

Extended-spectrum Beta-lactamases in Clinical Isolates of *Escherichia coli* and *Klebsiella spp.* in a Singapore Hospital: Clinical Spectrum

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Abstract

Introduction: The rising prevalence of extended-spectrum beta-lactamases in gram-negative bacillary pathogens is an important clinical problem resulting from the extensive use of broad-spectrum antibiotics. The emergence of the extended-spectrum beta-lactamases increases the possibility that traditional, empiric antimicrobial regimens may be ineffective. The aims of this study are: to determine the epidemiologic characteristics and clinical outcome of patients diagnosed with infection caused by *Klebsiella spp.* and *Escherichia coli* producing extended-spectrum beta-lactamases; to define a subgroup of patients who may benefit from early, empiric therapy; and to determine the local antibiotic sensitivity pattern in order to improve antibiotic utilisation in our hospital. **Materials and Methods:** A 4-month retrospective review of patients hospitalised in Changi General Hospital between November 2000 and February 2001 who were diagnosed with infection caused by isolates of *Klebsiella spp.* or *Escherichia coli* producing extended-spectrum beta-lactamases. **Results:** During the study period, 44% of *Klebsiella spp.* and 16.1% of *Escherichia coli* isolates were reported as producers of the extended-spectrum beta-lactamases. Sixty-eight patients were assessed to have clinically significant infection caused by 75 isolates. Most of them were elderly, had multiple medical problems and were recently treated with beta-lactam antibiotics. There was a trend toward better outcome in patients who received adequate initial, empiric therapy. **Conclusion:** Patients with infections caused by extended-spectrum beta-lactamase producing Enterobacteriaceae have certain identifiable, common clinical characteristics. In our institution, only carbapenems remain effective against all isolates of *Klebsiella spp.* or *Escherichia coli* producing extended-spectrum beta-lactamases. Further research is necessary to define a group of patients who can benefit from an early, broad-spectrum, empiric therapy.

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Introduction

Extended-spectrum beta-lactamases (ESBLs) in gram-negative bacillary pathogens are a growing and important problem in hospital practice and it is tied to extensive use of broad-spectrum antibiotics.¹⁻³ The emergence of ESBLs has increased the possibility that traditional, empiric antimicrobial regimens may be ineffective. A few studies have shown that inadequate empiric therapy may increase mortality.^{4,5} Unfortunately, indiscriminate broad-spectrum antibiotic therapy has been shown to result in increased antimicrobial resistance.^{6,7} Several strategies to optimise the use of antibiotics have been proposed in order to

balance the need to provide adequate initial antibiotic coverage to high-risk patients with avoidance of unnecessary antibiotic utilisation, which can promote resistance.⁸⁻¹¹ The use of antibiotic administration protocols, antibiotic rotation, de-escalation therapy in highly selected patients or restriction in hospital formulary are examples of strategies to optimise antibiotic utilisation.

In our institution, the increasing prevalence of gram-negative organisms producing ESBLs constitutes a growing clinical problem. Our study had the following objectives:

1. To determine epidemiologic characteristic and clinical outcome of patients who were diagnosed with infection

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caused by ESBL-producing Enterobacteriaceae.

2. To select the subgroup of patients who may benefit from empiric, broad-spectrum therapy.
3. To determine the antibiotic sensitivity pattern for our local Enterobacteriaceae producing ESBLs.

Such data would help us to choose an appropriate antimicrobial agent for empiric therapy and to optimise antibiotic utilisation^{12,13} in our hospital.

Materials and Methods

We reviewed the clinical records of patients admitted to our institution between November 2000 and February 2001, who had positive culture for ESBL-producing *Klebsiella spp.* or *Escherichia coli*.

The presence of ESBL was first determined by the double-disc diffusion test.¹⁴ Double-disc diffusion testing for keyhole phenomenon was performed with ceftazidime, amoxicillin/clavulanate and aztreonam discs. The discs were placed 25 mm apart with amoxicillin/clavulanate disc in the centre. If keyhole phenomenon was seen, the isolate was reported as an ESBL. If no keyhole phenomenon was seen but ceftazidime, aztreonam or ceftriaxone discs showed intermediate or complete resistance (using zone diameter sizes according to 1999 NCCLS disc diffusion criteria¹⁵), a further test for ESBL was performed using the E-test (AB BIODISK Sweden). If the E-test was positive for ESBL, then this was determined to be an ESBL. These tests were done on Mueller-Hinton agar with a bacterial inoculum McFarland 0.5 and overnight incubation.

The medical records of patients who met the stringent criteria of this study were analysed for demographic, clinical and microbiologic features and clinical outcome.

