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Original article

Prevalence and risk factors of infections caused by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae

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ABSTRACT

Objective: To study the clinical characteristics and associated risk factors of infections caused by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae.
Methods: A case–control study at a large university hospital in Japan, comparing patients who were infected or colonized with ESBL-producing Enterobacteriaceae (n = 212) and non-ESBL-producing Enterobacteriaceae (n = 2089) in 2010–2013. Data were collected from medical charts, retrospectively. Multivariate logistic regression analysis was used to explore risk factors of ESBL-producing Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*) infection or colonization for each pathogen, respectively.
Results: ESBL-producing Enterobacteriaceae [*E. coli* (n = 113), *K. oxytoca* (n = 46), *K. pneumoniae* (n = 41), *P. mirabilis* (n = 12)] were taken from patients were identified in 1409 outpatient and 892 inpatients. Infection or colonization caused by ESBL-producing Enterobacteriaceae was considered to be hospital-acquired, healthcare-associated and community-acquired in 60.4%, 17.9% and 21.7% patients, respectively. Independent risk factors for ESBL-producing Enterobacteriaceae infection or colonization were male sex, cerebrovascular disease, intubation/tracheostomy, major surgery within 60 days ($p < 0.001$). Moreover, antimicrobial usage (more than 4 days) during preceding 60 days, especially aminoglycoside, oxazolidinone, tetracycline, fluoroquinolone, trimethoprim/sulfamethoxazole, and second- and fourth-generation cephalosporin were risk factors ($p < 0.001$). However, acquisition location of infection (hospital-acquired and community-onset) was not a risk factor ($p > 0.05$).
Conclusion: The problem of ESBL production is no longer limited to hospital-acquired infections. The presence of chronic illness, such as cerebrovascular disease, and recent antimicrobial use were independent risk factors for ESBL-producing Enterobacteriaceae infection or colonization.

1. Introduction

As the prevalence of extended-spectrum β-lactamase (ESBL)-producing bacteria has increased worldwide sharply over the last decade, production of ESBL is increasingly important cause of resistance in gram-negative bacteria throughout the world [1–4]. The first ESBL was identified in Germany in 1983 [5]; ESBL are capable of degrading the β-lactam ring of most of the penicillins and cephalosporins [5,6].

ESBL-encoding plasmids frequently bear resistance genes for additional antimicrobial classes, such as sulfonamides, aminoglycosides, and fluoroquinolones [7,8]. Herein, treatment options for infections due to these multidrug-resistant organisms are therefore limited, and initial empirical therapy is often ineffective and associated with increases mortality. Consequently, infection by

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ESBL producers has been associated with increased mortality, prolonged hospitalization, and rising medical costs [9–11].

Additionally, it is not surprising that gram-negative bacteria that produce these ESBL are increasingly implicated as causes of community-onset infection [12–14]. The problem of ESBL-production is no longer limited to hospital-acquired infections.

Therefore, early recognition of patients who are at risk for infection with ESBL-producing bacteria is necessary to guide empirical treatment and to apply preventive measures to limit the dissemination of infection [15,16]. The aim of this study was to assess risk factors for infection or colonization with ESBL-producing Enterobacteriaceae.

2. Materials and methods

2.1. Study design and patients

The study population consisted of 2301 patients, including inpatients and outpatients, from whom Enterobacteriaceae was isolated at least once between 1 January 2010 and 30 June 2013 at Aichi Medical School University Hospital, a 955-bed tertiary care facility. We performed a detailed retrospective investigation of the clinical features of these patients, and extracted microbiological data for *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis* that were identified in clinical cultures. In cases in which any bacterial cultures were tested twice or more in this period in the same patients, we selected the case at the time of initial isolation of *Enterobacteriaceae*. The same patients were included more than once only if relevant species were identified at least six months apart.

This study was approved by Ethics Committee of the Faculty of Aichi Medical University, Japan (12-131).

2.2. Data collection

For each patient, the following putative risk factors were collected from the clinical records: age, gender, medical complication, and invasive procedure such as urinary catheter, intubation/tracheostomy, naso-gastric tube, central venous catheter, intravenous catheter, drain, artificial organ, and hospital admission during preceding two months, use of an antimicrobial agent for more than 4 days in the preceding 60 days [17], a major operation (any surgical procedure that involved anesthesia or mechanical ventilation) within 60 days. We investigated the history of antimicrobials usage with medical chart. Those for whom previous antimicrobial use could not be ascertained were excluded from the study. History of following associated diseases was documented: sepsis, cancer, cardiovascular disease, cerebrovascular disease, diabetes, diseases of the nervous system, psychiatric disorder, respiratory ailment, digestive system disease, urological diseases, skin and musculoskeletal disease, and blood dyscrasia. Antimicrobial susceptibility of the Enterobacteriaceae isolates was also recorded.

