Phenotypic Detection and Occurrence of Extended-Spectrum Beta-Lactamases in Clinical Isolates of *Klebsiella pneumoniae* and *Escherichia coli* at a Tertiary Hospital in Trinidad & Tobago

Patrick E. Akpaka and William H. Swanston
Department of Para-Clinical Sciences, Faculty of Medical Sciences, The University of the West Indies, St. Augustine; Trinidad & Tobago

The incidence and distribution of ESBL producing microorganisms such as *E. coli* and *K. pneumoniae* have been demonstrated and varies in different health care facilities and as well as other countries This study was carried out to determine the frequency of occurrence and the antimicrobial susceptibility pattern of ESBL producing *E. coli* and *K. pneumoniae* species from clinical isolates at a tertiary hospital in Trinidad & Tobago. Standard microbiological procedures and automated MicroScan System was used to identify, screen for putative ESBL production and determine antimicrobial susceptibility of 1,118 clinical isolates of *Enterobacteriaceae* species at the microbiology laboratory of the Eric Williams Medical Science Complex, Trinidad & Tobago over a 36 months period. All ESBL producing isolates flagged by the automated system were further confirmed by E-test method. The E-test confirmed a 15.2% ESBL rate among the *K. pneumoniae* isolates and 9.3% among the *E. coli* isolates. There was also a 1.8% rate of ESBL production in *K. pneumoniae* and 0.2% in *E. coli* isolates from specimens received from community health facilities into the laboratory. Isolates recovered from the intensive care unit of the hospital had 2.1% *E. coli* and 8.2% *K. pneumoniae* ESBL producers. Although all ESBL positive isolates were completely susceptible to imipenem and meropenem; and all positive *K. pneumoniae* isolates were susceptible to amikacin, there was a low susceptibility of ESBL positive *E. coli* to the aminoglycosides. However, susceptibility of these ESBL producing isolates to the fluoroquinolones varied. There is a high rate of ESBL production among isolates of *E. coli and K. pneumoniae* at this hospital that is linked to the extensive inappropriate use of third generation cephalosporins in the country. Further molecular studies are needed to characterize the types of these ESBL prevailing in the country.

Key-Words: ESBL detection, E-test methods, *E. coli, K. pneumoniae*, antimicrobial susceptibility, Trinidad & Tobago, Tertiary hospital.

Extended-spectrum β-lactamases (ESBL) are enzymes that hydrolyze broad-spectrum cephalosporins and monobactams including oxyimino-cephalosporins, as well as older β-lactams antimicrobial agents which are inhibited by clavulanic acids. Since these enzymes were initially recognized in *Klebsiella pneumoniae* in Germany in 1983, they have been increasingly described worldwide [1-5]. The incidence and distribution of ESBL producing microorganisms such as *E. coli* and *K. pneumoniae* have been demonstrated and varies in different health care facilities and as well as other countries [6,7].

In Trinidad and Tobago, the first report of incidence of ESBL was in a neutropenic patient at the Port of Spain General Hospital in an isolate of *Salmonella enteritidis*, which exhibited resistance to all penicillins and cephalosporins (including third generation cephalosporins), aminoglycosides and trimethoprim-sulphamethazole [8-10]. The several worldwide report of high prevalence rates and outbreaks of ESBL-producing microorganisms underscores the importance of searching and detecting their occurrence in all hospitals offering ambulatory services.

Given the difficulty in detecting ESBL production as well as inconsistencies in reporting [11], the prevalence of ESBL will most likely be underestimated in the developing countries. Infections by these microorganisms producing ESBL do pose therapeutic dilemma and challenging infection control problems in many hospitals. Therefore, this present study was conducted to determine the prevalence and antimicrobial susceptibilities among clinical isolates of ESBL producing *E. coli* and *K. pneumoniae* at one of the regional tertiary hospitals in the country.

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Address for correspondence: Dr. Patrick. E. Akpaka. Department of Para-Clinical Sciences, Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad & Tobago. Email: peakpaka@yahoo.co.uk. Phone (868) 736-0440; Fax: (868) 663-3797.

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Material and Methods

Bacterial Isolates

Consecutive non-duplicate clinical *Klebsiella pneumoniae* and *Escherichia coli* isolates recovered from specimens processed at the microbiology laboratory of the Eric Williams Medical Sciences Complex (EWMSC) were used. The EWMSC is a tertiary ambulatory hospital in Trinidad & Tobago, a two-island country located in the southern Caribbean Sea, off the coast of Venezuela. The study was done over a 3-year period, between December 2004 and November 2007.

