Isolation, Identification And Prevalence Of Pseudomonas Aeruginosa Isolates From Clinical And Environmental Sources In Onitsha Metropolis, Anambra State

C.O. Ezeador, P. C. Ejikegwu, S. N. Ushie, and N.R. Agbakoba

Abstract—This study was aimed to isolate and identify Pseudomonas aeruginosa and to determine the prevalence rate of isolated P. aeruginosa in Hospitals in Onitsha. Isolates of P. aeruginosa were recovered from both clinical and environmental sources using Cetrimide agar, Blood agar, Mueller-Hinton agar and MacConkey agar. All the inoculated plates were incubated at 37°C for 24-48 hours and growth was evaluated on these media. Isolates were identified on the basis of standard bacteriological methods like morphology, colonial characteristics, smell in culture, haemolysis, as well as pigment production on these media. All suspected isolates were further characterized and identified by many biochemical reactions. Results revealed that only 22 (18.3%) isolates were P. aeruginosa, while other 98 (81.7%) represented other bacterial genera. The 22 isolates included 19 (86.4%) environmental isolates and 3 (13.6%) clinical isolates. Pseudomonas aeruginosa was most commonly isolated from sink (13.6%), then mops and cleaning buckets (9.1%) and least from theatre bed, nasal swab, floor, disinfectant, car and wound swab (4.5%). The pigment varied from bluish-green to yellowish-green with a grape-like odor. All isolates were Gram negative, produced β-hemolysis on blood agar and were motile. The biochemical tests showed all the isolates to be strongly positive for catalase, oxidase, citrate, and casein hydrolysis. The prevalence rate of P. aeruginosa is relatively high and its isolation from sources like sinks and theatre bed could be suggestive of the role of this pathogen in nosocomial infections.

Index Terms—Cetrimide; Haemolysit; Pigment; Pseudomonas aeruginosa

I. INTRODUCTION

Pseudomonas aeruginosa emerged as a major human pathogen in the 1960s because of its ability to cause infections in immunocompromised hosts and fibrosis patients, all of whom were surviving much longer with modern medical treatments [1]. Since that time, Pseudomonas aeruginosa has become one of the most serious causes of nosocomial (acquired from hospital environments) bacterial infections, notably in the lung, blood, wound, burn and urinary tract [2]. P. aeruginosa strains are also found in various environmental habitats as well as in animal and human hosts, where they can act as opportunistic pathogens[3]. Pseudomonas aeruginosa is the major pathogenic species in the family Pseudomonadaceae and is readily identified as a Gram-negative straight or slightly curved rod with a length ranging from 1 to 3μm and a width of 0.5 to 1.0 μm [2]. It is an aerobic organism with polar flagella. It is motile but non-sporo forming. P. aeruginosa has parallel sides and rounded ends and are arranged singly, in small bundles or short chains. Fimbriae present on Pseudomonas aeruginosa differ from those on other gram-negative bacilli in their ability to cause haemagglutination [4]. Major morphologic characteristics on laboratory media include production of pigments. Four types of pigments are produced by P. aeruginosa strains including pyocyanin, pyoverdin, pyorubin and pyomelanin. Most commonly produced is a soluble blue-green coloured phenazine pigment called pyocyanin which diffuses into the surrounding medium [2]. This pigment is not produced by other species of this genus and hence its detection becomes diagnostic for Pseudomonas aeruginosa. Some strains produce red or black colonies because of synthesis of pigments termed pyorubin and pyomelanin, respectively, as well as yellow-green to yellow-brown pigment called pyoverdin (fluorescein). Colonies of Pseudomonas aeruginosa can have a highly varied morphology [2]. Cultures have a characteristic “grape-like” or “corn taco-like” odor due to the production of 2-aminoacetophenone from tryptophan by most strains as well and is helpful in confirming its presence on culture plates [5,6,7]. P. aeruginosais nutritionally versatile. It resists high concentrations of salt, dyes, weak antiseptics, and many commonly used antibiotics. Pseudomonas aeruginosa grows over a wide temperature range of 6 – 42°C; strongly positive in an indophenol oxidase test and can grow at 42°C, which differentiates this species from the rarely pathogenic Pseudomonas fluorescens and Pseudomonas putida [2]. Cetrimide agar, a commonly used selective medium for its isolation contains a detergent that inhibits growth of many other organisms, although with some exceptions and as well contains MgCl2 and K2SO4 to facilitate the production of the characteristic green pigmentation of Pseudomonas [2]. In blood agar, its colonies are large, flat, spreading and often haemolytic, whereas some strains produce small or mucoid colonies. In MacConkey agar, P. aeruginosa colonies are pale coloured and green colonies on cysteine lactose electrolyte deficient (CLED) medium. Pseudomonas aeruginosahas has been isolated from several hospital acquired infections from different clinical samples in many countries including Nigeria. A study in Kano reported P. aeruginosa one of the multi-drug resistant bacteria isolated from various clinical specimen [8]; whereas, in South-West, Akingbade et al. [9] reported isolating 110 isolates of P. aeruginosa form clinical wounds. It has also been isolated in other parts of Nigeria from various clinical specimens [10–16]. Hence, this study is aimed to isolate and identify Pseudomonas aeruginosa and to determine the

