam resistance. They were characterized by phenotypic approach and were not verified by a molecular analysis. Furthermore, this work was performed in a situation of routine: we compared two techniques on solid media directly from positive blood cultures. We did not compare our results to a standard obtained from a colony, which is a shortcoming of our study. For bacterial growth, bacteria like *Pseudomonas aeruginosa* do not grow fast enough to allow reading of diameters after 8 h of incubation. That is a drawback of this solid medium. In case of mixed bacteremia combining *Pseudomonas aeruginosa* and other bacteria, an infrequent event that we did not encounter during this study, it would be careful not to validate MHR-SIR results. Moreover, *S. aureus* cultures were occasionally unreadable at 8 h due to weak growth, representing 7.7% of all the strains tested (2 samples of the 26 positive blood cultures). It is important to emphasize that the MHR-SIR results of this study should be interpreted coupled with SIRscan® 2000 Automatic system. We did not evaluate here the reading without the SIRscan instrument. In addition, it could be interesting to know the number of positives that could be read at both 6 and 8 h. However, this information is not available due to the SIRscan setting in our study. The SIRscan system takes a picture of the agar from 6 h and, if unreadable, takes a new photo every 20 minutes, until 8 h. We only know that the MHR-SIR was read between 6 and 8 h. Nevertheless, from our experience, MHR-SIR for Enterobacteriaceae was in majority readable at 6 h whereas 8 h of incubation was necessary for *S. aureus*.

Considering antibiotics with a high rate of errors, ME observed with cephalexin against Enterobacteriaceae had no clinical consequences. Indeed, MHR-SIR could not detect natural cephalosporinases with an early reading using the cephalexin disk, unlike other cephalosporin disks. Amoxicillin-clavulanic acid has a high rate of VME (2.8%). However, as there is no intermediate zone defined (Jorgensen, 1993), a variation of 1 mm around the breakpoint diameter can lead to a ME or VME. Thus, caution must be observed before drawing conclusions and further evaluation are needed. Lastly, we underline that no carbapenemase producing strains were included in our study. Nevertheless, we tested MHR-SIR on NDM and OXA-48 producer strains from colonies with very good preliminary results at 6 and 8 h of incubation (unpublished data).

Despite some discrepancies, there are several benefits on the MHR-SIR method. AST by MHR-SIR is a rapid and reliable method for Enterobacteriaceae and *S. aureus* performed directly from positive blood cultures. The overall susceptibility profile is obtained on the same day the blood culture flags positive at a cost of less than US $6 for 16 antibiotics tested, which are chosen by the microbiologist.

Physicians can focus antibiotic therapy early, providing the laboratory is able to facilitate real-time reporting for MHR-SIR results. The ideal situation we propose would be to use the MHR-SIR in collaboration with an antimicrobial stewardship team.

5. Conclusion

Our results showed an excellent categorical agreement and correlations between diameters for MHR-SIR and standard MH methods. MHR-SIR can predict the result of overall AST profile in 6–8 h with reliable results. AST is obtained on the same day the blood culture becomes positive, with a moderate cost. Rapid AST by disc diffusion on MHR-SIR (i2a, France) could help antimicrobial stewardship teams in management of bloodstream infection by allowing earlier focusing of appropriate antimicrobial therapy.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diagmicrobio.2018.07.016.

Acknowledgments

We thank all laboratory technicians for their active participation in this study.

We gratefully acknowledge Erika Costanzo, Christian Curel and Amanda T Harrington for helpful discussions and Charlotte Duncan who helped us to correct the language.

Funding

None to declare.

Transparency declaration

None to declare.

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