Sites of acquisition of the organisms (community-onset or hospital-acquired), risk factors were also investigated in this study. Community-onset was defined outpatients or within 48 h of hospitalization, while hospital-acquired was defined hospital admission after 48 h or more. Moreover, the case in community-onset was classified as community-acquired and nursing and healthcare-associated.

Healthcare-associated infections were classified in accordance with the Japanese Respiratory Society [18]. Any of the following criteria were considered as healthcare-associated infections (i) resident of an extended care facility or nursing home; (ii) person who has been discharged from a hospital within the preceding 90 days; (iii) an elderly or disabled person who is receiving nursing care; (iv) person who is receiving regular endovascular treatment as an outpatient (dialysis, antimicrobial therapy, chemotherapy, immunosuppressive therapy).

2.3. Screening and confirmation of ESBL production

ESBL expression was screened with the disc diffusion method on Mueller–Hinton agar using cefotaxime, ceftazidime cefpodoxime and ceftriaxone with and without clavulanic acid (10 mg), as recommended by the Clinical and Laboratory Standards Institute (CLSI), and each set of samples was tested with CLSI quality control strains *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603.18 (M100-S21).

2.4. Antimicrobial susceptibility testing

All Enterobacteriaceae isolated from patients were tested for antimicrobial susceptibility. The minimum inhibitory concentration (MIC) was measured by the broth microdilution method based on the CLSI recommendations [19].

2.5. Statistical analysis

Patients were divided into two groups: the ESBL-positive group and the ESBL-negative group (non-ESBL), and categorical features of each group were compared respectively. Statistical analyses were performed using the χ^2-test, Fisher's exact test to compare categorical variables, and the *t*-test for comparing the age of patients in both groups. $p < 0.05$ was considered significant.

Univariate analyses were performed separately for each of the variables. Variables with a *p* value of <0.1 in the univariate analysis were candidates for multivariate analysis using a backward elimination method. Analysis of risk factors was performed according to species. All tests were 2-tailed, and a $p < 0.05$ was considered significant in the multivariable model. The odds ratio was calculated with a confidence interval (CI) of 95%. SPSS software package version 11.0.1J (LEAD Technologies, Inc) was used for statistical analysis.

3. Results

3.1. Patient population

During the study period, 168 blood cultures, 1182 urine cultures, 381 sputum cultures, 180 pus cultures and 390 others were taken from 1409 outpatients and inpatients ≦48 h of admission and 892 inpatients >48 h of admission to Aichi Medical School University hospital. Of the 2301 patients, 212 patients (9.2%) were detected as infected or colonized with an ESBL-producing Enterobacteriaceae during study period. One hundred twenty eight (60.4%) of the 212 patients yielding ESBL producers were male (Table 1). Ages ranged from 0 to 97 years (median: 63). Onset of infection caused by the ESBL-producing Enterobacteriaceae was considered to be hospital-acquired, community-acquired and nursing and healthcare-associated in 128 (60.4%), 46 (21.7%) and 38 (17.9%) patients, respectively (Table 1).

3.2. Epidemiological analysis

The 2301 episodes represented 1413 *E. coli*, 223 *K. oxytoca*, 543 *K. pneumoniae* and 122 *P. mirabilis*, including 212 ESBL producers (9.2%; 113 *E. coli*, 46 *K. oxytoca*, 41 *K. pneumoniae* and 12 *P. mirabilis*). Species distribution of ESBL-producing and non-ESBL-producing Enterobacteriaceae isolates were shown in Fig. 1. *E. coli* was the most frequent microbial isolated in both groups. However, the

Table 1
Univariate analysis of risk factors for ESBL-producing Enterobacteriaceae.

