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Evaluation of Expanded Spectrum Beta-lactamase and Carbapenemase Positiveness of *Enterobacter*iaceae Members Grown in Blood Cultures at Hacettepe University Adult Hospital Between 2004 and 2012

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ABSTRACT

The aim of this study was to determine extended-spectrum beta-lactamase (ESBL) and carbapenemase positivity of *Enterobacter*iaceae that grew in blood cultures and define the clinical properties of patients infected with carbapenemase positive strains. *E.coli*, *K. pneumoniae*, *Enterobacter* spp. and other *Enterobacter*iaceae in blood cultures between years 2004-2012 in Hacettepe University Faculty of Medicine were evaluated for ESBL and carbapenemase positivity. ESBL was defined with standard phenotypic method and carbapenemase with PCR for "VIM, IMP, KPC, NDM, OXA" carbapenemases. 382 of 1138 *E. coli* bacteremia (31.8%), 175 of 703 *Klebsiella* spp. bacteremias (24.8%) and 46 of 237 *Enterobacter* spp. bacteremias (24.8%) were ESBL positive. PCR was performed on 11 *E. coli* strains and 36 *Klebsiella* spp. strains. 7 of *E. coli* strains (63.6%) and 28 of *Klebsiella* spp. strains (77%) were carbapenemase positive. *Klebsiella* spp. subgroup analysis showed that 22 strains were OXA, 3 were VIM, 2 were IMP and one strain was OXA+VIM positive. For *E.coli* strains; 5 were OXA, 2 were OXA+VIM positive. NDM and KPC subtypes were detected. Our hospitals ESBL and/or carbapenemase positive *Enterobacter*iaceae frequency is similar with Europe and Turkey. In some years, there were sharp rises in ESBL producing bacteria rates , probably due to local epidemics. Resistance to antibiotics, increased mortality can be caused by these species. Since treatment options are limited, every precaution necessary should be taken to avoid spreading of resistant *Enterobacter*iaceae.

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INTRODUCTION

Members of *Enterobacter*iaceae are commonly isolated bacteria from clinical samples. Many well-known pathogens such as *E. coli*, *Klebsiella* spp., *Salmonella* spp., *Shigella dysenteriae*, *Citrobacter* spp, *Enterobacter* spp, *Kluvyera* spp., *Morganella morgagnii*, *Proteus* spp, *Serratia marcescens*, *Yersinia* spp. belong to this family (1). They are the causative agents of many infections, both community-acquired and hospital-acquired (2). In recent years, antimicrobial resistance observed, especially in *E. coli* and *K. pneumoniae* species of this family, has become a significant treatment problem. High mortality rates are observed in infections caused by these strains. The two main mechanisms of resistance that develop against broad-spectrum antibiotics in this family are extended-spectrum beta-lactamase (ESBL) and carbapenemase production (3). The many subtypes of ESBL and carbapenemase enzymes render these bacteria resistant or tolerant to carboxypenicillins (ticarcillin, carbenicillin)., ureidopenicillins (piperacillin)., and carbapenems (imipenem, meropenem, ertapenem, etc.). (4–6). Multicenter studies conducted in our country have revealed around 30% ESBL positivity in *E. coli* and *Klebsiella* spp (7,8). Bacteria producing carbapenemase have also become a significant problem due to

their increasing frequency in recent years. In our country, OXA-48 is considered the most important carbapenem-hydrolyzing enzyme (9,10). The antibiotic resistance created by these two mechanisms makes the treatment of infectious diseases difficult and increases mortality (11–13).

