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Microbiologic and Clinical Implications of Bacteremia Due to Extended-Spectrum-β-Lactamase-Producing *Klebsiella pneumoniae* with or without Plasmid-Mediated AmpC β-Lactamase DHA-1[▽]

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Bacteremias caused by *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase (ESBL-KP; *n* = 52) and producing both ESBL and AmpC-type DHA-1 beta-lactamase (ESBL-PMABL-KP; *n* = 20) were analyzed. Higher MIC_{50}s and MIC_{90}s for carbapenems, ciprofloxacin, and piperacillin-tazobactam were observed with ESBL-PMABL-KP than with ESBL-KP. Patients with oxyimino-β-lactam exposure and high modified Pitt bacteremia scores (HMPBSs) were at higher risk, while those with piperacillin-tazobactam and aminoglycoside exposure were at lower risk for ESBL-KP bacteremia. Patients with fluoroquinolone exposure, diabetes mellitus, and HMPBS were at higher risk, while those with aminoglycoside exposure were at lower risk, for ESBL-PMABL-KP bacteremia.

Enterobacteriaceae producing an extended-spectrum β-lactamase (ESBL) or a plasmid-mediated AmpC β-lactamase (PMABL) are resistant to broad-spectrum penicillins and oxyimino-β-lactams but usually remain susceptible to carbapenems (5, 11, 16). Strains producing PMABLs are also resistant to cephamycins. Flomoxef, a cephamycin unique in having a difluoromethylthioacetamido group at position 7, has better *in vitro* activity against ESBL-producing *Enterobacteriaceae* than other cephamycins (8) but is hydrolyzed by PMABLs (5, 8, 11).

In 2004, a cluster of in nosocomial bloodstream infections (BSIs) caused by flomoxef-resistant ESBL-producing *Klebsiella pneumoniae* isolates was noted at our hospital. In this work, we investigated those isolates and found that they also produced a PMABL. We also compared the clinical and microbiological features of bacteremic infections caused by ESBL plus PMABL-producing *K. pneumoniae* (ESBL-PAMBL-KP) with infections caused by *K. pneumoniae* producing only an ESBL (ESBL-KP) or lacking any of those enzymes.

ESBL production in *K. pneumoniae* was screened and then confirmed as recommended by the CLSI (1). Susceptibility testing was performed using the Etest (AB Biodisk, Solna, Sweden). *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as control strains. The breakpoints for susceptibility were ≤8 mg/liter for flomoxef (4) and according to the CLSI (2) for the other agents. As described previously, we identified the ESBL types using PCR amplification and sequencing for bla_{SHV}, bla_{TEM}, and $bla_{CTX\text{-}M}$ (7), detected PMABL genes by use of multiplex PCR (13), and genotyped the *K. pneumoniae* isolates by use of pulsed-field gel electrophoresis (PFGE) (3). The PFGE profiles were interpreted as previously proposed (18).

The studied bacteremic patients belonged in two groups, group 1 (ESBL-KP bacteremia; *n* = 52) and group 2 (ESBL-PMABL-KP bacteremia; *n* = 20). A control group of patients with nosocomially acquired cefazolin-susceptible *K. pneumoniae* bacteremia (*n* = 100) from the same ward and the same period was included for comparison. Demographic and clinical information for patients of the three groups are reported in Table 1. Comparison of contingency data was carried out using the Chi-square test or Fisher's exact test, while comparison of continuous data was performed using Student's *t* test or the Mann-Whitney U test. For comparison to the control group, variables in group 1 and group 2 with *P* values of <0.1 in univariate analyses were separately entered into a multiple logistic regression model to identify the independent risk(s) for BSIs caused by ESBL-KP and ESBL-PMABL-KP, respectively. All comparisons were performed using SPSS 15 software for Windows (SPSS, Inc., Chicago, IL).

PFGE patterns revealed multiclonality, indicating that each of the ESBL-KP and ESBL-PMABL-KP isolates was of an individual clone. One *K. pneumoniae* isolate might have more than one ESBL-encoding gene; a total of 62 ESBL-encoding genes were found in isolates of group 1, while 23 were found in those of group 2. CTX-M14 was predominant in group 2 (60.9% versus 30.6%; $P = 0.022$), while other ESBL enzymes, such as SHV-2, SHV-5, SHV-12, SHV-28, and CTX-M3 were not significantly different in each group. All ESBL-PMABL KP isolates produced the DHA-1 enzyme.