MicroScan Analysis

Automated micro dilution MicroScan WalkAway-96 System (Dade Behring) was used to perform the identification and antimicrobial susceptibility tests using Neg Combo 32 B1017-302 and Neg Combo 35 B1017-305 panels. All procedures were performed according to the manufacturer's instructions. The integrated LabPro 2.0 version that includes the Alert expert system uses growth in the presence of ceftazidime (1μg/mL) and cefotaxime (4μg/mL) at concentrations recommended by the CLSI for ESBL screening [12]. The MicroScan expert system software does not permit identification of ESBL producers among *Enterobacteriaceae*

organisms other than the *E. coli* and *Klebsiella species*. In addition, the system in our laboratory only alerts the user to the possibility of ESBL producers.

MICs obtained for several antimicrobial agents including cefotaxime, ceftriaxone, ceftazidime and aztreonam from the WalkAway system were interpreted according to CLSI breakpoints [12]. All isolates of *E. coli* and *Klebsiella pneumoniae* primarily indicated by the MicroScan system as possible ESBL producers with MIC breakpoints interpreted as resistant or intermediate by the system were regarded as having decreased susceptibility for the purpose of this study. These isolates are stored at -70°C in tripticase soy broth (Difco Laboratories, Detroit MI, USA) supplemented with 5% glycerol until further testing. All isolates that were not alerted as ESBL producers by the MicroScan had their antimicrobial susceptibility patterns compared with the ESBL positive isolates.

E-Test ESBL Confirmatory Tests

E-test strips, cefotaxime CT having a gradient concentration of 0.25 – 16μg/mL and ceftazidime TZ (0.5 – 32μg/mL) at one end; and cefotaxime plus clavulanic acid CTL (0.016 – 1μg/mL + 4μg/mL) or ceftazidime with clavulanic acid TZL (0.064 – 4μg/mL + 4μg/mL) at the other end were used in accordance with the protocols from the manufacturer [13]. ESBL production was determined if the microbial isolate had an MIC μg/mL of ≥ 0.5 for CT, ≥ 1 for TZ; and MIC μg/mL ratio of ≥ 8 for CT/CTL or TZ/TZL. ESBL production was also identified by the presence of phantom zone or a deformation of the cefotaxime or ceftazidime ellipse. A result was considered indeterminate when the MICs were outside the range of the MICs of the respective E-test ESBL test strip, and a MIC ratio could therefore not be calculated. Control strain for all the phenotypic testing were *E. coli* ATCC 25922 (negative control) and *K. pneumoniae* ATCC 700603 (ESBL positive).

Results

During the study period, 1,118 isolates of *K. pneumoniae* (402) and *E. coli* (716) were recovered and identified by the MicroScan machine from clinical specimens processed at the microbiology laboratory of the hospital. Urinary tract isolates 67.2% (752); specifically 72.2% *E. coli* and 27.8% *K. pneumoniae* were dominant. Isolates from soft tissues accounted for 23.6%, blood 5.3% and respiratory tract 3.1%. The performance of the automated MicroScan system and ESBL E-test in screening and confirming ESBL production in the *E. coli* and *K. pneumoniae* isolates is summarized in Table 1. The MicroScan putatively identified 275 isolates as ESBL producers comprising 15.2%*K. pneumoniae* and 9.4% *E. coli* respectively.

The E-test method confirmed ESBL production in 9.3% *E. coli* and 15.2% *K. pneumoniae* of the 1,118 isolates as evidenced by ratios of the MIC of ceftazidime and/or cefotaxime plus clavulanic acid of ≥8, as indicated by ESBL activity (Figures 1) according to E-test interpretative guidelines and CLSI criteria. The collective ESBL producing *E. coli and K. pneumoniae* isolates from specimens of patients coming from the community health facilities was 1.8% (21/1118); and from the intensive care unit of the hospital 4.2% (48/1118) comprising *E. coli* 2.1% (16/716) and *K. pneumoniae* 8.2% (33/402).

The antibiotic susceptibility patterns of the isolates confirmed as ESBL producers were analyzed and compared with non-ESBL producers (Table 2). All isolates producing ESBL were susceptible to the carbapenems (imipenem and meropenem). There was equally a high susceptibility rate of these isolates producing ESBL to cefotetan, 96.3% to *E. coli* and 98% to *K. pneumoniae*. It should be noted that microorganisms producing ESBL enzymes are also often resistant to quinolones, aminoglycosides and other antimicrobials via other mechanisms.

