OCCURRENCE AND ANTIBIOGRAM OF IMIPENEM RESISTANT BACTERIA IN SACHET WATER SOLD IN CALABAR METROPOLIS, NIGERIA.

*Archibong C. P. and Andy I. E.

Department of Microbiology, University of Calabar, Calabar.

*Corresponding Author: Archibong, C. P.

Department of Microbiology, University of Calabar, Calabar.

ABSTRACT

Sachet water is an affordable drinking water which is widely consumed within the Calabar metropolis. The microbial quality of this drinking water is of outmost significance to public health and can contribute to failure in the use of carbapenems for treatment of bacterial infections especially among the neonates, aged and immunocompromised individuals. This study was undertaken to investigate the occurrence and antibiotics resistance profile of bacterial isolates from the vended sachet water samples. Ten different brands of sachet water from various manufacturers were randomly collected on different occassions and transported to the laboratory for analysis within 18h using the surface plating technique for isolation of bacteria. The isolates were subjected to imipenem susceptibility testing and susceptibility to other antibiotics following Clinical Laboratory Standard Institute (CLSI, 2009) guidelines. Eleven (22%) of the isolates were imipenem resistant, 24(48%) showed intermediate resistance and 15 (30%) were susceptible to imipenem. The species composition and frequency of isolation were: *Salmonella spp* (9%), *Pseudomonas aeruginosa* (6%), *Aeromonas spp* (20%), *Proteus spp* (10%), *Citrobacter freundii* (17%), *Plesiomonas spp* (3%), *Serratia spp* (6%), *Enterobacter spp* (17%), *Acinetobacter spp*, (3%), *Klebsiella spp* (9%). All the imipenem resistant isolates showed multiple resistance to the antibiotics except *Citrobacter freundii* which was resistant to only one antibiotic. The number of antibiotics other species showed resistance were *Enterobacter spp* (10 antibiotics), *Acinetobacter spp* (9 antibiotics), *Plesiomonas spp* (8 antibiotics), *Pseudomonas aeruginosa* (8 antibiotics), *Salmonella spp* (2 antibiotics), *Proteus mirabilis* (5 antibiotics), *Serratia spp* (5 antibiotics) and *Klebsiella spp* (4 antibiotics). The multiple antibiotics phenotype of these isolates is a threat to an effective therapy and may contribute to increased mortality and morbidity rates as well as other outcomes. Consequently, monitoring of the water quality of these vended sachet water should be given a top priority.

KEYWORDS: Carbapenems, immunocompromised.

INTRODUCTION

The microbial examination of the different brands of sachet water showed the presence of bacteria with heteroterophic count not conforming to Environmental Protection Agency (EPA) and World Health Organisation (WHO) recommended standard of (1.0×10^2cfu/ml). Similar observation have been reported by several researchers who demonstrated the presence of opportunistic and pathogenic bacteria in sachet water samples (Awuah *et al*., (2014); Bukar *et al*., (2015); Ajayi *et al*., 2008; Adewoye (2013); Mgbakor *et al*., (2011); Adekunle *et al*., 2004). As expected natural water bodies present an aquatic habitat for microorganisms and is a source of water supply for the different sachet water manufacturers. Sachet water is one of the cheapest and affordable drinking water available to the public within Calabar metropolis. Various studies on assessment of the microbial quality of sachet water have shown the presence of antibiotics resistant bacteria, coliforms and other pathogenic bacteria which is a threat to public health (Addo *et al*., 2009; Olaoye and Onilude, 2009; Dada, 2009; Lateef *et al*., 2005; Tagoe *et al*., 2011). Distribution of such contaminated water to the public can facilitate the spread of water borne diseases (Oladipo *et al*., 2009; Clasen *et al*., 2007) and antibiotics resistant genes and bacteria from the environment (Edet *et al*., 2017) to human (Martínez, 2008). These antibiotics resistant genes can be taken up by human commensals and pathogens via transformation as well as horizontal and vertical genetic transfer mechanisms (Hooper and Gordon, 2001; Salyers *et al*., 2004). As a result of the widespread of antibiotics resistance, several antibiotics that were once effective in the treatment of bacterial infections are no longer effective (CDC, 2013).

