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Review article

An African perspective on the prevalence, fate and effects of carbapenem resistance genes in hospital effluents and wastewater treatment plant (WWTP) final effluents: A critical review

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ABSTRACT

This article provides an overview of the antibiotic era and discovery of earliest antibiotics until the present day state of affairs, coupled with the emergence of carbapenem-resistant bacteria. The ways of response to challenges of antibiotic resistance (AR) such as the development of novel strategies in the search of new antibiotics, designing more effective preventive measures as well as the ecology of AR have been discussed. The applications of plant extract and chemical compounds like nanomaterials which are based on recent developments in the field of antimicrobials, antimicrobial resistance (AMR), and chemotherapy were briefly discussed. The agencies responsible for environmental protection have a role to play in dealing with the climate crisis which poses an existential threat to the planet, and contributes to ecological support towards pathogenic microorganisms. The environment serves as a reservoir and also a vehicle for transmission of antimicrobial resistance genes hence, as dominant inhabitants we have to gain a competitive advantage in the battle against AMR.

1. Introduction

The major worry for the release of antimicrobials into the aquatic milieu is associated with the development of antimicrobial resistance genes (ARGs) (Fletcher, 2015). The spread of antimicrobial resistance genes (ARGs) is mainly detected with the help of antibiotic susceptibility testing which can be constantly reassessed (Duriez et al., 2016). For instance, a common mechanism of antibiotic resistance (AR) is carbapenemase production in some members of the Enterobacteriaceae group. The acquisition of carbapenem-resistance genes (CRGs) by Enterobacteriaceae is of global interest and public health importance (Sara et al., 2017) but it is unfortunate that the African continent lags behind in the developments of new antibiotics to tackle this unrelenting menace. Whole-genome sequencing (WGS) can play a significant role in rapid and accurate differentiation of the existing and emerging CRGs, which will be essential for surveillance and control of their spread with its source mainly from hospital wastewater effluents (Tang et al., 2017).

Wastewater treatment plants (WWTPs) receive sewage from various sources, including hospitals, which are important sources of antibiotics and antibiotic resistant bacteria (ARB) (Devarajan et al., 2016) and this menace is not unknown to the Sub-Saharan aquatic environments (Laffite et al., 2016). The release of antimicrobials into the natural environments select for ARB and in many cases may be associated with plasmids that contain one or more ARGs (Wellington et al., 2013). Plasmids are mobile genetic elements which are involved in transmitting ARGs between different bacterial species. These plasmids may also transfer virulence genes (VGs) from pathogenic bacterial strains to others, rendering non-pathogenic ones with virulence capabilities (Leimbach et al., 2013). High amounts of sub-lethal doses of most antibiotics administered to humans and animals are defecated as active substances, inevitably reaching WWTPs via hospital wastewater (Jelic et al., 2012; Vieno and Sillanpää, 2014). Alternatively, during therapeutic treatment procedures, the human intestinal microbiota is exposed to high concentrations of antimicrobials that can stimulate the generation of resistance phenotypes before its discharged into sewage through human excreta (Servais and Passerat, 2009). Globally, several findings on the presence of antibiotics, as well as the detection of ARB and ARGs in WWTPs final effluents samples have been reported including the study of Schwermer et al.

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(2018). A recent study by Barancheshme and Munir (2018) suggested some strategies to combat antimicrobial resistance (AMR) in WWTPs such as equipment upgrades with new developments, and they also pointed out that urban wastewaters may not ideally undergo appropriate treatment procedures due to broken down facilities in developing countries. Hence, the receiving environments are negatively impacted by wastewater discharges and this poses a significant public health risk.

1.1. Black box as a predominant source of ARGs and its negative impact on receiving freshwater ecosystem in Sub-Saharan Africa

Blackbox (any habitat that consist of WWTP final effluent) is a typical example of a reservoir for clinically relevant carbapenem-resistance Enterobacteriaceae (CRE). Carbapenemases of clinical significance are those enzymes with corresponding ARGs harboured on specific plasmids consequently favouring their transfer and spread among various bacterial strains (Gama et al., 2018). Subsequently, the carbapenem-resistance genes (CRGs) can easily be transferred via plasmids into other species, thus increasing the prospects of possible dissemination of new or emerging ARGs (Carattoli et al., 2018). WWTPs are regarded as hotspots for the procurement and spread of ARB into aquatic environments and according to several studies there are three main reasons set forth to sustain this idea: i) the chronic release of antibiotic residues, ARB, and ARGs collected in the community and hospital sewer systems; ii) the environmental conditions that are favourable for selection and/or transfer of ARGs among various bacterial species in WWTPs; and iii) the extensive observation that WWTP final effluents encompass high concentrations of diverse ARGs conferring AR against widely used antimicrobials and drugs of last resort (Lorenzo et al., 2018; Proia et al., 2018; Yee et al., 2019).

Receiving aquatic ecosystems (especially municipal rivers) may well play an imperative part in motivating the importunity and spread of ARB and ARGs as well as mobile genetic elements (MGEs) within bacterial communities. Szekeres et al. (2017) reported high levels of ARGs in river water samples hence, rivers offer a situation in which the horizontal exchange of MGEs encoding AR can occur between bacterial strains. Accordingly, WWTP final effluents are considered to be among the most significant conduits for the spread of ARGs into the environs. Several studies have investigated the occurrence of antimicrobials from wastewater (WW) treatment (Gothwal and Shashidhar, 2015; Margot et al., 2015), whereas countless other studies including the studies of Rizzo et al. (2013); Czekalski et al. (2014); Verlicchi et al. (2015); Ben et al. (2017) have focused on responses of ARGs to WW treatment and their impacts on the receiving surface waters. Likewise, some other studies have also analysed the incidence of ARB in final effluent samples of WWTPs using metagenomics analyses (Karkman et al., 2018; Chu et al., 2018). In spite of the significant number of studies carried out combining the investigation of antimicrobials and ARGs (Tong et al., 2019) along the WW treatment process (Lupan et al., 2017), comprehensive researches evaluating the fate of antibiotics, ARB, ARGs in WWTPs and their subsequent impacts on receiving water bodies are still deficient in South Africa. The continent Africa has its own share of the stigma with ARGs detected in different bacterial species. Bakour et al. (2015) reported the occurrence of bla_{NDM-1} and bla_{KPC} genes in other bacterial species like *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

Clinically, the most relevant carbapenemases are *Klebsiella pneumonia* carbapenemases (KPC), New Delhi metallo-β-lactamase-1 (NDM-1), and Oxacillinase-type carbapenemases (for instance, OXA-48-like) enzymes, whose genetic factors have been progressively reported globally in *Enterobacter* isolates recovered from different environmental samples such as rivers (Singh-Moodley and Perovic, 2016). A study by Proia et al. (2018) provides evidence of the occurrence of CRGs in samples from health-care centres and WWTPs and their further dissemination in receiving surface water. Concomitantly, a study in Tunisia carried out by Ben et al. (2017) reported the incidence of bla_{KPC} and $bla_{OXA\text{-}48\text{-}like}$ genes in *Escherichia coli* and *Klebsiella pneumoniae* isolates respectively. Unfortunately, many African countries employ the use of conventional WWT processes which inefficiently eliminate ARB (Di Cesare et al., 2016; Rafraf et al., 2016; Yang et al., 2016); it is paramount to evaluate the extent to which hospital wastewater effluents contribute to the distribution of ARGs, specifically those conferring resistance against carbapenems.

