

Faecal prevalence of extended-spectrum β -lactamase (ESBL)-producing coliforms in a geriatric population and among haematology patients

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Abstract

Antimicrobial resistance to the extended-spectrum cephalosporins is increasingly reported worldwide. In the local setting, nosocomial infections with multi-resistant Gram-negative bacilli are not uncommon and are a growing concern. However, there is limited data on the carriage rates of such organisms in the local setting. In May 2001, a prospective study was carried out to determine the enteric carriage rates of ceftazidime-resistant Gram negative bacilli (CAZ-R GNB) among residents of nursing homes and from in-patients of the geriatric and adult haematology wards of University Malaya Medical Centre. Ceftazidime-resistant Gram-negative bacilli (CAZ-R GNB) were detected in 25 samples (30%), out of which 6 were from nursing home residents, 5 from geriatric in-patients and 14 from the haematology unit. A total of 28 CAZ-R GNB were isolated and *Escherichia coli* (10) and *Klebsiella pneumoniae* (7) were the predominant organisms. Resistance to ceftazidime in *E. coli* and *Klebsiella* was mediated by extended-spectrum beta-lactamases (ESBLs). Although the majority of the CAZ-R GNB were from patients in the haematology ward, the six nursing home residents with CAZ-R GNB were enteric carriers of ESBL-producing coliforms. Prior exposure to antibiotics was associated with carriage of ESBL organisms and to a lesser extent, the presence of urinary catheters.

Key words: ESBL, geriatric population, Gram negative bacilli

INTRODUCTION

Antibiotic resistance among Gram-negative bacilli (GNB) is a rapidly expanding problem due to the organisms' ability to mutate, and to acquire and transmit plasmids and other mobile genetic elements encoding resistance genes¹. In the early 1960's, ampicillin, carbenicillin, and the narrow-spectrum cephalosporins were frequently used to treat GNB infections. However, plasmid-mediated β -lactamase mediated resistance has caused these antibiotics to lose their efficacy. In response, more stable β -lactams were developed in the late 1970s and 1980s, notably the expanded spectrum β -lactams (penicillins and cephalosporins) (ESBs). The extensive use of ESBs over time has also led to the selection of the resistant GNB, those capable of producing extended-spectrum β -lactamases. Extended-spectrum β -lactamases or ESBLs are most prevalent in *Escherichia coli* and *Klebsiella pneumoniae*, but have been

described in many other Enterobacteriaceae and in a few *Pseudomonas aeruginosa* isolates^{2,3}.

The selective pressures exerted by the expanded use of antimicrobial agents has enhanced the development and spread of resistant bacteria, not only in hospital settings but in areas outside the hospital including nursing homes^{4,5}. The growing number of geriatric patients harbouring ESBL-producing organisms indicate that besides hospitals, long-term care facilities and nursing homes are important reservoirs for these organisms. Geriatric patients, most of who are in an immunocompromised and debilitated state, are ideal targets for the infection and colonisation of multiply resistant organisms⁵. In Chicago, an outbreak of ceftazidime-resistant (CAZ-R) strains of *K. pneumoniae* and *E. coli* was associated with elderly, nursing home patients^{6,7}. An outbreak of ESBL-producing *K. pneumoniae* was also reported in a geriatric hospital in France⁸.

Other factors for development of resistance include patient's prior antibiotic use and number of antibiotics used^{9,10}.

Nosocomial infections with Gram-negative bacilli are not uncommon in the local setting and can be perceived as a growing threat to public health. However, there is limited data on the carriage rates of such organisms in the local setting. Thus, this prospective study was undertaken to evaluate the prevalence of ESBL-producers among a geriatric population and adult haematology in-patients. Evaluation of potential risk factors for the enteric carriage of ESBL producers was carried out to determine possible steps that could be taken to eliminate, or at least control, this problem.

