Rapid susceptibility testing by disk diffusion on MHR (i2a,**France**) agar in bloodstream infections

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Introduction

During episodes of bacteraemia, antibiotic treatment is started early and empirically. Direct exam of positive blood culture and identification of the pathogen can help clinicians to switch this probabilist antibiotherapy but the antimicrobial susceptibility testing (AST) represents a major issue in bloodstream infections. The susceptibility to clinically relevant antibiotics is unpredictable and can lead to therapeutic failures. Rapid results of AST can optimize the treatment and reduce mortality thank to earlier antibiotic adaptation [1] [2].

Material & Methods

This prospective pilot study was carried out in a clinical microbiology laboratory routinely between August 2016 and March 2017. AST was performed on positive blood samples by direct inoculation on Mueller-Hinton (MH) agar according to the indications of the British Society for Antimicrobial Chemotherapy (BSAC) [3]. Hundred and twenty one blood samples containing 99 *Enterobacteriacae* (82%) and 22 *Staphylococcus aureus* (18%) were tested in parallel by two methods : standard MH (Biorad, France, Marnes la Coquette), incubated 16 hours and MHR (Rapid MH, i2a, France, Montpellier), incubated between 6 and 8 hours. Two different panels of antibiotic discs were tested : 32 discs on Gram negative strains and 16 discs on *S. aureus*. Inhibition zones were read from digital images with the SIRscan 2000 automatic system (i2a, France) and were interpreted using EUCAST 2016 breakpoints. For each bacteria and each antibiotic, we compared the concordance of interpretation between the two methods: Susceptible (S), Intermediate (I) or Resistant (R).
The discrepancies were classified as follow :
minor error (me) : strain interpreted "S" or "I" with a method and respectively "I" or "R" with the other method
major error (ME) : strain interpreted "R" with MHR method and "S" with the standard method
very major error (VME) : strain interpreted "S" with MHR method and "R" with the standard method

Results

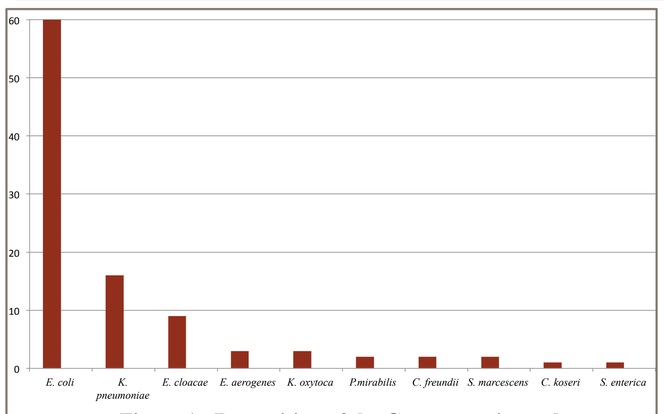


Figure 1 : Repartition of the Gram negative rods strains

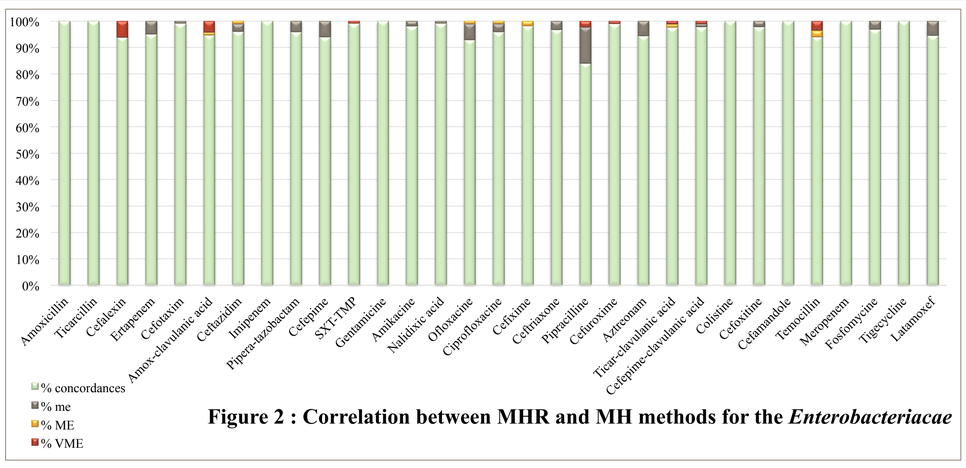
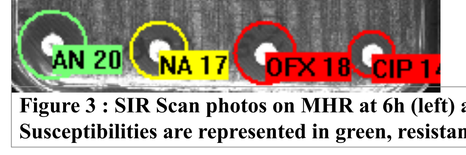


Figure 2 : Correlation between MHR and MH methods for the *Enterobacteriacae*



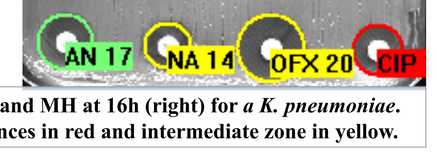


Figure 3 : SIR Scan photos on MHR at 6h (left) and MH at 16h (right) for *a K. pneumoniae*. Susceptibilities are represented in green, resistances in red and intermediate zone in yellow.

