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Detection and characterisation of extended-spectrum beta-lactamases among blood stream isolates of *Enterobacter* species in Hong Kong

Key Messages

1. Extended-spectrum beta-lactamase (ESBL) resistance in *Enterobacter* spp may be under-recognised.
2. Detection methods for ESBL resistance in *Enterobacter* spp may need to be modified.

Introduction

Among *Enterobacteriaceae*, production of extended-spectrum ß-lactamases (ESBLs) is increasingly recognised as a cause of resistance to expanded spectrum cephalosporins. In large hospitals, this resistance mechanism is found in 10-40% of *Klebsiella* spp and *Escherichia coli*. Less is known about the incidence of ESBL in *Enterobacter* because ESBL phenotypic screening of *Enterobacteriaceae* is commonly performed in the clinical laboratories only for *E coli* and *Klebsiella* spp. Detection of ESBL in *Enterobacter* spp is more complicated because high-level AmpC production interferes with tests that rely on synergism between clavulanic acid and a third-generation cephalosporin. Thus, recent studies have addressed this by using cefepime to replace the third-generation cephalosporins that were conventionally used. With this modification, a general hospital in Greece reported ESBL rates of 25% and 58% during 1998-1999 in a consecutive series of *Enterobacter cloacae* and *Enterobacter aerogenes* isolates, respectively.[1] Similarly, blood isolates of *Enterobacter* spp tested in a Korean tertiary hospital during 1994–2001 had an overall ESBL prevalence of 43%.[2] These findings highlight the potential for overlooking high ESBL rates in *Enterobacter* spp. In our study, we examined the production of AmpC and ESBL in blood isolates of *Enterobacter* spp collected over a 3-year period at two regional hospitals in Hong Kong.

Aims and objectives

To study the production of AmpC and extended-spectrum beta-lactamases in *Enterobacter* spp, with a view to improve surveillance of this type of emerging antibiotic resistance mechanisms.

Methods

Bacterial strains, identification, susceptibility testing and patient data

Blood isolates of *Enterobacter* isolated during 2000-2002 in two general regional hospitals were evaluated. Duplicate isolates were excluded by the first isolate per patient method. The VITEK GNI system was used for bacterial identification. For accurate identification at the species level, the glucose oxidation test was carried out as described. Antibiotic susceptibilities were tested by the disc diffusion method using Mueller–Hinton agar and interpreted according to the NCCLS.[3]

Detection of ESBL and AmpC

Three methods were used to detect ESBL production: modified double-disc synergy test, combined disc method, and the three-dimensional extract test.

Characterisation of ESBL-producing strains

Analytic IEF and filter mating were performed by standard methods. Beta-lactamase genes (TEM, SHV, CTX-M) were amplified and sequenced using class-specific primers.[4] The subset of ESBL-producing *Enterobacter hormaechei* was examined further by pulsed-field gel electrophoresis.

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Results

The ESBLs were identified in nine isolates (7%), including seven of 39 (18%) *E hormaechei*, one of 27 (4%) *E aerogenes* and the only *E intermedius* strain. The *E intermedius* strain was positive only in the three-dimensional extract test but not in the other two tests. The other eight strains were positive in all three tests. No ESBL was detected in other species, including non-hormaechei members of the *E cloacae* complex (n=61), *E agglomerans* (n=7), *E gergoviae* (n=4) and *E sakazakii* (n=1). For the detection of ESBL, the NCCLS screen method lacked specificity (63-72%). Discrimination could be improved using cefepime at a cut-off of ≤25 mm (sensitivity 90%, specificity 94%). The ESBL content included five different CTX-M enzymes (CTX-M-9, CTX-M-13, CTX-M-14, CTX-M-24 and a novel CTX-M-2-like ß-lactamase), SHV-12 (n=2) and unidentifiable ESBLs with a pI of 7.7 or 7.9 in two strains. The seven ESBL-producing *E hormaechei* were genotyped by pulsed-field gel electrophoresis and were found to be unrelated to each other. In three of the CTX-M-producing strains, ISEcp1-like elements, including promoters for the ß-lactamase gene, were found.

