Antibiotic Resistance Profile of Bacteria isolated from Septicaemia Cases in a Tertiary Health Care in Abeokuta, Nigeria

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ABSTRACT: The term septicaemia is often used in describing severe bacteraemic infections or a condition in which the blood serves as a site of bacteria multiplication. A total of 120 blood culture samples were collected during year 2011 to 2012. Bacteria isolated were characterized and the antibiotic sensitivity patterns were determined. The antibiotic sensitivity was carried out using Kirby-Bauer diffusion method. Bacteria isolated include *Escherichia coli, Klebsiella* sp, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. The highest number of bacteria was found among patient age Ò10 years. *Escherichia coli* accounted for 12(46.2%) of the bacteria isolated while *Pseudomonas aeruginosa, Klebsiella sp and Streptococcus pneumoniae* accounted for 6(23.1%), 6(23.1%) and 2(7.6%) respectively. The least prevalent isolate *Streptococcus pneumoniae* was found only in age group Ò10. Ceftazidime and Levoxin showed high sensitivity rate to most of the bacterial isolates. From this study, the uncontrolled use of antibiotics may have implication for emerging resistance of bacteria to commonly - used antibiotics.

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1. INTRODUCTION

Blood is normally sterile in healthy individuals. It is the main transport mechanism connecting all different parts of the body. As it serves as a transport system for oxygen, food materials, waste products and others round the body, it can also carry microbes (Eugene *et al*., 1998). However, it has no normal flora and the presence of microorganism in it indicates failure of the defence mechanisms to maintain its sterility. In many cases such a failure is transitory and of no clinical importance but in others, it is serious and life threatening (Murray, M. and Moosnick, 1941). Septicaemia, a symptomatic bacteriaemia, is a common condition with a resultant high morbidity and mortality (Odugbemi *et al.,* 1994; Ogunleye *et al*., 2005). Patient with septicaemia present with fever, difficulty in breathing, tachycardia, malaise, refusal of foods or lethargy. It is a medical emergency that requires urgent rational antibiotics therapy. The gold standard for diagnosis of septicaemia is the isolation of bacterial agent from blood culture (Iregbu *et al.,* 2006). Blood culture positive rates ranging from 25 ï 55% have been documented in previous studies carried out within Nigeria (Iregbu *et al*., 2006; Martins *et al*., 2005). In Nigeria, the outcome of treatment of septicaemia has remain poor, with reports of mortality of 33 to 41% from two tertiary hospitals in the country (Mokuola *et al*., 2002; Martins *et al*., 2005). As septicaemia is a life threatening emergency, the knowledge of epidemiological and antimicrobial susceptibility pattern of common pathogens in a given area helps to inform the choice of antibiotics. Predominance of either the gram- positive or gram- negative bacterial isolates is influenced by geographical location and changes in time; so also is the antibiotic susceptibility pattern influenced by location and time. Some bacteria commonly isolated include *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Nwadioha *et al*., 2010). The aim of this study was to identify bacterial agents from blood cultures and determine their antibiotic susceptibility profile.

2. MATERIALS AND METHODS

2.1. Sample Collection

Blood samples were collected with hypodermic needle and syringe following thorough cleaning of the venous site with 70% alcohol and followed by providone iodine. The rubber cap of each of the culture broths bottles was immediately cleaned with 70% alcohol. The used needle was replaced with

a new needle and then, the venous blood was injected into Brain Heart Infusion and Sodium thioglycolate broths in the ratio of one part of blood to five parts of the broth. The blood samples were categorized into different age ï groups of the individual patients. The blood culture broths were immediately sent to the laboratory. The subjects comprised one hundred and twenty (120) children and adults of both sexes aged between one day to 70 years having clinical features suggestive of septicaemia, who were on admission at the tertiary health care in Abeokuta.

2.2. Processing of Blood Culture

The already inoculated blood culture bottles were taken to the laboratory and incubated at 37^{o}C for 7 days, examined and subcultured later onto MacConkey agar, Blood agar and Chocolate agar (Cheesbrough, 2004).

2.3. Characterization and Identification of Isolates

Isolated pure cultures of bacteria were subjected to various morphological and biochemical tests. After which they were identified using Bergeyôs Manual of Systematic bacteriology. The following tests were carried out: Gram stain, Motility, Spore staining, Oxidase test, Urease test, Indole, Methyl red test, Citrate test, Vogue Proskauer test, Catalase test, Coagulase test, Fermentation of glucose, lactose and sucrose.

