

Emergence of Carbapenem-resistant Enterobacteriaceae among Extended-spectrum Beta-lactamase Producers in Accra, Ghana

Henry Kwadwo Hackman^{1*} Reuben Essel Arhin¹ Andrew Gordon¹ Samuel Nana Benyin Mensah²

1. Department of Science Laboratory Technology, School of Applied Sciences and Arts, Accra Technical University, Accra, Ghana
2. MDS-Lancet Laboratories, Accra, Ghana

Abstract

Previous studies in Accra had established that extended-spectrum beta-lactamase (ESBL) producers were increasingly becoming a public health nuisance. Since ESBL producers resulted in multi-drug resistance among most beta-lactams and non-beta-lactams, the antibiotic of choice for the treatment of these ESBL infections was carbapenems such as imipenem, meropenem and ertapenems. Hence the emergence and spread of carbapenem resistant bacteria may lead to therapeutically dead end for life-threatening infections. This study therefore focussed on the occurrence of carbapenem resistant Enterobacteriaceae among ESBL producers from clinical specimens analysed at MDS-Lancet Laboratories, Accra, Ghana. One thousand (1000) clinical isolates were identified and analysed for ESBL producers using the combined disk synergy method. In order to determine the carbapenemase producing ESBL bacteria and the antibiotic of choice for treating carbapenem resistant infections, antimicrobial susceptibility testing was performed to determine the minimum inhibition concentration of the antibiotics used against the ESBL producers. The antibiotics used included imipenem, meropenem ertapenem and other antibiotics. The results indicated that 600 (75%) of the clinical isolates were ESBL producers. Among the 600 ESBL producers, 43 (7.2) were carbapenem resistant bacteria including 7 different Gram negative bacterial species. Among the carbapenemase producers, *Escherichia coli* (34.9%) and *Klebsiella pneumoniae* (25.6%) were the dominant bacterial species. The carbapenem resistant bacteria indicated multi-drug resistance to penicillins (100%), cephalosporins (100%), amoxicillin-clavulanic acid (100%), piperacillin-tazobactam (100%), ciprofloxacin (83.7%), gentamycin (79.7%), amikacin (27.9%), colistin (18.6%) and fosfomycin (11.6%). Colistin seems to be is the drug of choice for treating carbapenem resistant strains. Although fosfomycin showed a higher activity, it is only recommended for urinary tract infections. Evidence based antibiotic usage and nosocomial infection control will help to control the emergence of carbapenem resistant strains in Accra, Ghana. Also, there is the need to intensify research in the use of natural products to treat resistant bacterial infections.

Keywords: Carbapenem, ESBLs, Antibiotics, Colistin

1.0 Introduction

One of the major public health challenges in the 21st century is the increasing dominance of antibiotic resistant strains in most bacterial infections. 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. Antibiotic resistant mechanisms are commonly due to enzymatic inactivation of antibiotics (Todar, 2008) such as the production of extended-spectrum beta-lactamases and carbapenemases by the Gram negative bacteria.

The misuse of antibiotics in humans and animals is accelerating the process of antibiotic resistance development. When antibiotics are misused or over prescribed, bacteria become resistant to their effects, making some infectious diseases difficult to treat. The problem of antimicrobial resistance is compounded by the principles of natural selection. 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.

Previous studies by Hackman and colleagues (2013) in Accra established that extended-spectrum beta-lactamase (ESBL) producers were increasingly becoming a public health nuisance due to community acquired and nosocomial infections. Since ESBL producers resulted in multi-drug resistance among most beta-lactams and non-beta-lactams (Hackman *et al.*, 2013a), carbapenems such as imipenem, meropenem and ertapenems are often the last resort for the treatment of ESBL infections (Pitout *et al.* 2008; Zilberberg *et al.* 2013).

Different classes of carbapenemases are produced by members of the Enterobacteriaceae family which lead to resistant carbapenem strains. Some of these include plasmid-mediated IMP-type carbapenemases, Verona integron-encoded metallo- β -lactamase (VIM) (Makena *et al.*, 2016), OXA carbapenemases (Santillana *et al.*, 2007), *K. pneumoniae* carbapenemase (KPC), CMY carbapenemases (Kim *et al.* 2006) and (New Delhi metallo- β -lactamase (NDM-1) (Walsh *et al.* 2011)

Most carbapenem-resistant strains are also ESBL producers (Bornet *et al.* 2000). Infections involving carbapenem-resistant Enterobacteriaceae are associated with significant morbidity and mortality (Makena *et al.*

2016). Since screening for ESBL is not a common practice in most health facilities in Ghana (Hackman *et al.*, 2013b), this laboratory-based study was undertaken to determine the occurrence of carbapenem-resistant bacteria among ESBL producers from clinical isolates and the antibiotic of choice for treating carbapenem-resistant strains at MDS-Lancet Laboratories, Accra, Ghana.

