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ORIGINAL ARTICLE

Risk factors of community-onset urinary tract infections caused by plasmid-mediated AmpC β-lactamase-producing *Enterobacteriaceae*

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Background: The AmpC β-lactamase (AmpC)-producing *Enterobacteriaceae* emerged worldwide. This study was conducted to determine the risk factors of community-onset urinary tract infections (UTIs) caused by plasmid-mediated AmpC-producing *Enterobacteriaceae*.
Methods: Patients who were diagnosed as community-onset UTIs caused by *Enterobacteriaceae* in a tertiary-care teaching hospital from December 2010 to January 2012 were included. Extended-spectrum β-lactamase (ESBL)-producing isolates were excluded. We identified plasmid-mediated AmpC-producing *Enterobacteriaceae* both phenotypically (by disk potentiation test and double-disk synergy test) and genotypically (by Multiplex polymerase chain reaction (PCR) assay). The demographic data, clinical characteristics, and risk factors of acquisition were described.
Results: Among the 323 non-ESBL-producing *Enterobacteriaceae* identified in community-onset UTIs, 50 isolates were phenotypically positive for AmpC. *Escherichia coli* was the most common AmpC-producing organism (60%), followed *by Klebsiella pneumonia* (8%), and

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Enterobacter cloacae and *Proteus mirabilis* (6% for each species). The independent risk factors for acquisition of AmpC-producing *Enterobacteriaceae* included prior history of cerebral vascular accident [odds ratio (OR) = 2.014; 95% confidence interval (CI) = 1.007–4.031; $p = 0.0048$], and prior use of fluoroquinolones (OR = 4.049; 95% CI = 1.759–9.319; $p = 0.001$) and cephamycin (OR = 9.683; 95% CI = 2.007–45.135; $p = 0.004$). AmpC-producing isolates were multidrug resistant. Carbapenems, cefepime, and piperacillin/tazobactam had the best *in vitro* efficacy. The most commonly identified plasmid-mediated AmpC gene was bla_{CIT}, followed by bla_{DHA}/bla_{EBC}, and bla_{MOx}.

Conclusion: For community-onset UTIs, AmpC-producing *Enterobacteriaceae* should be suspected in those with prior history of cerebral vascular accident and prior use of antimicrobials. To treat these multiple-resistant isolates, carbapenems, cefepime, and piperacillin/tazobactam may be considered.

Introduction

Urinary tract infections (UTIs) are common diseases in the community. Community-onset UTIs account for more than eight million visits to physicians' office and over one million hospital admissions in the United States annually, as well as significant morbidity and healthcare costs.[1,2] *Enterobacteriaceae* are the most common causative pathogens. Resistant *Enterobacteriaceae* have been associated with longer hospital stay and higher antibiotic costs.[3,4] The most common mechanism responsible for multidrug resistance is the production of extended-spectrum β-lactamase (ESBL) or AmpC β-lactamase.[5] The prevalence of ESBL in these uropathogens emerged in recent years, leading to many related clinical researches in UTIs.[6,7] However, few clinical studies on AmpC have been conducted in the UTIs. Multidrug resistance caused by AmpC has emerged among the members of *Enterobacteriaceae* family in the past decades. The prevalence of *Enterobacteriaceae* with AmpC genes had been reported in the United States, China, Korea, India, etc., with ranges between 1.2% and 2.79%.[8–10] A significant national increase in the proportion of isolates with AmpC genes had been observed in Canada, raising from 0.7% to 2.9% through 2007 to 2011.[11]

Bacteria expressing AmpC are resistant to many commonly used β-lactamases. Lack of awareness of such pathogens may result in the administration of inappropriate antimicrobials. The objective of this study was to determine the risk factors of community-onset UTIs caused by AmpC-producing *Enterobacteriaceae*. Because not all the bacteria harboring AmpC genes expressed AmpC and were resistant,[12] phenotypic methods were performed to identify plasmid-mediated AmpC-producing bacteria in this study. The microbiological and molecular characteristics were also described. We hope the information may help physicians identify patients at risk and increase the chance of administration of appropriate antimicrobials.