2.4. Antimicrobial sensitivity testing

Commercially available antimicrobial discs (Abtek Biological Ltd UK) were used to determine the drug sensitivity and resistance pattern of the isolates. A number of 15 different antibiotics with different disc concentration such as Gentamycin (Gen), Erythromycin (Ery), Levoxin (Lev), Ampicillin (Amp), Augmentin (Aug), Ceftriaxone (Cef), Cotrimoxazole (Cot), Ofloxacin (Ofl), Tetracycline (Tet), Streptomycin (Str), Ciprofloxacin (Cip), Cloxacillin (Cxc), Amoxicillin (Amx), Cefuroxime (Cxm), Ceftazidime (Caz), were used in this study. The antimicrobial sensitivity test of each isolate was carried out as described by the Kirby ï Bauer disc diffusion method (Bauer *et al*; 1966) as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2003). The turbidity of the bacterial suspensions was compared with 0.5Macfarlandôs barium sulfate standard solution. The standardized bacterial suspension was then swabbed and inoculated on to Muller Hinton Agar (Lab M Limited, UK) using sterile cotton swabs and left to dry for 10minutes, before placing the antimicrobial sensitivity discs. Antibiotic impregnated discs of 8mm diameter were used for the test. After incubation, the diameter of the zone of inhibition were measured and compared with zone diameter interpretative chart (CLSI / NCCLS, 2003 & 2007) to determine the sensitivity of the isolates to antibiotics. Standard strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 17853 were used as control.

3. RESULTS ANALYSIS

Out of the 120 blood culture samples collected, 26 (21.7%) were culture positive while 94(78.3%) blood samples were bacteriologically sterile. Figure 1 showed the age group prevalence of septicaemia among the culture-proven patients in Abeokuta. Fourteen (53.85%) children in age bracket of one day to Ò10 years were observed to be most vulnerable. Four (4) species of bacteria were identified from the blood cultures samples using various biochemical tests as specified by the Bergeyôs Manual of Systematic Bacteriology. The bacteria were *Escherichia coli*, *Klebsiella sp, Pseudomonas sp* and *Streptococcus pneumoniae.*

Figure 2, showed the distribution of the bacteria isolated from blood cultures among the age groups. The highest number of bacteria was found among patient with age Ò10years. *Escherichia coli* accounted for 12(46.2%) of the bacteria isolated while *Pseudomonas aeruginosa, Klebsiella sp and Streptococcus pneumoniae* accounted for 6(23.1%), 6(23.1%) and 2(7.6%) respectively. The most frequent bacteria isolates was *Escherichia coli* while the least prevalent isolate was *Streptococcus pneumoniae* found only in age group Ò10.

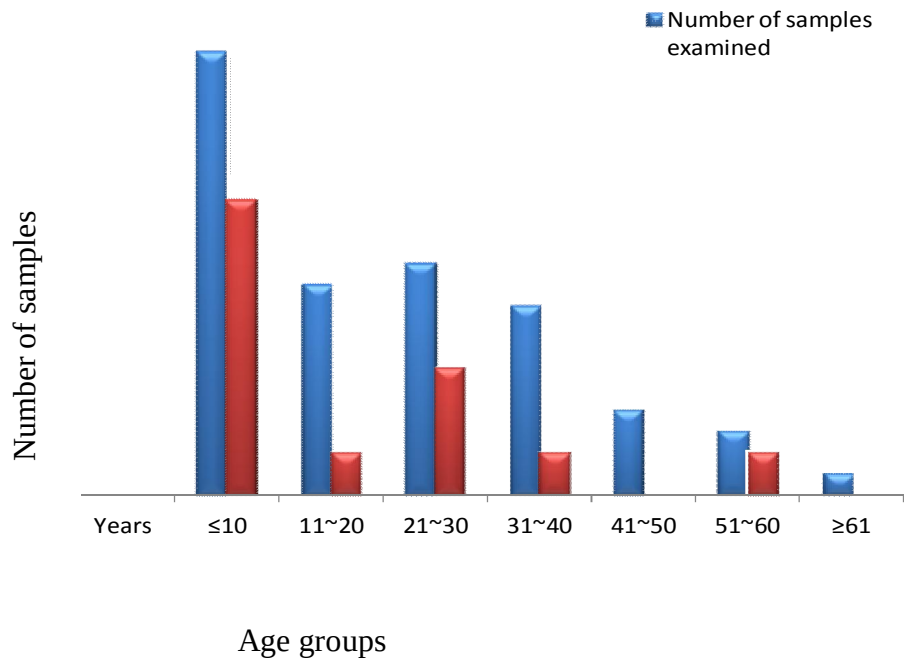

Figure 1: Prevalence of culture - proven bacteraemia according to Age group of the subjects.

