

Direct determination of antimicrobial susceptibility of Gram Negative Bacilli from respiratory samples on chromogenic agar plates with gradient antibiotic strips

C. d'Humières¹, M. Belhiba¹, F. Brocco², A. Andremont¹, N. Grall¹

¹ INSERM, IAME, UMR 1137, F-75018 Paris, France; Univ Paris Diderot, Sorbonne Paris Cité, F-75018 Paris, France; AP-HP, Hôpital Bichat, Service de Bactériologie, F-75018 Paris, France
² Liofilchem®, Roseto degli Abruzzi, Italy.

Introduction and Purpose

- In pneumonia, especially VAP (ventilator-associated pneumonia), shortening the time between empirical broad-spectrum antibiotic therapy and administration of appropriate antimicrobial agents improves the prognosis¹ and contributes to the control of multiresistant pathogens emergence². Several studies have shown the effectiveness of using E-test strips applied directly to clinical specimens like respiratory samples (tracheal aspirates/ bronchoalveolar lavage) in providing rapid determination of bacterial susceptibility to antibiotics³⁻⁶.
- In this study, we compared the results of direct determination of MICs of Gram Negative Bacilli (GNB) from respiratory samples using direct plating on chromogenic agar and gradient antibiotic strips "Liofilchem® MIC Test Strip" with the standard method.

Methods

- Hundred respiratory samples (52 tracheal aspirates, 17 expectorations, 23 bronchoalveolar lavage (BAL) and 8 mini-BAL) from different wards of Bichat Claude Bernard Hospital with GNB upon direct smear examination were each spread on 3 Muller-Hinton chromogenic agar plates (Liofilchem®, Roseto degli Abruzzi, Italy) and MICs to amoxicillin-clavulanic acid, ceftazidime, cefotaxime, imipenem, amikacin, ciprofloxacin were determined concomitantly by placing gradient strips of these antibiotics (Liofilchem® MIC Test Strip) on the plates (2 strips/plate).
- Bacteria present in the sample were species identified on the colour and phenotypic aspects of the clones (as recommended by the manufacturer) and their MICs were determined on the size of the inhibition zone after 18hrs incubation at 37°C. Results were compared with those obtained after 48hrs using a standard disk diffusion method after quantitative culture on respiratory samples (reference method).
- Antibiotic susceptibilities were interpreted as recommended by EUCAST. Each antibiotic/bacteria combination was categorized as :
 - total agreement when both methods gave the same result (Susceptible (S), Intermediate (I) or Resistant (R)),
 - major error when the test method resulted in S while the reference one indicated I or R,
 - minor error when the test method resulted I or R while the reference one indicated S.

Results

- Eight samples (8%) which had no GNB, more than 2 GNB in culture or a mismatch in the number of GNB between reference and test methods were excluded for further analysis. 78/92 (84.8%) samples were monomicrobial (30 enterobacteria, 37 *Pseudomonas aeruginosa*, 7 *Stenotrophomonas maltophilia* and 4 *Acinetobacter* spp.) and 14/92 (15.2%) were polymicrobial (2 different GNB).
- Antimicrobial susceptibility determined by Liofilchem® MIC Test Strip and standard methods were compared in 374 antibiotic/bacteria combinations (Table 1).
 - Total agreement between the two methods was observed for 96.5% (361/374) of the antibiotic/bacteria combinations studied.
 - Three major errors occurred (3/461, 0.7%), corresponding to *P. aeruginosa*/ceftazidim, *S. marcescens*/ ciprofloxacin and *K. pneumoniae*/amoxicillin-clavulanic acid combinations.
 - Eleven minor errors were observed (11/461, 2.4%) and affected combinations of *P. aeruginosa* with ceftazidime (4), imipenem (2), ciprofloxacin(1) or amikacin(1), *C. freundii* with cefotaxime (1) or ceftazidime (1) and *S. marcescens* with cefotaxime (1).

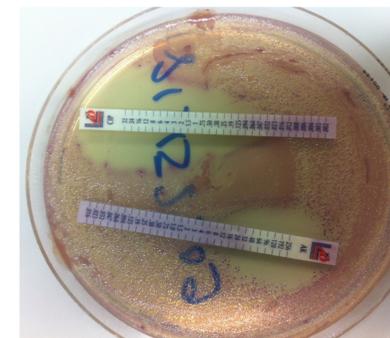
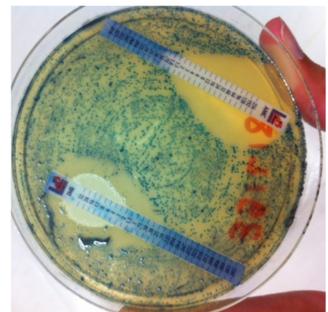


Table 1 : Performance of gradient antibiotic strips (Liofilchem ® MIC test strip) on GNB antimicrobial

antibiotic	antibiotic/bacteria combinations tested	Total agreement (%)	Minor error(%)	Major error (%)
Amoxicillin/Clavulanic-acid	47	46 (97,9)	0	1 (2,1)
Cefotaxim	45	43 (95,6)	2 (4,4)	0
Ceftazidim	94	88 (93,6)	5 (5,3)	1 (1,1)
Imipenem	92	90 (97,8)	2 (2,2)	0
Ciprofloxacin	91	89 (97,8)	1 (1,1)	1 (1,1)
Amikacin	92	91 (98,9)	1 (1,1)	0
total	461	447 (96,9)	11 (2,4)	3 (0,7)



Conclusion

- Determination of MICs of GNB from respiratory samples using direct plating on chromogenic agar and gradient antibiotic strips "Liofilchem® MIC Test Strip" is a promising method for providing clinicians with early information on the susceptibility of these bacteria. This may prove useful to best adapt antibiotic treatments.

References

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