	ESBL (n = 212)	Non-ESBL (n = 2089)	*p* Value
Age > 65 yrs	114 (53.8)	1321 (63.2)	0.0067
Gender (male)	128 (60.4)	812 (38.9)	<0.0001
Major surgery[a]	53 (25.0)	224 (10.7)	<0.0001
Acquisition of bacteremia			
HA	128 (60.4)	764 (36.6)	<0.0001
E. coli	56 (26.4)	396 (19.0)	
K. oxytoca	38 (17.9)	101 (4.8)	
K. pneumoniae	28 (13.2)	230 (11.0)	
P. mirabilis	6 (2.8)	37 (1.8)	
CA	38 (17.9)	898 (43.0)	<0.0001
E. coli	33 (15.6)	647 (31.0)	
K. oxytoca	2 (0.9)	44 (2.1)	
K. pneumoniae	3 (1.4)	161 (7.7)	
P. mirabilis	0 (0.0)	40 (1.9)	
NHCA	46 (21.7)	435 (20.8)	<0.0001
E. coli	24 (11.3)	257 (12.3)	
K. oxytoca	6 (2.8)	32 (1.5)	
K. pneumoniae	10 (4.7)	111 (5.3)	
P. mirabilis	6 (2.8)	33 (1.6)	
Medical complication			
Blood dyscrasia	8 (3.8)	38 (1.8)	0.0554
Cancer	38 (17.9)	319 (15.3)	0.3091
Cardiovascular disease	76 (35.9)	779 (37.3)	0.679
Cerebrovascular disease	35 (16.5)	124 (5.9)	<0.0001
Diabetes	46 (21.7)	410 (19.6)	0.4709
Digestive system disease	16 (7.6)	208 (10.0)	0.2594
Diseases nervous system	17 (8.0)	126 (6.0)	0.2535
Psychiatric disorder	21 (9.9)	225 (10.8)	0.6977
Respiratory ailment	31 (14.6)	298 (14.3)	0.8873
Sepsis	23 (10.9)	159 (7.6)	0.096
Skin and musculoskeletal disease	29 (13.7)	207 (9.9)	0.0847
Urological disease	57 (26.9)	898 (43.0)	<0.0001
None	1 (0.5)	40 (1.9)	0.096
Previous illness			
Blood dyscrasia	2 (0.9)	20 (1.0)	0.6693
Cancer	11 (5.2)	273 (13.1)	0.0009
Cardiovascular disease	11 (5.2)	214 (10.2)	0.0182
Cerebrovascular disease	46 (21.7)	306 (14.7)	0.0066
Digestive system disease	38 (17.9)	448 (21.5)	0.2314
Diseases of nervous system	0 (0.0)	29 (1.4)	0.0595
Psychiatric disorder	0 (0.0)	2 (0.1)	0.8242
Respiratory ailment	6 (2.8)	113 (5.4)	0.1062
Urological disease	14 (6.6)	223 (10.7)	0.0631
Sepsis	1 (0.5)	15 (0.7)	0.5583
Skin and musculoskeletal disease	9 (4.3)	167 (8.0)	0.0504
Medical device			
Artificial organ	33 (15.6)	198 (9.5)	0.0049
Central venous catheter	50 (23.6)	220 (10.5)	<0.0001
Drain	20 (9.4)	97 (4.6)	0.0025
Intubation/tracheostomy	57 (26.9)	123 (5.9)	<0.0001
Intravenous catheter	99 (46.7)	864 (41.4)	0.1333
Naso-gastric tube	64 (30.2)	312 (15.0)	<0.0001
Urinary catheter	94 (44.3)	609 (29.1)	<0.0001
Using steroids	12 (5.7)	140 (6.7)	0.5608

[a] Major surgery within 60 days, HA: healthcare-acquired, CA: community-acquired, NHCA: nursing and healthcare-associated.

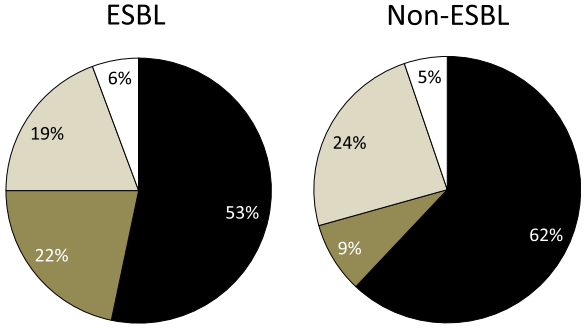

Fig. 1. Distribution of microbials in ESBL-producing and non-ESBL-producing Enterobacteriaceae isolates. ■ (black): *E. coli*, ■ (heavy gray): *K. oxytoca*, ■ (light gray): *K. pneumoniae*, □ (white): *P. mirabilis*.

species with the highest ESBL rate was *K. oxytoca* (20.6%: 46/223), followed by *P. mirabilis* (9.8%:12/122), *E. coli* (8.0%:113/1413) and *K. pneumoniae* (7.6%: 41/543) (Table 1).

3.3. Risk factors for ESBL-producing Enterobacteriaceae

Significant risk factors among underlying diseases included cerebrovascular disease and urological disease (Table 1). Additionally, cancer, cardiovascular disease, cerebrovascular disease and major surgery within 60 days were also significantly associated with ESBL production (Table 1).

Among medical devices, urinary catheterization, intubation/tracheostomy, naso-gastric tube, central venous catheter, drain, and artificial organ were risk factors for ESBL production.

Information on previous antimicrobial exposure was available, and 203 patients (8.8%) had a no-history of prior antimicrobial in the preceding 60 days (Fig. 2). With regards to previous antimicrobial exposure, the proportion of patients who had received one or more courses of antimicrobial therapy was significantly higher for the ESBL group compared with the non-ESBL group (142: 67.0% versus 752: 36.0%; $p < 0.001$) (Fig. 2). Cephalosporins were the most frequently used, followed by carbapenems, and fluoroquinolones in the ESBL group. Others included tetracycline, oxazolidinone and aminoglycosides.

