Global Spread of Carbapenem-Resistant *Enterobacteriaceae*: A Challenging Threat to the Treatment of Bacterial Diseases in Clinical Practice

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Abstract: **The increase in the occurrence of carbapenemase-producing *Enterobacteriaceae* constitutes a threat not only to treatment of bacterial infection but also to public health problems. Resistance to carbapenems is mostly due to the production of carbapenemases, which are capable of hydrolyzing not only carbapenems but also other groups of antibiotics like penicillins, cephalosporins, quinolones, etc. The most common carbapenemases include veronica integron Metallo-β-lactamases types (VIM), imipenemase (IMP) types, *Klebsiella pneumoniae* carbapenemase (KPC), oxacillinase-48 (OXA-48), and New Delhi metallo-β-lactamase-1 (NDM-1), encoded by carbapenem resistant determining genes *bla*VIM, *bla*IMP, *bla*KPC, *bla*OXA-48, and *bla*NDM, respectively. Carbapenemase activity can be investigated by phenotypic assay however carbapenemase encoding genes can also be part of the routine assay for diagnosis of bacterial infection. In Africa, there is limited data on the prevalence and distribution of carbapenem resistant *enterobacteriaceae* in clinical studies except in East Africa where a few studies have been done in Kenya and Tanzania.**

***Keywords*: Carbapenemase, Prevalence, Distribution, Resistance, Hydrolyze.**

I. INTRODUCTION

1.1 The Family Enterobacteriaceae

Enterobacteriaceae is a family of Gram-negative, facultatively aerobic and non-spore forming rod bacteria with the majority of the members of the family mainly inhabit the gut of humans and animals (Nordmann *et al*, 2011a & 2012). Currently, there are 51 known genera within the family of *Enterobacteriaceae* which include *Arsenophonus, Biostraticola, Citrobacter, Cronobacter, edwardsiella, Escherichia, Proteus, Morganella, Providencia, Salmonella, Photorhabdus, Serratia, Shigella, Yersinia, Xenorhabdus, Phaseollibacter, Samsonia, Saccharobacter, Erwinia, Plesiomonas Enterobacter,* etc (Iversen *et al* 2008; Halpern *et al*, 2013). Members of *Enterobacteriaceae* are generally motile with exception of *Klebsiella, Arsenophonus, Biostraticola* etc, catalase positive, and oxidase negative with exception of *Plesiomonas*. These members use the Embden-Meyerhof pathway for sugar metabolism and acid production from sugar fermentation. (Francino *et al*, 2006). Specifically, 16S rDNA and other gene sequences are required to identify members of this family (Francino *et al*, 2006). Most of the members of this family are pathogenic and cause infections in humans and animals such as gastrointestinal infections, sepsis, pneumonia, meningitis, peritonitis and urinary tract infections (Paterson, 2006; Nordmann *et al*, 2011b). Bacteria in the family *Enterobacteriaceae* are increasingly becoming multi-drug resistant bacteria.

1.2 Carbapenems

Carbapenems (currently ertapenem, imipenem, meropenem and doripenem, etc.) are the most effective and potent β lactam antibiotics, with the broadest spectrum and the least resistance. In the past, carbapenems have been active against all multiple drug-resistant (MDR) bacteria with little toxicity to the host, carbapenems became the preferred last choice of antibiotics for the treatment of MDR Gram negative bacterial infections. The development of carbapenem resistance (CR) in *Enterobacteriaceae* is of considerate concerns because there is no adequate next line of antibiotics to use against carbapenem resistant *Enterobacteriaceae* (Livermore *et al.*, 2011). There are less efficient antibiotics to treat life threatening infections caused by MDR organism (Nordmann *et al.,* 2011a and 2012; CDC, 2015, ECDC, 2013, 2016).

Currently, the high prevalence of carbapenem resistant *Enterobacteriaceae* (CRE) isolates especially in *Klebsiella pneumoniae* and *Escherichia coli* isolates in hospitals, community-associated infections and animals are a huge burden to the health care system (Bush & Fisher, 2011; Nordmann *et al.*, 2011a, 2012; ECDC, 2016). Carbapenem-resistant *Enterobacteriaceae* (CRE) are among the most severe threats to the treatment of infections. Many species can exhibit resistance due to diverse mechanisms, and many of these resistance genes are found on the mobile genetic elements thereby capable of transferring both horizontally and vertically within the bacteria population (Gustavo *et al.*, 2017).

