**Burkholderia cepacia Infections At A University Teaching Hospital In Lagos, Nigeria**

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**SUMMARY**

Twenty-five isolates of *B. cepacia*, representing 14.4% of all isolates, were obtained at the Microbiology Laboratory of a University Teaching Hospital in Lagos between January 1995 and December 1997. Identification of isolates was done using analytical profile index systems (Biomerieux, France) and sensitivity testing was by disc diffusion methods as recommended by the National Committee for Clinical Laboratory standards.

Majority of these isolates (24 out of 25) were cultured from in-patients, with most isolates from specimens which came in from the paediatric wards. Eighteen (72%) of the 25 isolates were obtained from blood, 4 (16%) were from urine and the remaining isolates were from wound swab (1) and sputum (1). Five (20.8%) of the blood isolates were obtained from neonates with symptoms and signs of septicaemia, 8 (44.4%) from neonates without features of septicaemia while diagnosis of septicaemia was uncertain in the remaining 5 blood isolates also from neonates. Factors that appeared to predispose to infection included intravenous fluid administration, catheterisation and surgery. Twelve (48%) of the 25 isolates were found to produce beta-lactamase by starch paper technique. *B. cepacia* showed reduced sensitivity to commonly used antibiotics like gentamicin (0%), and co-trimoxazole (0%). Majority of the isolates were sensitive to nalidixic acid (64%), cotrimoxazole (56.5%) and cefazidime (73.9%).

*B. cepacia* probably causes nosocomial infections in this environment. It may therefore be necessary to routinely carry out in-vitro antibiotic sensitivity testing for this organism in view of its resistance to commonly used antibiotic agents, so that appropriate antibiotic therapy can be instituted.

**INTRODUCTION**

Burkholderia in 1950 first described *Burkholderia cepacia* as the causative agent of bacterial onion bulb rot. This organism used to be grouped with the genus *Pseudomonas*. Later, based on nucleic acid homologies, *Pseudomonas* was divided into 5 groups, the rRNA homology group II being named *Burkholderia*. Previous synonyms were *Pseudomonas cepacia*, *Pseudomonas multivorans* and *Pseudomonas kingae*.  

Though well established as a plant pathogen, *B. cepacia* is said to be of low virulence in man. However, pathogenicity studies have shown that apart from capacity for cellular invasion and adherence, *B. cepacia* also produces extracellular products like elastase, gelatinase, haemolysin, protease and lipase, which are potential virulence factors. Lipopolysaccharides and plasmids, including resistance plasmids have been isolated from the organism. Apart from these, *B. cepacia* is ubiquitous in the environment because it is able to survive for long periods on environmental surfaces. The ability to survive in hostile aqueous environment enables it to contaminate soaps, equipment and solutions like distilled water, disinfectants and injectable medicines. It is therefore a common cause of hospital acquired infections like septicemia/bacteraemia, urinary tract infection, septic arthritis and peritonitis and outbreaks of these have been reported.

Recently, there was widespread concern over poor clinical outcome of respiratory tract infections in patients with cystic fibrosis due to resistance to antibiotics. Cystic fibrosis is rare in this environment, and there has been no documentation of *B. cepacia* infections. However, a preliminary report on glucose non-fermenting gram negative bacilli in Lagos University Teaching Hospital done between Jan. 1995 and June 1996 showed that 9% of all clinical isolates were *B. cepacia*. The isolates were obtained from wound swabs and urine but the study did not ascertain whether they caused infection or colonization. Although colonisation of patients is usually more endemic in hospitals and occurs more frequently in patients with severe disease, observation have shown that *B. cepacia* is transmissible indirectly between individuals, not only in hospitals and clinics, but also in social settings such as conferences and camps.

It is therefore considered necessary to establish the prevalence of *B. cepacia* infections in our environment. Information on antibiotic susceptibility and beta lactamase production should also be useful since *B. cepacia* has been found to harbour and transfer plasmids by conjugation.

**METHODOLOGY**

All bacteria isolated from urine, catheter tips, blood, cerebrospinal fluid, sputum, swabs pus and aspirates sent to the LUTH microbiology laboratory between January 1996 and December 1997 were subcultured on blood agar base (oxoid) supplemented with 7% human blood and MacConkey agar (oxoid).  

