Occurrence and molecular analysis of extended-spectrum \( \beta \)-lactamase-producing *Proteus mirabilis* in Hong Kong, 1999–2002

P. L. Ho\(^1\)*, Alex Y. M. Ho\(^1\), K. H. Chow\(^1\), River C. W. Wong\(^1\), R. S. Duan\(^1\), W. L. Ho\(^1\), Gannon C. Mak\(^1\), Kenneth W. Tsang\(^2\), W. C. Yam\(^1\) and K. Y. Yuen\(^2\)

\(^1\)Division of Infectious Diseases, Department of Microbiology and Centre of Infection, The University of Hong Kong, Queen Mary Hospital, Pokfulam Road, Pokfulam, Hong Kong Special Administrative Region, China; \(^2\)Department of Medicine, Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China

Received 8 February 2005; returned 17 March 2005; revised 23 March 2005; accepted 24 March 2005

**Objectives**: A study was conducted to evaluate the occurrence and characterization of extended-spectrum \( \beta \)-lactamases (ESBLs) among blood isolates of *Proteus mirabilis* collected over a 4 year period in Hong Kong.

**Methods**: Production of ESBLs among 99 consecutive and non-duplicate isolates was evaluated by the double-disc synergy test. The ESBLs were characterized by isoelectric focusing and PCR sequencing using specific primers. The epidemiological relationship of the isolates was studied by the Dienes test and PFGE.

**Results**: ESBLs were identified in 13 isolates, from none in 1999–2000 and up to 18.5% (5/27) in 2001 and 25.8% (8/31) in 2002. The ESBL-producing isolates were more resistant to ceftriaxone than to cefazidime, and were more likely than non-ESBL-producers to have resistance to ciprofloxacin (76.9% versus 14%) and gentamicin (38.5% versus 9.3%). The ESBL content included CTX-M-13 (8), CTX-M-14 (3), SHV-5 (2), TEM-11 (1), and an unidentified ESBL with a pI of 7.5. The Dienes test revealed that the genetic background in the 99 isolates was highly heterogeneous, with 54 distinct types among 92 isolates and seven were non-typeable. Among the 13 ESBL-producing isolates, five different backgrounds, including one cluster (Dienes-pulsotype A) with nine isolates, were identified by both Dienes test and PFGE, thus suggesting both clonal and multi-clonal spread of the CTX-M enzymes.

**Conclusions**: Our findings indicate the emergence of CTX-M enzymes among *P. mirabilis* in Hong Kong. More ESBL screening of this species is required to improve their recognition.

**Keywords**: antimicrobial resistance, epidemiology, cephalosporin resistance

**Introduction**

The emergence of extended-spectrum \( \beta \)-lactamases (ESBLs) in Gram-negative bacteria has become a major public health concern. It is clinically important to detect and specifically target ESBL because ESBL-producers may be clinically resistant to many \( \beta \)-lactams including the penicillins, aztreonam and oxyiminocephalosporins.\(^1\) Among the Enterobacteriaceae, the TEM- and SHV-derived ESBLs are often the predominant enzyme types among enterobacteria with an ESBL phenotype.\(^2\) In some South American countries, however, the CTX-M enzymes instead of the TEM- and SHV-derived enzymes are the predominant ESBL types.\(^3\) In a survey of 17 metropolitan public hospitals of Buenos Aires City, a CTX-M-2-related \( \beta \)-lactamase was found in 26 of 37 (70.3%) ESBL-producing enterobacteria.\(^4\) In Southeast Asia, a variety of CTX-M enzymes have been observed including: in mainland China, CTX-M-1, -3, -9, -13 and -14;\(^5-8\) in Taiwan, CTX-M-3, -14 and -15;\(^9-11\) in Hong Kong.
ESBL among *P. mirabilis* in Hong Kong