Initially, Carbapenems have been the most successful β-lactam antibiotics used in the treatment of infections caused by β-lactam resistant Gram negative bacteria. The clinical use of this antibacterial is under threat with the emergence of

carbapenemase, especially the class B Metallo β- lactamases (MBLs). MBLs can hydrolyze most β- lactams except for monobactams and confer a broad-spectrum resistance to the bacterial host, which is not reversible by conventional therapeutic β- lactamase inhibitors. The prevalence of MBLs has been increasing worldwide, notably among *Pseudomonas aeruginosa* and recently, reported among other Gram negative bacteria. MBLs producing Gram negative bacteria constitute an increasing public health problem globally because of their resistance to all β- lactam antibiotics except aztreonam. MBL genes are either carried on transferable plasmids or components of the bacterial chromosome. (Ahmad & Ali, 2014).

KPC producers have been reported in nosocomial *K. pneumoniae* isolates, *E. coli* (especially in Israel) and other enterobacterial species (Nordmann *et al.,* 2009). A single *K. pneumoniae* clone (sequence type [ST]-258) was identified extensively worldwide, indicating that it may have contributed to the spread of the *bla* KPC genes (Cuzon *et al.,* 2010). Within a given geographic location, several KPC clones are spreading with different multi-locus sequence types which includes: additional β-lactamase content; and by size, number, and structure of plasmids, but the KPC genes are associated with a single genetic element (transposon Tn4401) (Cuzon *et al.,* 2010). Although community-acquired KPC producers have been reported, they are rare, with the exception of isolates from Israel a few years ago (Nordmann *et al.,* 2009). The level of resistance to carbapenems by KPC producers may vary markedly; ertapenem is the carbapenem that has the lowest activity (Nordmann *et al.,* 2009). KPC producers are usually multidrug resistant (especially to all β-lactams), therefore the choice of therapy for the treatment of KPC - related infections remains limited (Nordmann *et al.,* 2012). It has been reported that death rates from infections with KPC producers are on the high side (>50%) (Borer *et al.,* 2009).

1.3 Genetic Variants of Carbapenem Resistance

Genetic determinants of CR have been classified into Ambler class A beta-lactamases which include; KPC, GES/IBC, SME, NMC-A, IMI and SFC (Patel & Bonomo, 2013; Woodford *et al.,* 2013), Ambler class B beta-lactamases which are termed as Metallo beta lactamases consisting of NDM, VIM, IMP, SPM, GIM, SIM, KHM, AIM, DIM, SMB, TMB and FIM (Patel & Bonomo, 2013). IMP, VIM and NDM plasmid-mediated Metallo beta lactamases are of global occurrence possibly because the encoding genes are located on mobile genetic elements (Patel & Bonomo, 2013) and carbapenem hydrolyzing class D β-lactamases (CHDLs) encompasses the various groups of oxacillinases (OXA) with the hydrolytic activity of amino and carboxy penicillins (Poirel *et al.,* 2010b), Studies have shown that the existence of CR bacteria in East Africa but in general, comprehensive data is still lacking about the molecular epidemiology of CR organisms (Manenzhe *et al.,* 2015; Ampaire *et al.,* 2016) and its menace on the health care system in Africa especially Nigeria.

1.4 Designs of the Review

The literature search used by the authors for this review was based on keywords related to the concept of the review, therefore terms such as antibiotic resistance, carbapenemases, and carbapenemase encoding genes, global epidemiology and future consideration of the treatment of infection associated with antibiotic resistant pathogens especially in Africa were used. Literature search on cross-sectional, observational randomized control studies and review published on the carbapenemases and related genes (both plasmid and chromosomally encoded) up to December 2019 were the main sources of information. The information was systematically searched in PubMed, Web of Science, Scopus, Elsevier Masson Consulte, Embase, Google scholar and African Journals Online, international conference proceedings, published theses and dissertations on carbapenemase-producing bacteria in Africa and the entire globe. Also, information for systematic reviews was obtained from websites of an international organizations which served as data sources for updates and contributions on the subject matter from experts.

II. RESULT AND DISCUSSION

2.1 Carbapenemases as a Carbapenem and other beta lactam antibiotic hydrolyzing Enzyme.

Carbapenemases are the most diverse and broad class of beta-lactamases. They are capable of efficiently hydrolyzing a wide range of beta-lactam antibiotics such as penicillins, cephalosporins, monobactams, and carbapenems. Carbapenemases represent the most versatile family of beta lactamases, with a broad spectrum unrivaled by other -lactam-hydrolyzing enzymes. Although it is recognized as "carbapenemases," many of these enzymes are capable of recognizing almost all hydrolysable beta lactams, and are most resilient against inhibition by all the commercially viable beta lactamase inhibitors (Nordmann and Poirel, 2002; Turton *et al.,* 2006). The enzymes that hydrolyze carbapenem are generally referred to as carbapenem-hydrolyzing enzymes or carbapenemases indicating carbapenems as their major substrate (Rasmussen and Bush, 1997; Francis *et al.,* 2018).