For several years, most developing countries make use of the conventional WWTPs and neighbouring water-bodies serve as receiving watersheds directly from hospitals and manufacturing companies (Shutes, 2001; Zhang et al., 2008). Many hospitals release effluents directly into water-bodies and even when effluents pass via WWTPs, they are poorly treated hence, this increases the likelihood of detecting ARGs. This unhygienic practice has resulted in the prevalence and dissemination of multidrug resistant (MDR) bacterial strains and this is of global concern to public health risk. Studies have confirmed that hospital WW effluents are major sources of ARGs, notwithstanding, conventional WWTPs do not have the capability to eliminate ARB. The dissemination of ARGs becomes even more perturbing because ARB strains are not constrained to the hospital environment but they are also found in other environmental niches (Dziri et al., 2018).

The acquisition rates of ESBL-Producing Enterobacteriaceae (ESBL-PE) as reported by Lääveri et al. (2018) proves the presence of another relevant ARGs detected in some sub-regions in Africa. Several investigations that focused on the prevalence of CRE have been carried out in some African countries including studies by Benie et al. (2017); Devarajan et al. (2017); Igbinosa et al. (2014), (2017); Isichei-Ukah and Enabulele (2018); Karkman et al. (2018); Lyimo et al. (2016); Moremi et al. (2016) and Tafoukt et al. (2017) as seen in Figure 1. However, there is still a lack of information that evaluates CRE from different environmental samples in other regions of Africa thus, more studies in this area should be encouraged.

1.2. Global epidemiology of carbapenem-resistant Enterobacteriaceae infections

The first report on CRE was in 1993 (Nordmann et al., 1993), a study that reported carbapenem resistance in *E. coli* and *Enterobacter cloacae* strains isolated from hospital samples in Paris, France. *Enterobacteria harbouring* carbapenem-resistance genes (CRG) have been increasingly reported for more than 2 decades now. *Klebsiella pneumoniae* carbapenemases also known as KPC have been reported in the United States of America as well as in Greece (Cuzon et al., 2008; Poirel et al., 2010). Studies on metalloenzymes such as Verona integron–encoded metallo-β-lactamase (VIM), Imipenemase (IMP) have been reported globally, with an increased incidence in Europe and also in Asia (Nordmann et al., 2011). Carbapenemase-producing genes of the oxacillinase-48-like type were previously reported in India, Mediterranean and some parts of Europe (Bakthavatchalam et al., 2016; Sharma et al., 2016). ARGs specifically bla_{NDM-1} has also been reported in the United Kingdom, Pakistan and India (Perry et al., 2011; Walsh and Toleman, 2011; Dimou et al., 2012) and are presently one of the most important carbapenemase of worldwide concern (Patel and Bonomo, 2013; Wang et al., 2015).

A study in South Africa carried out by Lowman et al. (2011) was the first reported case of bla_{NDM-1} gene detected in Enterobacteria isolated from clinical samples taken from patients that arrived from India. The gene bla_{KPC} was originally identified in bacterial strains recovered in USA in 1996 (Apisarnthanarak et al., 2013). Since then, these ARGs have been able to transfer genetic components and have demonstrated the tendency to spread rapidly among different bacterial species (Lawrence and Roth, 1996; Yong et al., 2009; Cuzon et al., 2008). Carbapenems remain the reliable last line of defence however, AMR is a major concern to researchers in view to uncovering ways to combat this public health challenge. Generally, resistance against antimicrobial agents is like a recurring nuisance to the health of humans and this has subsequently led to consistent research in this field. Due to indiscriminate use of antibiotics, the spread of AR has become a subject matter of trepidation with hospitals and WWTPs playing vital roles worldwide. The rapid emergence of CRE is presently a worry because the

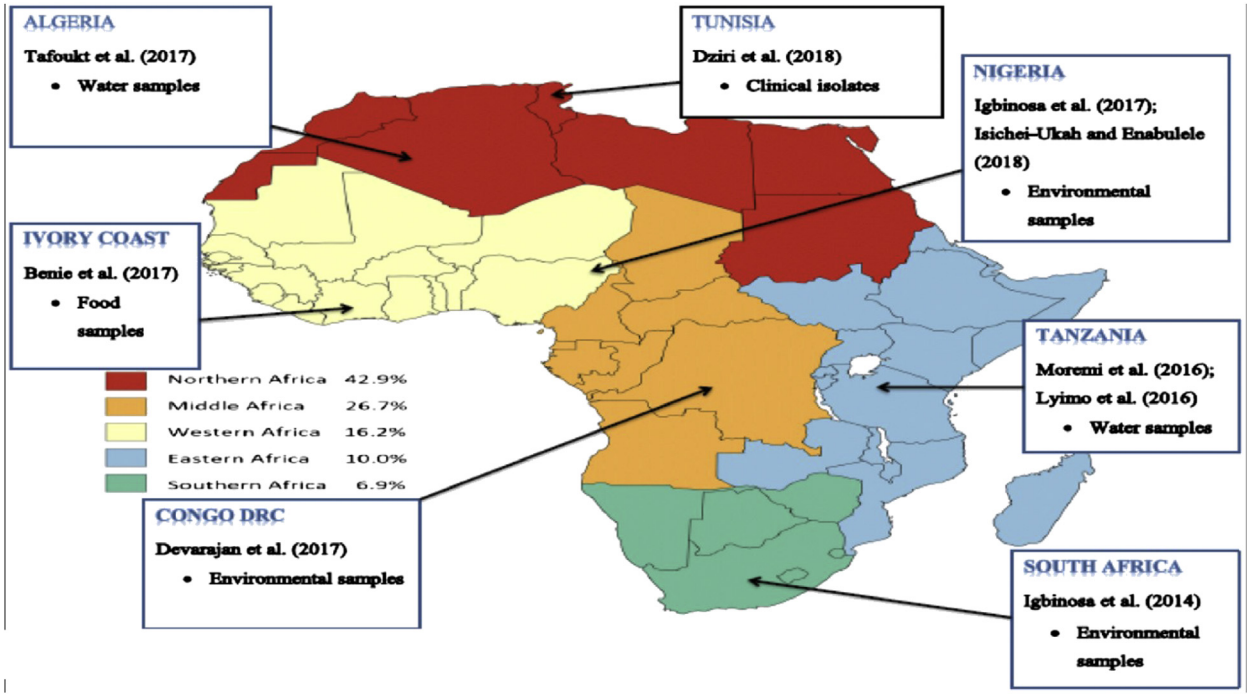

Figure 1. (a) ESBL-PE acquisition rates in five African sub-regions; joint data on 396 travellers from Finland and the Netherlands; Source: Lääveri et al. (2018) (b) prevalence of CRE in some African countries from different samples (2014–2018).

comparatively high minimum inhibition concentration (MIC) of carbapenems in these pathogenic bacteria along with exhibiting multiple ARGs make them difficult to eliminate (Seiffert et al., 2014). Moreover, some pathogenic strains of bacteria can acquire a silent gene, while exhibiting phenotypic resistance they are observed to lack ARGs (Abbassi et al., 2008).

A recent study by Mahon et al. (2017) in Ireland reported NDM-1-producing Enterobacteriaceae recovered from beach water samples. This study pointed out that it appears that there is a potential for CRE to exist in harsh environments thereby contributing to a transition of CRE from largely health care-associated to organisms affecting the general population and also the veterinary sector. Several reports on the predominance of Enterobacteriaceae isolates harbouring KPC-producing genes and other key CRGs have been on the rise in every continent even in Africa. Figure 2 represents a world map showing the global expansion of KPC over the years.

