Isolation and Characterization of Multi-drug Resistant Bacterial Pathogens from Hospital Effluents, South Eastern, Nigeria

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Abstract: This study was aimed to isolate and characterize public health important bacteria from waste water in hospital and evaluate the distribution of multiple drug resistance bacteria in the study area. The hospital waste water was taken from three different hospitals in Afikpo: Mater Misercordiae Hospital(MMH), Afikpo Medical Centre (AMC) and His Grace Hospital (HGH) between June and October 2015. Samples were aseptically collected, transported and processed within two hours following standard procedure. Identified organisms were assessed for different antibiotics following Kirby-Bauer disk diffusion method. A total of 60 wastewater samples were processed for the presence of drug resistant pathogens. Out of which 61 bacterial isolates were recovered. The most frequently identified bacterium was Pseudomonas spp 17 (27.9%) followed by E. coli 16(26.2%), Staphylococcus aureus 15(24.6%) and Salmonella spp 13(21.3%). The overall prevalence of multiple drug resistance (MDR) showed that all the bacterial isolates exhibited resistance to more than three (3) antibiotics, although their patterns of resistance varied. All isolated organisms were resistant to clindamycin and ampicillin. However, analysis of the performance of the different antibiotics against the isolated strains from different hospitals showed varied resistance profile. However, the most effective antibiotic was the quinolones where there was some level of susceptibility among many of the isolates. The presence of these multi-drug resistant strains from hospital waste waters could act as a vehicle to disseminate antibiotic resistant bacteria in the environment. Therefore, there is need for hospital wastewater to be treated before they are released into the environment.

Key words: Antibiotic • Afikpo • Multiple Drug Resistance • Waste Waters

INTRODUCTION

Antibiotics are used extensively to prevent or to treat microbial infections in human and animal beings; however uncontrolled and excessive use of antibiotics by human and animals results to an increase in antibiotic resistance and cause the spread of resistance genes in environmental samples such as hospital waste water [1]. Antibiotics exert a selection in favor of resistant bacteria by killing or inhibiting growth of susceptible bacteria; resistant bacteria can adapt to environmental conditions and serve as vectors for the spread of antibiotic resistance[2]. The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogen [2, 3]. The volume of antibiotics used in hospitals and private households and released into effluent and municipal sewage indicates a selection pressure on bacteria [4].

Hospital wastewater can be hazardous to public health and ecological balance since it can contain many kinds of pollutants such as radioactive, chemical and pharmaceutical wastes and high numbers of resistant bacteria and antibiotic residues at concentration able to inhibit the growth of susceptible bacteria [5]. Although sewage treatment processes reduce the numbers of bacteria in waste water the effluent will still generally contain large numbers of both resistant and susceptible bacteria [6]. Bacteria such as Bacillus, Staphylococcus and Streptococcus species, Pseudomonas aeruginosa and Candida albicans have been reported to be resistant to the commonly used antibiotic and as such have led to the outbreak of several diseases/infections [7].

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There is need to assess antibiotic susceptibility of microorganisms in our environment so as to be able to address the increasing problems of antibiotic resistance of microbes in human. This problem has been reported in various parts of the world, hence, various studies have evaluated the microbiological content of hospital effluents and their resistance factors to antibiotics quantitatively and qualitatively and found out that hospital effluents contain microorganisms with pathogenic potential for human comparable to household waste[7].

In view of the fact that no data have been reported about this within the study area. We proposed that due to their possible persistence in hospitals, multiple drug resistance (MDR) bacterial strains could be frequently found in untreated hospital wastewater (UHWW). To provide evidence for this hypothesis, we identified bacterial strains found in the untreated wastewater of selected hospitals in Afikpo area to determine if strains are capable of resisting common antibiotic in use in the hospital and also to confirm the fact that untreated hospital wastewaters contains MDR strains which are released into the environment.

**MATERIALS AND METHODS**

**Sample Collection and Procession:** A total of sixty (60) Hospital effluents used in this research work were collected aseptically from three major hospitals within Afikpo Metropolis, the hospitals are: Mater Misericordiae Hospital (MMH), His Grace Hopital (HGH) and Afikpo Medical Centre (AMC). With the aid of a sterilized sample container hospital samples were collected from the untreated wastewater outlet pipe of the selected hospitals before it enters the sewer system or discharged into the environment in the morning during the peak activities between 10.00 and 11:30 am. In brief, wastewaters were collected in 500 ml sterile microbiological containers mounted onto a handle of appropriate length. They were transported to the laboratory on ice and processed within 4 hours of collection.