Carbapenemases are a heterogeneous group of enzymes that can hydrolyze most beta-lactams including carbapenems (ECDC, 2016). In the literature, CRE is often named after the specific carbapenemases that they produce, such as *Klebsiella pneumoniae* carbapenemase (KPC)-producing CRE (KPC CRE), oxacillinase 48 (OXA-48)-producing CRE (OXA-48 CRE), and CRE that produce metallo-beta-lactamases such as the New Delhi metallo-betalactamase (NDM)-producing CRE (NDM CRE), Verona integron-encoded metallo-beta-lactamase (VIM)-producing CRE (VIM CRE), and IMP-type metallo-beta-lactamase-producing CRE (IMP CRE) (ECDC, 2016).

CRE typically harbor genes that encode carbapenem-hydrolyzing beta-lactamases or carbapenemases (Nordmann *et*

al., 2012). *Klebsiella pneumoniae* carbapenemases (KPCs) are the most common in the United States (Gustavo *et al*., 2017). KPCs are encoded by the blaKPC gene, which is classically plasmid-associated and located on the transposable elementTn4401. Although originally observed in *K. pneumoniae* strains, KPCs are found in the most variety of plasmids and observed in other *Enterobacteriaceae* (Conlan *et al.*, 2014). The increasing incidence of CRE has been largely credited to the spread of a single *K. pneumoniae* clone, ST258 (Munoz-Price *et al.*, 2013). In the United States, the majority of carbapenem resistance problem is due to this clone and other KPC-containing strains of *Enterobacteriaceae* (Gupta *et al.*, 2011). Apart from KPCs as most common in the United States (Gustavo *et al*., 2017), other carbapenemase genes have been reported to be common in other parts of the world, including the oxacillin hydrolyzing beta-lactamase gene blaOXA-48 in North Africa and West Europe, blaVIM in Mediterranean countries, and blaNDM in Pakistan, Bangladesh, and India (Nordmann *et al.*, 2011b). Carbapenemases belong to two major molecular families, distinguished by the hydrolytic mechanism at the active site.

The first carbapenemases described were from Gram-positive bacilli. Unlike other beta lactamases known at that time, these enzymes were inhibited by EDTA, thereby establishing them as metallo-enzymes. Later work has shown that all metallo-carbapenemases contain at least one zinc atom at the active site that serves to facilitate hydrolysis of a bicyclic beta lactam ring (Frere *et al.*, 2005). In the mid -to-late 1980s, another set of carbapenem-hydrolyzing enzymes emerged among the *Enterobacteriaceae* (Medeiros and Hare, 1986), in which EDTA did not inhibit their activity (Rasmussen *et al.*, 1996). Subsequent studies have shown that these enzymes utilized serine at their active sites and were inactivated by the β -lactamase inhibitors clavulanic acid and tazobactam (Rasmussen *et al.*, 1996; Yang *et al.*, 1990).

Before the early 1990s, all carbapenemases were initially described as species-specific and chromosomally encoded β -lactamases, each with a well-defined set of characteristics. However, the identification of plasmid that encodes IMP-1; a metallo-β-lactamase in *Pseudomonas aeruginosa*, ARI-1 (OXA-23); which is a class D carbapenemase in *Acinetobacter baumannii* (Paton *et al*., 1993) and KPC-1 and a class A carbapenemase in *Klebsiella pneumoniae* (Yigit *et al*., 2001) has changed the spreading patterns of carbapenemase. Also, several reports on carbapenemase have appeared recently, even including detailed compilations of the kinetic characteristics of these enzymes (Walsh, 2005; Walther-Rasmussen and Hoiby. 2006). There is less report on the epidemiology of carbapenemase and associated genes in Africa particularly Nigeria. The most common carbapenemases include veronica integron metallo-beta-lactamases types (VIM), imipenemase (IMP) types, *Klebsiella pneumoniae* carbapenemase (KPC), oxacillinase-48 (OXA-48), and New Delhimetallo-beta-lactamase-1 (NDM-1), which are encoded by carbapenem resistance determining genes VIM, IMP, KPC, OXA-48, and NDM, respectively (Nordmann *et al*., 2011c). It should be noted that what has been considered to be a problem of clonal spread has now become a global problem of interspecies dispersion. The proliferation of different members of carbapenemase families necessitates the vital need to understand the properties of these enzymes, with all their strengths and limitations at the molecular level.

