

BACTERIOLOGICAL PROFILE AND RESISTANCE PATTERN OF BLOOD CULTURE ISOLATES IN A TERTIARY HOSPITAL IN PORT-HARCOURT, NIGERIA

ABSTRACT

Blood stream infection can result in life threatened condition with high morbidity and mortality, hence prompt diagnosis and antimicrobial treatment is required. The gold standard of making a diagnosis of septicaemia is the isolation of the offending pathogen from a blood culture. A knowledge of the bacteriological profile and susceptibility patterns of organisms isolated from blood culture will help guide the choice of empirical antibiotic administration in that institution

The study aim to determine the bacteria profile of blood stream infections and the antibiotics susceptibility pattern Bacteria strains isolated from 623 blood cultures from various wards of the University of Port Harcourt Teaching Hospital, Nigeria, over a period of 24 months were retrospectively analyzed for frequency of isolation, susceptibility profile, age and gender distributions

Overall prevalence yield from blood culture was 22.5% and gram negative bacilli accounted for 81.6% of the total yield with *K. pneumoniae* being the most frequently isolated organism. *K pneumoniae* was mostly resistant to gentamicin and ceftriaxone (95.8%) while *S. aureus* showed the highest resistance to cefuroxime (91.7%) followed by ceftazidime (87.5%). Majority of the samples and isolates were from neonates representing 49.1% and 75.89% respectively and this was statistically significant $P < 0.05$ while ages between 1-18 months and adult accounted for 18.44% and 5.67% of the total isolates respectively.

The exhibition of multidrug resistance to the commonly used antibiotics buttresses the need for a review of the empirical antibiotic regimen used in sepsis. It also emphasizes the importance of the use of more sensitive and rapid methods of bacterial detection from blood culture.

KEY WORDS: blood culture, septicaemia, antibiotic resistance, Nigeria.

INTRODUCTION

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to bloodstream infection¹. In sepsis, there's active multiplication of bacteria with release of toxins into the bloodstream which triggers the release of cytokines, causing fever, chills, malaise and lethargy, with difficulty in breathing especially in children². It is common in health institutions worldwide with resultant significant morbidity and mortality³. In the United States alone, blood stream infections account for 10% of nosocomial infections with mortality rate of 4-5%^{2,3}. Though careful clinical assessment may provide a clue for making provisional diagnosis, the gold standard for the diagnosis of sepsis remains the isolation of the offending pathogen from a blood culture^{3,4}. Moreover, the isolated bacterial pathogen is tested against an array of antimicrobial agents to help select the most appropriate for patient management^{2,3,5}. Both gram negative and positive bacteria have been isolated from blood culture, and predominance of one type over the other and their susceptibility pattern vary

39 from place to place and even in the same place over time^{4,6,7}.

40 The ideal sample for a blood culture is best collected by venipuncture⁸. The collection from
41 intravascular devices is discouraged and only interpreted if paired with another sample from a
42 venipuncture. It is an aseptic procedure. False positive results are mostly due to
43 contamination from skin flora⁹. Therefore emphasis on adequate skin disinfection is an
44 important pre-analytical measure that will determine the accuracy of the blood culture result.
45 Skin disinfection can be accomplished by the use of either of, or a combination of:
46 chlorhexidine, tincture of iodine, povidone iodine and alcohol-based products¹⁰. Some studies
47 have compared their various efficacies judging by the contamination rates of blood
48 cultures^{11,12}. Over all, most authorities recommend that skin preparation be performed using
49 an alcohol swab followed by tincture of iodine or chlorhexidine and allowing the
50 disinfectants dry completely on the skin before proceeding with venipuncture¹³. In addition,
51 the culture bottles should also be prepared by swabbing the rubber septae with alcohol prior
52 to inoculation⁹.

