European Journal of
Health Sciences
(EJHS)

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AJP

European Journal of Health Sciences
ISSN 2520-4645 (online)
Vol.6, Issue 1, pp 35-44, 2021

www.ajpojournals.org

Urinary Tract Infections are becoming Multi-drug Resistant due to Extended Spectrum Beta-lactamases-producing *Klebsiella pneumonia*

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Abstract

Introduction: *Klebsiella pneumoniae* are among the most common cause of urinary tract infections such as cystitis and pyelonephritis. These multi-drug resistant *K. pneumoniae* are producers of extended spectrum beta-lactamases (ESBL) that are capable of hydrolyzing beta-lactams and non-beta-lactams. This laboratory-based study sought to establish the increase of ESBL-producing *K. pneumonia*e in multi-drug resistant urinary tract infections and determine the effective antibiotic treatment options.

Methods: One hundred and seventy five *K. pneumoniae* isolates obtained from urine cultures were randomly collected and the combined disc synergy method was used to determine the ESBL-producing *K. pneumoniae*. The Vitek 2 system (bioMérieux, France) was used to perform antimicrobial susceptibility testing of 17 commonly used antibiotics. 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.

Results: Of the 175 *K. pneumoniae* responsible for urinary tract infections, 73.7% were producing ESBL suggesting that most urinary tract infections caused by *K. pneumoniae* will be multi-drug resistant. The antimicrobial resistance differences between ESBL-producing and non-ESBL-producing *Klebsiella pneumoniae* indicated a significance difference with $p < 0.05$. This study indicated that imipenem and amikacin are the antibiotic of choice for the treatment of multi-drug resistant urinary tract infections caused by ESBL-producing *K. pneumoniae.* Cephalosporins and nitrofurantoin are suitable for the treatment of urinary tract infections due to non-ESBL-producing *K. pneumoniae*.

Conclusion: This study indicated that imipenem (carbapenem) and amikacin are the antibiotic of choice for the treatment of multi-drug resistant urinary tract infections caused by ESBL-producing *K. pneumoniae.* The third and fourth generation cephalosporins and nitrofurantoin are suitable for the treatment of urinary tract infections due to non-ESBL-producing *K. pneumoniae*. Rational use of antibiotics and evidence based antibiotic prescription will help to control the spread of resistance by ESBL-producing *K. pneumoniae*. There is the need to intensify research in the use of natural products to treat multi-drug resistant urinary tract infections emanating from ESBL-producing *K. pneumoniae*.

Keywords: *Extended spectrum beta-lactamase, Klebsiella pneumoniae, urinary tract infections, multi-drug resistant.*

Introduction

Klebsiella pneumoniae is a gram negative, lactose fermenting and rod-shaped bacterium, which forms part of the normal flora of the intestines, mouth and skin. In addition to pneumonia, meningitis and wound infections, they are among the most common cause of urinary tract infections such as cystitis (bladder infection) and pyelonephritis (kidney infection). Flores-Mireles *et al*., (2015) reported that about 150 million people develop Urinary Tract Infection (UTI) in every given year globally. Urinary tract infections are common in women than men (Salvatore *et al*., 2011). Infections caused by *K. pneumoniae* are mostly hospital acquired. These infections are not only difficult to treat but cause significant mortality all over the world.

The multi-drug resistant (MDR) *K. pneumoniae* are producers of extended spectrum beta-lactamases. Extended-spectrum beta-lactamases (ESBLs) are plasmid-mediated beta-lactamases that are capable of hydrolysing beta-lactams (penicillins and cephalosporins) except carbapenems and cephamycins (Paterson & Bonomo, 2005). They have been found in the *Enterobacteriaceae* and other Gram-negative bacilli. Most of these plasmids contain DNA encoding ESBL and carry genes conferring resistance to several non-beta-lactam antibiotics (Nordmann *et al*., 2011). ESBL-producing organisms appear susceptible to cephalosporins *in vitro* using conventional breakpoints but ineffective *in vivo* (Paterson & Bonomo, 2005).

Infectious diseases are the major cause of morbidity and mortality in Sub-Sahara Africa and Ghana is no exception. One of the major public health challenges confronting clinicians is the high occurrence of antibiotic resistance in most known bacterial pathogens (Falagas & Karageorgopoulos. 2009). Although antibiotic resistance occurs naturally, misuse of antibiotics in humans and animals is accelerating the process.

This laboratory-based study sought to establish the increase of ESBL-producing *Klebsiella pneumonia*e in multi-drug resistant urinary tract infections and determine the appropriate antibiotic treatment options in Accra, Ghana.

