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Antimicrobial Resistance and β-Lactamase Production among Hospital Dumpsite Isolates

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Abstract

Metallo-β-Lactamases (MBLs) and Extended Spectrum β-Lactamses (ESBLs) have emerged worldwide as a significant source of β-lactam resistance. The emergence of MBLs and ESBLs encoded on plasmids among Gram-negative pathogens in hospital dumpsites was investigated. Soils of different government and private hospitals were collected and processed following standard bacteriological techniques. Antimicrobial susceptibility testing was carried out by the disk-diffusion technique using Ceftazidime (30 µg), Cefuroxime (30 µg), Cefotaxime (30 µg), Cefixime (5 µg), Trimethprim-sulfamethoxazole (25 µg), Gentamycin (100 µg) Amoxicillin-Clavunalate (30 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg), Nitrofurantoin (300 µg) and Imipenem (10 µg). The role of plasmids in resistance was evaluated by subjecting isolates to curing using Sodium Dodecyl Sulfate (SDS). ESBLs production by Double-Disk Synergy Test (DDST) was carried out. Isolates resistant to Imipenem were subjected to a confirmatory test using Modified Hodge's test and to MBLs production by DDST. Eighty-two Gram-negative isolates comprising of 32 (39.02%) *Escherichia coli*, 20 (24.39%) *Serratia marcescens*, 14 (17.07%) *Klebsiella pneumonia*, 10 (12.28%) *Proteus mirabilis* and 6 (7.32%) *Enterobacter aerogenes* were obtained. Susceptibility results revealed a 100% resistance of all isolates to Ceftazidime, Cefuroxime, Cefixime, Amoxycillin-clavulanate and Cefotaxime. A total of 66 (80.48%) isolates harboured plasmids out of which 26 (31.71%) isolates were ESBL producers. MBLs production was observed in 8 (25.00%) *E. coli,* 2 (2.41%) *Klebsiella pneumonia* and 2 (2.41%) *Proteus mirabilis* isolates. All MBLs producing isolates were ESBLs producers. The finding of highly resistant isolates producing ESBLs and MBLs in a hospital environment is quite disturbing and should be addressed urgently.

Keywords

Metallo-Beta Lactamases (MBLs), Extended Spectrum Beta-Lactamses (ESBLs), Plasmids, Antimicrobial Resistance

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1. Introduction

Mismanaged hospital wastes contribute to the ever increasing resistant bacteria in our environment. Most of the substances such as pharmaceuticals and disinfectants used in hospitals for treatment and disinfection end up in dumpsites without any prior treatment. Antimicrobial resistance is usually found in hospital environment. Synthesis of β-lactamase is one of the major mechanisms by which bacteria develop antibiotic resistance [1]. These enzymes open up the beta lactam ring of the antibiotic by hydrolysis. The Extended Spectrum β-Lactamases (ESBLs) enzymes are β-lactamase that hydrolysis broader spectrum of antibiotics, including penicillin, oxyiminocephalosporins and monobactams [2] [3]. The dissemination of ESBLs had been reported to be rapid worldwide particularly in enterobacteriaceae [4]. Evidence abounds that ESBL-producing bacteria spread more in developing countries than in developed nations. Some credible reasons for this include poorer hygiene and impaired effective infection control [5]-[7]. The rate of increase in of ESBL-producing enterobacteriaceae in both hospital and community settings is a major clinical concern that demands urgent intervention due to obvious reasons.

The emergence of carbapenems, broad spectrum antibiotics with remarkable stability towards β-lactamases especially ESBLs was a huge relief to clinicians. However, this relief was short lived, because infections with carbapenemase-producing enterobacteriaceae quickly emerged. The carbapenemases hydrolyse not only carbapenem but also all hydrolysable β-lactams and are resistant against inhibition by β-lactamase inhibitors. Carbapenemases belong to two major groups distinguished by the hydrolytic mechanisms at the active site. The first group (those that belong to A, C and D) utilizes serine at the active site to carry out its hydrolytic ability; the second group uses at least one zinc atom at the active site to promote hydrolysis, establishing them as metalloenzymes (metallo β-lactamases) [8] [9].

Metallo β-lactamase genes are carried on mobile genetic elements, thus promoting the rapid dissemination of resistant genes [10]. The present study was therefore carried out to explore the environmental presence of ESBLs and metallo β-lactamases and to suggest proper hospital infection control measures that will prevent spread.