According to the CDC's 2011 data, the risk of developing nosocomial infection in America is around 4%. Based on this figure, it is estimated that 720,000 nosocomial infections develop in America every year. Similar figures have been reported from Europe as well (14,15). In recent years, many members of *Enterobacter*iaceae have become a significant problem in the treatment and control of infections with ESBL and/or carbapenemases (16,17). Carbapenem resistance was first detected in Pseudomonas aeruginosa in Japan in 1991. Subsequently, carbapenem resistance was reported in *K. pneumoniae* in 1997 and in *E. coli* in 1999. Since then, carbapenem-resistant strains have been reported from many countries including Korea, Singapore, Taiwan, Hong Kong, China, Malaysia, Brazil, the United Kingdom, Italy, Canada, the United States, Australia, Greece, and Colombia (11,19,20). Due to the high air traffic density today, it is estimated that carbapenem resistance will spread rapidly (21-23). According to CDC data, carbapenem resistance in *Klebsiella* species in the United States increased from 1% in 2000 to 4.2% in 2011 (24).

Table 1. Distribution of gram negative blood culture growths by type.

Bacteria	All cultures (n).	%
E.coli	1517	55.7
Klebsiella spp.	870	31.9
Enterobacter spp.	296	10.8
Proteus spp.	19	0.6
Other	19	0.6
Total	2721	99.6

The exact route of transmission of carbapenem-resistant microorganisms has not been fully understood. Limited information based on DNA fingerprinting and gel electrophoresis suggests inter-hospital cross-transmission. Furthermore, in some studies where environmental cultures were taken, carbapenem-resistant microorganisms were detected in sinks and stethoscopes; interestingly, no growth of carbapenem-resistant *E. coli* and *K. pneumoniae* was found in cultures taken from the hands of healthcare workers (25,26).

In light of this data, in order to take necessary measures against this public health problem and to outline a long-term combat plan, we aimed to determine the frequencies of ESBL and carbapenemase positivity in *Enterobacter*iaceae members isolated in blood cultures between 2004-2012 at Hacettepe University Adult Hospital, and to examine the clinical characteristics of patients infected with bacteria producing carbapenemase.

Table 2. Distribution of reproduction according to bacteria after correction.

Bacteria	All cultures	Corrected count	%
E.coli	1517	1138	53.9
Klebsiella spp.	870	703	33.3
Enterobacter spp.	296	237	11.2
Proteus spp.	19	15	0.07
Other	19	17	0.08
Total	2721	2110	100

METHODS

The list of *Enterobacter*iaceae members isolated from blood cultures between 2004 and 2012 at Hacettepe University Adult Hospital was obtained from the microbiology laboratory. Bacteria with less than 10 isolations per year were excluded.

The rates of ESBL positivity in *E.coli*, *K. pneumoniae*, *Enterobacter* spp., and other *Enterobacter*iaceae members isolated from blood cultures taken at Hacettepe University Hospitals between 2004 and 2012 were examined. ESBL positivity was identified using the BD Phoenix (BD, Sparks, USA). automated bacterial identification and antibiotic sensitivity system. The patients were between 18 and 98 years old. The number of isolates from the same infection episode taken from a patient's multiple blood cultures was counted as a single isolate, and the "corrected number of isolates" was calculated.

Samples were sent from internal, surgical services, intensive care units, operating theaters, hemodialysis units, burn units, and emergency departments.

Bacteria with a significant risk for carbapenemase positivity (imipenem or meropenem minimal inhibitory concentration (MIC) value > 4) were listed according to the CLSI 2010 guidelines (48). Archives were contacted for the files of these patients. The strains of 50 patients whose files could be accessed were evaluated for carbapenemase positivity using the PCR method. Carbapenemases named "VIM, IMP, KPC, NDM, OXA" were investigated.

Table 3. Bacteria ESBL positivity rates.

Bacteria	Corrected culture count	ESBL (+).	%
E.coli	1138	382	31.8
Klebsiella spp.	703	175	24.8
Enterobacter	237	46	19.4

ESBL: Extended-spectrum beta-lactamase.

Table 4. *E.coli* ESBL positivity rate distributed by years.