2.2 Class A carbapenemases

A number of class A carbapenemases have been described in which some are chromosome encoded (NmcA, Sme, IMI-1, SFC-1), and others are found to be plasmid- encoded (*Klebsiella pneumoniae* carbapenemases [KPC], IMI-2, GES, derivatives) (Nordmann *et al*., 2011c). Despite the different genomic locations, all the enzymes can effectively hydrolyze carbapenems and are partially inhibited by clavulanic acid. In light of this versatility, there is a serious effort in reducing the heavy reliance on carbapenems for the treatment of infections especially ESBL infections (Adodakpi *et al.*, 2019). KPCs are the most clinically common enzymes in the class A carbapenemases. The first KPC producer (KPC-2 in *K. pneumoniae*) was identified in 1996 in the Eastern United States (Spellberg *et al.*, 2011). Recently, KPC producing organisms had spread across the globe and have been reported in the places like the United States (still mostly in East coast states) and, in particular, in Puerto Rico, Colombia, Greece, Israel, and the People's Republic of China (Knapp *et al.*, 2001; Datta and Wattal, 2010). Similarly, outbreaks of KPC producers have been reported in many European countries and in South America (Knapp *et al.*, 2001; Datta and Wattal, 2010).

For many years, these enzymes were regarded as species-specific, chromosomally-encoded beta-lactamases, until many of the genes encoding these enzymes were detected on plasmids of some pathogenic bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii,* thus allowing the further spread of carbapenem resistance genes between bacterial species through horizontal or vertical transmission (Queenan and Bush 2007).

2.3 Class B Metallo-β-Lactamases

Class B Metallo-β-lactamases (MBLs) are mostly Veronica integron–encoded Metallo-β-lactamase (VIM) and IMP types while the most recent among the MBLs is the New Delhi Metallo-β-lactamase-1 (NDM-1) type (Mouton *et al*., 2000; WHO, 2014). The first acquired MBL is IMP-1, which was reported in *Serratia marcescens* in Japan in 1991 (Codjoe and Donkor, 2017). Since then, MBLs have been described worldwide (Mouton *et al*., 2000; WHO, 2014). Endemicity of VIM- and IMP-type enzymes has been reported in Greece, Taiwan, and Japan (Mouton *et al*., 2000; WHO, 2014) however, either outbreaks or single case of VIM and IMP enzymes has been reported in many other countries. These enzymes can hydrolyze all β-lactams except aztreonam (Mouton *et al*., 2000). Their activity is inhibited by EDTA

because of its affinity for zinc which constitutes the active site of the MBL but not by clavulanic acid (Codjoe and Donkor, 2017). MBL producers are mostly nosocomial especially multidrug-resistant *K. pneumoniae* (Codjoe and Donkor, 2017). Resistance levels to carbapenems of MBL producers may vary depending on the enzyme and death rates associated with MBL producers range from 18% to 67% (Codjoe and Donkor, 2017).

NDM-1 enzyme was discovered in 2008 in Sweden from an Indian patient that was hospitalized previously in New Delhi (Van Dan *et al.,* 2009), NDM-1–positive Enterobacteriaceae are now being given worldwide attention (Hayes & Orc, 1983; Bonfiglio *et* al 2002; Van Dan *et al.,* 2009). Since mid-August 2010, NDM-1 producers have been identified in all continents except in Central and South America. In most cases, countries with a direct link with the Indian subcontinent (Van Dan *et al.,* 2009). Few cases of NDM-1-producers have been reported from the United States and Canada (Van Dan *et al.,* 2009).

In contrast to several other carbapenemase genes, the blaNDM-1 gene is not associated with a single clone but rather with non-clonally related isolates and species (Hayes & Orc, 1983; Van Dan *et al.,* 2009). It has been identified mostly in *E. coli* and *K. pneumoniae* and to a lesser extent in other enterobacterial species (Hayes & Orc, 1983; Van Dan *et al.,* 2009). The magnitude of resistance to carbapenems by NDM-1 producing bacteria may vary. The plasmids that harbor the blaNDM-1 gene are vast and can possess a significant number of resistance genes such as other carbapenemase genes (oxacillinase-48 [OXA-48] types, VIM types), plasmid-mediated cephalosporinase genes, ESBL genes, macrolide resistance genes (esterase), rifampin (rifampin modifying enzymes), aminoglycoside resistance genes (16S RNA methylases) and sulfamethoxazole resistance genes. Therefore, these multi-genes carriers are the sources of multidrug and/or pan drug resistance (Hayes & Orc, 1983; Van Dan *et al.,* 2009). Such co-occurrence of such a high number of resistance genes in a bacterium has been rarely reported, even among carbapenemase producers. Many NDM-1 producers remain susceptible only to tigecycline, colistin and to a lesser extent fosfomycin (Hayes & Orc, 1983; Van Dan *et al.,* 2009).