Surprisingly, while all *K. pneumoniae* ESBL producers were fully susceptible to amikacin (100%), only 45% of *E. coli* was susceptible. There was low susceptibility of the *E. coli* producing ESBLs isolates to gentamicin (8.1%), tobramycin (9.2%) and trimethoprim/sulphamethazole (2.5%), while susceptibility rates of *K. pneumoniae* isolates occurred 22.2% in tobramycin, 39.3% gentamicin and 51.8% in trimethoprim/sulphamethazole.

As expected, there was complete resistance to the cephalosporins by the ESBL producing isolates. There was however slightly increased susceptibility of ESBL producing *E. coli* isolates to fluoroquinolones (ciprofloxacin 48.5%, levofloxacin 61.6% and moxifloxacin 68.9%). The *K. pneumoniae* isolates susceptibility to ciprofloxacin was 61.8%, levofloxacin 65.5%, and moxifloxacin 51.5%.

Discussion

The phenotypic data generated in this study indicate that at the Eric Williams Medical Sciences Complex, a significantly large number of the enterobacteriaceae isolates produce ESBL at the rate of 15.2% in *K. pneumoniae* and 9.3 in *E. coli*. This rate of ESBL production in *E. coli* isolates observed in this study is higher than the rates in Latin America and Europe at 8.5% and 5.3% respectively [14]; but similar to the rate observed in Hong Kong and lower than 24.5% in mainland China [15]. The *K. pneumoniae* rate in this study is however lower than the rates reported by Winokur et al. from Latin America [14] as well as rates reported by Hirakata Y. et al. from Singapore, mainland China, South Africa and Philippines [15].

The single most reason for this high rate of ESBL production among these *K. pneumoniae* and *E. coli* isolates in this hospital is the extensive inappropriate use of cephalosporins in the country as reported by Pinto Pereira et al. [16]. This selective pressure created by the use of this third generation cephalosporins has also been described as one of the most important factors elsewhere [17,18].

The specific 15.2% *K. pneumoniae* and 9.3% *E. coli* ESBL production prevalence rate encountered in the present study was also higher than *K. pneumoniae* and *E. coli* rate of 9.5%

Table 1. Results of MicroScan WalkAway system and E-test methods in detecting ESBL production from *K. pneumoniae* and *E. coli* isolates at Eric Williams Medical Sciences Complex Trinidad & Tobago from December 2004 – August 2007.

Species	N	MicroScan (%)		N	E-test (%)		
		Pos	Neg		Pos	Neg	Ind*
K. pneumoniae	402	170 (15.2)	232	170	162 (14.5)	0	8 (0.7)
E. coli	716	105 (9.4)	611	105	99 (8.9)	2	4 (0.4)
Total	1,118	275	843	273	261	2	12

N = number of isolates tested; Pos = positive; Neg = negative; Ind* = indeterminate, isolates observed to be indeterminate for ESBL production using the E-test method are regarded as positives for ESBL production.

Table 2. *In vitro* antibiotic susceptibility patterns of suspected or confirmed ESBL producing isolates of *K. pneumoniae* and *E. coli* recovered from clinical specimens processed at the microbiology laboratory of the Eric Williams Medical Sciences Complex Trinidad & Tobago from December 2004 to August 2007.