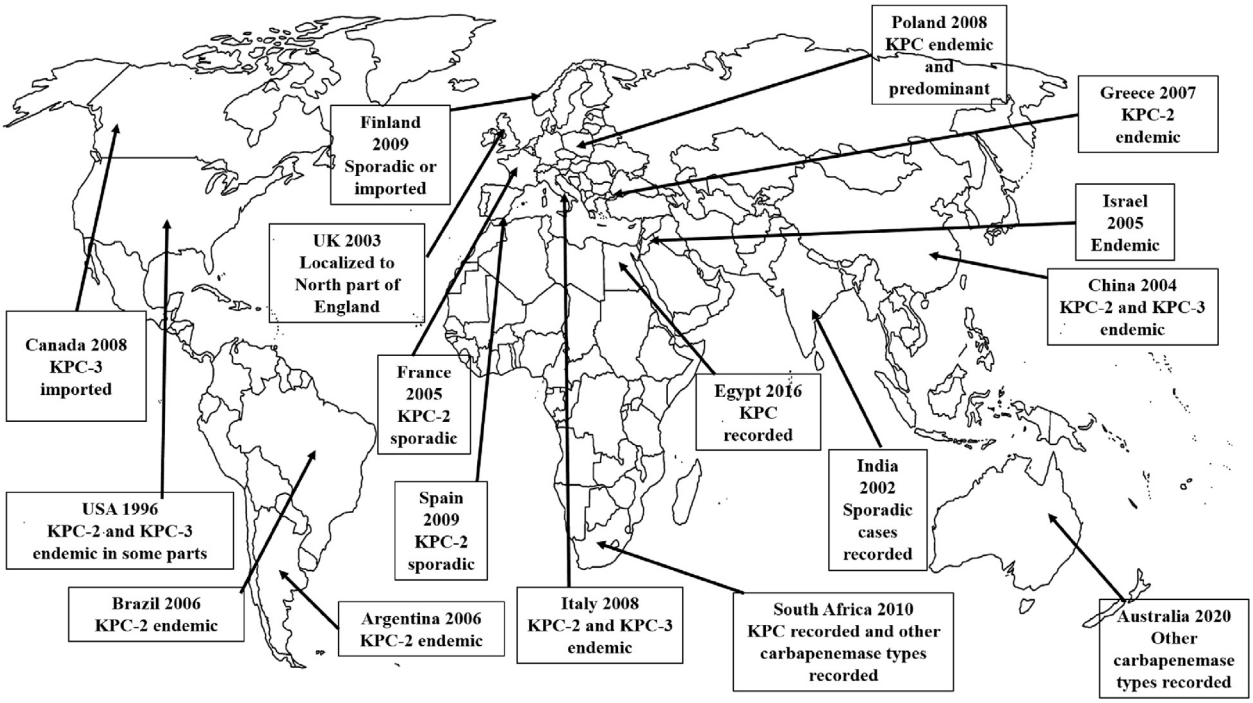

Figure 2. Clinical epidemiology of the universal expansion of *Klebsiella pneumoniae* carbapenemases (KPC).

1.3. Diversity of antimicrobial resistant bacteria in major hotspots and the removal efficiency

There is an abundance of carbon sources and other important nutrients which are favourable to the diversity of bacteria in WWTP final effluent (Mielczarek et al., 2013). The sewage microbiome is made up of environmental bacterial species (human and animal sources) with several of them harbouring ARGs (Li et al., 2018). Although there are various contributing factors that influence this microbiome (i.e. microbes in a particular environment including the human body), the consequence of unambiguous prospective discerning pressures like antimicrobial residues or metals, are apparently considered determinants that order the fate of ARB and ARGs during WWT processes (Manaia et al., 2018). Microbial pathogens cause infections (such as gonorrhoea) which remain virtually untreatable because of an increase in the strains harbouring emerging ARGs (Penchovsky and Traykovska, 2015). Coexistence of pathogenic microorganisms, antimicrobials and ARGs within WWTPs are responsible for the conducive conditions that enhance emerging ARB and facilitate horizontal genomic transfer amongst other species and it has been established that WWTP also serves as a hotspot for the transmission of ARB into the environment (Devarajan et al., 2015; Miller et al., 2016: Nnadozie et al., 2017). Advanced facilities of some WWTPs are effective in getting rid of pathogenic microbes and antibiotic residues largely during secondary and tertiary treatment processes (Uribe et al., 2015).

There have been reports of increased variations among ARB species in WWTP final effluents (Aali et al., 2014). Extended sludge retention time (SRT) along with hydraulic retention time (HRT) all through secondary treatment processes ease the removal of antibiotics by adsorption and biodegradation (Polesel et al., 2016). Nevertheless, those conditions previously mentioned can lead to the development of antimicrobial resistance (AMR) processes in microorganisms. Hence, there is lack of ideal conditions for the operation of conventional WWTPs for the effective elimination of antibiotics in developing countries (Michael-Kordatou et al., 2018). The removal of antibiotic deposits can be accelerated by means of coalescing conventional activated sludge (CAS) processes with a supplementary treatment technology that involves dosing with ozone treatment techniques (Helbling et al., 2012; Gerrity et al., 2013). The unconventional biological treatment system using membrane bioreactors (MBR) combined with coagulation seemingly possess the best removal efficiency (RE) of ARGs, and confiscate equally ARB and extracellular ARGs. Although some studies have predicted the fate of ARGs in WWTPs, the mechanisms of ARGs acquisition are still inconclusively established (Karam et al., 2016). RE represents the percentage of the number of molecules of a compound removed or destroyed (ARGs in this case). According to the report of Rafraf et al. (2016), the *RE* of WWTPs ranges from 45.5 to 100%. A report by Amábile-Cuevas (2016) stated that 90% of wastewater in US and Canada is treated while 66% of wastewater in Europe is treated. Additionally, 35% of wastewater in Asia is treated and 14% in Latin America but in Africa, less than 1% of wastewater is treated.

The spread of CRGs by CRE from hospital wastewater and WWTP final effluent to other non-pathogenic bacteria in the environment calls for a great deal of urgency to introduce new methods of elimination such as nucleic acid removal (Kardos, 2017). Figure 3 shows the design of a typical WWTP but unfortunately it is incapable of eliminating multidrug pathogens due to the mutations in genetic elements that lead to alterations in the target sites of antimicrobials. Out of all the stages involved in the WW treatment processes, both the final effluent and the activated sludge impact the environment negatively via farm practices as they are used for irrigation purposes and fertilizer respectively.

2. Enterobacteriaceae taxonomic history

The Family Enterobacteriaceae belongs to the Phylum: Proteobacteria > Class: Gamma Proteobacteria > Order: Enterobacteriales. Enterobacteriaceae group consists of several bacterial strains that are found in various environmental niches. Enterobacteriaceae members are generally rod-shaped, Gram-negative bacteria that are typical inhabitants of the abdominal flora, amid the most common pathogens to mankind that can cause infections ranging from pneumonia, meningitis, cystitis to pyelonephritis, septicaemia, peritonitis, as well as device-associated infections. They are the main causative agents of both community- and hospital-acquired infections (HAI), with *E. coli* being the most significant pathogen to man (Dramowski et al., 2016). For several years, Enterobacteriaceae members have succeeded in spreading easily among humans either by hand carriage or contaminated food/water and possess a proclivity to acquire genetic materials via horizontal gene transfer (HGT), usually facilitated by means of plasmids along with transposons (Xu et al., 2015).