53 In septicaemia, relatively few bacteria or fungi are present in blood, therefore, to improve the
54 yield of these pathogens, the volume of blood to be cultured should be quite significant¹³. Li
55 et al, in their study, reported increasing microbial yield with larger volumes of blood
56 cultured¹⁴. For adults, authorities recommend that 10-20ml of blood per bottle be cultured,
57 whilst 1-3ml per bottle is accepted for children under 3 years¹⁰. However, blood volumes
58 greater than 30ml are not recommended because there is a greater likelihood of clot formation
59 when it is inoculated into the bottle¹⁵. On the number of cultures, most authorities agree to the
60 use of two or more sets of blood cultures (a set consists of one aerobic bottle and one
61 anaerobic bottle). It has been reported that most septicaemia will be detected by at least two
62 sets of culture¹⁵. Furthermore, a single set would be difficult to interpret especially if
63 coagulase negative Staphylococci (which are common contaminants from the skin, yet can
64 also be a cause of serious infections) were to be cultured. The Task Force on the Prevention,
65 Diagnosis, and Treatment of Infective Endocarditis of the European Society of Cardiology
66 (ESC) recommend that 3 sets of blood be used to confirm the diagnosis of infective
67 endocarditis¹⁶. They also concluded that it was unnecessary to time collection of samples to
68 coincide with the fever peaks. Similarly, authorities advocate for blood culture sets to be
69 collected simultaneously from different venipuncture sites rather than serially^{14,17}.

70 Blood culture systems have evolved over the years from manual systems, to semi-automated

and eventually fully automated systems for bacterial identification and antimicrobial susceptibility testing. The manual systems, however, have remained the main stay of resource-limited settings due to their relative cheap costs for operation and procurement of consumables. A set, consisting of an aerobic and an anaerobic bottle containing their respective broth are inoculated with blood. The appropriate blood to broth ratio is between 1:5 and 1:10, dilutions greater or less than this range results in reduced microbial yield¹⁵. After the first overnight incubation, an aliquot of each broth is gram stained and sub-cultured unto appropriate agar plates. Following this, the bottles are examined daily for signs of microbial growth (turbidity, haemolysis, gas or visible growth on the surface of the broth)¹⁷. Blind or terminal subcultures are performed at the end of the incubation period. Manual systems are quite labour-intensive which is a major drawback especially in large hospitals with numerous samples to process. The Septi-Chek and Opticult blood culture systems (Becton Dickinson) are improvements of the manual system that limit contact with the blood samples during sub-culturing.

Extensive discussion on the automated blood culture systems like BACTEC system (Becton Dickinson Microbiology Systems), Bio Argos System (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France), BacT/Alert (Organon Teknica Corp.) and ESP (Difco Laboratories) are beyond the scope of this paper. The earlier versions of these systems detected the growth of microorganisms by measuring carbon dioxide production. This was done daily or twice daily by either the use of ¹⁴C-labeled CO₂ or infrared spectrophotometry. These gave way for the continuous-monitoring blood culture systems (CMBCS) which are able to detect carbon dioxide production in as little as 10-minute intervals as well as changes in pH, pressure and several other gases like oxygen, nitrogen and hydrogen. Thus microbial growth is detected much earlier and action taken.

The microbial spectra usually isolated from blood cultures include *Staphylococcus aureus*, *Escherichia coli* and other Enterobacteriaceae, *P. aeruginosa*, *S. pneumoniae*, and *Candida albicans*^{13,18}. *Klebsiella pneumoniae* and *Staphylococcus aureus* were the two most common isolates amongst neonatal blood culture specimens in a previous study in the University of Port Harcourt Teaching Hospital¹⁹. Similarly, another study in Calabar reported *Staphylococcus aureus* as the most common organism isolated from blood cultures of children²⁰. In yet another Nigerian study in Kano, *Escherichia coli* and *Staphylococcus aureus* were ranked amongst the most common isolates from blood²¹. Most of the *E. coli* isolates were susceptible to Ceftriaxone, Ciprofloxacin, Gentamycin and Co-amoxiclav;

while *S. aureus* showed comparable susceptibility to Cefuroxime, Ceftriaxone, Clindamycin and Co-amoxiclav¹⁹⁻²¹.

Despite the benefits of blood culture in the specific management of sepsis, the result may take 3-7 days to be available - a time span that may not be available for the desperately sick patient. Surviving Sepsis Campaign recommends that blood cultures should be obtained before instituting appropriate empirical antimicrobial agents which should be commenced within the first hour of recognizing severe sepsis^{2,22,23}. This is because, early administration of drugs to patients with sepsis drastically reduces mortality²³.

It is apparent that the early use of the most appropriate empirical antimicrobial agents is critical to the survival of sepsis patients. However, this is complicated by the changing pattern of the etiologic pathogens and their antimicrobial susceptibility over time even in a particular region. Hence, there is need for constant surveillance of this changing pattern periodically to guide empirical treatment.

This study is aimed at determining the current microbial profile of sepsis and their antimicrobial susceptibility pattern in University of Port Harcourt Teaching Hospital in the southern part of Nigeria. The result of this study is expected to guide empirical therapy and also influence infection control practices and rational antibiotics use thus mitigating against the emergence of antimicrobial resistance among common pathogens.

