

# Fecal carriage of CTX-M $\beta$ -lactamase-producing *Enterobacteriaceae* in nursing homes in the Kinki region of Japan

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**Abstract:** The detection rate of CTX-M-type  $\beta$ -lactamase-producing *Enterobacteriaceae* in Japan has significantly increased. Nursing homes may be a reservoir of antibiotic-resistant bacteria. Therefore, we determined the prevalence of, and risk factors associated with, fecal carriage of CTX-M-type  $\beta$ -lactamase-producing *Enterobacteriaceae* among nursing home residents. A total of 225 stool samples were collected for phenotypic and genotypic identification of extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Enterobacteriaceae*. Multivariate analysis was performed to identify the risk factors associated with fecal carriage of CTX-M producers. The prevalence of CTX-M-type ESBL-producing *Enterobacteriaceae*, as confirmed by phenotypic and genotypic methods, was 19.6% (44 of 225 samples). *Escherichia coli* was the predominant CTX-M-type ESBL-producing bacterium among these isolates (41 of 44 isolates). Genotyping of *bla*<sub>CTX-M</sub> gene-positive isolates showed that 30 (68.2%), 13 (29.5%), and 1 (2.3%) of 44 samples belonged to groups CTX-M-9, CTX-M-1 and CTX-M-2, respectively. Among the CTX-M-type ESBL-producing *Enterobacteriaceae* found in nursing homes, 95.5% (42 of 44 isolates) were co-resistant to quinolone antibiotics. In multivariate logistic regression analysis, inability to turn over in bed, diabetes, and invasive procedures within the last 2 years were the only variables independently associated with fecal carriage of CTX-M-type ESBL producers. Nursing home residents in Japan exhibit a high prevalence of CTX-M-type ESBL-producing *Enterobacteriaceae* carriage, with a high level of co-resistance to quinolones.

**Keywords:** *Enterobacteriaceae*, extended-spectrum beta-lactamase (ESBL), risk factors, fecal carriage, nursing homes, Japan

## Introduction

The detection rate of extended-spectrum  $\beta$ -lactamases (ESBLs) in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in the Kinki region of mid-western Japan significantly increased from 0.2% to 7.3% and from 0.0% to 2.4%, respectively, over a 10-year period from 2000 to 2009.<sup>1</sup> Our recent study among healthy adults in the same region showed 6.4% prevalence of ESBL producers, most of which were CTX-M-type ESBLs.<sup>2</sup> The reasons for the increased prevalence of ESBL producers in Japan have not been adequately investigated. In the USA and Europe, nursing homes have been identified as a reservoir of antibiotic-resistant bacteria and possibly play an important role in their emergence and spread.<sup>3-5</sup> Therefore, we studied the prevalence of, and risk factors associated with, fecal carriage of CTX-M-type ESBL-producing *Enterobacteriaceae* among the residents of nursing homes.

## Materials and methods

The study was conducted from March to September 2010 in three nursing homes located in mid-western Japan. Nursing home A was a privately owned 150-bed nursing home, whereas nursing homes B and C were 100- and 50-bed institutions, respectively, run by the local government.

We analyzed 225 stool samples from the three nursing homes, and one sample was obtained from each resident. Data on resident characteristics, including length of stay in the current nursing home, co-morbidities, and past and present antibiotic use, were collected from resident charts. Detailed information was also collected from resident charts in nursing home A, which had the highest prevalence of ESBL-producing *Enterobacteriaceae* carriers. The information included residents' physical and mental health status, history of invasive procedures within the last 2 years, past and present urinary tract infections, history of stroke, and other health conditions, such as diabetes.