Compared to other carbapenemases, NDM-1 has several characteristics that are deep of serious concern for public health worldwide. These characteristics are:

i. Occurrence of the NDM-1 gene not in a single species but many unrelated species and its widespread in the environment, at least in the Indian subcontinent (Papp-Wallace *et al*., 2011);
ii. Frequent acquisition of *K. pneumoniae* (a typical nosocomial pathogen), and *E. coli* (community-acquired human pathogen); and
iii. Level of the reservoir like the Indian subcontinent (>1.4 billion persons), certain areas in Pakistan (<20% of the population may carry NDM-1 producers) (Nordmann *et al.,* 2011b).

Of particular concern, NDM-1 has been identified in *E. coli* ST-type 131 as a source of community-acquired infection (Poirel *et al.,* 2010a), strain is known to mobilize efficiently the ESBL CTX-M-15 worldwide (Coque *et al.,* 2008). *E. coli* is the most common cause of diarrhea in children in India. Therefore, this organism may increase the risk of drug-resistant strains being released into the environment and further spread among humans. Accordingly, NDM-1 producers have been recently identified in tap and environmental water in New Delhi, among many unrelated Gram-negative species (Walsh *et al.,* 2011).

2.4 Class D Enzymes

OXA (Oxacillin-hydrolyzing) β -lactamases represented one of the most prevalent plasmid-encoded β -lactamase families in the late 1970s and early 1980s (Anne and Bush, 2007). The molecular class D OXA β -lactamases were placed in a separate molecular class different from the other serine β-lactamases (Anne and Bush, 2007). These classes of enzymes had been identified mainly in the *Enterobacteriaceae* and *P. aeruginosa* (Naas and Nordmann, 1999) and were functionally described as penicillinases that are capable of hydrolyzing antibiotics such as oxacillin and cloxacillin (Bush *et al*., 1995). These groups of enzymes are known to have a large amount of variability in amino acid sequences (Bush, 1988) and are poorly or not inhibited by clavulanic acid and EDTA (Anne and Bush, 2007).

Class D enzymes, also referred to as OXA type, can be subdivided into five families, namely the OXA-23, -24/40, -48 and -58 carbapenemases, which are mainly plasmid encoded, and the OXA-51 carbapenemase, which is chromosomally encoded and intrinsic found in *Acinetobacter baumannii* (Turton *et al.* 2006; Queenan and Bush, 2007; Rendani *et al.,* 2015;) Class D Enzymes of the OXA-48 type was first identified from a *K. pneumoniae* strain isolated in Turkey in 2003 (Poirel *et al.,* 2004). Since then, OXA-48 producers have been extensively reported from Turkey as a source of nosocomial outbreaks (Poirel *et al.,* 2004). Their worldwide distribution now includes countries in Europe, in the Southern and Eastern part of the Mediterranean Sea, and Africa (Benouda *et al.,* 2010; Cuzon *et al.,* 2011; Poirel *et al.,* 2011a).

Table 1: Molecular distribution of carbapenemase genes across the globe

Carbapenemases Group	Example	Description	Effect	Country
Class A	Sme, IMI-1, SFC-1, NmcA, KPC, IMI-2, GES derivatives	Plasmid and Chromosomally encoded.	All effectively hydrolyze carbapenems and are partially inhibited by clavulanic acid	KPC was first isolated in the Eastern United States but is now widely distributed in Puerto Rico, Colombia, Greece, Israel and China. European countries and in South America (Poirel *et al.*, 2011b; WHO, 2017).
Class B Metallo- β-lactamases (MBLs)	Veronica integron–encoded metallo-β-lactamase (VIM), IMP types and, recently isolated New Delhi metallo-β-lactamase-1 (NDM-1)	Plasmid-mediated.	These enzymes hydrolyze all β-lactams except aztreonam (Mouton *et al*., 2000). They are inhibited by EDTA because of its ability to bind zinc that is found in their active site	IMP-type in Greece, Taiwan, and Japan. NDM-1 in India, United States and Canada (Mouton *et al*., 2000; WHO, 2014).
Class D	OXA-48	Plasmid and chromosomally-encoded	Large amount of variability in amino acid sequences and are poorly or not inhibited by clavulanic acid and EDTA.	Isolated in Turkey. It has spread to countries in Europe Southern and Eastern parts of the Mediterranean Sea, and Africa (Cuzon *et al.*, 2011; Nordmann *et al*., 2011a).

