Epidemiology of *Escherichia coli*, *Klebsiella* Species, and *Proteus mirabilis* Strains Producing Extended-Spectrum \(\beta\)-Lactamases From Clinical Samples in the Kinki Region of Japan

Tatsuya Nakamura,1,2 Masaru Komatsu, PhD,3 Katsutoshi Yamasaki, PhD,4 Saori Fukuda,5 Yugo Miyamoto,6 Takeshi Higuchi,7 Tamotsu Ono,8 Hisaaki Nishio,9 Noriyuki Sueyoshi,10 Kenji Kida,11 Kaori Satoh,12 Hirofumi Toda,12 Masahiro Toyokawa, PhD,13 Isao Nishi,13 Masako Sakamoto,14 Masahiro Akagi,15 Isako Nakai,16 Tomomi Kofuku,17 Tamaki Orita,18 Yasunao Wada,19 Takuya Zikimoto,20 Chihiro Koike,1 Shohiro Kinoshita,20 Itaru Hirai,2 Hakuo Takahashi, MD,1 Nariaki Matsuura, MD,2 and Yoshimasa Yamamoto, MD2

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Abstract

In the present study, nonduplicate, clinical isolates of extended-spectrum \(\beta\)-lactamase (ESBL)-producing *Escherichia coli*, *Klebsiella* spp, and *Proteus mirabilis* were collected during a 10-year period from 2000 to 2009 at several hospitals in the Kinki region, Japan. The detection rate of *E coli* markedly increased from 0.24% to 7.25%. The detection rate of *K pneumoniae* increased from 0% to 2.44% and that of *P mirabilis* from 6.97% to 12.85%. The most frequently detected genotypes were the CTX-M9 group for *E coli*, the CTX-M2 group for *K pneumoniae*, and the CTX-M2 group for *P mirabilis*. *E coli* clone O25:H4-ST131 producing CTX-M-15, which is spreading worldwide, was first detected in 2007. The most common replicon type of *E coli* was the IncF type, particularly FIB, detected in 466 strains (69.7%). Of the *K pneumoniae* strains, 47 (55.3%) were of the IncN type; 77 *P mirabilis* strains (96.3%) were of the IncT type. In the future, the surveillance of various resistant bacteria, mainly ESBL-producing Enterobacteriaceae, should be expanded to prevent their spread.

Extended-spectrum \(\beta\)-lactamase (ESBL)-producing Enterobacteriaceae have been increasingly reported worldwide since their first description in 1983.1 Moreover, they have emerged worldwide as a significant cause of community and health care–associated infections.2 ESBLs are the major cause of resistance to oxyimino-cephalosporins in Enterobacteriaceae. ESBLs are mostly plasmid-mediated bacterial enzymes that can hydrolyze a wide variety of penicillins and cephalosporins.

Most ESBLs have evolved by genetic mutation from native \(\beta\)-lactamases, particularly TEM-1, TEM-2, and SHV-1. These parent enzymes are commonly found in gram-negative bacteria, particularly in Enterobacteriaceae.3 Until the 2000s, most of the ESBLs were structurally related to the narrow-spectrum TEM- and SHV-type \(\beta\)-lactamases, with one to several amino acid substitutions surrounding their active site. During the 1990s, they were described mainly as members of the TEM- and SHV-\(\beta\)-lactamase families in *Escherichia coli* and *Klebsiella pneumoniae* causing nosocomial outbreaks.3 Furthermore, in the late 1990s, a novel type of ESBL, the CTX-M enzymes, emerged worldwide, mostly from *E coli*.3,4 ESBL-producing *E coli* of the Toho-1-type were reported first in Japan in 1988.5 Nowadays, they are mostly found in *E coli* that cause community-acquired infections and, with increasing frequency, contain CTX-M enzymes. Moreover, *E coli* producing a CTX-M-type ESBL is an emerging cause of community-acquired urinary tract infection in young women in the United States,6 Europe,7 Hong Kong,8 and elsewhere. Increased community-acquired infection by ESBL-producing bacteria is complicating the selection of therapeutic drugs.