Several bacterial genera had been reported to develop resistance to some antibiotics since the 1940s such as

resistant *Staphylococcus* which showed resistance to the penicillins (Sabath *et al.*, 1977). Other bacterial genera reported were tetracycline-Resistant *Shigella* and methicillin-Resistant *Staphylococcus* in 1959 and 1962, erythromycin- Resistant *Streptococcus* (1968), gentamicin-Resistant *Enterococcus* (1979), imipenem-Resistant and Carbapenem resistant *Enterobacteriaceae* (1996) (Ventola, 2015). The development of resistance to various antibiotics can be attributed to the inappropriate use of antibiotics which is worsened by the slow pace at which new antibiotics are produced (Pechere, 2001; Piddock, 2012; Reads and Woods, 2014). The associated consequences of emergence of antibiotics resistant bacteria include poor therapeutic outcome, high cost of treatment, increased mortality and morbidity rates as well as extended hospital stay (Essack, 2001).

The beta-lactam antibiotics are widely used globally for treatment of bacterial infections due to the broad spectrum and efficacy as well as the availability of different derivatives (Livermore and brown, 2001). These antibiotics act on the bacterial cell wall and prevents the cross linkage of the tetrapeptides leading to a weakened cell wall. The different types of beta-lactam antibiotics grouped based on the ring structure include: penam, penem, carbapenem, cephem and monobactam (Fernandes and Prudêncio, 2013). The carbapenems (such as imipenems, meropenems, ertapenems), with a penem ring structure, represent a class of broad spectrum antibiotics used for the treatment of severe infections and infections caused by bacteria capable of producing extended spectrum beta-lactamases. They are regarded as drugs of last resort when other antibiotics are not effective for treatment (Hussein *et al.,* 2013; Esterly *et al.*, 2011; Tal-Jasper *et al.*, 2016). Pathogens that show resistance to the carbapenems are a major threat to health and an effective therapy because of limited antibiotics options available for treatment. Resistance to the carbapenems can be mainly via the following mechanisms: alteration of the penicillin binding proteins, expulsion through efflux pumps and production of carbapenemases such as metallo-beta-lactamases (Nordmann and Poirel, 2012). The metallo-beta-lactamases hydrolyze all beta-lactam antibiotics except the monobactams and cannot be inhibited by any known clinical inhibitors unlike other beta-lactamases (Rasmussen and Bush (1997).

The emergence of metallo-beta-lactamase (MBL) producing bacteria has been reported globally as well as the rapid spread to various regions of the world. Different factors have been shown to facilitate the widespread distribution of these bacteria. The major factors include over dependence and unregulated use of the carbapenems which has led to selection of carbapenem resistant bacteria (Bebell and Muiru, 2014). The spread can also be linked to drinking of contaminated water containing MBL producing bacteria. The effect is more pronounced among the immunocompromised, the neonates and the aged. To this end, this study was carried out to assess the imipenem resistance among bacterial isolates from different brands of sachet water as well as the antibiotics susceptibility patterns.

MATERIALS AND METHODS

Study area and Sample collection
This study was carried out within Calabar metropolis (with GPS coordinates: 4° 58' **58.3428**" N and 8° 20' 4.2108" E), a major city in Cross River State, located in the Southern part of Nigeria. Vended sachet water samples from ten different manufacturers were randomly selected for this study. The samples were collected from major streets and markets within Calabar metropolis over a period of three months (November to December). These samples were transported to the Microbiology laboratory, University of Calabar and analysed in duplicates within 18 hours of collection.

Enumeration, Isolation and Identification method
A one step ten-fold serial dilution and surface plating method was used in this study. One mililitre (1ml) of each of the sachet water samples was added to a 9ml sterile water and subsequently streaked on a freshly prepared Macconkey for isolation of Gram-negative bacteria and incubated aerobically at 35^0C for 24 h. The number of colonies was counted to determine the bacterial load of the sachet water samples. The distinct and discrete colonies were purified by sub-culturing onto Macconkey and blood agar and then stocked for biochemical identification following Bergey's manual of determinative bacteriology guidelines. The biochemical and cultural characteristics of the pure isolates were compared with Bergey's manual in order to determine the probable identity of the bacteria.