CTX-M-2, -9, -13, -14 and -24; in Korea, CTX-M-14; and in Japan, CTX-M-2, -3, -14, -M15, Toho-1 and Toho-2. In some of the above reports, the findings further indicated that the CTX-M group β-lactamases were already the predominant ESBL type in some Asian hospitals. In mainland China, one survey at the Peking Union Medical College hospital in 1999 identified CTX-M-3 among 19 of 27 (70.4%) ESBL-producing Enterobacteriaceae including three of five *Escherichia coli*, 11 of 17 *Klebsiella pneumoniae* and all of three *Enterobacter cloacae* and two *Citrobacter freundii*. This compared with another study in 1999 at the Huashan hospital that CTX-M-1 group was found in 13 of 58 (22.4%) *E. coli* and 41 of 81 (50.6%) *K. pneumoniae*. In Taiwan, at the National Cheng Kung University Hospital, a study of 1210 *E. coli* clinical isolates in 1999 identified 18 ESBL-producers, of which 10 isolates were found to harbour CTX-M-3. In Hong Kong, a study of 139 enterobacters in 2000 to 2002 by our group revealed CTX-M enzymes in five of nine unrelated ESBL producers.

Among *Proteus mirabilis*, strains harbouring CTX-M enzymes were previously observed in Italy (CTX-M-1); France (CTX-M-1 and -20); Brazil (CTX-M-8); and Argentina (CTX-M-2). Information on the prevalence of ESBL as well as the occurrence of CTX-M enzymes among *P. mirabilis* in Asian countries is lacking. Therefore, this study was conducted to evaluate the occurrence and characterization of ESBLs in blood isolates of *P. mirabilis* collected over a 4 year period in Hong Kong.

Materials and methods

**Bacterial strains, susceptibility testing and patient data**

Blood isolates of *P. mirabilis* isolated from 1999 to 2002 in eight hospitals in Hong Kong were evaluated. The eight hospitals include one 1400 bed university teaching hospital and seven district general hospitals (130–700 beds). The teaching hospital (QMH) provides acute care for all the major specialties for 12% of the 6.5 million population in Hong Kong, and has specialized centres for management of bone marrow, renal and liver transplant patients. Over the study period, there were altogether 114 consecutive blood isolates. By the first isolate per patient method, 19 repeat isolates from the study period, there were altogether 114 consecutive blood isolates. For the ESBL-producing strains, the patient records were reviewed as directed by the manufacturer (Roche Diagnostics, Mannheim, Germany). The primers TEM-F 5'-TTC TG TGC AAA ACC AGG GTT ATT CTT ATT TGT CGC-3' (position 882–900, accession no. AF126482) and TEM-R 5'-ACG CTC AGT GGA ACG AAA AC-3' (position 2089–2070, accession no. AF126482) were used to amplify a 1207 bp fragment containing the entire *bla*TEM gene and the upstream promoter. The nomenclature used for the *bla*TEM variants and promoters was as previously proposed. The primers SHV-1017F 5'-GCC CGG GTT ATT CTT ATT TGT CGC-3' (position 130–151, accession no. AF124984) and SHV-1017B 5'-TCT TTC TG TGC AAA ACC AGG GTT ATT CTT ATT TGT CGC-3' (position 1143–1120, accession no. AF124984) were used for the entire *bla*SHV gene. The CTX-M consensus primers MA1 forward 5'-SCV ATG TGC AGY ACC AGT AA-3' (position 270–289, accession no. X92506) and MA2 backward 5'-CCG CRA TAT GRT TGG TR-3' (position 794–813, accession no. X92506) were used as an initial screen. Subsequently, other primers specific for each CTX-M cluster were used to amplify the entire *bla*CTX-M gene. The CTX-M-9 group primers were M9U forward 5'-ATG GTG ACA AAG AGA GTG CA-3' (position 6288–6304, accession no. AF458080) and M9L backward 5'-CCC TTC TGC GGC GAT GAT TCT C-3' (position 957–975, accession no. D89862). For the CTX-M-positive strains, the region immediately upstream from *bla*CTX-M was explored by direct sequencing of amplicons obtained with forward primer ISEcp1U1 5'-AAAGAATTCTATGATAGAAGGTGT-3' (position 6285–6304, accession no. AF458080) and the consensus reverse MA2. All amplicons were generated and sequenced at least twice by automated PCR cycle sequencing with the BigDye dideoxynucleotide chain termination method in an ABI PRISM™ 377 Genetic Analyzer (Perkin-Elmer, Foster City, CA, USA). Amino acids in the β-lactamases were numbered according to Ambler et al. Positive (*E. coli* J35 Azii and water) controls were used in all reactions.