MATERIALS AND METHODS

Sample collection, screening and phenotypic confirmation of ESBL production

Over a 3-week period in May and June 2001, stool samples were collected from a geriatric population consisting of in-patients from the Geriatric unit in University Malaya Medical Centre (UMMC), and from randomly selected nursing homes for the elderly around Petaling Jaya, Selangor, as well as from in-patients in the adult hematology ward in UMMC. The study protocol was approved by the Ethics Committee of UMMC prior to any sample collection from patients. Data collected included patient demographics, prior antibiotic treatment, usage of urinary catheter, stay in nursing homes/wards and duration of stay/hospitalization. Stool samples were collected from 80 subjects who consented to participate in the study and were screened for the presence of CAZ-R Gram-negative bacilli (GNB) using MacConkey agar (OXOID, UK) containing 4 µg of ceftazidime. Resistance to ceftazidime was used as a marker of ESBL production. All samples were stored at room temperature and were centrally processed within 2 hours of collection. The CAZ-R isolates were subcultured and identified using the standard biochemical tests¹¹. Disc diffusion susceptibility testing as described by the NCCLS was used to determine the antibiograms of the isolates whereas the phenotypic confirmation of the production of ESBLs was determined using the double-disc synergy test (DDST) as described previously^{12,13}.

Antimicrobial susceptibility by MIC determination

The minimum inhibitory concentration of various antibiotics was determined using the standard agar dilution technique, in accordance with the National Committee for Clinical Laboratory Standards Guidelines¹². All the antibiotics which included ceftazidime, cephalexin, cefoperazone, cefotaxime and ceftriaxone, were obtained from Sigma (USA) with the exception of aztreonam (Bristol-Myers-Squibb, Italy), clavulanic acid (Smith-Kline and Beecham Ltd., UK) and imipenem (Merck and Co. Inc., USA). MIC values were also determined for the β-lactam and β-lactamase inhibitor combination of ceftazidime-clavulanic acid.

Isoelectric focusing

Analytical isoelectric focusing was carried out as previously described¹³ on ampholine polyacrylamide gels with broad pH range (3-9) (Pharmacia Biotech, Sweden) using a PhastSystem (Pharmacia Biotech, Sweden). Visualization of the β-lactamases were done by staining with nitrocefin, a chromogenic cephalosporin (OXOID, UK).

PCR amplification and PCR-SSCP (PCR-single stranded conformational polymorphism)

DNA template preparation and subsequent PCR amplification of the SHV gene was carried out based on the method of M'Zali *et al.* (1997)¹⁴. A pair of primers 5'-TCAGCGAAAAACACCTTG-3' and 5'-TCCCGCAGATAAATCACCA-3' were used to amplify the 475-bp sequence of the bla_{SHV} gene¹⁵. The 971-bp fragment of the TEM gene was amplified using primers 5'-TCGGGGAAATGTGCG-3' and 5'-TGCTTAATCAGTGAGGCACC-3', while the 396-bp fragment of the AmpC gene was amplified using primers 5'-ATTCGTATGCTGGATCTCGCCACC-3' and 5'-CATGACCCAGTTCGCCATATCCTG-3'. All primers were synthesized by Genemed Biotechnologies Inc., USA.

PCR-SSCP was carried out using the method of M'Zali *et al.* (1997)¹⁴. Briefly, the 475-bp SHV amplicons were digested with PstI, denatured with a formamide dye, prior to heating at 95°C for 5 mins. The denatured products were separated on a 12% denaturing polyacrylamide gel.

RESULTS

During the study period, 80 samples were collected with 35% (28 samples) being from nursing homes and the rest from the wards; 30% (24 samples) from the geriatric ward and 35% (28 samples) from the adult haematology ward. Ceftazidime-resistant gram-negative bacilli (CAZ-R GNB) were isolated from 26 samples out of which a majority were isolated from the haematology ward (51.7%). Five samples from the geriatric ward also contained ceftazidime-resistant (CAZ-R) GNB. Eight samples from five of the nursing homes contained CAZ-R GNB with one sample having two different CAZ-R GNB. Overall, 29 CAZ-R GNB were isolated from the 26 samples.