Previous use of aminoglycosides, second-generation cephalosporin, fourth-generation cephalosporin, chloramphenicol, fosfomycin, fluoroquinolones, clindamycin, tetracycline, oxazolidinone, sulfamethoxazole/trimethoprim were risk factors for ESBL production ($p < 0.001$). On the other hand, carbapenems and penicillins were protective ($p < 0.005$). The antimicrobial usage during the 60 days prior to inclusion in the study is shown in Table 3. Fourth-generation cephalosporin, fluoroquinolones, second-generation cephalosporins and fourth-generation cephalosporins were the most frequently used classes of agents before isolation of ESBL-producing *E. coli*, *K. oxytoca*, *K pneumoniae*, and *P. mirabilis*, respectively.

Results of the multivariate logistic regression analysis are presented in Tables. In the final model, independent risk factors for ESBL-producing *E. coli* were male sex (Odds ratio (OR), 0.39; 95% confidence interval (CI), 0.24–0.65; $p < 0.001$), major surgery within 60 days (OR, 2.89; 95% CI, 1.54–5.30; $p < 0.001$), previous use of tetracyclines (OR, 26.52; 95% CI, 12.58–57.28; $p < 0.001$), second-generation cephalosporin (OR, 3.04; 95% CI, 1.48–6.07; $p < 0.001$) and fourth-generation cephalosporin (OR, 50.19; 95% CI, 27.51–94.18; $p < 0.001$).

For ESBL-producing *K. oxytoca*, independent risk factors were history of cerebrovascular disease (OR, 3.92; 95% CI, 1.32–11.59; $p = 0.01$), intubation/tracheostomy (OR, 5.46; 95% CI, 2.05–14.89; $p < 0.001$), previous use of oxazolidinone (OR, 21.27; 95% CI, 3.95–171.47; $p = 0.001$), fluoroquinolones (OR, 2.10; 95% CI, 1.23–3.60; $p = 0.01$), fourth-generation cephalosporin (OR, 19.09; 95% CI, 5.41–76.41; $p < 0.001$).

For ESBL-producing *K. pneumoniae*, independent risk factors were previous use of aminoglycosides (OR, 5.17; 95% CI, 1.33–17.74; $p = 0.011$), ST (OR, 3.84; 95% CI, 1.20–1094; $p = 0.016$), tetracycline (OR, 5.84; 95% CI, 1.96–16.54; $p = 0.001$), second-generation cephalosporin (OR, 8.60; 95% CI, 3.69–19.55; $p < 0.001$).

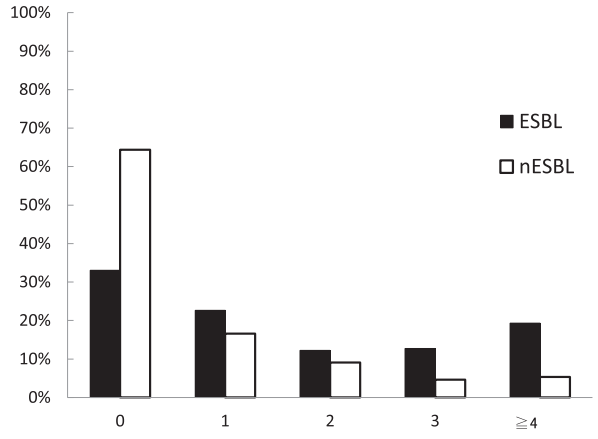

Fig. 2. The number of antimicrobial usage between infectious patients with and without- ESBL-producing Enterobacteriaceae proceeding 60 days. The frequency of antimicrobial usage in infectious patients with ESBL-producing Enterobacteriaceae was higher than that of in non-infectious patients with ESBL-producing Enterobacteriaceae.

Table 2
Comparison of the antimicrobial usage during the last 60 days prior to inclusion in the study population with and without ESBL-producing Enterobacteriaceae infection.