Presence of class I, II and III integrons in the strains were evaluated by PCR using primers published previously.

Detection of ESBL

Production of ESBL in the strains was screened by the double-disc synergy test. Two agar plates were inoculated as described for the standard disc diffusion test. In each plate, four 30 µg discs (ceftazidime, cefepoxide, ceftriaxone and cefepime) were placed at inter-disc distances (centre to centre) of 25 or 30 mm away from an amoxicillin/clavulanic acid disc (20/10 µg). A clear extension of the edge of the inhibition zone towards the disc containing clavulanic acid was interpreted as positive for ESBL production.

Molecular analysis of mechanism of resistance

A PCR and sequencing strategy was used to characterize enzymes related to the TEM, SHV and CTX-M families using primers previously described. A PCR and sequencing strategy was used to characterize enzymes related to the TEM, SHV and CTX-M families using primers previously described. A PCR and sequencing strategy was used to characterize enzymes related to the TEM, SHV and CTX-M families using primers previously described. A PCR and sequencing strategy was used to characterize enzymes related to the TEM, SHV and CTX-M families using primers previously described.
Isoelectric focusing (IEF)

Analytic IEF was performed in ampholine gel (pH 3.0 to 10.0; Pharmacia, Hong Kong, China) using lysozyme-based enzyme extract on an HE 950 isoelectric focusing apparatus (Hofer Scientific Instruments, CA, USA). Isolates were examined which had an ESBL phenotype in the screening tests but where the enzyme type remained unidentified by the PCR and sequencing strategies. The pl value of each enzyme was determined by spreading nitrocefin on the gel surface. Preparations from standard strains known to harbour TEM-1, TEM-8, SHV-4 and SHV-5 were used as markers. ESBL activity was detected by the substrate gel overlay method using cefotaxime at 1 mg/L and *Escherichia coli* ATCC 25922 as an indicator.

Antibiotic resistance transfer and plasmid content analysis

Plasmid DNA was extracted by two methods. Small plasmids (less than 10–20 kb) were extracted by the QIAprep Spin Miniprep Kit (Qiagen, Hong Kong) and analysed by conventional gel electrophoresis in accordance with manufacturer’s instructions. For detecting large plasmids, bacterial cells were embedded in agarose plugs and disrupted by alkaline lysis. Subsequently, the plasmids were converted to the linear forms by incubation with *Aspergillus oryzae* S1 nuclease (Sigma Chemical Co., St Louis, MO, USA) and were sized by pulsed-field gel electrophoresis (PFGE) as previously described. Filter conjugation was carried out in filters with *E. coli* J53Azr as the recipient. Cultures of the donor and recipient were collected on sterilized filters (Whatman International Ltd, Maidstone, UK) and the filter was incubated on the surface of an LB agar plate overnight. Inhibition of swarming was achieved by incubation at 30°C. Transconjugants were selected on Trypticase soy agar (TSA) plates containing sodium azide (150 mg/L; Sigma Chemical Co.) to select for plasmid-encoded resistance.

