Surveillance of Phenotypic Extended Spectrum Beta-Lactamase Resistance in Blood Isolates at a Hospital in East Trinidad

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ABSTRACT

Background

The Centres for Disease Control and Prevention (CDC) and World Health Organization (WHO) list extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* as serious threats and priority pathogens. This study identified phenotypic resistance patterns to these pathogens in east Trinidad, West Indies. We also aimed to set up and test a pilot surveillance system aligned to WHO's Global Antimicrobial Surveillance System (WHO-GLASS).

Methods

Two key bacterial isolates, *Escherichia coli* and *Klebsiella pneumoniae* were used and one specimen, blood, was used to test a pilot surveillance system. Data for resistance patterns, for Sangre Grande Hospital (SGH), for ESBL producing *E. coli* and *K. pneumoniae* were downloaded from the Microscan Autoscan© for the period 2013 - 2016. ESBL presence in bacteria resistant to Cefotaxime (CTX), Ceftazidime (CAZ) and Ceftriaxone (CRO) were recorded. Data were stored in a Microsoft Excel© spreadsheet and inputted into IBM© SPSSv22. Data were displayed as resistance percentages for the year. No patient data were collected. Simple descriptive statistics were used.

Results

The number of organisms recovered from the database for the period 2013 to 2016 were:134*E. coli* and 59*K. pneumoniae.* Phenotypic resistance rates for ESBLs for 2013 to 2016 were:
E. coli:

2013: Resistance ranged from 22.2-29.6% with maximum resistance seen for CTX.

2014: Resistance ranged from 12.9- 22.2%, with maximum resistance seen for CRO.

2015: Resistance ranged from 21.4- =26.2%, with maximum resistance seen for CTX.

2016: Resistance ranged from 29.4- 32.4%, with maximum resistance seen for CRO and CTX.

K. pneumoniae:

2013: Resistance was 40% for all 3rd generation Cephalosporins.

2014: Resistance was 16.7% for all 3rd generation Cephalosporins.

2015: Resistance was 16.7% for all 3rd generation Cephalosporins.

2016: Resistance ranged from 52.6 - 63.2%, with maximum resistance seen for CAZ.

Conclusion

Phenotypic resistance rates in *K. pneumoniae* and *E. coli* were generally high. There was an overall increase in resistance from 2013 to 2016 for both *K. pneumoniae* and *E. coli* with greater resistance being seen in K. *pneumoniae*.

Key Words: ESBL, *Escherichia coli*, *Klebsiella pneumoniae*, Resistance

INTRODUCTION

The World Health Organization (WHO) has developed a global priority pathogens list (global PPL) of antibiotic resistant bacteria to assist in prioritising the research and development of new and effective antibiotic treatments.[1] This includes the priority 1 pathogen *Enterobacteriaceae*, including phenotypic carbapenem resistant and third generation cephalosporin-resistant strains. The Centres for Disease Control and Prevention (CDC) lists extended spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella pneumoniae* as serious threats.[2] Hence, we chose to study ESBL production in these two bacteria. This data formed the rationale for choosing to study ESBL production in these two bacteria.

One study from Nepal indicated a high rate of extended spectrum beta-lactamase (ESBL) production was found in the *E. coli* and *K. pneumoniae* isolated from outpatients. This study suggested dissemination of ESBL producing bacteria in the community.[3] Chong et al have indicated that there is worldwide spread of ESBL producing bacteria which is of critical concern.[4] Heinz et al have found resistance genes circulating in the Caribbean region.[5] In a study from Jamaica by Nicholson et al, it was found that 18.2% of *K. pneumoniae* produced ESBLs, while there were no ESBL producing *E. Coli*.[6] This resistance in *K. pneumoniae* was also found by Christian et al in Jamaica.[7] In Trinidad and Tobago, ESBL producing *K. pneumoniae* have also been found.[8] In fact, Akpaka et al found ESBLs in *E. coli* and *K. pneumoniae* doing phenotypic studies using the Microscan automated system compared to the E-test.[9] Surveillance of antibiotic resistance is important for policy, infection prevention and control and antibiotic stewardship. This surveillance must first have a testing system at a designated laboratory and then a method of generation of key indicator data. This is seen in the WHO Global Antimicrobial Surveillance System (GLASS) methodology.[10]

In this paper, we examine ESBL resistance in *E. coli* and *K. pneumoniae* in blood isolates at the Sangre Grande Hospital. The aim was to initiate an antimicrobial resistance surveillance system, starting with these key bacterial isolates, which can be expanded to include other pathogens and resistance mechanisms in the future.