More than 50 CTX-M enzymes reported thus far can be grouped into 5 main subgroups according to the similarity of their amino acid sequence (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-10, and others).
In the present study, nonduplicate clinical isolates of ESBL-positive E coli, Klebsiella spp, and Proteus mirabilis were collected during a 10-year period from 1999 to 2009 at several hospitals in the Kinki region, Japan. Our study examined the prevalence and type of β-lactamase genes and plasmid replicon type among the isolates. Moreover, susceptibilities to oral antimicrobial agents were determined.

Materials and Methods

Bacterial Isolates

This laboratory surveillance was conducted with the cooperation of 18 institutions (17 clinical laboratories of various hospitals and 1 commercial laboratory) in the Kinki region, which is located in midwestern Japan. Specimens were collected from 2000 to 2009. A total of 40,522 isolates of gram-negative bacilli including E coli (25,320 isolates), K pneumoniae (11,582 isolates), Klebsiella oxytoca (2,933 isolates), and P mirabilis (1,187 isolates) were isolated from various clinical specimens, and antimicrobial sensitivity and genotypes were tested. A single isolate was selected from each patient and identified by the clinical procedures routinely used in each laboratory.

Screening for ESBL

The cefpodoxime minimum inhibitory concentration (MIC) criterion of more than 2 μg/mL was used to initially screen isolates. Strains that met the cefpodoxime MIC criterion were investigated by the double-disk synergy (DDS) test with amoxicillin–clavulanic acid (20 μg per disk), cefotaxime (30 μg per disk), ceftazidime (30 μg per disk), and cefepime (30 μg per disk) against the MIC criterion of more than 2 μg/mL, according to methods published previously.17 DDS-positive strains were subjected to polymerase chain reaction (PCR) analyses for the detection of ESBL genes.

PCR Amplification for the Detection of ESBL Genes

All DDS-positive strains were screened for the resistance genes SHV, TEM, and CTX-M by using a single PCR assay.1819 Genetic detection and genotyping of TEM, SHV, and CTX-M were performed by using PCR with bacterial DNA, which was extracted from the isolates by boiling the bacterial suspensions. A solution with an extracted DNA concentration of 0.1 ng/mL was used as the template for PCR analysis. In the case of genotyping of CTX-M genes, 4 primer sets that amplify group-specific CTX-M genes were used, as described previously: the CTX-M1 group includes CTX-M-1, CTX-M-3, CTX-M-10 to CTX-M-12, CTX-M-15, CTX-M-22, CTX-M-23, and CTX-M-28 to CTX-M-30; the CTX-M2 group, CTX-M-2, CTX-M-4 to CTX-M-7, CTX-M-20, and Toho-1; the CTX-M8 group, CTX-M-8; and the CTX-M9 group, CTX-M-9, CTX-M-13, CTX-M-14, CTX-M-16 to CTX-M-19, CTX-M-21, CTX-M-27, and Toho-2. The PCR products were analyzed by using 2% agarose gel electrophoresis and visualized by staining with ethidium bromide.

Detection of CTX-M-15 O25:H4-ST131

The serotyping of the CTX-M1 group was carried out by using E coli O and H antisera purchased from Denka Seiken (Tokyo, Japan), according to the manufacturer’s instructions. The complete nucleotide sequences of CTX-M1 group O25:H4 E coli genes were determined on both strands by direct sequencing of the PCR products. Multilocus sequence typing (MLST) was performed on 17 strains of CTX-M15 O25:H4 E coli by following the recommended procedure at the E coli MLST Web site (http://mlst.ucc.ie/dbs/Ecoli).

Plasmid Replicon Type Determination

PCR-based replicon typing was performed on 837 strains as described by Carattoli et al.20 Eighteen primer pairs targeting the FIA, FIB, FIC, HI1, HI2, II-Ic, I1-Ic, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FII replicons were used in separate PCR reactions.