Epidemiological typing

All strains were typed by the Dienes test using the procedure described by Skirrow. Conventional 5% horse blood agar plates were used. Discrimination of unique strains is based on the mutual inhibition of two different strains as they swarm towards each other on the agar surface. Initially, seven strains were spot inoculated onto one 9 cm plate to allow reading of 12 combinations. Inoculated plates were incubated at 35°C for 18 to 24 h before reading. The presence of mutual inhibition lines (i.e. Dienes lines) between two isolates indicates that the isolates are unrelated and represented distinct Dienes types. On the other hand, strains were assigned to the same type if Dienes lines were absent. Each pair of compatible reactions was always confirmed by repeat testing. In the confirmatory testing, each agar plate was spot inoculated with one pair of compatible isolates. Slowly swarming isolates were incubated for up to 48 h. If their spreading growths had not met, the test was repeated on a plate containing a thicker layer of agar. In this manner, each isolate was tested against all other isolates. The letters A, B, C and so on were assigned for each distinct Dienes type.

The subset of ESBL-producing strains was examined further by PFGE. *Nor1* (Amersham Pharmacia Biotech, Little Chalfont, UK) was used for macrorestriction digestion of genomic DNA. The fragments were resolved in 1% gel in a CHEP Mapper XA (Bio-Rad) for 22 h at 6 V/cm gradient and an included angle of 120°. The switching time ramped from an initial 2.2 s to a final 54.2 s.

Statistical analysis

The Chi-square, Fisher’s exact or Student’s *t*-test was used for statistical analysis. A *P* value of <0.05 was considered significant. For the purpose of analysis, non-susceptible including both intermediate and resistant categories was used in the calculation.

Nucleotide sequence accession number

The following β-lactamase genes have been submitted to the GenBank nucleotide sequence database under the following accession numbers: AY874537 (*bla*TEM-14, strain 217C5), AY870398 (*bla*CTX-M-13, strain 209A4), AY870399 (*bla*CTX-M-14, strain 25513).

Results

Frequency of ESBL production and antimicrobial susceptibility

The double-disc synergy test identified 13 (13.1%) isolates as ESBL-producers and 86 (86.9%) isolates as ESBL-non-producers. In terms of year of isolation, the ESBL rates were: 0% (0/26) in 1999, 0% (0/15) in 2000, 18.5% (5/27) in 2001 and 25.8% (8/31) in 2002. On the basis of the inhibition zone diameters alone, one of the 13 ESBL-producing isolates was susceptible to cefotaxime, six were susceptible to ceftriaxone (≥21 mm) and 11 were susceptible to ceftazidime (≥18 mm). In order to evaluate the effectiveness of extending the NCCLS *E. coli* and *Klebsiella* spp. ESBL breakpoints for initial screening of *P. mirabilis*, interpretation of the disc results for cefotaxime, ceftriaxone and ceftazidime were tabulated against the ESBL production status (not shown). The sensitivity and specificity of the NCCLS screening criteria were as follows: 92.3% and 100% for cefotaxime, 92.3% and 100% for ceftriaxone, and 15.3% and 100% for ceftazidime, respectively. One isolate (strain 217C5) escaped detection by all three discs (inhibition zone 23 mm for ceftazidime, 31 mm for cefotaxime and 35 mm for ceftriaxone).

Susceptibility of the isolates according to ESBL production is shown in Table 1. All isolates were susceptible to cefoxitin, suggesting that there were no AmpC-like enzymes. Strains with ESBL were more likely than those without to have resistance to gentamicin, tobramycin and ciprofloxacin. The non-susceptible rates for chloramphenicol, and co-trimoxazole were similar for the two groups. Multidrug resistance phenotypes for the non-β-lactam antibiotics were high in both groups. Most ESBL-producing strains were intermediate or resistant to ampicillin/sulbactam and amoxicillin/clavulinate while all were susceptible to piperacillin/tazobactam. All isolates were susceptible to amikacin and the two carbapenems.