Antimicrobials	ESBL (n = 212)	Non-ESBL (n = 2089)	*p* Value
Aminoglycoside	18 (8.5)	25 (1.2)	<0.0001
Carbapenem	50 (23.6)	239 (11.4)	<0.0001
Cephalosporin			
First generation	11 (5.2)	161 (7.7)	0.184
Second generation	42 (19.8)	120 (5.7)	<0.0001
Third generation	19 (9.0)	200 (9.6)	0.7724
Fourth generation	64 (30.19)	61 (2.9)	<0.0001
Chloramphenicol	9 (4.3)	1 (0.1)	<0.0001
Cyclic lipopeptide	1 (0.5)	11 (0.5)	0.6951
Fosfomycin	5 (2.4)	12 (0.6)	0.0156
Fluoroquinolone	48 (22.6)	160 (7.7)	<0.0001
Glycopeptide	18 (8.5)	112 (5.4)	0.0601
Clindamycin	21 (9.9)	67 (3.2)	<0.0001
Macrolide	1 (0.5)	37 (1.8)	0.1213
Oxazolidinone	30 (14.2)	38 (1.8)	<0.0001
Penicillin	6 (2.8)	224 (10.7)	0.0003
Penem	2 (0.9)	9 (0.4)	0.2691
ST[a]	15 (7.1)	61 (2.9)	0.0013
Tetracycline	43 (20.3)	38 (1.8)	<0.0001
Antifungal agent	25 (11.8)	89 (4.3)	<0.0001

[a] Trimethoprim/sulfamethoxazole.

For ESBL-producing *P. mirabilis*, independent risk factors were intubation/tracheostomy (OR, 26.20; 95% CI, 3.77–247.64; $p < 0.001$), previous use of oxazolidinone (OR, 21.30; 95% CI, 1.75–334.12; $p = 0.020$), fourth-generation cephalosporin (OR, 54.06; 95% CI, 6.64–632.31; $p < 0.001$).

Previous antimicrobial use was a significant risk factor for infection or colonization with ESBL-producing Enterobacteriaceae in general, but the specific agents depended on the species. On the other hand, sites of acquisition of the organisms (community-acquired, hospital-acquired or nursing and healthcare-associated) were not a risk factor for infection or colonization in any of the species studied (Tables 4–7).

3.4. Antimicrobial susceptibility

Antimicrobial susceptibility profiles for ESBL-producing *E. coli*, *K. pneumoniae*, *K. oxytoca*, *P. mirabilis* are shown in Fig. 3. There was no documented resistance to imipenem/cilastatin and meropenem against ESBL-producing *E. coli* (Fig. 3a). More than 80% ESBL-producing *E. coli* was susceptible to tazobactam/piperacillin, amikacin and fosfomycin. They had higher resistance rates not only to β-lactam antimicrobial agents, but also to non-β-lactam antimicrobial agents, including ciprofloxacin, levofloxacin and trimethoprim/sulfamethoxazole.

ESBL-producing *K. oxytoca* strains were also no documented resistance to imipenem/cilastatin and meropenem (Fig. 3b). Amikacin, minocycline and fosfomycin showed more than 80% of susceptibility rates to ESBL-producing *K. oxytoca*. They also had higher resistance rates to non-β-lactam antimicrobial agents, including ciprofloxacin, levofloxacin and trimethoprim/sulfamethoxazole compared with non-ESBL-producing *E. coli*. Compared with the results of *E. coli*, *K. oxytoca* was highly resistant to tazobactam/piperacillin and aztreonam.

ESBL-producing *K. pneumoniae* strains were also all susceptible to imipenem/cilastatin, meropenem and amikacin (Fig. 3c). On the other hand, ESBL-producing *K. pneumoniae* isolates had higher resistance rates to non-β-lactam antimicrobial agents, including ciprofloxacin, levofloxacin and fosfomycin.

ESBL-producing *P. mirabilis* strains were documented susceptible to imipenem/cilastatin and meropenem (Fig. 3d). More than

Table 3
The antimicrobial usage during the last 60 days prior to inclusion in the study population with each ESBL-producing Enterobacteriaceae pathogen.

Antimicrobials	Duration (day) of the antibiotic usage			
	E. coli (n = 113)	*K. oxytoca* (n = 46)	*K. pneumoniae* (n = 41)	*P. mirabilis* (n = 12)
Aminoglycoside	4 (3.5)	6 (13.0)	5 (12.2)	3 (25.0)
Carbapenem	17 (15.0)	18 (39.1)	11 (26.8)	4 (33.3)
Cephalosporin				
First generation	6 (5.3)	4 (8.7)	1 (2.4)	NA (0)
Second generation	20 (17.7)	8 (17.4)	13 (31.7)	1 (8.3)
Third generation	6 (5.3)	7 (15.2)	3 (7.3)	2 (16.7)
Fourth generation	42 (37.2)	11 (23.9)	6 (14.6)	5 (41.7)
Chloramphenicol	NA (0)	6 (13.0)	2 (4.9)	1 (8.3)
Cyclic lipopeptide	1 (0.9)	NA (0)	NA (0)	NA (0)
Fosfomycin	3 (2.7)	1 (2.2)	1 (2.4)	NA (0)
Fluoroquinolone	17 (15.0)	20 (43.5)	11 (26.8)	NA (0)
Glycopeptide	6 (5.3)	5 (10.9)	4 (9.8)	3 (25)
Clindamycin	5 (4.4)	11 (23.9)	5 (12.2)	NA (0)
Macrolide	NA (0)	NA (0)	1 (2.4)	NA (0)
Oxazolidinone	6 (5.3)	13 (28.3)	7 (17.1)	4 (33.3)
Penicillin	2 (1.8)	3 (6.5)	1 (2.4)	NA (0)
Penem	NA (0)	1 (2.2)	1 (2.4)	NA (0)
ST[a]	6 (5.3)	2 (4.3)	7 (17.1)	NA (0)
Tetracycline	23 (20.4)	9 (19.6)	9 (22.0)	2 (16.7)
Antifungal agent	10 (8.8)	7 (15.2)	8 (19.5)	NA (0)

