Title: Rapid 4 to 6 hour Detection of Extended Spectrum Beta-lactamases Using Quicolor Agar Medium with Disk Diffusion and Etest.

Running title: Rapid Detection of ESBL

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Abstract

With the growing frequency of extended spectrum beta-lactamases (ESBL) among Enterobacteriaceae, treatment of Gram negative nosocomial infections require rapid and reliable detection of this enzyme. Quicolor agar (QC agar) (Salubris Inc., Massachusetts, USA) is a novel chromogenic agar medium that changes color within 4 to 6 hours due to the metabolic activity of growing bacteria. The use of disk diffusion and Etest (AB BIODISK, Solna, SWEDEN) ESBL on QC agar based on color change seen after 4 to 6 hours rather than overnight visible growth may comprise a rapid way of detecting ESBL. This study investigated the use of QC agar compared to Mueller Hinton agar (MH) for the detection of ESBL using disk diffusion and Etest. One hundred Enterobacteriaceae isolated at Hacettepe University Hospital (Ankara, Turkey); of which 50 were predetermined to be ESBL positive and 50 as negative using the CLSI (formerly the NCCLS) disk diffusion ESBL (phenotypic confirmatory test) criteria. For disk diffusion and Etest, cefotaxime±clavulanate (CT/CTL) and ceftazidime±clavulanate (TZ/TZL) were used, and for Etest, cefepime±clavulanate (PM/PML) was also used. Disk diffusion was performed according to the CLSI method and Etest according to the manufacturer's instructions. On QC agar, inhibition zones and Etest inhibition ellipses demarcated by color change after 4 to 6h incubation were read and interpreted. QC agar rapid ESBL results for all strains were in agreement with the standard overnight procedure. All 50 ESBL positives were detected by both methods. For the 50 ESBL negatives, QC agar rapid results from Etest and disk diffusion were in complete agreement with the overnight MH results. Moreover, Etest detected 8 additional ESBL positive strains that disk diffusion missed. For disk diffusion, CT/CTL alone detected all 50 ESBL positives while TZ/TZL alone missed 5 ESBL positives. Etest CT/CTL alone

confirmed all 50 ESBL positives and identified 4 additional ESBL positive strains. When used together, Etest CT/CTL, TZ/TZL and PM/PML identified a total of 58 ESBL positives among the 100 strains tested. QC agar can be used for rapid and reliable ESBL detection within 4 to 6 hours using disk diffusion and Etest ESBL reagents. This rapid method should be further validated using genotype characterized ESBL and other beta-lactamase positive strains.

Keywords: Extended spectrum beta-lactamases (ESBL), Etest, rapid susceptibility.

Introduction

Resistance to broad spectrum beta-lactams, mediated by extended spectrum beta-lactamases (ESBL), is an increasing problem worldwide (1-3). ESBL-mediated resistance poses significant treatment problems and comprise a challenge for *in vitro* susceptibility testing methods and clinical reporting (4-7). Therefore, rapid and reliable detection of ESBL is mandatory to guide treatment of Gram-negative nosocomial infections and to initiate infection control measures.

Quicolor agar (QC agar) (Salubris Inc., Massachusetts, USA) is a novel chromogenic agar medium that changes color within 4 to 6 hours due to the metabolic activity of growing bacteria (8). There are two types of QC agar. Quicolor ES (OC ES) is for testing of Enterobacteriaceae and staphylococci. The red color of this medium turns to yellow due to bacterial growth and gives red inhibition zones. Quicolor NF is for testing of Gram negative non-fermenters like Pseudomonas spp. The yellow color of this medium turns to red due to bacterial growth and gives yellow inhibition zones. In this study, we used QC ES for testing the isolates belonging to Enterobacteriaceae.

The use of disk diffusion and Etest (AB BIODISK, Solna, SWEDEN) ESBL reagents with QC agar to obtain 4 to 6 hour same day results based on color change rather than overnight visible growth may comprise a valuable alternative for rapid detection of ESBL. This study compared this rapid QC agar alternative to the standard overnight ESBL detection methods.

Materials and Methods

Isolates

One hundred Enterobacteriaceae isolated at Hacettepe University Hospital (Ankara, Turkey) were included in the study. Fifty of these isolates were previously determined and reported as ESBL positive (*E. coli* n:45, *K. oxytoca* n:3 and *K. pneumoniae* n:2) and 50 ESBL negative (*E. coli* n:32, *K. pneumoniae* n:11, *K. oxytoca* n:3, *S. marcescens* n:2, *E. cloacae* n:1, *E. aerogenes* n:1) based on the CLSI (formerly the NCCLS) ESBL disk diffusion phenotypic confirmatory test criteria (9).