**Microbiological Analysis:** Isolation and identification of isolates from the Hospital effluent samples were aseptically carried out using standard microbiological techniques as described by Cheesbrough [8]. The effluent samples were aseptically inoculated onto culture media namely: Salmonella-Shigella agar (for the isolation of Salmonella spp), Pseudomonas Cetrimide selective agar for the isolation of (Pseudomonas aeruginosa), Mannitol salt agar (for the isolation of Staphylococcus aureus) and Eosin methylene blue agar (for the isolation of Escherichia coli). The agar plates were incubated at 37°C for 18-24 hours. All the bacterial isolates were identified based on their colony morphology, Gram’s reaction, catalase test, motility test and biochemical tests such as oxidase test, citrate test, indole test, Methylred-VogesProksaüer (MRVP) test, urease test, hydrogen sulphide production test and sugar fermentation.

**Total Heterotrophic Bacterial Counts:** This was determined with the nutrient agar using the spread plate technique as described by Cheesbrough [8]. Here 0.1 ml of the serially diluted samples was each inoculated onto different sterile nutrient agar plates in triplicates. The plates were incubated for 24 hours at 37°C. After incubation, colonies that appeared on the plates were counted and the mean expressed as CFU/ml.

**Antibiotic Susceptibility Test:** Bacteria isolates were subjected to in-vitro susceptibility test against commonly used antimicrobial agents using disk diffusion method following guidelines established by the Clinical and Laboratory Standards Institute [9]. In brief, by taking pure isolated colony, bacterial suspension was adjusted to 0.5 McFarland turbidity standards. The diluted bacterial suspension was then transferred to Mueller-Hinton agar plate using a sterile cotton swab and the plate was seeded uniformly by rubbing the swab against the entire agar surface and the plates were left at room temperature for 30 min and then antibiotic disks were applied to the surface of the inoculated plates using sterile forceps. The plates were then incubated aerobically at 37 °C for 24 h. Finally, the zone of inhibition was measured including the disk diameter. The susceptible, intermediate and resistant categories were assigned on the basis of the critical points recommended by the CLSI and according to the manufacturer’s leaflet attached to the disks. The used antibiotic discs contained; Erythromycin (10µg), Ceftriaxone (30µg), Ampicillin (5µg), Cefixime (5µg), Levofoxacin (5µg), Norfloxacaps (10µg), Ciprofoxacaps (5µg), Gentamicin (10µg), Ofloxacin (10µg) and Clindamycin (10µg). These antibiotics were chosen because they are either used in both human medicine and animal veterinary practice or because previous studies have reported microbial resistance to them.

**RESULTS**

A total of 60 wastewater samples were processed for the presence of drug resistant bacterial pathogens.
Among the total samples 61 bacterial isolates were recovered from the hospital effluents. Most frequently isolated bacterium was *Pseudomonas aeruginosa*, 17 (27.9%) followed by *Escherichia coli* 16 (26.2%), *Staphylococcus aureus* (24.6%), while the least number of isolates were *Salmonella* spp (21.3%) (Table 1). Also, it was observed that the highest number of isolated bacteria was from Mater Misericordiae Hospital (MMH), with twenty six (26) isolates followed by Afikpo Medical Centre (AMC), with eighteen isolates (18), while the least isolates was obtained was from His Grace Hospital (HGH), seventeen isolates (Table 1).

The results of the mean heterotrophic bacteria count obtained from the three hospitals are as follows: Mater Misericordiae hospital, had the highest number of bacterial counts with $4.25 \times 10^6$ CFU/mL followed by Afikpo Medical Centre, $1.86 \times 10^6$ CFU/mL, while the least number of counts were recorded from His Grace hospital, $2.80 \times 10^5$ CFU/mL (Table 2).

The results of the antibiotics susceptibility test showed that all the bacterial isolates exhibited resistance to more than three (3) antibiotics as seen in Fig.1, 2&3, although their patterns of resistance varied. All isolated organisms were completely resistant to clindamycin and 75% resistant to ampicillin. However, analysis of the performance of the different antibiotics against the isolated strains from the different hospitals showed varied degree of resistances. Among the isolates from Afikpo Medial Centre, there was 100% resistance to clindamycin, 75% resistance to ampicillin and gentamicin, 0% resistance was observed in ceftriaxone and ofloxacin, 0% in ceftriaxone, levofloxacin and norfloxacin, while the most effective antibiotic was erythromycin which had 0% susceptibility (Fig. 1).

Among the isolates from His Grace Hospital, the most effective antibiotics were the fluoroquinolones (levofloxacin and ciprofloxacin) where there was 100% susceptibility, however there was different resistance pattern as observed from the data obtained, 0% resistance was seen among the ceftriaxone and ampicillin, 50% resistance in ceftriaxone and clindamycin and 25% resistance among the gentamicin and erythromycin (Fig. 2). In Mater Misericordiae Hospital, the isolated strains showed similar pattern of resistances as observed in the other two hospitals, while levofloxacin is the most effective antibiotic against the isolates (Fig. 3).

