Prevalence of Multi-Resistant Strains of
Pseudomonas Aeruginosa Isolated At The
Lagos University Teaching Hospital Laboratory
from 1994 to 1996

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SUMMARY

A total of 114 strains of Pseudomonas aeruginosa
isolated from the Lagos University Teaching Hospital
(LUTH) Microbiology Laboratory, between November 1994
and December 1995, identified using an analytical profile
index system (API 20 NE) were checked for susceptibility
and resistance against various antimicrobial agents on
Mueller-Hinton agar using E test method (AB Biodisk,
Soll, Sweden). Ninety-two percent, 39.7%, 21.6% and
25.6% of isolates were found to be resistant to amoxicillin-clavulanate,
ceftazidime, cefotaxime and imipenem respectively.

Ninety-six percent, 54.2%, 62% and 95.5% of
isolates were susceptible to ciprofloxacin, ceftazidime, gentamycin
and amikacin respectively.

Twenty-seven percent of all isolates were found to be
multi-resistant and there was a gradual increase in the
percentage of multi-resistant strains observed from 1994
to 1996. A very high percentage of isolates resistant to
ceftazidime were also resistant to amoxicillin, ceftaxime and
gentamycin, among other antibiotics.

Forty percent of isolates were found to be resistant to
gentamicin and 69% of these gentamicin resistant isolates
were multi-resistant. Forty-five percent of all isolates were
found to be resistant to ceftazidime and 95.5% of those
ceftazidime resistant strains were found to be multi-resistant.
The high prevalence of multi-resistant strains in this
study probably emphasizes the need for antibiotic policies
in LUTH.

Key Words: Pseudomonas aeruginosa multi-resistant,
Gentamicin, Ceftazidime, LUTH.

INTRODUCTION

Pseudomonas aeruginosa is the most commonly
isolated non-fermentative Gram negative bacillus. It is
ubiquitous in the environment and is sometimes found as
part of the microflora of healthy individuals, the bowel
being colonized after ingestion of the organism.

It has great ability to survive in moist environment,
aqueous solutions, disinfectants, ointments, soaps, irrigation
fluids, eye drops, dialysis fluids and equipment, as
such, it is abundant in the hospital environment. In fact,
colonization of the respiratory tract is common in hospitalized
individuals, especially those who are intubated. It is also
one of the most prevalent organisms in Nosocomial
infections, in which it is isolated from most of the relevant
sites.

P. aeruginosa is an opportunistic pathogen which is
usually unable to cause disease in healthy immunocompetent
host. However, it's ability to cause diseases in
immuno-compromised hosts has been attributed to many
factors. These potential virulence factors that have been
studied and found to be important in causing diseases are
adhesins, neuraminidase, exo-enzyme S, exo-toxin A,
substrates, alginate, lipopolysaccharide and antibiotic resistance.

P. aeruginosa infection can be community or hospital
acquired. The community acquired infections tend to be
localized and associated with water or aqoues solutions.
In the hospital setting, P. aeruginosa has been associated
with respiratory tract infection, endocarditis, urinary tract
infection, wound infection, eye infections, ear infections,
bacteremia, and skin and soft tissue infections.

Fortunately, this organism can be detected easily in
the laboratory, using simple routine media. However,
antimicrobial sensitivity testing needs to be done routinely
and accurately because of the ease with which resistance
develops to traditionally used anti-pseudomonal antibiotics.
This can arise by mutation or the acquisition of plasmids.
Organisms resistant to gentamicin or to cefazidime or
resistant to both gentamicin and cefazidime have been
identified. This organism is also intrinsically resistant
to most antibiotics, a situation which favours its continued
existence in the hospital environment. There is therefore
the need to develop new antibiotics and to review continually
the trends in the susceptibility pattern in our locality.

The aims of this study are:

To isolate and identify P. aeruginosa from various
specimens in LUTH Laboratory.

To carry out antibiotic susceptibility testing on all P.
aeruginosa isolates.

MATERIALS AND METHODS

All bacterial pathogens isolated from all clinical speci-
ments in the Lagos University Teaching Hospital
Laboratory between January 1994 and December 1995
were subcultured on blood and MacConkey media and
carefully identified by proper laboratory methods. All Gram
negative bacilli that were oxidase positive, and did not
oxidize the but and slope of the Triple sugar iron agar
(DIFCO Laboratories, Detroit, Michigan, USA), were fur-
ther identified by the API 20 NE (Analytical Profile
index system, Biomérieux, SA 69200 Fétisole - France).

Susceptibility of all P. aeruginosa strains to Ticarcillin,
Ceftazidime, Imipenem, Gentamicin, Amikacin, Ciprofloxacin
d and Cefotaxime were determined on Mueller-Hinton agar using E test method (AB Biodisk,
Prevalence of Multi-Resistant Strains of Pseudomonas aeruginosa Isolated At The Lagos University Teaching Hospital Laboratory from 1994 to 1996.

Soll, Sweden) as recommended by the National Committee on Clinical Laboratory Standards (NCCLS).

