

# Comparative Analysis of Antimicrobial Resistance of Extended-Spectrum Beta-Lactamase Producers and Non-Extended-Spectrum Beta-Lactamase Producers among Bacterial Isolates in Accra, Ghana

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## Abstract

Antibiotic resistance may occur naturally but misuse of antibiotics in humans and animals is accelerating the process. One of the modes of resistant mechanism is the production of extended-spectrum beta-lactamases (ESBLs) by the bacteria. ESBLs are plasmid-mediated beta-lactamases that are capable of hydrolysing penicillins, cephalosporin and several non-beta-lactam antibiotics. This laboratory-based study sought to compare the rate of antimicrobial resistance between ESBL and non-ESBL-producers in Accra. Four hundred *K. pneumoniae* and *E. coli* isolates were collected at the Korle Bu Teaching Hospital and screened for ESBL and non-ESBL-producers using the combined disk method and Vitek 2 system. The minimal inhibition concentrations (MICs) for 17 commonly used antibiotics were determined using Vitek 2 System. The results indicated significant difference ( $P < 0.05$ ) between the antimicrobial resistance of ESBL-producers and non-ESBL producers except for amikacin and imipenem. The 198 non-ESBL phenotypes recorded relatively low antimicrobial resistance to cefotaxime 4(2%), ceftazidime 4(2%), nitrofurantoin 6(3%), amoxicillin/clavulanic acid 27(13.6%), gentamicin 34(17.2%) and ciprofloxacin 78(39.4%). In contrast, the 202 ESBL producers registered high antibiotic resistance to cefotaxime 197(97.5%), ceftazidime 175(86.6%), nitrofurantoin 94(46.5%), amoxicillin/clavulanic acid 64(31.7%), gentamicin 166(82.2%) and ciprofloxacin 161(79.7%). Cephalosporins and nitrofurantoin are suitable for the treatment of non-ESBL producers while imipenem and amikacin is the drug of choice for treating ESBL-producing infections. Evidence based antibiotic usage will help to control the spread of resistance by ESBL producers in Accra, Ghana. Also, there is the need to intensify research in the use of natural products to treat ESBL infections.

**Keywords:** Extended spectrum beta-lactamase, Resistance, Bacteria, Antibiotics

## 1.0 Introduction

Infectious diseases account for the major cause of morbidity and mortality in Sub-Sahara Africa and Ghana is no exception. The success of antimicrobials against pathogens is one of the remarkable achievements of medical science in the past decades. Large quantities of assorted antimicrobials are now available to developing countries due to economic development and technological advances. This remarkable achievement is accompanied by poor practices that promote drug resistance (Beitha, 2008).

One of the major public health challenges confronting clinicians, microbiologists, drug development experts and public health specialists is the prevalence of antibiotic resistance in most known bacterial pathogens. This public health threat led to the declaration of the first World Antibiotic Awareness Week from 16 to 22 November 2015 by the World Health Organization which was aimed to encourage best practices to avoid the further emergence and spread of antibiotic resistance. Antibiotic resistance in bacteria may be an inherent character of the organism that renders it naturally non-susceptible to specific antibiotics. Although antibiotic resistance occurs naturally, misuse of antibiotics in humans and animals is accelerating the process. When antibiotics are misused or over prescribed, bacteria become resistant to their effects, making some infectious diseases difficult -sometimes impossible- to treat. Other antibiotic resistances are acquired by means of mutation of the DNA of the bacteria or acquisition of resistance-conferring DNA from another source. The problem of antimicrobial resistance is compounded by the principles of natural selection. The most common mode of resistant mechanism is enzymatic inactivation of antibiotics (Todar, 2008) such as the production of extended-spectrum beta-lactamases by the bacteria.