2. Materials and Methods

2.1. Bacterial Isolates

A total of eighty two bacterial isolates of the family enterobacteriaceae were obtained from soils of different government and private hospital dump sites in Delta State, Nigeria.

2.2. Antibiotic Susceptibility

Susceptibilities to antimicrobial agents were determined by standard disk diffusion method on Mueller-Hinton Agar (MHA) according to Clinical Laboratory Institute (CLSI) guidelines [11]. The antibiotics used were Nitrofurantoin (NIT-300 µg), Ciprofloxacin (CPR-5 µg), Ceftazidine (CAZ-30 µg), Cefuroxime (CRX-5 µg), Gentamicin (CN-10 µg), Ofloxacin (OFL-5 µg). Amoxicillin-Clavulanic acid (Amx-ClA-30 µg), Cefixime (CXM-5 µg), Cefotaxime (CTX-30 µg), Trimethoprime-Sulfamethoxazole (SXT-25 µg), Imipenem (10 µg), (Oxoid UK).

2.3. Detection of Extended Spectrum β-Lactamase (ESBL) Production

ESBL production was detected phenotypically by the Double Disc Synergy Test (DDST) method, in accordance with Clinical Laboratory Standard Institute, CLSI guidelines [11].

A 0.5 McFarland suspension of the test isolate was prepared and inoculated onto Muller Hinton Agar (MHA) plates. Amoxicillin-clavulanic acid disc (30 µg) was placed at the center of the MHA plate inoculated with the test isolate, a ceftazidime disc (30 µg) and a cefotaxime disc were each placed 15mm apart from the center disk. The plates were incubated at 37˚C for 24 hrs. Enhancement of zone diameters of cefotaxime or ceftazidime toward the amoxicillin-clavulanic acid disc were recorded as ESBL positive.

2.4. Phenotypic Confirmation of Carbapenemases (Modified Hodges Test)

Screening of isolates for carbapenemases were first carried out according to the guideline of CLSI. In this method, an imipenem disc was placed on the surface of an inoculated Mueller Hinton Agar using a sterile forcep.

This was incubated at 37˚C for 24 hrs. Isolates that showed reduced susceptibility of ≤23 mm were subjected to a confirmatory test by the modified Hodges test.

In the modified Hodges test, a 0.5 McFarland suspension of *E. coli* ATCC 25922 was evenly inoculated with a sterile cotton swab on the surface of MHA plates. An imipenem disc (10 µg, Oxoid England) was placed on the surface of the MHA using a sterile inoculating wire loop, the test isolate was streaked on a straight line from the edge of the imipenem disc to the edge of the plate. The plates were incubated at 37˚C for 24 hrs.

A clover lead type indentation or flattening at the intersection of the test organism and *E. coli* ATCC 25922 within the zone of inhibition of the imipenem disc is considered positive for carbapenemase production.

2.5. Plasmid Curing

Plasmid curing experiment was carried out according to the procedures described by [12] using sodium dodecyl sulphate (SDS) as the curing agent. The isolates were incubated on nutrient broth at 37˚C for 24 hrs. This overnight culture was inoculated into 4.5 ml sterile nutrient broth, 0.5 ml of SDS was added. This was incubated for 48 hrs at 37˚C. Thereafter, 0.5 ml of the broth was added into 4.5 ml sterile nutrient broth. Incubation followed for another 24 hrs at 37˚C. Antimicrobial susceptibility test was again carried out. Results of antimicrobial resistance were interpreted as either plasmid or chromosomal borne, depending on if resistance was lost or not.

3. Result and Discussion

Beta-lactamase producing bacteria have properties of enzyme production and are currently increasing worldwide in the environment. A cocktail of different active compounds may be present in lower concentrations in hospital dumpsite than they are in therapy. Consistent, excessive dumping of these active compounds exerts pressure on bacterial evolution, creating minor modifications or mutations on an already existing gene leading to new genes that will further be a challenge to therapy.

Eighty-two gram negative bacteria (**Table 1**) were isolated from soils of hospital dump sites in some General and private hospitals located in Warri, Nigeria. All isolates were multi drug resistant. In addition, 100% resistance was observed in all isolate to ceftazidine, cefuroxime, Amoxicillin-clavulanic acid and Cefixime (**Table 2**). This reflects the likely antimicrobial compositions of bacterial clones carried by the population of the sampling area. Though this study did not correlate antibiotic usage in the different hospitals sampled with resistance of isolates obtained from their dumpsite.