METHODS

The site of the study was the Sangre Grande Hospital

(SGH) which has 120 beds and serves a population of 120,000 persons. This is the smallest population served by a hospital in Trinidad and Tobago.[11] This study was a descriptive surveillance study utilising retrospective data. The objective was to use one key, but clinically important specimen. Thus, blood was chosen. In addition, two key Gram-negative organisms were tested against third generation cephalosporins and using the third generation cephalosporin plus a beta-lactamase inhibitor as outlined below. This study was aligned to the WHO-GLASS methods and recommendations and methods previously published by Akpaka *et al* in 2008. Data for resistance patterns for SGH for ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* were downloaded from the Microscan Autoscan© for the period 2013 to 2016. This was for blood isolates only, in line with WHO-GLASS methods. This data represented isolates from the community and hospital. The study utilised isolates only and no demographic or patient data were collected. Simple descriptive statistics were used.

Quality Control

The integrated LabPro© 2.0 version from the Microscan included the Alert expert system. This system used growth in the presence of ceftazidime and cefotaxime compared with cefotaxime/clavulanate and ceftazidime/ clavulanate. This comparison is used in the Gram-negative panel for ESBL screening. ATCC 25922, an *E. coli,* was used for quality control during the years 2013 to 2016. The data was inspected in a Microsoft Excel© spreadsheet to ensure that the data quality was met. Duplicate isolates were removed to ensure that there were no repeat samples from the same patient. The LabPro generates reports according to international standard criteria. The software was last updated in 2016. Once an ESBL was detected in LabPro, all the third generation cephalosporins were flagged as resistant.

Data Management

Extended Spectrum Beta-Lactamase (ESBL) production was screened by the Microscan. It measured changes in the microtiter wells. All positive ESBL producers were recorded. Once an ESBL was detected, cefotaxime (CTX), ceftazidime (CAZ) and ceftriaxone (CRO) were flagged as resistant by the Microscan's software. This study showed almost 100% concordance between the E-test and Microscan. Data were stored in a Microsoft Excel© spreadsheet and inputted into IBM© SPSSv22. Data were displayed as resistance percentages for the year. WHONET software was also used for data analysis of resistance patterns. Clinical Laboratory Standards Institute (CLSI) interpretative criteria were used for comparison. The period 2013 to 2016 was examined to determine if there was any change in resistance patterns of ESBLs.

RESULTS

The number of organisms recovered from the database for the period 2013 to 2016, was 134 *E. coli* and 59 *K. pneumoniae* as distributed in Table 1. For the year 2013, 27 *E. coli* and 10 *K. pneumoniae* were isolated. For the years 2014, 2015 and 2016, 31 *E. coli* and 12 *K. pneumoniae*, 42 *E. coli* and 18 *K. pneumoniae* and 34 *E. coli* and 19 *K. pneumoniae* were isolated, respectively (see Table 1). Phenotypic resistance rates for ESBLs for 2013 to 2016 were:

Table 1: Number of Organisms for the period 2013 to 2016

Organism	Year				Total
	2013	2014	2015	2016	
Escherichia coli	27	31	42	34	134
Klebsiella pneumoniae	10	12	18	19	59

***E. coli*:**

The resistance, as indicated by phenotypic ESBL presence, for cefotaxime (CTX), ceftazidime (CAZ) and ceftriaxone (CRO) fluctuated from 2013 to 2016. There, however, was a small increase from 22.2%-29.6% in 2013 to 29.4%- 32.4%, in 2016. This is highlighted in Table 2 and Figure 1.