These isolates were observed for colonial morphology, gram stain morphology and oxidase test. Triple sugar iron (DIFCO laboratories, Detroit Michigan, USA) was used to distinguish between glucose fermenting and non-fermenting gram negative bacilli.
Burkholderia cepacia infections.

All oxidase positive glucose nonfermenting gram
negative bacilli were further identified using the Analytical
Profile Index systems API 20 NE (Biomerieux, 69280
March L'Etoile, France). Beta lactamase production of all
organisms thus identified as B. cepacia was determined
using the starch paper technique6.

Antibiotic sensitivity testing was done on Mueller-
Hinton agar, using the disc diffusion method in accordance
with the National Committee for Clinical Laboratory
Standards4.

Pseudomonas aeruginosa (ATTC 27853) and
Escherichia coli (ATCC 35218) were used appropriately
for quality control of all tests. Each isolate was followed up
to the wards and the affected patients examined so as to
determine infection and/or colonisation of patients, using
the following designed criteria.

Wound/ear swabs

Infection: - Infection documented in case note.
- Dirty wound with pus

Contamination/colonisation - Clean wound
Uncertain role: - Wound swab sent as
routine investigation.

Blood culture

Septicaemia/Bacteraemia: - Clinical diagnosis of
septicaemia
- Clinical signs of
septicaemia like fever,
Tachycardia, hypotension,
hypothermia
- Intravenous fluid in place
- Isolate obtained from more
than one blood culture

Contamination Pseudo/
bacteraemia
- No clinical signs of
septicaemia
- Patient not on intravenous
infusion

Uncertain role: - Isolate obtained in mixed
cultures.

Sputum

Pneumonia - Clinical diagnosis of
pneumonia
- Clinical signs of
pneumonia on chest
examination
- Pus cells on Gram stain of
sputum
- Pure culture of B. cepacia
from sputum

Contamination
- No clinical evidence of
pneumonia
- No pus cells in sputum
- Mixed culture

Urine

Infection: - Classical symptoms of
urinary tract infection
- Patient catheterized or
had undergone genito-
urinary surgery
- Significant bacteriuria.

Results

A total of 1,764 organisms were isolated in LUTH
between January 1996 and December 1997. Out of these,
25 isolates were B. cepacia representing a prevalence rate
of 1.4%.

Majority of these B. cepacia isolates (24 out of 25)
were cultured from inpatients. Most (20 of 25) were from
specimens from the paediatric wards with 18 of them
coming from the neonatal unit, while the other wards
altogether contributed only five isolates. The remaining 2
isolates were each obtained from specimens sent from
the obstetrics wards and the outpatient clinic.

Table 1 shows the distribution of B. cepacia in various
species. Eighteen (72%) were obtained from blood
while 4 (16%) were obtained from urine. The remaining
isolates were obtained from wound swabs (1), ear swab
(1) and sputum (1).

The clinical status of each affected patient is presented
on Table II. Five (28%) of blood isolates were obtained
from patients with signs and symptoms of septicaemia, 8
(44.4%) were from patients without signs and symptoms
of septicaemia while diagnosis of septicaemia was
uncertain in 5.

All the 4 urine isolates obtained in pure cultures were
from patients with laboratory evidence of urinary tract
infection. Isolates from wound and ear swabs were
obtained in pure cultures from patients with clinically
evident wound and ear infections.

Factors that appeared to predispose to infection or
colonisation of patients included intravenous fluid
administration, catheterisation and surgery (Table III). Twelve
(48%) of the isolates were found to produce beta
lactamase by the starch paper technique. The antibiotic
susceptibility pattern of B. cepacia (Table IV) revealed low
sensitivity to the commonly used antibiotics in our
environment, like gentamicin (8%) and ceftriaxone (0%).

On the other hand, 20 of 23 (86.9%) isolates were
sensitive to Nalidixic acid. The percentage sensitivity to
the cephalosporins varied from 56.5% (13 of 23 to
cetiraxone, to 73.9% (17 of 23) to cefotaxime.

Isolates were generally distinguishable by biotyping
as shown in Table V. Thirteen biotypes were seen. One
group (1477577) was the largest with four isolates out of
Burkholderia cepacia infections.

which only 2 were obtained within 4 days interval. There was another group (1047577) of three isolates, which were cultured, in different months and years. The remaining groups were made of one or two isolates each. The fifteen blood isolates from the same neonatal ward (D1) were distributed into 10 biotypes.