Most studies have shown that the β-lactam antibiotics are the most common treatment for bacterial infections in hospital today [13] [14]. Production of β-lactamases are the major cause of resistance of bacteria to β-lactam antibiotics. β-lactamases such as extended spectrum β-lactamases (ESBLs) [15] [16] and carbapenemases such as metallo-beta-lactamases (MBLs) are usually hospital acquired [17] [18]. Most literature in Nigeria [19] [20] and all over the world [21] [22] has reported different prevalence levels of ESBLs and carbapenemases in hospitals. Organisms producing ESBLs and carbapenemases are known to have multiple resistance capability. These enzymes are able to hydrolyse all β-lactam antibiotics and are usually plasmid borne. These capabilities increase selection pressure and persistence.

To detect if resistance pattern was plasmid mediated, plasmid curing was carried out using sodium dodecyl sulfate (SDS). Result indicated that over 80% of the isolates harboured plasmids. The presence of antibiotic resistance genes on plasmids of pathogenic bacteria isolates has further helped in the fast spread of resistance among bacteria, causing diversity in plasmid profile, resulting in multi and pan drug resistant bacteria. This limits

Table 1. Distribution of isolates obtained from soil of hospital dumps it.

Isolate	No. of isolate (%)
E. coli	32 (39.02)
Serratia marcescens	20 (24.39)
Klebsiella pneumonia	14 (17.07)
Proteus mirabilis	10 (12.20)
Enterobacter aerogenes	6 (7.32)
Total	**82**

therapeutic options, puts a challenge for the present day clinician, and demands the development of more antibiotics which sadly are no longer readily produced. To overcome this situation, there should be real change. There should be proper policy for preventive medicine. Appropriate ways of managing hospital wastes should be adopted and enforced.

ESBLs production was observed in 31.71% while 14.63% of isolates carried MBLs genes (**Table 3**). This indicates that the *β*-lactamases have made their way out of the clinics and are now in hospital environment. However previous studies have isolated ESBLs in the community; from sachet water [23] from healthy human specimens of community individuals [24].

It is difficult to give valid epidemiological information on carbapenemases in different parts of the world. This information is dependent on investigators. Countries with active investigators have large number of reports though their countries may not necessarily have higher prevalence than others. However, high prevalence has been found in Greece [25], Italy [26], Turkey [27] and Israel [28]. The enzymes have been found in both hospital and environmental settings.

Currently carbapenemases in Enterobacteriaceae are mainly found in *K. pneumonia* and *E. coli*. This makes the whole situation more worrying. In this study, 25.00% of *E. coli*, 14.29% of *Klebsiella pneumonia* and 10.00% *Proteus mirabilis* isolates were MBLs producers. These isolates harboured plasmids. Most MBL genes are often associated with transferable plasmids and some of them constitute cassettes in class 1 integrons (VIM-, IPM- and GIM-) [29]. Integrons always carry resistance to sulphonamides [30]. All isolate positive for MBLs production in this study were resistant to trimethoprim-sulfamethoxazole. Thus the MBLs genes in this study were probably carried on integrons. Several reports dealing with integrons and gene cassettes have revealed their role in the spread of resistance [30]-[32]. The VIM-type enzyme appears to be the most prevalent. It was found repeatedly in western (Belgium, Germany, Greece, Italy, Portugal, Spain) and Eastern Europe (Croatia, Poland, Russia), United States, Latin America and Asia (China, India, Japan, Korea, Taiwan). This implies that bacteria of similar resistance genes may be rapidly spreading in clinics and are gradually translating into the environment.

Table 2. Resistance pattern of isolates obtained from hospital dump site.