2.0 Materials and Methods

2.1 Materials

Chocolate, Colombia agar, MacConkey, Dex & Chlor, TSA, CLED, Mueller Hinton agar were prepared according to manufacturers' guidelines. API 20E was used to identify isolates. (bioMérieux, France) was also used to determine MIC. MAST ID™ ESβL Detection Disks (Mast Group, UK) were used for ESBL screening and confirmation according to CLSI standards on a Mueller Hinton agar plate.

2.2 Study Sites

Bacterial isolates obtained from clinical specimens collected at satellite centres affiliated to MDS-Lancet laboratories, Accra, Ghana.

2.3 Sample Size

A sample size of 1000 bacterial isolates 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 ESBL producing bacteria were used in the work.

2.5 Exclusion Criteria

All non-ESBL producing bacteria were not used in this study.

2.6 Culturing, Gram Staining and Identification of Bacterial Isolates

The clinical specimens were cultured on Chocolate, Colombia, MacConkey, Dex & Chlor, TSA, CLED agar using aerobic and anaerobic incubation techniques and incubated at 37°C. All the plates were incubated for 24 hours with the exception of TSA which was incubated for 48hours. The pure colonies were gram-stained to confirm their Gram negative reaction. API 20E was used to identify the isolates.

2.7 Detection of ESBL Phenotype using Combined Disc Synergy Method

MAST ID™ ESβL Detection Discs (Mast Group, UK) were used to screen and confirm the ESBL phenotypes. The MAST ID™ ESβL Detection Discs 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 Discs 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 Discs indicates the presence of ESBL in the test organism.

2.8 Detection of Carbapenem-resistant Enterobacteriaceae (CRE), Determination of Minimal Inhibition Concentration (MIC) and Antibiotic Susceptibility Testing

The selected 16 antibiotics used were ampicillin, cefuroxime, cefepime, augmentin, piperacillin/tazobactam, nalidixic acid, cotrimoxazole, nitrofurantoin, ertapenem, ciprofloxacin, gentamicin, imipenem, meropenem, amikacin, colistin and fosfomycin. The Kirby-Bauer method of antibiotic susceptibility testing was used to determine the susceptibility of the isolates to carbapenems and other antibiotics. Etest system (bioMérieux, France) was also used to determine the minimum inhibition concentration (MIC) of the antibiotics used. The therapeutic significance of the zones of inhibition and MICs was based on the Clinical and Laboratory Standards Institute (CLSI) breakpoints.

3.0 Results

3.1 Occurrence of ESBL-producers among Bacterial Isolates

The combined disk synergy method detected 600(75%) ESBL producers among the 1000 total bacterial isolates with *E. coli* and *Klebsiella pneumoniae* being the dominant species as indicated in table 1.

Table 1: Occurrence of ESBL producers among clinical isolates

Bacterial species	ESBL producers (n=600) Number (%)
<i>Escherichia coli</i>	195 (32.5)
<i>Providencia rettgeri</i>	90 (15)
<i>Citrobacter koseri</i>	45 (7.5)
<i>Acinetobacter baumannii</i>	45 (7.5)
<i>Pantoea species</i>	45 (7.5)
<i>Klebsiella pneumonia</i>	135 (22.5)
<i>Proteus mirabilis</i>	45 (7.5)

3.2 Occurrence of Carbapenem-resistant Enterobacteriaceae among ESBL-producers

The results showed among the 600 ESBL producers, 43 (7.2) were carbapenem-resistant Enterobacteriaceae (CRE) including 7 different Gram negative bacterial species as indicated in figure 1. Among the carbapenemase producers, *Escherichia coli* (34.9%) and *Klebsiella pneumoniae* (25.6%) were the dominant bacterial species.

