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Original Article

Clinical Experiences of the Infections Caused by Extended-Spectrum β-Lactamase-Producing *Serratia marcescens* at a Medical Center in Taiwan

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SUMMARY: CTX-M-3 has become the most common extended-spectrum β-lactamase (ESBL) produced by *Serratia marcescens* in Taiwan. An expanded effort to detect ESBL among 123 nonrepetitive isolates of *S. marcescens* was made and 15 (12%) ESBL-producers were identified, all revealing CTX-M-3. Without routinely detecting the ESBL for *S. marcescens* in clinical laboratories, 80% of the ESBL-producers were reported to be susceptible to cefepime. The clinical spectrum of ESBL-producing *S. marcescens*-related infections included febrile urinary tract infection ($n = 3$); afebrile pyuria ($n = 2$); pneumonia ($n = 3$); spontaneous bacterial peritonitis ($n = 3$); secondary bacteremia ($n = 2$) and one each with primary bacteremia and colonization of the central catheter tip. Overall, the 30-day mortality rate was 33.3% (5/15) and the outcome depended on the severity of the underlying disorder and infection per se. In conclusion, although our case numbers were limited, due to the substantial incidence and associated mortality of ESBL-producing *S. marcescens* and its potential treatment failure by an apparently susceptible cephalosporin, we recommend that the detection and report of ESBL production for *S. marcescens* in clinical laboratories be made mandatory.

INTRODUCTION

Serratia marcescens is a common cause of nosocomial infection in Taiwan (1), where strains producing extended-spectrum β-lactamases (ESBLs) have recently been reported (2,3). CTX-M-3, which confers characteristic phenotypes of high-level cefotaxime resistance but is susceptible to ceftazidime, has become the predominant ESBL identified among the *S. marcescens* strains tested (2). Nevertheless, the clinical impact of infection with CTX-M-3-producing *S. marcescens* remains undefined. Based on previous clinical experiences with ESBL-producing *Enterobacteriaceae* strains, treatment with an apparently susceptible cephalosporin may potentially lead to clinical failure due to the abilities of these strains to become resistant during therapy (4,5). However, as is well known, the clinical spectrum and outcome of the infections caused by ESBL-producing *S. marcescens* have not been described. Since routine surveying for ESBL production in *S. marcescens* has not commonly been performed at clinical microbiology laboratories (6), physicians may still use an apparently susceptible cephalosporin to treat the infection caused by *S. marcescens* without knowing whether ESBLs are present. Therefore, the objectives of this study were to analyze the epidemiology, clinical spectrum and outcome of infections caused by ESBL-producing *S. marcescens* recovered from a medical center in Taiwan.

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MATERIALS AND METHODS

Organisms: From August 2001 through January 2002, a total of 123 nonrepetitive clinical isolates of *S. marcescens* were consecutively collected at a medical center in Taiwan. Each isolate was recovered from a different patient. All isolates were identified on the basis of routine microbiologic methods, and the species were confirmed using the VITEK system (BioMerieux Vitek Inc, Hazelwood, Mo., USA).

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was performed using a disk diffusion method (7). The control strains included *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853. Routine antibiotic disks (Becton Dickinson Microbiology Systems, Cockeysville, Md., USA) included the following: amikacin (30 μg), amoxicillin/clavulanic acid (20/10 μg), ampicillin (10 μg), cefotaxime (30 μg), cephalothin (30 μg), ciprofloxacin (5 μg), piperacillin (100 μg), gentamicin (10 μg) and imipenem (10 μg). The breakpoints for the susceptible and resistant categories of additional flomoxef (30 μg) were 18 mm or more and 12 mm or less, according to the manufacturer's instruction (BD Biosciences, Franklin Lakes, N. J., USA).

Confirmation test of ESBL phenotype: MICs were determined by an agar dilution method, and a final bacterial inoculum of 10^4 CFU per spot of 5 to 8 mm in diameter was delivered with a multipoint inoculator (8). The ESBL phenotype was confirmed by a reduction of ≥ 3 $\log_2$ dilutions in an MIC for either cefotaxime or ceftazidime (Sigma Chemical Co., St. Louis, Mo., USA) in the presence of clavulanic acid (The United States Pharmacopoeia Convention, Inc., Rockville, Md., USA) 4 μg/ml (6). The MIC ranges included 0.06 - 512 μg/mL for either ceftazidime or cefotaxime alone,

and 0.06 - 64 μg/mL for ceftazidime or cefotaxime plus 4 μg/mL clavulanic acid (2). Quality control was assured by *Klebsiella pneumoniae* ATCC 700603.