Susceptibility to Oral Antimicrobial Agents

The susceptibilities to oral antimicrobial agents, amoxicillin-clavulanic acid (AMC), minocycline, levofloxacin, fosfomycin, colistin, and trimethoprim-sulfamethoxazole (SXT), were determined by the broth diffusion method on Mueller-Hinton agar (Eiken Chemical, Tokyo, Japan), according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).21 The quality control strains used in this study were E coli ATCC 25922 and E coli ATCC 35218. Throughout this study, the results were interpreted using CLSI criteria for broth dilution.22

Results

ESBL Detection

Between 2000 and 2009, a total of 40,522 strains, including 25,320 E coli, 11,582 K pneumoniae, 2,933 K oxytoca, and 1,187 P mirabilis strains, were analyzed. ESBL isolation rates are shown in Figure I and Table I. The detection of ESBL-producing E coli markedly increased.
from 2 strains (0.24%) in 2000 to 224 strains (7.25%) in 2009. The detection of ESBL-producing K pneumoniae increased from 0 (0.00%) to 30 strains (2.44%) and that of ESBL-producing K oxytoca increased from 0 (0.00%) to 3 strains (1.18%). The detection of ESBL-producing P mirabilis increased from 14 (6.97%) to 23 strains (12.85%) in a survey conducted since 2004. In the whole of the Kinki region, the detection rate increased from 0.42% (between 2000 and 2004) to 3.4% (between 2005 and 2009).

Molecular Detection of ESBL

The changes in the genotypes detected during the survey period are shown in Figure 2 for E coli and in Figure 3 for K pneumoniae. The most frequently detected genotypes in the 10 years were the CTX-M9 group for E coli (374 strains [55.9%]), the CTX-M2 group for K pneumoniae (38 strains [44.7%]), and the CTX-M2 group for P mirabilis (79 strains [98.8%]). The detection number of E coli of the CTX-M9 group increased from 25 strains to 144 strains from 2005. In addition, the detection number of E coli of the CTX-M1 group increased from 11 strains to 57 strains from 2005. The detection number of K pneumoniae of the CTX-M9 group increased from 2006. The detection rates...
Prevalence of Plasmid Replicons

The most common replicon type of *E. coli* was the IncF type, particularly FIB, detected in 466 strains (69.7%), followed by FIA in 336 strains (50.2%), I1-1 in 83 strains (12.4%), and N in 65 strains (9.7%) in Table 2. Of the *K. pneumoniae* strains, 47 (55.3%) were of the N type. Most of the bacteria carried a single plasmid, but some bacteria carried multiple plasmid types. The average number of plasmids carried by the bacteria was from 1 to 1.05. Of the *P. mirabilis* strains, 77 (96.3%) were of the IncT type.

Susceptibility for Oral Drug

Oral drug susceptibility rates in Table 3 for *E. coli* were 96.0% for colistin, 93.7% for fosfomycin, 62.6% for minocycline, 47.2% for AMC, 44.3% for SXT, and 32.2% for levofloxacin. The levofloxacin susceptibility rate was the highest for the CTX-M2 group genotype. The drug susceptibility rates for *K. pneumoniae* were 86.9% for levofloxacin, 86.9% for colistin, 83.3% for fosfomycin, 51.2% for AMC, 46.4% for SXT, and 21.4% for minocycline. The SXT susceptibility rate was the lowest (0%) for the TEM/SHV group genotype. Drug susceptibility rates for *P. mirabilis* were 100% for AMC, 64.6% for SXT, 50.6% for fosfomycin, 15.2% for levofloxacin, 0.0% for colistin, and 0.0% for minocycline. The AMC susceptibility rate was 100%.