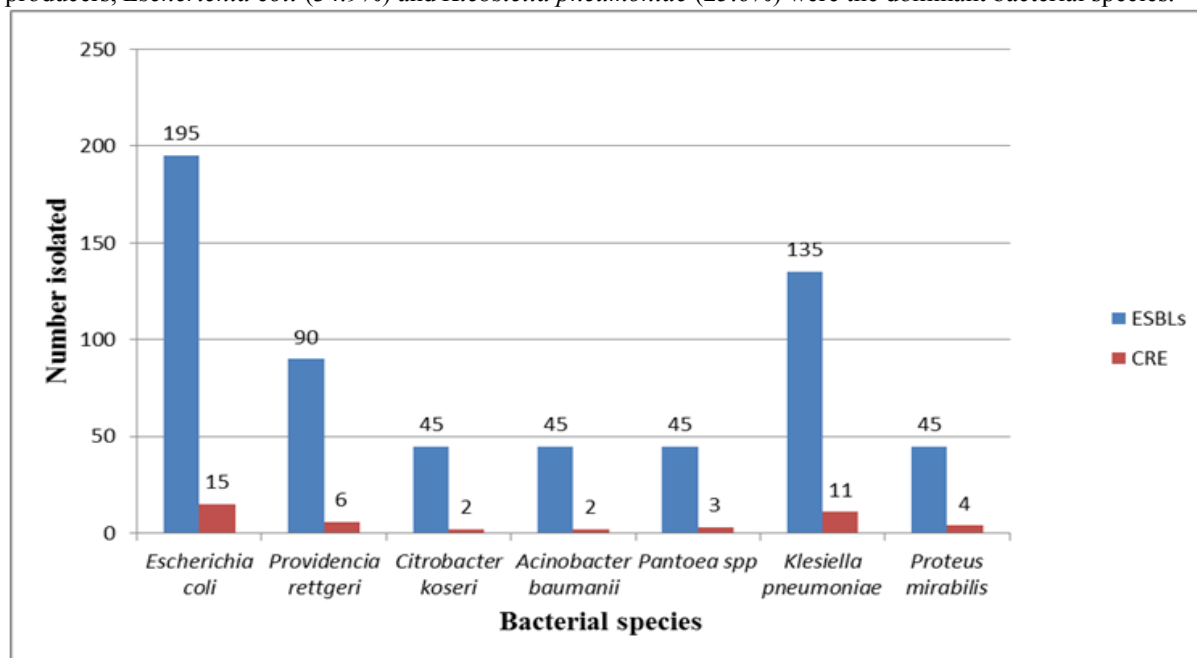


Figure 1: Occurrence of carbapenem-resistant Enterobacteriaceae among ESBL producers

3.3 Antimicrobial Resistance of Carbapenem-resistant Enterobacteriaceae

The carbapenem-resistant bacteria indicated multi-drug resistance to penicillins (100%), cephalosporins (100%), amoxicillin-clavulanic acid (100%), piperacillin-tazobactam (100%), ciprofloxacin (83.7%), gentamycin (79.7%), amikacin (27.9%), colistin (18.6%) and fosfomycin (11.6%) as indicated in figure 2.