Plasmid extraction and purification: Plasmid DNA was extracted from the *S. marcescens* isolates based on the rapid alkaline lysis method as previously described (9), and was purified using a Plasmid Miniprep Purification Kit (Amersham Biosciences, Uppsala, Sweden).

PCR amplification and DNA sequencing: To amplify the CTX-M-, TEM- and SHV- related gene from the plasmid DNA of clinical isolates with the ESBL phenotype, the following oligonuleotide primers were used: TEM-forward (5′-ATA AAA TTC TTG AAG ACG AAA-3′), TEM-reverse (5′-GAC AGT TAC CAA TGC TTA ATC-3′) were used to amplify bla_{TEM} (10); SHV-forward (5′-TGG TTA TGC GTT ATA TTC GCC -3′), SHV-reverse (5′-GGT TAG CGT TGC CAG TGC T -3′) were used to amplify bla_{SHV} (11); and CTX-M-forward (5′- TGT TGT TAG GAA GTG TGC CGC -3′), CTX-M-reverse (5′- TCG TTG GTG GTG CCA TAG TC -3′) were used to amplify $bla_{CTX\text{-}M\text{-}3}$ (12). The complete coding sequences of the $bla_{CTX\text{-}M\text{-}3}$ genes were amplified with the primers CTX-M-3-forward (5′-GGA TCC ATG GTT AAA AAA TCA CTG CG-3′) and CTX-M-3-reverse (5′-AAG CTT TTA CAA ACC GTC GGT GAC-3′), as described in our previous report (2). PCR conditions for all these genes were 3 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C; and finally, 7 min at 72°C. Purified amplicons were sequenced with the same primers by use of an automated DNA sequencer (ABI PRISM 377; Applied Biosystems, Foster City, Calif., USA).

Pulsed-field gel electrophoresis (PFGE): PFGE was performed and interpreted as previously described (13,14). Whole chromosomal DNA was digested in agarose with *Xba*I, and the restriction fragments were separated in a CHEF MAPPER XA apparatus (Bio-Rad Laboratories, Hercules, Calif., USA). All bands had to match exactly to classify isolates as indistinguishable. Patterns differing by one to 3 bands were designated as highly related. Isolates that differed by 4 - 6 bands were considered possibly related. Isolates with more than 6 bands different were considered different types (14). Strains were designed as non-typeable if repeated attempts at DNA preparation and electrophoresis failed to provide a banding pattern (13).

Data collection: The demographic and clinical data of patients with ESBL-producing *S. marcescens* were collected retrospectively. The following information was recorded: age, sex, date of admission, onset of infection, specimen and site of infection, ward patient was staying in when the positive sample was collected, underlying disease, co-pathogen or concurrent other infection and antimicrobial therapy and ultimate outcome. Nosocomial infection was defined as an infection which developed ≥48 h after hospitalization, while an infection which developed without recent (<30 days) hospitalization was considered community-acquired. An antibiotic regimen was considered to be appropriate if the infecting organism was susceptible to at least one of the drugs administered.

RESULTS

Sources of the organisms and their antimicrobial susceptibility testing: A total of 123 clinical isolates were included in the experiments. The sources of the organisms and their antimicrobial susceptibilities by the disk diffusion method are shown in Table 1. The most common sources were various respiratory secretions (bronchoalveolar lavage, 24; endotracheal aspirates, 3; and sputum, 1), followed by urine, pus, ascites, blood and central venous catheter tips. Overall, the organisms that were most susceptible to antibiotics included imipenem (99%), cefepime (89%), flomoxef (80%) and amikacin (76%). The susceptibility rates of other compounds were less than 60%, including cefotaxime (57%) and ciprofloxacin (54%). The susceptibility rates for cefotaxime of isolates from urine (8/26, 31%), blood (2/13, 15%) and catheter tips (1/11, 9%) were less than those from bronchoalveolar lavage (21/24, 88%), ascites (14/17, 82%) and pus (18/24, 75%).