Discussion

In this study, long-term surveillance between 2000 and 2009 demonstrated that ESBL-producing *E. coli* increased about 30 times from 0.24% to 7.25% in 10 years. According to the figures, the number of isolates with different ESBL genes among ESBL-positive *K. pneumoniae* isolates detected in hospitals and associated health care facilities, 2000-2009. The CTX-M1 group includes CTX-M-1, CTX-M-3, CTX-M-10 to CTX-M-12, CTX-M-15, CTX-M-22, CTX-M-23, and CTX-M-28 to CTX-M-30; the CTX-M2 group, CTX-M-2, CTX-M-4 to CTX-M-7, CTX-M-20, and Toho-1; the CTX-M8 group, CTX-M-8; and the CTX-M9 group, CTX-M-9, CTX-M-13, CTX-M-14, CTX-M-16 to CTX-M-19, CTX-M-21, CTX-M-27, and Toho-2.


![Table 2](https://academic.oup.com/ajcp/article-abstract/137/4/620/1761063/25) Number of Replicons in Parental Strains of ESBL-Producing Strains*
to Fang et al. ESBL-producing E. coli increased by about 10 times between 2001 and 2006 in Sweden, a result comparable with that from a different survey conducted in the same period. Recently, many reports have been made on intestinal bacteria that acquired genes such as KPC and NDM-1. Until now, ESBL-producing E. coli have rarely been detected in Japan. This study may be useful for the prediction of resistant bacteria in the future.

Our study revealed the changes in the ESBL genotypes of ESBL-producing E. coli, Klebsiella spp, and P. mirabilis in the Kinki region of Japan. Shibata et al. investigated ESBL-producing intestinal bacteria of the CTX-M group in Japan. They reported that 89 of 168 E. coli strains belonged to the CTX-M9 group. In particular, the CTX-M9 group, frequently detected in E. coli, is presumably the most common genotype in Japan. Similarly, in this study, 374 of 669 E. coli strains were of the CTX-M9 group. In particular, contrary to the decreased CTX-M2 group, the CTX-M9 group has increased since 2007. The CTX-M2 group is frequently detected in food products such as meat. It was thought that usage restrictions of the antimicrobial agent to domestic animals had influenced a decrease of CTX-M-2. On the other hand, the CTX-M1 group has slightly increased. In Japan, the trend of the E. coli clone O25:H4-ST131 producing CTX-M-15, which causes problems worldwide, is unknown. Hawkey reported trends of increase in Asia. In addition, detection in India and Pakistan were reported. The problem is that this strain is detected in hospital- and community-acquired urinary tract infections and develops multidrug resistance. This study demonstrated that the E. coli clone O25:H4-ST131 producing CTX-M-15 was detected in 2007 or later, suggesting the continuing existence of this strain in Japan. At present, community-acquired infection with this strain is uncommon. However, attention should be given to future trends.

The study of plasmid replicon types provides information about the spread and risks of ESBL-producing bacteria. The genes responsible for CTX-M β-lactamases are encoded by plasmids belonging to the narrow host-range incompatibility types (ie, IncFI, IncFII, IncHI1, and IncI) or the broad host-range incompatibility types (ie, IncN, IncP-1-a, IncL/M, and IncA/C). In this study, the IncF group predominated among the E. coli strains, regardless of their genotypes. Many strains acquired multiple plasmids. Similar results have been obtained in other studies. About 10% of the strains were of the 11-1- and N-types, suggesting the existence of various E. coli clones. The spread of community-acquired infection indicates the potential spread of these various clones in different forms. On the other hand, the N-type predominated among K. pneumoniae strains and the T-type among the P. mirabilis strains, suggesting the spread of a single clone or plasmid. Many reports have been published particularly on these 2 strains in hospital-acquired infection. In addition, in this study, both species were occasionally detected in the same facility and ward, suggesting an epidemiology different from that of E. coli. Bacterial properties vary with species. Thus, measurements should be done carefully, according to the species.

