Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.4, No.14, 2014

Antibiotic Resistance Profile of Non-Extended Spectrum Beta-Lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Accra, Ghana

*Henry Kwadwo Hackman[1], Charles A Brown[2], Kingsley Twum-Danso[3]
1. Department of Science Laboratory Technology, School of Applied Sciences and Arts, Accra Polytechnic
2. Department of Medical Laboratory Technology, School of Allied Health Sciences, University of Ghana, Korle Bu
3. Department of Microbiology, University of Ghana Medical School, Korle Bu
*Email of corresponding author: h.k.hackman@gmail.com

Abstract
One of the major challenges facing health professionals is the prevalence of antibiotic resistance. Most Gram-negative bacteria produce beta-lactamases which are enzymes that in-activate β-lactams. Recent publications suggested that extended spectrum beta-lactamase production in *E. coli* and *K. pneumoniae* is one of the main causes of antimicrobial resistance in penicillins, cephalosporins and some non-beta-lactam antibiotics in Accra. This present work sought to determine the resistance profile of antimicrobials to non-ESBL-producing isolates in Accra. The 400 *K. pneumoniae* and *E. coli* isolates were screened for non-ESBL-producing strains using the combined disk method. The minimum inhibition concentration for 17 antibiotics was determined using Vitek 2 Compact System (bioMérieux, Marcy I'Etoile, France). Among the 400 total bacterial isolates, 198 (49.5%) were non-ESBL producers. Co-resistances to ampicillin (66.7%), piperacillin (59.1%), tetracycline (77.8%) and trimethoprim/sulphamethoxazole (68.2%) have been collaborated in this work. The increasing rise in resistance to the beta-lactam/beta-lactamase inhibitor combination antibiotics such as amoxicillin/clavulanic acid (13.6%) and piperacillin/tazobactam (18.7%) is problematic since they have become the empirical drug of choice for treating most infections. The steady increase in resistance to gentamicin (17.2%) as well as the floroquinolones such as ciprofloxacin (39.4%) and norfloxacin (34.9%) is alarming. In the absence of ESBLs, cephalosporins generally have been effective in treating infections caused by enterobacteria. Nitrofurantoin remains reliable for managing non-life threatening urinary tract infections. Amikacin and imipenem continue to be effective third-line treatment options for Gram-negative bacteria infections. As antibiotic resistance increases and the development of new antimicrobials declines, it is imperative that we use antimicrobials that are still effective rationally. Evidence based antibiotic prescriptions and usage as well as regular evaluation of antibiotic resistance will help to control the spread of antibiotic resistance in Accra, Ghana.

Keywords: Extended spectrum beta-lactamase, Resistance, Antibiotic

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 (Plotkin and Shnayerson, 2003). 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. Antibiotic resistance in bacteria may be an inherent character of the organism that renders it naturally non-susceptible to specific antibiotics. 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 genes for drug resistance may be located on the bacterial chromosome, plasmid or transposons. Once the resistance genes have developed, they can be vertically transferred to the progeny of the parent bacterium during DNA replication. The resistant genome can also be horizontally transferred to bacteria of the same species or even different species through the process of conjugation, transduction and transformation (Todar, 2008). The problem of antimicrobial resistance is compounded by the principles of natural selection. The most common mode of resistant mechanism is enzymatic inactivation of the antibiotic (Todar, 2008).

Recent work published by Hackman and colleagues (2013) suggested that extended spectrum beta-lactamase production in *E. coli* and *K. pneumoniae* is one of the major causes of antimicrobial resistance in

penicillins, cephalosporins and some non-beta-lactam drugs in Accra. This present work seeks to determine the resistance profile of antimicrobials to non-ESBL-producing isolates in Accra.

2.0 Materials and Methods

2.1 Materials

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

2.2 Study Sites

Lactose fermenting bacterial isolates were collected from the Central Laboratory of the Korle Bu Teaching Hospital (KBTH) and Advent Clinical Laboratories; both in the Accra Metropolis, 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*.

