Antimicrobial susceptibility study

Acceptable performance of VITEK 2 system to detect extended-spectrum β-lactamases in clinical isolates of *Escherichia coli*: a comparative study of phenotypic commercial methods and NCCLS guidelines

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Abstract

The disk approximation method, Etest (AB Biodisk, Solna, Sweden), and VITEK 2 system (bioMérieux, Marcy l’Etoile, France) were used to study 399 extended-spectrum β-lactamase (ESBL)-producing (115 strains) and non-ESBL-producing (284 strains) clinical isolates of *Escherichia coli* after recommended procedures. Comparative study of the phenotypic findings yielded data on the sensitivity, specificity, and positive and negative predictive values and performance of each method. The sensitivity (100% using 2 substrates), specificity (99.3–100%), and predictive values of the disk approximation, Etest, and VITEK 2 methods were similar.

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1. Introduction

Extended-spectrum β-lactamases (ESBLs) are enzymes produced by Gram-negative bacteria, providing these with resistance to all cephalosporins, penicillins, and aztreonam (Bradford, 2001). Infected subjects commonly have greater morbidity and mortality when treated by antibiotics with inadequate in vitro activity (Paterson et al., 2002). Control of infection by these microorganisms is a major challenge that requires accurate and fast ESBL detection in clinical microbiology laboratories. Rapid characterization of isolates is especially important in the treatment of hospitalized patients.

Because of the characteristics of these enzymes, the isolates that produce them can appear falsely sensitive to many cephalosporins (Paterson and Yu, 1999) and therefore escape identification. The National Committee for Clinical Laboratory Standards (NCCLS, 2004) published norms for detecting ESBLs in vitro in *Escherichia coli*, *Klebsiella pneumoniae*, and *K. oxytoca*, establishing cutoff points for reduction of sensitivity to drugs usually hydrolyzed by these enzymes, with confirmation based on enzyme inhibition by clavulanic acid.

Methods evaluated for ESBL detection include disk approximation (Jarlier et al., 1988), Etest (AB Biodisk, Solna, Sweden) (Cormican et al., 1996), combination disk method with cephalosporins alone plus cephalosporins with clavulanic acid (Carter et al., 2000), and automated antibiotic interpretation systems such as VITEK 2 (bioMérieux, Marcy-l’Etoile, France) (Sanders et al., 2000). However, these methods have not been compared using the same clinical isolates. The present study was designed to compare the capacity of disk approximation method, Etest, and VITEK 2 to detect ESBLs in clinical isolates of *E. coli*.

2. Material and methods

A study was conducted of 115 ESBL-producing (group 1) and 284 non-ESBL-producing (group 2) clinical isolates of *E. coli* identified by biochemical tests (Murray et al., 2003). Clinical isolates were obtained during 2002 from patients treated at San Cecilio University Hospital, Granada; 71% of isolates were of outpatient origin, and 63% came from women.

The phenotypic detection of ESBLs and the sensitivity to cefoxitin (FOX) and cefepime (FEP) were characterized by NCCLS disk diffusion method (NCCLS 2004). Strains were inoculated onto Mueller-Hinton agar plates (bioMérieux)
and the following disks were used: cefpodoxime (CPD, 10 μg), CPD plus clavulanic acid (CD01, 10/1 μg), cefazidime (CAZ, 30 μg), CAZ plus clavulanic acid (CD02, 30/10 μg), cefotaxime (CTX, 30 μg), CTX plus clavulanic acid (CD03, 30/10 μg), FOX (30 μg), and FEP (30 μg, Oxoid, England). ESBL presence was suspected when inhibition haloes of ≤ 17, 22, or 27 mm were obtained for CPD, CAZ, or CTX, respectively. ESBL presence was confirmed by demonstration of synergy between cephalosporin with reduced halo and clavulanic acid (defined by increase of ≥ 5 mm in inhibition halo of disks containing cephalosporin plus clavulanic acid versus disks with cephalosporin alone). Molecular typing was not performed. Group 1 included 54 isolates resistant to CTX and CPD and 61 isolates also resistant to CAZ, all showing synergy with clavulanic acid; 5 isolates were also resistant to FOX and 10 to FEP. Group 2 included 24 isolates resistant to CPD but without synergy with clavulanic acid and 36 resistant to FOX; none were resistant to FEP.