An infection was presumed to be caused by ESBL Enterobacteriaceae if all of the following criteria were fulfilled:

- a) The symptoms, signs or laboratory/imaging studies were suggestive of an infection.
- b) The organism was cultured from an adequate specimen or a primarily sterile site (sputum, urine and blood) or non-sterile site using the best available technique (that is, from pressure sores after surgical debridement).¹⁶
- c) Patient improved only with adequate antibiotic regimen.

Urine specimen was considered adequate if the microscopic examination showed ≥ 10 white blood cells (WBC) and ≤ 3 epithelial cells per high power field. The sputum specimen was considered adequate if the microscopic examination showed ≥ 25 neutrophils ($\geq 2+$) and ≤ 10 epithelial cells ($\leq 1+$) per high power field.¹⁶

Pneumonia was defined as new onset of fever, cough, dyspnoea or tachypnoea, new finding of localised rales on physical examination and new pulmonary infiltrate on the

chest radiograph while there was no evidence of another pathology which could explain the above findings.^{17,18}

Urinary tract infection (UTI) was defined as^{16,17} fever, urgency, frequency, dysuria or suprapubic tenderness associated with pyuria of ≥ 10 white blood cells per high power field and urine culture growing 100,000 colonies/mL with no more than 2 organisms.

An infection was defined as early if it occurred within 48 hours of admission. Infection occurring more than 48 hours from admission was considered to be late infection. Inadequate antibiotic regimen was defined as the absence of antimicrobial agent directed against a specific class of microorganism or the administration of the antimicrobial agent to which the microorganism responsible for infection was resistant.⁸

Results

During the period of study, our laboratory reported 1046 isolates of *Escherichia coli* and 800 isolates of *Klebsiella spp.* One hundred and sixty-eight *Escherichia coli* isolates (16.1%) and 352 *Klebsiella spp.* isolates (44.0%) were reported as producers of the extended-spectrum beta-lactamases. Only 75 of these isolates (14.4%) were clearly responsible for clinical infection in 68 patients. Six patients had urine culture positive for both *Klebsiella spp.* and *Escherichia coli*. One patient with prolonged admission had recurrent UTI and the second infection was caused by a different ESBL-producing isolate. Infections caused by ESBL-producing *Klebsiella spp.* were approximately twice more common than with ESBL-producing *Escherichia coli* (Table 1). Thirteen patients had positive blood cultures. Six patients had bacteraemia, which originated from the urinary tract, 3 from the respiratory tract, 2 from the hepatobiliary system and 1 from a pressure sore. In 1 case, the source of bacteraemia remained undetermined.

The patients were admitted to the following departments: Medicine (n = 38, 55.9%), Geriatric Medicine (n = 12, 17.6%), Surgery (n = 8, 11.8%), Urology (n = 5, 7.3%) and Orthopedics (n = 5, 7.3%). The typical patient who suffered from this type of infection was elderly, with multiple

Table 1. ESBL-producing Isolates – Source of Positive Culture

	Total number of isolates	<i>Escherichia coli</i>	<i>Klebsiella spp.</i>
Total number of strains	75	26	49
Blood cultures	13	6	7
Urine cultures	54	18	36
Sputum/tracheostomy/ endotracheal tube	6	1	5
Bile	1	1	0
Pleural fluid	1	0	1

Table 2. Characteristics of Patients (n = 68)

Characteristic	No. of patients (%)
>65 years old	52 (76.5)
Male	29 (42.6)
Female	39 (57.4)
Underlying medical condition:	
Stroke	38 (55.9)
Hypertension	30 (44.1)
Diabetes mellitus	29 (42.6)
Ischaemic heart disease	16 (23.6)
Chronic renal failure	10 (14.7)
Congestive heart failure	9 (13.2)
Malignancy	7 (10.3)
Neutropaenia	0
Premorbid condition:	
Bed-bound	26 (38.2)
Bedsore	10 (14.7)
Nursing home resident	7 (10.3)
Contractures	5 (7.4)
Urinary catheter	49 (72)
Prior use of beta-lactams	44 (64.7)
Central line	13 (19.1)

comorbidities and recent treatment with beta-lactam antibiotics. Very frequently, these patients were bed-bound and had a temporary or permanent urinary catheter (Table 2). The average patient was anaemic (mean haemoglobin level, 11.3 ± 2.1 mg/dL) and had hypoalbuminaemia (25.6 ± 6.9 g/L), which was consistent with a poor premorbid condition.

In our study, patients with early and late infections had similar clinical characteristics and outcomes. Thirty-seven patients in our study had early infection and 9 of them died (mortality 24.3%). Thirty-one patients had late infection and 9 of them died (mortality 29%). This difference was small and statistically insignificant.