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prevalence rate of isolated *P. aeruginosa* in Hospitals in Onitsha.

II. METHODS

A. Study Area

This study was carried out in ten hospitals randomly selected from Onitsha, Anambra State, South-Eastern Nigeria. Onitsha is the largest city in Anambra State; it is a commercial, educational, and religious centre with a river port on the eastern bank of the Niger River in Anambra State.

B. Sampling/Sample Collection

A total of 152 samples were collected from environmental and clinical sources and examined. Multiple hospital environmental samples were collected from various sites of intensive care unit (ICU), operation theatres (OT) and wards of ten different hospitals in Onitsha. These sites included patients table, trolley, sink, floor, nurses’ tray, sphygmomanometer, theatre beds, patients’ bed, buckets, mops, water taps, laboratory work bench and disinfectants/antiseptics (chloroxylenol/cetrimide and chlorhexidine gluconate). Clinical specimens collected included ear swab, wound swab, nasal swab and hand swab from attending medical personnel.

Single use sterile cotton swabs sticks were used to collect samples from both animate and inanimate sources. The sterile swab sticks were moistened in sterile water and rolled over the sample area for at least 3 – 5 seconds on both clinical and environmental sites in order to obtain the sample. Swabs sticks were returned aseptically into their containers and samples were transported immediately in an ice box to the Microbiology laboratory for processing within one hour of collection.

C. Isolation, Identification and Biochemical Characterization of *Pseudomonas aeruginosa*

In the laboratory, respective non-duplicate swab samples from hospital environment and clinical specimen were cultured first on a selective agar, Cetrimide agar and MacConkey agar; then, suspected colonies were sub-cultured on Blood agar and on Muller Hinton agar (MHA) to observe for haemolysis and pigmentation. All the inoculated plates were incubated at 37°C for 18-24 hours and growth was evaluated on these media. Isolates were identified on the basis of standard bacteriological methods like morphology, colonial characteristics, haemolysis, as well as pigment production on these media [17]. Further identification were done by their Gram stain reaction, motility by hanging drop, odour in cultures, and biochemical tests such as catalase test, oxidase test using oxidase strips (Oxoid Ltd Basingstoke, UK), citrate utilization, starch hydrolysis, casein hydrolysis, indole production, urea hydrolysis, production of acid from glucose (O/F test) and growth at 42°C [18,19].

D. Antimicrobial Susceptibility Testing

The antibiotscissusceptibility of the twenty-two isolates of *P. aeruginosa* to the following antibacterial agents, representing 8 classes of antimicrobial agents was tested by the Kirby-Bauer disc diffusion method [20] using disks (Oxoid Ltd., Baslow, Hants, England) on Mueller Hinton agar and interpreted as recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines [21]: (drug:concentrations in µg) piperacillin (PRL: 30 µg), cefuroxime (CXM: 30 µg), ceftazidime (CAZ: 30 µg), cefotaxime (CTX: 30 µg), cefepime (FEP: 30 µg), amikacin (AK: 30 µg), imipenem (IPM: 10 µg), ciprofloxacin (CIP: 30 µg), chloramphenicol (C: 30µg), tetracycline (TE: 30 µg), and amoxicillin - clavulanic acid (AMC: 30 µg) [22].

III. RESULTS

A total of 152 samples were processed out of which 130 were from the environmental samples and 22 were clinical samples. One hundred and twenty (120) bacteria were isolated from the samples and *Pseudomonas aeruginosa* accounted for 22 (18.3%) with 81.7% representing other bacteria including *E. coli, B. cereus, K. pneumonia and S. aureus*. The twenty-two *Pseudomonas aeruginosa* isolates included 19 (86.4%) environmental isolates and 3 (13.6%) clinical isolates. The clinical samples were swabs obtained from a medical staff, a patient with wound infection and a child with otitis externa. Only three *Pseudomonas aeruginosa* strains were isolated from clinical specimens. Out of ten nasal and hand swabs collected respectively from medical personnel, only one *Pseudomonas aeruginosa* was recovered from the nasal swab of medical personnel while none was recovered from the hand swab.