Reference strains K. pneumoniae ATCC 700603 and E. coli ATCC 25922 were used. Assays were performed in duplicate and intraobserver agreement was tested.

2.1. Disk approximation method

The method of Jarlier et al. (1988) was followed, using 30-μg disks of CTX, CAZ, CPD, and ATM (BBL, Becton Dickinson, Franklin Lakes, NJ) at 30 mm from amoxicillin/clavulanic acid (20/10 μg) disk (BBL). Positive synergy and ESBL presence were defined by increase of ≥ 5 mm in the inhibition halo of disks containing cephalosporin or ATM or appearance of an area of intermediate synergy in presence of clavulanic acid. If no such increase was shown, the test was repeated using a distance of 20 mm to increase the sensitivity.

2.2. Etest

Paper strips containing CAZ/CD02 and CTX/CD03 were used. ESBL presence was defined by reduction in CAZ or CTX minimum inhibitory concentration (MIC) of 3 or more dilutions in presence of clavulanic acid (Bradford, 2001). FEP ESBL tests were not used because no isolate was resistant to both cephalosporins and their combination with clavulanic acid.

2.3. VITEK 2 system

VITEK 2 is an automated susceptibility testing system enabling rapid determination of MICs by analysis of bacteria growth kinetics with antibiotics in test cards. VITEK cards for susceptibility testing (AST-N020) were inoculated and incubated following manufacturer’s recommendations (Livermore et al., 2002).

2.4. Statistical analysis

The diagnostic capacity of each phenotypic method was studied by analyzing the sensitivity, specificity, and positive and negative predictive values.

3. Results

Table 1 lists results obtained from the comparative phenotypic study using VITEK 2, Etest, and disk approximation method in the 399 strains. The sensitivity, specificity, and positive and negative predictive values are shown in Table 2.

4. Discussion

E. coli is the most frequently isolated enterobacterium in clinical microbiology laboratories, and possible resistances to antibiotics must be accurately detected. Table 3 summarizes the behavior in the laboratory of the studied methods. The Jarlier disk approximation approach is easy to perform and interpret but requires correct placement of the disks. The NCCLS method is also easy to use and interpret but the disks are used exclusively for this task. The Etest is again easy to use and interpret but colonies can appear in
the halo (although not in the present study), increasing the MIC and complicating interpretations. Moreover, the strips are only used for this analysis. Although the CTX/CD03 strip detected all strains in the present study, this does not always occur, and the manufacturer recommends simultaneous use of CTX/CD03 and CAZ/CD02. According to Vercauteren et al. (1997), the CAZ/CD02 Etest detects 81% of ESBL-producing clinical isolates of E. coli, higher than the percentage detected in this study.

The above methods are specific procedures that involve a diagnostic delay of 36–48 h. In contrast, VITEK 2 can diagnose ESBL presence 4 h after card inoculation. Furthermore, the behavior of VITEK 2 in this study suggests that confirmation of E. coli results may not be necessary. Sensitivity and specificity values obtained were somewhat better than those reported by Leverstein-van Hall et al. (2002) (100% sensitivity, 87% specificity), Sanders et al. (2000) (91% sensitivity), and Livermore et al. (2002) (93% sensitivity), although these other studies evaluated ESBL-positive strains belonging to various species (and producing different types of ESBL), not only E. coli. Moreover, VITEK 2 is performed as part of routine laboratory work and does not require a specific procedure.

In conclusion, VITEK-2 showed an acceptable capacity to detect ESBL-producing E. coli in our setting.

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**References**