Forty-four (64.7%) patients received beta-lactam antibiotics within the 3 months prior to developing infection caused by ESBL Enterobacteriaceae. Seven patients were treated more than once with beta-lactam antibiotics. Ceftriaxone was prescribed to 27 (39.7%) patients and penicillins (cloxacillin, penicillin and amoxicillin) to 21 (30.9%) patients. Ceftazidime was used in only 3 (4.4%) patients.

Overall mortality was 26.5% (18 patients). In 13 of them, infection was caused by *Klebsiella spp.* and in 5 by *Escherichia coli*.

We analysed our data to determine how a delay in adequate therapy influences clinical outcome. To simplify data collection, we counted the time from the collection of positive culture to the administration of the adequate therapy. By the end of the fourth day, 42 (61.8%) patients had

Table 3. Outcomes, Complications and Interventions

Complication/intervention	All patients (n = 68)	Fatal infections (n = 18)	ICU admission (n = 15)
Death	18 (26.5%)	-	10 (66.7%)
ICU admission	15 (22.1%)	10 (55.6%)	-
Septic shock	15 (22.1%)	10 (55.6%)	10 (66.7%)
Mechanical ventilation	13 (19.1%)	8 (44.4%)	13 (86.7%)
Inotropic support	12 (17.6%)	8 (44.4%)	10 (66.7%)
Acute renal failure	10 (14.7%)	7 (38.9%)	8 (53.3%)

ICU: intensive care unit

received adequate therapy. In the remaining 26 patients, therapy was delayed or (in some cases) they never received adequate treatment. Mortality in the former group was 19% (8 of 42 patients) as opposed to the latter group where mortality reached 38.5% (10 of 26 patients). This difference approached statistical significance ($P = 0.078$).

The mortality was highest among patients with septic shock – 60% (9 of 15 patients). Patients with septic shock who received adequate therapy within the first 4 days had 50% mortality (4 of 8 patients). Patients who did not receive such therapy within the first 4 days had higher mortality of 71.4% (5 of 7 patients). Due to the small number of patients with septic shock, this difference was not statistically significant.

Patients with infections caused by ESBL Enterobacteriaceae had a prolonged mean hospital stay of 24 days (range, 2 to 139 days). The hospitalisation was particularly long for patients with late infection. For survivors with early infection (28 patients), the mean hospitalisation was 11.3 days (range, 4 to 29 days). Late infection among survivors (22 patients) occurred at a mean of 23.1 days (range, 4 to 54 days) after admission and their mean hospitalisation lasted 39.8 days (range, 4 to 139 days).

Patients with infections caused by ESBL-producing Enterobacteriaceae developed multiple complications and frequently required treatment in the intensive care unit (Table 3).

Imipenem was the only antibiotic that covered all isolates of ESBL-producing Enterobacteriaceae. Amikacin was effective against 72.9% and piperacillin/tazobactam against 66.7% of isolates. Amoxicillin/clavulanate and gentamicin covered only about half of the isolates (Table 4).

Discussion

In our institution, the prevalence of ESBL-producing Enterobacteriaceae is high. Forty-four per cent of *Klebsiella spp.* and 16.1% of *Escherichia coli* isolates were reported as ESBL-producers. Similar prevalence was reported in several countries around the world.¹⁹ A review of the

Table 4. Antibiotic Sensitivity Profile

Antibiotic tested	Isolates		
	Sensitive (%)	Intermediate (%)	Resistant (%)
Imipenem	71 (100)	0	0
Amikacin	43 (72.9)	8 (13.6)	8 (13.6)
Piperacillin/tazobactam	12 (66.7)	2 (11.1)	4 (22.2)
Amoxicillin/clavulanate	40 (57.1)	23 (32.9)	7 (10)
Gentamicin	37 (49.3)	3 (4)	35 (46.7)
Nitrofurantoin	22 (46.8)	3 (6.4)	22 (46.8)
Trimethoprim/ sulfamethoxazole	19 (26)	2 (2.7)	52 (71.2)
Ciprofloxacin	16 (22.5)	12 (16.9)	43 (60.6)

clinical records of the study patients allowed us to make several observations. Our study largely confirmed previous observations that ESBL-producing Enterobacteriaceae predominantly affect a specific group of patients.^{8,16,18} These are usually elderly patients with multiple comorbidities who were treated previously with beta-lactam antibiotics. Very frequently, they were bed-bound and had temporary or permanent urinary catheter. Often, these infections were serious with several patients requiring admission to the intensive care unit.