Escherichia coli was the most commonly isolated CAZ-R GNB (34.5% of all CAZ-R isolates). *Klebsiella spp.* was the second most frequently isolated (24.1%) followed by *Enterobacter spp.*, *Proteus spp.* and *Citrobacter spp.* (10.3%) and *P. aeruginosa* (6.9%), while *Acinetobacter spp.* was the least commonly isolated organism (3.5%).

From the 29 CAZ-R GNB, 17 extended-spectrum β -lactamase (ESBL)-producing organisms were detected through the double-disc synergy test. The 17 organisms consisted of 10 *E. coli* and 7 *K. pneumoniae*, which meant that all the isolated *E. coli* and *K. pneumoniae* were ESBL producers. Of the 15 CAZ-R GNB isolated from haematology ward 53.3% were ESBL-producers while 40% of those from the geriatric ward were ESBL-producers. 77.8% of CAZ-R GNB from nursing homes were ESBL-producers.

In this study, association of putative risk factors such as prior antibiotic treatment, usage of catheter, staying in nursing homes/wards and duration of stay with development of ceftazidime resistance and ESBL-production in GNB were determined using Pearson's Chi-square test. 84% of the ESBL-producing GNB came from patients with prior antibiotic treatment ($p=0.05$). However, there was not much difference in those patients who acquired resistant GNB (10.5%) with those who had sensitive isolates in terms of catheter usage (8.3%).

To determine the effect of duration of stay, three patients with initially susceptible isolates were followed-up within a week and results show that they did not acquire resistant organisms.

Further analyses was carried out on the ESBL-producing *E. coli* and *K. pneumoniae* isolates.

All isolates displayed high level resistance to ceftazidime (128 $\mu\text{g/ml}$), but were susceptible to the carbapenem and imipenem. Two of the *E. coli* isolates were resistant to the cephamycin and cefoxitin. PCR analyses using AmpC specific primers confirmed the presence of the AmpC gene.

IEF results are summarized in Table 3. Most of the isolates were found to produce more than one β -lactamase with pI values ranging from 5.1 to > 9.0, out of which all of these isolates produced a β -lactamase with a pI value of 8.2, which is the similar pI value of the SHV-5 β -lactamase. Most of the isolates also produced a β -lactamase with the pI value of between 5.1 to 6.0 which are characteristic of TEM enzymes. Two of the isolates produced a β -lactamase with the pI value of >9.0 which was indicative of the AmpC type β -lactamase.

The identity of the β -lactamases were further confirmed via amplification of the genes using primers specific to the bla_{SHV} and bla_{TEM}. The results from the PCR correlated with the data obtained from the IEF assay (Table 2).

PCR-SSCP analysis of the SHV gene was carried out on the 17 isolates which had the pI value of 8.2. Amplification of the SHV gene was consistently obtained from all 17 isolates, confirming the presence of the SHV gene. The isolates were further analysed using PCR-SSCP. 13 of the isolates gave an identical pattern to that of SHV-5 (Figure 1), while 4 isolates had almost identical patterns to that of the SHV-5 with the exception of an additional band. This band did not correspond to any of the other SHV controls (SHV-1 to -5).

DISCUSSION

A majority of the extended-spectrum β -lactamases belong to the TEM or SHV family of β -lactamase genes and are generally located on plasmids. These enzymes differ in only a few amino acids which, however, result in alterations that vary the spectrum of activity of these enzymes. ESBL-producing isolates are generally resistant to extended-spectrum cephalosporins and aztreonam¹⁶ as well as to many older β -lactam antibiotics. This has caused a major shift from more easily eradicated GNB towards more resistant phenotypes, with fewer options for treatment.

In this study, ceftazidime was selected as the representative third-generation cephalosporin for the detection of resistant GNB that are

TABLE 1. Distribution of ceftazidime-resistant gram negative bacilli (GNB) according to source.

Sources (no. of samples)	Samples containing ceftazidime- resistant GNB (no. of isolates)	% Samples with resistant GNB
Haematology ward (28)	13 (15)	46.4
Geriatric ward (24)	5 (5)	20.8
Nursing homes (28)	8 (9)	28.6
Total (80)	26 (29)	32.5

TABLE 2. Summary of results of IEF, PCR and PCR-SSCP of ceftazidime-resistant *K. pneumoniae* and *E. coli* isolated from geriatric populations in UMMC and nursing homes.