ESBL production was identified by phenotypic and genotypic methods, as described elsewhere.<sup>2</sup> Collected fecal specimens were plated on MacConkey agar supplemented with 2 µg/mL cefotaxime (CTX-MacConkey) using the streak culture method. ESBL expression was confirmed using cefotaxime and ceftazidime, with and without clavulanic acid, as recommended by the Clinical and Laboratory Standards Institute (CLSI), and each set of samples was tested with CLSI quality control strains *E. coli* ATCC25922 and *K. pneumoniae* ATCC700603.<sup>6</sup> Isolates were identified using conventional biochemical tests. Drug susceptibility tests for imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, norfloxacin, and tetracycline were performed with the disc diffusion method using SN Discs (Nissui Pharmaceutical Co, Ltd, Tokyo, Japan).

Bacterial DNA was extracted from the isolates by boiling the bacterial suspensions. DNA samples with a concentration of 0.1 ng/µL were used as a template for polymerase chain reaction (PCR) analysis. The universal primers CTX-M-U1 (5'-ATG TGC AGY ACC AGT AAR GTK ATG GC-3') and CTX-M-U2 (5'-TGG GTR AAR TAR GTS ACC AGA AYC AGC GG-3') were used to detect the *bla*<sub>CTX-M</sub> gene, as described previously.<sup>7</sup> DNA from the reference *E. coli* *bla*<sub>CTX-M</sub>-positive strain was used as a positive control. To genotype the *bla*<sub>CTX-M</sub> genes, we used four primer sets that amplify group-specific *bla*<sub>CTX-M</sub> genes, as described elsewhere.<sup>8</sup> To identify the *qnrA*, *qnrB*, and *aac(6)-Ib* genes, PCR analysis was performed on the samples collected from nursing home A, as described elsewhere.<sup>9,10</sup> PCR products were visualized on 2% agarose gel electrophoresis followed

by staining with GelRed Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA).

Statistical analysis was performed using SPSS v.16.0 (SPSS, Chicago, IL, USA). All qualitative variables were analyzed using the  $\chi^2$  or Fisher's exact tests, and quantitative variables were analyzed with the Mann-Whitney *U* test, as appropriate. Factors identified in univariate models as being statistically significant were included in the multivariate logistic regression model and are presented as odds ratios with 95% confidence intervals. Statistical significance was set at  $P < 0.05$ .

## Results

The characteristics of the 225 study residents from the three nursing homes are listed in Table 1. The median age of the residents was 85 years (range 52–100 years). The majority (74.7%) of the residents were women. The median length of stay in the three nursing homes varied from 87 to 300 days.

Bacterial growth on CTX-MacConkey agar was observed in 103 of 225 samples (45.8%). The prevalence of CTX-M-type ESBL-producing *Enterobacteriaceae*, as confirmed by phenotypic and genotypic methods, was 22.9% (33 of 144 samples) in nursing home A, 18.8% (9 of 48 samples) in nursing home B, and 6.1% (2 of 33 samples) in nursing home C (Table 2). *E. coli* was the predominant CTX-M-type ESBL-producing bacterium among these isolates (41 of 44 isolates), followed by other *Escherichia* spp. (3 of 44 isolates). Genotyping of *bla*<sub>CTX-M</sub> gene-positive isolates showed that 30 (68.2%), 13 (29.5%), and 1 (2.3%) of the 44 samples belonged to groups CTX-M-9, CTX-M-1, and CTX-M-2, respectively.

Disc diffusion test for multidrug resistance revealed high levels of co-resistance of CTX-M-type ESBL producers to quinolone antibiotics (33 of 33, 8 of 9, and 1 of 2 isolates in nursing homes A, B, and C, respectively). More than half of these isolates (25 of 42) were resistant to tetracycline in addition to quinolones. However, all the 44 CTX-M-type

**Table 1** Characteristics of the nursing home residents

| Variable                | Nursing home |           |          |
|-------------------------|--------------|-----------|----------|
|                         | A            | B         | C        |
| Capacity                | 150          | 100       | 50       |
| No of residents studied | 144          | 48        | 33       |
| Age, years              | 85.0         | 86.5      | 84.0     |
| (median [range])        | (53–100)     | (52–100)  | (60–98)  |
| Female sex, %           | 71.5         | 83.3      | 75.8     |
| Length of stay, days    | 300 ± 345    | 105 ± 194 | 87 ± 136 |
| (median ± SD [range])   | (2–1,490)    | (2–1,015) | (8–630)  |