2.5 Phenotypic Prevalence of ESBL, AmpC and Carbapenemase in Enterobacteriaceae

The increased number of MDR strains of the members of *Enterobacteriaceae* producing the ESBLs, AmpC enzymes and carbapenemase has limited therapeutic options, therefore, creating difficulty in the treatment of infections caused by these bacteria clinically. ESBLs are widespread all over the globe in which Africa is not left out, but the prevalence of the phenotypic traits among the clinical isolates may vary from one geographical location to the others (Navon-Venezia *et al.*, 2003; Winokur *et al.*, 2001). In one of the studies carried out in India, it was observed that the occurrence of ESBLs in *E. coli* isolates was 14.3% while that of *K. pneumoniae* was 24.5% (Shahid *et al.*, 2008). Similar findings have earlier been reported by Ghatole *et al*., (2004) and Navon-Venezia *et al.*, (2003). Consequently, the prevalence of AmpC enzymes was reported as 9.9% in *E. coli* and 31.1% in *K. pneumoniae* isolates in the same study carried out in India. The prevalence of AmpC enzymes as found by Shahid *et al.*, (2008) was observed to be higher than the reports from some other countries (Shahid *et al.*, 2008).

Some hospitals have reported various phenotypic prevalence of ESBL, AmpC and MBLs and carbapenemase production in many states of Nigeria. Aibinu *et al*., (2007) and Enwuru *et al*., (2011) have reported the incidence of ESBL and MBLs phenotypically in some members of *Enterobacteriaceae* isolated from clinical samples in Lagos, Southwest Nigeria. Also, phenotypic reports of ESBLs, AmpC, carbapenemase and MBLs have been reported by Yusuf *et al*., (2015). The phenotypic occurrence of ESBLs AmpC, carbapenemase and MBLs in *E. coli*, *P. aeruginosa*, *Proteus spp.* and *Klebsiella pneumonia* has been majorly reported globally. Despite the widespread of antibiotic resistance genes among the members of *Enterobacteriaceae*,, there are scarce or limited data on the prevalence of resistance genes especially ESBLs, Amp C, MBLs and carbapenemase genes in Nigeria.

2.6 Distribution of Carbapenem - Resistant Bacteria

In most sub-Saharan Africa, there are little data on the prevalence and distribution of carbapenem resistance bacteria among Enterobacteriaceae. In East Africa, a few studies have been done in Kenya and Tanzania. A surveillance study in Kenya reported the isolation of NDM-1 producing *Klebsiella pneumoniae* from urine samples (Nordmann *et al.*, 2011b) while in Tanzania; a study reported a prevalence of 35.24% carbapenemase genes among multi-drug resistant Gram negative bacteria based on the PCR assays (Mushi *et al.*, 2014). Also, isolation of carbapenemase-producing bacteria among ESBL isolates was reported in South Africa (Coetzee and Brink, 2011; Brink *et al.*, 2012).

It has been reported that in Uganda, CR genetic determinants in non-glucose fermenting bacteria reported at Mulago hospital were IMP-like (36%), VIM-like (32%), SPM-like (16%), NDM-1-like (4%) for *P. aeruginosa* and OXA-23- like (60%), OXA-24-like (7%), OXA-58-like (13%), and VIM-like (13%) for *A. baumannii* (Okoche *et al.*, 2015). Carbapenemase genes in CRE at Mulago and Mbarara hospitals were also documented (Okoche *et al.*, 2015; Ampaire *et al.*, 2014). At Mulago, the genes characterized included; VIM (10.7%), followed by OXA-48 (9.7%), IMP (6.1%), KPC (5.1%) and NDM-1 (2.6%). The highest number of genes appeared in *Klebsiellapneumoniae* (52.2%), followed by *E. coli* (28.4%), *Enterobacter* spp (7.5%), *Serratia marcescens* (4.5%), *Proteus mirabilis* (3.0%), *Citrobacter freundii*, *Klebsiella oxytoca*, and *Pantoea agglomerans* (Ssekatawa *et al*., 2018), table 2.

Tanzania Molecular analysis of CRE at a tertiary hospital in Mwanza established by multiplex PCR revealed that the principal CR genes were IMP (21.6%), followed by VIM (12.3%), OXA-48 (4.9%), then KPC (3.5%), and NDM (3.1%). CP *E. coli* had the highest prevalence (14%), followed by *K. pneumoniae* (10.57%), trailed by *P. aeruginosa* (10.13%), then *Klebsiella oxytoca* (1.76%), *A. baumannii* (1.3%), *C. freundii* (0.88%), *Serratia marcescens* (0.88%) and *Salmonella* spp. (0.44%) (Mushi *et al.*, 2014) while CP *P. aeruginosa* harboring VIM CR gene were identified from Muhimbili National Hospital, using PCR (Moyo *et al*., 2014), Table 2.

