Community spread of extended-spectrum $\beta$-lactamase-producing *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*: a long-term study in Japan

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COMMUNITY-ACQUIRED INFECTIONS CAUSED BY EXTENDED-SPECTRUM $\beta$-LACTAMASE (ESBL)-PRODUCING BACTERIA, PARTICULARLY CTX-M-PRODUCING *ESCHERICHIA COLI*, ARE A RISING CONCERN WORLDWIDE. THERE ARE FEW DATA FROM JAPAN ON THE ACQUISITION OF ESBLS IN THE COMMUNITY OR THE INFLUX OF THESE BACTERIA INTO HOSPITALS. THEREFORE, WE EXAMINED THE PREVALENCE OF ESBL CARREIAGE IN OUTPATIENTS, IN ORDER TO ESTIMATE THE SPREAD OF ESBLS IN COMMUNITY SETTINGS. WE ANALYSED BACTERIAL ISOLATES FROM OUTPATIENT SAMPLES AT OUR INSTITUTION OVER A 9-YEAR PERIOD FROM 2003 TO 2011, WITH RESPECT TO EPIDEMIOLOGICAL DATA ON ESBL-PRODUCING BACTERIA AND THEIR GENOTYPIC FEATURES. OUT OF 5137 ISOLATES, 321 (6.3 %) WERE ESBL PRODUCERS, INCLUDING *E. coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. THE DETECTION RATES OF THE ESBL-PRODUCING ISOLATES GRADUALLY INCREASED AND REACHED 14.3, 8.7 AND 19.6 % FOR *E. coli*, *K. pneumoniae* AND *P. mirabilis* STRAINS, RESPECTIVELY, IN 2011. GENOTYPING ANALYSIS SHOWED THAT MANY OF THE STRAINS PRODUCED MULTIPLE $\beta$-LACTAMASES, INCLUDING TEM, SHV AND CTX-M, RATHER THAN JUST CTX-M. THE CTX-M-9 GROUP WAS DOMINANT AMONG THE CTX-M GENOTYPES; FURTHER, THE CTX-M-1 AND M-2 GROUPS WERE ALSO DETECTED (~30 %). THIS IS BELIEVED TO BE THE FIRST REPORT FROM JAPAN SHOWING A DEFINITE INCREASE IN ESBL DETECTION IN OUTPATIENTS. IN ADDITION, OUR FINDINGS SUGGEST THE SIMULTANEOUS COMMUNITY SPREAD OF DIVERSE ESBL GENOTYPES, NOT AN EXPANSION OF PARTICULAR ESBL GENES.

INTRODUCTION

The emergence of extended-spectrum $\beta$-lactamase (ESBL)-producing bacteria is of concern worldwide (Pitout & Laupland, 2008). ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* are now listed as two of the six drug-resistant pathogens for which few potentially effective drugs are available (Talbot et al., 2006). Until the end of the 1990s, most of the ESBLs detected were either TEM or SHV types as a main genotype, and were usually associated with nosocomial outbreaks caused by *K. pneumoniae* (Paterson & Bonomo, 2005). Since 2000, the number of ESBL-producing *E. coli* isolates has been dramatically increasing (Oteo et al., 2010). A recent global surveillance database showed that the detection rates for ESBL-producing *K. pneumoniae* and *E. coli* isolates were ~50 % and ~15 %, respectively (Rossi et al., 2006; Reinert et al., 2007). The ESBL-producing *E. coli* predominantly carry CTX-M types, which belong to a new ESBL family distinct from the TEM and SHV types (Oteo et al., 2010; Pitout & Laupland, 2008). Notably, the origin of the CTX-M ESBLs is completely different from that of TEM or SHV ESBL, and CTX-M-producing *E. coli* strains usually spread in the community. This epidemiological situation is becoming a new concern for outpatient therapy because of the multi-antibiotic resistance of these community-spreading ESBL-producing bacteria.

The first ESBL-producing bacterium was isolated in Japan in 1993 (Ishii et al., 1995). The detection rates of ESBL-producing *E. coli* strains in hospitalized patients reached around 5 % in a nationwide survey conducted in 2009 (Yamaguchi et al., 2011), suggesting that organisms with
ESBLs have been spreading gradually throughout Japan. Recently, we reported a longitudinal observation of the nosocomial spread of ESBL-producing bacteria in our hospital (Hara-Sanshin Hospital) (Chong et al., 2011). ESBL-producing E. coli strains have been frequently isolated in recent years. Intriguingly, CTX-M ESBLs have been more frequently detected than the TEM or SHV types. Considering the origin of CTX-M genes, the influx of ESBL-producing bacteria from the community may be associated with the spread within our hospital. In fact, no data have been reported from Japan regarding community-acquired ESBL infections, which is reflected in part by the prevalence of ESBL carriage in outpatients. Thus, in Japan, little is known about the dissemination of ESBLs in community settings or the influx of ESBL carriers into hospitals.