Imipenem and Antibiotics susceptibility testing
The pure isolates were screened for imipenem resistance following Clinical Laboratory Standards Institute (2006) guidelines. Pure broth culture of the isolates equivalent to 0.5 McFarland standard was swabbed on a Muller Hinton agar. Imipenem disks (10µg) were aseptically placed on the inoculated agar and incubated overnight at 35^0C. The zones of inhibition were measured and compared with CLSI standards to determine the degree of resistance. The following benchmarks were used to determine the level of susceptibility: ≤13mm (resistant); 14-15mm (intermediate resistance) and ≥16 mm (susceptible). The imipenem resistant isolates were tested against 13 antibiotics following CLSI (2006) procedures. The following antibiotics disks (Oxoid) were used in this study: sulfamthoxazole-trimethoprim (25µg), septrin (30µg), chloramphenicol (30µg), ampicillin (30µg), augmentin (30µg), gentamycin (10µg), pefloxacin (10µg), orflaxacin (10µg), ciprofloxacin (5µg), ceftazidime (30µg), aztreonam (30µg), cefuroxime (30µg), streptomycin (10µg).

Statistical analysis

Descriptive statistics using IBM SPSS 21 were used in analyzing the data obtained from this study.

RESULTS

The results of the study are presented in the tables and figures below. Table 1 shows the number of isolates from the ten brands of sachet water samples from ten different manufacturers (SW1-SW10). A total of 50 gram-negative bacilli were isolated; SW7 had the highest colony forming units count of 15, others ranged from 1-10 CFU/ml (table 1). The number and percentages of bacterial isolates resistant and susceptible to imipenem is shown in table 2. From the 50 isolates, 11(22%) showed resistant to imipenem, 24(48%) were intermediate resistant and 15(30%) were susceptible to imipenem. The frequency of isolation and the genera of the isolates were: *Salmonella spp* (9%)*, Pseudomonas aeruginosa*

(6%)*, Aeromonas spp* (20%)*, Proteus spp* (10%)*, Citrobacter freundii* (17%)*, Plesiomonas spp* (3%)*, Serratia spp* (6%)*, Enterobacter spp* (17%)*, Acinetobacter spp,* (3%)*, Klebsiella spp* (9%) (figure 1). Table 3 and table 4 show the Species resistant pattern and antibiotics resistant pattern of the imipenem resistant isolates against 13 antibiotics. All the isolates were resistant at least two antibiotics except *Citrobacter freundii*. All the *Enterobacter spp* showed resistance to 10 antibiotics followed by *Acinetobacter spp* (9 antibiotics), *Pseudomonas spp* and *Plesiomonas spp* (8 antibiotics); *Citrobacter freundii* was resistant to 1 antibiotic and *Aeromonas spp* was susceptible to all the antibiotics. The number of antibiotics other bacterial species were resistant to were: *Salmonella spp* (two antibiotics), *Proteus mirabilis* and *Serratia spp* (5 antibiotics), *Klebsiella spp* (4 antibiotics).

Table 1: Number of Gram-negative bacteria colony forming units.

Sachet water brands (code names)	Number of colony forming units
SW1	10
SW2	10
SW3	1
SW4	3
SW5	1
SW6	2
SW7	15
SW8	2
SW9	5
SW10	1
total no of isolates	50

Table 2: Imipenem susceptibility of isolates from sachet water samples.