K. pneumonia, *A. baumannii* and *Pseudomonas aeruginosa* possessing NDM and VIM-2 genes respectively were isolated in Nairobi (Poirel *et al.*, 2011a; Warnes *et al.*, 2012) while Whole Genome sequencing (WGS) was employed to identify NDM-1like CR genes in *K. pneumoniae* isolates at Kilifi County Hospital (Henson*et al.*, 2017), table 2.

Table 2: Occurrence of Carbapenem Resistance genetic determinants in East Africa

S/N	CR genetic determinant	Genomic occurrence	Bacteria	Country	Reference
1.	blaIMP, blaVIM, blaSPM and blaNDM	36% 32% 16% 4%	*P. aeruginosa*	Mulago hospital , Uganda	Okoche *et al.*, 2015;
2.	blaOXA-23, blaOXA-24, blaOXA-58, and blaVIM	60% 7% 13% 13%	*A. baumannii*	Mulago hospital , Uganda	Okoche *et al.*, 2015;
3.	blaVIM, blaOXA-48 blaIMP, blaKPC and blaNDM-1.	10.7% 9.7% 6.1% 5.1% 2.6%	*Klebsiellapneumoniae*, *E. coli*, *Enterobacter* spp, *Serratia marcescens*, *Proteus mirabilis*, *Citrobacter freundii*, *Klebsiella oxytoca*, and *Pantoea agglomerans*	Mulago and Mbarara hospitals	Okoche *et al.*, 2015; Ampaire *et al.*, 2014
4	IMP, VIM, OXA-48, KPC, and NDM.	21.6% 12.3% 4.9% 3.5% 3.1%	*K. pneumoniae*, *P. aeruginosa Klebsiella oxytoca A. baumannii C. freundii*, *Serratia marcescens* and *Salmonella* spp	Tertiary hospital in Mwanza, Muhimbili National Hospital, Tanzania	Mushi *et al.*, 2014
5	NDM and VIM-2 genes NDM-1	Nil Nil Nil	*K. pneumonia*, *A. baumannii* and *Pseudomonas aeruginosa*	Nairobi Kilifi County Hospital, Kenya.	Warnes *et al.*, 2012; Poirel *et al.*, 2011a, Henson *et al.*, 2017

2.7 Mechanism of resistance

Unfortunately, resistance has emerged in many bacteria treated with carbapenems. The most common mechanisms of resistance are the acquisition of carbapenem hydrolyzing β-lactamases of Ambler class D enzymes (oxacillinases) (CDO) (Poirel & Nordmann, 2002), β -lactamases belonging to class B (metallo-enzymes) (MBLs) (Walsh *et al*., 2005) and a few class A β -lactamases such as the KPC enzymes in *Klebsiella* species. Often these β -lactamases do not act alone and are often accompanied by mutations in genes encoding penicillin-binding proteins and alteration in outer-membrane permeability; for example, the loss of porins CarO and Omp33–36 in *A. baumannii* (Gehrlein *et al*., 1991).

In *A. baumannii*, the spread of carbapenem resistance largely results from the clonal dissemination of a resistant strain where a crucial combination of a mobile carbapenem resistance gene (often encoding the class D b-lactamases OXA-23 or OXA-58) has entered a congenial host (Brown & Amyes, 2006). The spread of these resistant bacteria is due as much to cross-infection as to antibiotic usage. These genes have migrated to the congenial host because they are closely linked to insertion sequences, which have promoted their mobility (Poirel & Nordmann, 2006; Turton *et al*., 2006). A further complication is that all *A. baumannii* possess an inherent class D b-lactamase, collectively known as OXA- 51-like, which can provide weak hydrolytic activity on the carbapenems, though currently only rarely produces clinical resistance (Amyes, 2011).

2.8 Detection of Carbapenemase Producers

The detection of carbapenemase producers in clinical infections is based first on susceptibility assay using disk diffusion or by automated systems (Nordmann *et al.*, 2011a). The standard of the European Committee on Antimicrobial Susceptibility Testing has been lowered compared to the standard of 2008 (EUCAST, 2018; Nordmann *et al.*, 2011a).The reduction in the breakpoints of carbapenems has been further lowered substantially in 2018 for better detection of carbapenem resistant isolates and carbapenemase producers. However, susceptibility standards according to CLSI are now lower than those of the European guidelines (Table 3). Therefore, emphasis should be placed on the CLSI standards to make treatment decisions. There should be special tests for carbapenemase detection to monitor the efficacy of the antibiotic (Carbapenem) and the epidemiology of carbapenemase producers.

