Journal of Microbiology, Immunology and Infection (2018) 51, 565—572

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

Citrobacter freundii bacteremia: Risk factors of mortality and prevalence of resistance genes

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Received 26 April 2016; received in revised form 25 August 2016; accepted 26 August 2016
Available online 22 June 2017

KEYWORDS
Bacteremia;
Citrobacter freundii;
Mortality risk factors;
Polymicrobial infection;
TEM-1 gene

Abstract *Background/purpose:* Multidrug-resistant strains of *Citrobacter* have emerged, which carry Amp-C β-lactamase (Amp-C), broad-spectrum β-lactamase, extended-spectrum β-lactamase (ESBL), and other resistance mechanisms. These strains are associated with a higher rate of in-hospital mortality. The object of this study is to determine the mortality risk factors, susceptibility pattern to antibiotics, and prevalence of resistance genes in patients with *Citrobacter freundii* bacteremia.
Methods: From January 2009 to December 2014, blood isolates of *C. freundii* were collected in MacKay Memorial Hospital, Taipei, Taiwan. PCR technique and sequencing were performed for resistance genes. Pulsed-field gel electrophoresis (PFGE) was done using *Xba*I restriction enzyme. The clinical characteristics and risk factors for mortality are demonstrated.
Results: The 36 blood isolates of *C. freundii* belonged to 32 different PFGE pulsotypes, and 15 isolates (41.7%) were polymicrobial. The most common source of infection was intra-abdominal origin (61.1%), followed by unknown sources (22.2%), the urinary tract (8.3%), intravascular catheter (5.6%), and soft tissue (2.8%). High degree of antibiotic resistance was noted for cefazolin (100%), cefoxitin (97.2%), and cefuroxime (66.7%). The bla_{TEM-1} resistance gene was present in 16.7% isolates. 72.2% isolates carried bla_{AmpC} and 5.6% isolates carried ESBL

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http://dx.doi.org/10.1016/j.jmii.2016.08.016

genes (bla_{SHV-12} or $bla_{CTX-M-15}$). Multivariate analysis indicated that the independent risk factor for 28-day mortality was carrying the bla_{TEM-1} resistance gene.
Conclusion: For patients with *C. freundii* bacteremia, carrying the bla_{TEM-1} resistance gene was an independent risk factor for 28-day mortality. Carbapenems, fourth-generation cephalosporins, amikacin, and quinolones are still reliable agents for drug-resistant strains.

Introduction

Citrobacter species belong to a group of facultative, anaerobic, Gram-negative bacilli within the family Enterobacteriaceae. They are frequently found in water, soil, food, and the intestines of animals and humans. Previously recognized as environmental contaminants or colonizers with low virulence, they are now known to cause a wide spectrum of infections involving the urinary tract, liver, biliary tract, peritoneum, intestines, bone, respiratory tract, endocardium, wounds, soft tissue, meninges, and the bloodstream.[1–4] *Citrobacter* species account for 0.8% of all Gram-negative infections in one large observational study and have represented approximately 3–6% of the isolates of Enterobacteriaceae in a hospital setting.[1,5,6] This genus is classified into 11 genospecies (*Citrobacter freundii*, *C. koseri*, *Citrobacter amalonaticus*, *Citrobacter farmeri*, *Citrobacter youngae*, *Citrobacter braakii*, *Citrobacter werkmanii*, *Citrobacter sedlakii*, *Citrobacter rodentium*, *Citrobacter gillenii*, and *Citrobacter murliniae*), of which *Citrobacter freundii* and *Citrobacter koseri* have been recognized as significant pathogens in patients with underlying diseases or immunocompromised status.[7–9]