All the *Pseudomonas aeruginosa* isolates recovered from the selective media, Cetrimide agar appeared as circular mucoid smooth colonies with a grape-like odour and showed β-haemolysis on blood agar. All the isolates grew on MacConkey agar but did not ferment lactose sugar, while on Muller-Hinton agar; the isolates produced a diagnostic pigment (Figure 1). The pigment varied from bluish-green to yellowish-green with a grape-like odour. Gram reaction showed the isolates to be Gram negative while motility test by hanging drop showed all isolates to be motile. The biochemical tests showed all the isolates to be strongly positive for catalase, oxidase, citrate, casein hydrolysis (Figure 2), as well as oxidation of glucose and xylose to acid; whereas the isolates were negative for indole, urease, starch hydrolysis as well as oxidation of lactose and maltose.

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Fig. 1. The bluish–green pigmentation of *Pseudomonas aeruginosa*. Plate A is on Cetrimide agar while Plate B is on Blood agar with β-haemolysis.

Fig. 2. Casein Hydrolysis by *Pseudomonas aeruginosa* isolates. This plate shows ability to hydrolyse casein by the exoenzyme, casease in *Pseudomonas aeruginosa* as indicated by a clear zone around the bacteria.

*Pseudomonas aeruginosa* was most commonly isolated from sinks (13.6%), then mops and cleaning buckets (9.1%) and least from theatre bed, nasal and hand swabs, and floor, disinfectant, ear and wound swabs (4.5%) (Table I).

### Table I: Distribution of *P. aeruginosa* isolates from different sources.

<table>
<thead>
<tr>
<th>Type of specimen</th>
<th>Total No. of specimen</th>
<th><em>P. aeruginosa</em> isolates No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theatre bed</td>
<td>9</td>
<td>1</td>
<td>4.5</td>
</tr>
<tr>
<td>Sink</td>
<td>12</td>
<td>3</td>
<td>13.6</td>
</tr>
<tr>
<td>Patients’ bed</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mops</td>
<td>12</td>
<td>2</td>
<td>9.1</td>
</tr>
<tr>
<td>Hands swab</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nasal swab</td>
<td>10</td>
<td>1</td>
<td>4.5</td>
</tr>
<tr>
<td>Nurses’ tray</td>
<td>10</td>
<td>1</td>
<td>4.5</td>
</tr>
<tr>
<td>Floor</td>
<td>11</td>
<td>1</td>
<td>4.5</td>
</tr>
<tr>
<td>Disinfectant</td>
<td>9</td>
<td>1</td>
<td>4.5</td>
</tr>
<tr>
<td>Patients’ table</td>
<td>10</td>
<td>2</td>
<td>9.1</td>
</tr>
<tr>
<td>Trolley</td>
<td>10</td>
<td>2</td>
<td>9.1</td>
</tr>
<tr>
<td>Sphygmonomanometer</td>
<td>7</td>
<td>2</td>
<td>9.1</td>
</tr>
<tr>
<td>Water tap</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Buckets</td>
<td>11</td>
<td>2</td>
<td>9.1</td>
</tr>
<tr>
<td>Lab work bench</td>
<td>9</td>
<td>2</td>
<td>9.1</td>
</tr>
<tr>
<td>Ear swab</td>
<td>1</td>
<td>1</td>
<td>4.5</td>
</tr>
<tr>
<td>Wound swab</td>
<td>1</td>
<td>1</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>152</strong></td>
<td><strong>22</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

IV. DISCUSSION

*Pseudomonas aeruginosa* is ranked second among Gram-negative bacteria isolated in hospital environment, and a leading cause of nosocomial infections responsible for high morbidity and mortality rate [17]. High prevalence of *Pseudomonas* infections is common among critically ill patients on admission on intensive care unit and those with underlying clinical conditions [18].

Of the 152 samples collected, twenty-two *Pseudomonas aeruginosa* isolates were obtained (3 clinical and 19 environmental isolates). This is a contrast to a study from Egypt which reported a total of 57 *Pseudomonas aeruginosa* strains (54 clinical and 3 environmental isolates) isolated from 287 clinical specimens and 40 environmental specimens (17) and a total of 60 clinical and environmental isolates of *P. aeruginosa* [23]. The high number of *Pseudomonas aeruginosa* isolated from previous study could be as a result of differences in sample size and due to the fact that many of the samples were from clinical sources.