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TABLE 1. Demographic and clinical information of bloodstream infections due to *K. pneumoniae*[a]

Variable	Value for patients			*P*		
	Group 1 (n = 52)	Group 2 (n = 20)	Controls (n = 100)	Group 1 vs. controls	Group 2 vs. controls	Group 1 vs. group 2
Demographics						
Male sex	30 (57.6)	13 (65.0)	60 (60.0)	0.729	0.804	0.657
Median age (range)	45 (22–91)	42 (30–91)	37 (18–91)	0.009	0.120	0.863
Clinical information						
Neutropenia[b]	3 (5.7)	1 (5.0)	25 (25.0)	0.009	0.013	1
Malignancy	11 (21.2)	6 (30.0)	54 (54.0)	<0.001	0.085	0.537
Renal failure[c]	28 (53.8)	12 (60.0)	32 (32.0)	0.003	0.023	0.837
Diabetes mellitus	17 (32.6)	10 (50.0)	23 (23.0)	0.246	0.026	0.277
Hepatic dysfunction[d]	16 (30.8)	4 (20.0)	30 (30.0)	1	0.428	0.535
CVC insertion[e]	25 (48.1)	11 (55.0)	57 (57.0)	0.309	1	0.792
Foley catheter indwelling[e]	36 (69.2)	17 (85.0)	65 (65.0)	0.718	0.114	0.289
Mechanical ventilatory support[e]	27 (51.9)	13 (65.0)	34 (34.0)	0.037	0.014	0.462
Median (range) modified Pitt bacteremia score[f]	5 (0–11)	6 (1–10)	3 (0–10)	0.003	0.001	0.761
High modified Pitt bacteremia score[f] (≥7 points)	16 (30.7)	11 (55.0)	10 (10.0)	0.003	<0.001	0.103
LOS ≧ 14 days before infection	40 (76.9)	15 (75.0)	63 (63.0)	0.027	0.442	1
Previous hospitalization[g]	31 (59.6)	16 (80.0)	58 (58.0)	0.862	0.082	0.177
Recent antibiotic therapy[h]	44 (84.6)	17 (85.0)	76 (76.0)	0.302	0.564	1
Recent exposure to:						
Carbapenems[i]	11 (21.2)	6 (30.0)	19 (19.0)	0.831	0.364	0.537
Fluoroquinolones[j]	14 (26.9)	10 (50.0)	18 (18.0)	0.214	0.007	0.113
β-Lactam/β-lactamase-inhibitor[k]	18 (34.6)	6 (30.0)	47 (47.0)	0.168	0.219	0.786
Piperacillin-tazobactam	5 (9.6)	5 (25.0)	42 (42.0)	<0.001	0.625	0.127
Aminoglycosides[l]	20 (38.5)	8 (40.0)	65 (65.0)	0.002	0.046	0.881
Oxyimino-β-lactams[m]	37 (71.2)	14 (70.0)	45 (45.0)	0.003	0.051	0.847
Flomoxef	7 (13.4)	4 (20.0)	6 (6.0)	0.135	0.061	0.484
Appropriate empirical therapy[n]	30 (57.7)	5 (25.0)	100 (100)	<0.001	<0.001	0.026
Initial therapy failure at 72 h[o]	17 (33.3)	12 (60.0)	23 (23)	0.245	0.002	0.065
Mortality within 14 days	20 (38.5)	14 (70.0)	32 (32)	0.473	0.002	0.033

[a] Results are shown for ESBL-producing *K. pneumoniae* without DHA-1 (group 1), ESBL-producing *K. pneumoniae* with DHA-1 (group 2), and non-ESBL-producing *K. pneumoniae* (controls). A patient was included once if culture of blood sampled at different time points all grew *K. pneumoniae*, and the first bloodstream infection (BSI) episode and the pathogen in this episode were counted. Values are numbers (percentages) of patients unless stated otherwise. Abbreviations: CVC, central venous catheter; ICU, intensive care unit; LOS, length of stay.