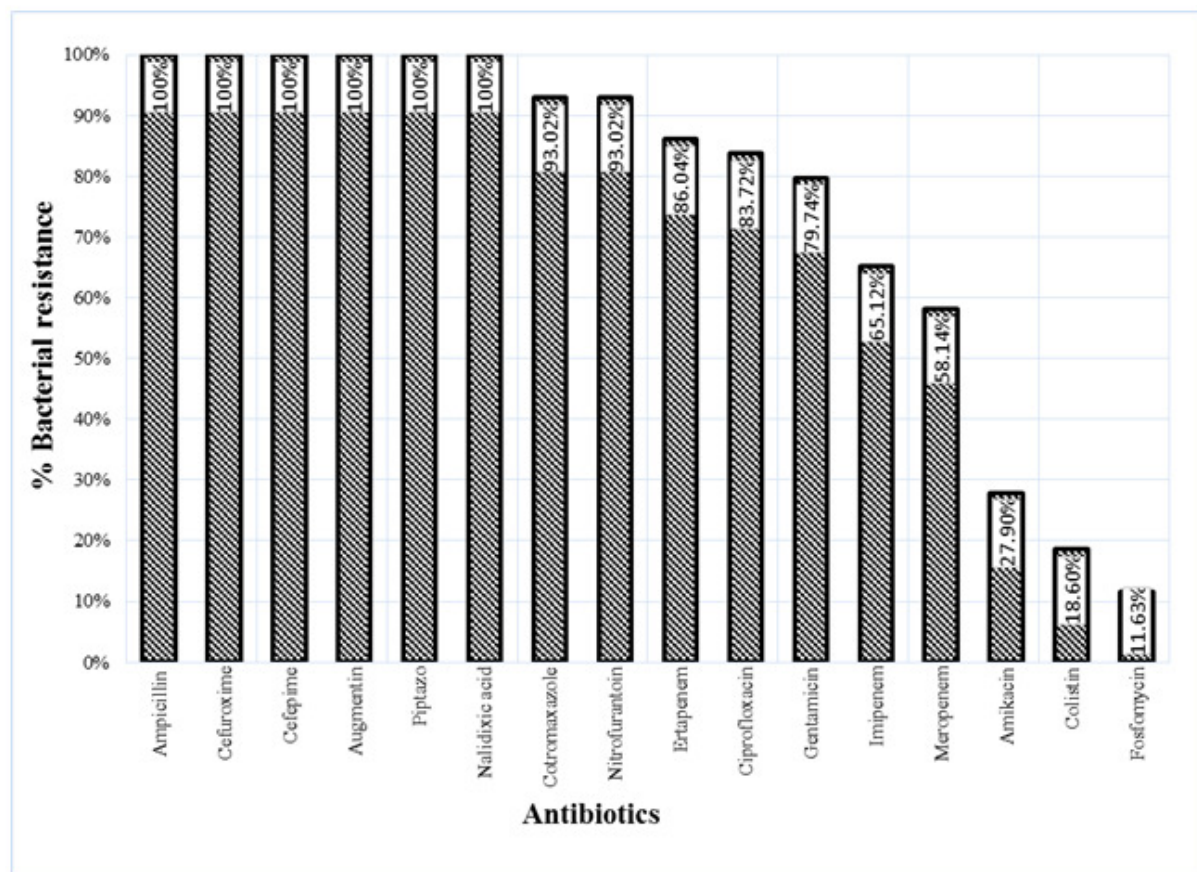


Figure 2: Resistance of carbapenem-resistant Enterobacteriaceae to antibiotics

4.0 Discussion and Conclusions

4.1 Occurrence of Carbapenem-resistant Enterobacteriaceae among ESBL producers

A previous study of antimicrobial resistance of ESBL producers recommended carbapenems as the drug of choice for treating ESBL-producing infection (Hackman *et al.*, 2013a). The magnitude of emerging resistance of carbapenem-resistant Enterobacteriaceae (CRE) is currently poorly known in Ghana because of the lack of appropriate studies. It is possible that this is the first study of carbapenem-resistant Enterobacteriaceae among ESBL producers in Ghana. It is there appropriate to assess the occurrence of CRE to inform institutional and national prevention efforts. The study showed that the proportion of carbapenem-resistant Enterobacteriaceae (CRE) among 600 ESBL producers was 7.2%. The emergence of carbapenem-resistant Enterobacteriaceae in Accra may be due to the overuse and abuse of carbapenems to treat bacterial resistant infections. The rapid transmission of plasmid-mediated beta-lactamase (Paterson & Bonomo, 2005) among related species require urgent actions to implement nosocomial infection control measures to curb the spread of ESBL and CRB strains in Accra.

This relatively low rate of CRB among ESBLs in Accra was comparable with studies in Belgium, France (Huang *et al.* 2013) and USA (Guh *et al.*, 2015). Two large studies conducted in 2009–10 in Spain and Canada found proportions of carbapenemase-producing Enterobacteriaceae of 0.04% and 0.02% respectively (Miro *et al.* 2013).

4.2 Resistance of Carbapenem-resistant Enterobacteriaceae to Antibiotics

Infections involving carbapenemase-producing bacteria are associated with significant morbidity and mortality (Makena *et al.*, 2016) due to its multi-drug resistance tendencies.

Fosfomycin provides an appropriate treatment option for uncomplicated UTIs as shown in this study. Given its low rates of resistance and the available evidence, fosfomycin may serve as a useful option for oral treatment of carbapenem-resistant uropathogens. Further research into the most appropriate dosing regimen and duration of fosfomycin for MDR UTIs is needed.

There is relatively low resistance of bacterial isolates to colistin is indicated in this study. The first plasmid mediated colistin-resistance gene was isolated and described in 2016 (Lui, 2016). Colistin was known for its nephrotoxicity. It remains one of the last-resort antibiotics for multidrug-resistant *Pseudomonas aeruginosa*,

Klebsiella pneumoniae, and *Acinetobacter* (Falagas *et al.* 2008). NDM-1 metallo- β -lactamase multidrug-resistant Enterobacteriaceae have also shown susceptibility to colistin (Kumarasamy *et al.* 2010).

Colistin is therefore established as the drug of choice for treating carbapenem-resistant Enterobacteriaceae in Accra. Other studies in USA has reported high susceptibility of carbapenem-resistant Enterobacteriaceae to tigecycline (Guh *et al.*, 2015)

5.0 Conclusion

This work has established the emergence of carbapenem-resistant Enterobacteriaceae (7.2%) among ESBL producing phenotypes in Accra, Ghana. Colistin seems to be is the drug of choice for treating carbapenem-resistant strains. Although fosfomycin showed a higher activity, it is only recommended for urinary tract infections. Evidence based antibiotic usage and nosocomial infection control will help to control the emergence of carbapenem-resistant strains in Accra, Ghana. Efforts should be made to develop rapid diagnostic systems for the routine detection of CRE in the laboratory. Further work must be done to characterize the carbapenemase-producing genes and there is the need to intensify research in the use of natural products to treat antibiotic resistant infections.

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