Antimicrobial resistant (AMR) has remained a prolonged challenge to global therapeutic options and this menace has existed and persisted since the 1960's, ever since then AMR has spread swiftly worldwide. AMR bacterial strains are able to withstand attack by antimicrobial agents and this ability threatens the effective prevention of infections (Alanis, 2005). Figure 4 represents examples of the common members of family Enterobacteriaceae.

3. Common members of Enterobacteriaceae

The family Enterobacteriaceae includes many genera and species with members being the most commonly encountered microorganisms

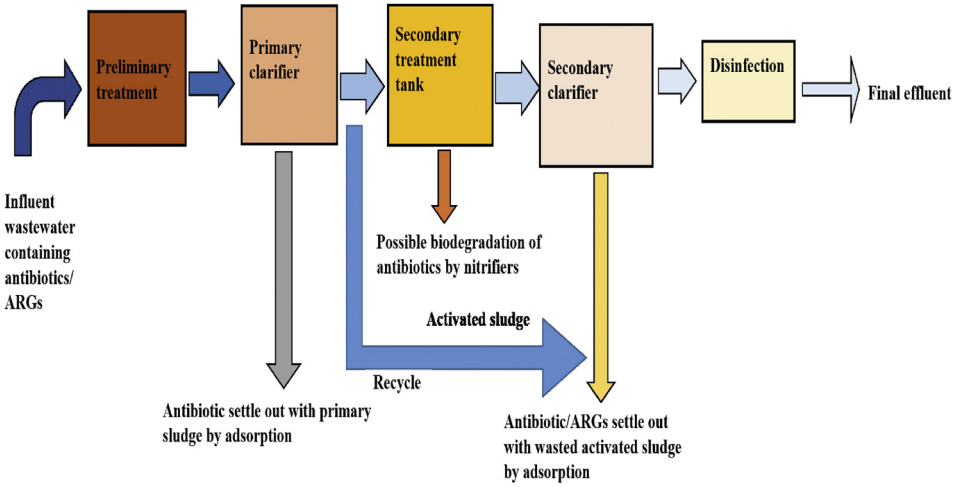

Figure 3. A flowchart of treatment stages involved in a typical conventional wastewater treatment plant.

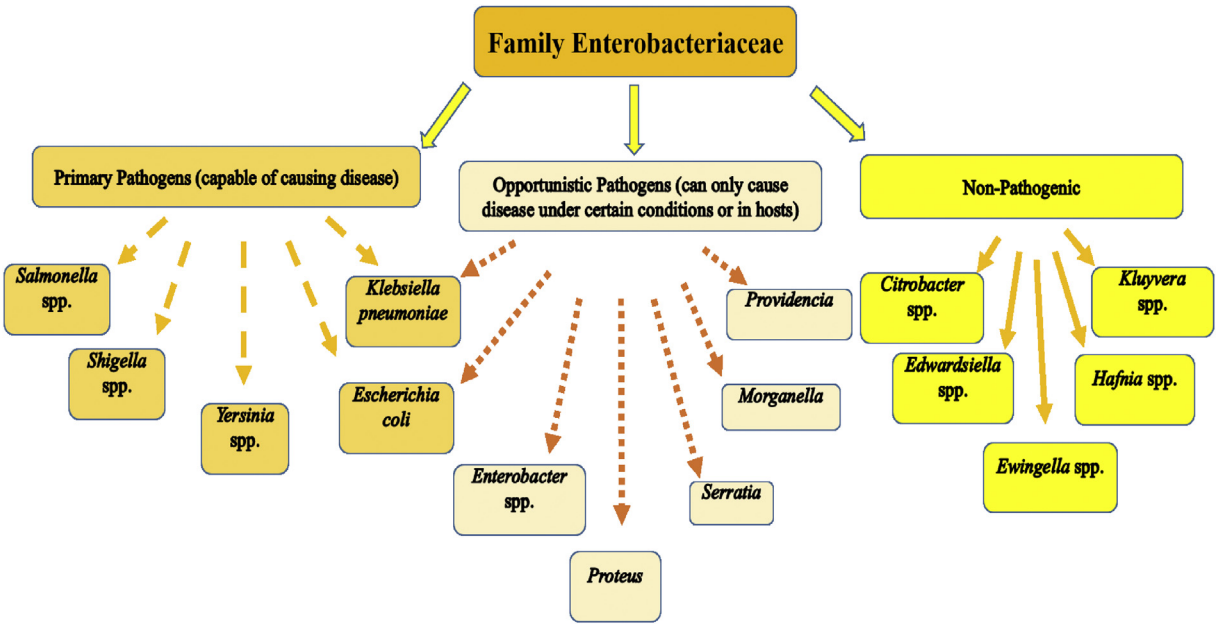

Figure 4. Common members of the Family Enterobacteriaceae.

previously isolated from clinical specimens. There are about 176 named species among 44 different genera however, clinically relevant species comprise primarily of *E. coli*, *K. pneumoniae* and *Proteus mirabilis* (Coudron et al., 2000). Other Enterobacteria include *Enterobacter* species, *Klebsiella* spp., *Morganella* spp., *Proteus* spp., *Providencia* spp., and *Serratia* spp. The obligate pathogens include *Salmonella* species, *Shigella* spp. and *Yersinia* spp (Kocsis and Szabó, 2013). Members of Enterobacteriaceae are distributed worldwide and are naturally found in soil, water, farm crops, fruits, meats, flowering plants and trees.

3.1. *Salmonella spp.*

The genus Samonella are rod-shaped, Gram-negative bacteria which are characterized into two species namely *Salmonella enterica* and *Salmonella bongori*. *S. enterica* is further classified into six subspecies that comprise over 2,500 serotypes. Generally, *Salmonella* spp. reside in the digestive tract of human and gut of warm-blooded animals (Wen et al., 2017). However, less than 100 serotypes (predominantly *S. typhi*, and *S. paratyphi* A and B) are responsible for human infections (Schellack et al., 2018).

Salmonella enterica serovar Typhimurium is regarded as a major food-borne pathogen and can cause Salmonellosis with several reports of the detection of antibiotic resistant (AR) strains. A specific strain of *S. typhi* was reported by Kopecko et al. (2009) to exhibit a silent gene. Some studies reported that some genes were compared and found to be similar to 11 genome sequences of various *Salmonella* serovars (Allard et al., 2013; Alikhan et al., 2018). Typhoid and paratyphoid fever are severe and frequently lethal feverish sicknesses caused by the enteric *Salmonella enterica*, *serotype typhi*i and *paratyphii*. Typhoid remains a contagious ailment that spreads via the faecal–oral route and consists of various ways of occurrence which include: (i) the consumption of food/drinks (if handled by an asymptomatic carrier of the pathogen), (ii) oral transmission (faecal contaminated water), (iii) the ingestion of shellfish (from contaminated water), (iv) poor sanitation practices and (v) the consumption of raw farm produce such as fruits and vegetables (Schellack et al., 2018).

A study carried out in India by Guerra et al. (2014) reported that *Salmonella* spp. harboured carbapenem-resistance gene (CRG) bla_{NDM-1} in clinical samples from faeces and urine specimens recovered from some patients. Nevertheless, studies by Le Hello et al. (2013) and Seiffert et al. (2014) reported the detection of OXA-48-producing *Salmonella enterica* in different regions across the world. Multidrug resistant (MDR) *Salmonella* spp. may silently spread into the environment with the probability of causing illness, and this poses a grave risk to the health of the general public (Guerra et al., 2014).

