Efficacy of practised screening methods for detection of cephalosporin-resistant Enterobacteriaceae


Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 5EQ, UK

Received 3 May 2006; returned 10 July 2006; revised 5 September 2006; accepted 2 October 2006

Objectives: Enterobacteriaceae with extended-spectrum β-lactamases (ESBLs) are now widespread and simple phenotypic tests are required to detect them in diagnostic laboratories. We investigated the performance of screening methods at 16 hospitals in South-East England.

Methods: Sixteen laboratories in South-East England submitted 1195 consecutive Enterobacteriaceae isolates found to be resistant, by their routine methods, to any or all of cefpodoxime, ceftazidime and cefotaxime. These isolates were re-tested centrally with various cephalosporin/clavulanate combinations and with multiplex PCR for bla_{CTX-M} and bla_{AmpC} alleles.

Results: Screening methods among the laboratories were the following: cefpodoxime discs alone (1 site); cefpodoxime, cefotaxime and ceftazidime discs (9 sites) or agar dilution (1 site); Phoenix (2 sites), Vitek 1 (1 site) and Vitek 2 (2 sites). A total of 8% of isolates submitted based on disc tests proved fully cephalosporin-susceptible, compared with 3% sent based on tests with automated systems and none of those sent based on agar dilution tests. Among isolates submitted solely on cefpodoxime resistance 256/372 (69%) proved cephalosporin-susceptible or had only borderline resistance with no clear mechanism demonstrable; this proportion decreased to 28/160 (18%) for those submitted on the basis of resistance to ceftazidime, 18/122 (15%) for those resistant to cefotaxime and 26/496 (5%) for those resistant to both cefotaxime and ceftazidime. The inference of ESBL production by Vitek 2 had the best agreement with reference laboratory results.

Conclusions: 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, as did the automated systems. Cefpodoxime disc tests nevertheless remain a useful primary screen for laboratories prepared only to test one agent.

Keywords: ESBL detection, β-lactamases, diagnostic tests

Introduction

Cephalosporin resistance among Enterobacteriaceae is changing in nature and prevalence worldwide, largely owing to the proliferation of CTX-M β-lactamases. In the UK, CTX-M extended-spectrum β-lactamases (ESBLs) were unknown before 2000, but are now the predominant mechanism among cephalosporin-resistant Escherichia coli and Klebsiella pneumoniae. These shifts present laboratory challenges, as resistance is not always obvious and it is widely considered that cephalosporins should be avoided in therapy of infections caused by ESBL producers, irrespective of direct susceptibility test results. It follows from this that ESBL producers should be sought by indicator tests and, to this end, the Health Protection Agency (HPA) published guidelines in collaboration with the British Society for Antimicrobial Chemotherapy (BSAC). The HPA recommendations are that either (i) cefpodoxime or (ii) both cefotaxime and ceftazidime should be used as screening agents, with clavulanate synergy tests done on isolates found resistant to any of these.

This work reports on the performance of these and other screening methodologies for cephalosporin resistance and possible ESBL producers, as used in NHS laboratories of South-East England.

Methods

Collection of isolates and centralized testing

Consecutive Enterobacteriaceae isolates found to be resistant to any or all of cefpodoxime, ceftazidime and cefotaxime were collected...
ESBL testing methods

from 16 clinical diagnostic laboratories in London and South-East England (see the Acknowledgements section) participating in a resistance survey. Laboratories using automated systems were also allowed to submit isolates that their systems had inferred as having ESBLs regardless of cephalosporin resistance detected. The identification, susceptibility testing and β-lactamase characterization of these resistant isolates received at the Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) have been described in full elsewhere. Among 1195 Enterobacteriaceae isolates collected from 16 clinical diagnostic laboratories in London and South-East England (see the Acknowledgements section) participating in a resistance survey, laboratories using automated systems were also allowed to submit isolates that their systems had inferred as having ESBLs regardless of cephalosporin resistance detected. The identification, susceptibility testing and β-lactamase characterization of these resistant isolates received at the Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) have been described in full elsewhere.

Statistical analysis

Differences between proportions were analysed using χ² tests, or by Fisher’s exact test if sample sizes were small or unbalanced. The odds of substantive resistance mechanisms (i.e. a well-characterized resistance mechanism ESBL, AmpC, etc.) being present versus not present in isolates referred for cephalosporin resistance testing at sentinel laboratories was modelled via logistic regression. (Excel 2002, analysis tool pack, Microsoft and the SAS System, SAS Institute Inc.).