2.6 Culturing and Gram Staining of the Bacterial Isolates

The lactose fermenting bacterial isolates 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.

2.7 Identification of Bacterial Isolates, Determination of Minimal Inhibition Concentration (MIC) and Antibiotic Sensitivity Testing

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. The Vitek 2 system (bioMérieux, Marcy I'Etoile, France) performs antimicrobial sensitivity 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 each antibiotic.

Each AST card contains dried antibiotics with a microbiological culture medium in varying concentrations. The 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 Non-ESBL Phenotype using Combined Disk Method

MAST ID™ ESβL Detection Disks (Mast Group, UK) were used to screen the ESBL phenotypes. The MAST ID™ 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.

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™ 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 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 <5mm in the presence of clavulanic acid from any or all of the sets of MAST ID™ ESβL Detection Disks indicates the absence of ESBL in the test organism.

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

3.0 Results
3.1 Bacterial Isolates
Of the 400 bacterial isolates collected, 175 (43.7%) were *K. pneumoniae* and 225 (56.3%) were *E. coli*.

3.2 Occurrence of Non-ESBL-producing Phenotypes
The combined disk synergy method detected 198 (49.5%) of non-ESBL producers among the 400 total bacterial isolates of which 46 (26.3%) were *K. pneumoniae* and 152 (67.5%) were *E. coli* isolates as shown in table 1.

Table 1: Distribution of Non-ESBL-Producing Phenotypes

	Number (%)		
ESBL Detection Method	*K. pneumoniae* n=175	*E. coli* n=225	All Isolates n=400
CDM	46(26.3)	152(67.5)	198(49.5)

CDM: Combined Disk Synergy Method

3.3 Antimicrobial Susceptibility among Non-ESBL-producing Isolates
Of the 198 non-ESBL-producing organisms, 21.2%, 21.7%, 31.8% and 34.8% were susceptible to ampicillin, tetracycline, trimethoprim/sulfamethoxazole and piperacillin respectively as indicated in table 2. Approximately 59% and 72% of the 198 non-ESBL producers were susceptible to amoxicillin/clavulanic acid and piperacillin/tazobactam respectively as demonstrated in table 3. Cefazolin, cefotaxime, ceftazidime and cefepime recorded susceptibility percentages of 75.3%, 93.9%, 92.9% and 97.0% respectively. Cefoxitin demonstrated significant susceptibility of 86.4% to the non-ESBL producers. Of the 198 non-ESBL producers, 99.5%, 98.5%, 81.3% and 86.9% of imipenem, amikacin, gentamicin and nitrofurantoin were susceptible to the non-ESBL-producing organisms respectively. Approximately 60% of the non-ESBL producers were susceptible to ciprofloxacin and norfloxacin.

Table 2: Antimicrobial Susceptibility among Non-ESBL-Producers (n=198)

		MIC (µg/ml)	
Antimicrobial Agent	No. (%) of Susceptible Isolates	MIC_{50}	MIC_{90}
Ampicillin	42(21.2)	≤2	≤2
Amoxicillin/Clavulanic acid	116(58.6)	≤2	8
Piperacillin	69(34.8)	≤4	16
Piperacillin/Tazobactam	142(71.7)	≤4	8
Cefazolin	149(75.3)	≤4	8
Cefoxitin	171(86.4)	≤4	≤4
Cefotaxime	186(93.9)	≤1	≤1
Ceftazidime	184(92.9)	≤1	≤1
Cefepime	192(97.0)	≤1	≤1
Imipenem	197(99.5)	≤1	≤1
Amikacin	195(98.5)	≤2	16
Gentamicin	161(81.3)	≤1	≤1
Ciprofloxacin	119(60.1)	≤0.25	≤0.25
Norfloxacin	119(60.1)	≤0.5	2
Tetracycline	43(21.7)	≤1	≤1
Nitrofurantoin	172(86.9)	≤16	32
Trimethoprim/Sulfamethoxazole	63(31.8)	≤20	40