NA: not available. The number of patients used some kinds of antimicrobial more than 3 days (%).
[a] Trimethoprim/sulfamethoxazole.

Table 4
Multivariate analysis of risk factors for ESBL-producing *E. coli*.

	Odds ratio	95.0% CI	*p* value
Sex	0.39	−0.72 to −0.23	<0.001
Major surgery[a]	3.11	0.25–0.87	<0.001
Antimicrobial use[b]			
Cephalosporin			
Second generation	2.98	0.18–0.89	0.002
Fourth generation	54.73	1.70–2.32	<0.001
Tetracycline	26.80	1.27–2.03	<0.001

[a] Major surgery within 60 days.
[b] Antimicrobial use within 60 days.

Table 5
Multivariate analysis of risk factors for ESBL-producing *K. oxytoca*.

	Odds ratio	95.0% CI	*p* value
Cerebrovascular disease (1)	4.61	1.31–1.53	0.006
Intubation/tracheostomy	5.18	0.33–1.33	0.001
Antimicrobial use[a]			
Cephalosporin			
Fourth generation	26.26	0.97–2.39	<0.001
Oxazolidinone	21.76	0.69–2.59	0.001
Fluoroquinolone	5.59	0.33–1.40	0.002

Cerebrovascular disease (1): medical complication.
[a] Antimicrobial use within 60 days.

Table 6
Multivariate analysis of risk factors for ESBL-producing *K. pneumoniae*.

	Odds ratio	95.0% CI	*p* value
Antimicrobial use[a]			
Aminoglycoside	5.17	0.14–1.43	0.012
Cephalosporin			
Second generation	8.60	0.65–1.48	<0.001
ST[b]	3.84	0.09–1.20	0.016
Tetracycline	5.84	0.34–1.40	0.001

[a] Antimicrobial use within 60 days.
[b] Trimethoprim/sulfamethoxazole.

80% of ESBL-producing *P. mirabilis* was susceptible to piperacillin, tazobactam/piperacillin, sulbactam/cefoperazone, ceftazidime, cefozopran, amikacin, aztreonam, levofloxacin, and trimethoprim/sulfamethoxazole.

Antimicrobial susceptibility profile against ESBL-producing Enterobacteriaceae therefore varied across the species, but they were susceptible to carbapenems and aminoglycosides.

4. Discussion

The increasing prevalence of infections caused by antimicrobial-resistant bacteria makes empirical treatment of these infections more difficult [20]. In addition, local antimicrobial susceptibility patterns should be known in order to prescribe appropriate empiric antimicrobials. In this study, therefore, we investigated the resistance rates of Enterobacteriaceae (*E. coli*, *K. pneumoniae*, *K. oxytoca*, *P. mirabilis*) isolated from inpatients and outpatients, and described risk factors contributing to this resistance. Since the objective of this study was to identify risk factors for the acquisition of ESBL-producing organisms, we did not differentiate between infection and colonization in the main analysis. Colonization with a resistant organism is often the precursor to infection and the duration of medical devices increases the risk of a patient developing an infection [2,3,8,21,22].

ESBL producers had a prevalence of 9.2% among Enterobacteriaceae. The proportion of ESBL-producing strains was higher in bloodstream isolates (13.7%: 23/168), followed by urinary tract infection (8.9%: 105/1182), respiratory isolates (7.6%: 29/381). The species with the highest ESBL production rate was *K. oxytoca*

Table 7
Multivariate analysis of risk factors for ESBL-producing *P. mirabilis*.

	Odds ratio	95.0% CI	*p* value
Intubation/tracheostomy	72.7	1.04–3.76	<0.001
Antimicrobial use[a]			
Cephalosporin			
Fourth generation	224.4	1.48–4.49	<0.001
Oxazolidinone	25.5	0.23–3.39	0.037

[a] Antimicrobial use within 60 days.