Results

Summary of collection

The resistance mechanisms identified in the collection are fully described elsewhere. Among 1195 Enterobacteriaceae isolates submitted, 73 (6%) proved susceptible to all the three indicator cephalosporins (cefotaxime, ceftazidime and cefpodoxime) on MIC testing. The remaining 1122 were categorized as follows: 647 (55%) were ESBL producers, 502 of them with CTX-M enzymes; 186 (16%) were AmpC hyperproducers; nine were Klebsiella oxytoca hyperproducing K1 enzyme; and four (two Klebsiella spp. and two Enterobacter spp.) were inferred to have both ESBLs and hyperproduced AmpC enzymes based on phenotypes. The remaining 276 isolates had only borderline resistance, with MICs of one or more cephalosporins—most often (143/276 cases) cepodoxime—raised by only one or two dilutions above the breakpoint, and with no specific resistance mechanism (i.e. ESBL, AmpC or K1 enzyme) identified.

Cephalosporin testing methods at sentinel laboratories

The sentinel laboratories adopted a variety of methods to screen for cephalosporin resistance: cepodoxime (5 μg), cefotaxime (30 μg) and ceftazidime (30 μg) discs were all used by nine laboratories whilst one used cepodoxime (5 μg) discs only, and one used breakpoint plates. The remaining five sites used automated systems, namely Phoenix (two sites) (Becton, Dickinson, Oxford, UK), Vitrek (one site) or Vitek 2 (two sites) (bioMérieux, Basingstoke, UK). The breakpoints adopted by the sentinel laboratories were those of the BSAC except at four of the five sites with automated systems (one Phoenix and all Viteks) which used the Clinical and Laboratory Standards Institute (CLSI) breakpoints and their ESBL screening criteria.

Among isolates submitted as resistant on the strength of disc tests—all of which were by BSAC methodology—8% (59/732) proved cephalosporin-resistant on reference testing, while automated systems miscalculated cephalosporin resistance in only 3% (11/308) of cases. This apparent difference may, however, be artefactual, as four of five automated systems were calibrated against CLSI cephalosporin breakpoints which are higher than those of the BSAC, thus militating against submission of isolates with borderline resistance at the BSAC breakpoints. No cephalosporin-susceptible isolates were mis-referred by the centre using agar dilution breakpoint plates.

Table 1 relates the number of isolates with substantive resistance mechanisms, as determined by the reference laboratory, with the screening method used by the source laboratory. Among 85 isolates submitted by the site that screened only with cepodoxime 5 μg discs, 43 proved to have substantive resistance mechanisms, including 31 with ESBLs; the remaining 42 either proved susceptible or required cephalosporin (generally only cepodoxime) MICs one or two dilutions above the breakpoint without evidence of a substantive mechanism based on synergy studies or PCR.

Sub-analysis of those isolates tested with multiple cephalosporin discs at their source laboratory but submitted solely based on cepodoxime resistance also suggested poor specificity with this compound. Among 242 such isolates, only 55 (22.7%) proved to have a substantive mechanism, as against 70% of those found to be resistant to cepodoxime and cefotaxime, 83% of those found resistant to cepodoxime and ceftazidime and 95% of those found resistant to all three of these cephalosporins. Estimated odds ratios were used to measure relative reliability of resistance to the three indicator cephalosporins as markers for detection of isolates with substantive resistance mechanisms compared with sole detection of cepodoxime resistance. We found that isolates with resistance to all three cephalosporins were nearly 80 times more likely (OR = 78.7, 95% CI: 42.6–158.3) to have a substantive resistance mechanism (Table 2). A similar improvement (OR = 31.9, 95% CI: 9.2–152.7) was seen with the screening using the automated systems.

Prediction of ESBL production by automated systems

The Phoenix and Vitek systems infer ESBL production by analysis of antibiograms by their respective (and rather different) expert systems. ESBLs were confirmed by reference investigation in 97 (77%) isolates out of 125 isolates inferred to have ESBLs by the Phoenix system; the corresponding proportions increased to 102/111 (91%) for the Vitek 2 (P < 0.05). The site using a Vitek sent just eight isolates as ESBL producers and ESBL production was confirmed in six of these. The automated systems incorrectly inferred ESBL production in a few (11) AmpC hyperproducers and in isolates that proved sensitive on reference testing; worryingly, all these systems failed to detect ESBLs in a few (14) cephalosporin-resistant isolates (mostly Klebsiella pneumoniae or Enterobacter cloacae) that were found to have these enzymes by reference testing.

Assessment of methods used for detection of cephalosporin resistance and ESBLs

Comparison of screening results and reference data showed that the disc diffusion method was more liable to miscall resistance than the automated systems. This finding is not surprising, as the disc method is prone to experimental variation arising from disparity of antibiotic loading during the manufacture of the discs and in the precision of measuring zone of inhibitions. Moreover the use of CLSI breakpoints by four of the five laboratories with automated systems militated against submission of isolates with borderline resistance at BSAC breakpoints unless these borderline
isolates were also inferred by the automated systems to be ESBL producers.