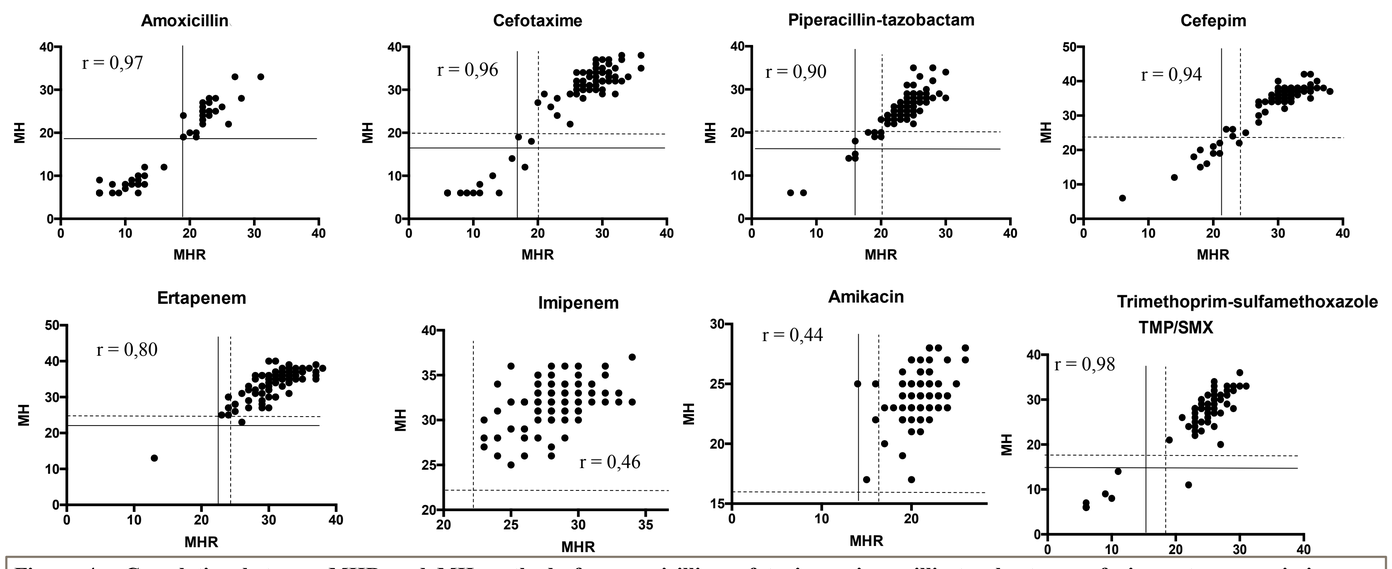


Figure 4 : Correlation between MHR and MH methods for amoxicillin, cefotaxime, piperacillin-tazobactam, cefepim, ertapenem, imipenem, amikacin, trimethoprim-sulfamethoxazole.
Full lines represent the R/I breakpoints, dottted lines represent the I/S breakpoints.

Tests were performed on 121 blood samples from patients with bloodstream infections due to an *Enterobacteriacae* or *S. aureus*.
In total, 3206 antibiotic-microrganims combination were tested.

Among the 99 *Enterobacteriacae*, 60 isolates (61%) were identified as *E. coli,* 16 isolates (16%) as *K. pneumoniae* and 9 isolates (9%) as *E. cloacae* (Figure1). 2861 tests were performed. The results showed 2772 (96.9%) concordances, 62 (2.2%) me, 8 (0.3%) ME and 19 (0.7%) VME. The minor errors were observed for piperacillin (19% of the minor errors). The very major errors were observed for the amoxicillin-clavulanic acid, temocillin and on group 3 *Enterobacteriacae* for cefalexin.

Concerning the group containing 22 *S. aureus* strains, we found 345 (98,0%) concordances of the 352 tests performed and we observed 6 (1,7%) me, no ME and 1 (0,3%) VME. The minor errors were mainly observed for cefoxitin (83% of the minor errors).

Discussion & Conclusion

Good categorical agreement between MHR and standard MH methods was observed (Figure 2). Bacterial growth showed an easy reading between 6 and 8 hours on MHR (Figure 3). Although discordances were reported, MHR can predict the result of the antibiotic susceptibility test allowing earlier modification of the empirical treatment. Nevertheless, cefalexin was not reliable on group 3 *Enterobacteriacae*, which had no clinical consequences. Moreover, for temocillin and amoxicillin-clavulanic acid, where no intermediate zone is defined, it can be difficult to conclude when the diameter is near to the limit zone. The high level of minor errors observed with the piperacillin disc is probably explained by the poor sensibility of this antibiotic to detect penicillinase phenotype. Good correlation coefficients were obtained for diameters between both MH and MHR methods (Figure 4), except for imipenem and amikacin, even though neither ME nor VME were observed, because all of our strains were susceptible to these two antibiotics. Concerning our assays on *S. aureus*, methicillin resistance was not systematically predictable with cefoxitin diameter, due to an uncertain zone. Using the new rules of EUCAST 2017 (breakpoint at 22 mm), 100% correlation for cefoxitin was observed.
This rapid susceptibility testing by disc diffusion on MHR (i2a, France) agar could improve antibiotic stewardship in bloodstream infection by allowing earlier appropriate initial antimicrobial treatment.

References

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2.Van den Bijllardt 2017 « Shortening the incubation time for antimicrobial susceptibility testing by disk diffusion for Enterobacteriaceae: how short can it be and are the results accurate ? » J. Antimicrob. Agents 2017 May;49(5):631-637. doi:10,1016/j.ijantimicag.2016,12,019. Epub 2017 Mar 3
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