[b] Neutropenia was defined as an absolute peripheral neutrophil count of <500 cells/μl.

[c] Renal failure was defined as a serum creatinine level of >2.0 mg/dl or as kidney functions in patients receiving regular hemodialysis.

[d] Hepatic dysfunction was defined as high serum bilirubin level (>2.5 mg/dl) and alanine aminotransferase level (>80 IU/liter).

[e] Invasive procedure was defined as central venous catheter implacement, endotracheal intubation, or urinary catheterization within 72 h before the emergence of *K. pneumoniae* BSI.

[f] The modified Pitt bacteremia score was calculated according to the following criteria: (i) oral temperature, 2 points for a temperature of ≤35°C or ≥40°C 1 point for a temperature of 35.1 to 36.0°C or 39.0 to 39.9°C, and 0 points for a temperature of 36.1 to 38.9°C; (ii) for hypotension, 2 points for an acute hypotensive event with decreases in systolic (>30 mm Hg) and diastolic (>20 mm Hg) blood pressures, use of intravenous vasopressor agents, or a systolic blood pressure of <90 mm Hg; (iii) for mechanical ventilation, 2 points; (iv) for cardiac arrest, 4 points; and (v) for mental status, 0 points for alert, 1 point for disoriented, 2 points for stuporous, and 4 points for comatose. A modified Pitt bacteremia score of ≥7 points was considered high (12).

[g] Prior hospitalization was defined as a hospital stay within the 3 months before BSI developed.

[h] Antibiotic exposure within 1 month.

[i] Including imipenem, meropenem, and ertapenem.

[j] Including ciprofloxacin, levofloxacin, and moxifloxacin.

[k] Including amoxicillin-clavulanic acid, ampicillin-sulbactam, and piperacillin-tazobactam.

[l] Including amikacin and gentamicin.

[m] Including aztreonam, ceftazidime, cefotaxime, and ceftriaxone.

[n] Appropriate empirical antibiotic therapy refers to an empirical antimicrobial treatment with the regimen containing at least one antibiotic to which the *K. pneumoniae* that subsequently grew from blood culture was susceptible *in vitro*.

[o] The initial response was considered favorable if clinical conditions and laboratory data improved; otherwise, it was regarded as initial therapy failure at 72 h.

Compared to the ESBL-PMABL-KP isolates, the ESBL-KP isolates not harboring DHA-1 had significantly higher flomoxef susceptibility (98.1% versus 0%) and ciprofloxacin susceptibility (67.3% versus 10%) rates. While the ESBL-KP and ESBL-PMABL-KP isolates were universally susceptible to meropenem, a number of them were ertapenem intermediate (EI), ertapenem resistant (ER), imipenem intermediate (II), and/or imipenem resistant (IR). Specifically, among the 52 ESBL-KP isolates, 1 (1.9%) was EI and another 1 (1.9%) ER and II; among the 20 ESBL-PMABL-KP isolates, 14 (70%) were exclusively EI, 3 (15%) were exclusively ER, 2 (10%) were ER and II, and 1 (5%) was ER and IR. The MIC_{50} and MIC_{90} values of meropenem, imipenem, ertapenem, ciprofloxacin, and piperacillin-tazobactam for isolates in group 1 were overall lower than those for isolates in group 2 (Table 2).

Thirty patients (57.7%) in group 1 and five (25.0%) in group 2 received appropriate empirical antibiotic therapy (P = 0.026). Compared to what was found for group 1, there was a trend suggesting a higher percentage of initial therapy failure (60% versus 33.3%; P = 0.065) and a significantly higher 14-

TABLE 2. Antimicrobial susceptibility of ESBL-producing *K. pneumoniae* isolates with or without DHA-1

Antibiotic	MIC (μg/ml) for ESBL-producing *K. pneumoniae* isolates					
	Without DHA-1 (n = 52)			With DHA-1 (n = 20)		
	Range	50%	90%	Range	50%	90%
Meropenem	0.015–0.5	0.03	0.06	0.06–1	0.12	0.5
Imipenem	0.12–2	0.25	0.5	0.25–4	0.5	2
Ertapenem	0.015–4	0.015	0.5	0.5–4	0.5	2
Ciprofloxacin	0.03–>32	0.125	8	0.5–>32	>32	>32
Amikacin	1–>256	4	32	1–>256	>256	>256
Piperacillin-tazobactam	0.25–>256	8	32	0.25–>256	8	64

day mortality rate in group 2 (70% versus 38.5%; $P = 0.033$), which might result from the lower frequency of carbapenem-containing antibiotics (15% versus 32.7%) used on an empirical basis.

