Rapid 4 to 6 hour detection of extended spectrum beta-lactamases (ESBL) using



Quicolor agar medium with disk diffusion (DD) and Etest.

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Abstract

BACKGROUND: With the growing frequency of ESBL among Enterobacteriaceae, treatment of Gram negative nosocomial infections require rapid and reliable detection of ESBL. Quicolor ES agar (QE) (Salubris Inc., USA) is a novel chromogenic agar media that changes color within 4 to 6 hours due to the metabolic activity of growing bacteria. The use of DD and Etest ESBL on QE based on color change rather than overnight visible growth may comprise a rapid way of detecting ESBL. This study compared this rapid media to the standard ESBL procedure for both phenotypic methods. **METHOD**: One hundred Enterobacteriaceae isolated at Hacettepe University Hospital (Ankara, Turkey); 50 ESBL positive and 50 negative based on the CLSI (NCCLS) disk diffusion ESBL (phenotypic confirmatory test) criteria were used. For DD and Etest, cefotaxime±clavulanate (CT/CTL) and ceftazidime±clavulanate (TZ/TZL) were used, and for Etest, cefepime±clavulanate (PM/PML) was used in addition. DD was performed according to the CLSI criteria and Etest was done according to the manufacturer's instructions. QE was set up in the same way and inhibition zones and Etest ellipses demarcated by color change after 4 to 6h incubation was read and interpreted. RESULTS: QE rapid results for all strains were in agreement with the standard overnight procedure. All 50 ESBL positives were detected by each of the method. For the 50 ESBL negatives, rapid results of both Etest and DD on QE within 4 to 6 hours were in complete agreement with the overnight 16-18h MHA results. Moreover, Etest detected 8 strains that DD was not able to identify. For DD, 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. CONCLUSIONS: QE can be used for rapid and reliable ESBL detection within 4 to 6 hours using DD and Etest ESBL reagents. This rapid method should be further validated using genotype characterized

Background

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

Quicolor ES agar (QE) (Salubris Inc., 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 DD and Etest (AB BIODISK, Solna, Sweden) ESBL reagents with QE with 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 alternative based on QE to the standard ESBL procedure for both phenotypic methods.

Method

Isolates

One hundred Enterobacteriaceae isolated at Hacettepe University Hospital (Ankara, Turkey) were included in the study. Fifty of these isolates were previously determined as ESBL positive and 50 negative based on the CLSI (NCCLS) ESBL disk diffusion phenotypic confirmatory test criteria.

Culture Media

QE agar and Mueller Hinton agar (MH) were used for DD and Etest. For QE, the color change from red to yellow was indicative of bacterial growth.

Detection of ESBL

DD was performed according to the CLSI criteria. Bacterial suspensions were prepared from overnight cultures to obtain a turbidity of 0.5 McFarland. The suspensions were streaked onto MH and QE agar.

For DD, cefotaxime±clavulanate (CT/CTL) and ceftazidime±clavulanate (TZ/TZL) were used and for Etest, cefepime±clavulanate (PM/PML) was additionally used.

All plates were incubated at 35°C for 16-18h. QE plates were read at 4-6h and 16-18h. At 4-6h, interpretation of DD and Etest was based on color change of QE from red to yellow where the demarcation of inhibition zones and Etest ellipses were read. At 16-18h, reading and interpretation was according to CLSI criteria.