Figure 1: Percent ESBL Resistance for *Escherichia coli* isolated from blood in Sangre Grande Hospital 2013 - 2016

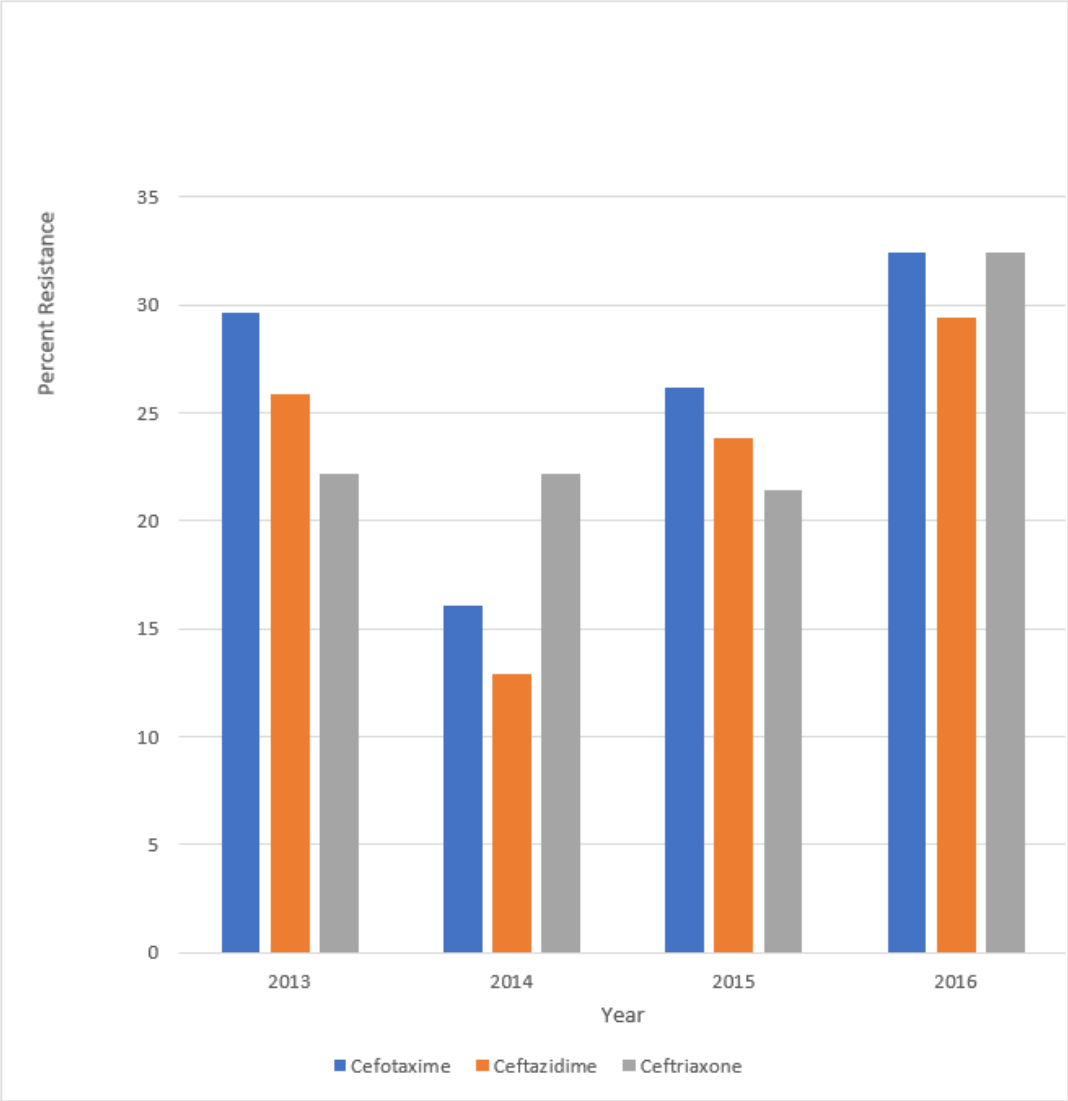

Table 2: Phenotypic ESBL Positive *Escherichia coli* isolated from blood in Sangre Grande Hospital 2013 - 2016

Year	Antibiotic Resistance in *Escherichia coli*		
	Cefotaxime (%)	Ceftazidime (%)	Ceftriaxone (%)
2013	29.6	25.9	22.2
2014	16.1	12.9	22.2
2015	26.2	23.8	21.4
2016	32.4	29. 4	32.4

***K. pneumoniae*:**

The resistance, as indicated by phenotypic ESBL presence, for cefotaxime (CTX), ceftazidime (CAZ) and ceftriaxone (CRO) fluctuated from 2013 to 2016. It was, however, quite high overall and showed a trend of increased resistance from 40% in 2013 to 63.2% in 2016. This is high-lighted in Table 3 and Figure 2.

