Resistance of Clinical Isolates to Generation of Cephalosporins in a Tertiary Hospital in Ogbomoso, South-Western Nigeria

E.K. Oladipo, J.O. Ogunsola, B.S. Akinade and E.H. Awoyelu

INTRODUCTION

Antimicrobial drugs vary considerably in their range of effectiveness. Many are narrow-spectrum drugs; they are effective only against a limited variety of pathogens. Others are broad-spectrum drug that attacks many different kinds of pathogens.

Commonly used antibiotics include the penicillin, cephalosporins, aminoglycosides, chloramphenicol, tetracyclines, polymyxins and erythromycin and the common synthetic antimicrobials are the sulphonylamides, trimethoprim and nalidixic acid (Ochei and Kolhatkar, 2007). Cephalosporins are grouped into generation by their antimicrobial properties, categorized chronically and are therefore divided into first, second and third generation. Each newer generation
of cephalosporin has greater Gram negative antimicrobial properties than the preceding
generation. The later generation of cephalosporin has greater effect against resistant bacteria
(Forbes et al., 1998; Cheesbrough, 2006). Cephalosporins are used to treat, pneumonia, strep
throat, staph infections, tonsillitis, bronchitis, otitis media, various types of skin infections and
gonorrhea. Cephalosporins are closely related to the penicillin and have a bactericidal effect by
inhibiting the synthesis of the bacteria cell wall (Forbes et al., 1998; Cheesbrough, 2006). The
classification of cephalosporins into generations is a common practice although the exact
categorization is often imprecise.

Resistance of Gram negative bacteria to cephalosporins as with other beta-lactam antibiotics
is a function of a site (penicillin-binding proteins). Permeation through the outer-membrane is
largely governed by the presence and properties of porins which are water filled channels
facilitating the movement of hydrophilic molecules across the membrane. The properties of porins
vary considerably between wild-type bacteria species and their members (and hence the ability of
a bacterial cell to exclude antibiotics) may be reduced in strains with acquired resistance. In the
case of cephalosporin, ability to cross the outer membrane is related to physiochemical properties
such as molecular size, hydrophobicity and the number and charge of ionized group. Thus, for
example, permeability rate than dipolar cephalosporins. The phenotypically expressed
susceptibility of a particular bacterial strains to cephalosporin is brought about by a dynamic
combination of permeation, the ability of the agent to resist degradation of binding to the
beta-lactamase in the periplasmic space which act upon the relatively low concentration of
cephalosporin present their and target affinity (Pfeifer et al., 2010). Antimicrobial resistance has
been reported worldwide and increasing rates of resistance among clinical isolates is a great
concern in both developed and developing countries. A rise in bacteria resistance to antibiotics
complicates treatments of infections. Because of the prevailing antibiotics resistance in
microorganisms, broad spectrum cephalosporins are used empirically and specifically in both
developed and developing countries. Therefore, the study was designed to determine the
antimicrobial susceptibility pattern of selected clinical isolates to cephalosporin using different
antibiotics in various generations.

MATERIALS AND METHODS
Study area: This study was carried out in Ogbomoso, Oyo State, South Western part of Nigeria,
in order to determine resistance of clinical isolates to cephalosporins among the patients at Ladoke
Akintola University Teaching Hospital Ogbomoso and Bowen University Teaching Hospital
Ogbomoso, Oyo State, Nigeria over a period of five months (March to August, 2013).

Bacterial isolates: A total number of 105 isolates made up of 31 Pseudomonas aeruginosa, 31
Escherichia coli, 19 Proteus mirabilis and 24 Klebsiella spp., isolated from different clinical
specimens including urine, blood culture, wound swab, eyes swab, ear swab, high virginal swab,
abscess, catheter tips, aspirate, sputum and cerebrospinal fluid were used for this study. These
samples were collected from both Universities with 90 isolates from Ladoke Akintola University
Teaching Hospital Ogbomoso and 18 isolates from Bowen University Teaching Hospital Ogbomoso
at the Department of Medical Microbiology and Parasitology.

Identification of bacterial isolates: The bacterial isolates were identified based on their
morphological behaviour on various differential media. Media were prepared according to the
manufacturer’s instructions and sterilized at 121°C for 15 min at 15 lb pressure. Further identification was then carried out by standard biochemical test as described by Jolt et al. (1994) and Cheesbrough (2006).