Year	Corrected culture count	ESBL (+). cultures	ESBL positivity rate (%).
2004	132	43	32.6
2005	221	68	30.7
2006	210	87	41.4
2007	163	42	25.7
2008	181	82	45.3
2009	152	58	38.1
2010	151	50	33.1
2011	139	48	34.5
2012	168	48	28.6
Total	1138	382	31.8

ESBL: Extended-spectrum beta-lactamase.

The clinical characteristics and antibiotic therapies of patients with identified carbapenemase positivity and subtype, for whom sufficient data (clinical characteristics, physical examination, antibiotics used and duration, treatment response) were available in their files, were examined. The MIC values of the isolates to the antibiotics used by the patients were determined. Whether the patients were alive or not on January 2014 was obtained from the "National death notification system."

RESULTS

Among other *Enterobacter*iaceae, Serratia marcescens was isolated in 5 patients, Morganella morganii in 4 patients, *Citrobacter freundii* in 2 patients, Citrobacter *braakii* in 1 patient, *Salmonella* group D in 2 patients, *Burkholderia cepacia* in 1 patient, and *Kluyvera ascorbata* in 1 patient.

A total of 2721 *Enterobacter*iaceae isolates were obtained from blood cultures at Hacettepe University Adult Hospital between 2004 and 2012. These isolates included 1517 (55.7%) *E.coli*, 870 (31.9%) *Klebsiella* spp, 296 (10.8%) *Enterobacter* spp., 19 (0.6%). Proteus spp., and 19 (0.6%). other bacteria (Table 1).

Among the *Klebsiella* spp. isolates (870), 732 (84.1%) were *K. pneumoniae*, 130 (14.8%) were *K. oxytoca*, and 8 (0.1%) were *K. ozaneae* subtypes.

Among the *Enterobacter* spp. isolates (296), 225 (76.0%) were *E. cloacae*, 62 (21.1%) were *E. aerogenes*, and 9 (0.3%) were other *Enterobacter* subtypes.

After obtaining these data, the number of ESBL-positive isolates was determined by counting a single isolate from a patient's multiple blood cultures taken during the same infection episode (corrected number of isolates). This prevented the same bacterium from being counted multiple times and falsely increasing the rate. After correction, the total number of isolates was 2110, with 1138 (53.9%) *E.coli*, 703 (33.3%) *Klebsiella* spp., 237 (11.2%) *Enterobacter* spp., 15 (0.7%) *Proteus* spp., and 17 (0.8%) other bacteria (Table 2).

Following the correction, ESBL-positive bacteria were identified based on data obtained from the microbiology laboratory. Of the 1138 *E. coli* isolates, 382 (31.8%), of the 703 *Klebsiella* spp. isolates, 175 (24.8%), and of the 237 *Enterobacter* spp. isolates, 46 (24.8%) were ESBL-positive (Table 3).

When examining ESBL positivity in *E. coli* isolates by year, the number of ESBL-positive isolates ranged from 42 (2007) to 87 (2006). The percentage of ESBL positivity ranged from 25.7 (2007) to 45.3 (2008). There was no significant increase in ESBL positivity over the years (Table 4).

When examining ESBL positivity in *Klebsiella* spp. isolates by year, the number of ESBL-positive isolates ranged from 8 (2004 and 2005). to 42 (2010). The percentage of ESBL positivity ranged from 10.9 (2007) to 50.6 (2010). After a significant and sudden increase in 2010, the rates returned to those of previous years (Table 5).

When examining ESBL positivity in *Enterobacter* spp. isolates by year, the number of ESBL-positive isolates ranged from 1 (2004, 2005, and 2011) to 13 (2009). The percentage of ESBL positivity ranged from 3.3 (2005) to 39.3 (2009). The variability in ESBL positivity rates by year was remarkable.

When isolates with an imipenem or meropenem MIC value > 4 were considered at risk for carbapenemase positivity, 25 *E. coli* and 45 *Klebsiella* spp. strains were identified as risky. Among these, 11 *E. coli* and 36 *Klebsiella* spp. strains were selected for PCR analysis (Table 7).