Citrobacter infection can occur in sporadic cases or as nosocomial spread. Documented outbreaks in the ward or intensive care unit (ICU) resulted in colonization, surgical wound infection, and meningitis. Therefore, a well-established hospital policy is essential for infection control.[10–13] A mortality rate of 6.8% has been reported among hospitalized patients with *Citrobacter* infections, but it can significantly increase to 17.8–56% with *Citrobacter* bacteremia.[8,14–16] Public concern has risen in regard with longer hospital stays and higher antibiotic costs due to the recent emergence of multidrug-resistant strains of *Citrobacter*. These strains carry Amp-C β-lactamase (Amp-C), broad-spectrum β-lactamase, extended-spectrum β-lactamase (ESBL), plasmid-mediated quinolone resistance determinants, or even carbapenemase.[8,9,17–23] Multidrug-resistant strains of *Citrobacter* are more commonly acquired among patients who have received previous antibiotic treatments, and this fact results from the inducible expression of Amp-C and the selection of ESBL strains.[18] Multidrug-resistant *C. freundii* strains have been associated with a higher rate of in-hospital mortality compared to susceptible strains.[24]

Although serious septicemias in neonatal patients have been reported, bacteremia due to *Citrobacter* is less common in adults.[10,25] Bloodstream infection of *Citrobacter* in adults can originate from the abdominal cavity, the urinary tract, lungs, or intravascular catheters. Polymicrobial bacteremia frequently occurs if the infection source is intra-abdominal.[16] Many poor prognostic factors for *C. freundii* bloodstream infections have been reported, such as pneumonia, altered mental status, hypothermia, oliguria, septic shock, hyperbilirubinemia, azotemia, and thrombocytopenia.[16] In this study, we focus on isolates of *C. freundii* from bloodstream infection and surveying the mortality risk factors, susceptibility pattern to antibiotics, and the prevalence of resistance genes.

Methods

Bacterial isolates

From January 2009 to December 2014, blood isolates of *C. freundii* were collected in MacKay Memorial Hospital, a 2200-bed tertiary medical center in northern Taiwan. For patients with more than one positive blood culture, only the first isolate was included. Those isolates were identified in the microbiology laboratory using a Vitek 2 system (bio-Me'rieux Vitek Systems Inc., Hazelwood, MO, USA). Susceptibility tests were also performed using this system. Antibiotics susceptibility testing against cefazolin, cefuroxime, ceftazidime, cefotaxime, cefoxitin, flomoxef, cefpirome, co-trimaxazole, gentamicin, amikacin, ertapenem, imipenem/cilastatin, tigecycline, ciprofloxacin, and moxifloxacin were interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines.[26] Isolates were kept frozen at −70 °C in trypticase soy broth (BD, Sparks, MD, USA) containing 20% glycerol (v/v) until further testing.

Gene detection

Bacteria were boiled in sterile water for 10 min, and the supernatant was collected and used for PCR as DNA sources. The 25-μl reaction mix consisted of 1X S-T Gold buffer, 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 20 pmol of each primer, and 0.4 units of Super-Therm polymerase. Bacteria isolates were screened by PCR for the presence of the carbapenemase genes (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{SPM}, bla_{GIM}, bla_{SIM}, bla_{KPC}, bla_{OXA}, bla_{BIC}, bla_{AIM}, bla_{DIM}) and β-lactamase genes (bla_{SHV}, bla_{TEM}, bla_{CTX-M}, bla_{AmpC}). Products were visualized on agarose gel. Sequence analysis of the resulting amplicons was performed with an ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Pulsed-field gel electrophoresis (PFGE)

The *C. freundii* isolates were typed by PFGE following digestion of intact genomic DNA with *Xba*I (Biolabs,

Beverly, MA, USA). The DNA fragments were separated on 1% (w/v) SeaKem GTG agarose gels in 0.5% Tris-borate-EDTA buffer in a CHEF Mapper apparatus (Bio-Rad, Hercules, CA, USA). This was performed with a potential of 6 V/cm pulsed from 5 to 30 s for 22 h at 14 °C. The completed gels were stained with ethidium bromide and photographed with UV light. The *Xba*I restriction profiles were initially compared with each other by visual inspection, and isolates were considered to be closely related if they showed differences of less than three bands. Computer-assisted analysis was also performed using BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium). Cluster analysis was performed by the unweighted pair group method with mathematical averaging, and DNA relatedness was calculated using the band-based dice coefficient with a band tolerance setting of 1.5% and optimization setting of 1.5% for the whole profile. Isolates were considered to belong to the same cluster when the similarity coefficient reached 90%.