Table I: Distribution of various specimens yielding B. cepacia

<table>
<thead>
<tr>
<th>SPECIMEN</th>
<th>NUMBER (%) OF ISOLATES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>18(72)</td>
</tr>
<tr>
<td>Urine</td>
<td>4(16)</td>
</tr>
<tr>
<td>Wound swab</td>
<td>1(4)</td>
</tr>
<tr>
<td>Ear swab</td>
<td>1(4)</td>
</tr>
<tr>
<td>Sputum</td>
<td>1(4)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>25(100)</td>
</tr>
</tbody>
</table>

Total number of isolates from Jan. 1996 to Dec. 1997 = 1784
Prevalence rate of B. cepacia = 1.4%

Table II: Possible roles of B. cepacia in disease process

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Infection (%)</th>
<th>No Infection (%)</th>
<th>Role Uncertain (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>5(27.77)</td>
<td>8(44.44)</td>
<td>5(27.77)</td>
<td>18</td>
</tr>
<tr>
<td>Urine</td>
<td>4(100)</td>
<td>0</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Wound swab</td>
<td>1(100)</td>
<td>0</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Ear swab</td>
<td>1(100)</td>
<td>0</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Sputum</td>
<td>-</td>
<td>4(100)</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>11</td>
<td>9</td>
<td>9</td>
<td>25</td>
</tr>
</tbody>
</table>

Key:
Infection = Clinical or laboratory evidence of infection in the patient
No infection = No clinical evidence of infection in the patient
Role uncertain = Isolate obtained in mixed culture.

Table III: Factors which appeared to pre-dispose to B. cepacia infection in the patients

Predisposing Factors

<table>
<thead>
<tr>
<th>Clinical Condition</th>
<th>No of Cases</th>
<th>Intra-venous Infusion</th>
<th>Urinary Catheter</th>
<th>Surgery</th>
<th>No Factors</th>
<th>Apparent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salient or bacteremia</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Pseudobacteremia</td>
<td>8</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Urinary tract Infection</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Wound Infection</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Ear Infection</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>19</strong></td>
<td><strong>5</strong></td>
<td><strong>3</strong></td>
<td><strong>1</strong></td>
<td><strong>10</strong></td>
<td><strong>10</strong></td>
</tr>
</tbody>
</table>

Discussion and Conclusion

The 25 isolates of B. cepacia obtained in this study represent 1.4% of all bacteria isolated in the hospital laboratory between January 1996 and December 1997. This low figure which falls within the range obtained from previous studies carried out in the hospital and Ohio in which prevalence rates were between 0.5% and 2.0%, may actually be an under-estimate. This is because there are many patients who can not afford laboratory test in our environment.
Majority of isolates were from specimens sent in from the wards while only one out of 25 was from an out-patient specimen. This probably confirms the opportunistic nature of this organism, which has been attributed to its ability to survive for long periods in air, intravenous fluids, disinfectants, hospital sinks, drains and other wet environment. Molecular typing techniques have also demonstrated its transmission from the environment to immunodeficient patients.

Seventy percent of the isolates in this study were from the neonatal wards which are high risk areas for nosocomial infection because neonates are relatively immunosuppressed and when ill in the hospital usually require invasive procedures like exchange of blood transfusion and the use of medical devices like intravenous lines. Isolation of B. cepacia from intravenous fluid has also been previously reported in LUTH. In 1996, the contamination rates for in-use infusion in LUTH neonatal wards was found to be 25.6% (100 of 360 infusions) and B. cepacia accounted for 21.4% of the 140 organisms isolated from the contaminated infusions. Other liquids or solutions, which have been implicated as sources of B. cepacia in the hospital include 5-fluorouracil injections and ethacridine, lactate solutions.