Methods

Patients and hospital setting

This study was prospectively conducted at the Taipei Veterans General Hospital, a 2900-bed tertiary-care teaching hospital located in Taipei, Taiwan. Adult patients were eligible if they were admitted to the Taipei Veterans General Hospital between December 1, 2010 and January 31, 2012 with the diagnosis of community-onset UTIs caused by *Enterobacteriaceae*. Patients were initially enrolled in the study based on a positive urinalysis result at the Emergency Department and then evaluated only if they met the criteria for a positive urine culture (i.e., $\geq 10^5$ CFU/mL of *Enterobacteriaceae* to be fully evaluable). Only the first infection episode of each patient was included in the analysis. Patients with ESBL-producing *Enterobacteriaceae* were also excluded. Other exclusion criteria included pregnancy or lactation in women, complete obstruction of the urinary tract, perinephric, or intrarenal abscess, and prostatitis suggestive by history of physical findings.[13] This study was approved by the Institutional Review Board of Taipei Veterans General Hospital (VGHIRB No.:201003025IC).

Definitions and variables

Infections diagnosed within the first 48 hours of hospitalization were defined as community-onset infections. It was further classified as healthcare-associated and community-acquired infections. Episodes were considered healthcare-associated infections according to the criteria as follows[14]: the patient received intravenous therapy, wound care, or specialized nursing care at home within 30 days prior to the diagnosis of UTIs; the patient attended a hospital or hemodialysis clinic, or received intravenous chemotherapy within 30 days prior to the diagnosis of UTI; the patient had been hospitalized for 2 or more days within 90 days prior to the diagnosis of UTI; or the individual resided in a nursing home or long-term care facility. Patients who did not meet the above criteria about health care-associated infections were considered to be community-acquired.

Potential risk factors for plasmid-mediated AmpC-producing *Enterobacteriaceae* in community-onset UTIs were ascertained by reviewing the medical charts. The following variables were collected: age and sex; comorbidities; recent steroid use, chemotherapy, or operation; urinary catheter or nasogastric (NG) insertion; recurrent UTIs or antimicrobial exposure; and severity of illness at presentation, including shock, acute kidney injury, acute respiratory failure, disseminated intravascular coagulation, and intensive care unit admission.

Bacteria identification and antimicrobial susceptibility

Identification of *Enterobacteriaceae* was performed using the VITEK 2 system (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibilities were tested by the agar dilution method and interpreted according to the Clinical and Laboratory Standards Institute (CLSI).[15]

Testing for ESBL phenotype

ESBL confirmatory test involved testing cefotaxime (CTX, 30 μg) and ceftazidime (CAZ, 30 μg) alone and in combination with clavulanate (CLA, 10 μg) on Mueller—Hinton agar.[16] If the zone diameter increased 5 mm or more in the presence of CLA, the isolate was considered ESBL-producing. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as quality controls. To avoid false positive result from ESBL confirmatory test,[17] a modified disk potentiation test using 3-aminophenylboronic acid (APB, 400 μg), an AmpC enzyme inhibitor, was conducted. An increase of ≥5 mm in the zone diameter of CTX/CLA and/or CAZ/CLA disks tested in combination with APB (CTX/CLA/APB and/or CAZ/CLA/APB) versus CTX and/or CAZ disks containing APB (CTX/APB and/or CAZ/APB) was considered positive for ESBLs.[18—20]

Testing for AmpC phenotype

In contrast to ESBL confirmatory test recommended by CLSI, there was no standardized method to detect AmpC. In our study, AmpC production was phenotypically detected by the disk potentiation test that contains a CAZ or CTX disk alone or in combination with APB (300 μg), and the double-disk synergy test, which contains a CAZ, a CTX, and an APB (300 μg) disk.[21] An enlargement of the growth-inhibitory zone diameter ≥ 5 mm in a CAZ/APB or CTX/APB disk in the former test or a discernible expansion of the zone around the CAZ or the CTX disk toward a APB disk was indicative of AmpC production.