Extended-spectrum beta-lactamases (ESBLs) are plasmid-mediated beta-lactamases that are capable of hydrolysing beta-lactams (penicillins and cephalosporins) except carbapenems and cephamycins. They are inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. They have been found in the *Enterobacteriaceae* and other Gram-negative bacilli (Paterson and Bonomo, 2005). ESBL-producing organisms appear susceptible to cephalosporins *in vitro* using conventional breakpoints but ineffective *in vivo*. The improper usage of extended-spectrum antibiotics exerts a selective pressure for emergence of ESBL-producing strains. The resistant plasmids can then be transferred to other bacteria. Most of these plasmids not only

contain DNA encoding ESBL but also carry genes conferring resistance to several non-beta-lactam antibiotics (Paterson and Bonomo, 2005).

This present work seeks to compare the antimicrobial resistance between ESBL and non-ESBL-producing isolates based on their minimal inhibition concentration in Accra, Ghana.

## 2.0 Materials and Methods

### 2.1 Materials

Glycerol broth, blood agar and MacConkey agar were prepared according to manufacturers' guidelines. MAST ID™ ESBL Detection Disks (Mast Group, UK) were used for ESBL screening and confirmation according to CLSI standards on a Mueller Hinton agar plate. Vitek 2 Compact System (bioMérieux, France) was used to identify the isolates, determine minimum inhibition concentration of selected antibiotics and interpret the MICs according to CLSI breakpoints.

### 2.2 Study Sites

Lactose fermenting bacterial isolates was collected from the Central Laboratory of the Korle Bu Teaching Hospital (KBTH), Accra, Ghana.

### 2.3 Sample Size

A sample size of 400 *K. pneumoniae* and *E. coli* corresponds with the standard techniques used to calculate the minimum sample size based on the expected prevalence and using appropriate levels of precision at 95% confidence level.

### 2.4 Inclusion Criteria

Non-duplicate pure cultures of *K. pneumoniae* and *E. coli* were used in the work.

### 2.5 Exclusion Criteria

All isolates not confirmed as *K. pneumoniae* and *E. coli* were not used in this study.

### 2.6 Identification of the Bacterial Isolates

The lactose fermenting bacterial colonies isolated from urine specimens and stored in glycerol broth were sub-cultured on blood and MacConkey agar and incubated at 35°C for 24 hours. The pure colonies were gram-stained to confirm their Gram negative reaction. The isolates were identified as *K. pneumoniae* and *E. coli* based on their Gram stain reaction and biochemical reaction characteristics in the ID test cards wells using Vitek 2 system.

### 2.7 Determination of Minimal Inhibitory Concentration (MIC)

The Vitek 2 system (bioMérieux, France) performs antimicrobial susceptibility testing (AST) based on kinetic analysis of growth data and uses the micro-dilution method to determine the therapeutic significance of the MICs of the antibiotics. At the end of the incubation cycle, MIC values and their interpretations (susceptible, resistant and indeterminate) were generated for the selected antibiotics.

Each AST card contains dried antibiotics with a microbiological culture medium in varying concentrations. The selected 17 antibiotics used were ampicillin, amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefazolin, cefoxitin, cefotaxime, ceftazidime, cefepime, imipenem, amikacin, gentamicin, ciprofloxacin, norfloxacin, tetracycline, nitrofurantoin, trimethoprim/sulfamethoxazole.

### 2.8 Detection of ESBL Phenotype using Combined Disc Synergy Method

MAST ID™ ESBL Detection Disks (Mast Group, UK) were used to screen and confirm the ESBL phenotypes. The MAST ID™ ESBL Detection Disks comprise of cefpodoxime 30µg disks, cefpodoxime 30µg + clavulanic acid 10µg disks; ceftazidime 30µg disks, ceftazidime 30µg + clavulanic acid 10µg disks and cefotaxime 30µg disks, cefotaxime 30µg + clavulanic acid 10µg disks.