Strain	IEF	Detection of resistance genes via PCR			PCR- SSCP
		TEM	SHV	AmpC	
Ec A (NH1)	6.5, 8.2	+	+	-	SHV-5
Ec F (NH2)	6.5, 8.2	+	+	-	SHV-5
Ec J (6D)	6.8, 8.2	+	+	-	SHV-5
Kp N (13U)	8.2, 9.0	-	+	+	SHV-5
Ec O (6D)	8.2	-	+	-	SHV-5
Ec 15 (NH3)	6.5, 8.2, 9.0	+	+	+	SHV-5
Ec19-1 (NH4)	5.1, 8.2	+	+	-	SHV-5
Kp 19-2 (NH4)	6.0, 8.2	-	+	-	-
Ec 20 (NH4)	6.0, 8.2	+	+	-	SHV-5
Ec 21 (NH4)	8.2	-	+	-	SHV-5
Ec 51b (6D)	8.2	-	+	-	SHV-5
Kp 53a (6D)	8.2	-	+	-	-
Ec 57b (13U)	5.4, 8.2	+	+	-	SHV-5
Kp 63 (6D)	6.5, 8.2	+	+	-	SHV-5
Kp 64 (6D)	5.4, 8.2	+	+	-	-
Kp K2a (6D)	6.5, 8.2	+	+	-	-
Kp L1a (6D)	5.4, 8.2	+	+	-	SHV-5

NB. Ec – *Escherichia coli*; Kp – *Klebsiella pneumoniae*; Isolates were isolated from the following :
i. UMMC wards : 6D – haematology ward; 13U - geriatric ward.
ii. Nursing homes : NH1 – NH4.

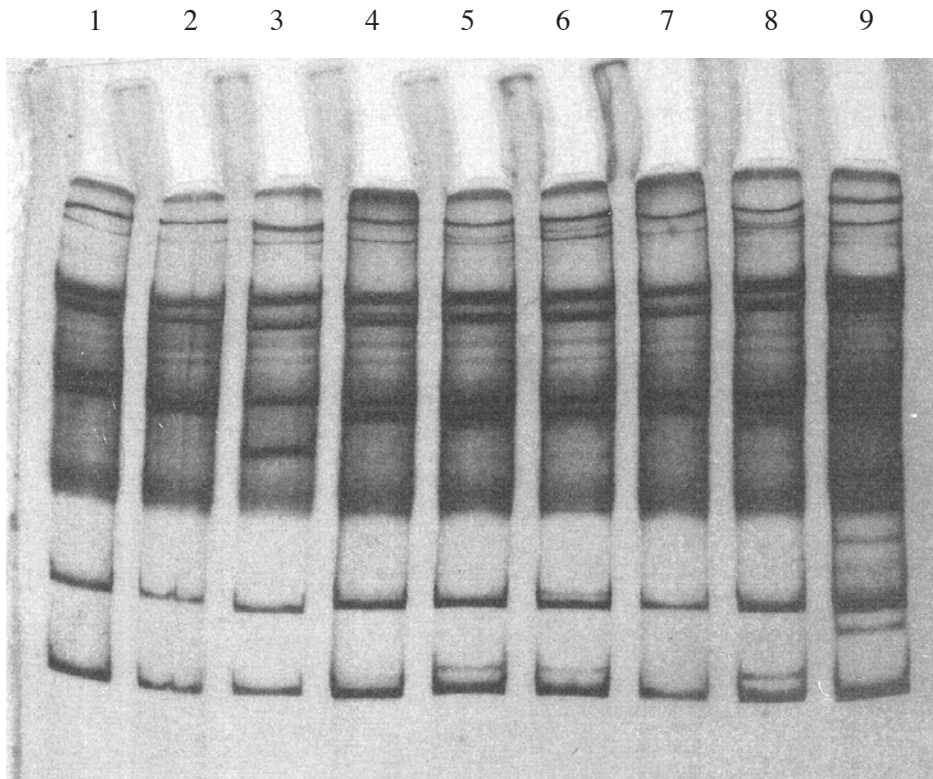


FIG. 1: PCR-SSCP of amplified SHV gene products from SHV controls and SHV genes isolated from ESBL-producing *E. coli*. (Lane 1 – SHV-1 control; Lane 2 – SHV-2 control; Lane 3 – SHV-4 control; Lane 4 – SHV-5 control; Lanes 5 - 10 – isolates)