Figure 2: Percent ESBL Resistance for *Klebsiella pneumoniae* isolated from blood in Sangre Grande Hospital 2013 - 2016

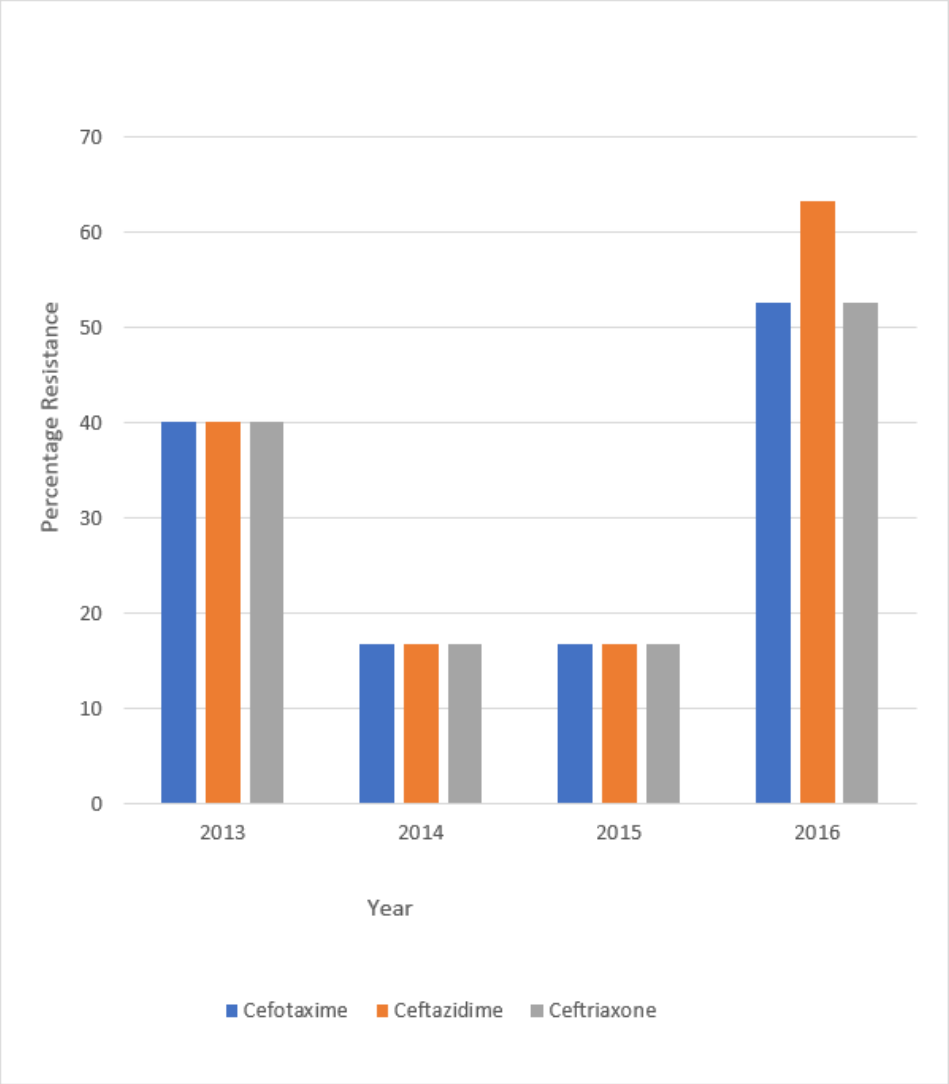

Table 3: Phenotypic ESBL Positive *Klebsiella pneumoniae* isolated from blood in Sangre Grande Hospital 2013 - 2016

Year	Antibiotic Resistance in *Klebsiella pneumoniae*		
	Cefotaxime (%)	Ceftazidime (%)	Ceftriaxone (%)
2013	40	40	40
2014	16.7	16.7	16.7
2015	16.7	16.7	16.7
2016	52.6	63.2	52.6

DISCUSSION

E. coli resistance to the third generation cephalosporins tested and thus ESBLs was over 30% by 2016 indicating a trend of a high level of resistance. Similarly, *K. pneumoniae*, also showed increasing ESBL detection from 2013 to 2016. This is consistent with *K. pneumoniae* resistance in Trinidad and elsewhere in the Caribbean.[8-9] In a phenotypic study from 2004 to 2007, Akpaka et al (study year?) showed that the ESBL rate among the *K. pneumoniae* isolates was 15.2% and 9.3% among the *E. coli* isolates.