3.2. *Escherichia coli*

Escherichia was named after Theodore Escherich, a German paediatrician who was the first to define the colon bacillus under the previous name Bacterium coli commune in the year 1885 (Shulman et al., 2007). Escherichia has five common human pathogenic species including *E. coli*, *E. blattae, E. fergusonii*, *E. hermanii* and *E. vulneris*. *Escherichia coli* can naturally be found in the intestines (Sokurenko et al., 1998). Several studies across the world including the studies of An et al. (2002); Edge and Hill (2005); Servais et al. (2009) and Chigor et al. (2010) have reported the occurrence of *E. coli* in surface water samples. Generally, *E. coli* exists as a non-pathogenic microorganism and essentially plays an important role in the gut of a healthy human. However, some pathogenic strains of *E. coli* harbour virulence genes (VGs) thus causing common infections like chronic diarrhoea, gastroenteritis, urinary tract infections (UTI), sepsis and also respiratory illnesses (Sidhu et al., 2013).

Pathogenic strains of *E. coli* comprise a varied group of bacteria consisting of strains which are further categorized into respective pathotypes (Chapman et al., 2006). According to Gomi et al. (2015), *E. coli* consists of intestinal pathogenic *E. coli* strains i.e. InPEC and extra-intestinal pathogenic *E. coli* i.e. ExPEC. Extra-intestinal infections are majorly caused by three *E. coli pathotypes*: (i) uropathogenic *E. coli* (UPEC) strains that cause UTI in humans and other domestic animals (ii) neonatal meningitis *E. coli* (NMEC); and (iii) strains responsible for septicemia in both humans and animals.

Another six *E. coli* pathotypes are related to diarrhoea and are referred to as diarrheagenic *E. coli* (DEC) (Akbar and Anal, 2011) and these comprise the following (i) Shiga toxin-producing *E. coli* (STEC) also known as Verocytotoxin-producing *E. coli* (VTEC) or enterohemorrhagic *E. coli* (EHEC) (ii) Enteroinvasive *E. coli* (EIEC) as well as (iii) diffusely adherent *E. coli* (DAEC) and (iv) Enterotoxigenic *E. coli* (ETEC) along with (v) Enteropathogenic *E. coli* (EPEC) (vi) Enteroaggregative *E. coli* (EAEC).

ETEC is one of the important bacterial agent that causes chronic diarrhoea which can lead to death (Walker et al., 2007). Specific

virulence factors like enterotoxins are responsible for its pathogenicity. Amongst *E. coli* pathotypes, ETEC harbours at least lt1 and lt2 (heat-labile) or sta and stb (heat-stable) toxins and reports have linked it with various infections affecting humans (Suez et al., 2013).

EPEC is another significant pathotype of DECs and has been linked to child diarrhoea. EPEC strain is one of the major DEC that acquires a plasmid called EAF (EPEC adherence factor) which encrypts a type IV pilus known as the bundle-forming pilus (bfp) (Miri et al., 2017) and many types of EPEC have the eae chromosomal genes (Alikhani et al., 2013).

EHEC causes infections such as bloody or non-haemorrhagic diarrhoea and haemolytic uremic syndrome (HUS) (Rivas et al., 2006). A wide variety of food items have been linked with these infections (Akbar and Anal, 2011). The main virulence gene of EHEC is called Shiga toxins (stx genes) also known as Verotoxin (Vtx) and it contains two subdivisions namely stx1 and stx2 (Dänicke et al., 2015). However, EHEC strains of O157:H7 serotypes are the commonest serotypes of this group and Miri et al. (2017) reported that EHEC O157:H7 arose from the locus of enterocyte effacement-containing (LEE-containing) O55 EPEC strains that developed bacteriophage encrypting stx. The majority of these serotypes lacks the LEE pathogenicity island (Nielsen and Andersen, 2003). EHEC causes traveller's diarrhoea in both children and adults in developing and developed countries. This pathologic group is made up of intestinal bacteria that caused several global outbreaks (Thapar and Sanderson, 2004). EAEC is another *E. coli* pathotype which clings to HEp-2 cells and abdominal mucosa by virtue of fimbria structure aggregative adherence fimbriae (*AAf*) encoded by aggR gene which is located in the main virulence plasmids of typical EAEC (Kaper et al., 2004; Miri et al., 2017).

EIEC is the causative agent of invasive inflammatory colitis and dysentery, but usually, it causes watery diarrhoea and can be fatal among immunosuppressed individuals. The ability to invade the epithelium of the colon is dependent on a 220kb plasmid, called pInv, which harbours the genes meant for a type III discharge system that is used as the virulence factor. EIEC strains are very similar to pathogenic Shigella strains (Berglund, 2015). Although many strains of *E. coli* used to be considered as harmless commensals other strains of EIEC harbour ARGs and a study by Gülmez et al. (2008) reported carbapenem-resistance genes (CRGs) in *E. coli*.

3.3. Enterobacter spp.

Pathogenic strains of *Enterobacter* spp. can cause an extensive variety of nosocomial infections which include invasive and also device-associated diseases (Rosenthal, 2008). MDR strains have been reported to be responsible for several outbreaks in clinics and intensive care units (ICU) worldwide. *Enterobacter* spp. can cause community-acquired infections while becoming resistant against antimicrobials because of their colonization within clinical environments. AR against carbapenems impends our preceding effective therapeutic options and this phenomenon constitutes a concern to public health globally (Paterson, 2006).

Enterobacter genus has 13 species although *E. aerogenes* and *E. cloacae* are the two key species. Additionally, both species are significant nosocomial pathogens that can cause various opportunistic infections such as bacteremia, skin infections, soft-tissue infections, UTIs, endocarditis, intra-abdominal infections, lower respiratory tract infections, septic arthritis, osteomyelitis, ophthalmic infections, among many others (Foxman, 2010). There are four other species that can be found in the environment or as plant pathogens but have not been found in human clinical specimens and these include *E. intermedius*, *E. dissolvens*, *E. nimipressuralis* and *E. pyrinus* (Brady et al., 2013). Although reports on carbapenem resistance (CR) are increasingly reported among Enterobacteriaceae members, there are a few reports on ARGs harboured by *Enterobacter* spp. despite being the first member reported to harbour CRGs (Neuwirth et al., 1996; Coudron et al., 1997).

An alternative CR strategy for these pathogens is to combine ESBLs, increased efflux pump, porin modification and a sturdily expressed (derepressed) endogenous AmpC enzyme (Flury et al., 2016). In *E. cloacae*, an extensive variety of carbapenem MICs have been ascribed to these options. However, the loss of porins appeared to be the foremost contributor to CR (Stürenburg et al., 2002).

3.4. Klebsiella spp.

Klebsiella was initially classified into three main species based on their biochemical reactions. Presently seven major species which demonstrated similarities in DNA homology have been identified. The other six species include *K. ornithinolytica, K. oxytoca*, *K. ozaenae*, *K. rhinoscleromatis*, *K. planticola*, and *K. terrigena* (Brisse and Verhoef, 2001). *Klebsiella pneumoniae* is the main species existing as a facultative anaerobic, non-motile bacillus and its natural habitat comprises surface water, soil and plants. *K. pneumoniae* can also live as commensal resident of the mammalian nasopharynx and digestive tract (Flemming and Wingender, 2010).