Culture Media

Quicolor ES agar and Mueller Hinton agar (MH) (Merck, Darmstadt, Germany) were used for disk diffusion and Etest. For QC ES agar, the color change from red to yellow was indicative of bacterial growth.

Detection of ESBL

Disk diffusion was performed according to the CLSI criteria. Bacterial suspensions were prepared from overnight cultures to obtain a turbidity equivalent to 0.5 McFarland standard. The suspensions were streaked onto MH and QC ES agar (9). Cefotaxime±clavulanate (CT/CTL) and ceftazidime±clavulanate (TZ/TZL) were used for disk diffusion and Etest, and additionally cefepime±clavulanate (PM/PML) for Etest.

All plates were incubated at 35℃ for 16-18 h. QC ES agar plates were read both at 4-6 h and 16-18 h. At 4-6 h, interpretation of disk diffusion and Etest was based on color change of QC ES agar from red to yellow at the demarcation of inhibition zones and Etest inhibition ellipses were read. Reading and interpretation at 16-18 h was according to the CLSI criteria.

For disk diffusion, an inhibition zone difference of ≥ 5 mm for CT/CTL or TZ/TZL was considered confirmatory for ESBL. For Etest ESBL, MIC results for the double-sided CT/CTL and TZ/TZL and PM/PML antibiotic gradient strips were interpreted either by MIC ratios of ≥ 8 or the presence of a phantom zone or deformation of ellipse as ESBL positive (9).

Results

Rapid results on QC ES agar for all strains (ESBL positives and negatives) were in agreement with the overnight procedure. All 50 ESBL positives were detected by both methods (Figures 1-3). Etest detected 8 additional strains that disk diffusion was not able to identify. For disk diffusion, CT/CTL alone detected all 50 ESBL positives while TZ/TZL alone missed 5 ESBL positives. Etest CT/CTL alone confirmed all 50 ESBL positives and identified 4 additional ESBL positive strains. Etest CT/CTL, TZ/TZL and PM/PML used together identified a total of 58 ESBL positives among the tested strains.

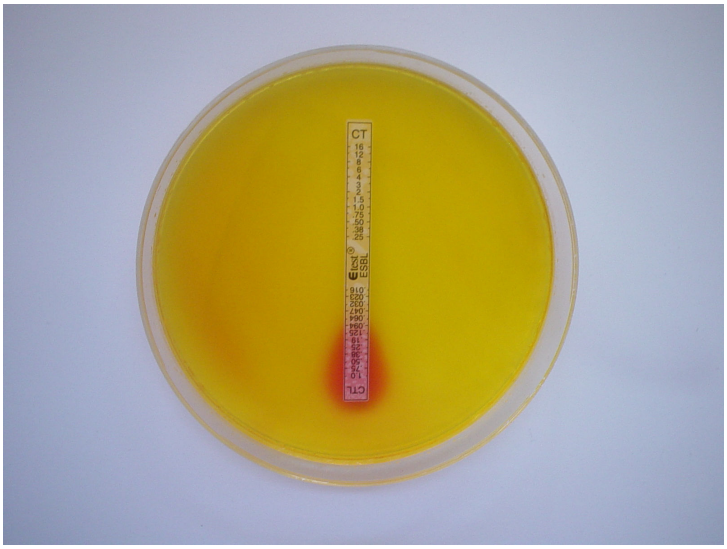

Figure 1: ESBL positive isolate with Etest on QC ES agar at 4-6h

(CT:Cefotaxime; CTL:Cefotaxime-clavulanic acid)

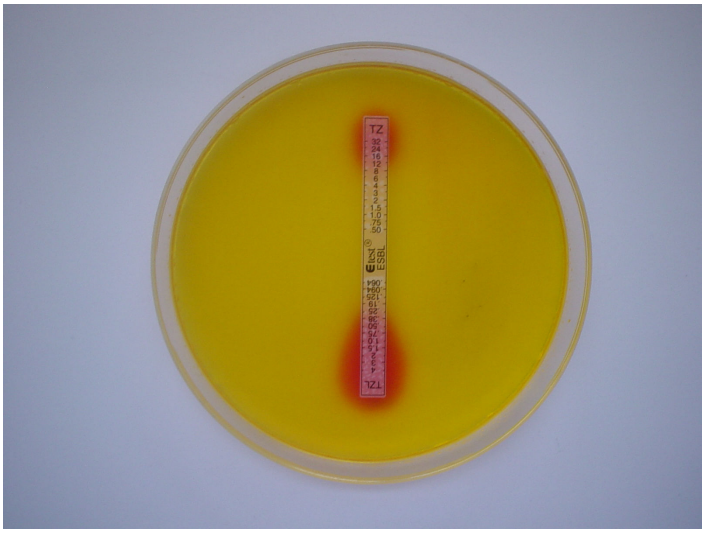

Figure 2: ESBL positive isolate with Etest on QC ES agar at 4-6h

(TZ: ceftazidime; TZL: ceftazidime-clavulanic acid)