Determination of plasmid-mediated AmpC genes

The isolates with AmpC-producing phenotype were sought for AmpC genes using a multiplex PCR assay.[22] In contrast to the chromosomal AmpC genes that are usually expressed in the low level, the plasmid-mediated AmpC beta-lactamase genes are usually highly expressed and therefore were our target.[12] This multiplex nucleic acid amplification assay utilizes PCR primers specific for bla_{DHA}, bla_{ACC}, bla_{MOx}, bla_{CIT}, bla_{FOx}, and bla_{EBC} and is capable of detecting six phylogenetic families of bla_{AmpC} on the basis of amplicon size.

Statistical analysis

Descriptive statistics are used to describe clinical variables in patients with plasmid-mediated AmpC-producing or non-AmpC-producing *Enterobacteriaceae* in community-onset UTIs. Continuous and categorical variables were compared by use of the two-sided Student *t* tests and Chi-square tests, respectively. Univariate analysis followed by multivariate logistic regression analysis was performed to identify the risk factors of community-onset UTIs caused by plasmid-mediated AmpC-producing *Enterobacteriaceae*. Variables introduced into the multivariate analysis included those with $p < 0.1$. A two-tailed $p < 0.05$ was considered significant. All statistical analysis was conducted using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).

Results

During the study period, a total of 376 patients with community-onset UTIs caused with *Enterobacteriaceae* were identified. Fifty-nine patients with ESBL-producing isolates were excluded and 317 were enrolled (Table 1). Three hundred and twenty-three *Enterobacteriaceae* were isolated; 218 (67.5%) were *E. coli*, 32 (9.9%) were *P. mirabilis*, 25 (7.7%) were *K. pneumoniae*, and 12 (3.7%) were *Serratia marcescens*. AmpC-producing *Enterobacteriaceae* were identified in 49 patients. *E. coli* was the most common AmpC-producing organism (30 of 50 isolates, 60%), followed *by K. pneumoniae* (four isolates, 8%), and *Enterobacter cloacae*/*P. mirabilis* (three isolates for each species, 6%) and *Citrobacter freundii*/*Morganella morganii*/*S. marcescens* (two isolates for each species, 4%).

Patients with AmpC-producing *Enterobacteriaceae* were older and were more likely to have had previous episodes of UTIs compared to those with negative phenotype (Table 2). They more frequently had a prior history of cerebral vascular accident and bed-ridden status, and urinary catheter in place. Most of the UTIs caused by AmpC-producing and non-AmpC-producing *Enterobacteriaceae* were community

Table 1 The AmpC-producing and non-AmpC-producing *Enterobacteriaceae* in this study

Bacteria species	Phenotype	
	AmpC-producing ($n = 50$)	non-AmpC-producing ($n = 273$)
Citrobacter amalonaticus	0	1
Citrobacter diversus	0	6
Citrobacter freundii	2	2
Enterobacter aerogenes	1	1
Enterobacter cloacae	3	3
Enterobacter gergoviae	0	1
Escherichia coli	30	188
Klebsiella oxytoca	0	1
Klebsiella pneumoniae	4	21
Morganella morganii	2	3
Proteus mirabilis	3	29
Proteus penneri	0	1
Proteus vulgaris	1	0
Providencia alcalifaciens	0	1
Providencia rettgeri	0	3
Providencia stuartii	1	2
Serratia marcescens	2	10
Serratia species	1	0

acquired in origin but AmpC-producing ones were more commonly found in healthcare-associated UTIs. Patients with AmpC-producing *Enterobacteriaceae* also had prior antimicrobial exposure more frequently, especially fluoroquinolones, cephalosporins, and carbapenems (Table 3).

Multivariate analysis (Table 4) revealed the independent risk factors for the acquisition of AmpC-producing *Enterobacteriaceae* included prior history of cerebral vascular accident (OR = 2.014; 95% CI = 1.007–4.031; $p = 0.048$), and prior use of fluoroquinolones (OR = 4.049; 95% CI = 1.759–9.319; $p = 0.001$) and cephamycin (OR = 9.683; 95% CI = 2.007–45.135; $p = 0.004$).