MIC_{50}: MIC at which 50% of the non-ESBL-phenotypes were susceptible to a particular antimicrobial agent

MIC_{90}: MIC at which 90% of the non-ESBL-phenotypes were susceptible to a particular antimicrobial agent

3.4 Antimicrobial Resistance among Non-ESBL-producing Isolates

Of the 198 non-ESBL-producing organisms, 66.7%, 59.1%, 77.8% and 68.2% were resistant to ampicillin, piperacillin, tetracycline, and trimethoprim/sulfamethoxazole respectively as indicated in table 3. Approximately 14%, 19%, 16% and 17% of the non-ESBL producers were resistant to amoxicillin/clavulanic acid, piperacillin/tazobactam, cefazolin, and gentamicin respectively. Cefotaxime, ceftazidime, cefepime, imipenem, amikacin and nitrofurantoin recorded resistance percentages of less than 5%. Approximately 39% of the non-ESBL producers were resistance to ciprofloxacin and norfloxacin.

Table 3: Antimicrobial Resistance among Non-ESBL-Producers (n=198)

		MIC (µg/ml)	
Antimicrobial Agent	No. (%) of Resistant Isolates	MIC_{50}	MIC_{90}
Ampicillin	132(66.7)	≥32	≥ 32
Amoxicillin/Clavulanic acid	27(13.6)	≥32	≥ 32
Piperacillin	117(59.1)	≥128	≥ 128
Piperacillin/Tazobactam	37((18.7)	≥128	≥ 128
Cefazolin	31(15.7)	≥64	≥ 64
Cefoxitin	14((7.1)	≥64	≥ 64
Cefotaxime	4(2.0)	≥64	≥ 64
Ceftazidime	4(2.0)	32	32
Cefepime	4(2.0)	32	32
Imipenem	0(0.0)	-	-
Amikacin	2(1.0)	≥64	≥ 64
Gentamicin	34(17.2)	≥16	≥ 16
Ciprofloxacin	78(39.4)	≥ 4	≥ 4
Norfloxacin	78(39.4)	≥16	≥16
Tetracycline	154(77.8)	≥16	≥16
Nitrofurantoin	6(3.0)	256	256
Trimethoprim/Sulfamethoxazole	135(68.2)	≥320	≥320

MIC_{50}: MIC at which 50% of the non-ESBL-phenotypes were resistant to a particular antimicrobial agent

MIC_{90}: MIC at which 90% of the non-ESBL-phenotypes were resistant to a particular antimicrobial agent

4.0 Discussion

Antibiotics are among the most commonly prescribed drugs in hospitals and studies on their resistance patterns ensure quality healthcare. Antibiotics are widely and inappropriately used in Ghana resulting to antibiotic resistance. Newman and colleagues (2006) who studied bacterial isolates from various clinical specimens in Ghana, recorded high resistance rates for tetracycline, co-trimoxazole, ampicillin and chloramphenicol. Though the work of Newman and colleagues (2006) did not specify the ESBL phenotypes of the bacterial isolates, their findings were consistent with this present study with high resistances of non-ESBL-producing bacterial isolates to ampicillin, tetracycline and trimethoprim/sulphamethoxazole.

In Zimbabwe (Mbanga *et al*., 2010), the high resistance to ampicillin, co-trimoxazole and trimethoprim/sulphamethoxazole reported correlate to this study. Also, the findings of a study in Ethiopia (Kibret and Abera, 2011) with high resistance rate to tetracycline are confirmed in this study. It was reported in Nigeria that Gram-negative isolates showed high resistance to ampicillin (90%) and co-trimoxazole (85%) (Clarence *et al*., 2007) which were slightly higher than rates recorded in this study. 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 study with non-ESBL-producers resistant rates of 2% to cefotaxime, ceftazidime and cefepime (which are third generation cephalosporins) and 1% for amikacin. However, there was increased resistant rate of 39.4% to ciprofloxacin and norfloxacin.