2.0 Materials and Methods

2.1 Materials

Glycerol broth and MacConkey were prepared according to manufacturers' guidelines. MAST ID^{TM} ESβL Detection Disks (Mast Group, UK) were used for ESBL screening and confirmation according to CLSI standards on a Muellar Hinton agar plate. Vitek 2 Compact System (bioMérieux, France) was used to identify the isolates, determine the antimicrobial susceptibility testing and interpret the MICs according to CSLI breakpoints.

2.2 Study Sites

Lactose fermenting bacterial isolates from urine specimen was collected from Advent Clinical Laboratory, Accra, Ghana.

2.3 Sample Size

One hundred and seventy five (175) *K. pneumoniae* isolates obtained from urine cultures were randomly collected and analyzed in the clinical laboratory.

2.4 Inclusion Criteria

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

2.5 Exclusion Criteria

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

2.6 Identification of *Klebsiella pneumoniae* Isolates

The lactose fermenting bacterial colonies isolated from urine specimen cultures, stored in glycerol broth were sub-cultured on MacConkey agar, and incubated at 35°C for 24 hours. The isolates were identified as *K. pneumoniae* based on their Gram-negative reaction and biochemical reaction characteristics using Vitek 2 system (bioMérieux, France).

2.7 Detection of ESBL Phenotype using Combined Disc Synergy Method

The combined disc synergy method was used to determine the ESBL-producing *K. pneumoniae* [4]. MAST ID^{TM} ESβL Detection Discs (Mast Group, UK) were used to screen and confirm the ESBL phenotypes. The MAST ID^{TM} ESβL 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 (Paterson & Bonomo, 2005).

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^{TM} ESβL 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 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 ≥5mm in the presence of clavulanic acid from any or all of the sets of MAST ID^{TM} ESβL Detection Disks indicates the presence of ESBL in the test organism (Paterson & Bonomo, 2005).

2.8 Determination of Antimicrobial Susceptibility Testing

The Vitek 2 system (bioMérieux, France) was used to perform antimicrobial susceptibility testing (AST) by determining the minimal inhibition concentration (MIC) of 17 commonly used antibiotics to treat urinary tract infections. The Vitek 2 system (bioMérieux, France) used 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.

The commonly used antibiotics for urinary tract infections included ampicillin, cefazolin, cefoxitin, cefotaxime, ceftazidime, cefepime, imipenem, amikacin, gentamicin, ciprofloxacin, norfloxacin, tetracycline, nitrofurantoin, trimethoprim/sulfamethoxazole, piperacillin. piperacillin/tazobactam and amoxicillin/clavulanic acid.

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.

3.0 Results

3.1 ESBL-producing *Klebsiella pneumoniae*

Of the 175 *K. pneumoniae* isolates, 129 representing 73.7% were ESBL producers and 46 representing 26.3% were non-ESBL producers. As indicated in table 1, there was a significant difference (p<0.05) between the ESBL-producing *K. pneumoniae and* non-ESBL producing *K. pneumoniae*.

Table 1: Occurrence of ESBL-producing and Non-ESBL producing *K. pneumoniae*

Bacteria/ESBL phenotype	**ESBL-producer Number (%)**	**Non-ESBL-producer Number (%)**
K. pneumoniae (n=175)	129 (73.7)	46 (26.3)

3.2 Antimicrobial Resistance of ESBL-producing and Non-ESBL-producing *Klebsiella pneumoniae*

The difference between antimicrobial resistance of ESBL-producing *K. pneumoniae* and non-ESBL-producing *K. pneumoniae* is significant (P<0.05) as indicated in table 2 below. The non-ESBL- *K. pneumoniae* recorded limited antimicrobial resistance to cephalosporins such as cefotaxime (2.2%), ceftazidime (2.2%), cefepime (2.2%); amoxicillin/clavulanic acid (13.0%), gentamicin (17.4%) and ciprofloxacin (39.1%) as against the high resistance rates by ESBL-producing *K. pneumoniae* as shown in figure 1.

Table 2 Comparison of Antimicrobial Resistance between ESBL-producing *Klebsiella pneumoniae* and Non-ESBL producing *Klebsiella pneumoniae*

Antibiotics	**ESBLs (n=129)**	**Non-ESBLs (n=46)**
	Number (%)	**Number (%)**
Ampicillin	129 (100)	31(67.1)
Piperacillin	129 (100)	27 (58.7)
Amoxicillin/Clavulanic acid	41 (31.8)	6 (13.0)
Piperacillin/Tazobactam	68 (52.7)	9 (19.6)
Cefazolin	129 (100)	7 (15.2)
Cefoxitin	23 (17.8)	3 (6.5)
Cefotaxime	129 (100)	1 (2.2)
Ceftazidime	129 (100)	1(2.2)
Cefepime	129 (100)	1 (2.2)
Imipenem	1 (0.8)	0 (0.0)
Amikacin	1 (0.8)	0 (0.0)
Gentamicin	106 (82.2)	8 (17.4)
Ciprofloxacin	103 (79.8)	18 (39.1)
Norfloxacin	102 (79.2)	18 (39.1)
Tetracycline	91 (70.5)	36 (78.3)
Nitrofurantoin	60 (46.5)	1 (2.2)
Trimethoprim/Sulphamethoxazole	125 (96.9)	31 (67.4)

ESBL-producing *Klebsiella pneumoniae* were deemed to be resistant to all penicillins and cepaholosprins according to CLSI guidelines.