Therefore, the aim of this study was to examine the epidemiological data obtained from outpatients, including the prevalence of ESBL-producing bacteria and their genotypic features. We longitudinally analysed 5137 bacterial isolates from outpatients at our hospital over a 9-year period from 2003 to 2011.

METHODS

Samples. The present study was conducted at Hara-Sanshin Hospital, a general tertiary hospital with 359 beds, in south-western Japan. To detect ESBL-producing bacteria, all bacterial isolates from outpatients were analysed during the period from January 2003 to December 2011. More than 500 urine samples are cultured every year in the urology department of our hospital. Out of 5137 isolates, 4628 (90.1%) were from urine. The types of isolated samples other than urine were stool (5.3%) and sputum (2.7%). Bacterial isolates from hospitalized patients were also analysed during the same period. In total, 3101 isolates were obtained throughout the hospital, including 334 in 2003, 302 in 2004, 330 in 2005, 319 in 2006, 305 in 2007, 327 in 2008, 387 in 2009, 427 in 2010 and 370 in 2011. The samples isolated from hospitalized patients were of the following types: urine (66.0%), stool (15.1%), sputum (9.4%), blood (3.8%) and others (5.7%). During this period, the main ESBL producers were E. coli, K. pneumoniae and P. mirabilis; therefore, the detection rates of these species were calculated in terms of their isolation numbers. Information on the isolated strains, including aetiology and susceptibility to antibiotics, was obtained from a microbiology laboratory computer database.

Microbiology. Urine samples were cultured, chiefly using CLED Agar medium (BD). The species were identified using the Vitrek system (bioMérieux). When several ESBL-producing strains were detected from the same patient, only one sample was counted as an ESBL isolate. Antibiotic susceptibilities were determined using the breakpoints standardized by the Clinical and Laboratory Standards Institute (CLSI) (Jacoby & Munoz-Price, 2005). Screening and confirmatory tests on the ESBL-producing bacteria were conducted according to CLSI recommendations. In addition, β-lactamase producers were confirmed using a Cica β test I/MBL kit (Kanto Chemical Company). Strains positive for ESBLs in the confirmatory test were then examined for the genotypes. PCR analysis was conducted using five sets of primers to amplify type-specific ESBL genes, including the CTX-M, TEM and SHV genes (Muratani et al., 2006). Three sets of primers were used to detect group-specific CTX-M genes (Muratani et al., 2006).

Statistical analysis. Categorical variables were analysed using a Fisher’s exact test. P<0.05 was considered to be statistically significant. All statistical calculations were performed using the SAS software (SAS Institute, Cary, NC, USA).

RESULTS

Identification of ESBL-producing bacteria from outpatients

From 2003 to 2011, the main ESBL-producing bacteria isolated from outpatients were E. coli, K. pneumoniae and P. mirabilis, and 321 (6.3%) of 5137 isolates were identified as ESBL producers (Table 1). The detection rates of ESBL-producing E. coli, K. pneumoniae and P. mirabilis strains were 6.4, 5.1 and 7.2%, respectively. These rates gradually increased and reached 8–20% for all three bacteria in 2011. The detection rate of the E. coli strains increased first, and this was followed by increases in the detection rates of the K. pneumoniae and P. mirabilis strains.

Molecular characterization of ESBL-producing bacteria

The ESBL-producing bacteria were genotyped for β-lactamase genes. The genotypes were classified into the TEM and/or SHV (TEM/SHV), CTX-M, or TEM/SHV and CTX-M (TEM/SHV + CTX-M) genotypes, because many strains produced multiple β-lactamases (Table 2). The TEM/SHV + CTX-M genotype was the most prevalent in all three bacteria.

Among the ESBL genotypes, the CTX-M genotype has been reported to spread rapidly worldwide, and specific genetic groups have been characterized in different geographical areas (Cantón & Coque, 2006). The CTX-M genotypes detected in our hospital could be divided into three groups: CTX-M-1, CTX-M-2 and CTX-M-9 (Table 3). Most of the E. coli isolates carried the CTX-M-9 group. The K. pneumoniae as well as the E. coli isolates predominantly expressed the CTX-M-9 group. In contrast, the CTX-M-2 group was the most prevalent in the P. mirabilis isolates.