Using a pure culture of the test organism, a suspension in distilled water equivalent in density to a McFarland 0.5 opacity standard was prepared. Using a sterile swab, the suspension was spread uniformly across the surface of Mueller-Hinton agar plate. Using a sterile forceps, one of each MAST ID™ ESBL Detection Disks was placed onto the inoculated medium ensuring that they were evenly spaced. The plates were incubated aerobically at 35-37°C for 18 – 20 hours. The diameter of any zones of inhibition that were observed were measured and recorded. The zone of inhibition for the cefpodoxime, ceftazidime and cefotaxime was compared to that of the cefpodoxime, ceftazidime and cefotaxime plus clavulanic acid combination disks. An increase in zone diameter of  $\geq 5$ mm in the presence of clavulanic acid from any or all of the sets of MAST ID™ ESBL Detection Disks indicates the presence of ESBL in the test organism.

## 2.9 Statistical Analyses

The data from the work was collated and statistically analysed using the chi-square test and Mann-Whitney U test. *P* values < 0.05 were considered significant.

## 2.10 Ethics Approval and consent to participate

Ethics approval (MS-Et/M.9 – P.4.14/2010-11) was obtained from the Ethical and Protocol Review Committee prior to the start of this study.

## 3.0 Results

### 3.1 Occurrence of ESBL-producers and Non-ESBL-producing Phenotypes

The combined disk synergy method detected 202(50.5%) ESBL producers among the 400 total bacterial isolates of which 129 (73.7%) were *K. pneumoniae* and 73 (32.4%) were *E. coli* isolates as shown in table 1. Of the 400 isolates, 198 (49.5%) were detected to be non-ESBL producers of which 46 (26.3%) were *K. pneumoniae* and 152 (67.5%) were *E. coli* isolates as indicated in table 1.

**Table 1** Occurrence of ESBL producers and Non-ESBL producers

Bacteria/ESBL phenotype	ESBLs (n=202) Number (%)	Non-ESBLs (n=198) Number (%)	All Isolates (n=400) Number (%)
<i>K. pneumoniae</i> (n=175)	129(73.7)	46(26.3)	175(43.7)
<i>E. coli</i> (n=225)	73(32.4)	152(67.5)	225(56.3)
All Isolates (n=400)	202(50.5)	198(49.5)	400(100.0)

### 3.2 Antimicrobial Resistance of ESBL-Producing and Non-ESBL-Producing Isolates

The results indicated significant difference ( $P < 0.05$ ) between the antimicrobial resistance of ESBL-producers and non-ESBL producers except for amikacin and imipenem as indicated in table 2. Non-ESBL phenotypes recorded limited antimicrobial resistance to cefotaxime (2%), ceftazidime (2%), nitrofurantoin (3%), amoxicillin/clavulanic acid (13.6%), gentamicin (17.2%) and ciprofloxacin (39.4%) as against the high resistance rates (97.5%, 86.6%, 46.5%, 31.7%, 82.2% and 79.7% respectively) registered by ESBL-producers as shown in figure 1.

**Table 2** Comparison of Antimicrobial Resistance between ESBL Producers and Non-ESBL Producers

Antimicrobial Agent	ESBLs (n=202) Number (%)	Non-ESBLs (n=198) Number (%)	P-value
Ampicillin	202(100)	132(66.7)	0.001
Piperacillin	202 (100)	117 (59.1)	0.001
Amoxicillin/Clavulanic acid	64 (31.7)	27 (13.6)	0.001
Piperacillin/Tazobactam	104 (52.5)	37 (18.7)	0.001
Cefazolin	197 (97.5)	31 (15.7)	0.001
Cefoxitin	36 (17.9)	14 (7.1)	0.001
Cefotaxime	197 (97.5)	4 (2.0)	0.001
Ceftazidime	175 (86.6)	4 (2.0)	0.001
Cefepime	50 (24.8)	4 (2.0)	0.001
Imipenem	2 (1.0)	0 (0.0)	0
Amikacin	1 (0.5)	2 (1.0)	0.466
Gentamicin	166 (82.2)	34 (17.2)	0.001
Ciprofloxacin	161 (79.7)	78 (39.4)	0.001
Norfloxacin	160 (79.2)	78 (39.4)	0.001
Tetracycline	143 (70.8)	154 (77.8)	0.001
Nitrofurantoin	94 (46.5)	6 (3.0)	0.001
Trimethoprim/Sulphamethoxazole	196 (97.0)	135 (68.2)	0.001