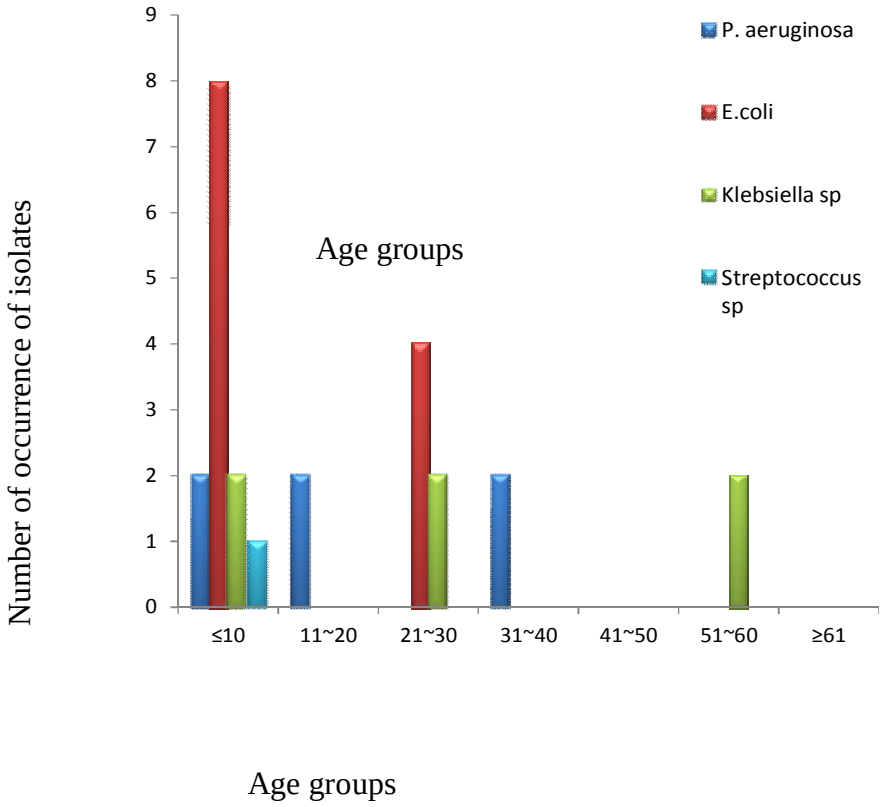

Figure 2: Distribution of the bacteria isolated from blood cultures among the age Groups

The susceptibility studies showed that most of *Escherichia coli* were susceptible to ceftazidime (83.3%), levoxin (66.7%), but were 100% resistance to cloxacillin, cotrimoxazole and tetracycline. The *Pseudomonas aeruginosa* isolates were 100% resistance to ampicillin, amoxicillin, cloxacillin, cotrimoxazole and tetracycline but 66.7% resistance were recorded to augmentin, ceftriaxone, ciprofloxacin, erythromycin and gentamycin (66.7%) (Table 1). *Klebsiella sp* were resistance to Ampicillin, Amoxicillin, Augmentin, Cefuroxime, Cloxacillin, Cotrimoxazole, Erythromycin, Gentamycin, Streptomycin and Tetracyline (100%) and were highly sensitive to Cefuroxime, Ceftazidime and Ofloxacin (66.7%).