Comparison of ESBL detection among out- and inpatients

To examine whether the increase in ESBL detection from outpatients was related to the spread of ESBLs within our hospital, the number of each ESBL-producing bacterium detected was compared between out- and inpatients in our hospital (Fig. 1). In both out- and inpatients, ESBL-producing E. coli were isolated from the year 2003. These strains steadily increased in number, and reached ~20% in 2011. During the same period, out- and inpatients also carried ESBLs in other bacteria, including K. pneumoniae and P. mirabilis. Interestingly, the frequent detection of ESBLs was first observed in the E. coli strains, and was followed by the K. pneumoniae and P. mirabilis strains, irrespective of...
whether the patients were out- or inpatients. The detection rates of ESBL-producing *K. pneumoniae* and *P. mirabilis* strains increased during recent years, similarly to that of the *E. coli* strains, and reached ~10 and ~30 %, respectively.

**DISCUSSION**

In the new millennium, the mode of ESBL infection dramatically changed throughout the world, and CTX-M-producing *E. coli* became the primary ESBL producers associated with community-acquired infections (Oteo *et al.*, 2010; Pitout & Laupland, 2008). More recently, community-acquired infections due to ESBL-producing *K. pneumoniae* and *P. mirabilis* have also been reported (Ben-Ami *et al.*, 2006; Hansen *et al.*, 2012; Luzzaro *et al.*, 2006; Roux *et al.*, 2012; Soge *et al.*, 2006; Tumbarello *et al.*, 2011; Valverde *et al.*, 2008). In 2011, the prevalence of ESBL-producing *K. pneumoniae* and *P. mirabilis* isolates in our outpatients was 14.3, 8.7 and 19.6 %, respectively (Table 1). The detection rates of ESBL-producing bacteria in outpatients and inpatients on admission were shown to be ~15 % (Ben-Ami *et al.*, 2006; Luzzaro *et al.*, 2006). In terms of faecal carriage of ESBL-producing bacteria, studies have reported a frequency of ~10 % (Oteo *et al.*, 2010; Woerther *et al.*, 2010). The prevalence of healthy carriers with ESBLs in the community was recently shown to be 6.4 % in Japan (Luvsansharav *et al.*, 2011).

In Japan, the CTX-M-2 group was thought to be the main CTX-M group; toho-1, belonging to the CTX-M-2 group, was the first ESBL isolated in Japan (Hawkey, 2008). Therefore, the pattern of the CTX-M genotypes in Japan was considered to be quite different from that observed in the surrounding East-Asian countries (Bonnet, 2004; Hawkey, 2008). However, recent nationwide surveillances, which were conducted on hospitalized patients, reported that the CTX-M-9 group was predominant in *E. coli* (Shibata *et al.*, 2006; Suzuki *et al.*, 2009), which is consistent with the genotypic pattern observed in other Asian countries. The genotypic features of CTX-M in our study were similar to those observed in the nationwide surveillances for all three bacteria (Table 3), suggesting that similar CTX-M types are spreading throughout Japan, regardless of in- or outpatient status. The dissemination of CTX-M-15-producing bacteria has been reported

<table>
<thead>
<tr>
<th>Year</th>
<th>Isolates, total</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
<th>P. mirabilis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>With ESBL</td>
<td>Total</td>
<td>With ESBL</td>
</tr>
<tr>
<td>2003</td>
<td>522</td>
<td>5 (1.0)*</td>
<td>432</td>
<td>5 (1.2)*</td>
</tr>
<tr>
<td>2004</td>
<td>531</td>
<td>15 (2.8)</td>
<td>429</td>
<td>15 (3.5)</td>
</tr>
<tr>
<td>2005</td>
<td>515</td>
<td>15 (2.9)</td>
<td>432</td>
<td>14 (3.2)</td>
</tr>
<tr>
<td>2006</td>
<td>569</td>
<td>19 (3.3)</td>
<td>468</td>
<td>16 (3.4)</td>
</tr>
<tr>
<td>2007</td>
<td>516</td>
<td>21 (4.1)</td>
<td>426</td>
<td>20 (4.7)</td>
</tr>
<tr>
<td>2008</td>
<td>575</td>
<td>40 (7.0)</td>
<td>468</td>
<td>32 (6.8)</td>
</tr>
<tr>
<td>2009</td>
<td>575</td>
<td>46 (8.0)</td>
<td>465</td>
<td>36 (7.7)</td>
</tr>
<tr>
<td>2010</td>
<td>640</td>
<td>65 (10.2)</td>
<td>525</td>
<td>54 (10.3)</td>
</tr>
<tr>
<td>2011</td>
<td>694</td>
<td>95 (13.7)*</td>
<td>533</td>
<td>76 (14.3)*</td>
</tr>
<tr>
<td>Total</td>
<td>5137</td>
<td>321 (6.3)</td>
<td>4178</td>
<td>268 (6.4)</td>
</tr>
</tbody>
</table>

