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Antibiotics susceptibility profile of Metallo-beta-lactamase producing bacterial isolates harboring blaIMP and blaNDM genes from selected sachet water samples sold in Calabar Metropolis, Nigeria

[1]Andy, I. E., [2]Archibong, C.P.

Department of Microbiology, University of Calabar, Calabar

Abstract: **Bacterial infections associated with metallo-beta-lactamase (MBL) production are often difficult to manage with existing antibiotics. This is as a result of the broad antibiotics hydrolytic spectrum of metallo-beta-lactamases which are known to hydrolyze all beta lactams except the monobactam antibiotics. this investigation was carried out to assess the antibiogram of metallo-beta-lactamase (MBL) producing bacteria from selected sachet water samples sold in Calabar Metropolis. Isolates from a previous study confirmed to harbour the blaIMP and blaNDM genes were subjected to antibiotics susceptibility testing following Clinical Laboratory Standard Institute guidelines. The antibiotics resistant spectrum of the MBL producing isolates were: *Plesiomonas shigelloides* strain 187 (OFX-CN-AU-CH-CAZ), *Enterobacter cloacae* strain S20504 (OFX-CN-AU-CH-S-CAZ), *Photobacterium ganghwense* strain ZR07 (CAZ), *Bacillus licheniformis* strain 60 (OFX-CN-AU-CH-S-AZT-CAZ), *Klebsiella pneumoniae* strain DSM 30104 (OFX-CN-AU-CH-S-SXT-CAZ), *Plesiomonas spp* strain TIL_TAL_1 (OFX-PEF-CN-AU-S-SXT-AZT-CAZ), *Comamonas testosterone* strain 1 (OFX-CN-AU-S-SXT-AZT-CAZ), *Enterobacter sacchari* SP1 (OFX-PEF-CN-AU-AM-CH-S-AZT), *Acinetobacter soli* strain MBR7 (CAZ), *Photobacterium ganghwense* strain ZR07 (AZT-CAZ). The multiple antibiotics resistance indices (MARI) of the MBL producing isolates was in the range of 0.2-0.8. The multidrug resistance nature of these MBL producing isolates showed that they are a potential threat to health. Hence, measures to reduce the spread of these bacteria from the environment to human should be developed and implemented.**

1. INTRODUCTION

Antibiotics are chemotherapeutic agents used for treatment of bacterial infections. After the discovery of penicllins by Alexander Fleming in 1929, other antibiotics were discovered and developed (Birnbaum *et al.*, 1985; Fischbach and Walsh, 2009). These antibiotics are classified into groups based on the mode of action and targets on the bacterial cell. The various types of antibiotics include the aminoglycosides, beta lactams, fluoroquinolones, macrolides, polymyxins (Russell, 2004). Due to the high selective toxicity of antibiotics, they were suitable for management and treatment of bacterial infections. This had a huge positive impact on health leading to reduced morbidity and mortality rates (Davies and Davies, 2010;). Unfortunately, the excessive and unregulated use of antibiotics has resulted in the development of bacterial resistance to these agents (Liu *et al*., 2001; Smith *et al.*, 2002).

Antibiotics resistance is a natural survival strategy for bacterial growth and proliferation (de la Fuente-Núñez *et al.*, 2013). The problem of antibiotics resistance is major challenge to human health. It has been linked to increased morbidity and mortality rates as well as increased cost of treatment of bacterial infections (Hart and Kariui, 1998; Cosgrove, 2006). Different resistance mechanisms have been identified such as alteration of antibiotics target sites, efflux pump mechanism and secretion of enzymes which inactivates the antibiotics (Benveniste *et al*., 1973; Blair *et al.*, 2015). The ability of bacteria to secrete antibiotics-inactivating enzymes is encoded in the genetic constitution. There are different types of enzymes that degrade antibiotics, such enzymes include: aminoglycosidases and beta lactamases. Within the beta lactamases, there are subclasses of the enzymes that mediate resistance to different type of beta lactam antibiotics (Bush *et*

al., 1995). The metallo-beta-lactamase represent a subclass of beta lactamases which hydrolyses various types of beta-lactam antibiotics and other classes of antibiotics used for treatment of bacterial infections (Mendes *et al.*, 2006).