Discussion

The simple application of NCCLS criteria designed for ESBL detection in *E coli* and *Klebsiella* spp lead to many false-positive results in *Enterobacter* spp, in contrast to specificities of 97 to 99% reported for screening of *E coli* and *Klebsiella* spp.[5] In our collection of *Enterobacter* spp, it appeared to be possible to increase the test specificity by including cefepime in the initial screening. The high diversity of ESBLs among *Enterobacter* spp in Hong Kong is intriguing. In addition to having CTX-M and SHV classes of ESBL, each ß-lactamase combination in the nine strains was distinct. For the SHV class of ESBL, only SHV-12 was present among these strains. Elsewhere, this ESBL is known to exist among *E aerogenes* (in Italy) and among *E cloacae* (in Beijing, Taiwan, Korea and Thailand).[6] In agreement with previous studies, we demonstrated that four of the five isolates carrying *bla*CTX-M genes were able to transfer the ESBL phenotype in mating experiments, suggesting the spread of *bla*CTX-M genes by conjugative plasmids.[6] Interestingly, similar ß-lactamase combinations involving SHV-12, CTX-M-9, CTX-M-13 and CTX-M-14 were known to exist among *Enterobacteriaceae* collected from a hospital in Guangzhou. Millions of passengers travel between Guangzhou and Hong Kong annually. Our finding thus indicates a probable cross-boundary spread of ESBL-producing organisms or their determinants. The IS*Ecp1*-like element observed upstream of the *bla*CTX-M genes in the present study may involve in the translocation and dissemination of these ß-lactamase genes.[7]

Conclusions

The prevalence of ESBL among *Enterobacter* spp in Hong Kong is high and that their ß-lactamase content is diverse. Our finding adds to the increasing recognition of CTX-M enzymes in the Far East and further emphasises the need for screening ESBL in clinical isolates of *Enterobacter* spp.

The detection of ESBL in *Enterobacter* spp could be enhanced by: (1) inclusion of the glucose oxidation test for more accurate speciation of *E hormaechei*, and (2) inclusion of cefepime as an indicator in the ESBL screen.

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References

1. Tzelepi E, Giakkoupi P, Sofianou D, Loukova V, Kemeroglou A, Tsakris A. Detection of extended-spectrum beta-lactamases in clinical isolates of *Enterobacter cloacae* and *Enterobacter aerogenes*. J Clin Microbiol 2000;38:542-6.
2. Pai H, Hong JY, Byeon JH, Kim YK, Lee HJ. High prevalence of extended-spectrum beta-lactamase-producing strains among blood isolates of *Enterobacter* spp. collected in a tertiary hospital during an 8-year period and their antimicrobial susceptibility patterns. Antimicrob Agents Chemother 2004;48:3159-61.
3. Katz O, Peled N, Yagupsky P. Evaluation of the current NCCLS recommendations for screening and confirming ESBL production. 103rd American Society for Microbiology General Meeting, Washington, DC: American Society for Microbiology; 2003.
4. Ho PL, Shek RH, Chow KH, et al. Detection and characterization of extended-spectrum beta-lactamases among bloodstream isolates of *Enterobacter* spp. in Hong Kong, 2000-2002. J Antimicrob Chemother 2005;55:326-32.
5. Ho PL, Tsang DN, Que TL, Ho M, Yuen KY. Comparison of screening methods for detection of extended-spectrum beta-lactamases and their prevalence among *Escherichia coli* and *Klebsiella* species in Hong Kong. APMIS 2000;108:237-40.
6. Bonnet R. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 2004;48:1-14.
7. Poirel L, Decousser JW, Nordmann P. Insertion sequence ISEcp1B is involved in expression and mobilization of a bla(CTX-M) beta-lactamase gene. Antimicrob Agents Chemother 2003;47:2938-45.