Confirmation of ESBL phenotype by MIC- and PCR-based methods: The MIC-based method of ESBL phenotypic confirmation recommended by the NCCLS document was not issued for *S. marcescens* (6). This method suggests MIC ranges of 0.25 - 128 μg/mL for ceftazidime + clavulanic acid

Table 1. Number of isolates susceptible to each antimicrobial agent by disk diffusion method for *Serratia marcescens* isolated from various clinical specimens

Specimens (n = 123)	Antibiotics										
	GM	AM	CF	AMC	CIP	CTX	PIP	AN	FLO	FEP	IPM
Urine (26)	10	0	1	1	14	8	7	13	16	22	26
Pus (24)	20	2	0	3	21	18	20	24	24	23	24
Blood (13)	2	0	0	0	8	2	3	10	7	10	13
Tip (11)	1	0	0	2	6	1	1	5	6	10	11
Sputum (1)	0	0	0	0	1	0	0	0	1	1	1
ETA (3)	2	0	0	0	2	3	2	3	3	3	3
BAL (24)	21	1	1	7	7	21	22	24	21	22	23
PE (2)	2	0	0	0	0	2	2	2	2	2	2
CSF (2)	2	0	0	0	1	1	2	2	2	2	2
Ascites (17)	11	1	0	1	6	14	12	11	16	14	17
Total	71	4	2	14	66	70	71	94	98	109	122
S (%)	58	3	2	11	54	57	58	76	80	89	99

GM, gentamicin; AM, ampicillin; CF, cephalothin; AMC, amoxicillin/clavulanic acid; CIP, ciprofloxacin; CTX, cefotaxime; PIP, piperacillin; AN, amikacin; FLO, flomoxef; FEP, cefepime; IPM, imipenem; ETA, endotracheal aspirate; BAL: bronchoalveolar lavage; PE, pleural effusion; CSF, cerebrospinal fluid; S, susceptibility.

Table 2. *Serratia marcescens* with ESBL phenotype confirmed by MIC- and PCR-based methods

Strain no.	MIC (μg/mL)				ESBL
	CAZ	CAZ/CLA	CTX	CTX/CLA	
62	0.5	0.12	128	1	CTX-M-3
66	0.5	0.25	256	1	CTX-M-3
67	0.12	0.12	64	1	CTX-M-3
68	0.25	0.25	256	8	CTX-M-3
72	8	4	256	8	CTX-M-3
73	2	2	256	16	CTX-M-3
74	0.5	0.12	256	2	CTX-M-3
78	2	0.5	256	1	CTX-M-3
80	0.5	0.5	64	1	CTX-M-3
86	0.5	0.12	128	1	CTX-M-3
87	0.5	1	512	32	CTX-M-3
92	0.5	0.12	128	0.5	CTX-M-3
97	0.5	0.25	256	1	CTX-M-3
103	0.5	0.25	64	1	CTX-M-3
116	0.5	0.06	64	0.5	CTX-M-3

ESBL, extended-spectrum β-lactamase; CAZ, ceftazidime; CLA, clavulanic acid; CTX, cefotaxime.

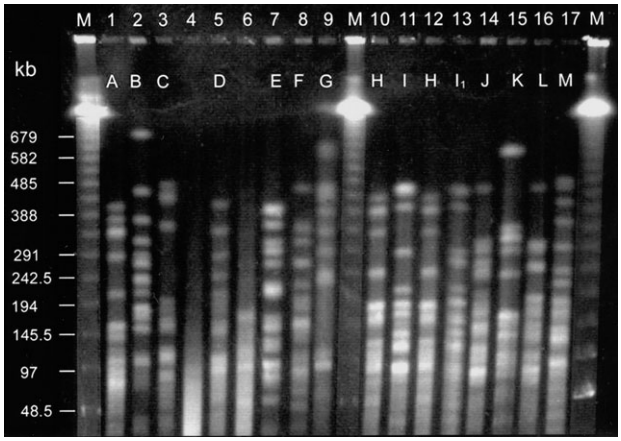

Fig. 1. Pulsed-field gel electrophoresis revealing 13 different types (patterns A to M) among 17 *Serratia marcescens* producing extended-spectrum β-lactamases. M, molecular size markers of lambda DNA ladder (in kilobase [kb]). Lanes 1 to 15, isolates from Patients 1 to 15 in current report. Lanes 16-17, ESBL-producing *S. marcescens* from another hospital as controls. Lanes 4 and 6 are non-typeable. Lanes 10 and 12 are indistinguishable (pattern H). Lanes 11 and 13 are highly related (patterns I and I_1).