Characteristics of the ESBL-producing strains

All ESBL-producing strains were isolated from patients with healthcare-related infections. All 13 patients had one or more underlying diseases such as stroke (*n* = 4), dementia (*n* = 2), diabetes mellitus (*n* = 2) and chronic lung disease (*n* = 2). Nine patients were residents in old age homes. In most patients (9 of 13), the source of bacteraemia was urinary catheter-related infection. The β-lactamase-specific PCR and sequencing revealed the following ESBL enzymes (Table 2): CTX-M-13 (*n* = 8), CTX-M-14 (*n* = 3), SHV-5 (*n* = 2), and TEM-11 (*n* = 1). In addition,
ESBL among *P. mirabilis* in Hong Kong

| Table 1. Antimicrobial susceptibility of *P. mirabilis* with and without ESBL |
|-----------------------------------------------|--------------------|-----------------|-----------------|
| **Agent(s)**                               | **No. (%) non-susceptible** |
| **ESBL-positive** (n = 13) | **ESBL-negative** (n = 86) | **P value** |
| Ampicillin                                  | 13 (100%)           | 54 (62.8%)      | 0.008           |
| Cefoxitin                                   | 0 (0%)              | 0 (0%)          | –               |
| Imipenem                                    | 0 (0%)              | 0 (0%)          | –               |
| Meropenem                                   | 0 (0%)              | 0 (0%)          | –               |
| Amoxicillin/clavulanate                     | 9 (69.2%)           | 21 (24.4%)      | 0.001           |
| Ampicillin/sulbactam                        | 11 (84.6%)          | 30 (34.9%)      | 0.001           |
| Piperacillin/tazobactam                     | 0 (0%)              | 1 (1.2%)        | 0.7             |
| Ciprofloxacin                               | 10 (76.9%)          | 12 (14%)        | <0.001          |
| Co-trimoxazole                              | 5 (38.5%)           | 23 (26.7%)      | 0.4             |
| Chloramphenicol                             | 8 (61.5%)           | 38 (44.2%)      | 0.3             |
| Tobramycin                                  | 7 (53.8%)           | 18 (20.9%)      | 0.01            |
| Kanamycin                                   | 6 (46.2%)           | 30 (34.9%)      | 0.4             |
| Gentamicin                                  | 4 (30.8%)           | 8 (9.3%)        | 0.004           |
| Netilmicin                                  | 0 (0%)              | 4 (4.6%)        | –               |
| Amikacin                                    | 4 (30.8%)           | 16 (18.6%)      | 0.3             |
| MDR                                         | 6 (46.2%)           | 19 (22.1%)      | 0.06            |

MDR, multidrug resistance was defined as resistance to at least one aminoglycoside plus two or more of ciprofloxacin, co-trimoxazole and chloramphenicol.

TEM-2 was found in nine isolates and TEM-1b in one isolate. The gene encoding the TEM-11 had nucleotide sequences (including C at 226, A at 317, G at 346, T at 436, G at 604, C at 843 and A at 925 according to Sutcliffe numbering) that were characteristic of the *bla*<sub>TEM</sub> gene in *E. coli* and differed from TEM-2 in Arg-164 (CGT) being replaced by His-164 (CAT). One strain had an unidentified ESBL with a pI of 7.5 in addition to a TEM-682 and aCHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; SUZ, sulfathiazole; TET, tetracycline; TOB, tobramycin.