Upon separate comparison to the control group (Table 1), we found (i) significantly higher proportions of renal failure, mechanical ventilation, and high modified Pitt bacteremia scores (HMPBSs) and lower proportions of neutropenia and recent aminoglycoside exposure in group 1 and group 2, (ii) significantly older ages, longer hospital stays prior to bacteremia, higher proportions of recent exposure to an oxyimino-β-lactam(s), and lower proportions of underlying malignancy and recent piperacillin-tazobactam exposure in group 1, and (iii) significantly higher proportions of recent exposure to a fluoroquinolone(s) and underlying diabetes mellitus in group 2.

The multiple logistic regression model revealed that patients with recent oxyimino-β-lactam exposure (odds ratio [OR] = 3.22; 95% confidence interval [CI] = 1.45 to 7.45; $P = 0.004$) and HMPBS (OR = 3.13; 95% CI = 1.18 to 8.33; $P = 0.022$) were at higher risk, while those with recent piperacillin-tazobactam (OR = 0.17; 95% CI = 0.06 to 0.49; $P = 0.001$) and aminoglycoside exposure (OR = 0.31; 95% CI = 0.14 to 0.67; $P = 0.003$) were at lower risk for ESBL-KP bacteremia. On the other hand, patients with recent fluoroquinolone exposure (OR = 11.10; 95% CI = 2.64 to 46.61; $P = 0.001$), diabetes mellitus (OR = 4.64; 95% CI = 1.27 to 17.03; $P = 0.021$), and HMPBS (OR = 16.38; 95% CI = 4.00 to 67.05; $P < 0.001$) were at higher risk, while those with recent aminoglycoside exposure (OR = 0.22; 95% CI = 0.05 to 0.77; $P = 0.019$) were at lower risk for ESBL-PMABL-KP bacteremia.

This study highlighted some risk factors for bacteremia caused by ESBL-KP and ESBL-PMABL-KP isolates and the difference in antibiotic susceptibilities between these isolates. Previous reports suggested that substitution of piperacillin-tazobactam for expanded-spectrum cephalosporins reduced the prevalence of ESBL-producing *Enterobacteriaceae* in settings where ESBL is endemic (6, 10, 15) and that piperacillin-tazobactam could be clinically effective in the treatment of infections caused by ESBL-producing isolates showing MICs of the antibiotic of ≤16 μg/ml (14). Given this, it is not surprising to find that patients with prior exposure to piperacillin-tazobactam were reported to be at lower risk for acquisition of bacteremia caused by ESBL-producing *Enterobacteriaceae* (11, 19). Fluoroquinolone exposure has been a well-known risk factor for acquisition of infections due to ESBL-producing *Enterobacteriaceae* (11, 17, 20). Our data further indicated that patients with fluoroquinolone exposure were also at higher risk for ESBL-PMABL-KP bacteremia.

Additional remarkable susceptibility data in our series were the 100% resistance to flomoxef and the higher overall MIC values of ertapenem with the ESBL-PMABL-KP isolates than with *K. pneumoniae* isolates with ESBL alone. These findings might in part result from the acquisition of the $bla_{\text{DHA-1}}$ gene and from the presence of porin (e.g., OmpK36) deficiency in some isolates (9).

The findings that piperacillin-tazobactam and aminoglycoside-containing antibiotic regimens were associated with a lower risk for acquisition of ESBL-KP bacteremia and ESBL-PMABL-KP bacteremia, respectively, suggest that to minimize the emergence of infections caused by these multidrug-resistant microbes, piperacillin-tazobactam and/or aminoglycoside-containing regimens should be given priority over oxyimino-β-lactams and/or fluoroquinolones.

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