Study population and data collection

We retrospectively reviewed medical charts of all patients with *C. freundii* bloodstream infection. Bloodstream infection of *C. freundii* was defined as the isolation of *C. freundii* from one or more blood cultures with clinical features of infection. The following data were collected: patient characteristics, source of bacteremia, comorbidities, intravascular catheter, major operation, whether or not they were admitted to the ICU at the onset of bacteremia, and outcome. An adequate empirical therapy was defined as the administration of at least one antimicrobial agent, to which a pathogen was sensitive in vitro, within 48 h of bacteremia, with an approved route and dosage appropriate for end organ function. Otherwise, a therapy that did not meet these criteria was considered inadequate. This retrospective study was approved by the MacKay Memorial Institutional Review Broad (protocol numbers 16MMHIS002).

Statistical analysis

Categorical variables were characterized as numbers and percentages (or positive rates). Continuous variables were characterized as the mean ± standard deviation (SD) and the median with inter-quartile range (IQR). Categorical variables were analyzed using the chi-square test with Yates' continuity correction or Fisher's exact test as appropriate. Continuous variables were analyzed by a Student's two-sample *t*-test for parametric methods or Mann–Whitney *U* test for nonparametric methods as appropriate. Binary logistic regression was used to identify independent risk factors for the 28-day mortality. Odds ratios (OR) and 95% confidence intervals (CI) were analyzed separately for each of the risk factor variables by the univariate analysis. Subsequently, all significant variables with p value ≤ 0.05 in the univariate analysis were carried into the multivariate analysis of the binary logistic regression. All data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 21.0 (SPSS Inc., Chicago, IL, USA).

Results

During the study period, a total of 36 blood isolates of *C. freundii* were collected from 36 patients (Table 1). Nineteen (52.8%) patients were male, while 17 (47.2%) were female. The age of patients ranged from 28 to 87 years with a mean of 60.78 ± 15.28 years. There was no difference in age between male and female groups (59.11 ± 15.79 and 62.65 ± 14.94 years, respectively). Of all 36 isolates of *C. freundii*, 15 (41.7%) were polymicrobial infections, including *Enterobacter*, *Enterococcus*, *Klebsiella*,

Table 1 Comparison of clinical characteristics among 36 patients with *Citrobacter freundii* bacteremia.

Characteristics	No.	Percentage
Age	60.78 ± 15.28	
Sex(Male)	19	52.78%
ICU stay	6	16.67%
Resistance genes		
bla_{TEM-1}	6	s16.67%
$bla_{CTX-M-15}$	1	2.78%
bla_{SHV-12}	1	2.78%
bla_{AmpC}	26	72.22%
28-day mortality	6	16.67%
Polymicrobial infection	15	41.67%
Comorbidities		
Hypertension	12	33.33%
Chronic kidney disease	6	16.67%
Diabetes mellitus	10	27.78%
Coronary artery disease	5	13.89%
COPD	2	5.56%
Malignancy	18	50.00%
Biliary stones	8	22.22%
Liver cirrhosis	5	13.89%
Major operation	6	16.67%
Peptic ulcer disease	10	27.78%
Shock	7	19.44%
Invasive procedures		
Foley catheter	3	8.33%
PCN	1	2.78%
Mechanical ventilator	3	8.33%
CVC	6	16.67%
Hemodialysis catheter	5	13.89%
ERCP	3	8.33%
PTCD/PTGBD	4	11.11%
Port-a-catheter	5	13.89%
Source of infection		
Urinary	3	8.33%
Abdominal	22	61.11%
Intravascular catheter	2	5.56%
Soft tissue	1	2.78%
Unknown	8	22.22%
Adequate empirical therapy	25	69.44%

COPD = chronic obstructive pulmonary disease, CVC = central venous catheter, ERCP = endoscopic retrograde cholangiopancreatography, ICU = intensive care unit, PCN = percutaneous nephrostomy, PTCD = percutaneous transhepatic cholangial drainage, PTGBD = percutaneous transhepatic gall bladder drainage.