K. pneumoniae is presently considered as one of the most imperative opportunistic microorganism because it can cause nosocomial and community infections, predominantly amongst immune-compromised people, with amplified rates in health-care settings associated with antibiotic use (Holt et al., 2015). The gastrointestinal tract serves as a reservoir for certain microbes and is often regarded as the concealed source of common infections (Martens and Demain, 2017).

K. pneumoniae can potentially cause respiratory infections, UTI, blood and wound infections. In addition, *K. pneumonia* has been reported to cause diabetes related liver abscess syndrome in some Asian countries (Siu et al., 2012; van Crevel et al., 2017). *Klebsiella* spp. has been reported to be responsible for severe nosocomial infections with few pharmacological therapeutic options which can bring about a high mortality rate (Ackerman et al., 2018). The pathogenicity of *K. pneumoniae* is as a result of several virulence elements that permit it to overwhelm innate host immunity maintaining infections in a mammalian host.

In some developed and developing countries, carbapenem-resistant *Klebsiella pneumonia* (CRKP) has been reported with molecular epidemiology based on multilocus sequence typing (MLST) and this proves that several sequence types (STs) are common in this microorganism (Karampatakis et al., 2016; Alotaibi et al., 2017; uz Zaman et al., 2018). For instance, *K. pneumoniae* ST258 is the most commonly related strain with CR in the US and Greece (Kitchel et al., 2009; Andrade et al., 2014). The ST258 clone has been reported to belong to the MDR clonal complex (CC) 258 of *K. pneumoniae* which is associated with KPC-producing *K. pneumoniae* strains (KPC-Kp).

Interestingly, some studies carried out in Brazil and several Asian countries reported other members of CC258 (especially ST11) to be the major clones in dissemination of bla_{KPC} and bla_{NDM-1} genes (Peirano et al., 2017). A study carried out in Russia also described ST340 as another member of CC258 which can be potentially linked with NDM-1-producing *K. pneumoniae* (Netikul and Kiratisin, 2015). As previously discussed, these AR profiles can as well be attributable to the expression of different enzymes like Extended-spectrum β-lactamases (ESBL) along with the overexpression of efflux schemes. Colistin remains one of the rare antimicrobials still effective against KPC-Kp, but regrettably, evolving data on colistin-resistance have further constrained therapeutic alternatives (Bassetti et al., 2017).

4. Antibiotic resistance in water-food nexus

The epidemiologic report and phenotypic findings of carbapenem-resistant Enterobacteriaceae (CRE) are complex probably because Enterobacteriaceae members may well be unsusceptible (i.e. intermediate or resistant) against carbapenems. Moreover, studies have reported various microbes isolated from the aquatic environment with tendencies of harbouring silent genes (Lehn et al., 1996; Nguyen et al., 2019), these

are inactive genes considered to be part of cryptic genetic systems. Alternatively, studies have shown plasmids carrying the bla_{OXA-48} gene in different enterobacterial strains investigated in different countries but shared very dissimilar features (Falagas et al., 2014). Ribot et al. (2006), Mediavilla et al. (2016), Poirel et al. (2016) and Fernandes et al. (2016) reported the occurrence of some Enterobacteriaceae members that harboured *mcr-1* colistin resistance genes and if not contained may spread to other strains thereby compounding the existing concerns to public health.

In 2014, in a workshop organized by the World Health Organization (WHO), the speakers explored microbial and genomic movements across agriculture, health and environmental compartments. A call was made to generate a real-time worldwide databank of prevailing data sets that included antibiotic resistance (AR), infections, sequencing, climate, wildlife movement, and migrating birds. The databank was assembled, assimilated, and analysed to recognise gaps, encourage novelty, and take collective action against AR. A recommendation was made to frame this work inside a new paradigm—the Collective Antimicrobial Resistance Ecosystem (CARE). The paradigm was established on incessant exposures to numerous forms of resistance determining factors at the interface of humans, animals, and also the environment. Slayton et al. (2015) reported on a similar approach intended for the reduction of AR infections in hospitals which are considered as major hotspots for antibiotic resistant bacteria (ARB).

Every gram of soil sample inside the native resistome (i.e. the collection of all the ARGs and their precursors present in the chromosomes of both pathogenic and non-pathogenic bacteria), is a peculiar collection of all the potential ARB in a microbial milieu and can be driven towards a problematic direction through anthropogenic factors for instance aquaculture, animal husbandry, antibiotic production, WWTPs and general pollution. Horizontal gene exchange (HGE) arises in the commensal relationship that briefly occurs out of this environmental selection leading to the transfer of ARGs and spread of mobile genetic elements (MGE) between strains (Lozupone et al., 2012). Nevertheless, the rudiments involved that remain vital in driving the rapid emergence of ARB are selection, growth conditions and cell contact as well as cell density. Management of these elements is crucial in order to abate HGE and ARB (von Wintersdorff et al., 2016). Ample evidence demonstrates this process of HGE. A study carried out by Li et al. (2015) reported on the abundance of ARGs in the metagenome of diverse environs stating the loads in natural environmental niches, such as soils and river water. Contrariwise, more studies are necessary to analyse hospital wastewater effluents which is the key source of ARB. Figure 5 represents the CARE model showing antimicrobial agents exposure linkage with the human-animal-environment interface.

4.1. Occurrence of carbapenem resistance Enterobacteriaceae (CRE) in different environmental niches

The reuse of wastewater for irrigation purposes in order to improve soil fertility may lead to various public health implications (Castro et al., 2015). River water serves as a reservoir for different bacterial species and pollution may be from direct contact with either wastewater or animal dung (Araújo et al., 2017). Undercooked or raw farm products such as vegetables and fruits eventually serve as reservoirs of pathogenic microorganisms due to a switch in ecological niche. Enterobacteria are the most common causative agents of food poisoning and a host of various infections. However, there is a need for more studies investigating CRE isolates recovered from different environmental niches in South Africa. Certainly, surface waters are exposed to discharges from various sources, in receipt of chemical and microbial contaminants that originate from agricultural, domestic and industrial origins. Pollution of waterbodies has been reported to modulate the antibiotic resistome (Tacão et al., 2012; Tafoukt et al., 2017). Another source of contamination from WWTPs arises from the use of activated sludge as organic fertilizer to treat farm soil and the use of WWTPs final effluents and/or receiving watersheds as irrigation sources for vegetable farms and also drinking for farm animals (Kümmerer, 2004; Vaz-Moreira, 2014). Effluents from WWTPs are made up of diverse wastewater which comprises those from households, pharmaceutical industries and hospitals (Hernández et al., 2011; Birch et al., 2015; Bueno et al., 2016). AR is a major and growing public health concern and surveillance of the rise of this AR phenomenon in the environments is improbably inadequate (Marti et al., 2014). As earlier mentioned, several studies have shown that most WWTPs final effluents consist of MDR bacterial strains and another study of Marti et al. (2013) reported the detection of ARGs and concentration of antibiotics in both WWTP effluent samples and river water samples. Figure 6 is a schematic diagram showing the process involved in the spread of antibiotic resistant bacteria.