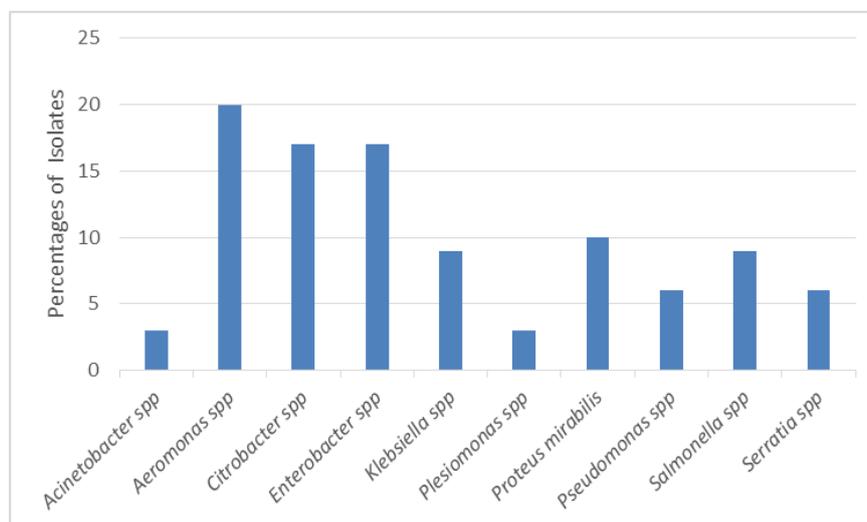| Imipenem susceptibility	Total number of isolates (n=50)
Resistance	11(22%)
Intermediate	24(48%)
Susceptible	15(30%)

Figure 1: Frequency of Imipenem resistant bacterial isolates.

Table 3: Species resistance pattern of Imipenem resistant bacteria to other antibiotics.

Antibiotics	*Salmonella spp* (n=3)	*Pseudomonas aeruginosa* (n=2)	*Aeromonas spp* (n=7)	*Proteus mirabilis* (n=4)	*Citrobacter freudii* (n=6)	*Plesiomonas spp* (n=1)	*Serratia spp* (n=2)	*Enterobacter spp* (n=6)	*Acinetobacter spp* (n=1)	*Klebsiella spp* (n=3)
Orfloxacin (OFX)	3(100)	2(100)	5(71)	4(100)	6(100)	1(100)	2(100)	6(100)	1(100)	3(100)
Pefloxacin (PEF)	0(0)	1(50)	3(43)	2(50)	3(50)	0(0)	0(0)	6(100)	1(100)	3(100)
Gentamycin (CN)	1(33)	2(100)	2(29)	2(50)	3(50)	1(100)	0(0)	3(50)	1(100)	1(33)
Augmentin (AU)	2(67)	2(100)	5(71)	3(75)	5(83)	1(100)	2(100)	6(100)	1(100)	2(67)
Ampicillin (AM)	1(33)	1(50)	2(29)	3(75)	5(83)	0(0)	1(50)	6(100)	1(100)	2(67)
Ciprofloxacin (CPX)	0(0)	0(0)	1(14)	2(50)	5(83)	0(0)	0(0)	6(100)	0(0)	2(67)
Streptomycin (SP)	1(33)	0(0)	2(29)	3(75)	4(67)	0(0)	1(50)	3(50)	0(0)	2(67)
Chloramphenicol (CH)	2(67)	2(100)	2(29)	3(75)	5(83)	0(0)	1(50)	6(100)	1(100)	2(67)
Septrin (S)	3(100)	2(100)	3(43)	4(100)	3(50)	1(100)	2(100)	6(100)	1(100)	1(33)
Sulfamethoxazole-Trimethoprim (SXT)	1(33)	1(50)	3(43)	4(100)	5(83)	1(100)	1(50)	6(100)	0(0)	2(67)
Aztreonam	1(33)	2(100)	4(57)	4(100)	5(83)	1(100)	2(100)	6(100)	1(100)	3(100)
Ceftazidime (CAZ)	2(67)	2(100)	4(57)	4(100)	5(83)	1(100)	2(100)	6(100)	0(0)	3(100)
Cefuroxime (CFX)	2(67)	2(100)	1(14)	2(50)	3(50)	1(100)	0(0)	4(67)	1(100)	2(67)

Table 4: Antibiotics resistance pattern of the imipenem resistant bacteria.