Recently, the spread of community-acquired infection by ESBL-producing strains of the CTX-M type is causing problems. Reportedly, more ESBL-producing strains have been detected in females with urinary tract infection. The spread of community-acquired infection complicates the selection of antibacterial agents for outpatient care. Few oral antibacterial agents effective against ESBL-producing bacteria are available. According to recent reports, quinolone resistance is regarded as a serious problem. In addition, in this study, quinolone-resistance rates were high among the E. coli strains: about 70% of the strains were resistant. In particular, the resistance rate of the CTX-M1 group was the highest (about 85%). Thus, quinolones cannot be used for the treatment of those infective diseases. The susceptibility of E. coli to fosfomycin is being maintained. The reason for it will be that 3 g/d is recommended, as described in the Sanford guidelines. On the other hand, 80% of the K. pneumoniae strains are susceptible.
to quinolones. Thus, quinolones should be effective for *K. pneumoniae*. Antibacterial susceptibility varies with strains and genotypes. Thus, antibacterial agents for areas should be selected on the basis of the results obtained by studies on the epidemiologic backgrounds of those areas.

The detection rate of the ESBL-producing *E. coli* in our institutions was low, in comparison with that in Western countries and other areas in Asia. One of the reasons for this may be the difference in the type of antibiotics used in these countries: carabapenems and oxacephems, in particular, have often been used in Japan. Because ESBL-producing bacteria are susceptible to these drugs, they might have suppressed the diffusion of these bacteria. However, recently, reduced use of these drugs is recommended in clinical settings to avoid the overall resistance to these antibiotics. This may lead to an increase in the ESBL-producing bacteria in the near future in Japan.

This report described the epidemiologic trends of ESBL-producing *E. coli*, *Klebsiella* spp., and *P. mirabilis* in Japan. Various causative genes are known for β-lactam-resistant intestinal bacteria. Many reports have been published on resistant bacteria (eg, KPC, NDM-1, CTX-M-15) worldwide. In the future, the surveillance of various resistant bacteria, mainly ESBL-producing bacteria, should be expanded to prevent their spread.

From the Departments of Clinical Laboratory, 1Kansai Medical University Hirakata Hospital, Osaka, Japan; 2Wakayama Rosai Hospital, Wakayama, Japan; and 3Osaka Police Hospital, Osaka; 4Division of Biomedical Informatics, Course of Health Science, Graduate School of Medicine, Osaka University, Osaka; 5Bacteriological Testing Section of Central Laboratory, FALCO Biosystems, Kyoto, Japan; 6Department of Clinical Pathology, Tenri Hospital, Nara, Japan; Clinical Laboratory, 6National Hospital Organization Minami Wakayama Medical Center, Wakayama, Japan; 7Koyo Second Red Cross Hospital, Kyoto; 8Shiga Medical Center for Adults, Shiga, Japan; 9Social Insurance Shiga Hospital, Shiga; 10Japanese Red Cross Otsu Hospital, Shiga; 11The Research Foundation for Microbial Diseases of Osaka University, Osaka; 12Sumitomo Hospital, Osaka; 13Hyogo Prefectural Amagasaki Hospital, Hyogo, Japan; 14The Research Foundation for Microbial Diseases of Osaka University, Osaka; 15Osaka Police Hospital, Osaka; 16Division of Biomedical Informatics, Course of Health Science, Graduate School of Medicine, Osaka University, Osaka; 20Kobe University Hospital, Tokyo; 19Hyogo Medical University Hospital, Hyogo; and 21Kobe University Hospital, Hyogo; 2Laboratory for Clinical Investigation, Kobe University Hospital, Hyogo; 3Laboratory for Clinical Investigation, Kyoto University Hospital, Kyoto; 12Department of Medical Technology, Kinki University School of Medicine, Osaka; and 1Laboratory for Clinical Investigation, Osaka University Hospital, Osaka.

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Address reprint requests to Tatsuya Nakamura: Dept of Clinical Laboratory, Kansai Medical University Hirakata Hospital, 2-3-1 Shinmachi, Hirakata City, Osaka, 573-1191, Japan.

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**References**