The resistance rate of AmpC-producing *Enterobacteriaceae* against most of the cephalosporins (cefazolin, cefuroxime, cefmetazole, flomoxef, cefotaxime, ceftazidime) and penicillins (ampicillin and ampicillin/sulbactam) were over 50% (Table 5). These AmpC-producing isolates were also resistant to other classes of antimicrobials including fluoroquinolones and trimethoprim/sulfamethoxazole. Carbapenems had the best *in-vitro* activity against AmpC-producing *Enterobacteriaceae* followed by cefepime and piperacillin/tazobactam. Non-AmpC-producing isolates were generally susceptible to most antimicrobial agents, except ampicillin.

Multiplex PCR showed the most commonly detected plasmid-borne AmpC genes was bla_{CIT} (28 of 50 isolates), followed by bla_{DHA} (six isolates), bla_{EBC} (three isolates), and bla_{MOx} (one isolate). Three isolates harbored two different types of AmpC genes. Fifteen isolates did not have detectable AmpC genes.

Table 2 Demographic and clinical characteristics of patients with AmpC-producing *Enterobacteriaceae* and those with non-AmpC-producing ones

Characteristics	AmpC-producing ($n = 49$)	Non-AmpC-producing ($n = 268$)	p
Age	83 (78–87.5)	80 (65–84)	0.003
Male	26 (53.1)	103 (38.4)	0.079
Intensive care unit admission	1 (2.1)	7 (2.6)	> 0.99
Prior UTIs within 1 y	21 (42.9)	61 (22.8)	0.005
Infection type			
Healthcare-associated	18 (36.7)	63 (23.5)	0.076
Community-acquired	31 (63.3)	205 (76.5)	0.076
Initial presentation			
Consciousness change	10 (20.4)	38 (14.2)	0.367
Fever	28 (57.1)	163 (60.8)	0.745
Shock	2 (4.1)	6 (2.2)	0.358
Shaking chills	11 (22.4)	80 (29.9)	0.378
Disseminated intravascular coagulation	0 (0.0)	3 (1.1)	> 0.99
Inotropic agent use	0 (0.0)	4 (1.5)	> 0.99
Acute respiratory failure	1 (2.0)	12 (4.5)	0.700
Acute renal failure	11 (22.4)	48 (17.9)	0.582
Acute urine retention	2 (4.1)	9 (3.4)	0.681
Underlying diseases			
Diabetes mellitus	22 (44.9)	97 (36.2)	0.319
Uremia	2 (4.1)	7 (2.6)	0.634
Chronic kidney disease	10 (20.4)	40 (14.9)	0.450
Urolithiasis	9 (18.4)	27 (10.1)	0.151
Hypertension	25 (51.0)	143 (53.1)	0.884
Coronary artery disease	9 (18.4)	40 (14.9)	0.691
Congestive heart failure	6 (12.2)	33 (12.3)	> 0.99
Cerebral vascular accident	20 (40.8)	63 (23.5)	0.018
Chronic obstructive pulmonary disease	4 (8.2)	22 (8.2)	> 0.99
Autoimmune diseases	0 (0.0)	10 (3.7)	> 0.99
Steroid use within 2 mo	6 (12.2)	16 (6.0)	0.126
Solid or hematological malignancies	10 (20.4)	55 (20.5)	> 0.99
Chemotherapy within 2 wk	0 (0.0)	10 (3.7)	0.371
Operation within 1 mo	3 (6.1)	6 (2.2)	0.148
Bed-ridden	21 (42.9)	63 (23.5)	0.008
Invasive procedures			
Nasogastric tube	15 (30.6)	53 (19.8)	0.131
Urinary catheter	24 (49.6)	71 (26.5)	0.003

Data are presented as *n* (%) except for age, which is shown as median (interquartile range).
UTIs = urinary tract infections.