MATERIALS AND METHODS

This is a retrospective cross-sectional study. We reviewed the results of a total of 623 blood cultures collected from patients of various ages admitted in different wards in University of Port Harcourt Teaching Hospital over a period of two years from January 2016 to December 2017. The University of Port Harcourt teaching hospital is one of the two tertiary health institutions in Rivers state and serves a large proportion of people living in the south- south geo-political zone of Nigeria.

The indications for blood cultures were clinical features adjudged by the attending clinicians to be indicative of sepsis. Blood culture samples were aseptically collected by the doctors into blood culture bottles containing trypticase soy broth and thioglycolate broth and immediately transported to the Microbiology laboratory for microscopy, culture and sensitivity. These were incubated aerobically and anaerobically at 35⁰C- 37°C and monitored for evidences of growth (haemolysis, turbidity, clot formation, gas production and cotton ball

effect). Bottles showing evidence of growth were promptly sub-cultured on blood, chocolate and MacConkey agar media and incubated in appropriate temperature and atmospheric pressures according to established methods^{13,18}. Isolates were identified through standard microscopic and biochemical procedures. Antimicrobial susceptibility tests were carried out by modified Kirby Bauer method in accordance with CLSI criteria and similarly interpreted²⁴. *Escherichia coli* ATTC 25922, *Staphylococcus aureus* ATTC 25923, *Pseudomonas aeruginosa* ATTC 27853, *Enterococcus faecalis* ATTC 2921 and *Streptococcus pneumoniae* ATTC 49619 were used as control strains.

RESULTS

A total of 623 blood culture requests were received and processed in the Department of Medical Microbiology during the study period. Majority of this came from the neonatal age group with 306 blood samples (49.1%) followed by children 245(39.9%) and then adults 72 (11.6%) as shown in Table 1. Also, there was a slight male preponderance in the patients with 361 males and 262 females giving a sex distribution of 1.38:1.

The bacterial isolation rate from all the patients in this study was 22.5% with the highest rate recorded among neonates with 34.6% having culture positivity as shown in Table 2. Bacterial isolation was more in the females 28.2% than in males 18%, table 3.

141 bacterial organisms were isolated from 140 patients including *K. pneumoniae* (72), *E. coli* (37), *S. aureus* (24), *P. aeruginosa* (5), *S. pyogenes* (2), *S. marcescens* (1) in decreasing order of number isolated as shown Table 2. There was a case of mixed growth of 2 organisms from a neonatal patient. The isolated organisms were predominantly gram negatives (81.6%).

The isolated bacteria were tested against ampicillin, gentamicin, ofloxacin, cefuroxime, ceftazidime, ciprofloxacin, amoxicillin clavulanic acid, ceftriaxone and meropenem with their resistance pattern shown in Table 3. Most of the isolates exhibited multidrug resistance. The most isolated organism *Klebsiella pneumoniae* showed the highest resistance to both gentamicin and ceftriaxone (95.8%) followed by amoxicillin clavulanic acid (94.4%) and ceftazidime (91.7%) while the least resistance was to ofloxacin (18.1%). *Staphylococcus aureus* showed the highest resistance to cefuroxime (91.7%) followed by ceftazidime (87.5%) with least resistance to ampicillin (41.7%). *Pseudomonas aeruginosa* showed total resistance to 4 out of the 6 tested antimicrobial agents.

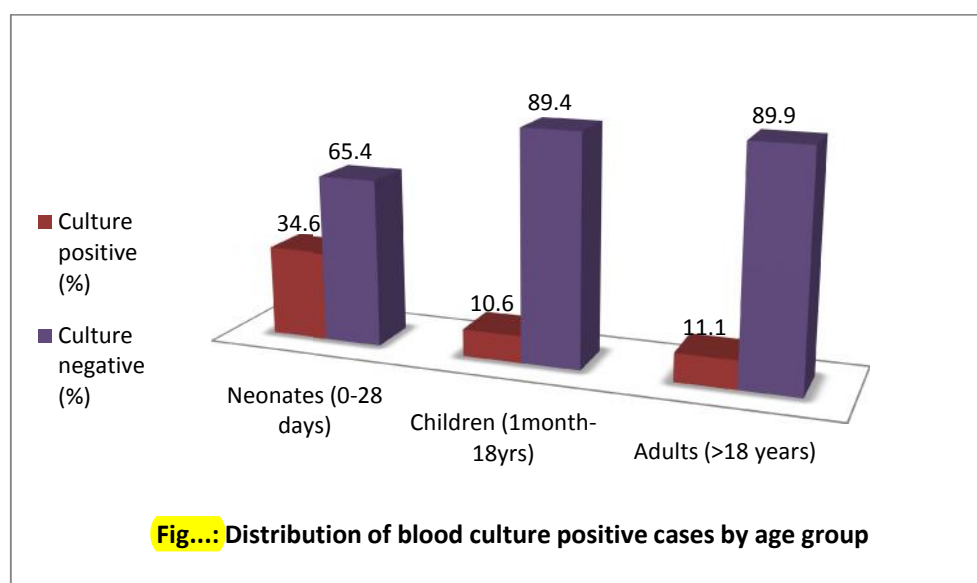
Table 1: Age groups and gender distribution of patients