**Susceptibility test:** The susceptibility test was conducted using the Kirby-Bauer method of sensitivity determination. Petri-dishes of Mueller Hinton agar were prepared according to the manufacturer’s instruction. The 0.1 mL of the bacterial isolates equivalent to 0.5 McFarland standard was seeded into each of the Petri-dishes containing Mueller-Hinton agar using sterile swabs. These were allowed to stand for 45 min to enable the inoculated organisms to pre-diffuse. The bacterial isolates were tested for their sensitivity to antibiotics by means of disc diffusion method using prepared antibiotics discs containing different μg of antibiotics; cefadroxil (30 μg), cefotaxim (30 μg), cefamandole (30 μg), cefadroxil (30 μg), cefpodoxime (10 μg) and cefixime (5 μg); all are products of Oxoid, UK. The antibiotic discs were aseptically placed on the surfaces of the sensitivity agar plates. These were incubated for 18-24 h at 37°C and the radial zone of inhibitions were taken. The results were expressed as susceptible, intermediate or resistant according to criteria developed by Clinical Laboratory Standard Institute (CLSI., 2007).

**RESULTS**

From Table 1 *Escherichia coli* isolates from urine has a low susceptible to cefadroxil, cefotaxims and cefamandole while resistant to cefador and intermediate cefpodoxime and cefixime. *Escherichia coli* isolate from an abscess showed an intermediate effect of cefadroxil, susceptible to cefotaxims and cefpodoxime; resistant to cefamandole, cefador and cefixime. Catheter’s tip, aspirate and wound isolates of *E. coli* were resistance to cefadroxil, cefotaxims, cefamandole, cefador, cefpodoxime and cefixime.

*Escherichia coli* isolates obtained from stool culture were resistant to cefadroxil, cefotaxims, cefamandole, cefador, cefpodoxime and cefixime. One isolate obtained from blood culture was completely resistant to all the antibiotics used. While two isolates obtained from High Vaginal Swab (HVS), was susceptible to cefadroxil but the other was resistant; these two isolates were resistant to cefotaxims, cefamandole, cefador, cefpodoxime and cefixime. The *Escherichia coli* isolated from eyes swab were resistant to cefadroxil but susceptible to cefotaxims and

| Table 1: Susceptibility pattern of *Escherichia coli* to different generations of cephalosporin using CLSI (2007) criteria |
|---------------------------------------------------------------|---------------------------------------------------------------|
| **Isolation site** | **First generation** | **Second generation** | **Third generation** |
|                  | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R |
| Urine             | 4 | 0 | 12 | 3 | 0 | 13 | 3 | 0 | 15 | 3 | 0 | 13 | 2 | 1 | 13 | 15 | 2 | 79 |
| Abscess           | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 2 | 1 | 3 |
| Catheter’s tip    | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 6 |
| Wound swab       | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 30 |
| Stool culture     | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 8 | 4 |
| Blood culture     | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 6 |
| HVS               | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 11 |
| Eye swab          | 0 | 0 | 2 | 1 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | 11 |
| Aspirate          | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 6 |
| Total             | 5 | 1 | 25 | 5 | 0 | 26 | 3 | 0 | 28 | 4 | 0 | 28 | 2 | 1 | 28 | 19 | 11 | 156 |
| Susceptibility (%) | 16.1 | 3.2 | 80.6 | 16.1 | 0 | 83.6 | 9.7 | 0 | 90.3 | 0 | 3.2 | 96.8 | 12.9 | 0 | 90.3 | 6.5 | 3.2 | 90.3 | 10.2 | 5.9 | 83.9 |

remaining were resistant to cefamandole, cefador, cefpodoxime and cefixime. The overall susceptibility rate of *Escherichia coli* to different generations of cephalosporin is 12.9% sensitive, 4.1% intermediate and 82.9% resistant as shown in Table 1.

Table 2 shows the susceptibility of *Pseudomonas aeruginosa* obtained from urine, some were susceptible to cefadroxil and cefamandole but were resistant to cefotaxims, cefador, cefpodoxime and cefixime. The isolates obtained from blood culture were susceptible to cefadroxil while resistant to cefotaxims, cefamandole, cefador, cefpodoxime and cefixime. The isolates obtained from ear swab were susceptible to cefadroxil and cefamandole while resistant to cefadroxil, cefpodoxime and cefixime. Isolate obtained from eyes swab was resistant to cefadroxil and cefotaxims; intermediate for cefamandole, resistant to cefadroxil, cefpodoxime and cefixime while isolate from high vaginal swab was susceptible to cefadroxil and resistant to cefotaxims, cefamandole, cefador, cefpodoxime and cefixime. The *Pseudomonas aeruginosa* isolated from urine were susceptible to cefadroxil, cefotaxims and cefamandole while the remaining isolates were resistance to cefadroxil, cefpodoxime and cefixime. The overall susceptibility pattern of *Pseudomonas aeruginosa* to different generations of cephalosporin is 14.7% sensitive, 3.2% intermediate and 82.2% resistant.

From Table 3 *Proteus mirabilis* isolates obtained from sputum were susceptible to cefadroxil, cefamandole and cefotaxims while resistant to other antibiotics. Isolates from ear swab were

### Table 2: Susceptibility pattern of *Pseudomonas aeruginosa* to different generations of cephalosporins using CLSI (2007) criteria

<table>
<thead>
<tr>
<th>Isolation site</th>
<th>First generation</th>
<th>Second generation</th>
<th>Third generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFP</td>
<td>CTX</td>
<td>MA</td>
</tr>
<tr>
<td>Wound swab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood culture</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Ear swab</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Eye swab</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>HVS</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Urine</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sputum</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Abcess</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>1</td>
<td>13</td>
</tr>
</tbody>
</table>

Susceptibility (%)


### Table 3: Susceptibility pattern of *Proteus mirabilis* to different generations of cephalosporin using CLSI (2007) criteria

<table>
<thead>
<tr>
<th>Isolation site</th>
<th>First generation</th>
<th>Second generation</th>
<th>Third generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFP</td>
<td>CTX</td>
<td>MA</td>
</tr>
<tr>
<td>Sputum</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Ear swab</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Susceptibility (%)

Table 4: Susceptibility pattern of Klebsiella spp., to different generations of cephalosporin using CLSI (2007) criteria

<table>
<thead>
<tr>
<th>Isolation site</th>
<th>First generation</th>
<th>Second generation</th>
<th>Third generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFP</td>
<td>CTX</td>
<td>MA</td>
</tr>
<tr>
<td>Urine</td>
<td>S</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>Wound swab</td>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Sputum</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>HVS</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Ear swab</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Stool culture</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Eye swab</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Throat swab</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Cather’s tip</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CSF</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>Susceptibility</td>
<td>29.4</td>
<td>5.9</td>
<td>64.7</td>
</tr>
</tbody>
</table>

CFP: Cefadroxil, CTX: Cefotaxims, MA: Cefamandole, CEC: Cefador, CPD: Cefpodoxime, CFM: Cefixime, S: Sensitive, I: Intermediate, R: Resistant, CLSI criteria; for cefotaxims, S: $\geq 23$, I: $15-22$ and R: $\leq 14$, cefamandole, S: $\geq 18$, I: $15-17$ and R: $\leq 14$, for cefadroxil, S: $\geq 18$, I: $15-17$ and R: $\leq 14$, for cefpodoxime, S: $\geq 21$, I: $18-20$ and R: $\leq 17$, for cefador, S: $\geq 18$, I: $15-17$ and R: $\leq 14$, for cefixime, S: $\geq 20$, I: $17-19$ and R: $\leq 16$

resistant to all antibiotics. The overall susceptibility rate of Proteus mirabilis to different generations of cephalosporins is 20.6% sensitive, 4.8% intermediate and 74.6% resistant.

From Table 4 isolates of Klebsiella spp., obtained from urine were susceptible to cefadroxil, cefotaxims, cefpodoxime and cefamandole while some were resistant to cefador and cefixime. Isolates obtained from wound swab has a low susceptibility to each of cefotaxims, cefamandole, cefador, cefpodoxime and cefixime. Sputum isolates was susceptible to both cefadroxil and cefotaxims while resistant to other antibiotics. Isolates obtained from High Vaginal Swab (HVS), stool culture, eyes swab and ear swab was susceptible to cefadroxil and were resistant to cefotaxims, cefamandole, cefador, cefpodoxime and cefixime. The isolates obtained from catheter’s tip, Cerebrospinal Fluid (CSF) and throat swab were resistant to all the antibiotics used. The overall susceptibility rate of Klebsiella spp., to different generations of cephalosporin is 18.1% sensitive, 2.9% intermediate and 79.0% resistant.

DISCUSSION

Cephalosporin is one of the most widely used antibiotics in the treatment of both Gram positive and Gram negative organisms. In this study, Escherichia coli isolates showed a highest resistant to cefadroxil, followed by cefamandole, cefpodoxime and cefixime which are second and third generation of cephalosporins, this agree with the study by Jawetz et al. (2010) that second generations agents are active against organisms covered by first generation drugs. Also in support with observation made on third generations that they have broad spectrum of activity and further increased activity against Gram-negative rods (Jawetz et al., 2010). From this study the organism showed a highest susceptibility rate to cefadroxil which is a first generation, this confirmed its effectiveness over the others.

Result from this study also revealed that Pseudomonas aeruginosa showed a highest resistant rate to cefpodoxime and cefixime which are third generation cephalosporins followed by cefador, cefamandole and cefotaxims which are second generation. This organism showed minimal resistance rate to cefadroxil a first generation cephalosporin.

This agrees with Jawetz et al. (2010), that second generation agents are active against organisms covered by first generation drugs but have extended coverage against Gram-negative
rods including *Klebsiella* spp., but not *Pseudomonas aeruginosa* but disagree with the study of Pichichero (2006) that reported that third generation have a broad-spectrum of activity and further increased activity against Gram-negative organisms which may be due to the fact that microorganisms may lose the specific target structure for a drug for several generations and thus be resistant (Jawetz *et al.*, 2010).

*Pseudomonas aeruginosa* showed highest susceptibility rate to cefadroxil which is first generation and this may occur due to the abuse use of various generations of cephalosporins which leads the mutation in the genetic make-up of pathogens and thus drug resistant. In line with this study, *Proteus mirabilis* have a highest inhibitory concentration to cefotaxims and cefadroxil followed by cefpodoxime and cefixime which are second and third generations, respectively. This disagrees with the observation of Jawetz *et al.* (2010) that second generation agents are active against organisms covered by first generation drugs but have extended coverage against Gram-negative rods including *Klebsiella* spp., but not *Pseudomonas aeruginosa*.

It also disagrees with the study of Pichichero (2006), that third generation cephalosporins have a broad-spectrum of activity and further increased activity against Gram-negative organisms and this may be due to the fact that Gram-negative bacteria pursue various molecular strategy the presence and properties for development of resistance to these antibiotics (Pfeifer *et al.*, 2010). The most effective of all this antibiotics is cefadroxil which is a first generation and cefamandole have moderate effects on this organism than the rest of the antibiotics expect cefadroxil which it may be due to the misuse or abuse use of different generations of cephalosporins.

As it is revealed from the result of this study, the resistant of *Klebsiella* spp., increased according to different generations of antibiotics used. Cefadroxil also showed high rate of effectiveness than the rest of all the generations of cephalosporins used. This study is not in support with the study of Jawetz *et al.* (2010) that second generation agents are active against organisms covered by first generation drugs but have extended coverage against Gram-negative rods including *Klebsiella* spp., but not *Pseudomonas aeruginosa*. It also disagrees with the study by Jawetz *et al.* (2010) that third generations have a broad-spectrum of activity and further increased activity against Gram-negative organisms.

Analysis from this study showed that, a significant difference was observed in the mean effect of the first generation cephalosporin on the clinical isolates; with a mean value ranging between 1.94 effect on *Pseudomonas aeruginosa* and this may be due to the ability of the different isolates to produce beta-lactamase that reduced the activity of the first generation cephalosporin. This result agrees with the study of Jawetz *et al.* (2010), who from a study noted that the first generation cephalosporins are only moderately effective against some aerobic rod and anaerobic cocci. With no significant different observed in the mean effect of the second generation cephalosporin on the isolates, this result disagrees with the claims of Jawetz *et al.* (2010) that these agents are active against *Klebsiella* spp., *Proteus mirabilis* but not *Pseudomonas aeruginosa*. There is no significant difference in the mean effect of fourth generation cephalosporin used on clinical isolates tested; this may be due to misuse and abuse of cephalosporins which leads the mutations in the genetic makeup of pathogens and thus drug resistant. The resistance of clinical isolates to different generations of cephalosporins could be as a result of abuse of the antibiotics and there should be proper monitoring by qualified personnel in the field to curb the trends of antibiotics misuse. There should be proper monitoring of antimicrobial susceptibility both in the community and hospital settings.

Despite the resistance of bacteria to first and second generation cephalosporins they could still be used if appropriate laboratory sensitivity testing done on the isolates. However, in the absence
of that, third generation cephalosporins should be recommended. In order to avoid the crisis of drug resistance, the efficacy of antibiotic should be checked from time to time by carrying out comparative studies as done in this study.

REFERENCES
CLSI., 2007. Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. M100-S17, Vol. 27, No. 1, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA., USA.