Isolates were considered multi-resistant if they showed resistance to three or more antibiotics.

RESULTS

A total number of 793 various organisms were isolated between November 1994 and December 1996 and 114 (14.4%) were Pseudomonas aeruginosa (Table I). As shown on this table, 32.99%, 39.9%, 37.82%, 12% and 35.2% of these isolates were found to be resistant to amoxillin-clavulanate, cefotaxime, ceftazidime, imipenem and gentamicin respectively.

Susceptibilities to antibiotics of isolates from patients in the wards of the laboratory were studied and shown in Table I. All strains of Pseudomonas aeruginosa isolates were susceptible to amoxillin-clavulanate, cefotaxime, ceftazidime, imipenem and gentamicin respectively.

Twenty seven percent of isolates were found to be multi-resistant (Table II). A breakdown shows a prevalence rate for multi-resistant isolates of 7.5% in 1994, 14.3% and 46.5% in 1995 and 1996. 21.6% of all the strains were found to be resistant to Pseudomonas aeruginosa isolates, 96%, 84.2% and 63% respectively white 1994, 1995 and 1996. On the other hand, 83% of isolates were found to be moderately susceptible to ceftazidime and 5.8% to gentamicin.

Forty five percent of isolates were found to be resistant to ceftazidime and 95.9% of these ceftazidime resistant isolates were found to be multi-resistant. All these ceftazidime resistant strains were also resistant to cefotaxime and gentamicin but none was resistant to ciprofloxacin. Of the Pseudomonas aeruginosa resistant isolates, 95%, 83.3% and 95.8% were also resistant to cefotaxime, gentamicin and ticarcillin respectively.

Table I

<table>
<thead>
<tr>
<th>Antimicrobial Agents</th>
<th>Number tested</th>
<th>Number susceptible</th>
<th>Number (%) moderately susceptible</th>
<th>Number resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxillin-clavulanate</td>
<td>40</td>
<td>28(70)</td>
<td>(2.5)</td>
<td>37(92.5)</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>114</td>
<td>68(59.8)</td>
<td>0</td>
<td>46(40.4)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>111</td>
<td>28(25.3)</td>
<td>4(35.4)</td>
<td>44(39.7)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>48</td>
<td>26(54.2)</td>
<td>4(8.3)</td>
<td>18(37.5)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>105</td>
<td>63(60)</td>
<td>5(4.8)</td>
<td>37(35.3)</td>
</tr>
<tr>
<td>Piperaclillin</td>
<td>111</td>
<td>87(78.4)</td>
<td>0</td>
<td>24(21.6)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>112</td>
<td>77(68.7)</td>
<td>21(18.8)</td>
<td>14(12.5)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>105</td>
<td>100(95.3)</td>
<td>1(0.9)</td>
<td>4(3.9)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>105</td>
<td>101(96.2)</td>
<td>1(0.9)</td>
<td>3(2.9)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>57</td>
<td>50(87.7)</td>
<td>6(10.5)</td>
<td>1(1.8)</td>
</tr>
</tbody>
</table>

Total number of P. aeruginosa Isolated = 114
Total number of various Organisms = 793

Table II

Prevalence of multiresistant strains of P. aeruginosa from November 1994 to December 1996.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>114</td>
</tr>
<tr>
<td>1995</td>
<td>112</td>
</tr>
<tr>
<td>1996</td>
<td>114</td>
</tr>
</tbody>
</table>

1. No (%) of multiresistant isolates

<table>
<thead>
<tr>
<th>Year</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>1(7.1)</td>
</tr>
<tr>
<td>1995</td>
<td>22(19.5)</td>
</tr>
<tr>
<td>1996</td>
<td>60(52.7)</td>
</tr>
</tbody>
</table>

2. No (%) of isolates resistant to Pseudomonas aeruginosa:

<table>
<thead>
<tr>
<th>Year</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>0</td>
</tr>
<tr>
<td>1995</td>
<td>2(18.2)</td>
</tr>
<tr>
<td>1996</td>
<td>62(54.3)</td>
</tr>
</tbody>
</table>

Table

Resistance Patterns of Gentamicin-resistant, Ceftazidime-resistant and Piperacillin-resistant strains of P. aeruginosa

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Number (%)</th>
<th>Cefotaxime</th>
<th>Gentamicin</th>
<th>Piperacillin</th>
<th>Ciprofloxacin</th>
<th>Imipenem</th>
<th>Aztreonam</th>
<th>Gentamicin-resistant isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Piperacillin</td>
<td>24(21.6)</td>
<td>23(95.8)</td>
<td>22(83.3)</td>
<td>23(95.8)</td>
<td>22(92.9)</td>
<td>0</td>
<td>0</td>
<td>20(83.3)</td>
</tr>
<tr>
<td>2. Gentamicin</td>
<td>42(10.0)</td>
<td>33(78.6)</td>
<td>21(50)</td>
<td>22(95.6)</td>
<td>8(36.3)</td>
<td>22(100)</td>
<td>21(95.5)</td>
<td>2(5)</td>
</tr>
<tr>
<td>3. Ceftazidime</td>
<td>24(27.3)</td>
<td>22(100)</td>
<td>22(100)</td>
<td>17(73.3)</td>
<td>8(36.3)</td>
<td>0</td>
<td>0</td>
<td>22(100)</td>
</tr>
</tbody>
</table>