Age groups	Male (%)	Female (%)	Total (%)	Chi-square (p-value)
Neonates (0-28 days)	195 (63.7)	111 (36.3)	306 (49.1)	8.24 (0.004)*
Children (1month – 18 years)	138 (56.3)	107 (43.7)	245 (39.3)	0.43 (0.659)**
Adults (>18 years)	28 (38.9)	44 (61.1)	72 (11.6)	3.48 (0.001)*
	361 (58)	262 (42)	623	

*Statistically significant $P < 0.05$

** not statistically significant $P < 0.05$

Figure 1: Distribution of blood culture positive cases by age group



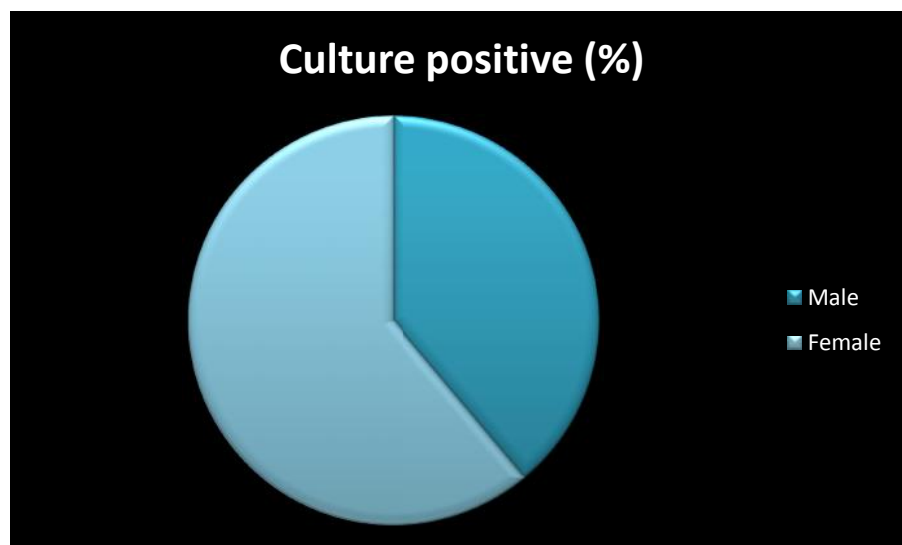
187 **Table 2: Distribution of isolates among the different age groups**

Isolates	Neonates	Children	Adults	Total	Chi-square (P-value)
<i>K. pneumonia</i>	60 (56.1)	8 (30.8)	4 (50.0)	72 (51.1)	5.36 (0.068)**
<i>E. coli</i>	24 (22.4)	9 (34.6)	4 (50.0)	37 (26.2)	4.07 (0.130)**
<i>S. aureus</i>	18 (16.8)	6 (23.1)	0 (0.0)	24 (17.0)	2.89 (0.088)**
<i>P. aeruginosa</i>	4 (3.7)	1 (3.8)	0 (0.0)	5 (3.5)	0.01 (0.979)**
<i>S. pyogenes</i>	0 (0.0)	2 (7.7)	0 (0.0)	2 (1.4)	8.36 (0.003)*
<i>S. marcescens</i>	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.7)	4.33 (0.037)*
TOTAL	107 (100.0)	26 (100.0)	8 (100.0)	141 (100.0)	

188 *Statistically significant $P < 0.05$

189 ** Not statistically significant $P < 0.05$

190 **Figure 2: Distribution of blood culture positive cases by gender**



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194 **Table 3: Antibiotic resistant pattern of the isolates**