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

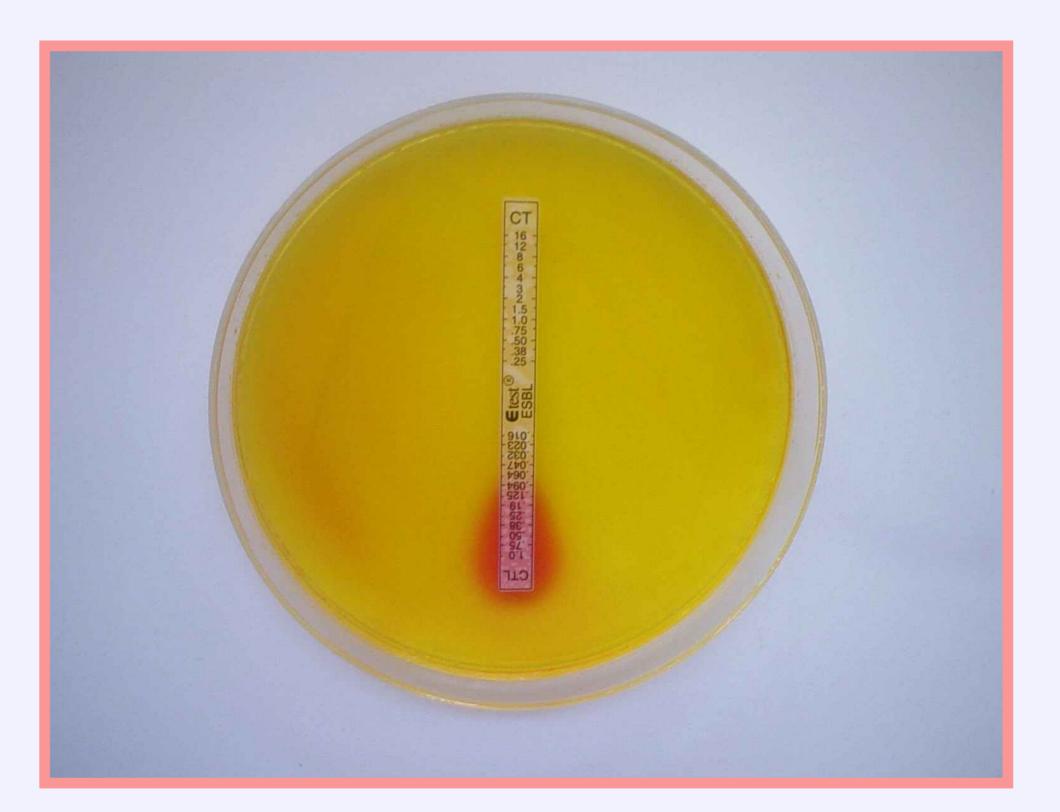


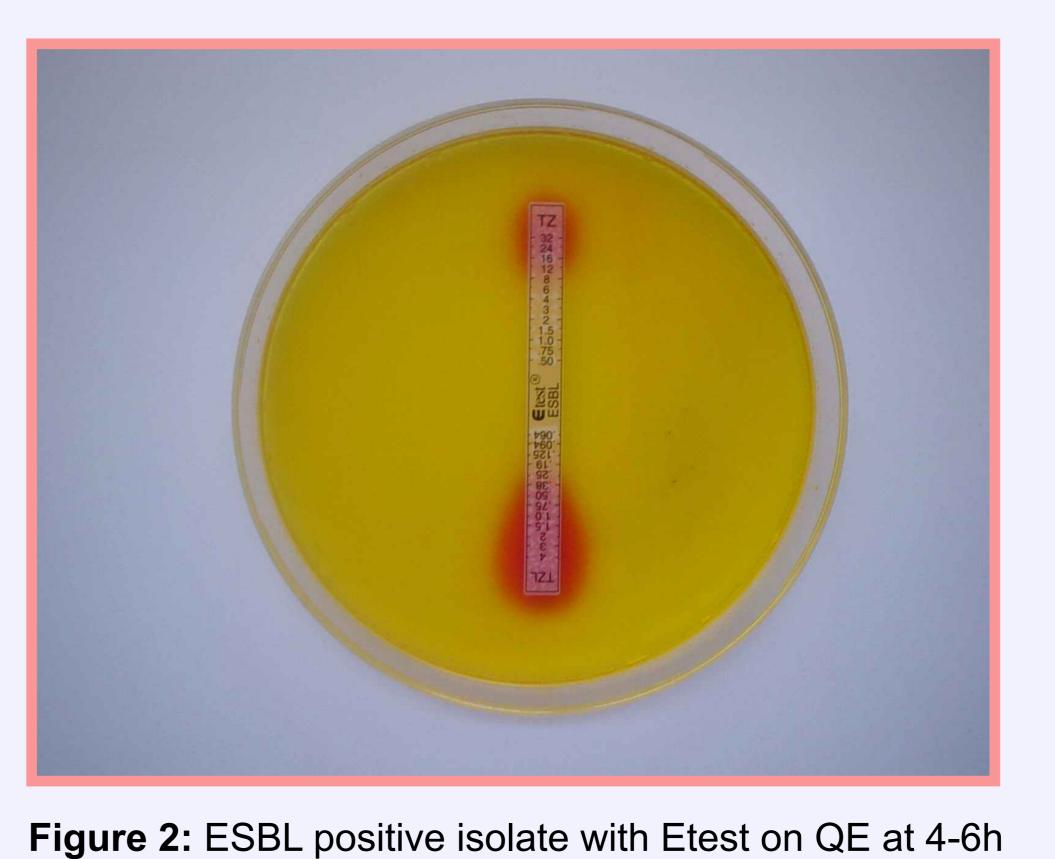
Figure 1: ESBL positive isolate with Etest on QE at 4-6h (CT:Cefotaxime; CTL:Cefotaxime-clavulanic acid)



Figure 3: ESBL positive isolate with DD on QE at 4-6h (CTX:cefotaxime; CD03:cefotaxime/clavulanate; CAZ:ceftazidime; CD02:ceftazidime/clavulanate)

Results

- QE rapid results for all strains were in agreement with the overnight procedure. All 50 ESBL positives were detected by both methods.
- For the 50 ESBL negatives, results of Etest and DD on QE within 4 to 6h were in complete agreement with the 16-18h MH results.
- Etest detected 8 additional strains that DD was not able to identify. For DD, 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.



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

Conclusions

- The rapid results of ESBL confirmatory testing using QE were in agreement with the standardised CLSI overnight method.
- QE can be used for rapid and reliable ESBL detection within 4 to 6h using DD and Etest ESBL reagents.
- Etest ESBL reagents were more sensitive than DD in identifying ESBL production in more strains.
- The QE chromogenic agar for rapid ESBL detection should be further validated using genotype characterized ESBL and other beta-lactamase producers to ascertain sensitivity and specificity of this method.