Of the 11 *E. coli* strains analyzed by PCR, 7 (63.6%) and of the 36 *Klebsiella* spp. strains, 28 (77%) were found to be carbapenemase-positive (Table 7).

Table 5. *Klebsiella spp.* ESBL positivity rate distributed by years.

Year	Corrected culture count	ESBL (+). cultures	ESBL positivity rate (%)
2004	51	8	15.6
2005	73	8	10.9
2006	63	12	19.0
2007	110	40	36.3
2008	118	18	25.2
2009	72	19	26.3
2010	83	42	50.6
2011	67	15	22.3
2012	66	13	19.6
Total	703	175	24.8

ESBL: Extended-spectrum beta-lactamase.

Table 6. Suspicious carbapenemase strains and those for which PCR was performed.

Bacteria	At risk	PCR performed
E.coli	25	11
Klebsiella spp.	45	36

When carbapenemase subtypes were examined, it was found that 22 *Klebsiella* spp. strains were OXA-positive, 3 were VIM-positive, 2 were IMP-positive, and 1 strain was positive for both OXA and VIM. Among the *E.coli* strains, 5 were OXA-positive, and 2 were positive for both OXA and VIM. NDM and KPC subtypes were not detected (Table 8).

When the files of patients with carbapenemase-positive strains were requested, files were obtained for 3 out of 7 patients with *E. coli* isolates and 19 out of 28 patients with *Klebsiella* spp. isolates. It was observed that all *Klebsiella* spp. isolates with available files were *Klebsiella pneumoniae*.

Among the examined files, 10 *Klebsiella pneumoniae* strains had sufficient data, while no *E. coli* strains had sufficient data. Of the 10 *Klebsiella pneumoniae* strains with sufficient data, 4 were ESBL-positive, while 6 were negative. Carbapenemase was detected in 8 patients, with OXA detected in 8 patients, VIM in 1 patient, and OXA + VIM in 1 patient.

The files of 10 patients with sufficient data were examined. Their ages ranged from 28 to 81 years (mean: 56 years). Seven patients had hematological malignancies. Of the remaining 3 patients, two (patient no: 5 and 10) had been hospitalized in the neurology intensive care unit for a long time. The other patient without hematological malignancy (patient no: 4) had a case of bacteremia following a prostate biopsy (The patient was later diagnosed with prostate cancer.). Four patients had focal lung infections, 4 had neutropenic fever, and 2 had gastrointestinal tract infections. Eight of the 8 patients who started antibiotic therapy with piperacillin-tazobactam died without receiving other treatment, and the antibiotic therapy had to be changed in the remaining 6 patients, 3 of whom died. Of the 2 patients who started treatment with carbapenems, one died after 1 day of treatment, and the other was discharged after recovery but was later lost due to progression of the underlying disease (Table 9).

DISCUSSION

In our study, we aimed to determine the epidemiology of *Enterobacter*iaceae members isolated from blood cultures, the frequencies of ESBL and carbapenemase, and the clinical characteristics of patients infected with a carbapenemase-producing strain.

When we look at the frequency distribution of *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and other *Enterobacter*iaceae isolated in blood cultures, dominance of *E. coli* and *Klebsiella* spp. is evident. This situation is consistent with data from America, Europe, and Turkey (27–30).

Table 7. PCR results.

Bacteria	PCR performed	CPM positive	%
E.coli	11	7	63.6
Klebsiella spp.	36	28	77

CPM: Carbapenemase.