The steady rise in resistance of bacterial 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 of 39.4% as observed in this study.

The observed increase in resistance in non-ESBL producers to the beta-lactam/beta-lactamase inhibitor combination antimicrobials such as amoxicillin/clavulanic acid and piperacillin/tazobactam is worrying since these beta-lactam/beta-lactamase inhibitor combination antibiotics have become the empirical drug of choice for some clinicians for treating infectious diseases in Ghana. As antibiotic resistance increases and the development of new antimicrobials declines, it would seem prudent to take the caution of Kimang'a (2012) seriously. Nitrofurantoin continues to be effective against *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 antibiotics for third-line treatment options.

5.0 Conclusion

Co-resistances to ampicillin (66.7%), piperacillin (59.1%), tetracycline (77.8%) and trimethoprim/sulphamethoxazole (68.2%) have been collaborated in this work. The increasing rise in resistance to the beta-lactam/beta-lactamase inhibitor combination antibiotics such as amoxicillin/clavulanic acid (13.6%) and piperacillin/tazobactam (18.7%) is very worrying. The steady increase in resistance to gentamicin (17.2%) as well as the floroquinolones such as ciprofloxacin (39.4%) and norfloxacin (34.9%) is alarming. In the absence of ESBLs, cephalosporins have generally been effective in treating infections caused by enterobacteria. Nitrofurantoin remains reliable for managing non-life threatening urinary tract infections. Amikacin and imipenem continue to be effective non first-line treatment options for Gram-negative bacteria infections. Evidence based guidelines and clinical practices and systematic implementation of antimicrobial stewardship programs should be encouraged to improve antimicrobial use.

Acknowledgements
The authors appreciate the support from the management of Advent Clinical Laboratories and Central Laboratory, Korle Bu Teaching Hospital.

References

Beitha A (2008). Mapping factors that drive drug resistance (with a focus on resource-limited settings): a first step towards better informed policy. Center for global development.
Clarence S, Helen U, Nosakhare O (2007). Multi-antibiotics-resistance plasmid profile of enteric pathogens in pediatric patients from Nigeria. Biokemistri; 19(1): 35-42.
Hackman HK, Osei-Adjei G, Ameko E, Kutsanedzie F, Gordon A, Brown CA, Twum-Danso K. (2013). Phenotypic determination and antimicrobial resistance profile of extended spectrum beta-lactamases in *Escherichia coli* and *Klebsiella pneumoniae* in Accra, Ghana. Journal of Natural Sciences; 3(12): 75-83.
Kibret M and Abera B (2011). Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. African Health Sciences; 11(S1): S40 - S45.
Kimang'a NA (2012). A situational analysis of antimicrobial drug resistance in Africa: are we losing the battle? Ethiop J Health Sci; 22:2.
Mbanga J, Dube S and Munyanduki H (2010). Prevalence and drug resistance in bacteria of the urinary tract infections in Bulawayo province, Zimbabwe. East Afr J Public Health. 7(3): 229-32.
Nankanishi N, Yoshida S, Wakebe H, Inoue M, Mitsuhashi S (1999). Mechanisms of resistance to fluoroquinolones in *Enterococcus faecalis. Antimicrob Agents Chemother;* 35: 1053-1059.
Newman MJ and Seidu A (2002). Carriage of antibiotic resistant E coli in adult intestinal flora. *WAMJ*; 21:48-50.
Newman M, Frimpong E, Asamoah-Adu A and Sampane DE (2006)**.** Resistance to antimicrobial drugs in Ghana. Ghanaian-Dutch Collaboration for Health Research and Development, Project Number 2001/GD/07, Technical Report Series NO.5.
Plotkin MJ and Shnayerson M (2003). The killers within: the deadly rise of drug-resistant bacteria.
Todar K (2008). Textbook of bacteriology: bacterial resistance to antibiotics. University of Wisconsin, USA; 1-4

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar