MULTIDRUG RESISTANCE PROFILES OF CLINICAL ISOLATES OF PSEUDOMONAS AERUGINOSA AND ESCHERICHIA COLI OF CLINICAL ORIGIN

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ABSTRACT

The indiscriminate use of antibiotics contributes to the dissemination of multiple antibiotic resistances in bacterial pathogens in the community and hospital environment; and this development is of serious public health importance. This study was conducted to determine the incidence of Pseudomonas aeruginosa and Escherichia coli in certain clinical samples as well as to determine their susceptibility patterns to some commonly used antibiotics. The organisms were isolated using standard microbiological techniques and the antibiotic susceptibility study was determined using the Kirby-Bauer disc diffusion method as per the guidelines of Clinical Laboratory Standard Institute (CLSI). The result of this studies showed that most of the clinical isolates were highly resistant to amoxicillin and amoxycillin-clavulanic acid. It was also observed that these clinical isolates showed least resistance to gentamicin, ofloxacin, and ciprofloxacin. There was a significant difference (P≥ 0.05) in the percentage resistance patterns between the clinical isolates of P. aeruginosa and E. coli. Eleven isolates that were resistant to more than one antibiotic were subjected to
plasmid curing using 1 % and 5 % sodium dodecyl sulphate (SDS). It was observed that at treatment with 1 % SDS, some of the isolates became resistant to more than one antibiotic. But when SDS was increased to 5 %, some of the isolates that were resistant became completely sensitive to all the antibiotics used. However, one of the *P. aeruginosa* that was initially sensitive to chloramphenicol became completely resistant at 5 % SDS and another isolate of *P. aeruginosa* that was initially sensitive to septrin, sparflinoxacin and ciprofloxacin became completely resistant at 1 % and 5 % SDS. Conclusively, the clinical isolates of *P. aeruginosa* and *E. coli* used in this study were multiply resistant to some commonly used antibiotics; and the transfer of resistance gene amongst organisms could be responsible for the growing development and spread of resistance in this environment.

**KEYWORDS:** Multiple antibiotic resistance, *Pseudomonas aeruginosa, Escherichia coli*, Plasmid curing, Antimicrobial susceptibility testing

**INTRODUCTION**

Microbial resistance to antimicrobial agents has resulted in morbidity and mortality from treatment failures of some infectious diseases and this development has also increased the health care costs of patients especially in developing economies like Nigeria. Appropriate use of antimicrobial agents coupled with proper susceptibility test results prior to the prescription of antibiotics to sick patients has unquestionable benefit – since antimicrobial susceptibility testing’s gives physicians a clue as to which drugs to use for a given ailment. Nevertheless, the general public and physician’s alike still use these agents inappropriately. Antibiotic resistance genes in most bacteria are frequently found in extra chromosomal elements such as plasmids; and these genetic materials aid in the transfer of resistance genes amongst bacterial population. *Pseudomonas aeruginosa* is naturally resistant to many of the widely used antibiotics, so chemotherapy is often difficult (Dubois *et al.*, 2001, Khan and Malik, 2001). *P. aeruginosa* is a ubiquitous bacterium that is found in water, soil, on plants and it has been implicated in wound infection (Banerjee and Stableforth, 2000; Oteo *et al.*, 2002). Antibiotic resistant bacteria are widespread; and several antibiotic resistant genes can be carried by genetic elements like plasmids – which transfer these genes to susceptible bacteria. *Escherichia coli* are Gram negative bacterium; and its natural environment is the gastrointestinal tract of warm-blooded animals (Alhaj, 2007; Von and Marre, 2005; Poole, 2004). Antibiotic resistant *E. coli* may pass on the genes responsible for antibiotic resistance to other species of bacteria through horizontal gene transfer. It has been observed that
antibiotic susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment (Salyers et al., 2004). *E. coli* are one of the major opportunistic pathogen in neonatal and immune-compromised patients where it is implicated in a variety of bacterial infections (Annette, 1998; Raina et al., 1999; Okeke et al., 2000; Olowe et al., 2003; Tobih et al., 2004). Infections with antibiotic resistant bacteria make the therapeutic options for treatment extremely difficult (El-Astal, 2004). Therefore, the determination of antimicrobial susceptibility patterns of clinical isolates is often crucial for optimum treatment of infected patients because such protocols will guide physicians on the proper type of drugs to administer to a given patient. Occurrence and prevalence of these resistant strains in the environment is more common in developing countries; and this is due in part to the indiscriminate use of drugs and the possibility of obtaining antibiotics over-the-counter even without a doctor’s prescription (Bataineh et al., 2007; Hernandez et al., 1997; Berrouane et al., 2000; DuBois et al., 2001). *P. aeruginosa* populations are biofilm forming in nature; and this contribute to their antibiotic resistance prowess in the hospital environment (Cornelis, 2010). In view of this, this present study determined the incidence and antimicrobial susceptibility patterns of *P. aeruginosa* and *E. coli* isolates of clinical origin.

**MATERIALS AND METHODS**

**Sample site:** A total of 170 clinical samples were examined, 140 samples of urine (from in- and out-patients with urinary tract infection), and 30 wound swabs (from patients with wound, burns or surgical patients). These specimens were collected from both urban and rural areas of Nsukka, Enugu State, Nigeria. Urine specimens were aseptically collected with sterile containers. Wound specimens were collected with sterile swab sticks.

**Isolation and characterization of bacteria isolates:** Urine samples were mixed thoroughly by inverting the containers several times. Using a sterile wire loop, the samples were inoculated on MacConkey agar and Cystein lactose electrolyte deficient (CLED) medium (Oxoid, UK), and incubated at 37°C for 24 hrs. The swab sticks were inoculated into tubes of nutrient broth and incubated at 37°C for 24 hrs. Ten-fold serial dilutions of the culture broth were prepared. Diluents were plated out on MacConkey agar and Cystein lactose electrolyte deficient (CLED) medium (Oxoid, UK), using the spread plate method. The plates were incubated at 37°C for 24 hrs. Suspected colonies were further purified to obtain pure cultures. The pure isolates were stored on agar slants for further use. Pure cultures of *E. coli* and *P. aeruginosa* isolates were identified biochemically using conventional identification
techniques including oxidase test, sugar fermentation, indole, citrate, catalase, methyl red test, voges-proskauer test and Gram staining.

**Antibiotic susceptibility test:** The antimicrobial susceptibility test was carried out by the Kirby-Bauer disk diffusion method in line with the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (now Clinical Laboratory Standard Institute, CLSI) (NCCLS, 2004). The antibiotics used include: amoxicillin-clavulanic acid (30 µg), Gentamicin (10 µg), Pefloxacin (30 µg), Ofloxacin (10 µg), Streptomycin (30µg), Chloramphenicol (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (10 µg), Amoxacillin (30µg), septrin (30 µg) (Oxoid, UK). Pure cultures of the bacterial isolates standardized to 0.5 McFarland turbidity standards were aseptically streaked on Mueller Hinton Agar plates using sterile cotton swab sticks. The antibiotic disks were aseptically placed on the MH agar plates; and these were incubated at 37°C for 24 hrs. The inhibition zone diameters were measured to the nearest millimeter using meter rule (NCCLS, 2004).

**Plasmid curing:** Resistance curing was conducted on multidrug resistant isolates. This was done to determine whether the gene coding for resistance is carried in the chromosomes or plasmids. Plasmid being an extra chromosomal DNA molecule is eliminated from host bacteria after exposure to sub-lethal concentrations of intercalating agents such as Acridine orange, Ethidium bromide and detergents such as sodium dodecyl sulphate (SDS). The curing agent used in this work was SDS. The experiment was done according to a previously used methodology (Tomoeda, 1968).

**Use of sodium dodecyl sulphate (SDS):** Two concentrations (1% and 5%) of SDS in nutrient broth were used in this experiment. Nutrient broth was prepared and supplemented with 1 g of SDS in one batch of 99 ml and 5 g of SDS in the second batch of 95 ml to achieve a final concentration of 1 % and 5 % (w/v) SDS respectively. It was then sterilized by autoclaving at 121°C for 15 min. Selected overnight cultures of isolates were standardized to 0.5 McFarland turbidity standards using sterile saline. From these, 0.1 ml of each culture was inoculated separately into 5 ml of SDS supplemented nutrient broth in test tubes and incubated at 37°C for 24 h. After incubation, cultures were standardized and spread on Mueller Hinton agar and susceptibility testing was carried out on each of the cured isolates.

**Statistical analysis:** The data obtained was analyzed using one way and two way analysis of variance (ANOVA).
RESULTS

*E. coli* and *P. aeruginosa* were isolated from wound and urine using standard bacteriological procedure. The results of the number of isolates from Urban and Rural hospitals are shown in Table 1.

Table 1: Isolates from samples from urban and rural hospitals

<table>
<thead>
<tr>
<th>Site</th>
<th>Sample</th>
<th>No. of samples</th>
<th><em>E.coli</em></th>
<th><em>P.aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban hospital</td>
<td>Urine</td>
<td>70</td>
<td>43(61.4%)</td>
<td>30(42.9%)</td>
</tr>
<tr>
<td></td>
<td>Wound</td>
<td>15</td>
<td>7(46.7%)</td>
<td>8(53.3%)</td>
</tr>
<tr>
<td>Rural hospital</td>
<td>Urine</td>
<td>70</td>
<td>37(52.9%)</td>
<td>24(34.28%)</td>
</tr>
<tr>
<td></td>
<td>Wound</td>
<td>15</td>
<td>4(26.7%)</td>
<td>6(40%)</td>
</tr>
</tbody>
</table>

Figure 1 shows the antibiotic resistance patterns of *E. coli* and *P. aeruginosa* isolated from urine samples from urban hospital. The results showed that both *E. coli* and *P. aeruginosa* exhibited the highest resistance to septrin with percentage resistance of 83.7 % and 90 %, respectively. *E. coli* showed the least resistance to ciprofloxacin with percentage resistance of 41.9 % while *P. aeruginosa* showed least resistance to Sparfloxacin with percentage resistance of 23.3%. The results of the antibiotic resistance of the wound isolates from urban hospital to different antibiotics are presented in Figure 2. The isolates showed 100 % resistance to amoxicillin-clavulanic acid and amoxicillin. *E. coli* also showed 100 % resistance when chloramphenicol and perfloxacin were used. *P. aeruginosa* showed 87.5 % resistance when perfloxacin was used and also showed high resistance to septrin and chloramphenicol with percentage resistance of 87.5 % and 75 %, respectively.

*E. coli* showed the least resistance to gentamicin, ofloxacin and streptomycin with percentage resistance of 16.7 % for each of them. *P. aeruginosa* exhibited the least resistance when Streptomycin was used with percentage resistance of 12.5 %. The result showed there was a significant difference (p≥0.05) in the percentage resistance exhibited by the isolates to the different antibiotics used.
Figure 1: Percentage antibiotics resistance of urine isolates from urban hospital.

**Key:** AU-augmentin (amoxicillin-clavulanic acid), CN-gentamycin, PEF-perfloxacin, OFX-ofloxacin, S-streptomycin, SXT-septrin, CH-chloramphenicol, SP-sparfloxacin, CPX-ciprofloxacin, AM-amoxicilin

Figure 2: Percentage antibiotics resistance of wounds isolates from urban hospital.

**Key:** Au-augumentin,CN-gentamycin,PEF-perfloxacin,OFX-ofloxacin,S-streptomycin,SXT-septrin,CH-chloramphenicol,SP-sparfloxacin, CPX-ciprofloxacin, AM-amoxicilin

The result of antibiotics resistance of urine and wound isolates from rural hospital is shown in Figure 3 and Figure 4.
In Figure 3, the result of antibiotics resistance by the urine isolates is presented. The result showed that *E. coli* has the highest resistance to Amoxacillin with percentage resistance of 67.6 % while *P. aeruginosa* recorded the highest resistance when Amoxacillin is used with percentage resistance of 83.3 %, *E. coli* also recorded 62.2 % resistance when Augmentin is used while *P. aeruginosa* recorded 79.2 % when each of Augmentin (amoxicillin-clavulanic acid) and Perfloxacin is used. *E. coli* and *P. aeruginosa* recorded the least resistance when Septrin is used with percentage resistance of 10.8 % and 12.5 %, respectively. Figure 4 showed the resistance of isolate from wound to different antibiotics. Both of the isolates recorded 100 % resistance to Augmentin and Amoxicillin, *P. aeruginosa* also recorded 100% resistance to perfloxacin while *E. coli* recorded 75% to the same Perfloxacin. *E. coli* recorded least resistance to Gentamicin, Ofloxacin, Septrin, Streptomycin and Chloramphenicol with each having percentage resistance of 25%, while *P. aeruginosa* recorded the least resistance to Septrin, Gentamycin and Streptomycin with each having percentage resistance of 33.3%.

The result showed that there is significant different (P≤ 0.05) between the resistance of the isolates from urine and the isolates from wound.

![Figure 3: Percentage antibiotics resistance of urine isolates from rural hospital.](image)

AU-augumentin,CN-gentamycin,PEF-perfloxacin,OFX-ofloxacin,S-streptomycin,SXT-septrin,CH-chloramphenicol,SP-sparfloxacin,
CPX-ciprofloxacin, AM-amoxacilin
Figure 4: Percentage antibiotics resistance of wound isolates from rural hospital.

AU-augmentin,CN-gentamycin,PEF-perfloxacin,OFX-ofloxacin,S-streptomycin,SXT-septrin,CH-chloramphenicol,SP-sparfloxacin,
CPX-ciprofloxacin, AM-amoxacilin

The effect of SDS mediated plasmid curing on antibiotic resistance pattern of *E. coli* and *P. aeruginosa* is also evaluated. The results are presented in Table 2. The result showed that there is no significant difference ($P \leq 0.05$) in the effect of SDS mediated plasmid curing when their resistance patterns were compared.

### Table 2: effect of sds mediated plasmid curing on resistant bacteria isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Resistance</th>
<th>Effect of curing at 1% concentration</th>
<th>Effect of curing at 5% concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>Resistant to all</td>
<td>All resistance except CN, S</td>
<td>Sensitive to all</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Resistant to all except S</td>
<td>Resistant to all except CN, S, OFX</td>
<td>All sensitive</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Resistant to all</td>
<td>Resistant to all except CN, S</td>
<td>Sensitive CN, S</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Resistant to all CH,</td>
<td>All resistance except CN, S, OFX, CH</td>
<td>All resistance except CN, S, OFX, CH</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Resistant to all CN, OFX,CH</td>
<td>All resistant except CN, OFX, S</td>
<td>All sensitive</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>All resistant except CN, OFX, S</td>
<td>All resistant except CN, OFX, S, CPX</td>
<td>All sensitive</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>All resistant except CN, S</td>
<td>All resistant except CN, OFX, S</td>
<td>All resistant except CN, OFX, S</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>All resistant except CN</td>
<td>All resistant except CN, OFX, S</td>
<td>All resistant except CN, OFX, S</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>All resistance except</td>
<td>All resistance except</td>
<td>All resistance except</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th></th>
<th>CPX</th>
<th>CN,S,CPX</th>
<th>CN,OFX,S,CPX</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>All resistance except CN,OFX,CH</td>
<td>All resistance except CN,OFX,CH</td>
<td>All resistant except CN,OFX,S,CH</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>All resistance except S,CH</td>
<td>All resistance except to CN,OFX, S,CH</td>
<td>All resistance except CN,OFX,S</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Multiple antibiotic resistances in bacteria population is currently one of the greatest challenges in the effective management of infections. In this study, several clinical samples were examined for the presence of multidrug resistance *E. coli* and *P. aeruginosa*; and the effect of SDS mediated plasmid curing on the antibiotics resistance potential of the isolates were also evaluated using 1% and 5% concentrations of SDS. The result of this showed that there is higher prevalence of *E. coli* in urine than wound. But when wound was analyzed there was higher prevalence of *P. aeruginosa* recorded. This agreed with the findings of Anuratha *et al.*, 2008 who reported that *P. aeruginosa* was the most frequent of isolated from burn wound infections. The isolates were tested for resistance to different antibiotics; and the result showed that *E. coli* isolated from urine from urban hospital showed higher resistance to Septrin with percentage resistance of 41.9%. This isolate also showed higher resistance to Amoxicillin and Augumentin (amoxicillin-clavulanic acid) with percentage resistance of 76.7% and 72.1% respectively. *P. aeruginosa* recorded highest resistance when Amoxicillin was used and least resistance when Sparfloxacin was used. The result showed that there is a significant difference (p≤ 0.05) when the resistance of the Isolates to different antibiotics are compared. The isolates showed some degree of resistance to all the antibiotics. This agrees with the result of Manikandan *et al.*, 2011 which reported multidrug resistance amongst uropathogens. This is also in line with the result of Uchenna, 2005 who reported multidrug resistance in Gram-negative bacteria. The evaluation of resistance of isolates of wounds from urban hospital also indicated different degrees of resistance to the antibiotics. *E. coli* showed complete resistance to Augumentin, Perflxacin, Chloramphenicol and Amoxicillin with percentage resistance of 100%. There is a significant difference (p≤ 0.05) in the percentage resistance by the isolates. These isolates recorded the least resistance when Streptomycin is used against *E. coli* and *P. aeruginosa*. Harbottle *et al*; 2006 reported that the overuse of antibiotics has become the major factor for the emergence and dissemination of multi-antibiotics resistance strain of several pathogenic bacteria. In a different study, Gehan *et al.*, (2011) reported complete resistance to Amoxicillin by *P. aeruginosa*. The resistance pattern of the urine isolates from rural hospital was not indifferent from the ones from urban...
hospital – as they also displayed varying degrees of resistance to different antibiotics. The results showed that there is no significant difference (p≥ 0.05) in the percentage resistance when the isolates from urban and rural urine were compared. This result agrees with the findings of Olowe et al, 2008 who reported multidrug resistance by *E. coli*. Fred C., (2006) also reported multidrug resistance by *P. aeruginosa*. This study also investigated the resistance of wound isolates from rural hospital to different antibiotics. All the isolates showed different levels of resistance to the antibiotics. *E. coli* showed complete resistance to Augumentin and Amoxacillin while *P. aeruginosa* showed complete resistance to Augumentin, Pefloxocin and Amoxacillin. This agrees with the work of Iheanyi et al., (2009) who reported 100% resistance to several antibiotics by bacteria isolates. *E. coli* also showed low resistance to Septin, Ofloxacin, Gentamicin and Chloramphenicol with each of the antibiotics having percentage resistance of 25%. Eleven isolates of *E. coli* and *P. aeruginosa* that were resistant to at least seven antibiotics were subjected to plasmid curing. The result showed that when the isolates were treated with 1% SDS, some of the isolates become susceptible to most of the antibiotic, but when it was increased to 5% SDS, the number of organisms that was susceptible increased but it was also noticed that some of the bacteria become resistant at this high increase. This result showed that some of the bacteria had resistant plasmid which can promote the transfer of resistance to other susceptible bacteria. Conclusively, this study show that *E. coli* and *P. aeruginosa* isolates of clinical origin are multidrug resistant; and that they possess plasmids through which resistance traits of bacteria can be exchanged between one organism and another. There is need for instituting an antimicrobial resistance surveillance system that provides clinician with up to date data on the prevalence and resistance of commonly encountered bacterial pathogens (inclusive of *P. aeruginosa* and *E. coli*) in our hospitals – so that antibiotic usage in these settings can be properly guided.

REFERENCES