**Table 3** Mann-Whitney U test of antimicrobial resistance between ESBL Producers and Non-ESBL Producers

	Statistics
Mann-Whitney U	59.500
Wilcoxon W	212.500
Z	-2.929
Asymp. Sig. (2-tailed)	.003
Exact Sig. [2*(1-tailed Sig.)]	.003 <sup>b</sup>

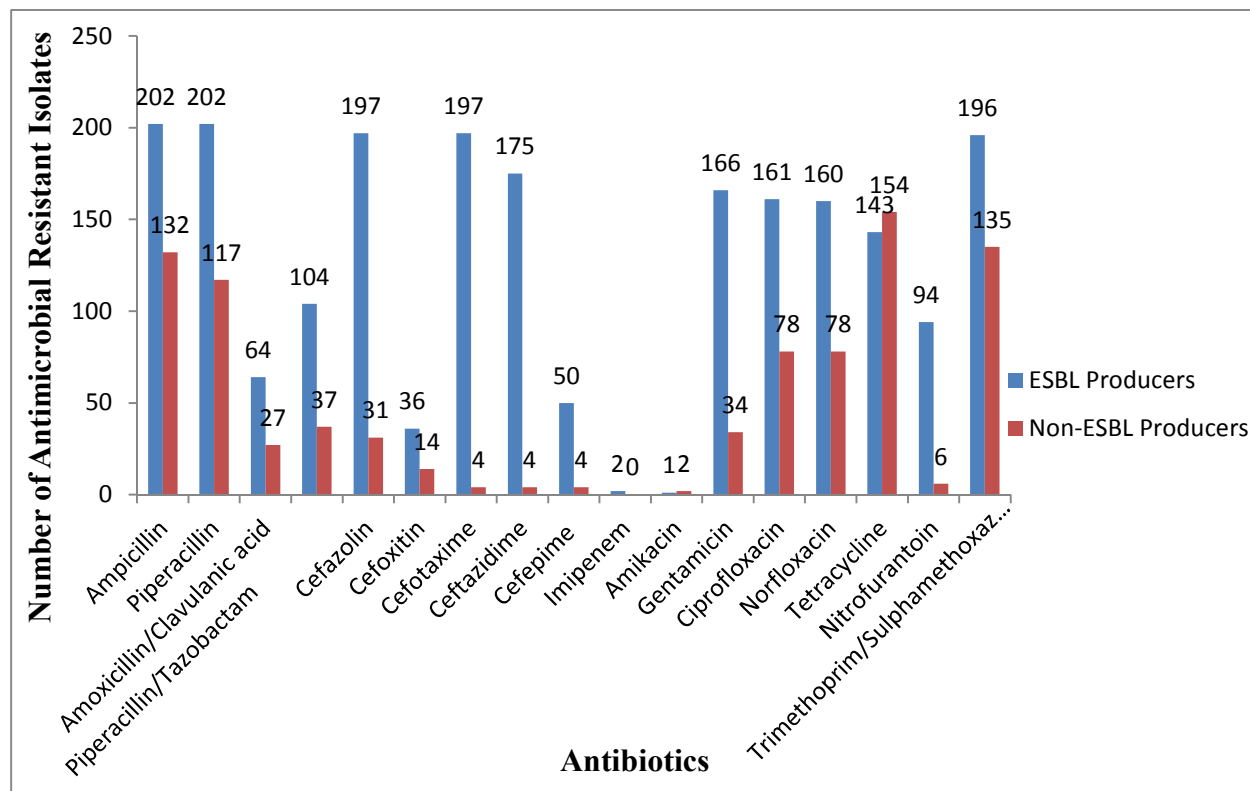


Figure 1: Resistance of ESBL and Non-ESBL producers to selected Antibiotics

#### 4.0 Discussion

##### 4.1 Antimicrobial Resistance of Non-ESBL Producers

Antibiotics are among the most commonly prescribed drugs in hospitals and studies on their resistance patterns ensure quality healthcare. Antibiotics are widely and indiscriminately used in Ghana resulting to antimicrobial resistance (Newman *et al.*, 2006). The observed resistance of non-ESBL producers to the beta-lactams, non-beta-lactams and beta-lactam/beta-lactamase inhibitor combination antimicrobials may be due to an inherent character of the organism that renders it naturally non-susceptible to specific antibiotics. Although antibiotic resistance occurs naturally, misuse of antibiotics in humans and animals may be accelerating the process due to other resistance mechanisms activated by indiscriminate use of antibiotics. Newman and colleagues (2006) who studied bacterial isolates from various clinical specimens in Ghana, recorded high resistance rates for ampicillin, tetracycline and co-trimoxazole, though their work did not specify the ESBL phenotypes of the bacterial isolates. In Zimbabwe (Mbanga *et al.*, 2010) and Ethiopia (Kibret and Abera, 2011) the high resistance of bacterial isolates to ampicillin, co-trimoxazole and trimethoprim/sulphamethoxazole and tetracycline are consistent with this current study.

This study recorded resistant rate of 39.4% to ciprofloxacin and norfloxacin. The steady rise in resistance of non-ESBL-producing isolates to the fluoroquinolones such as ciprofloxacin and norfloxacin is alarming as cautioned by (Newman and Seidu, 2002). The rise in resistance to fluoroquinolones may be due to the ease with which mutations in the DNA gyrase are transferred to other fluoroquinolones (Nankanishi *et al.