

Efficacy of Practiced Screening Methods for Detection of Cephalosporin-Resistant Enterobacteriaceae

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BACKGROUND

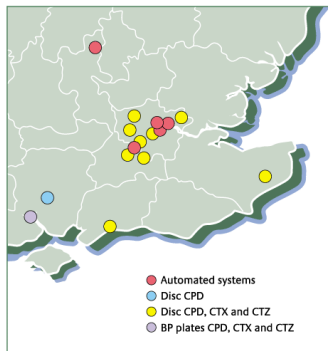
- Enterobacteriaceae with extended-spectrum β -lactamases (ESBL) particularly CTX-M enzymes are now widespread in the UK.
- CTX-M ESBLs were unknown before 2000, but are now the predominant mechanism among cephalosporin-resistant *E. coli* and *K. pneumoniae*¹ in the UK.
- Simple phenotypic tests are required to identify ESBLs in diagnostic laboratories; the simplest method is to screen with cefpodoxime and to do clavulanate synergy tests on isolates found resistant though, many laboratories include further cephalosporins in the initial screen.
- We investigated the screening methods used by 16 hospitals in South-East England and their effectiveness.

METHOD

- Sixteen laboratories in the South-East of England (figure 1) submitted a total of 1195 consecutive Enterobacteriaceae isolates that they found to be resistant by their routine methods to any or all of cefpodoxime, ceftazidime and cefotaxime.
- Isolates were tested centrally by the British Society for Antimicrobial Chemotherapy (BSAC)'s agar dilution method² using various cephalosporin/clavulanate combinations.
- Resistance genes were sought by multiplex PCR for *bla*_{CTX-M} and *bla*_{AmpC} alleles.^{3,4}

Figure 1. Location of the 16 survey laboratories. Colour depicts method and cephalosporins used in screening.

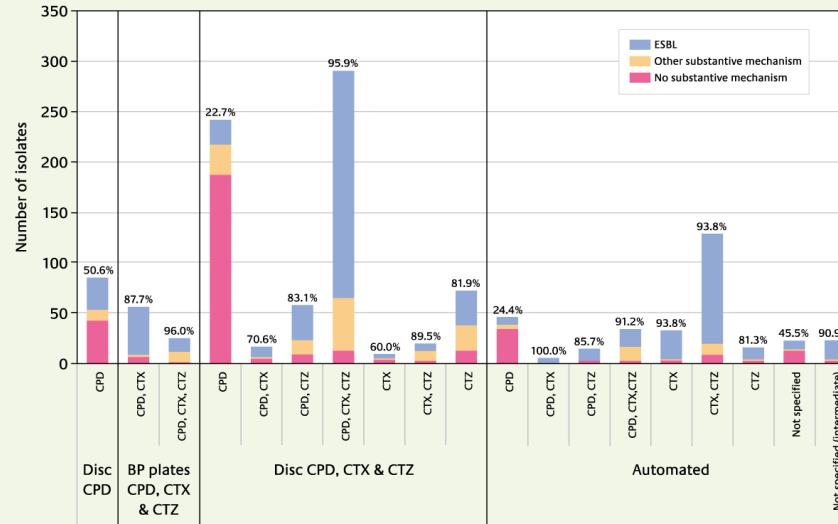
Red - automated systems cefpodoxime, cefotaxime and ceftazidime (Vitek, 1 site; Vitek 2, 2 sites and Phoenix, 2 sites); Blue - disc cefpodoxime (5 μ g); Yellow - discs cefpodoxime (5 μ g), cefotaxime (30 μ g) and ceftazidime (30 μ g); Purple - breakpoint plates, cefpodoxime, cefotaxime and ceftazidime.



CPD = cefpodoxime, CTX = cefotaxime, CTZ = ceftazidime

RESULTS

Figure 2. Proportion of isolates with substantive resistance mechanism as determined by the reference laboratory in relation to sentinel laboratory screening method and cephalosporins used in screening.



- Among 1195 Enterobacteriaceae isolates submitted:

- 73 proved sensitive to all three indicator cephalosporins on MIC testing.
- 647 (54%) were ESBL producers, 502 of them with CTX-M enzymes
- 186 (15%) were AmpC hyperproducers,
- 9 were *Klebsiella oxytoca* hyperproducing K1 enzyme
- 2 *Klebsiella* spp. and *Enterobacter* spp. had phenotypes suggesting both ESBL and AmpC enzyme activity.
- 276 isolates had only borderline resistance to any cephalosporin; in most (203/276) cases the cephalosporin affected was cefpodoxime
- Survey site screening methods were;
 - Cefpodoxime (5 μ g), cefotaxime (30 μ g) and ceftazidime (30 μ g) discs (9 sites)
 - Cefpodoxime (5 μ g) discs only (1 site)
 - Automated systems (Phoenix - 2 sites, Vitek 2 - 2 sites, and Vitek - 1 site)

- Cefpodoxime, cefotaxime and ceftazidime by agar dilution (1 site)

- Figure 2 relates the proportion of isolates with substantive resistance mechanisms, as determined by the reference laboratory, with the screening method used at the source laboratory.
- 8% of isolates submitted from laboratories using disc tests proved fully cephalosporin susceptible, compared with 3% sent based on tests with automated systems (Phoenix, Vitek 1 and 2) and none of those sent based on agar dilution tests.
 - The performance of the automated systems may partly reflect calibration against higher CLSI breakpoints, which would mitigate against submission of isolates with borderline resistance at lower BSAC breakpoints.
- Among 85 isolates submitted by the site that screened only with cefpodoxime 5 μ g discs, 43 had substantive resistance mechanisms (50%), including 31 with ESBLs; 42 had only borderline resistance

- Among 242 isolates tested with multiple cephalosporin discs at their source laboratory but submitted solely based on cefpodoxime resistance showed that only 55/242 (22.7%) had a substantive mechanism.
 - By contrast, 70% of isolates submitted as resistant to cefpodoxime and cefotaxime, 83% of those resistant to cefpodoxime and ceftazidime and 95% of those resistant to all three of these cephalosporins had a substantive mechanism.
- Among automated systems used at source laboratories the Vitek 2 had the best agreement with reference results (Table 1).
 - Nevertheless both the Phoenix and Vitek 2 mis-called a few AmpC-hyperproducing *Enterobacters* as ESBL producers.

Table 1. Resistance mechanisms found by the reference laboratory in isolates with ESBL production inferred by automated systems.

| Automated system | Substantive Resistance Mechanisms | | | | Non-substantive Resistance Mechanisms | | Total |
|------------------|-----------------------------------|-----------|-------|----|---------------------------------------|-----------|-------|
| | ESBL | | Other | | Other/ borderline | Sensitive | |
| | CTX-M | Non CTX-M | AmpC | K1 | | | |
| Phoenix | 74 | 23 | 7 | 2 | 16 | 3 | 125 |
| Vitek | 6 | | | | 2 | | 8 |
| Vitek 2 | 51 | 51 | 4 | | 1 | 4 | 111 |

CONCLUSION

- Many isolates found resistant only to cefpodoxime at the source sites proved not to have ESBLs or AmpC
- Screening with cefotaxime and ceftazidime allowed better specificity for identification of mechanism-based resistance than did cefpodoxime screening, as did the automated systems
- Cefpodoxime nevertheless remains a useful, but poorly specific, single primary screen before confirmatory testing if only a single disc can be accommodated, e.g. in testing of community urines.

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