coagulase-negative *Staphylococcus*, *Acinetobacter baumannii*, and other Enterobacteriaceae. Six patients died within 28 days, the overall mortality rate was 16.7%, and there was no statistical difference in the 28-day mortality between polymicrobial and monomicrobial infections (18.8% and 15%, $p = 0.677$). Eighteen patients (50%) had underlying malignancy, including eight (22.2%) with hepatobiliary or pancreatic cancer, two (5.6%) with rectosigmoid cancer, two (5.6%) with urothelial cancer, two (5.6%) with cervical or vaginal cancer, two (5.6%) with breast cancer, one (2.8%) with thyroid cancer, and one (2.8%) with retroperitoneum cancer. Twelve patients (33.3%) had hypertension, 10 patients (27.8%) had diabetes mellitus, and 10 patients had peptic ulcer disease (27.7%).

The most common source of infection was intra-abdominal origin (61.1%), followed by unknown sources (22.2%), the urinary tract (8.3%), intravascular catheters (5.6%), and soft tissue (2.8%). For the 22 patients with intra-abdominal infection as the source of bacteremia, eight (36.3%) had intra-abdominal cancer, six (27.2%) had hepatobiliary stones, six (27.2%) received abdominal surgery (mainly appendectomy), five (22.7%) had liver cirrhosis, three (13.6%) received endoscopic retrograde cholangiopancreatography (ERCP), and three (13.6%) received percutaneous transhepatic cholangial drainage (PTCD) or percutaneous transhepatic gall bladder drainage (PTGBD). Among the three patients with urinary tract infection, one had bladder cancer and another had cervical cancer. Two patients had intravascular catheter-related bacteremia, including one port-a-catheter infection and one central venous catheter infection. For patients with polymicrobial *C. freundii* bacteremia, 53.3% of infections originated from intra-abdominal routes, followed by 26.7% from an unknown source, 13.3% from an intravascular catheter, and 6.7% from the urinary tract.

Varying degrees of resistance to different antibiotics were observed, including cefazolin (100%), second-generation cephalosporin (66.7% for cefuroxime), third-generation cephalosporins (25% and 22.2% for ceftazidime and cefotaxime, respectively), cephamycins (97.2% for cefoxitin and 27.7% for flomoxef), co-trimaxazole (27.8%), and gentamicin (19%). Antibiotic agents with a low level of resistance included imipenem/cilastatin (0%), amikacin (0%), ertapenem (2.8%), tigecycline (2.8%), cefpirome (2.8%), ciprofloxacin (5.6%), and moxifloxacin (5.6%), as shown in Fig. 1. Fig. 2 shows the dendrogram and PFGE of *Xba*I-digested genomic DNAs from the 36 *C. freundii* blood isolates. 32 unique pulsotypes were determined. bla_{AmpC} was detected in 26 isolates (72.2%). The bla_{TEM-1} presented in six patients (16.7%) with pulsotypes 2, 3, 6, 12, 19, and 30. bla_{SHV-12} presented in one patient (2.8%) with pulsotype 31, and $bla_{CTX-M-15}$ presented in one patient (2.8%) with pulsotype 12. No carbapenemase genes were detected among these isolates.

In univariate analysis, the independent risk factors for mortality included carrying bla_{TEM-1}, ICU stay, chronic kidney disease, and hemodialysis catheter placement. The multivariate analysis showed that carrying bla_{TEM-1} was an independent risk factor for 28-day mortality (odds ratio = 20.84%, CI = 1.285−338.000, $p = 0.033$) (Table 2). For patients carrying isolates of *C. freundii* with bla_{TEM-1} resistance gene, the 28-day mortality rate reached 50% (Table 3). The mortality rate decreased to 10% if bla_{TEM-1}-negative.

