Evaluation of Direct E-test on Lower Respiratory Tract Samples using a Chromogenic Agar Medium: a Rapid Procedure for Antimicrobial Susceptibility Testing

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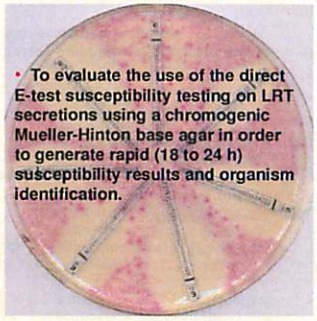
INTRODUCTION

- Ventilator-associated pneumonia (VAP) is associated with elevated morbidity and mortality rates.
- Patients with VAP who receive initial appropriate microbiologic workup and antimicrobial treatment have a better prognosis and a lower mortality rate.
- Quantitative cultures of lower respiratory tract (LRT) secretions with conventional bacterial isolation, identification, and antimicrobial susceptibility testing takes no less than 48 to 72 h.
- We have previously demonstrated that direct E-test on LRT samples from ICU patients is an accurate procedure that provides susceptibility results in 18 to 24 h (Cercenado et al., Diagn. Microbiol. Infect. Dis. 2007; 58:211).
- In this study we evaluate a modification of this technique using a chromogenic Mueller-Hinton agar medium to generate an indication of the pathogen involved.

OBJECTIVE

- To evaluate the use of the direct E-test susceptibility testing on LRT secretions using a chromogenic Mueller-Hinton base agar in order to generate rapid (18 to 24 h) susceptibility results and organism identification.



MATERIAL AND METHODS

Study period: Over a period of 6 months, all tracheal aspirates received in our microbiology laboratory from patients admitted to the ICUs and suspected of having VAP were examined by Gram stain.

Microbiological management of samples: After Gram staining, those samples showing microorganisms were directly spread with a swab onto the surface of a chromogenic Mueller-Hinton agar plate (Colorex Mueller-Hinton. Sclavo Diagnostics, Italy) (15 cm ø). Six E-test strips were placed onto the plates that were incubated at 35ºC. MIC readings were performed at 18 to 24 h. All samples were also processed by the standard quantitative culture (≥10^4 cfu/ml were considered significant) and identification and susceptibility testing of the microorganisms isolated were performed by standard procedures (MicroScan, microdilution method, CLSI guidelines).

Definitions and interpretation of results. Comparisons: Individual organism-antimicrobial agent comparisons were made between direct and standard tests. **Total agreement:** Same susceptibility category with standard and direct E-test method (S/S, I/I, R/R). **Major error:** Resistant by direct E-test method and susceptible by standard procedures (R/S). **Very major error:** Susceptible by direct E-test method and resistant by standard procedures (S/R). **Minor error:** An error involving the intermediate category.

Antimicrobial agents tested for the comparison between the direct E-test and the standard method: - **Gram positive:** Oxacillin, and ciprofloxacin. - **Gram negative:** ciprofloxacin, cefepime, piperacillin/tazobactam, imipenem, and amikacin.

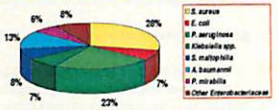
RESULTS

- No. respiratory samples received: 272
- No. samples evaluated (significant counts by quantitative culture): 143
  - 94 monomicrobial
  - 49 polymicrobial

Total No. microorganisms evaluated: 192
Total No. microorganism-antibiotic combinations evaluated: 731

- For evaluation purposes, *Haemophilus* spp. *S. pneumoniae*, and *M. catarrhalis* were excluded since they do not grow on chromogenic Mueller-Hinton agar

Distribution of microorganisms isolated



Colour of microorganisms on chromogenic Mueller-Hinton agar

1. *K. pneumoniae*
2. *E. coli*
3. *E. faecalis*
4. *S. aureus*
5. *S. maltophilia*
6. *P. aeruginosa*
7. *E. cloacae*
8. *A. baumannii*

Microorganisms not detected on chromogenic Mueller-Hinton agar at 18 h. of incubation

Microorganisms	No. Isolates
S. maltophilia	12 (9 polymicrobial) (3 monomicrobial)
S. aureus (MS)	1 (polymicrobial)
Hafnia alvei	1 (polymicrobial)

- 92.7% of the Isolates (178) were recovered at 18 h. of incubation
- All isolates (192) were detected at 24 h. of incubation

Comparison between the direct E-test and the standard susceptibility testing method

Comparisons	No. (%)
Total agreement:	693 (94.8%)
Minor error:	4 (0.5%)
Major error:	29 (3.9%)
Very major error:	5 (0.68%)

Total No. of comparisons : 731
All VME were polymicrobial cultures

Direct E- test *vs.* standard method

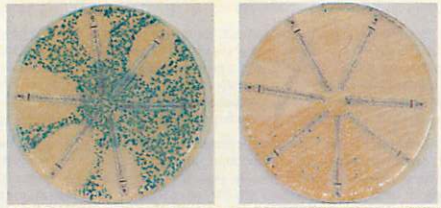
Antimicrobials	No. comparisons	(%) TA	mE	No. ME	VME[a]
Oxacillin	53	98.2	-	1	-
Ciprofloxacin	178	97.8	-	2	2
Amikacin	125	97.6	-	1	2
Piperacillin/Taz	125	96	-	5	-
Cefepime	125	94.4	3	3	1
Imipenem	125	85.6	1	17	-

[a]TA: total agreement; mE: minor error; ME: major error; VME: very major error

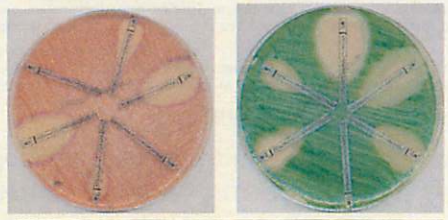
Direct E-test on respiratory samples using chromogenic Mueller-Hinton agar. Monomicrobial cultures

E. cloacae *K. pneumoniae* *E. coli* *P. aeruginosa* *A. baumannii*

Direct E-test on respiratory samples using chromogenic Mueller-Hinton agar. Polymicrobial cultures



P.aeruginosa + *K. pneumoniae* *S. maltophilia* + *S. aureus* (MS)



E. coli + MRSA *P. aeruginosa* + *E. cloacae*

- Total agreement was observed in the detection of MRSA.
- One methicillin-susceptible *S. aureus* isolate was misinterpreted as MRSA (polymicrobial culture with a *Moraxella* spp. isolate).
- Discrepancies corresponded to 20 monomicrobial and 18 polymicrobial cultures.
- The majority of discrepancies occured with imipenem (14.4%) and cefepime (5.6%), and with *A. baumannii* and *P. aeruginosa*.
- Major errors were observed mainly with imipenem and *A. baumannii* (9 cases).
- The detection of *S. maltophilia* required 24 hours of incubation.

CONCLUSIONS

➢ Direct antimicrobial susceptibility testing with the E-test method on LRT samples is a rapid technique which provides results at 18-24 h comparable to those of standard methods.

➢ Total agreement with the standard procedure was 94.8%.

➢ The chromogenic medium allowed identification by colours and facilitated readings especially in polymicrobial cultures.