Table 3 Previous antimicrobial exposure in patients with AmpC-producing *Enterobacteriaceae* and those with non-AmpC-producing ones

Previous antimicrobial exposure	AmpC -producing (n = 49)	Non-AmpC-producing (n = 268)	p
Antimicrobial exposure within 3 mo	30 (61.2)	57 (21.3)	<0.001
Fluoroquinolones	14 (28.6)	19 (7.1)	<0.001
Cephalosporins	24 (49.0)	39 (14.6)	<0.001
Oxyimino-cephalosporin	6 (12.2)	24 (9.0)	0.434
Cephamycin	6 (12.2)	3 (1.1)	<0.001
Aminoglycosides	2 (4.1)	3 (1.1)	0.172
Penicillins	6 (12.2)	22 (8.2)	0.409
Carbapenems	3 (6.1)	3 (1.1)	0.073
Others[a]	3 (6.1)	9 (3.4)	0.407

[a] Including clindamycin, metronidazole, vancomycin, teicoplanin, and trimethoprim/sulfamethoxazole.
Data are presented as n (%) of patients treated with specific antimicrobials.

Discussion

Our study revealed that the prior history of cerebral vascular accident and prior fluoroquinolones and cephamycin use were associated with acquisition of plasmid-mediated AmpC-producing *Enterobacteriaceae*. AmpC-producing isolates were resistant to most of the cephalosporins and penicillins, and to fluoroquinolones and trimethoprim-sulfamethoxazole. The most commonly identified plasmid-borne AmpC gene was bla_{CIT}, followed by bla_{DHA}, bla_{EBC}, and bla_{MOx}.

There have been numerous studies evaluating risk factors for UTIs with ESBL-producing organisms.[23,24] However, little was known about the risk factors of UTIs caused by plasmid-mediated AmpC-producing *Enterobacteriaceae*. In our study, we found that the prior history of cerebral vascular accident was one of the independent risk factors for UTIs caused by AmpC-producing *Enterobacteriaceae*. Resistant strains, such as those with ESBL or carbapenemases, were more commonly isolated in the vulnerable patients because of the impaired survival fitness.[7,25] An early case-control study suggested an association between the prior use of an oxyimino-cephalosporin and the emergence of AmpC-producing *Enterobacteriaceae* infections.[26] Our study revealed the prior use of cephamycin was an independent risk factor. The association between the resistance genes and the prior use of the antimicrobials these genes were against has been well documented.[27] For example, the prior use of carbapenems was associated with subsequent carbapenem resistance in *Acinetobacter baumannii*, whereas the use of cephalosporins has been associated with the subsequent presence of ESBL in *Enterobacteriaceae*.[28] Surprisingly, the prior use of fluoroquinolones was also the independent factor. This may be due to the collateral damage, which described the selection and unwanted development of multidrug-resistant organisms, most commonly linked to cephalosporins and quinolones.[29] The use of multiple antimicrobials may select for isolates equipped with multiple resistance mechanisms. Collateral damage may also be the reason that AmpC-producing *Enterobacteriaceae* were resistant to non-β-lactam antibiotics, particularly fluoroquinolones and trimethoprim-sulfamethoxazole. Because these AmpC-producing isolates were multi-resistant, management of related UTIs were expected to be more difficult. The *in vitro* susceptibility in our study showed carbapenems, followed by cefepime and piperacillin/tazobactam, may be prescribed to critically ill patients with the higher risk of being infected by AmpC-producing *Enterobacteriaceae*. However, carbapenem resistance can arise in AmpC-producing bacteria by mutations that reduce influx or enhance efflux.[30] Similarly, cross-resistance or inducible

Table 4 Multivariable analyses of risk factors for the acquisition of AmpC-producing *Enterobacteriaceae*

Characteristics	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p	OR (95% CI)	p
Healthcare associated UTIs	1.889 (0.991–3.604)	0.053		
Age	1.032 (1.007–1.057)	0.012		
Cerebral vascular accident	2.244 (1.188–4.238)	0.013	2.014 (1.007–4.031)	0.048
Bed-ridden	2.440 (1.297–4.593)	0.006		
Urinary catheter insertion	2.664 (1.430–4.963)	0.002		
Diagnosis of UTI in 1 y	2.545 (1.350–4.797)	0.004		
Antimicrobial exposure within 3 months[a]	5.845 (3.067–11.139)	<0.001		
Fluoroquinolones	5.242 (2.413–11.387)	<0.001	4.049 (1.759–9.319)	0.001
Cephamycin	12.326 (2.971–51.139)	<0.001	9.683 (2.077–45.135)	0.004
Carbapenems	5.761 (1.128–29.422)	0.035		

[a] Not included in the multivariate analyses.
CI = confidence interval; OR = odds ratio; UTI = urinary tract infection.

