Interpretation of Results of ESBL Detection Using E-test® Needs in Consensus

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INTRODUCTION

Rapidly emerging resistance to the broad-spectrum β-lactams, mediated by extended-spectrum β-lactamases (ESBLs), is an increasing problem worldwide. This problem has been characterised by difficulties in the recognition of ESBL-producing strains in the laboratory, particularly by rapid susceptibility test methods.

In many cases routine susceptibility testing fails to detect resistance due to ESBL production in strains that remain apparently susceptible to ceftazidime or cefotaxime at the breakpoints advocated by the NCCLS (8 to 16 µg/ml). At the same time, clinical data demonstrate *in vivo* resistance of ESBL producers to oxyimino-β-lactams regardless of the actual susceptibility found. This bags the question of the best way to detect ESBL producers.

Several tests based on on the sensitivity of class A ESBLs to β-lactamase inhibitors such as clavulanic acid have been used in laboratory practice, including a double-disk synergy test and E-test® ESBL (AB BIODISK, Sweden). The latter method allows detection of ESBL producers using the MIC ratio of ceftazidime alone (TZ) and its combination with clavulanic acid (TZL) as a discriminative criterion.

OBJECTIVE

- To evaluate the possibility to use flexible interpretive criteria for the E-test ESBL screening of clinical isolates of *Klebsiella pneumoniae* with high and low level of resistance to ceftazidime.

MATERIALS AND METHODS

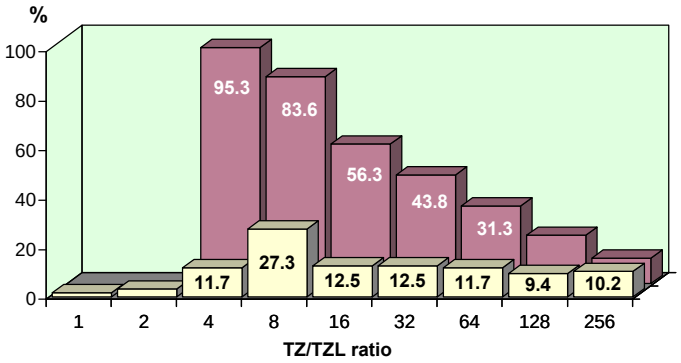
The strains used in this study were collected from intensive care units of 11 geographically distant hospitals throughout Russia. Identification of the strains was done using the API strips (Bio-Mérieux, France). The MICs of ceftazidime and other extended-spectrum β-lactams were determined using the E-test method.

128 clinical strains of *K.pneumoniae* with decreased susceptibility to ceftazidime (MIC ≥ 1 µg/ml) were tested for the production of ESBLs.

The standard E-test® strips, containing of two gradients aligned in opposing directions for the detection of ESBLs were used. One side of the strip has ceftazidime (TZ) MIC range of 0.5-32 µg/ml and the other a ceftazidime MIC range of 0.125-8 µg/ml overlaid with a constant level of 4 µg/ml clavulanic acid (TZL). MIC ratios of TZ/TZL have been used as a discriminative criteria for the presence or absence of ESBLs.

RESULTS AND DISCUSSION

The following figure shows the results of ESBL detection in clinical isolates of *K.pneumoniae*.



TZ/TZL Ratio	Number of isolates	Cumulative Frequency
256	13	13
128	12	25
64	15	40
32	16	56
16	16	72
8	35	107
4	15	122
2	4	126
1	2	128

□ - Percent of isolates with current TZ/TZL ratio ■ - Percent of ESBL producers according to the corresponding TZ/TZL ratio

The cumulative data are indicative of the total number/percent of the suspected ESBL-producing strains according to the corresponding MIC ratio. If the previously suggested MIC ratio of ≥ 16 has been used, the cumulative number was 72 (56.3%). However, if newly proposed MIC ratio of ≥ 8 has been considered, the cumulative number increased up to 107 (83.6%). If we use the MIC ratio starting from 4 it will give us an additional 11.7% of potentially ESBL-producing strains. These strains were characterised by low-level resistance to ceftazidime with the MICs ranged from 1 to 4 µg/ml and were ESBL-positive according to the double-disk synergy test. The detection of low-level ESBL production in such clinical strains is one of the most difficult diagnostic challenges so far as the results may be affected by the number of factors: impermeability of the outer membrane, presence of additional β-lactamases, etc.

CONCLUSIONS

- The above data suggest that the currently accepted criteria of TZ/TZL MIC ratio ≥ 8 may need reassessment for the detection of ESBLs in clinical isolates of *K.pneumoniae* with low-level resistance to ceftazidime.
- Detection of ESBL by E-test method is simple and convenient. However, we need in consensus for interpretation of results of ESBLs detection.

20th ICC, Sydney (Australia), 29 June – 3 July, 1997