The consumption of sachet water can be a potential means of spread of the metallo beta lactamase producing bacteria from the environment to human (Heuer *et al.*, 2009). This can be associated with absence of effective treatment treatment techniques and poor sanitary measures adopted in handling and processing of these sachet water (Oladipo *et al.*, 2009; Dada, 2009; Omalu et al.,2011). Bacterial infections associated with the MBL producing pathogens are often difficult to treat due to the broad antibiotics hydrolytic spectrum of metallo-beta-lactamases which often result in poor therapeutic outcomes (McGowan Jr, 2006; Nordmann *et al.*, 2011). The consequence of this can be seen among the immunocompromised and neonates who are prone to bacterial infections because of a weakened immune response (Shirani *et al.*, 2016). Consequently, this investigation was carried out to assess the antibiogram of bacterial isolates confirmed to harbor the MBL genes.

2. MATERIALS AND METHODS

Antibiogram and Multiple Antibiotics Resistance Indices (MARI) of the Metallo-beta-lactamase (MBL) producing bacteria

The bacterial isolates from a previous study by Archibong and Andy (2018) confirmed to harbor the MBL genes were subjected to antibiotics susceptibility test. The agar diffusion method, in accordance to the procedures outlined in CLSI manual (2006), was used for determination of the antibiotics susceptibility pattern of the isolates. A broth culture of the bacteria which corresponds to 0.5 Macfarland was swapped on a Mueller Hinton agar plate and antibiotics disks were placed on the agar surface. The antibiotic disks contained the following antibiotics: sulfamthoxazole-tremethoprim (25µg), septrin (30µg), chloramphenicol (30µg), ampicillin (30µg), augmentin (30µg), gentamycin (10µg), pefloxacin (10µg), ofloxacin (10µg), ciprofloxacin (5µg), ceftazidime (30µg), aztreonam (30µg), and imipenem (10µg). After 24h incubation period, the diameter of the zones of inhibition were measured and compared with CLSI standards to determine the degree of sensitivity and resistance to each of the antibiotics. The multiple antibiotics resistant indices were calculated using the formular: MARI = x/y, where "x" is the number of antibiotics the isolates were resistant to and "y" is the total number of antibiotics the isolates were tested against (Kelsey *et al.*, 2003).

3. RESULTS

The specific antibiotics to which the MBL producing isolates exhibited resistance is shown in Table 1. The highest resistance was observed in *Enterobacter sacchari* SP1 isolate which *was* resistant to 9 antibiotics including aztreonam; this was followed by *Plesiomonas sp* strain TIL_TAL_1 which showed resistance to 8 antibiotics. *Acinetobacter soli* strain MBR7 was susceptible to all the antibiotics while one of the *Photobacterium ganghwense* strain ZR07 was resistant to only ceftazidime, the other strain showed resistance to aztreonam and ceftazidime. Table 2 shows the multiple resistance indices of the MBL producing isolates and the number of antibiotics to which the isolates showed resistance. *Enterobacter sacchari* SP1 showed the highest MARI value of 0.8 with resistance to 9 nine antibiotics while *Acinetobacter soli* strain MBR7 was susceptible to all the 12 antibiotics. Other isolates, Pleisomonas *shigelloides* strain 187, *Enterobacter cloacae* strain S20504, *Photobacterium ganghwense* strain ZR07, *Bacillus licheniformis* strain 60, *Klebsiella pneumoniae* strain DSM 30104, *Plesiomonas sp* strain TIL_TAL_1, *Comamonas testosterone* strain 1, and *Photobacterium ganghwense* strain ZR07 had MAR values ranging from 0.2 - 0.7 and showed resistance to 2 - 7 antibiotics.