*P<0.0001.
†P<0.001.
‡P<0.01.
§P<0.1.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Total isolates</th>
<th>TEM/SHV</th>
<th>CTX-M</th>
<th>TEM/SHV+CTX-M</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>223</td>
<td>18 (8.1)</td>
<td>85 (38.1)</td>
<td>120 (53.8)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>31</td>
<td>5 (16.1)</td>
<td>2 (6.5)</td>
<td>24 (77.4)</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>12</td>
<td>0 (0.0)</td>
<td>4 (33.3)</td>
<td>8 (66.7)</td>
</tr>
<tr>
<td>Total</td>
<td>266</td>
<td>23 (8.6)</td>
<td>91 (34.2)</td>
<td>152 (57.2)</td>
</tr>
</tbody>
</table>
worldwide, and the mode of spread is epidemic in every area (Cantón & Coque, 2006; Peirano & Pitout, 2010). CTX-M-15 belongs to the CTX-M-1 group. Thus, the spread of CTX-M-15 is less characteristic in Japan, including the south-western region where our hospital is located. Recently, Khanna et al. (2012) reported the community spread in the UK of several different CTX-M groups showing no clonality. In our findings, all the CTX-M groups, CTX-M-1, 2 and 9, were detected in the E. coli strains which were strongly suggested to be linked to community-acquisition of ESBL genes. This finding likely reflects the simultaneous spread of multiple specific CTX-M clones, known as an allodemic pattern, which could accelerate the dissemination of CTX-M ESBL genes.

### Table 3. CTX-M types of ESBL-producing bacteria isolated from outpatients

Data are shown as number of isolates with percentage of the total number of isolates in parentheses.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Total isolates</th>
<th>CTX-M group</th>
<th>CTX-M-1</th>
<th>CTX-M-2</th>
<th>CTX-M-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>205</td>
<td>CTX-M group</td>
<td>43 (21.0)%</td>
<td>13 (6.3)%</td>
<td>149 (72.7)%</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>26</td>
<td>CTX-M group</td>
<td>12 (46.2)%</td>
<td>0 (0.0)%</td>
<td>14 (53.8)%</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>12</td>
<td>CTX-M group</td>
<td>2 (16.7)%</td>
<td>8 (66.6)%</td>
<td>2 (16.7)%</td>
</tr>
<tr>
<td>Total</td>
<td>243</td>
<td></td>
<td>57 (23.5)%</td>
<td>21 (8.6)%</td>
<td>165 (67.9)%</td>
</tr>
</tbody>
</table>

Fig. 1. Frequencies of extended-spectrum β-lactamase (ESBL)-producing strains in the isolates obtained from outpatients (black bars) and hospitalized patients (grey bars) at Hara-Sanshin Hospital in Fukuoka (Japan). The detection rates of ESBL-producing bacteria were determined using the samples isolated from outpatients and hospitalized patients. *P<0.05.
The results from outpatients in our hospital showed that the combined pattern of TEM/SHV + CTX-M was dominant for all three bacteria (Table 2). Notably, the detection of ESBL-producing bacteria from out- and inpatients initiated at about the same period (Fig. 1). Community-acquired infections due to SHV-producing K. pneumoniae have been reported, suggesting the spread of SHV genotypes in the community (Lee et al., 2011). In addition, CTX-M-producing K. pneumoniae have been reported to be transmitted between the hospital and the community (Calbo et al., 2011; Woerther et al., 2011). Cross-transmission of ESBL genes to other bacteria within the hospital and the efflux of those genes into the community may accelerate the dissemination of ESBL-producing bacteria in the community, regardless of the bacterial species or ESBL genotypes.

ESBL-producing bacteria are resistant to almost all β-lactam antibiotics, except carbapenems, as indicated by their definition (Paterson & Bonomo, 2005). In addition, bacteria carrying ESBL genes, particularly those with CTX-M genotypes, exhibit co-resistance to fluoroquinolones (Cantón & Coque, 2006). The dissemination of ESBL-producing bacteria in the community has an effect on outpatient therapy. Community-acquired bacteraemia, due to ESBL-producing E. coli strains, is becoming a critical concern for outpatients, because inappropriate use of empirical antibiotics, such as cephalosporins and fluoroquinolones, has resulted in high mortality (Rodriguez-Baño et al., 2006, 2010). Fosfomycin has been reported to be effective against infections caused by ESBL-producing E. coli (Falagas et al., 2010); however, one study has already reported increased fosfomycin resistance in CTX-M-producing E. coli strains isolated from the community (Oteo et al., 2009). In the near future, we may be forced to use carbapenems as the first choice for empirical therapy of patients with community-acquired infections due to ESBL-producing bacteria. Constant and careful surveillance for the emergence of ESBL-producing bacteria is strongly needed not only in hospitalized patients, but also in outpatients.

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