Table 5 Resistance rate to different antimicrobials between AmpC-producing and non-AmpC-producing *Enterobacteriaceae*[a]

Antimicrobials	Resistance rate		
	AmpC-producing (n = 50)	Non-AmpC-producing (n = 273)	p
Gentamicin	14 (28.0)	46 (16.8)	0.096
Ampicillin	49 (98.0)	196 (71.8)	<0.001
Ampicillin/sulbactam	36 (73.5)	49 (17.9)	<0.001
Piperacillin/tazobactam	5 (10.2)	0 (0.0)	<0.001
Cefazolin	43 (86.0)	50 (18.3)	<0.001
Cefuroxime	32 (65.3)	23 (8.9)	<0.001
Cefmetazole	33 (67.3)	11 (4.2)	<0.001
Flomoxef	24 (49.0)	4 (1.5)	<0.001
Cefotaxime	30 (60.0)	6 (2.2)	<0.001
Ceftazidime	24 (49.0)	4 (1.5)	<0.001
Cefepime	2 (4.1)	3 (1.1)	0.168
Ciprofloxacin	26 (53.1)	35 (12.9)	<0.001
Levofloxacin	24 (49.0)	35 (12.9)	<0.001
TMP/SMX	28 (57.1)	103 (39.8)	0.036
Ertapenem	1 (2.0)	0 (0.0)	0.155
Imipenem	0 (0.0)	0 (0.0)	

[a] Not all isolates were tested.
Data are presented as n (%) of isolates resistant to specific antimicrobials.
TMP/SMX = trimethoprim/sulfamethoxazole.

resistance to β-lactamase inhibitor during therapy has also been reported.

Six different groups of AmpC were categorized based on their similarities, including ACC (origin *Hafniaalvei*), FOX (origin unknown), MOX (origin unknown), DHA (origin *Morganella morganii*), CIT (origin *C. freundii*), and EBC (origin *E. cloaca*).[22] In our study, bla_{CIT} was the most commonly detected AmpC genes. Bla_{CIT} included the genes encoding LAT-1 to LAT-4, CMY-2 to CMY-7, and BIL-1.[22] The exact AmpC gene in our study was not identified. In a national surveillance study in Canada, the dominant genotype in AmpC-producing *E. coli* was CMY-2.[11] One study in Japan also showed the predominant type in the Kinki region is CMY-2.[31] Other studies in Spain and Thailand also revealed the same result.[32,33]

The strength of our study included the large number of patients and prospective design. Our study had its limitations. First, phenotypic methods may underestimate the prevalence of AmpC-producing *Enterobacteriaceae*. Western blot and quantitative PCR may identify them more sensitively. However, the overexpression of protein and RNA transcript may not necessarily lead to phenotypic change. Second, some thought that the exclusion of ESBL-producing bacteria may incur some concerns that these patients may not represent the whole population. However, the risk factors of those with ESBL-producing bacteria, including previous antibiotics use, were similar to those for AmpC-producing ones in our study.[34,35] We excluded ESBL-producing bacteria to avoid confounding. Third, we excluded patients with severe urinary tract comorbidities such as the complete obstruction of the urinary tract, perinephric or intrarenal abscess, and prostatitis, which may underestimate the prevalence of AmpC-producing bacteria.

In conclusion, we demonstrated that independent risk factors for community-onset UTIs caused by plasmid-mediated AmpC-producing *Enterobacteriaceae* were the prior history of cerebral vascular accident and prior use of cephamycin and fluoroquinolones. These isolates were multiple resistant to cephalosporins, penicillins, fluoroquinolones and trimethoprim-sulfamethoxazole. Carbapenems, cefepime, and piperacillin/tazobactam have the best *in vitro* efficacy.

Conflicts of interest

Dr. Chang-Phone Fung received a research grant from the Investigator-Initiated Studies Program of Merck & Co., Inc. This does not alter the authors' adherence to all of the *Journal of Microbiology, Immunology and Infection's* policies on sharing data and materials.

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