Isolates (No.)	*E. coli* (32)	*S. marcescens* (20)	*K. pneumonia* (14)	*P. mirabilis* (10)	*E. aerogenes* (6)
NIT	18.75	50.00	14.29	20.00	33.33
CPR	6.25	20.00	78.52	20.00	0.00
CAZ	100.00	100.00	100.00	100.00	100.00
CRX	100.00	100.00	100.00	100.00	100.00
GEN	31.25	50.00	28.5	20.00	66.67
OFL	0.00	10.00	14.29	0.00	33.33
AMX-CLA	100.00	100.00	100.00	100.00	100.00
CXM	100.00	100.00	100.00	100.00	100.00
SXT	56.25	70.00	57.14	40.00	33.33
CTX	100.00	100.00	100.00	100.00	100.00
IMI	46.88	15.00	42.86	0.00	0.00

Nitrofurantoin (NIT), Ciprofloxacin (CPR), Ceftazidine (CAZ), Cefuroxime (CRX), Gentamicin (CN), Ofloxacin (OFL). Amoxicillin-Clavulanic acid (Amx-ClA), Cefixime (CXM), Cefotaxime (CTX), Trimethoprime -Sulfamethoxazole (SXT), Imipenem (IMI).

Table 3. Prevalence of *β*-lactamases in the isolates (% prevalence).

Isolate	No. positive for ESBLs	No. of isolates resistant to imipenem (zone of inhibition ≤ 23 mm)	No positive for MBLs
E. coli (32)	10 (31.25)	8 (25.00)	8 (25.00)
S. marcescens (20)	8 (40.00)	0 (0.00)	0 (0.00)
k. pneum (14)	4 (28.57)	12 (100.00)	2 (14.29)
P. mirabilis (10)	2 (20.00)	8 (60.00)	2 (10.00)
E. aerogenes (6)	2 (33.33)	0 (0.00)	0 (0.00)
Total 82	**26 (31.71)**	**28 (34.15)**	**12 (14.63)**

The potential of the *Klebsiella pneumoniae* carbapenemase (kpc)-positive Enterobacteriaceae and VIM producers isolates to spread outside hospital settings and to circulate in the community has been reported [33] [34] Varying prevalence figures of MBLs have been reported in Nigeria [34] yusuf and co-authors while working on clinical isolates reported 16.7% in *K. pneumoniae*, 16% in *Proteus* and 13.5% in *E. coli*. They also observed MBL producers in both hospital and community isolates [35]. Many metallo-betalactamase genes constitute reservoirs in the environment. During the course of evolution, some environmental bacteria (*B. cereus*, *B. anthracis*) produced metallo-enzymes to protect themselves against beta-lactams produced naturally by some soil-dwelling bacteria (*Streptomyces* sp.) or fungi. The dissemination of carbapenemase genes therefore proceeds in two directions: environmental sources may provide the genetic source of the enzyme, and clinical strains may spread these enzymes both within hospital and into the environment.

In the past, all ESBLs were sensitive to carbapenem such as imipenem or meropenem. It was actually used to define ESBLs. Carpabenems were the last resort for infections caused by ESBLs. This is changing as revealed in this study. Though a very low prevalence figure of ESBL resistant to the carbapenem, imipenem was observed, it is emerging. The control of spread is now or never because cumulative experience with ESBL supports the idea that once the prevalence goes beyond a critical level, their eradication from bacterial communities is nearly impossible. Bearing the frightening properties of metallo-enzymes in mind, it is important that a great effort should be made in pursuance of preventive medicine

It is necessary to consider the hospital immediate environment outside the hospital in dealing with issues bordering on nosocomial infection. Nosocomial infections should not only be looked at as infections whose development is enhanced by the inside of a hospital environment. A patient hospitalized for some time and later discharged still has a weak immune system and as such can be easily re-infected on discharge, if the immediate environment outside the hospital is polluted with MDR isolates. This kind of infection should be included as nosocomial infections. This may be the reason for many discharged patient to return back to the hospital after a couple of days. A better health care quality and minimizing nosocomial infections is achievable if we also pay attention to the immediate environment outside the hospital. Information on the health implications of improperly managed healthcare wastes should be provided. Additionally, the implementation of measures that will check the importation of carbapenemases from endemic countries is of utmost importance. It is equally important that all involved in the management of health; from medical clinicians to cleaners play a part in hospital waste management. There should be behavioural change by healthcare givers, patients and visitors. The cleaners in particular have fundamental roles to play in providing a hygienic and clinically clean hospital environment.

4. Conclusion

The irresponsible dumping of wastes around the dust bin was observed more in government owned hospital than in the private hospitals. This underlines the need to make governments, as well as governmental bodies on health matters aware of this irresponsibility, and the implications so that they work on it. This should be a matter of priorities. Local governments where these government hospitals are located should allocate more financial resources for effective disposal of hospital wastes. The development of a conducive atmosphere in a hospital environment is imperative.

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