possible ESBL producers. Ceftazidime was used because it has a lower rate of being hydrolysed as compared to other broad-spectrum β -lactams. Therefore it can be concluded that if the GNB are capable of hydrolysing ceftazidime, they are most likely able to hydrolyse other β -lactams. This is due to the mutations that have increased the spectrum of ESBL, but also tend to lower their efficacy as enzymes. This, in turn, often results in enhanced but low-level resistance to β -lactams.

Studies have shown that among the risk factors for acquiring ESBL-producing GNB were, staying in nursing homes/wards, longer hospital stay, prior use of antibiotics and presence of catheters¹⁷. The population studied consisted of a geriatric population (including those from nursing homes) and haematology patients because previous studies indicated that these populations are the most likely targets and possible reservoirs of ESBL-producing GNB and VRE. This could be due to the fact that most of these patients are immunocompromised and debilitated and have often used multiple drugs and invasive devices.

Pearson's chi-square test was carried out to determine the risk factors for emergence of ESBL-producing GNB. 84.2% of the ESBL-producing GNB was detected in patients who had taken antibiotic beforehand ($p=0.05$). Emergence of these ESBL-producing GNB might be due to selection of these organisms.

In this study, 58.6% of the CAZ-R GNB isolated were ESBL-producers. This percentage is alarmingly high and shows that nursing homes/wards may be reservoirs for CAZ-R GNB. Usage of urinary catheter was not identified as a major risk factor here with only 9% of patients with resistant GNB having used a catheter. However, this contradicting result might be due to the small sample number studied. In addition, the data also showed that there was no apparent association between the duration of hospitalization and the acquisition of resistant organisms. However, the small number of patients observed had a short stay (1 week), therefore no definite correlation could be made. Thus, catheter usage and duration of stay cannot be ruled out as possible risk factors for the acquisition of resistant organisms.

The preceding studies suggested that various antibiotics (gentamicin, imipenem) can be used to treat patients with serious infections due to CAZ-R GNB but third generation cephalosporins should not be used. Only 41.9% of the resistant GNB were sensitive to ciprofloxacin and thus it should not be used as an empirical treatment when a ceftazidime-resistant organism is suspected. This is compounded by reports of association of ciprofloxacin use and CAZ-R which could be a result of plasmid linkage of these resistance determinants¹¹.

Further analyses of the ESBL-producing isolates all of which were *E. coli* and *K. pneumoniae*, revealed high resistance to ceftazidime, cephalexin, and cefoperazone, as well as aztreonam, intermediary to high level resistance to cefotaxime, ceftriaxone, and susceptibility to the carbapenem, imipenem. This is indicative of a characteristic ESBL-producer¹⁸. All the isolates produced a β -lactamase with the pI value of 8.2, indicative of the SHV-5 gene, which is the prevalent in UMMC, as indicated in previous studies¹⁹. PCR-SSCP confirmed the presence of the SHV-5 gene. Two of the *E. coli* isolates displayed resistance to cefoxitin and the β -lactamase-inhibitor, clavulanate, indicative of an AmpC hyperproducer¹⁸. Further analyses revealed the presence of the AmpC gene, which together with the ESBL activity of these two isolates suggests the presence of a dual mechanism of resistance.

In conclusion, prior antibiotic exposure may predispose to carriage of ESBL-producing GNB. Therefore third-generation cephalosporins should not be used as empirical treatment in patients with possible serious GNB infection and have had recent exposure to broad-spectrum antibiotics. Organisms with new mutations or genes would not survive if there was no selective pressure to encourage the emergence of these new variants.

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