European Journal of Health Sciences
ISSN 2520-4645 (online)
Vol.6, Issue 1, pp 35-44, 2021

www.ajpojournals.org

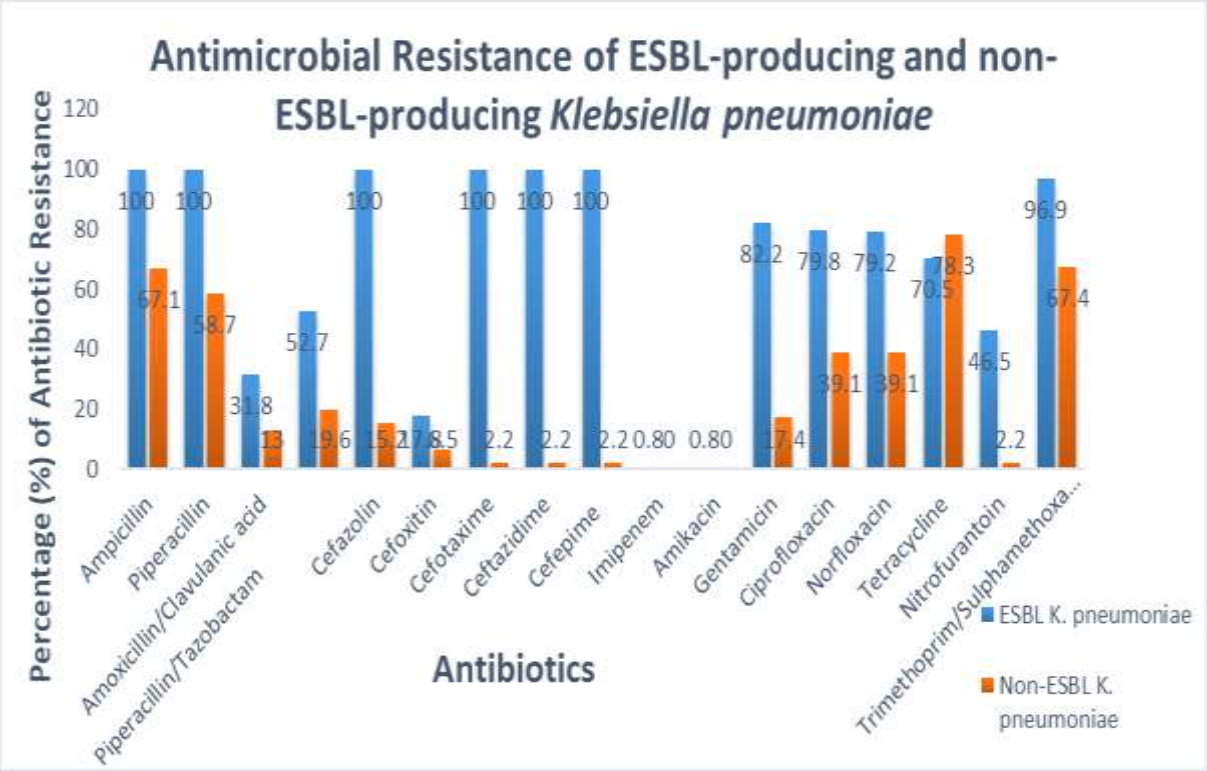

Figure 1: Antibiotic resistance of ESBL-producing and non-ESBL-producing *K. pneumoniae*

4.0 Discussion

Klebsiella pneumoniae is one of the plasmid mediated producers of extended spectrum beta-lactamases. In this study, of the 175 *K. pneumoniae* responsible for urinary tract infections, 73.7% were producing extended-spectrum beta-lactamases suggesting that most urinary tract infections caused by *K. pneumoniae* will be multi-drug resistant. This is consisted with a study by Adu-Sarkodie in 2010 which reported high levels of ESBL production in *K. pneumoniae* than *Escherichia coli* in Kumasi.

The rate of ESBL-producing bacteria has assumed alarming rates in many parts of Africa and the world as a whole. 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.