5. Mechanisms of carbapenem resistance in Enterobacteriaceae

Bacterial strains can exhibit antibiotic resistance (AR) against carbapenems through several mechanisms and these include production of

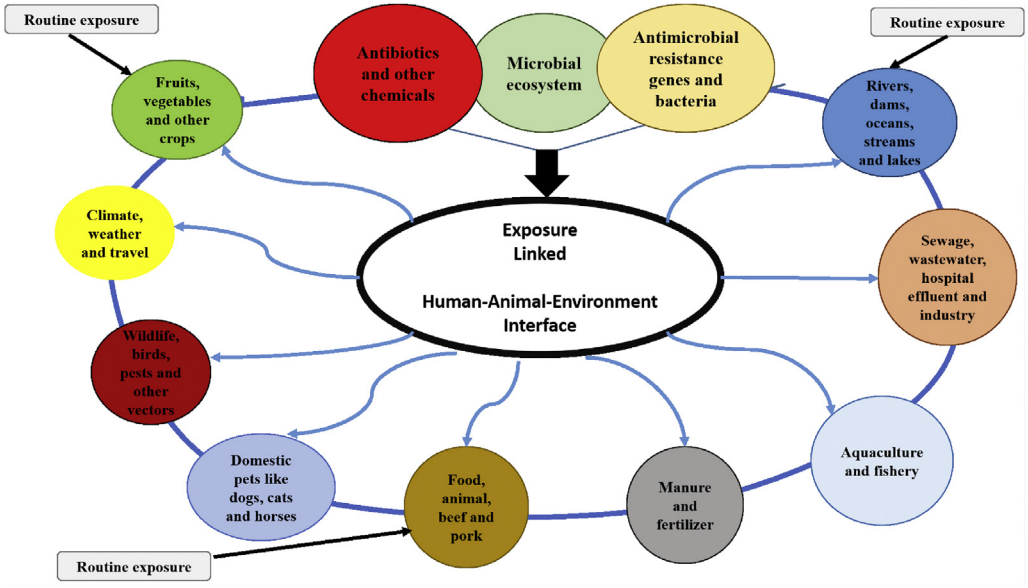

Figure 5. The CARE model (Collective Antimicrobial Resistance Ecosystem).

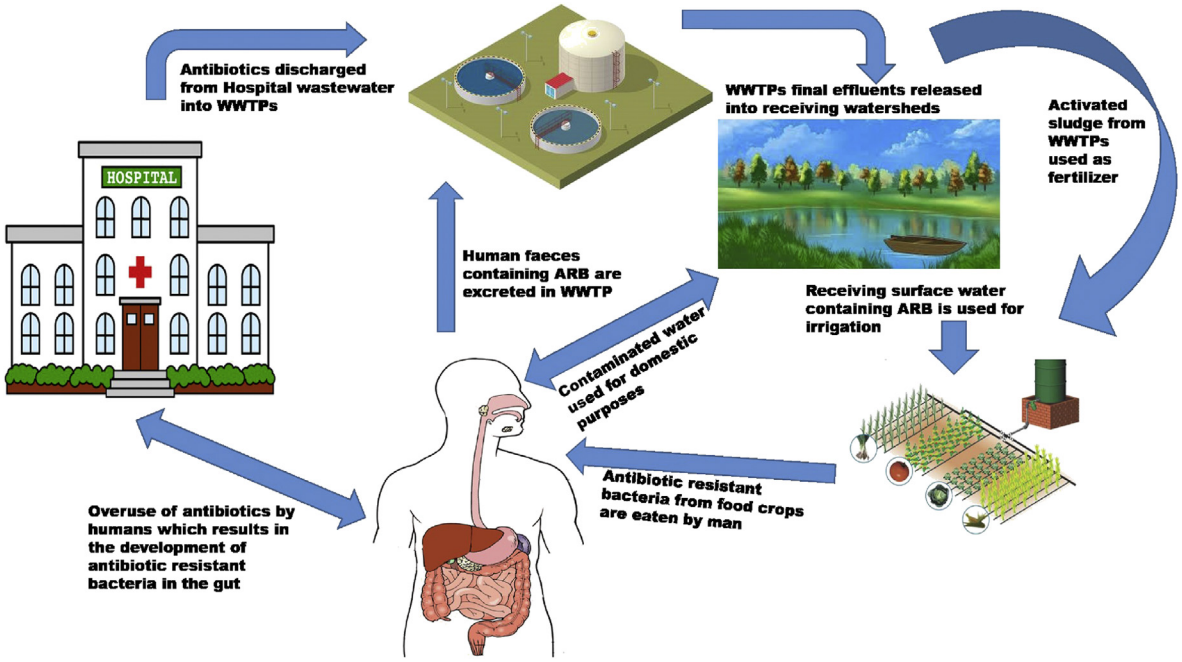

Figure 6. A schematic diagram showing the process involved in the spread of antibiotic resistant bacteria.

β-lactamases (carbapenemases), efflux pumps and mutations that alter the expression and/or function of porins and penicillin-binding proteins (PBPs). Carbapenemase producing (CP) microorganisms are usually only susceptible to polymyxins (e.g. colistin), fosfomycin and variably susceptible to tigecycline, although colistin resistance in CP *Klebsiella pneumoniae* isolates has also been reported (Nordmann et al., 2009). Carbapenemases are able to hydrolyze carbapenems effectively, while other β-lactamases hydrolyze them very slowly (Temkin et al., 2014). Furthermore, carbapenemases hydrolyze a variety of β-lactams, including cephalosporins, penicillins and aztreonam but these enzymes can be inhibited by β-lactamase inhibitors, such as clavulanic acid, tazobactam and sulbactam (Drawz and Bonomo, 2010). Associated with the rise in carbapenem resistance is the deficiency of new antimicrobial agents. This has triggered different initiatives globally to develop novel or more effective antimicrobial compounds and also to develop novel delivery-targeting strategies (Baptista et al., 2018). Among the common mechanisms of resistance (MOR) are target protection, decreased cell permeability, target overproduction, altered target site/enzyme, enzyme inactivation, increased efflux due to over-expression of efflux pumps, amongst others (Baptista et al., 2018). There are limitations in the development of new antibiotics, nevertheless combination therapy has been considered and there are ongoing studies to analyse the effectiveness of polytherapy. Every class of antibiotics has its mode of action and sequentially the target pathogens develop resistance mechanisms thereby conferring AR against the existing antibiotics. Table 1 summarizes different classes of antibiotics, the modes of action and common MOR exhibited by ARB.

Microorganisms usually exhibit bioreduction, which involves accumulation of metallic ions in order to reduce their toxicity. They can either bioreduce intracellularly with the support of various reducing species present inside the cell and on the cell wall, or bioreduce extracellularly by different metabolites (Singh et al., 2018). Some bacterial strains exhibit MOR in the form of other more complex phenotypes (such as biofilm formation and quorum sensing) which can also be induced by antibiotics. AR against carbapenems can arise as a result of modifications in membrane absorptivity through mutations in bacterial efflux pumps otherwise porins attached with ESβL expression, or by the procurement of carbapenem-resistance genes (CRGs) which encode specific carbapenemase (Potter et al., 2016). The latter mechanism has been reported globally in Enterobacteriaceae isolated from clinical samples. Nonetheless, the perseverance of the corresponding ARGs in environmental bacterial strains is of great concern as a result of its implication to public wellbeing (Walsh, 2010).

According to Nordmann et al. (2012), in some members of Enterobacteriaceae group, CR arises as a result of two main mechanisms: (i) acquisition of CRGs encoding for enzymes that can degrade carbapenems, or (ii) a reduction in the uptake of carbapenems by a qualitative or quantitative deficit of porin expression associated with overexpression of carbapenemases that have very low affinity for carbapenems (Netikul and Kiratisin, 2015; Shields et al., 2017).