Total number of isolates found to be multi-resistant: 114
DISCUSSION

A total of 114 strains of *Pseudomonas aeruginosa* were isolated from clinical specimens between 1964 and 1965. This represents 14.4% of all pathogens during this time, a figure which falls within the range obtained in previous studies.\(^1,\,2\)

Thirty-five percent of all isolates were resistant to gentamicin, which is traditionally considered in this environment as the first-line drug against gram-negative bacilli in the hospital setting. There is little information on the mechanisms of resistance to *P. aeruginosa* to aminoglycosides. However, aminoglycoside-resistance modifying enzymes, the genes for which are encoded on either plasmids or chromosomes, have been found to be the most commonly responsible for aminoglycoside resistance in aerobic bacteria\(^3\), and the distribution of these enzymes may, in part, be a function of antibiotic selection pressure.

On the other hand, 92% of isolates were found to be resistant to tetracycline-clavulanic acid. This is not surprising since *P. aeruginosa* is said to be intrinsically resistant to the penicillins and most other antibiotics, due to a combination of cell membrane impermeability and production of chromosomally determined beta-lactamas\(^e\). The penicillins (ampicillin, cloxacillin, amoxicillin-clavulenate) have been the most commonly prescribed antimicrobials in LUTH, even as far back as 1965\(^5\). It is thus possible that their overuse selected for the emergence of these resistant strains by inducing production of beta-lactamase especially since majority of *P. aeruginosa* strains isolated in LUTH were found to produce beta-lactamase\(^6\). Inducible beta-lactamases of *Pseudomonas* species are not inhibited by clavulanic acid\(^7\).

Seventy-four percent of isolates were found to be resistant to ceftazidime, a third generation cephalosporin with poor activity against *P. aeruginosa*. It is therefore advised that ceftazidime should not be used for suspected *Pseudomonas* infections, even when the organism appears to be sensitive in-vitro, because this is not necessarily translated to in-vivo action.\(^7\) Resistance to ceftazidime has been found to be due to hyper-production of inducible enzymes\(^8\),\(^10\).

In this study, 54.2% of isolates were sensitive to cefotaxime, which is one of the very few cephalosporins useful in the treatment of *P. aeruginosa* infections. There has been no recent study in this environment on the use or prescription of cefotaxime, but anecdotal reports suggest that this antibiotic is usually the first-line antibiotic prescribed in the neonatal wards, along with gentamicin, for neonatal sepsis. Reports also suggest that cephalosporins, especially cefotaxime, are used often in the surgical wards and that most patients go outside LUTH to purchase the antibiotics prescribed by their doctors. It is thus possible that overuse of cefotaxime has induced hyper-production of chromosomally mediated beta-lactamase to which it has been found to be stable\(^9\). This probably also explains the fact that all strains of *P. aeruginosa* that were resistant to cefotaxime were also resistant to ceftriaxone.

In this study, 29% of all isolates were multi-resistant and 69% of these multi-resistant isolates exhibited resistance to piperacillin. A very large percentage of these piperacillin-resistant isolates were also resistant to three other antibiotics, namely ticarcillin, cefotaxime, and gentamicin. The high percentage of these piperacillin resistant *P. aeruginosa* and the gradual increase in their prevalence may suggest the presence of a plasmid coding for this resistance pattern in our environment. In other studies, the prevalence of plasmid mediated beta-lactamase resistance has been found to be low, so this may be an area for future investigation to determine the actual mechanism of this resistance pattern\(^13\).

Over 65% of strains were found to be sensitive to amikacin and ciprofloxacin and these can therefore be used as reserve drugs to deal with multi-resistant organisms. Although, gentamicin is popular in this environment for treating *Pseudomonas* infection, amikacin though not available right now in this country is preferred to gentamicin in most parts of the world because it is more resistant to many of the more common plasmid-mediated enzymes\(^13\).

Ciprofloxacin has been found useful in treating infection with multi-resistant strains but its use is generally not recommended in prepubertal children because it has been found to cause cartilage damage and arthropathy in young animals\(^13\), though this has not been seen in children so not absolutely contraindicated. Therefore, in life-threatening infections, like septicaemia, caused by a *P. aeruginosa* strain resistant to both gentamicin and ceftazidime, ciprofloxacin may be the only viable alternative in the country.

There are no strict rules concerning the use or prescription of antibacterial agents in LUTH at this time, except the limitations imposed on choice by the existing hospital formulary. The search for rational and effective policies of antibiotic usage arises from increasing concern about the spread of resistant strains, the burden of unwanted effects from antimicrobial drugs and the mounting cost of the agents. The high prevalence of multi-resistant strains in this study emphasizes the need for antibiotic policies in LUTH.

REFERENCES