Klebsiella oxytoca is a bacterium that has been gaining importance in recent years. It is one of the common causes of antibiotic-associated hemorrhagic colitis independent of Clostridium difficile (31). With the frequency of 14.8% we detected in our study, it is seen that it is not a rare pathogen. In three studies conducted in our country to investigate ESBL and carbapenemase, a lower number of *K. oxytoca* strains were detected (32,33). This difference may be attributed to hospital outbreaks that have increased the number of isolates over the years. Further epidemiological studies are needed to determine the frequency of *K. oxytoca* in our country.

When *Enterobacter* spp. isolates were examined, it was found that 76.0% were *E. cloaceae* and 24.1% were *E. aerogenes*. This distribution is consistent with the international literature (34).

When the number of isolates of the mentioned bacteria was determined by counting only one growth of the same bacterium in the same infection episode (corrected number of isolates)., it was observed that the frequency of these bacteria did not change. The number of isolates decreased from 1517 to 1138 for *E. coli*, from 870 to 703 for *Klebsiella* spp., and from 196 to 237 for *Enterobacter* spp.

After the correction, based on the data obtained from the microbiology laboratory, the bacteria that were ESBL positive were identified, and their frequencies were calculated. Of the 1138 *E. coli* isolates, 382 (31.8%)., 175 (24.8%). of the 703 *Klebsiella* spp. isolates, and 46 (24.8%). of the 237 *Enterobacter* spp. isolates were ESBL positive. The HITIT-I study, conducted multicentrically between June 2004 and January 2005, investigated the presence of ESBL in a total of 1196 gram-negative hospital isolates, including 457 *Escherichia coli*, 390 *Klebsiella pneumoniae*, 194 *Pseudomonas aeruginosa*, and 155 *Acinetobacter baumannii*. In this study, where the minimum inhibitory concentration (MIC). values of antibiotics and the production of ESBL were determined by the E-test method (AB Biodisk, Solna)., ESBL positivity was found in 26% of *E. coli* strains and 32% of *K. pneumoniae* strains; these rates were 31.7% and 33.3% in blood isolates of *E. coli* and *K. pneumoniae*, respectively (8). The rate of ESBL production among *Enterobacter*iaceae species is rapidly increasing worldwide.

The increasing antibiotic resistance is causing significant problems in Europe (35). Rates of up to 60% for *Klebsiella* spp. have been reported from Latin America (36). In light of these studies, it can be said that the ESBL positivity rates of around 30% for *E. coli* and around 25% for *Klebsiella* spp. in our hospital are similar to those in Europe and Turkey.

Table 8. Carbapenemase subtypes.

Carbapenemase subtypes	OXA	VIM	IMP	OXA+VIM	NDM	KPC	Total
Klebsiella spp.	22	3	2	1	-	-	28
E.coli	5	-	-	2	-	-	7

When isolates with an MIC value of >4 for imipenem or meropenem were considered risky for carbapenemase positivity, 25 *E. coli* and 45 *Klebsiella* spp. strains were found to be risky. It was decided to perform PCR on 11 *E. coli* and 36 *Klebsiella* spp. strains selected from the patients from whom the files could be accessed. Carbapenemase was detected in 7 (63.6%) of the 11 *E. coli* strains and 28 (77%) of the 36 *Klebsiella* spp. strains tested by PCR. When carbapenemase subtypes were examined, it was found that 22 of the *Klebsiella* spp. strains were OXA, 3 were VIM, 2 were IMP, and one strain was positive for both OXA and VIM. Of the *E.coli* strains, 5 were OXA, and 2 were positive for both OXA and VIM. NDM and KPC subtypes were not detected. All OXA group carbapenemases were of the OXA-48 subtype. After being first identified in a *Klebsiella pneumoniae* strain in our country (37)., OXA-48 has been found in many countries. It is the most commonly detected carbapenem-hydrolyzing enzyme in our country, as reported in many national and international studies (38). VIM and IMP subtypes have been previously reported in our country (39). There is not enough study on their frequencies. The NDM subtype, which was not detected in our study, was recently reported to cause an outbreak in a neonatal service in Istanbul (45). The KPC subtype was not detected, as in a study conducted with samples from Hacettepe University Ihsan Dogramaci Children's Hospital (45).