Discussion

In this study, we demonstrated that the independent risk factor for 28-day mortality in patients with *C. freundii* bacteremia was carrying the bla_{TEM-1} resistance gene, and bla_{TEM-1} presented in 16.7% of all isolates with different pulsotypes. Polymicrobial infection occurred in 41.7% of patients with *C. freundii* bacteremia, and 53.3% of polymicrobial infections originated from intra-abdominal routes. Intraabdominal infection (61.1%), especially biliary tract infection, was the leading cause of *C. freundii* bacteremia, and this result can be explained by the relatively high rates of hepatobiliary or pancreatic malignancy (22%), hepatobiliary stones (22%), and intra-abdominal

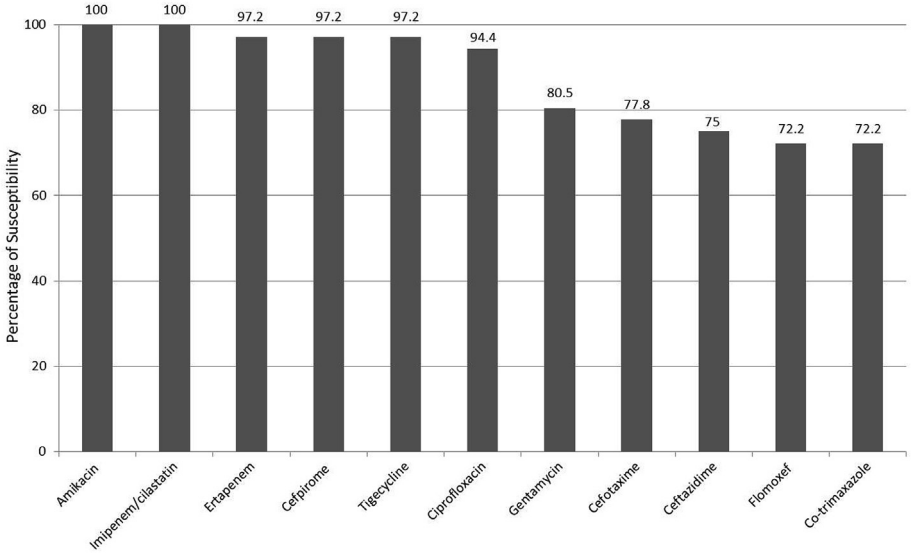

Figure 1. Percentage of susceptibility to antibiotics of 36 isolates of *C. freundii*.