and 0.25 - 64 μg/mL for cefotaxime + clavulanic acid. These MIC ranges are not appropriate for *S. marcescens*. Because the coexistent AmpC enzyme in an ESBL-producer probably maintains a high MIC level for ceftazidime or cefotaxime in the presence of clavulanic acid, the MIC-based method should use broad MIC ranges to avoid off-scale MIC levels and nondeterminable results. Our previous experiences have suggested that the MIC ranges included 0.06 - 512 μg/mL for either ceftazidime or cefotaxime alone, and that 0.06 - 64 μg/mL for ceftazidime or cefotaxime plus 4 μg/mL clavulanic acid could overcome the probable AmpC confounding effect and the ESBL phenotype for *S. marcescens* could thus successfully be determined (2). However, there is the possibility for a false-negative ESBL interpretation if the net activity of ESBL is relatively weaker than that of AmpC in terms of conferring to elevated MICs for cefotaxime/cefotaxime + clavulanic acid such as 128/64 or 64/16 μg/mL (AmpC phenotype) (2). In fact, the PCR-based method did not detect an ESBL gene among our cefotaxime-nonsusceptible *S. marcescens* isolates with characteristics of the AmpC phenotype. Overall, a total of 12% (15/123) of the isolates were confirmed to be an ESBL-related phenotype and were all identified as producing CTX-M-3 by PCR and subsequent DNA sequencing (Table 2). The susceptibility rate of cefepime for the ESBL-producers was 80% using the disk diffusion method.

Molecular epidemiology of ESBL-producing *S. marcescens*: The demographic data, clinical spectrum and outcome of patients infected by ESBL-producing *S. marcescens* is tabulated in Table 3. There were 9 women and 6 men with a mean age of 69 years (range, 36 - 84 years). The sources of the 15 ESBL-producers included urine (n = 5); blood (n = 4); ascites (n = 2); sputum (n = 2); a central venous catheter tip (n = 1); and bronchoalveolar lavage (n = 1). There were 12 patients with nosocomial infections, including 2 patients directly transferred from other hospitals with an infection that had developed after ≥48 h hospital stay. Only 2 cases (Patients 10 and 13) acquired infections in the same ward (Respiratory Care Center [RCC]), implicating no obvious epidemiological linkage between the other patients. Although the isolates from Patients 4 and 6 were poorly visualized on PFGE, they were acquired from different sources (Patient 4, community-acquired; Patient 6, nosocomially acquired), probably implying no epidemiologic correlation. The PFGE of the remaining 13 isolates revealed 11 different types (patterns A to K). Of these, only two isolates from Patients 10 and 12, respectively, were indistinguishable (Fig. 1). Among 3 patients with community-acquired infections, one (Patient 4) had no prior history of hospitalization, one (Patient 1) had been hospitalized due to renal stone disease and had undergone a prostatectomy 1 year ago, and the third (Patient 2) had multiple hospitalizations due to acute exacerbation of chronic obstructive pulmonary disease within a 1-year period. The ESBL-producing *S. marcescens* has been repetitively isolated from the urine specimens of Patient 1 for 1 year, even after several courses of therapy with imipenem were administered during prior hospitalizations.

Clinical spectrum and outcome of ESBL-producing *S. marcescens* infections: The clinical spectrum of infections caused by ESBL-producing *S. marcescens* included febrile urinary tract infection (n = 3), afebrile pyuria (n = 2), pneumonia (n = 3), spontaneous bacterial peritonitis (n = 3), and one each with primary bacteremia, cholangitis with bacteremia, bed sore infection with bacteremia, and colonization of a central catheter tip (without concurrent bacteremia and local exit site infection). Overall, the 30-day mortality rate was 33.3% (5/15). Except for one patient (Patient 10), who rapidly died within 24 h after onset due to fulminant sepsis including spontaneous bacterial peritonitis, bacteremia and pneumonia, 14 patients received >3 days of antimicrobial therapy, resulting in 4 deaths within 1 month. Among 6 patients receiving inappropriate antimicrobial therapy (such as cefotaxime, cephradine and piperacillin), one patient (Patient 2) died of pneumonia, while of 8 patients who received an appropriate antibiotic regimen (such as imipenem, flomoxef, and ciprofloxacin), 2 patients died of spontaneous bacterial peritonitis (Patients 3 and 7) and one died of an intracerebral hemorrhage (Patient 5). Overall, the causes of death of the 3 patients were attributed to infections that were uncontrolled even under antimicrobial therapy.