The design of our study does not allow us to conclude if any of the above clinical features constitute a risk factor for acquiring an infection caused by ESBL-producing Enterobacteriaceae. Only case control studies with a control group of patients sharing similar clinical characteristics would be able to clarify this problem.

The majority of ESBL-producing isolates did not fulfill the inclusion criteria for our review. Most of them were excluded because they were cultured from inadequate specimens. A significant proportion of the ESBL-producing Enterobacteriaceae isolated from urine or sputum were excluded because patients had no clinical and laboratory evidence of the urinary or respiratory tract infection. Most of the wound specimens yielded multiple organisms and were also excluded.

We chose 48 hours as a cut-off between early and late infections. This time limit is rather arbitrary but frequently used by other authors to differentiate between community-acquired and nosocomial infections.²⁰ It is very likely that patients who presented with symptoms of infections within the first 48 hours did not have infections which was truly community-acquired. They frequently had a history of previous hospitalisations and previous courses of beta-lactam antibiotics and they could have been colonised with the strain of the ESBL-producing Enterobacteriaceae during the previous hospital stay. Such strain could cause subsequent invasive infections.

In our study, patients with early and late infections had similar clinical characteristics and outcomes. Thirty-seven patients in our study had early infection and 9 of them died (mortality rate, 24.3%). Thirty-one patients had late infection and 9 of them died (mortality 29%). This difference was small and statistically insignificant.

In contrast to previous studies, only a small minority of patients with infection caused by ESBL-producing Enterobacteriaceae received ceftazidime in the preceding 3 months. The majority were treated with ceftriaxone or penicillins. The reason may be related to the methodology of our study. We relied on our hospital case records to determine prior exposure to antibiotics. We could not document prior use of the beta-lactams in one third of our patients. The above numbers may underestimate true, prior use of beta-lactam antibiotics since they reflect only antibiotics prescribed in our hospital. It is very likely that during the 3 months preceding hospitalisation, our patients were also treated with antibiotics (including ceftazidime) in another hospital or in outpatient clinics.

A few trials have shown that inadequate antimicrobial treatment may influence mortality of the critically ill patients.^{4,5} In our study patients, there was a trend towards increased mortality among patients who initially received inadequate antimicrobial therapy.

Patients who received adequate therapy within 4 days of the collection of the specimen yielding positive culture had lower mortality (19%). Mortality among those who received delayed adequate therapy was higher (38.5%). This difference approached statistical significance ($P = 0.078$). The main reason why we chose the cut off of 4 days for the above calculations is that in clinical practice, it may take up to 4 days from collection of the specimen to institution of the antibiotic therapy. This entire process consists of several stages (collection of specimen, culture incubation, organism identification, sensitivity testing, reporting and institution of the therapy). Even if minor delays occur at each of these stages, they should not exceed 4 days.

We do not suggest that all patients (with clinical parameters discussed above) admitted with symptoms of infection should receive empiric, broad-spectrum antibiotic coverage. In fact, that would lead to the emergence of multiresistant bacterial strains causing difficult-to-treat infections. However, the results of our review suggest that further research should be done in order to define a subgroup of patients who would benefit most from such early, aggressive, antimicrobial therapy. Patients with the above clinical characteristics and septic shock on presentation could have a better likelihood of survival if they were treated aggressively from the beginning.

The choice of agents that can be used for the therapy of infections caused by the ESBL-producing Entero-

bacteriaceae is very limited. All ESBL-producing Enterobacteriaceae were susceptible to imipenem. Only two thirds of the isolates were susceptible to amikacin and piperacillin/tazobactam. It means that the use of these antibiotics for an early, empiric therapy may result in failure in a significant proportion of patients. Some properties of amikacin and piperacillin/tazobactam further limit their use. Amikacin can be used as a single agent for the treatment of the urinary tract infections but may be ineffective (as a monotherapy) in the therapy of the other infections.²¹ Therapy with piperacillin/tazobactam may occasionally fail even when the isolate shows in vitro sensitivity to this antibiotic (inoculum effect).²²

Our study also showed that trimethoprim/sulfamethoxazole and ciprofloxacin covered only a quarter of isolates. It means that, in most cases, there is no effective oral antibiotic for step-down continuation of therapy. These results are similar to those reported in other parts of the world.^{19,23-25}

In summary, our study shows that patients with infections caused by ESBL-producing Enterobacteriaceae have certain identifiable common clinical characteristics. These infections are associated with high morbidity and mortality. The choice of antimicrobial agents effective against ESBL-producing Enterobacteriaceae is currently very limited. Further research is necessary to define a group of patients who can benefit from an early, broad-spectrum, empiric therapy.

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