Table 3. Breakpoint values for the susceptibility to carbapenems according to guidelines in Europe (EUCAST) and the United States (CLSI), (EUCAST, 2018; CLSI, 2018)

		EUCAST		CLSI		
		Inhibition Zone Diameter (mm)				
Carbapenem	Disk Content µg	Target	Range	S	R	Disk Content µg
Doripenem	10	34	31 - 37	≥ 23	≤ 19	10
Ertapenem	10	31	28 - 34	≥ 22	≤ 18	10
Imipenem	10	27	24 - 30	≥ 23	≤ 19	10
Meropenem	10	34	30 - 38	≥ 23	≤ 19	10

*EUCAST, European Committee on Antimicrobial Susceptibility Testing (www.eucast.org/clinicalbreakpoints); CLSI, Clinical and Laboratory Standards Institute; S, sensitive; R, resistant.

There has been an effort on identifying phenotypically a carbapenemase activity however some of the tests. The modified Hodge test based on *in vivo* production of carbapenemase has been suggested for detecting carbapenemase producers (Miriagou *et al.*, 2010). However, this test is time consuming and may sometimes lack specificity (especially AmpC producers) and sensitivity (weak detection of NDM producers) (Castanheira *et al.*, 2011). Although, this test is useful for detecting KPC and OXA-48 producers. Worthy of note is the Boronic acid–based inhibition testing has been reported being specific for KPC detection in *K. pneumonia* when performed with imipenem or meropenem however not with ertapenem once the isolate in question produces a plasmid - mediated AmpC β-lactamase (Miriagou *et al.*, 2010). The E test MBL strip (bioMérieux, Solna, Sweden) is one of the methods advocated for the detection of MBL producers on the basis of inhibition of MBL activity by EDTA (Walsh *et al.*, 2005). The E test MBL, using imipenem and imipenem/EDTA, is efficient for the detection of MBL producers with high resistance (Walsh *et al.*, 2005), but maybe deficient for detecting MBL producers with a low resistance to imipenem. Although there is an inhibition test for AmpC ß-Lactamase, ESBL resistance, Glycopeptide Resistance Detection (GRD) and Metallo ß-Lactamase. It is vital to mention that there is no inhibition test for detection of OXA-48/OXA-181 producers which are the class D carbapenemases.

The use of spectrophotometric assay for the detection of carbapenemase activity is time-consuming and requires specific training. The standard for the identification of carbapenemases is based on the use of molecular techniques, mostly PCR (Nordmann *et al.*, 2011a). Molecular detection of carbapenemase genes on bacterial colonies may give results within 4–6 hours with a high level of sensitivity and specificity. Other molecular techniques such as the Check-Points DNA technology and sequencing of PCR products may be very useful if employ in the detection of the mechanisms of carbapenem and other antibiotic resistance. However, the main disadvantages of molecular techniques for the detection of carbapenemases are their cost, personnel training and other contingency. Therefore, there is an urgent need for an inexpensive, rapid, sensitive, and specific test for the detection of carbapenemase activity.

III. CONCLUSION

The prevention of the spread of carbapenemase producers relies on the early detection of carriers. Early identification of carbapenemase producers in clinical infections will be vital especially to forestall and prevent the development of those hospital-based outbreaks. The low frequency of the discovery of novel antibacterial drugs is an indication that there is a need to conserve the efficacy of existing antibacterial drugs in clinical practice as much as possible. Worthy of note is that carbapenemase producers in *Enterobacteriaceae* are different from other multidrug-resistant bacteria because of their susceptibility to only a few antibacterial drugs and high level of spreading the resistance genes. Also, there are no vaccines for the prevention of infections associated with carbapenemase-producing *enterobaceriaceae*. Therefore, significant attention must be placed on the associated infections so that such infections will not become life - threatening due to the lack of any effective treatment.

Some of the infections caused by carbapenemase-producing pathogens being an enzyme that hydrolyze all β-lactam antibiotics such as penicillins, cephalosporins, monobactams, and carbapenems. This condition, therefore, renders such infections difficult to treat and thereby responsible for the high mortality rates. The descriptions of these enzymes as species-specific chromosomal carbapenemases have been recently followed by the appearance of carbapenemase genes that are easily transferred on mobile elements among species. While the carbapenemase - associated infection is being considered relatively rare, reports of their occurrence in outbreak settings have steadily increased both in hospital and community - acquired. It will be vital to evaluate the effective antibiotic of choice before prescription in clinical practice. Also, as an intervention, there should be more awareness on the right use of antibiotics and rigorous infection control measures both in the hospital and community just as it is being employed in the control of epidemic diseases like virus-cause disease.

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