Table 3. Demographic data, clinical spectrum and outcome of patients with infections caused by ESBL-producing *Serratia marcescens*

Patient	Source[1)]	Infection[2)]	Ward[3)]	Underlying disease[4)]	Associated infection or co-pathogen	Therapy[5)]	Outcome (hospitalized day)
1[6)]	urine	afebrile pyuria	OPD	BPH renal stone	none	ciprofloxacin (S)	prolonged bacteruria
2[6)]	sputum	pneumonia	ER	COPD	none	cefotaxime (R)	died (day 7)
3	blood	SBP	18C	cirrhosis uremia	none	imipenem (S)	died (day 12)
4[6)]	blood	cholangitis	ER	duodenal diverticulum	none	cephradine (R) gentamicin (R)	discharged (day 9)
5	urine	febrile pyuria	SICU	ICH	none	imipenem (S)	died (day 18)
6	blood	bed sore infection	ER*	DM old CVA	DM foot	piperacillin (R)	discharged (day 27)
7	ascites	SBP	11C	cholangiocarcinoma	*Pseudomonas aeruginosa*	imipenem (S) ciprofloxacin (S)	died (day 9)
8	urine	febrile pyuria	ER*	spinal cord injury	none	piperacillin (S)	discharged (day 18)
9	BAL	pneumonia	RCC	dementia	*Pseudomonas aeruginosa*	piperacillin (R)	discharged (day 12)
10	ascites	SBP	RCU	cirrhosis DM	pneumonia bacteremia	none	died (day 1)
11	urine	febrile pyuria	SICU-1	ICH	none	cephradine (R)	discharged (day 31)
12	blood	bacteremia	RCW	DM ICH COPD	pneumonia	imipenem (S)	discharged (day 42)
13	urine	afebrile pyuria	RCU-1	DM neurogenic bladder	none	cephradine (R)	discharged (day 42)
14	sputum	pneumonia	SICU-2	DM	*Escherichia coli*	flomoxef (S)	discharged (day 52)
15	CVC tip	colonization	RCC	traumatic fracture	pneumonia *Acinetobacter baumannii*	imipenem (S)	discharged (day 60)

[1)]: BAL, bronchoalveolar lavage; CVC, central venous catheter.
[2)]: SBP, spontaneous bacterial peritonitis.
[3)]: Ward: at which positive samples were collected. OPD, outpatient department; ER, emergency room; SICU, surgical intensive care unit; RCC, respiratory care center; RCU, respiratory care unit; RCW, respiratory care ward. *: directly transferred from other hospitals with nosocomial infection.
[4)]: BPH, benign prostate hypertrophy; COPD, chronic obstructive pulmonary disease; ICH, intracerebral hemorrhage; DM, diabetes mellitus; CVA, cerebrovascular accident.
[5)]: S, susceptible; R, resistant.
[6)]: Community-acquired infection.

DISCUSSION

This study demonstrated a prevalence of 12% for ESBL production (all CTX-M-3) among *S. marcescens* isolates. The successful detection of ESBL by the MIC-based method implies that the net activity of CTX-M-3 among our isolates is stronger than that of AmpC in terms of conferring resistance to cefotaxime. This result is in accordance with our previous report demonstrating that the agar dilution method using broader MIC ranges may achieve a highly predictive value compared to the PCR-based method (2). Also, the most common type of ESBL (CTX-M-3) produced by *S. marcescens* in this study was the same as that in our previous report (2,3). A total of 53 (43%) isolates in the current study were resistant to cefotaxime. The alternative mechanism of resistance in the 38 cefotaxime-resistant isolates lacking an ESBL was presumed to be due to the AmpC β-lactamases, as in our previous report (2). However, the rate of ESBL production among *S. marcescens* apparently was not in accordance with our previous report, which described 22 (65%) of 34 cefotaxime-resistant isolates containing $bla_{\text{CTX-M-3}}$ genes (2). In fact, a recent molecular epidemiology experiment using PFGE for those previously described ESBL-producers revealed a major clone implicating intra-hospital outbreaks (data not shown), thus accounting for its high rate of ESBL production, which may not reflect the true prevalence of ESBL production among *S. marcescens*. In contrast, PFGE for the ESBL-producers of the current report did not reveal clonal relatedness, hence explaining the discrepancy in the ESBL rates between the two studies.