**Abbreviation:** SD, standard deviation.

**Table 2** Prevalence of ESBL-producing *Enterobacteriaceae* in nursing homes

| Variable                                 | Nursing home (n [%]) |          |           | Total (n [%]) |
|--|----------------------|----------|-----------|---------------|
|  | A                    | B        | C         |               |
| No of residents studied                  | 144                  | 48       | 33        | 225           |
| ESBL-producing <i>Enterobacteriaceae</i> | 37 (25.7)            | 9 (18.8) | 3 (9.1)   | 49 (21.7)     |
| CTX-M-type ESBL                          | 33 (22.9)            | 9 (18.8) | 2 (6.1)   | 44 (19.6)     |
| Bacterial species                        |                      |          |           |               |
| <i>Escherichia coli</i>                  | 31 (93.9)            | 8 (88.9) | 2 (100.0) | 41 (93.2)     |
| Other <i>Enterobacteriaceae</i>          | 2 (6.0)              | 1 (11.1) | 0 (0.0)   | 3 (6.8)       |

**Abbreviation:** ESBL, extended-spectrum  $\beta$ -lactamase.

ESBL producers identified were susceptible to carbapenems and amikacin. Only one isolate was susceptible to all the antibiotics tested, and another isolate was resistant to gentamicin and tetracycline only. In nursing home A, where 100.0% (33 of 33 isolates) quinolone co-resistance was observed, we detected the *aac(6)-Ib* gene in 22 of the 33 isolates (66.7%); however, none of the isolates carried the *qnrA* or *qnrB* genes.

Analysis of the data on resident characteristics showed no significant differences in terms of age, gender, or length of stay between carriers and non-carriers of CTX-M-type ESBL producers. Furthermore, carriers and non-carriers of CTX-M-producing bacteria did not differ in the current and past antibiotic use (data not shown). However, univariate analysis of the data collected from the resident charts in nursing home A revealed the following risk factors for CTX-M-type ESBL-producing *Enterobacteriaceae* carriage: (1) resident's condition that requires the highest level of care; (2) inability to turn over in bed; (3) diaper use; (4) diabetes; (5) urinary tract infection; (6) history of hospitalization within the last year; and (7) invasive procedures within the last 2 years (Table 3). In multivariate logistic regression analysis, inability to turn over in bed, diabetes, and invasive procedures within the last 2 years were the only variables independently associated with fecal carriage of CTX-M-type ESBL producers.

## Discussion

Our findings show that nursing homes in mid-western Japan have an almost three times higher prevalence (19.6%) of CTX-M-type ESBL producers than that reported in the previous clinical study and among healthy people in the same region.<sup>1,2</sup> Thus, as predicted, nursing homes may play the important role of reservoir in the rapid spread of CTX-M-type ESBLs in Japan. However, the prevalence of fecal carriage of ESBL producers in Japanese nursing homes is low compared

**Table 3** Univariate and multivariate logistic regression analyses of risk factors associated with CTX-M-type ESBL-producing *Enterobacteriaceae* carriage in nursing home A