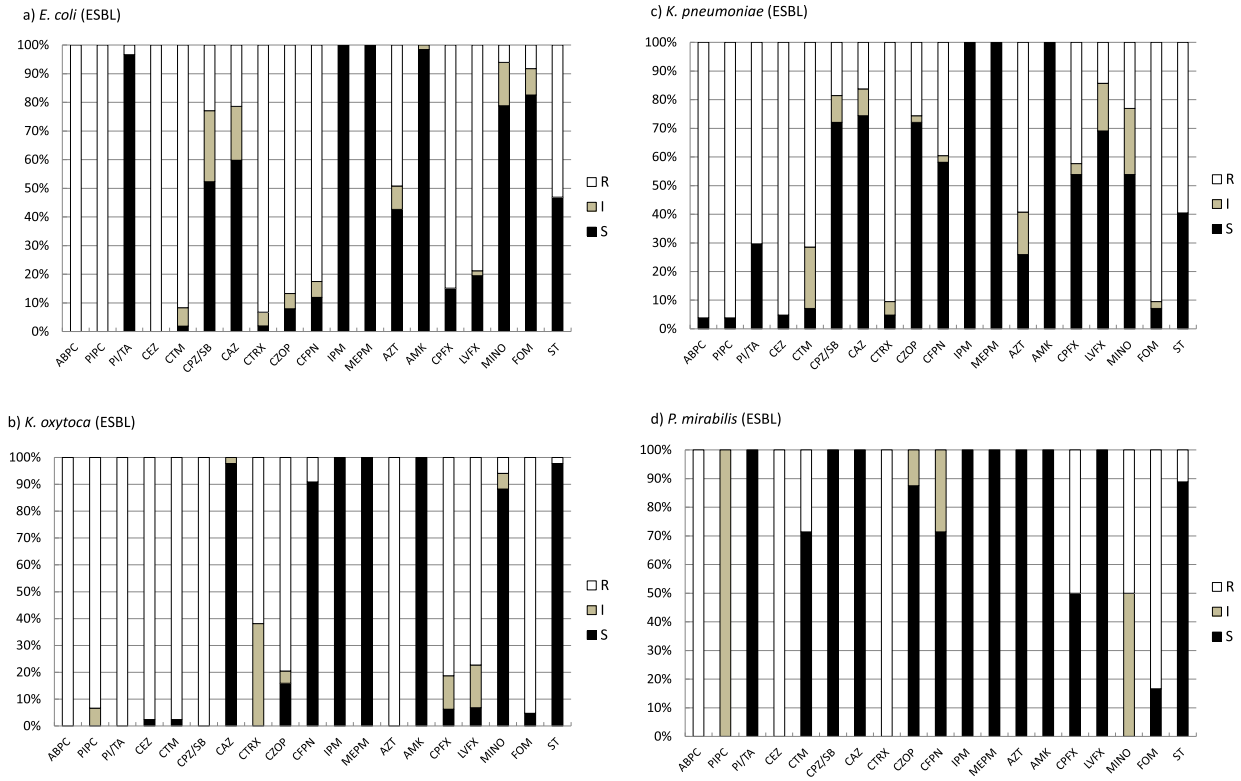

Fig. 3. Susceptibility profiles of ESBL Enterobacteriaceae isolates (a: *E. coli*, b: *K. oxytoca*, c: *K. pneumoniae*, d: *P. mirabilis*) against various antimicrobials. ABPC: ampicillin, PIPC: piperacillin, PI/TA: piperacillin/tazobactam, CEZ: cefazolin, CTM: cefotiam, CPZ/SB: Cefoperazone/Sulbactam, CAZ: ceftazidime, CTRX: ceftriaxone, CZOP: cefozopran, CFPM: cefepime, IPM: imipenem, MEPM: meropenem, AZT: aztreonam, AMK: amikacin, CPFX: ciprofloxacin, LVFX: levofloxacin, MINO: minomycin, FOM: fosfomycin, ST: sulfamethoxazole/trimethoprim.

(46/223: 20.6%), followed by *P. mirabilis* (12/122: 9.8%), *E. coli* (113/1413: 8.0%) and *K. pneumoniae* (41/543: 7.6%) (Table 1). Compared with previously reported data, our data showed similar proportion of ESBL-producing isolates [23–25].

Multivariate analysis identified male sex, cerebrovascular disease as medical complication and major surgery within 60 days, previous antimicrobial use the preceding 60 days, as risk factor for ESBL-producing Enterobacteriaceae infection (Tables 4–7). Our study confirms findings in previous studies [26,27]. Moreover, intubation/tracheostomy was a risk of having an ESBL organism in this population (Tables 5 and 7). Association of prolonged ventilation and acquisition of resistant organisms has been well established as these patients tend to be more debilitated, have greater exposure to acid suppressors, antimicrobial agents and have opportunities for aspiration and nosocomial acquisition. As the presence of chronic illness was risk factors for the acquisition of ESBL [28], especially for *K. oxytoca*, some chronic illness caused by cerebrovascular disease related with infection caused by ESBL producers.