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>MMH</th>
<th>AMC</th>
<th>HGH</th>
<th>Total (%)</th>
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<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>15 (24.6)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>17 (27.9)</td>
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<tr>
<td><em>Escherichia coli</em></td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>16 (26.2)</td>
</tr>
<tr>
<td><em>Salmonella</em> spp</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>13 (21.3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>26</td>
<td>18</td>
<td>17</td>
<td>61 (100)</td>
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</table>

KEY: MMH = Mater Misericordiae Hospital; AMC = Afikpo Medical Center; HGH = His Grace Hospital

<table>
<thead>
<tr>
<th>S/N</th>
<th>Sample point/location</th>
<th>colony count (CFU/mL) ($\times 10^6$)</th>
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<tbody>
<tr>
<td>1</td>
<td>MMH</td>
<td>4.25</td>
</tr>
<tr>
<td>2</td>
<td>AMC</td>
<td>1.86</td>
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<tr>
<td>3</td>
<td>HGH</td>
<td>2.80</td>
</tr>
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Fig. 1: Antibiotic susceptibility pattern of bacteria isolates from Afikpo Medical Centre  
Key: LEV= Levofloxacin; NFX= Norflaxacin; CIP= Ciprofloxacin; GNT= Gentamicin; OFC= Ofloxacin; CLD= Clindamycin; ERY= Erythromycin; CET= Ceftriaxone; CIF= Cefixime; AMP= Ampicillin

Fig. 2: Antibiotic susceptibility pattern of bacteria isolates from HIS GRACE Hospital.  
Key: LEV=Levofloxacin; NFX= Norflaxacin; CIP= Ciprofloxacin; GNT= Gentamicin; OFC= Ofloxacin; CLD= Clindamycin; ERY= Erythromycin; CET= Ceftriaxone; CIF= Cefixime; AMP= Ampicillin
of detection of pathogenic bacteria in this study might be Enterobacter cloacae, Citrobacter spp and Klebsiella pneumoniae from hospital waste water were spectrum beta-lactamase (ESBL) producing isolates reported that the most common multi-resistant extended effluents respectively. Also, study in Rio de Janeiro, Brazil bacteria such as Enterobacteriaceae Enterobacter cloacae and E. coli[13]. The high frequency of detection of pathogenic bacteria in this study might be due to the admission of cases with these bacterial infections, which are common in developing countries. Also the high number of pathogenic bacteria obtained from Mater Misericordiae hospital might likely be as a result of the high number of patients that the hospital received, this is because hospitals present an environment for a concentrated source of resistant bacteria, which may be released into the environment or sewer system.

The result of the antibiotics susceptibility studies showed that all the bacterial isolates exhibited resistance to more than three (3) antibiotics as seen in Figs.1, 2&3, although their patterns of resistance varied. All isolated organisms were completely resistant to clindamycin and 75% resistant to ampicillin. The study finding is consistent with previous reports by other authors such as [11, 12] where multiple drug resistance was common in Gram negative isolates such E. coli, Klebsiella spp, Citrobacter spp and Enterobacter spp recovered from Ethiopian hospitals. However, direct comparison of study findings with those of other scholars was difficult because of differences in types of antibiotics used, types of wastewater samples collected and types of organisms isolated. The susceptibility pattern of the antibiotics against Pseudomonas aeruginosa revealed that the organism was resistant to ampicillin, cefixime, gentamicin and clindamycin. Pseudomonas spp. is the main cause of nosocomial infections causing morbidity and mortality and these infections are difficult to eradicate. There is a global emergence of multidrug resistant strains of Pseudomonas and they are naturally resistant to many antibiotics. Their antimicrobial susceptibility is limited to only a few drugs and the emergence of resistance during therapy against initially susceptible strains occurs at relatively high frequency[11].

Multiple drug resistance was equally observed among the Grampositive isolates like S. aureus in the study area. All isolates of S. aureus were resistant to ampicillin (100%), while there were various degrees of resistances among other antibiotics like ceftriaxone, cefixime and gentamicin. This is in line with other studies that reported that the organism is resistant to antiseptics, disinfectants and antibiotics and survives in the sewage system for long periods of time [10, 14& 15]. Also Abulreesh [16] contended that multidrug resistant staphylococci (S. aureus and coagulase negative Staphylococci) have been a common problem and recovered from diverse environmental sources, such as drinking water supplies, foodstuffs, the mucosa of humans and farm animals and hospital environments which can be important public health concern.