*, 1999). This may explain why both ciprofloxacin and norfloxacin have similar high resistance rates as observed in this current study. The observed resistance of non-ESBL producers to the beta-lactam/beta-lactamase inhibitor combination antimicrobials such as amoxicillin/clavulanic acid and piperacillin/tazobactam is worrying since amoxicillin/clavulanic acid has become the empirical drug of choice for some clinicians for treating infectious diseases in Ghana. The over-the-counter sales and empirical prescription of ciprofloxacin and amoxicillin/clavulanic acid to treat various infections in Ghana may be blamed for the alarming rates of resistance for these antimicrobial agents. It would therefore seem prudent to take the caution of Kimang'a (2012) to manage infectious diseases with evidence based treatment seriously.

On the other hand, lower rate of resistance was observed for ceftriazone (a third generation cephalosporin) and amikacin in Ghana by Newman and colleagues in 2006. This is consistent with this current study with non-ESBL-producers resistant rates of 2% to cefotaxime, ceftazidime and cefepime (which are third generation cephalosporins) and 1% for amikacin. Nitrofurantoin continues to be effective against non-ESBL-producing *K. pneumoniae* and *E. coli* infections especially in non-life threatening urinary tract infections. Considering the

resistant rate of 1% and 0% for amikacin and imipenem respectively, it is appropriate to reserve these two antimicrobials for third-line treatment options for non-ESBL producers.

Although the rates of resistance in non-ESBL producing isolates is worrying, the negative effects of ESBL production on the resistance of bacterial isolates to beta-lactams, non-beta-lactams and beta-lactam/beta-lactamase inhibitor combination antimicrobials requires urgent attention.

#### **Increased Antimicrobial Resistance of ESBL Producers**

As shown in table 3, a Mann-Whitney U test conducted to determine whether there was a difference between ESBL Producers and Non-ESBL Producers indicate a significance difference,  $z = -2.929$ ,  $p < 0.05$ . The Mann-Whitney U test indicated that antimicrobial resistance was greater for ESBL Producers ( $Mdn = 160$ ) than for non-ESBL Producers ( $Mdn = 31$ ). Antimicrobial resistance was increased in ESBL-producers than non-ESBL producers except for amikacin and imipenem which serve as the antibiotic of choice for treating ESBL infections. ESBL phenotypes recorded high resistance to cefotaxime (97.5%), ceftazidime (86.6%), nitrofurantoin (46.5%), amoxicillin/clavulanic acid (31.7%), gentamicin (82.2%) and ciprofloxacin (79.7%) as against the limited resistance rates (2%, 2%, 3%, 13.6%, 17.2% and 739.4% respectively) registered by non-ESBL-producers.