Eighteen (72%) of the isolates in this study were obtained from blood cultures which all came from the neonatal wards. Five (27.8%) of the blood isolates were from patients with clinical features of septicaemia and B. cepacia was also cultured from the intravenous fluid in two of these cases with exactly the same biotypes suggesting that the intravenous fluids were the source of the septicaemia. The role of B. cepacia in the disease process of another 52.7% of the blood isolates was not clear because the patients had multiple organisms, while the remaining 8 (44.4%) cases had no features of septicaemia and isolates were suspected to be contaminants. These findings are not surprising because B. cepacia has been associated with bacteremia and sepsis.

Four isolates were obtained from urine, three from catheterized in-patients and one from a pregnant woman attending the antenatal clinic. Although B. cepacia has been known to cause urinary tract infection, especially in patients who had genito-urinary surgery or instrumentation, clinical features of UTI could not be confirmed in the patients in this study because they were lost to follow up. However, despite the fact that all affected patients with UTI in this study were discharged without appropriate medication, it is likely that their infection will resolve spontaneously because previous studies have shown that once the catheter was removed (except in patients with renal calculi), infection often subsided in patients with normal host defences.

Chronic suppurative otitis media caused by B. cepacia has been well documented. This organism was isolated in pure culture from one patient with otitis media in this study and the infection appeared to respond to the antibiotic to which the organism was sensitive in-vitro. The isolation of B. cepacia in pure culture in this study from the wound swab of a patient who had had abdominal surgery correlated with a previous report in which the organism was ultimately traced to a contaminated disinfectant. Unfortunately, the source of the infection in this study was not determined.

Using API 20 NE to type all the 25 isolates, thirteen biotypes were seen and the fifteen blood isolates from the same neonatal wards were distributed into ten biotypes suggesting that most isolates were probably not from the same source and therefore not epidemic strains. The few isolates that were of the same biotypes were cultured at different periods of time from specimens in different wards and probably just represented the endemic hospital strains.

The antimicrobial susceptibility pattern of B. cepacia to various antibiotics showed resistance to the commonly used antibiotics like co-trimoxazole and gentamicin. This is not surprising since B. cepacia, like Pseudomonas aeruginosa to which it is related, has been found to be intrinsically resistant to many antibiotics including the penicillins. Nevertheless, sensitivity to aminoglycosides like gentamicin and tobramycin has been reported. None of the isolates was sensitive to gentamicin. This is worrisome because gentamicin is the usual drug of choice against gram-negative bacilli infections in this environment. Resistance to aminoglycosides has been documented but there appears to be improved sensitivity when combined with polymyxins. Co-trimoxazole is another drug used commonly in this environment as a first-line drug against infection. Even though susceptibility to co-trimoxazole has been documented both in-vitro and in-vivo, only 2 (8%) isolates were sensitive in this study. Sixty-four percent of the 25 isolates were sensitive to nalidixic acid. This suggests that the drug could probably be used to treat cystitis caused by B. cepacia when in-vitro sensitivity has been confirmed. Other quinolones are however likely to be of more empiric usefulness as twenty (80%) of 23 isolates were found to be sensitive to ofloxacin. Quinolones have been found useful in the treatment of B. cepacia infections although resistance to ciprofloxacin, which is the gold standard, has been recorded recently. The high susceptibility rate obtained in this study is probably due to the fact that these drugs are very expensive and are not so readily prescribed in this environment.

Only 3 out of 25 isolates were tested for sensitivity to the third generation cephalosporins. In this study, while 17 (73.9%) of 23 isolates were sensitive to cefazidime, 13 (56%) were sensitive to ceftriaxone and 13 (55%) of 22 isolates to cefuroxime. The response of B. cepacia to the third generation cephalosporins varied in different
reports. In-vitro sensitivity was reported in some studies while clinical failures was recorded in others. Resistance to the cephalosporins has been associated with production of beta-lactamases. Twelve (48%) isolates were found to produce beta-lactamase using the starch paper technique in this study. These enzymes, which were reported in previous studies to be penicillinases and carbapenemases, have been found to reduce the activities of penicillins, cephalosporins and carbapenems to which B cepacia have been found previously sensitive. B. cepacia should therefore not be immediately discarded as a contaminant in clinical specimens rather; it is necessary to attempt to identity to the species level as it is probably an important cause of nosocomial infection in LUTH. It is also necessary to carry out in-vitro antibiotic sensitivity testing in view of its resistance to commonly used antibiotic agents so that appropriate therapy can be instituted for infections caused by this organism.

REFERENCES