| Characteristics                                 | Univariate analysis  |         | Multivariate analysis |         |
|---|----------------------|---------|-----------------------|---------|
|   | OR (95% CI)          | P value | OR (95% CI)           | P value |
| Required care level                             |                      |         |                       |         |
| Level 5   | 3.2<br>(1.29–7.914)  | 0.009   | –                     |         |
| Level 1–4                                       | 1                    |         |                       |         |
| Ability to turn over in bed                     |                      |         |                       |         |
| Incapable                                       | 3.50<br>(1.56–7.86)  | 0.002   | 2.81<br>(1.18–6.70)   | 0.019   |
| Capable   | 1                    |         | 1                     |         |
| Use of diapers                                  |                      |         |                       |         |
| Yes   | 2.61<br>(1.14–5.99)  | 0.021   | –                     |         |
| No  | 1                    |         |                       |         |
| Diabetes  |                      |         |                       |         |
| Yes   | 2.78<br>(1.11–6.99)  | 0.025   | 3.22<br>(1.11–9.32)   | 0.031   |
| No  | 1                    |         | 1                     |         |
| Past and present urinary tract infection        |                      |         |                       |         |
| Yes   | 4.13<br>(1.61–10.56) | 0.002   | –                     |         |
| No  | 1                    |         |                       |         |
| History of hospitalization within the last year |                      |         |                       |         |
| Yes   | 3.16<br>(1.37–7.30)  | 0.006   | –                     |         |
| No  | 1                    |         |                       |         |
| Invasive procedures within the last 2 years     |                      |         |                       |         |
| Yes   | 4.73<br>(2.06–10.85) | <0.001  | 4.54<br>(1.87–11.01)  | 0.001   |
| No  | 1                    |         | 1                     |         |

**Notes:** Required care level is assessed by the authorized professional people according to the Long-Term Care system of Japan.<sup>15</sup> Five levels of care are distinguished: care level 1 is defined as requiring partial care, whereas care level 5 is defined as impossible to live without care.

**Abbreviations:** CI, confidence interval; ESBL, extended-spectrum  $\beta$ -lactamase; OR, odds ratio.

with findings in some other countries. In Northern Ireland, UK, 40.5% (119 of 294 samples) of nursing home residents were gut carriers of ESBL-producing *E. coli*, which were also resistant to fluoroquinolones;<sup>3</sup> in Bolzano, Italy, 41.4% of 111 residents in a long-term-care facility (LTCF) were colonized with ESBL-producing *E. coli*.<sup>11</sup>

The dominance of CTX-M among the ESBLs in nursing homes is consistent with the global trend. The prevalent identification of *E. coli* (93.2%; 41 of 44 isolates) among the ESBL producers and the CTX-M-9 group dominance (68.2%; 30 of 44 isolates) is similar to previous findings in Japan, both in clinical settings and among asymptomatic individuals.<sup>2,12,13</sup>

Among the CTX-M-type ESBL-producing *Enterobacteriaceae* found in nursing homes, 95.5% (42 of 44 isolates) were co-resistant to quinolone antibiotics. It is

suggested that *bla*<sub>CTX-M</sub> genes are directly linked to quinolone-resistance (*qnr*) genes. However, we did not identify isolates with *qnrA* or *qnrB* genes in this study.<sup>14</sup> A hospital study in Japan reported that most of the CTX-M-producing isolates were resistant to fluoroquinolones,<sup>13</sup> whereas fluoroquinolone resistance was observed in only 7.1% (1 of 14 isolates) of asymptomatic ESBL carriers in Japan.<sup>2</sup> However, all the CTX-M-producing *Enterobacteriaceae* isolated at the nursing homes were susceptible to carbapenems and amikacin.

Poor functional status, diabetes, and invasive procedures have also been previously identified as risk factors for ESBL colonization or infection both among residents of LTCFs and among general patients.<sup>4,5</sup> Not only residents, but also 11.6% of 69 staff members in an LTCF were colonized with ESBL-producing *E.coli*.<sup>11</sup> This may imply contact transmission of CTX-M-type ESBL-producing *Enterobacteriaceae*. Furthermore, among the three studied nursing homes, fecal carriage of CTX-M-type ESBL producers was the lowest (6.1%) in nursing home C (50-bed unit). Infection-control measures may have been better implemented in the nursing home with fewer residents. Therefore, infection control must be closely monitored in nursing homes to prevent resident-to-resident, staff-to-resident, and vice versa transmission of ESBL producers.

In summary, nursing home residents in Japan have a high prevalence of CTX-M-type ESBL-producing *Enterobacteriaceae* carriage, with a high level of co-resistance to quinolones. Further monitoring and public health efforts focusing on nursing homes are needed to control the spread of ESBL-producing bacteria in Japan's aging society.

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## Disclosure

The authors report no conflicts of interest in this work.

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