Secondly, in a study carried out by Cherian et al (study year?), the authors also found resistance in *K. pneumoniae* and *E. coli*, but at rates lower than the study by Akpaka et al (study year?) and ~~thus~~ the current study.[9, 12] Thus, the rates of phenotypic ESBL production were much higher in our setting for both organisms. The increasing resistance from the study by Cherian et al in 1998 and Akpaka et al (study year?) to the current study period indicates that there is a temporal trend where resistance increases with time. The other notable fact is that with all studies done in Trinidad and Tobago, the *K. pneumoniae* shows greater resistance over the periods studied compared to *E. Coli*.[9, 12] This is an essential epidemiological trend. Importantly, the study by Akpaka et al was conducted over a similar time to this current study, but involved a much larger hospital serving a larger population and thus, they would have expectedly retrieved more isolate information. Our study was therefore limited by the smaller population served.

Additionally, *K. pneumoniae* and *E. coli* resistance are both quite concerning globally. The Global Antimicrobial Resistance Surveillance System (GLASS) Report from 2017 – 2018 shows high rates of resistance in *K. pneumoniae* and *E. coli* globally.[13] Trinidad and Tobago has enrolled in WHO GLASS in 2020. A study from Taiwan showed that 19.7% of patients with community onset bacteraemia had third generation cephalosporin resistance signifying that ESBL resistance in *E. coli* and *K. pneumoniae* is not just a local problem, but a regional and international issue requiring robust surveillance and testing systems which are simple to implement, perform and sustain.[14]

Importantly, an ESBL is an enzyme produced by certain *Enterobacteriae* (*E. coli*, *Klebsiella pneumoniae*) enabling them to hydrolyse all penicillins, aztreonam, cephalosporins, but not cephamycins like (cefoxitin, cefotetan) or carbapenems.[15] This study looked at screening methods for ESBLs and its use for surveillance. Confirmatory methods include the double disc diffusion, combination disc test, E-test, and molecular methods.[16-17] The Microscan did use ceftazidime with clavulanic acid and cefotaxime with clavulanic acid for screening.[18] When reporting results, it should indicate resistance to all penicillins, all cephalosporins and aztreonam ~~as resistant~~, even if they are susceptible in vitro. In confirmed or screening positive ESBL producing bacteria, reports should have no change in susceptibility interpretations for cephamycins (e.g., cefoxitin), beta-lactam/beta-lactamase inhibitor combinations (e.g., piperacillin/ tazobactam) and carbapenems.[19] However, it may not be practical to use beta-lactam/beta-lactamase inhibitor combinations in vivo as they may be ineffective.[20]

Lastly, this study was limited because there were no confirmatory ESBL tests-which can be either phenotypic or molecular based. This may have resulted in an overestimation of resistance. However, the study by Akpaka et al (2004 to 2007) shows that there was an almost 100% concordance between the confirmatory test, the E-test, and the Microscan results. Thus, there has been previous validation by Akpaka et al (2004 to 2007), even though it was not stated, of Microscan results for ESBL testing in surveillance organisms used in our study. This study also did not look at diseases such as septicaemia as it was a laboratory-based study and only dealt with laboratory isolate data which consisted of blood isolates and no other samples or clinical information. Thus, resistance from other sample types was not considered. This would have led to a lower level of resistance reported overall and lower number of isolates recovered for the time period. Funding was also limited and thus, we were unable to procure reagents and material for confirmatory testing. There were a relatively small number of organisms retrieved from the database but the hospital was also small serving a small population.

CONCLUSION

In conclusion, this study conducted 10 years after the last phenotypic report by Akpaka et al 2004 to 2007, shows

that phenotypic ESBL resistance is still a problem, at least in east Trinidad. Thus, clinicians should be mindful of this in assessing microbiology reports and treating patients. Most of the regular work done in laboratories is phenotypic. This sets out a practical approach to finding ESBL resistance for under-resourced laboratories. We believe that this pilot surveillance study, supported by the previous validation of the Microscan results, will contribute to the knowledge needed to combat the evolving global problem of antimicrobial resistance and will help inform rational antibiotic treatment. This is important for policy and guideline development in Antimicrobial Resistance (AMR) in Trinidad and Tobago, the Caribbean region and globally.

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