*Pseudomonas aeruginosa* showed a prevalence rate of 18.3% of the total bacteria pathogens isolated in this study and also represented 13.6% of all clinical specimens and 14.6% of all environmental specimens. A similar study carried out in Zaria, North Central Nigeria by Olayinka et al. [13] revealed a prevalence rate of 10.4% while a study in Egypt by Mahmoud et al. [17] revealed a prevalence rate of 19.5%. In addition, a study by Yusuf et al. [8] in North-West Nigeria recovered 83 isolates of *P. aeruginosa* from various clinical samples with a prevalence of 13.1%. This difference could also be attributed to the differences in the study population and the larger number of specimens in these studies. However, Gad et al. [24] in Minia, Egypt, Naqvi et al. [18] in Pakistan and Haleem et al. [5] in Babylon revealed a prevalence rate of 19.5%, 23.1%, and 31.57% respectively.

This study showed that *Pseudomonas aeruginosa* was isolated from a nasal swab, a wound and ear swab samples; thus, indicating it could suggest its involvement in causing...
nosocomial infections. This is in line with the report by Olayinka et al. [13] who isolated P. aeruginosa strains from clinical samples comprising mainly urine (51.1%) and wounds (41.3%) obtained from the surgical units of a University Teaching Hospital in Zaria, North Central, Nigeria. Moreso, Akingbade et al. [9] recovered P. aeruginosa isolates from clinical wound samples in three tertiary hospitals in South West, Nigeria, as Nworie et al., [25] recovered 6 P. aeruginosa isolates from wound patients in Abakaliki, Eastern Nigeria. Several other studies have also shown that Pseudomonas aeruginosa has been frequently isolated from clinical specimens such as urine, sputum, burns, blood and seminal fluid [17, 18, 24].

Pseudomonas aeruginosa was also isolated from environmental sites such as sinks, cleaning mops, buckets and others like trolley, floor, etc. This is in line with the report from Corona et al. [26] that P. aeruginosa was recovered from hospital environments such as sinks, mops, bath taps and medical equipment such as catheters, ventilation tubes, etc. Furthermore, Haleem et al. [5] in his study reported that P. aeruginosa was isolated most from catheter tips, disinfectant (chloroxylenol/cetrimide) and beds for environmental samples as well as, burns, wound and ear swab for clinical samples. In this study, a high number of P. aeruginosa was not isolated from patient’s bed whereas a study by Haleem et al. [5] also recorded that 4 (2.63%) isolates of Pseudomonas aeruginosa were recovered from patient’s bed. This variation in the prevalence could be attributed to maintaining simple personal hygiene by patients and environmental factors or other intrinsic factors.

The increase in the prevalence of P. aeruginosa recovered from both clinical and environmental samples as shown in this study depicts the alarming rate of nosocomial infection caused by P. aeruginosa. This has been well reported in other parts of the world leading to long hospitalization of patients, antibiotic resistance due to the intrinsic resistance nature of this pathogen as well as increase in morbidity and mortality due to this pathogen.

V. CONCLUSION
Pseudomonas aeruginosa accounts for 10% of all hospital-acquired infections. Specifically, this bacterium is the second most frequently recovered pathogen from intensive care unit (ICU) patients and the infections due to this organism are often difficult to treat due to antibiotic resistance. Owing to the menace caused by the high prevalence rate of Pseudomonas aeruginosa producing β-lactamase enzymes as revealed in this study, this underscores a crucial need for effective control measure within this environment.

VI. RECOMMENDATION
To combat the problems associated with high prevalence rate of P. aeruginosa, these recommendations are suggested:
1. Proper diagnosis and early detection of these β-lactamase producing isolates in a routine laboratory which could help to avoid treatment failure.
2. Epidemiological studies should be undertaken in hospital settings to monitor the source of infections, control of nosocomial infections and therein, design model strategies for the use of broad-spectrum antibiotics in the hospitals.
3. The government should enact strict laws to prohibit indiscriminate use and prescription of antibiotics (e.g: cephalosporins and carbapenems) in the community and hospital environment and improve on public enlightenment programs to stop drug abuse.

VII. FINANCIAL & NON-FINANCIAL COMPETINGINTEREST
The authors declare no financial or non-financial competing interest.

VIII. CONFLICT OF INTEREST
Authors declare they have no conflict of interest.

IX. REFERENCES

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