Furthermore, a previous study revealed that previous antimicrobials use may contribute to dissemination of ESBL-producing strains but the selective pressure may vary among classes of agents [26]. Our multivariate analysis identified that second- and fourth-generation cephalosporin, aminoglycosides, tetracycline, oxazolidinone, ST and fluoroquinolones were the antimicrobial classes independently associated with ESBL-producing organisms infection or colonization, while the risk of specific classes many differ for each pathogen (Tables 4–7).

Thirty-eight point eight percent of patients (894/2301) had exposure to at least one of the antimicrobial agents. We found that previous antimicrobial treatment with fourth-generation cephalosporin was a major risk factor for infection with ESBL-producing Enterobacteriaceae (Tables 4, 5 and 7). As our findings confirm previous findings, prior exposure to aminoglycosides, cephalosporins, trimethoprim/sulfamethoxazole and fluoroquinolones were independent risk factors for ESBL organisms [9,29–31].

However, contrary to our expectations based on previous studies, third-generation cephalosporin use was not a risk factor for ESBL-producing *Enterobacteriaceae* infection or colonization [2,12]. This may be due to the relatively small number of patients who received third-generation cephalosporins in our study population (Table 2). Hence, our results suggested that the antimicrobial history of the other generation cephalosporin could also be its risk factor.

On the other hand, tetracycline was selected as the risk factor for acquisition of ESBL-producing pathogens (Tables 4 and 6). Minocycline and tigecycline can be used in our hospital. Our study showed that ESBL-producing Enterobacteriaceae had various susceptibility to minocycline for each specie respectively (Table 3). Hence, this observation indicated that, in the case of patients with complicated infection, prior use of tetracycline, which has relatively broad antimicrobial spectrum, may cause bacterial selection at the infected tissues. Therefore, prior tetracycline use might be an independent risk factor for the acquisition of ESBL-producing gene.

E. coli and *Klebsiella* spp have variable antimicrobial resistance mechanisms, which may include the production of ESBL. Prior antimicrobial was described as potential control points, whereas other authors thought that these were useful risk factors regarding suspicion of ESBL-producing Enterobacteriaceae infection [2,14,22].

Of note, the problem of ESBL production is no longer limited to community-onset or hospital-acquired infections [12–14]. Some

previous studies revealed that ESBL production was likely to be a surrogate of healthcare exposure, while our multivariate logistic regression analysis did not identified healthcare-associated, community-acquired and nursing and healthcare-associated as independent risk factor for acquisition of ESBL-producing Enterobacteriaceae (Tables 4–7). Our results suggested that previous medical history regard to antimicrobial was more critical risk factor for infection or colonization of ESBL-producing Enterobacteriaceae.

Consisted with previous study [27], our study revealed that ESBL-producing Enterobacteriaceae have various antimicrobial susceptibility profiles, except carbapenems and aminoglycosides. Depend on the bacterial isolates (Fig. 3). By the result of other analysis, the empirical use of carbapenems is suggested only for patients having some risk factors [22]. Similar with previous reports, carbapenems seem to be appropriate for the empirical treatment of ESBL infection, because of its vary high rate of susceptibility. However, our data suggested that Enterobacteriaceae were also highly susceptible to aminoglycosides. Hence, aminoglycosides could also be used cautiously as antimicrobial empiric therapy, especially for urinary tract infection.

There are several limitations to this study. This is a retrospective study and we report the results of a single medical center with a restricted number of ESBL-producing isolates. It is important to recognize that the current study was conducted only in one hospital of Japan and the findings may not be representative of the whole country.

Other limitation is that this is a case–control study (with the controls being patients without Enterobacteriaceae infection), the controls in this study (non-ESBL-producing Enterobacteriaceae) are generally unlikely to be previously exposed to antimicrobials (because if they were, then the strains would be dead); therefore, the risk of previous antimicrobial exposure tended to be overestimated in the case group.

Finally, we did not extract the data of autoimmune illness which is usually included in this type of risk factor study (there were not post-transplant patients in this population).

In conclusion, the problem of ESBL-producing Enterobacteriaceae infection is no longer limited to hospital-acquired infections, while some previous studies revealed that ESBL production was likely to be a surrogate of healthcare exposure. The presence of chronic illness was risk factors for the acquisition of ESBL, such as cerebrovascular disease. In addition, antimicrobial treatments with aminoglycoside, oxazolidinone, tetracycline, fluoroquinolone, trimethoprim/sulfamethoxazole, second- and fourth-generation cephalosporin were potent risk factor for ESBL-producing Enterobacteriaceae infection or colonization.

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Competing interests

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