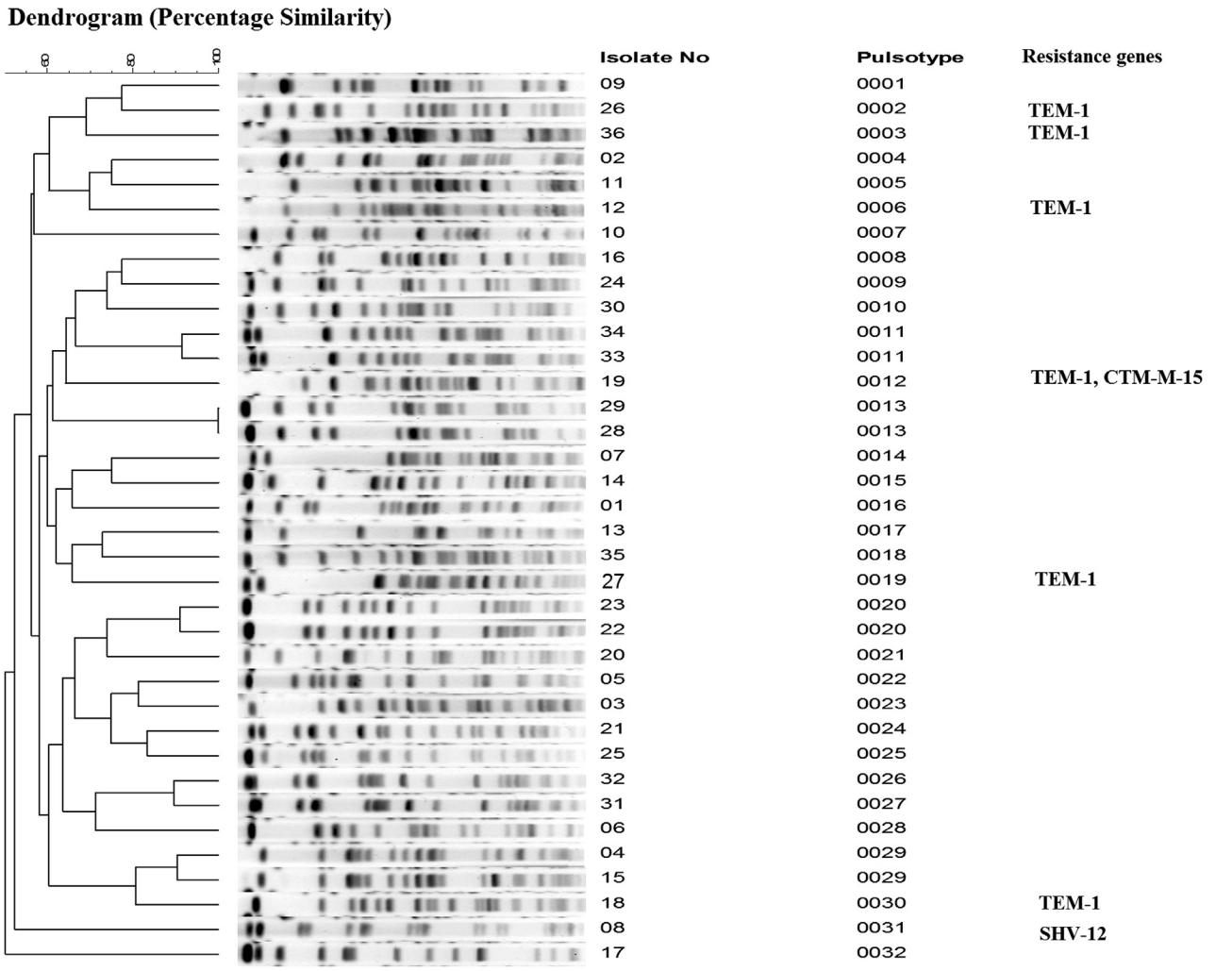

Figure 2. Dendrogram of 36 *Citrobacter freundii* PFGE pulsotypes and resistance genes. PFGE = pulsed-field gel electrophoresis.

surgery (16.7%) in our study population in Taiwan. In another retrospective study of a 700-bed tertiary university hospital in Greece, *Citrobacter* species were frequently associated with polymicrobial infection, and the length of hospital stay was reported to be longer in patients with polymicrobial compared to monomicrobial infections.[9] Moreover, polymicrobial bacteremia of *C. freundii* was reported to be a significant risk factor of mortality in one study.[27]

Most of our patients were aged and had significant underlying illnesses, including malignancy (50%), hypertension (33.3%), diabetes mellitus (27.8%), hepatobiliary stones (22.2%), chronic kidney disease (16.7%), and coronary artery disease (13.9%). We also found that a large percentage of malignancy were intra-abdominal, including hepatobiliary, pancreatic, or rectosigmoid cancer. This correlation between *C. freundii* bacteremia and elderly patients with underlying diseases are consistent with previous studies.[1,8,16] It is worth mentioning that four younger patients (mean age 43.7 years) without significant systemic illness were diagnosed with *C. freundii* bacteremia due to acute appendicitis and underwent appendectomy. Thus, we can point out that *C. freundii* bacteremia mostly occurs in older patients with systemic illnesses like malignancy, but there is still a small portion of sporadic cases in younger persons due to acute appendicitis.

Owing to the wide use of broad-spectrum antibiotics, *C. freundii* has become increasingly resistant. In an early study in Taiwan, isolates of *C. freundii* in two different time periods (from 1987 to 1988 and from 1997 to 1998) were collected and showed decreasing susceptibility to piperacillin, cefuroxime, ceftazidime, aztreonam, gentamicin, tobramycin, amikacin, and ciprofloxacin.[17] A tertiary care hospital in India reported even higher degrees of resistance to ceftazidime (85%), cefotaxime (85%), piperacillin (65%), amikacin (30%), ciprofloxacin (60%), and imipenem (15%) in isolates of *C. freundii*.[8] In our study, a high rate of resistance (66.7—97.2%) to second-generation cephalosporin and cephamycin and around 22.2—25% resistance to third-generation cephalosporins were observed. Carbapenems, cefpirome, amikacin, and quinolones are still reliable agents for treating *C. freundii* bacteremia. Although most isolates of *C. freundii* were susceptible to tigecycline, it is not recommended for bacteremia because of its low serum concentration and lack of clinical trials in bacteremic patients.[28,29] In our

Table 2 Risk factors associated with 28-day mortality in 36 patients with *Citrobacter freundii* bacteremia (mortality:6; survival: 30).