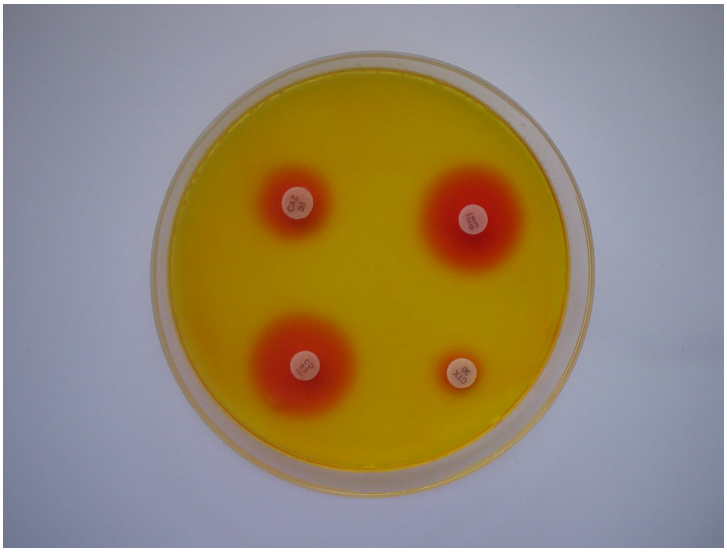

Figure 3: ESBL positive isolate with disk diffusion on QC ES agar at 4-6h (CTX:cefotaxime; CD03:cefotaxime/clavulanate; CAZ:ceftazidime; CD02:ceftazidime/clavulanate)

Discussion

Early detection of ESBL mediated resistance is very important especially among ICU related infections in order to implement effective antibiotic treament as early as possible. It is important to start with the appropriate empiric treatment and correct it as soon as possible to efficiently manage these types of infections, reduce side effects and minimise resistance selection and spread and thus decrease health care costs (10-13).

The ESBL mediated resistance mechanism poses a therapeutic dilemma as it is difficult to acurrately detect in the clinical laboratory (1,14). There are several methods used for ESBL detection and these include biochemical and molecular based methods. The latter methods are more time-consuming and expensive to carry out (15-18).

Among Enterobacteriaceae, ESBL producers are mostly seen among *E.coli* and *Klebsiella* spp. Since the incidence of ESBL producers have been increasing, it is very important to screen for these enzymes and thereafter rapidly confirm their presence to verify correct treatment of Gram-negative nosocomial infections.

Phenotypic screening and confirmation of ESBL are the most cost-effective and widely used methods in clinical microbiology laboratories today (9,17,18). Etest ESBL is a unique predefined gradient technology that compares the reduction of the MIC of the parent cephalosporin when overlapped with a constant level of clavulanic acid (6,17,19). Three substrates (cefotaxime, ceftazidime and cefepime) are easily tested across 7 MIC dilutions, allowing both high level and lower levels of ESBL enzymes to be efficiently detected as exemplified by the additional 8 ESBL strains missed by the disk diffusion method. However, both phenotypic methods require at least 16 to 18 h for detection of ESBL (6,9,19).

Using QC ES agar, a color change from red to yellow induced by the metabolic activity of bacteria growing on the agar surface allows the endpoint to be visible within 4 to 6 hours i.e. long before bacterial colonies are formed. Thus, disk diffusion and Etest results can be read as changes of color around the demarcation of the endpoint rather than a grow - no growth result.

This study showed that QC ES agar can be efficiently used for both disk diffusion and Etest ESBL reagents to perform rapid 4 to 6 h same day ESBL testing. Etest ESBL reagents were found to be more sensitive than disk diffusion in identifying additional ESBL strains due to the unique design of this gradient method. Therefore, the use of QC ES agar with Etest ESBL comprise a valuable alternative to help manage efficient treatment of ESBL associated infections. This may have significant impact on

reducing the duration of hospital stay, cost of patient care and mortality and morbidity by contributing to an early implementation of the most appropriate treatment.

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