Table 1: Antibiotics resistance spectrum of Metallo-beta-lactamase (MBL) producing bacteria

Isolates	Antibiotics resistance pattern
Plesiomonas shigelloides strain 187	OFX-CN-AU-CH-CAZ
Enterobacter cloacae strain S20504	OFX-CN-AU-CH-S-CAZ
Photobacterium ganghwense strain ZR07	CAZ
Bacillus licheniformis strain 60	OFX-CN-AU-CH-S-AZT-CAZ
Klebsiella pneumoniae strain DSM 30104	OFX-CN-AU-CH-S-SXT-CAZ
Plesiomonas spp strain TIL_TAL_1	OFX-PEF-CN-AU-S-SXT-AZT-CAZ
Comamonas testosterone strain 1	OFX-CN-AU-S-SXT-AZT-CAZ
Enterobacter sacchari SP1	OFX-PEF-CN-AU-AM-CH-S-AZT-
Acinetobacter soli strain MBR7	CAZ
Photobacterium ganghwense strain ZR07	AZT-CAZ

Table 2: Multiple antibiotics resistance indices (MARI) of the MBL producing isolates

ISOLATES	MAR INDICES	NUMBER OF RESISTANT ANTIBIOTICS
Plesiomonas shigelloides strain 187	0.4	5
Enterobacter cloacae strain S20504	0.5	6
Photobacterium ganghwense strain ZR07	0.1	1
Bacillus licheniformis strain 60	0.6	7
Klebsiella pneumoniae strain DSM 30104	0.6	7
Plesiomonas spp strain TIL_TAL_1	0.7	8
Comamonas testosterone strain 1	0.6	7
Enterobacter sacchari SP1	0.8	9
Acinetobacter soli strain MBR7	0	0
Photobacterium ganghwense strain ZR07	0.2	2

4. DISCUSSION

In this study, the MBL producing isolates were tested against several antibiotics commonly used for treatment of bacterial infections and a multiple antibiotics resistance was observed. From these findings, it can be speculated that the MBL producing bacteria also harbour genes encoding resistance to other group of antibiotics apart from the beta lactam antibotics (Shirani *et al.*, 2016). This explains the treatment failures sometimes encountered in the management of infections associated with MBL producing bacteria (Nordmann *et al*., 2011). Khosravi *et al* (2012) also reported MBL producing bacteria exhibiting multidrug resistance to at least six antibiotics. The highest resistance was observed against ceftazidime, with 90% of the isolates showing resistance to this antibiotic. However, all the isolates were susceptible to ciprofloxacin and streptomycin which is consistent with findings from other studies (Navaneeth *et al*., 2002; Marchiaro *et al*., 2005; Varaiya *et al*., 2008; Bashir *et al*., 2011; Aghamiri *et al* 2014). Different from our observation, Franco *et al* (2010) observed aztreonam as the second most active antibiotic. Contradicting the popular notion that all MBL producing bacteria are susceptible to aztreonam, the results from this study showed some MBL producing bacteria were resistance to aztreonam.

The multiple antibiotics resistance indices (MARI) were computed for the MBL producing bacteria. The MARI indices are often used as an indicator to evaluate multidrug resistance profile of bacterial species. A high index is an indication of resistance to multiple antibiotics and constitutes a major threat to chemotherapy. It is also an indication of the degree of exposure of the bacteria to antibiotics (Krumperman, 1983; Kaspar *et al.*, 1990; Chitanand *et al.*, 2010). Bacterial populations exposed to various classes of antibiotics are bound to develop resistance to these antibiotics via selective pressure which results in the emergence of species harboring resistance genes. Given the high rate of mutation that occurs in bacterial genetic make-up, it is not surprising to observe resistance to antibiotics that were hitherto active against the bacterial population. In this study a high MARI in the range 0.2 – 0.7 was recorded among the MBL producing bacteria and this an indication of the presence of other genes apart from the MBL genes encoding resistant traits to other family of antibiotics. Results from this study agrees with the result of MARI of *E. coli* isolates in a study by Chika *et al* (2017) who recorded a MARI average of 0.4 for the isolates.

This indicates that MBL producing bacteria are associated with multidrug resistance. The MBL producing bacterial isolates in this study were all resistant to at least three antibiotics mostly used for treatment of bacterial infections. This observation shows that MBL producing pathogens constitute a threat to health and an effective chemotherapy. Early detection of carbapenem resistant bacteria during diagnosis and regulating the usage of the carbapenems can contribute in curbing the spread and emergence of the MBL producing bacteria.

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