There were geographic discrepancies in the prevalence rates and genotypes of ESBLs produced by *S. marcescens* among different countries. For example, in one region of Poland, Naumiuk et al. reported a prevalence rate of 19% for ESBL-producing *S. marcescens* predominated by CTX-M-3 with evidence of clonal dissemination (15). However in one region of France, De Champs et al. reported a prevalence rate of about 1.2 to 2.5% of *S. marcescens* producing ESBLs, which were all identified as TEM-3 like β-lactamases (16). This TEM-3 like enzyme has been the most prevalent ESBL (51.2%) produced by *Enterobacteriaceae* with evidence of a clonally epidemic spread in that particular region of France (16). It is difficult to explain the reasons for the relatively lower ESBL rates in comparison to the rates obtained in our study. When using the same indicators of cefotaxime and ceftazidime to survey ESBL as those used in our study, however, ESBL prevalenc can potentially be underestimated if the net activity of the TEM-3 like enzyme is weaker than that of AmpC in terms of conferring ceftazidime or ceftotaxime resistance, which would probably result in a false-negative ESBL test. The PCR-based method for all cefotaxime or ceftazidime nonsusceptible strains might reflect an accurate

rate of ESBL prevalence. The relatively high prevalence rate of CTX-M-3-producing *S. marcescens* in our non-clonally related isolates may be attributed to the insertion sequence IS*Ecp1*, which can be involved in the mobilization and expression of the $bla_{CTX\text{-}M\text{-}3}$ gene (17).

The clinical spectrum of ESBL-producing *S. marcescens* varied, and included colonization, asymptomatic pyuria (or in a chronic urinary carrier), primary bacteremia, urosepsis, peritonitis and pneumonia. The severity of the infections also varied from mild and self-limited entities (survived on inappropriate antimicrobial therapy) to life-threatening events. For example, Patient 4 with suspected cholangitis-related bacteremia recovered quickly on therapy with resistant cephradine and gentamicin, suggesting the mild severity of infection. Patient 9 had pneumonia with probable co-pathogens of *P. aeruginosa* and *S. marcescens*, but survived on therapy with piperacillin susceptible to the former, but resistant to the latter, implicating the minor pathogenic role of *S. marcescens* in this case. In contrast, Patient 10 rapidly died of multiple ESBL-producing *S. marcescens*-related infections, including spontaneous peritonitis, bacteremia and pneumonia. In addition, 5 patients were treated with imipenem, resulting in 3 deaths (Patients 3, 5 and 7), suggesting difficult-to-treat infections associated with severe underlying disorders (Patients 3 and 7) or a severe, original, non-infectious disease (Patient 5).

Although carbapenem has been regarded as the drug of choice for severe infections caused by ESBL-producing *Enterobacteriaceae* (4,5), for severe ESBL-producing *S. marcescens* infections, there is a great need for alternative, more effective therapeutic modalities than the use of carbapenem. At present, too few such patients have been treated to permit meaningful assessments of outcomes. In addition, despite the apparently high susceptibility of cefepime (89% for all strains and 80% for ESBL-isolates), none of the patients herein were treated with this broad-spectrum oxyimino-cephalosporin. Thus, the impact of ESBL on the clinical effect of this compound can be difficult to discern. However, attention should be devoted to the detection of ESBL-producing *S. marcescens* to avoid potential treatment failure. Nowadays, due to increasingly known ESBLs in *S. marcescens*, physicians are forced to increase their indiscriminative use of carbapenems, which may possibly further promote carbapenem-resistant strains. However, although our case numbers were limited, it seems unethical to let physicians continuously treat those patients suffering from ESBL-producing *S. marcescens* infections without enabling the physicians to find out if ESBL is present.

In conclusion, the clinical spectrum of these ESBL-producing *S. marcescens* varied, with different severity and outcomes. At present, carbapenem remained the most active agent tested. Due to the substantially high mortality rate associated with ESBL-producing *S. marcescens* and to avoid potential treatment failure of an apparently susceptible cephalosporin as well, we suggest that the detection and report of ESBL production for *S. marcescens* in clinical laboratories be made mandatory.

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