Table 2. Characteristics of 13 ESBL-producing *P. mirabilis* isolates in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Year</th>
<th>Dienes type</th>
<th>Pulsotype</th>
<th>Associated resistance markers&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Integrons</th>
<th>β-Lactamase content&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Plasmid profile (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>209A4</td>
<td>2001</td>
<td>A</td>
<td>A</td>
<td>TET-SUZ-CIP-CHL-GEN-TOB</td>
<td>inl 1</td>
<td>TEM-2, CTX-M-13, SHV-5</td>
<td>45, 280</td>
</tr>
<tr>
<td>210A9</td>
<td>2001</td>
<td>A</td>
<td>A</td>
<td>TET-SUZ-CIP-GEN-TOB</td>
<td>inl 1</td>
<td>TEM-2, CTX-M-13, SHV-5</td>
<td>45, 300</td>
</tr>
<tr>
<td>216D9</td>
<td>2001</td>
<td>A</td>
<td>A</td>
<td>TET-SUZ-CIP-TOB</td>
<td>inl 1</td>
<td>TEM-2, CTX-M-13</td>
<td>45, 97</td>
</tr>
<tr>
<td>223H5</td>
<td>2002</td>
<td>A</td>
<td>A</td>
<td>TET-SUZ-CIP-CHL-GEN-TOB</td>
<td>inl 1</td>
<td>TEM-2, CTX-M-13</td>
<td>45, 97</td>
</tr>
<tr>
<td>224H9</td>
<td>2002</td>
<td>A</td>
<td>A</td>
<td>TET-CIP</td>
<td>inl 1</td>
<td>TEM-2, CTX-M-13</td>
<td>45, 97</td>
</tr>
<tr>
<td>231F5</td>
<td>2002</td>
<td>A</td>
<td>A</td>
<td>TET-SUZ-CIP-TOB</td>
<td>inl 1</td>
<td>TEM-2, CTX-M-13</td>
<td>45, 97, 280</td>
</tr>
<tr>
<td>249I8</td>
<td>2002</td>
<td>A</td>
<td>A</td>
<td>TET-SUZ-CIP</td>
<td>none</td>
<td>TEM-2, CTX-M-13</td>
<td>45, 97, 280</td>
</tr>
<tr>
<td>252D3</td>
<td>2002</td>
<td>A</td>
<td>A</td>
<td>TET</td>
<td>inl 1</td>
<td>TEM-2, CTX-M-13</td>
<td>45, 97, 280</td>
</tr>
<tr>
<td>217C5</td>
<td>2001</td>
<td>A</td>
<td>A</td>
<td>TET-SUZ-CHL</td>
<td>none</td>
<td>TEM-11</td>
<td>45, 97, 280</td>
</tr>
<tr>
<td>228C1</td>
<td>2002</td>
<td>B</td>
<td>C</td>
<td>TET-CIP-CHL</td>
<td>inl 1, intl 2</td>
<td>CTX-M-14</td>
<td>2, 3.5</td>
</tr>
<tr>
<td>189D6</td>
<td>2001</td>
<td>C</td>
<td>E</td>
<td>TET-SUZ-CHL</td>
<td>none</td>
<td>CTX-M-14</td>
<td>1.5, 2.5, 6, 15</td>
</tr>
<tr>
<td>232C6</td>
<td>2002</td>
<td>D</td>
<td>B</td>
<td>TET-SUZ-CIP-CHL-GEN-TOB</td>
<td>int 1, intl 2</td>
<td>TEM-1, pl 7.5</td>
<td>220</td>
</tr>
<tr>
<td>255I3</td>
<td>2002</td>
<td>E</td>
<td>D</td>
<td>TET-SUZ-CIP-CHL-GEN-TOB</td>
<td>int 1</td>
<td>TEM-2, CTX-M-14</td>
<td>45, 220</td>
</tr>
</tbody>
</table>

<sup>a</sup>CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; SUZ, sulfathiazole; TET, tetracycline; TOB, tobramycin.

<sup>b</sup>*Pa*Pa was the promoter for all TEM-2 and TEM-1.

Epidemiological typing of strains

Seven isolates did not produce sufficient swarming on blood agar surface and were not typeable in the Dienes test. The remaining 92 isolates constituted 54 different Dienes types (A to BB). The distribution of the Dienes types according to ESBL status is shown in Table 3. Fifty-seven formed 13 clusters of 2 to 11 isolates each. There was only one isolate for each of the remaining 41 Dienes types. The 13 ESBL-producing isolates fell into five Dienes types: nine isolates for Dienes type A and one each for Dienes type B to E. Further analysis of the 13 isolates producing ESBL using PFGE identified five distinct pulsortypes (Table 2) and the results were concordant with that obtained by the Dienes test. The nine isolates of Dienes type A had PFGE patterns that were identical or differed by one to four fragments (pulsotype A). The four isolates with Dienes type B to E had four unrelated pulsortypes B to E.