When the files of patients with strains producing carbapenemase were requested, files of 3 (42%) of 7 patients with *E. coli* isolates and 19 (67.8%) of 28 patients with *Klebsiella* spp. isolates were accessed.

It was understood that all *Klebsiella* spp. isolates for which files could be accessed were *Klebsiella pneumoniae*. Due to the inability to access these files and probably because the patients whose files were accessed were likely to be alive, our study is expected to show a slightly higher mortality rate.

Among the files examined, the number of files containing sufficient data was 10 (52.6%) for *Klebsiella pneumoniae* strains, while there were no files containing sufficient data for *E. coli* strains. This finding should be considered as a warning that we as physicians need to record patients' clinical information more meticulously.

When the clinical information of the patients was examined, it was observed that they had risk factors known in the literature for being infected with a strain producing ESBL or carbapenemase, such as hematological malignancy, prolonged hospital stay, and ICU admission (48,49). The fact that 6 (60%) of 10 patients died due to these infections once again highlights how high the mortality can be. We attribute the finding of a higher value than the mortality rates reported in the literature, up to 50%, to patient selection bias (11,12,47,50) (file availability). As of January 2014, 9 of 10 patients had died, indicating that the patients had underlying conditions with a poor prognosis.

Table 9. Clinical characteristics of the patients whose files were examined.

Hasta no	Age / Gender	Diagnosis	Foci	Empiric	Response	MIC (mg/L).	2nd AB	MIC (mg/L).	Hosptial	January 2014
1	55 M	ALL	Lung	P/T	Ex	>64	-	-	Ex	-
2	28 M	AML	GIT	P/T	Ex	>64	-	-	Ex	-
3	55 M	Lymphoma	NPF	MEM	Cure	-	-	-	Cure	Ex
4	81 M	BPH	GIT	P/T	None	>64	IMP	2	Cure	Ex
5	63 F	CVE	Lung	P/T	None	>64	DOR	4	Ex	-
6	58 F	AML	Lung	P/T	None	>64	-	>64	Ex	-
7	63 F	AML	NPF	P/T	None	>64	IMP	8	Ex	-
8	73 F	ALL	NPF	IMP	Ex	>4	-	-	Ex	-
9	25 F	Lymphoma	NPF	P/T	None	>64	IMP	2	Cure	Alive
10	70 F	ALL	Lung	P/T	None	>16	CIP	1	Cure	Ex

MIC: Minimum inhibitory concentration, AB: Antibiogram, M: Male, F: Female, ALL: Acute Lymphoblastic Leukemia, AML: Acute myeloid leukemia, BPH: Benign prostatic hyperplasia, CVE: Cerebrovascular event, GIT: Gastrointestinal tract, P/T: Piperacillin tazobactam, MEM: Meropenem, IMP: Imipenem, DOR: Doripenem, CIP: Ciprofloxacin.

The frequency of *Enterobacter*iaceae producing ESBL and/or carbapenemase in our hospital is similar to that in Turkey and Europe. There are probably sudden increases in these values in some years, likely due to local or country-wide outbreaks.

The most important carbapenemase detected in our hospital is OXA-48. In our study, subtypes such as VIM and IMP, which have been reported from our country before, were also detected. When encountering a bacterium with a high MIC value for carbapenems, we believe that this information should be taken into account.

The victims of bacteria producing ESBL and/or carbapenemase are individuals with serious immunosuppression, who have been hospitalized for a long time and are in the most risky areas of hospitals for nosocomial infections, intensive care units. Considering the limited treatment options available to us, it is clear how vital primary prevention of these agents' transmission is. We believe that adherence to the general recommendations for preventing carbapenem-resistant *Enterobacter*iaceae by the CDC and ESCMID is necessary.

Conflicts of Interest

None to declare.

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