**Value of indicator cephalosporin resistance as a marker for substantive mechanisms**

Cefpodoxime has been proposed as the best single screening cephalosporin to detect those isolates warranting further investigation as plausible ESBL producers. The present data (Table 1) nevertheless shows that cefpodoxime screens, used alone, have poor specificity. Thus, approximately half of the isolates submitted by the laboratory screening only for cefpodoxime resistance had no substantive resistance mechanism. In contrast, isolates found resistant to either cefotaxime or ceftazidime during disc screening mostly (>89%) had confirmed cephalosporin resistance and a demonstrable mechanism. However, screening based on cefotaxime and ceftazidime requires that both of these drugs are tested, so as to reliably detect both CTX-M producers and those with ceftazidimase-type TEM variants, a fact underscored by the observation that 82/502 of the CTX-M β-lactamase-producing isolates appeared resistant to cefotaxime but not ceftazidime in screening. These findings are in keeping with those of other, smaller studies.8

**Automated prediction of ESBLs**

Previous performance analyses of automated systems have largely stressed their sensitivity, by challenging them with known ESBL producers;9,10 in this study we tested the predictive value of a positive result, assessing what proportion of the isolates identified as ESBL producers could be confirmed by reference testing. The Vitek 2 achieved the best agreement with the reference data, whereas the Phoenix systems gave more false positive results (9/111 for Vitek 2 versus 28/125 for Phoenix, \(P < 0.05\)). Many of the false positives were for isolates without substantive resistance mechanisms. Four of ten AmpC hyperproducers tested by the Vitek 2 were inferred to have ESBL production; this proportion was slightly higher for the Phoenix system, at 7 of 13 \(P = 0.07\). All of the automated systems misinterpreted some cephalosporin-resistant ESBL producers as ESBL-negative; most of these had CTX-M enzymes (9/14).

**Discussion**

We conclude that cefpodoxime is a useful, if poorly specific, primary screen for oxyimino cephalosporin resistance and possible ESBL production for laboratories willing only to test
one agent. Parallel cefotaxime and ceftazidime testing is superior, capturing a slightly greater number of genuinely cephalosporin-resistant isolates, and miscalling considerably fewer of those with only borderline cephalosporin resistance and no substantive mechanism; nevertheless it demands the testing of two intravenous cephalosporins, and not all laboratories are prepared to undertake this, for example for community urines.

Acknowledgements

Members of the Steering Group: G. Duckworth, N. A. C. Potz, A. P. Johnson (HCAI & AMR Dept, HPA, London), D. M. Livemore, R. Hope (ARMRL, HPA, London), G. Fraser (LARS, HPA, London), E. Haworth (LARS, HPA, South East). Participating laboratories: Ashford (Kent) Microbiology Laboratory—M. Baker, G. Calver; Epsom Hospital—S. Chambers, P. Jackson, R. Prosser; Frimley Park Hospital, Camberley—R. Sharkey; Harold Wood Hospital—R. Reeve; Hillingdon Hospital—P. Kumari; Kingston Hospital—J. Leach, S. Patel; Milton Keynes General Hospital—D. Bardell; Northwick Park Hospital, Harrow—A. O’Connor, R. Wall; Royal Free Hospital, London—I. Balakrishnan, A. Ghafur; Royal Hampshire County Hospital, Winchester—M. Dryden, M. Grover, S. Lowden; Queen Elizabeth Hospital, Woolwich—M. Millett, G. Vosper; St Peter’s Hospital, Chertsey—S. Baillie; St Thomas Hospital, London—G. French, K. Shannon; Southampton HPA Laboratory—H. Humphrey; University College Hospital, London—C. Palmer, N. Shetty; Worthing Hospital—H. Plumb. This study was part-sponsored by Merck, Sharp & Dohme (US & UK).

Transparency declarations

All authors work in the field of antibiotic resistance and could be considered to have vested interests in investment in this area, whether by governments or charities or industry. D. M. L. is on a speakers bureaux for bioMerieux and has undertaken contract research for Oxoid, both of which have major commercial interests in susceptibility testing.

References


Table 2. Relative efficacy as represented by odds ratios of different combinations/ permutations of the three indicator cephalosporins for detection of isolates with a substantive resistance mechanism compared with sole cefpodoxime resistance for the laboratories using the disc method and testing all three cephalosporins in parallel.

<table>
<thead>
<tr>
<th>Cephalosporin resistances detected in screening</th>
<th>Odds ratio</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime</td>
<td>5.1</td>
<td>1.4–20.5</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>15.4</td>
<td>8.1–31.3</td>
</tr>
<tr>
<td>Cefpodoxime and cefotaxime</td>
<td>8.2</td>
<td>2.8–26.5</td>
</tr>
<tr>
<td>Cefpodoxime and ceftazidime</td>
<td>16.7</td>
<td>8.2–36.8</td>
</tr>
<tr>
<td>Cefotaxime and ceftazidime</td>
<td>29.0</td>
<td>7.9–185.8</td>
</tr>
<tr>
<td>Cefpodoxime, cefotaxime and ceftazidime</td>
<td>78.7</td>
<td>42.6–158.3</td>
</tr>
</tbody>
</table>

ESBL testing methods