Analysis of the plasmid contents and transfer of antibiotic resistance

Attempts to transfer ESBL phenotype from *P. mirabilis* to *E. coli* recipients were unsuccessful despite repeated testing. The ESBL-producing strains had one to four plasmids with size ranging from ~1.5 to 300 kb (Table 2). A common 45 kb plasmid was found in all the CTX-M-13-producing strains. The three CTX-M-14-producing strains had plasmids of different sizes.

Discussion

Our data revealed a high prevalence of ESBL among *P. mirabilis* that caused invasive infection in Hong Kong. Since ESBL screening of *P. mirabilis* was not routinely performed in the clinical laboratories, the ESBL status for 12 of the 13
ESBL-producing isolates was not reported at the time they were isolated. Despite a dramatic increase in the ESBL rate from 0% in 1999 to 25.8% in 2002, the change over time within this species and the clonal spread of Dienes-pulsotype A have gone completely unnoticed. Antibiotic resistance problems such as ESBL can vary greatly depending on the patient populations, time periods, geographic regions as well as the methods for collecting and testing strains. In Europe, 15.2% of 688 P. mirabilis isolates collected by the ICARE project during 1996 to 1999 from 53 hospital laboratories in the United States.

For detecting ESBL, screening with both ceftriaxone and ceftazidime was useful for a small sample of P. mirabilis. However, the number of ESBL-positive isolates potentially missed by the NCCLS selection criteria remains unknown because they were not tested. Further studies to evaluate the utility of NCCLS guidelines for identifying ESBLs in P. mirabilis are warranted.

The diversity of ESBLs among the 13 P. mirabilis in this study is intriguing. To the best of our knowledge, this is also the first time CTX-M-13, CTX-M-14 and TEM-11 have been found in P. mirabilis. Among our ESBL-producing strains, TEM-1 was the major ESBL type. This is in contrast to the situation in other parts of the world in which TEM-type enzymes were the major ESBL type reported in this bacterial species. In P. mirabilis, the first ESBL, TEM-3 was found in a strain isolated in France in 1989. Subsequently, TEM-8, TEM-15, TEM-20, TEM-21, TEM-24, TEM-52, TEM-57, TEM-66, TEM-72, TEM-87 and TEM-92 were described in Europe and mainly in France and Italy. In the United States, TEM-10 was also known to occur in P. mirabilis. In Argentina where the CTX-M enzymes are widespread, one study found that CTX-M was also prevalent among a small sample of P. mirabilis. As was reported previously, our finding indicates that TEM-2 but not TEM-1 was the major penicillinase in P. mirabilis.

The Dienes test is a simple and highly discriminatory tool for epidemiological typing of P. mirabilis, and is easy to perform. In this study, typing by the Dienes test revealed both clonal and multi-clonal spread of the CTX-M enzymes. As Dienes type A included multiple ESBL- as well as non-ESBL-producing strains, it is possible that strains belonging to this type represent a highly successful lineage. Interestingly, five distinct resistance patterns for the non-β-lactam antibiotics can be recognized among the nine ESBL-producing strains that shared the same Dienes type. As different plasmids were seen in the Dienes A isolates, this may indicate variations in plasmid-mediated resistance determinants.

It is known that genes encoding the CTX-M-type enzymes are often found in transferable plasmids and are readily transmissible by conjugation. However, the ESBL phenotype in the strains did not transfer in this study. This may be explained by previous observations that the efficiency of conjugation from P. mirabilis to E. coli is low. In agreement with the association of the CTX-M with integrons, most of our CTX-M-producing strains had one or two integrons.

In conclusion, our data indicate a high prevalence of ESBL among P. mirabilis in Hong Kong and their enzyme types are diverse. Our findings add to the increasing recognition of CTX-M enzymes in Southeast Asia and emphasize the need for enhanced surveillance of ESBL in this species.