Antibiotics	<i>K. pneumoniae</i> (n=72)	<i>E. coli</i> (n= 37)	<i>S. aureus</i> (n= 24)	<i>P. aeruginosa</i> (n=5)	<i>S. pyogenes</i> (n=2)	<i>S. marcescens</i> (n=1)
Ampicillin	NT	NT	10 (41.7)	NT	NT	NT
Gentamicin	69 (95.8)	30 (81.1)	18 (75)	5 (100)	2 (100)	0 (0)
Ofloxacin	13 (18.1)	10 (27.0)	12 (50)	3 (60)	NT	0 (0)
Cefuroxime	65 (90.3)	31 (83.7)	22 (91.7)	NT	1 (50)	1 (100)
Ceftazidime	66 (91.7)	32(86.5)	21 (87.5)	5 (100)	1 (50)	0 (0)
Ciprofloxacin	39 (54.2)	15 (40.5)	19 (79.2)	2 (40)		0 (0)
Amoxicillin-clavulanic acid	68 (94.4)	37 (100)	20 (83.3)	5 (100)	1 (50)	1 (100)
Ceftriaxone	69 (95.8)	33 (89.2)	21 (87.5)	NT	NT	0 (0)

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196 **DISCUSSIONS**

197 The findings of this study revealed that septicemia still remains very prevalent in Nigeria
 198 affecting all age groups but with a higher prevalence in neonates. The isolation rate of
 199 bacteria from blood culture in this study was 22.5% which agrees with a rate of 23.4% from a
 200 study in Uyo³, but is lower than 31.4% reported in Ile-Ife²⁵ both in Nigeria. It also falls in
 201 range with 20.5% reported by Atul **et al** in India⁶. Neonates were more affected with an
 202 isolation rate of 34.6% which agrees with an earlier study done in this center 10 years ago
 203 among neonates which had an isolation rate of 33.1%¹⁹ and also corroborates with other
 204 studies in Nigeria^{7,20,25,26}. This higher prevalence of septicemia in neonates is generally
 205 attributed to their immature immune system, obstetric practices, bottle feeding (in some
 206 cases) and poor hygiene. Also, in this study, females had more positive blood culture. This
 207 may be attributed to genetic factors. However, other studies both within and outside Nigeria
 208 have shown conflicting results in this regard^{2-6,20-22,25-29}.

209 There were more gram negative bacteria isolated than gram positive. This observation was
 210 also made in some studies in Nigeria^{19,21}, India⁶ and Iran³⁰. However, this contrasts with other
 211 studies where gram positive organisms are predominant^{2-5,7,20,22,31}. The most common isolate
 212 from this study was *Klebsiella pneumoniae* (51%), followed by *Escherichia coli* (26%) and

Staphylococcus aureus (17%). This is comparable to an earlier study done in this center¹⁹. However, some other studies within and outside Nigeria showed *Staphylococcus aureus* as the predominant organism isolated^{3,4,20,27}.

This study confirms reports that multidrug resistant isolates (resistant to at least 3 drugs) from blood culture are on the rise. Resistance to gentamicin and the cephalosporins were high across all the isolates whereas resistance was least to ofloxacin across board for the isolates. Similar pattern of resistance have been reported in some studies in Nigeria^{3,19}. However, this pattern contrasts with other studies done within and outside Nigeria^{2,5,7,20–22,26}. This attests to the fact that antimicrobial resistance pattern varies with geography and time and underscores the need for constant surveillance. The high level of resistance to gentamicin and cephalosporins is expected since these have been used over the years as the first line for empirical treatment of septicemia and other bacterial infections in the study population. Also the ease with which prescription drugs are obtained over the counter in the country may also contribute to this. Thus there is need to review the antimicrobial agents currently used for empirical treatment of sepsis in the study population. Also efforts should be made to regulate access to prescription medicines including antibiotics. The principles of antimicrobial stewardship should be promoted as part of the efforts to mitigate the emergence of drug resistance. Most of the isolates which are multidrug resistant are acquired primarily from the hospital. Hence, infection control practices will go a long way in reducing the incidence of sepsis.

CONCLUSION

This study has shown the microbial and resistance profile of blood culture isolates in Port-Harcourt, Nigeria in the last two years. Most of the isolates exhibited multidrug resistance to commonly used drugs, hence the need for urgent review of drugs used for empirical treatment. Results from this study will provide the much needed information to guide such review. Periodic studies are recommended in order to make necessary changes in the future. We also recommend that tertiary health institutions such as ours should acquire better and rapid testing technologies for blood culture testing in order to improve upon the yield and turnaround time of blood cultures.

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