DISCUSSIONS

Antibiotic resistance is a major public health threat and the presence of resistant organisms in environmental waters is an emerging concern around the world. In Nigeria, the challenge of antibiotic resistance has been a major public health issue resulting to high cost of medical treatment, therapeutic failures and economic loss to the nation. In view of these challenges, the present study was an attempt to investigate the incidence of antibiotic resistant bacteria from hospital effluents samples of major hospitals and clinics within Afikpo area.

The result of the present study showed the presence of pathogenic bacteria such as Pseudomonas aeruginosa, S. aureus, E. coli and Salmonella spp as indicated in Table 1. In the present study frequently identified bacterium was Pseudomonas spp. 17(27.9%) followed by Escherichia coli. 16(26.2%), Staph. aureus 15(24.6%) and Salmonella spp 13(21.3%), Similar results have been reported in Nigeria and elsewhere; Ekhaide and Omavwoya [10] in Benin hospital showed that the bacterial genera, Klebsiella, Pseudomonas and Serratia were the most frequently distributed isolates in the hospital wastewater. While similar study conducted by Moges et al. [11] and Fekadu et al. [12] from hospitals in Ethiopia reported similar high prevalence of pathogenic bacteria such Klebsiella spp, Pseudomonas spp, E. coli, Citrobacter spp and Staph. aureus from hospital effluents respectively. Also, study in Rio de Janeiro, Brazil reported that the most common multi-resistant extended spectrum beta-lactamase (ESBL) producing isolates from hospital waste water were Klebsiella pneumoniae, Enterobacter cloacae and E. coli[13].

![Fig. 3: Antibiotic susceptibility pattern of bacteria species from Mater Misericordiae Hospital](Image)

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<table>
<thead>
<tr>
<th>Antibiotic</th>
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<tr>
<td>ERY</td>
<td>30%</td>
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<tr>
<td>AMP</td>
<td>40%</td>
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<tr>
<td>CET</td>
<td>50%</td>
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<td>CIF</td>
<td>55%</td>
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In view of these findings, there is a need for urgent measures to address antibiotic resistance in Nigeria. The high number of pathogenic bacteria obtained from Mater Misericordiae hospital might likely be as a result of the high number of patients that the hospital received, this is because hospitals present an environment for a concentrated source of resistant bacteria, which may be released into the environment or sewer system.

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The susceptibility pattern against E. coli revealed that the organism was resistant to ampicillin and the quinolones (norfloxacin, levofloxacin, ciprofloxacin and ofloxacin). A similar study conducted in Bangladesh showed that all E. coli isolates were multidrug resistant to the antibiotics used against them [17]. Antibiotics such as Ciprofloxacin, Ofloxacin belonging to Quinolones family are considered to be best medicines in case of MDR infections and have broad spectrum effects because of the mechanism of its action and less prone to development of resistance by bacteria unless there is development of MDR efflux pump [18,19]. However, the volume of antibiotics used in hospitals and private households released into effluent and municipal sewage and the indiscriminate use of antibiotics by individuals at the present time naturally causes selective pressure on bacteria and consequently causing drug sensitive organisms becoming multidrug resistant [20].

The result of the susceptibility pattern of the Salmonella spp showed similar pattern as observed in E. coli strains, the bacteria recovered from the three hospitals were resistant to the quinolones, gentamicin and clindamycin. Salmonella has been reported to be an environmentally persistent pathogen capable of surviving and proliferating in diverse environments [21]. Previous scholars have reported multiple antibiotics resistance of Salmonella. A study conducted in California cities showed that a set of Salmonella serovars were resistant to multiple antibiotics, with many serovars sharing the resistant phenotypes [22].

In a study of this nature there were limitations the researcher encountered, some of them are: wastewater samples was taken over a short period of time and may not have depicted seasonal variation and the number of isolates must have been below the expected number. Secondly, because only wastewater was used, the study was unable to differentiate the source of resistant bacteria (clinical isolates or sewage system) and the study failed to select all antimicrobial agents commonly used for resistance evaluation due to the fact that some of them were not available during the period of study.

CONCLUSIONS

The present investigation was able to prove the existence of some antibiotic resistant bacteria (Salmonella spp., Pseudomonas spp., S. aureus and Escherichia coli) from hospital wastewater from the study area. There were differences in the detection of pathogenic bacteria in the 3 hospitals, with higher frequency in the Mater Misericordiae effluents. In conclusion, the presence of antibiotic resistant organisms in this waste water should not be overlooked. Wastewaters from hospital effluents should be properly treated before they are discharged into the environment.

REFERENCES