Isolate	Antibiotics	Number of antibiotics
Salmonella spp	OFX-S	2
Pseudomonas aeruginosa	OFX-CN-AU-CH-S-AZT-CAZ-CFX	8
Proteus mirabilis	OFX-S-SXT-AZT-CAZ	5
Citrobacter freundii	OFX	1
Plesiomonas spp	OFX-PEF-CN-S-SXT-AZT-CAZ-CFX	8
Serratia spp	OFX-AU-S-AZT-CAZ	5
Enterobacter spp	OFX-PEF-AUG-AMP-CPX-CH-S-SXT-AZT-CAZ	10
Acinetobacter spp	OFX-PEF-CN-AUG-AMP-CH-S-AZT-CFX	9
Klebsiella spp	OFX-PEF-AZT-CAZ	4

Orfloxacin (OFX), Streptomycin (S), Gentamycin (CN), Augmentin (AU), Chloramphenicol (CH), Aztreonam (AZT), Ceftazidime (CAZ),
Pefloxacin (PEF), Ampicillin (AMP), Ciprofloxacin (CPX), Cefuroxime (CFX), Sulfamethoxazole-trimethoprim (SXT), Septrin (S)

DISCUSSION

Out of the 50 Gram-negative bacterial isolates subjected to imipenem susceptibility testing, 70% of the isolates were resistant to imipenem. The imipenem resistant isolates were of the following bacterial genera: *Acinetobacter, Aeromonas, Citrobacter, Enterobacter, Esherichia, Klebsiella, Plesiomonas, Proteus, Pseudomonas, Salmonella and Serrratia.* Reports from different studies have shown imipenem resistance among non-fermentative bacteria and *Enterobacteriaceae.* Yousefi *et al* (2010) reported 58.1% imipenem resistance among *Pseudomonas aeruginosa* isolates while Khosravi *et al* (2010) observed a resistant rate of 41%. Also, Ampaire *et al* (2015) and Mate *et al* (2014) reported 37.15% and 30% imipenem resistant rates respectively among *Pseudomonas, Klebsiella, Enterobacter* and *Esherichia species*. Different resistance mechanisms can be implicated to underlie the observed imipenem resistance. These include efflux pump mechanism, alteration of outer membrane permeability, secretion of beta-lactamases and presence of acquired genetic elements such as class 1 integrons and gene cassettes which facilitate spread of genes encoding resistance (Fuste *et al*., 2013). Among the isolates of *Enterobacteriaceae* family, carbapenem resistance can

be attributed to plasmid AmpC beta-lactamases in combination with the extended spectrum beta-lactamases (Bedenic *et al*., 2014; Bradford *et al*., 1997) In *Klebsiella, Enterobacter, Escherichia coli,* mutation in the porins and efflux pump mechanisms have been associated with imipenem resistance (Codjoe and Donkor, 2017); (Walsh *et al*., 2000). While in *Pseudomonas aeruginosa*, the overexpression of OprD porins appears to be the most common resistance mechanism.

In addition to being resistant to imipenem, the isolates in this study also showed resistance to other antibiotics within the beta-lactam class and other classes of antibiotics like the fluoroquinolones, aminoglycosides and cephalosporins. The same phenomenon was reported by Yousefi *et al* (2010) who observed high level resistance among imipenem resistant bacteria and also noted that there is a significant difference between antibiotics resistance rate of imipenem susceptible and resistant bacteria. **G**utiérrez *et al* (2007) noted resistance among carbapenem resistant bacteria to beta-lactams and non beta-lactams than carbapenem susceptible bacteria. As reported by Fonseca (2005), the multiple antibiotics resistance nature of these bacteria can be linked to the presence of class 1 integron, which not only harbour

genes encoding resistance to different classes of antibiotics, they also enhance the rapid spread and transfer of antibiotics resistance genes.

Infections associated with the carbapenem resistant bacteria are often difficult to treat due to the high level resistance and limited antibiotics options available (Ampaire *et al.*, 2015). Various risk factors for carbapenem resistant infections include suppressed immune system, age, long hospital stay and excessive use of carbapenems (Cadjoe and Donkor, 2017). Among the immunocompromised, the aged and the neonates, drinking of sachet water contaminated with carbapenem resistant bacteria can further compromise the outcome of treatment of bacterial infections which can result in death, morbidity and economic burden.

CONCLUSION

The high percentage and widespread imipenem resistance among different genera of Gram-negative bacteria is an indication of rapid emergence of imipenem resistant bacteria. this can be attributed to lack of regulation of the use and misuse of imipenem which are very useful in the overall treatment and management of severe infections. Considering the importance, antibiotics surveillance policies and usage regulations should be instituted and followed as well as proper monitoring of sachet water sold to the public.

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