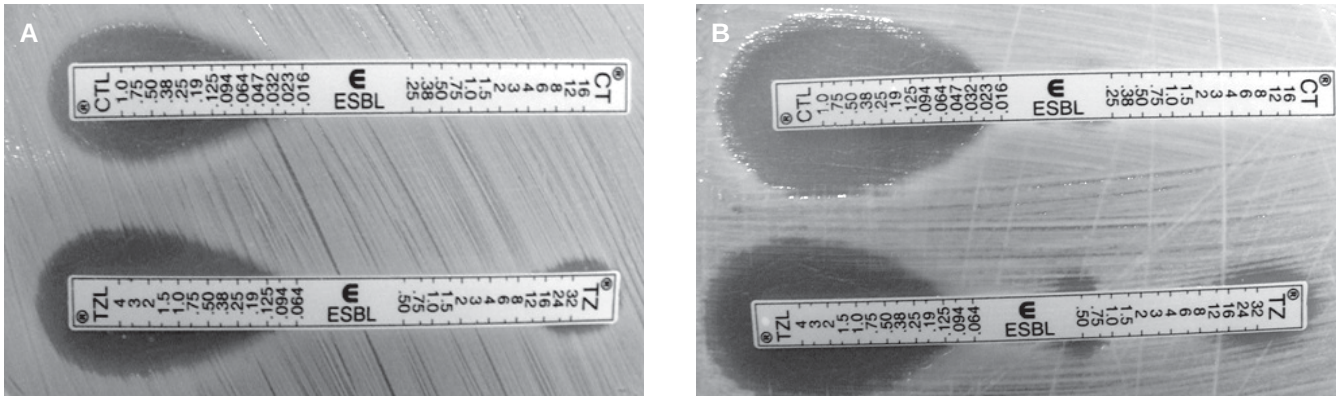
Antimicrobial	Percentage of susceptible isolates			
	E. coli		*K. pneumoniae*	
	ESBL^{-ve}	ESSBL $^{+ve}$	ESBL^{-ve}	ESBL $^{+ve}$
Ampicillin	6.7	0	0	0
Amp/Sulbactam	13.3	0	30	0
Piperacillin	20	0	8.5	0
Pip/Tazo	86.7	80	78	62.5
Aztreonam	67	0	70	0
Meropenem	100	100	100	100
Imipenem	100	100	100	100
Ciprofloxacin	75	48.5	75.5	61.8
Levofloxacin	70	61.6	70	65.5
Moxifloxacin	87	68.9	78.5	51.5
Cefotetan	100	96.3	100	98
Cefuroxime	40	0	53.8	0
Cefotaxime	37	0	75	0
Ceftriaxone	42	0	58.5	0
Ceftazidime	18	0	30.5	0
Cefepime	45	0	69.2	12.5
Amikacin	86.7	45	75	100
Gentamicin	40	8.1	77	39.3
Tobramycin	47	9.2	68.8	22.2
Trimeth/Sulfa	26.6	2.5	78.4	51.8

and 2.9% in 1998; and 6.3% and 0.7% in 2001 by Cherian BP et al. [10] in another tertiary hospital in the country. Our rate is also lower than 18.2% encountered in *K. pneumoniae* from Jamaica, another Caribbean country [19]. The reason for this variation between the results could largely be due to difference in study designs. This is evident in the studies done by Cherian et al. and Nicholson et al. [10,19] where less number of isolates, collected over a shorter period of time were was used.

Conversely, the frequency of ESBL producers from isolates from specimens of patients who attended the community health centers in this present study was lower than what was reported in India [20] but higher than 1.48% reported from Brazil [21]. The high rates of ESBL seen among isolates of patients treated in the community is not because of over the counter sales of antibiotics practiced in India [20] but mostly because of poorly directed therapy among our general practitioners in which there is indiscriminate practice of antibiotics prescription for patients with viral infections in the community in Trinidad [22]. Although the ESBL producing isolates from the intensive care hospital of our study 2.1% *E. coli* 2.1% and 8.2% *K. pneumoniae* respectively, yet the rates are still lower than 11.2% in *E. coli* and 16.2% *K. pneumoniae* reported in USA [23] or 80% in *K. pneumoniae* isolates reported in India [24].

There is significantly high rate of resistance of all the *K. pneumoniae* isolates to the fluoroquinolones. This clearly demonstrates that these antimicrobial agents are to be used with caution in infections such as urinary tract infections or intra abdominal infections as has been documented elsewhere [25]. All isolates of *E. coli* and *K. pneumoniae* in ESBL phenotypes in this study were susceptible to the carbapenems (meropenem and imipenem).

Figure 1. Detection of ESBL carriage with an E-test ESBL strips. Ceftazidime MIC against *E. coli* isolate in A is > 32µg/mL in the absence of clavulanate and 0.125µg/mL in the presence of clavulanate. Ceftazidime MIC against *K. pneumoniae* isolate in B is > 32 µg/mL in the absence of clavulanate and 0.125µg/mL in the presence of clavulanate. Observe the phantom zone production in B. As the ratio of ceftazidime with and without clavulanate is ≥8, the isolates were phenotypically determined as ESBL producers.



This result is in agreement with reports from Spain, North America and United Kingdom [26-28]. This augurs well in this hospital because these antimicrobials are licensed in the country, easily and readily available; and can be recommended as drug of choice for treatment of infections by these organisms. The main limitation of this study is the inability to characterize the types of ESBL prevailing in the country.

Conclusion

Despite the inability to characterize the ESBL types in this study, there is a high rate of ESBL among the *E. coli and K. pneumoniae* isolates encountered in this hospital that may be attributed to the extensive inappropriate use of third generation cephalosporins. There is a strong need to carry out further molecular studies to delineate the prevailing types of ESBL in this hospital and country.

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