Studies have reported other non-enzymatic mechanisms associated with CR such as reduction in the expression of outer membrane proteins (OMPs) (particularly OmpK35 and OmpK36 porins) to be related to an increase in MICs of both cephalosporins and carbapenems when exposed to *K. pneumonia* (Arora and Jagdale, 2009; Poulou et al., 2013; uz Zaman et al., 2014; Hamzaoui et al., 2018). Nevertheless, there may be other factors responsible for CR, factors such as carbapenemase production with no defective porin expression can strongly raise carbapenem MICs (Gomez-Simmonds et al., 2018). CR also encompasses numerous collective mechanisms which could include alterations or mutations in OMP and up-regulation of efflux systems—linked with hyper-production of AmpC β-lactamases or ESBLs—or creation of particular carbapenem-hydrolysing-β-lactamases (Rubin and Pitout, 2014; Russotto et al., 2015; Van Duin and Doi, 2017).

The widespread use of prescribed drugs such as antibiotics and antivirals including anti-retrovirals (ARVs) culminates in WWTP final effluents across the world (K'oreje et al., 2018; Myllyniemi Maldonado, 2018) and this is a major health concern due to its potential to encourage AMR. Mosekiemang et al. (2019) also investigated the occurrence of ARVs in environmental samples. Recent studies by Hocquet et al. (2016) and Christou et al. (2017) described AMR as a result of the ineffectiveness of different antibiotics which is a consequence of the mutations developed at the various target sites in bacterial cell organelles. The aftermath of this scourge is disastrous hence swift actions are needed to tackle this consistent phenomenon.

6. Non-carbapenemase mediated CRE

The outer membrane (OM) of bacteria has the ability to form a hydrophobic barrier that gives protection to the cell against external

Table 1. Antibiotics class, modes of action and common resistance mechanisms (Burch et al., 2013; Butaye et al., 2015).

Antimicrobial class	Mode of action	Mechanism of resistance (MOR)
Beta-lactam antibiotics ***Penicillins:*** Penicillin G, Penicillin V Methicillin, Oxacillin Ampicillin, Amoxycillin Piperacillin	Hinder cell wall production. Binds enzymes (Penicillin-Binding Proteins-PBPs) which help form peptidoglycans	1. Beta-lactamase production primarily – *bla* genes. 2. Changes cell wall protein enzymes so that they cannot bind to PBPs. *MecA* gene for methicillin resistance - *S. aureus*. 3. TEM-1, TEM-2, SHV-1 type beta-lactamase (*bla*) producing genes-transfer of plasmid 4. Cephalosporinases
Cephalosporins: *1st and 2nd generation* Cephalexin, Cephradine *3rd and 4th generation* Ceftiofur, Cefquinome Cefotaxime, Ceftazidime ***Monobactams:*** Aztreonam ***Carbapenems:*** Imipenem, Meropenem, Doripenem, Ertapenem Beta-lactamase inhibitors Clavulanic acid, Sulbactam Tazobactam	Inhibits/binds to beta-lactamase enzymes	Extended-spectrum beta lactamases (ESBLs) CTX-*M beta*-lactamase (*bla*) genes-transfer of plasmid (better term- expanded spectrum cephalosporinases – ESCs) 1. Carbapenemases – NDM-1, OXA-48-like, KPC, (*bla*) genes – serine based; IMP and VIM – metallo beta-lactamase (*bla*) enzyme genes (Zn dependent) 2. *AmpC* gene (bla_{CMY} sub-group) – cephalosporinase; Metalloenzyme genes – inhibitor antimicrobial resistant genes *mecA* gene – cannot bind to PBPs;
Polymixin Colistin	Action on cell membrane-disrupts permeability	Unclear – reduction of bacterial permeability
Tetracyclines Chlortetracycline, oxytetracycline, Doxycycline, minocycline	rRNA-binds to 30S subunit and interferes with amino acid transfer Prevents protein production	Inducible efflux in *E. coli* etc (*tetA, tetB, tetC*) Binding site mutations (*tetO*, *tetM* genes) Rare, changes to tetracycline molecule
Aminoglycosides Streptomycin, Neomycin, Kanamycin, Apramycin, Gentamicin, Amikacin **Aminocyclitol** Spectinomycin	rRNA-binds to 30S subunit, so misreads genetic code. Inhibits protein production. Effect on permeability of cell membrane	Phosphorylation, adenylation and acetylation of aminoglycoside (*aph*, *aad*, *aac* genes) inhibits binding. Streptomycin – single binding site Others – multiple binding sites, slower resistance, primarily plasmid transfer

agents like detergents, heavy metals and other antimicrobials (Spengler et al., 2017). This OM contains definite proteins, known as porins that can also form hydrophilic channels in order to permit the selective uptake of crucial nutrients and other compounds (e.g. antibiotics), moreover, the principal porins in Enterobacteriaceae involved in uptake of antimicrobials belong to the OmpF/OmpC families (James et al., 2009). Hence, any fluctuations in the amount or activity of porins might be reliant on AR. Chromosomal mutations of the central channel or gatekeeping loop in the OMP, shift in the types of porins or a loss of porin expression can increase resistance against antimicrobials (Nordmann et al., 2012). Additionally, breakdown of porin can be controlled in swift response to antimicrobials or aromatic products thru numerous cascades including the mar and sox operons, by means of a successive reduction in the quantity of porins in the bacterial OM (Shimizu, 2015). CR was first observed among *Enterobacter* spp. which over-expressed a chromosomal *ampC* gene encrypting an inherent cephalosporinase and displayed alterations in the OmpC or OmpF porins and according to a study by Bello and Dingle (2018), similar mechanisms were reported in *Serratia* spp., *Citrobacter freundii*, and *Morganella morganii*. Furthermore, CR mechanisms have also been reported in other members of Enterobacteriaceae that do not exhibit an intrinsic cephalosporinase (Poole, 2004; Su et al., 2008). From the several studies reviewed, various reports have necessitated the urgency to act fast in order to curtail the emergence and spread of ARGs in the environment. There is the need to develop new antibiotics or new ways to combat the defence metabolic mechanisms displayed by CRE.

Different approaches, such as the use of nanostructured materials, are being developed to overcome the various MOR. Nanostructured materials can possess antimicrobial activity by themselves. In addition, nanoparticles which include metallic, organic and carbon nanotubes may circumvent MOR in pathogenic bacterial strains and, associated with their antimicrobial potential, inhibit biofilm formation or other important microbial processes (Singh et al., 2018). Other strategies to oppose AR, for instance, the combined use of plant-based antimicrobials and nanoparticles to overcome toxicity issues, are also being investigated. On the other hand, the use of nanoparticles still presents a challenge to treatment options and more studies in this area are also needed.

7. Conclusion

Antimicrobial resistance (AR) has been increasing globally and bacterial infections are predominantly treated with antibiotics but the antibacterial activities have been compromised. The major routes of transmission of carbapenem-resistant bacteria to humans are generally through consumption of contaminated foods or water as well as poor domestic hygiene in many developing countries. The introduction of more metagenomics studies and use of risk assessment techniques in analysing different environmental samples will be essential for the prevention of infections caused by carbapenem-resistant Enterobacteriaceae. This calls for proper treatment of WWTP final effluent before being discharged into receiving watersheds and the development of new antibiotics that will assist in tackling AR strains. The manufacture of new and effective antibiotics for better treatment of bacterial infections will also help to reduce the prevalence of CRE and the dissemination of antimicrobial resistance genes.

Declarations

Author contribution statement

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The authors declare no conflict of interest.

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