It may seem that the rate of ESBL-producing bacteria is assuming alarming rates in Ghana and West Africa. Of the 400 total bacterial isolates used in this study, 202 (50.5%) were ESBL producers which is consistent with the work published by Olysegun and colleagues in 2006 which observed 50% ESBL production rate in clinical isolates studied from north-western Nigeria. These infections may be either hospital-acquired or community-acquired ESBL-producers. This justifies the need for routine ESBL phenotype screening in health facilities and the institution of ESBL infection control measures in health facilities and the general population as a whole.

The high prevalence of ESBL-producers may be attributed to indiscriminate antibiotic exposure (over-the-counter sales, self-medication and empirical treatment) to extended-spectrum beta-lactam antibiotics (cephalosporins) used for the treatment of blood infections, respiratory infections, urinary tract infections and other infectious diseases (Bonnet, 2004). This exerts antibiotic selective pressure for the emergence of ESBL-producing organisms in the population (Du Bois *et al.*, 2005). Since extended spectrum beta-lactamases are plasmid mediated, the genes encoding these enzymes are easily transferable among other bacteria population thereby increasing the occurrence of ESBL-producing organisms (Bonnet, 2004). More so, *Klyuvera* species which serve as the reservoir of CTX-M ESBL genes (Bonnet, 2004) are present in the environment. This serves to heighten public health concerns since CTX-M ESBL genes are easily transferred to other bacteria plasmids in the Enterobacteriaceae family (Bonnet, 2004).

This present work recorded high resistance of ESBL-producers to gentamicin (82.2%), ciprofloxacin (79.7%), norfloxacin (79.2%), tetracycline (70.8%) and trimethoprim /sulfamethoxazole (97%). A study by Aibinu and colleagues (2003) in hospitals in Lagos reported significant co-resistance of ESBL phenotypes to ciprofloxacin (75%), streptomycin (89%), amikacin (63%) and trimethoprim-sulfamethoxazole (100%). However, their reported resistant rate for amikacin (63%) contradicted the outcome in this present work. The high resistance rates in non-beta-lactams may be due to the fact that the ESBL genes are plasmid mediated and most of these plasmids not only contain DNA encoding ESBL but also carry genes conferring resistance to several non-beta-lactam antibiotics (Paterson and Bonomo, 2005).

Oteo and colleagues (2010) recommended the treatment of severe ESBL-producing *E. coli* infections to include the use of carbapenems, amikacin, tigecycline, amoxicillin/clavulanic acid and piperacillin/tazobactam. Results from this work support aspects of this assertion especially for imipenem and amikacin. However, resistant rates of 31.7% and 52.5% recorded by amoxicillin/clavulanic acid and piperacillin/tazobactam respectively in this study will limit their use for treating ESBL infections. Fluoroquinolones used to be regarded as the treatment of choice for complicated urinary tract infections due to ESBL-producing organisms (Paterson and Bonomo, 2005). Unfortunately, increasing *in vitro* resistance of ESBL producers to fluoroquinolones will limit its role in treating ESBL infections as demonstrated in figure 1.

#### **5.0 Conclusions**

ESBL-producers registered more antimicrobial resistance than non-ESBL producers. The continuous spread of ESBL infections in the population is detrimental to quality healthcare. The outcome of this work recommends third generation cephalosporins and nitrofurantoin as suitable for the treatment of non-ESBL producers with imipenem and amikacin as the drug of choice for treating infectious diseases caused by ESBL-producing organisms. Evidence based antibiotic usage will help to control the spread of resistance by ESBL producers in Accra, Ghana. The possible development of carbapenem-resistant-enterobacteriaceae will be detrimental to the clinical management of ESBL producing infections. Also, there is the need to intensify research in the use of natural products to treat ESBL infections.

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