Demographic and characteristics	Univariate analysis			Multivariate analysis		
	Odds ratio	(95% CI)	*p* value	Odds ratio	(95% CI)	*p* value
$bla_{\text{TEM-1}}$	9.000	1.223–66.231	0.031	20.840	1.285–338.000	0.033
ICU stay	9.000	1.223–66.231	0.031	4.839	0.147–158.937	0.376
Chronic kidney disease	9.000	1.223–66.231	0.031	1.488	0.069–32.160	0.800
Hemodialysis catheter	14.000	1.632–120.087	0.016	7.614	0.112–518.365	0.346
Malignancy	2.286	0.362–14.431	0.379	–	–	–
Polymicrobial	1.500	0.258–8.711	0.651	–	–	–
Adequate empirical therapy	0.857	0.132–5.552	0.872	–	–	–
Age	1.025	0.962–1.092	0.440	–	–	–
Sex(Male)	0.875	0.151–5.054	0.881	–	–	–
Urinary source	–	–	–	–	–	–
Abdominal source	0.250	0.039–1.605	0.144	–	–	–
Intravascular catheter source	–	–	–	–	–	–
Soft tissue source	–	–	–	–	–	–
$bla_{\text{CTX-M-15}}$	–	–	–	–	–	–
$bla_{\text{SHV-12}}$	–	–	–	–	–	–
bla_{AmpC}	0.727	0.111–4.768	0.740	–	–	–

CI = confidence interval, ICU = intensive care unit.

Table 3 The clinical characteristics among 6 patients harboring $bla_{\text{TEM-1}}$ *Citrobacter freundii* bacteremia.

No	Age	Sex	28-Day mortality	ICU stay	Co-morbidity	Invasive procedures	Poly-microbial infection	Other β-lactamase genes	Adequate empirical therapy
1	72	F	No	No	Bladder cancer	No	No	$\boldsymbol{bla_{\text{AmpC}}}$	Yes
2	68	M	Yes	No	Bladder cancer	No	No	$\boldsymbol{bla_{\text{AmpC}}}$	No
3	41	M	No	No	HTN, CKD, DM	No	No	$\boldsymbol{bla_{\text{AmpC}}}$, $\boldsymbol{bla_{\text{CTX-M-15}}}$	No
4	66	M	Yes	Yes	CKD, cirrhosis	HD catheter	Yes	$\boldsymbol{bla_{\text{AmpC}}}$	No
5	65	F	No	No	CAC, HTN, CAD, cirrhosis	PTCD	No	$\boldsymbol{bla_{\text{AmpC}}}$	No
6	57	F	Yes	No	Breast cancer	Port-a-cath	No	$\boldsymbol{bla_{\text{AmpC}}}$	Yes

CAC = Cholangiocarcinoma, CAD = coronary artery disease, CKD = chronic kidney disease, DM = diabetes mellitus, HD = hemodialysis, HTN = hypertension, ICU = intensive care unit, PTCD = percutaneous transhepatic cholangial drainage.

previous retrospective study in the same hospital from January 2002 to December 2003, 58% of *C. freundii* bloodstream infection isolates were resistant to cefotaxime. Among the cefotaxime-resistant strains, 43% carried ESBL and Amp-C genes.[30] In this study conducted from January 2009 to December 2014, the rate of cefotaxime-resistant strains of C. *freundii* decreased to 22.2%, despite the fact that the MIC interpretive criteria for resistance to cefotaxime had modified from ≥64 μg/mL in 2007 to ≥4 μg/mL in 2014 by CLSI.[26,31] Among these cefotaxime-resistant strains, 25% carried ESBL and Amp-C genes. The mortality rate also decreased from 41 to 16.7% during these two periods. In order to trace the clonal relationship of the 36 isolates of *C. freundii*, we performed PFGE and found 32 different pulsotypes. All the six isolates carrying $bla_{\text{TEM-1}}$ had different pulsotypes, which reflected no outbreak observed in our hospital. These results demonstrate a trend of decreasing antibiotic resistance and mortality rate in patients with *C. freundii* bacteremia in our hospital during the past ten-year period. A recent study showed that reduced fluoroquinolone usage was associated with reversal in resistance prevalence in ESBL-producing *E. coli* within months.[32] We had applied strict infection control and isolation policies for multiple drug resistant organisms as well as antibiotic steward program during the past decade, and these might have attributed the decline of resistance and ESBL strains.