Acknowledgements

Part of the work was submitted by A. Y. M. H. to the University of Hong Kong in partial fulfilment of the requirement for Master in Medical Sciences. The work is supported by research grants from the Research Fund for the Control of Infectious Diseases of the Health, Welfare and Food Bureau of the Hong Kong SAR Government and from the University Development Fund Project-Research Centre of Emerging Infection Diseases of the University of Hong Kong.

References

sequence information and proposed nomenclature for bla

Presentation of Cumulative Antimicrobial Susceptibility Test Data: J Clin Microbiol
Klebsiella pneumoniae healthy animals.

Escherichia coli

Informational Supplement M100-S12.

ance Standards for Antimicrobial Susceptibility Testing: Twelfth
detection of extended-spectrum inhibitor-potentiated disc-diffusion test with other methods for the
associated with integrons, the extended-spectrum
isolated in three Parisian hospitals.


Yamasaki K, Komatsu M, Yamashita T et al. Production of CTX-

Saladin M, Cao VT, Lambert T et al. Diversity of CTX-M β-

Bonnet R, Sampaio JL, Labia R et al. A novel CTX-M β-
lactamase (CTX-M-8) in cephalotaxine-resistant Enterobacteriaceae iso-

Bauernfeind A, Stempler I, Jungwirth R et al. Sequences of β-
lactamase genes encoding CTX-M-1 (MEN-1) and CTX-M-2 and relationship of their amino acid sequences with those of other β-


National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Susceptibility Testing: Twelfth
Informational Supplement M100-S12. NCCLS, Wayne, PA, USA, 2002.

Ho PL, Chow KH, Yuen KY et al. Comparison of a novel, inhibitor-potentiated disc-diffusion test with other methods for the
detection of extended-spectrum β-lactamases in Escherichia coli and


Brinas L, Zarazaga M, Saenz Y L et al. Lactamases in ampicillin-resistant β-lactamase isolates from foods, humans, and

Leflon-Guibout V, Heym B, Nicolas-Chanoine MH. Updated sequence information and proposed nomenclature for blatem genes


Kerr MB, Klemmensen T, Frimodt-Moller N et al. Susceptibility of Danish Escherichia coli strains isolated from urinary tract infections
and bacteremia, and distribution of sul genes conferring sulphon-

Reyes A, Bello H, Domínguez M et al. Prevalence and types of

Siu LK, Lo JYC, Yuen KY et al. β-Lactamases in Shigella flexneri isolates from Hong Kong and Shanghai and a novel OXA-1-like


Barton BM, Harding GP, Zuccarelli AJ. A general method for

Coetzee JN. Extension of a chromosome linkage group of

Skirrow MB. The dienes (mutual inhibition) test in the investi-

Mutnick AH, Turner PJ, Jones RN. Emerging antimicrobial
resistances among Proteus mirabilis in Europe: report from the
MYSTIC Program (1997-2001). Meropenem Yearly Susceptibility Test

Schwaber MJ, Raney PM, Rasheed JK et al. Utility of NCCLS
guidelines for identifying extended-spectrum β-lactamases in non-

Mariotte S, Nordmann P, Nicolas MH. Extended-spectrum β-


de Champs C, Monne C, Bonnet R et al. New TEM variant (TEM-92) produced by Proteus mirabilis and Providencia stuartii


Pagani L, Migliavacca R, Pallecchi L et al. Emerging extended-

Perilli M, Segatore B, de Massis MR et al. TEM-72, a new extended-spectrum β-lactamase detected in Proteus mirabilis and

Perilli M, Segatore B, Massis MR et al. Characterization of a

Palzkill T, Thomson KS, Sanders CC et al. New variant of TEM-

Cullmann W, Flensberg T, Opferkuch W et al. Correlation of β-
lactam production and resistance to β-lactam antibiotics in