Infections emanating from *K. pneumoniae* isolates include urinary tract infection, pneumonia, bacteremia, thrombophlebitis, cholecystitis, upper respiratory tract infection, wound infection, osteomyelitis, meningitis and nosocomial infections (Umeh and Berkowitz, 2009). These infections may be either hospital-acquired or community-acquired ESBL-producers. The high occurrence of ESBL-producers may be attributed to prolong hospital admission and indiscriminate antibiotic exposure especially to extended-spectrum beta-lactam antibiotics used for the treatment of blood, urinary tract infections and other infectious diseases. This exerts antibiotic selective pressure for the emergence of ESBL-producing organisms in the population. 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.

A Mann-Whitney U test conducted in this study to determine antimicrobial resistance differences between ESBL-producing and non-ESBL-producing *K. pneumoniae* indicated a significance difference with $p < 0.05$. Antibiotic resistance was increased in urinary tract infections emanating from ESBL-producing *K. pneumoniae* than non-ESBL producers except for amikacin and imipenem. Amikacin and carbapenems will serve as the antibiotics of choice for the effective treatment for multi-drug resistant urinary tract infections due to ESBL-producing *K. pneumoniae*. This was corroborated by Yasin and colleagues who recommended a combination therapy of amikacin and meropenem for urinary tract infections caused by multi-drug resistant *K. pneumoniae*.

Previously, fluoroquinolone was considered as the drug of choice for complicated urinary tract infections due to ESBL-producing organisms. Unfortunately, increasing *in vitro* resistance of ESBL producers to fluoroquinolones will limit its role in treating ESBL infections as demonstrated in figure 1. Studies by Oteo *et al*. (2010) recommended the use of amikacin, carbapenems, tigecycline, amoxicillin/clavulanic acid and piperacillin/tazobactam to treat severe urinary tract infections caused by ESBL-producers.

Results from this work supported aspects of this assertion especially for imipenem and amikacin. However, resistant rates of 31.8% and 52.7% recorded for amoxicillin/clavulanic acid and piperacillin/tazobactam respectively in this study will limit their use as treatment options for urinary tract infections caused by ESBL-producing *K. pneumoniae*.

Conventionally, the CLSI recommends that all penicillins and cephalosporin should be considered as resistant for all ESBL phenotypes. This is because ESBL-producing organisms appear susceptible to cephalosporins *in vitro* using conventional breakpoints but ineffective *in vivo*. Hence all cephalosporins including third and fourth generation cephalosporins such as cefotaxime, ceftazidime, ceftriaxone and cefepime will be ineffective when used to treat urinary tract infections caused by ESBL-producing *K. pneumoniae*.

Most extended-spectrum beta-lactamases produced by *K. pneumoniae* are plasmid mediated which also carry genes conferring resistance to several non-beta-lactam antibiotics. This explains the high antibiotic resistance rates of ESBL-producing *K. pneumoniae* to non-beta-lactams such as gentamicin (82.2%), ciprofloxacin (79.8%), norfloxacin (79.2%), tetracycline (70.5%) and trimethoprim-sulfamethoxazole (96.9%) as against the limited resistance rates registered by non-ESBL-producing *K. pneumoniae*.

The resistant rate of non-ESBL-producing *K. pneumoniae* to ciprofloxacin and norfloxacin was 39.1%. The steady rise in resistance of non-ESBL-producing isolates to the fluoroquinolones is worthy of concern. The reported resistance of non-ESBL-producing *K. pneumoniae* to the beta-lactam/beta-lactamase inhibitor combination antibiotics such as amoxicillin/clavulanic acid and piperacillin/tazobactam is a public health concern since amoxicillin/clavulanic acid has become the empirical drug for some clinicians for treating urinary tract infections 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 be prudent to use evidence-based therapy to manage urinary tract infections.

Although the rates of resistance in non-ESBL-producing *K. pneumoniae* is worrying, the increase in multi-drug resistant urinary tract infections due to ESBL-producing *K. pneumoniae* requires urgent mitigating actions.

5.0 Conclusions

The increase of multi-drug resistant urinary tract infections can be attributable to ESBL-producing *K. pneumoniae*. The continuous spread of ESBL-producing infections in the population is a drawback to quality healthcare. The apparent development of carbapenem-resistant-*K. pneumoniae* (CRKP) will be detrimental to the clinical management of urinary tract infections caused by ESBL-producing *K. pneumoniae*.

6.0 Recommendations

The following are recommended:

1. Imipenem (carbapenem) and amikacin are the antibiotic of choice for the treatment of multi-drug resistant urinary tract infections caused by ESBL-producing *K. pneumoniae.*

2. The third and fourth generation cephalosporins and nitrofurantoin are suitable for the treatment of urinary tract infections due to non-ESBL-producing *K. pneumoniae*.

3. Evidence-based antibiotic prescription should be practiced by clinicians to control the spread of resistance by ESBL-producing *K. pneumoniae*.

4. There is the need to intensify research in the use of natural products to treat multi-drug resistant urinary tract infections emanating from ESBL-producing *K. pneumoniae*.

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