Increased morality rate among patients with *Enterobacter* sp. or *C. freundii* bloodstream infections had correlation with antibiotic resistance.[24] Our study further revealed that isolate of *C. freundii* carrying $bla_{\text{TEM-1}}$ was a significant risk factor for 28-day mortality according to multivariate analysis. Increased resistance to cephalosporins, gentamicin, quinolones, or even ertapenem was observed in these six $bla_{\text{TEM-1}}$ positive isolates, resulting in ineffective antibiotic treatment. TEM-1 is a broad-spectrum β-lactamase with limited resistance against penicillins and only a narrow spectrum of cephalosporins. Thus, the co-existence of Amp-C and other mechanisms may broaden the spectrum of resistance. For *C. freundii*, antimicrobial resistance phenotypes cannot accurately distinguish the resistance mechanisms caused by Amp-C or ESBL, especially

for combinations of ESBL and Amp-C.[33] Because of the inducible expression of Amp-C, isolates carrying only the Amp-C gene can have a variety of levels of resistance, from cefuroxime-susceptible to both cefotaxime-resistant and flomoxef-resistant. Among our 36 isolates of *C. freundii*, one isolate carrying $bla_{CTX\text{-}M\text{-}15}$, $bla_{TEM\text{-}1}$, and bla_{AmpC} showed increased minimum inhibitory concentration (MIC) to cefpirome, but it was still susceptible to carbapenem. Therefore, carbapenems are suggested for treatment if MIC for fourth-generation cephalosporin is high. However, since the emergence of carbapenem resistance in *C. freundii* has been reported worldwide, the reasonable usage of carbapenem in daily practice must be evaluated as part of antibiotic stewardship and infection control programs.[13,34–36] In our study, one strain of *C. freundii* showed elevated MIC to ertapenem, but no carbapenemase genes were detected by PCR. This resistance might be attributed to other mechanisms, such as loss of outer membrane protein or up-regulation of the efflux pump.

Our study had its limitations. First, only one isolate carrying $bla_{SHV\text{-}12}$ and another with $bla_{CTX\text{-}M\text{-}15}$ were collected in our study, so we do not find that these two ESBL genes have an influence on patient outcomes. Second, univariate analysis showed that ICU stay, chronic kidney disease, and hemodialysis catheter placement were other mortality risk factors, but they were not statistically significant in multivariate analysis. Due to limited number of cases in our study, it may not be possible to identify all the possible mortality risk factors for *C. freundii* bloodstream infection.

In conclusion, the $bla_{TEM\text{-}1}$ resistance gene was present in 16.7% of *C. freundii* isolates, and ESBL genes ($bla_{SHV\text{-}12}$ or $bla_{CTX\text{-}M\text{-}15}$) were detected in 5.6% isolates of *C. freundii*. Carrying the $bla_{TEM\text{-}1}$ resistance gene was an independent risk factor for 28-day mortality in patients with *C. freundii* bacteremia. Currently, carbapenems, fourth-generation cephalosporins, amikacin, and quinolones are still reliable agents for drug-resistant strains. Appropriate and early antibiotic therapies for patients at risk of mortality along with proper infection control measures are essential for decreasing *C. freundii* bacteremia mortality as well as drug-resistant strains.

Conflicts of interest

All authors declare that they have no competing interests.

Acknowledgments

This study was supported by grants MMH-104-15 and MMH-105-16 from MacKay Memorial Hospital, Taipei, Taiwan.

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