Table 1: *In - vitro* susceptibility patterns of isolates from blood cultures

		Antibiotic														
		Amp	Amx	Aug	Cef	Caz	Cxm	Cip	Cxc	Cot	Ery	Gen	Lev	Ofl	Str	Tet
E. coli	S	2(16.7)	4(33.3)	65(0)	6(50)	10(83.3)	6(50)	4(40.0)	0(0)	0(0)	2(16.7)	4(33.3)	8(66.7)	2(16.7)	2(16.7)	0(0)
(n=12)	R	10(83.3)	8(66.7)	6(50)	6(50)	2(16.7)	6(50)	8(66.7)	12(100)	12(100)	10(83.3)	8(66.7)	4(33.3)	10(83.3)	10(83.3)	12(100)
Klebs sp	S	0(0)	0(0)	0(0)	10(33.3)	10(33.3)	0(0)	4(66.7)	0(0)	0(0)	0(0)	0(0)	4(66.7)	2(33.3)	0(0)	0(0)
(n=6)	R	6(100)	6(100)	6(100)	4(66.7)	4(66.7)	6(100)	2(33.3)	6(100)	6(100)	6(100)	6(100)	2(33.3)	4(6.7)	6(100)	6(100)
Pseudo sp	S	0(0)	0(0)	2(33.3)	2(33.3)	6(100)	6(100)	2(33.3)	0(0)	0(0)	2(33.3)	2(33.3)	4(66.7)	4(66.7)	4(66.7)	0(0)
(n=6)	R	6(100)	6(100)	4(66.7)	4(66.7)	0(0)	0(0)	4(66.7)	6(100)	6(100)	4(66.7)	4(66.7)	2(33.3)	2(33.3)	2(33.3)	6(100)
S.pneum.	S	0(0)	0(0)	2100)	2100)	2100)	0(0)	2(100)	0(0)	0(0)	0(0)	0(0)	2(100)	0(0)	2(100)	2(100)
(n=2)	R	2(100)	2(100)	0(0)	0(0)	0(0)	2(100)	0(0)	2(100)	2(100)	2(100)	2(100)	0(0)	2(100)	0(0)	0(0)
Overall (%) (nt = 26)	R	24 (92.3)	22 (84.6)	16 (62.5)	14 (43.9)	6 (33.1)	14 (53.9)	14 (53.9)	26 (100)	26 (100)	22 (84.6)	20 (76.91)	8 (30.9)	18 (69.2)	18 (69.2)	24 (92.3)

Keys:Gen = Gentamycin, Ery = Erythromycin, Lev = Levoxin, Amp = Ampicillin, Aug = Augmentin, Cef = Ceftriaxone, Cot = Cotrimoxazole, Ofl = Ofloxacin, Tet = Tetracycline, Str = Streptomycin, Cip = Ciprofloxacin, Cxc = Cloxacillin, Amx = Amoxicillin, Cxm = Cefuroxime, Caz = Ceftazidime. S ï Sensitive R ï Resistant n - Number of bacterial isolates nt - Total number of bacterial isolates

4. DISCUSSION

The rate (21.7%) of bacterial isolation in the blood culture in this study was relatively low compared to some previous studies done in Nigeria, namely; Calabar (44.9%) (Martins *et al*., 2005), Ilorin (30.8%) (Mokuola *et al*., 2002) and Ife (55%) (Ako-Nai*et al*, 1999). India (Madhu *et al*., 2002) recorded a relatively low rate (22.9%) of positive blood cultures.

This study has established that the septicaemia affects nearly all age groups but it was observed that children were more vulnerable than adults as children in age bracket of one day to 10 years were most infected. This vulnerability was most prominent, pronounced and apparent among the age group Ó10 because they accounted for the majority (53.85%) of the patients that had cultureï proven septiceamia in this study. The higher occurrence in childhood septicaemia has been reported from different parts of Nigeria (Ako-Nai *et al*., 1999).

Findings revealed that septicaemia still remains the major killer disease in Nigeria (Eugene, 1998). The high occurrence of children septicaemia recorded in Abeokuta in this study may probably be adduced to their low immune response, socio-economic status of the parents, poor hygiene practices, bottle feeding and high incidence of delivery at home (Komolafe and Adegoke, 2008).

The bacterial isolates obtained in this study were resistant to gentamycin (76.91%) which is in contrast to a study done in Calabar in which 80% effectiveness was recorded against bacterial isolates (Martins *et al*., 2005). Also in contrast, to a research carried out in Kano metropolis in which gentamycin recorded 70.7% effectiveness against bacterial isolates (Nwadioha *et al*, 2010). Gentamycin is routinely used synergistically with a beta-lactam antibiotic or vancomycin for empirical therapy in infective endocarditis (Madhu *et al*., 2002). Ciprofloxacin which recorded 82.9% effectiveness across all the bacterial isolates tested in vitro in a study (Nwadioha *et al*, 2010) in Kano, was less effective on bacterial isolates obtained in this study. Ciprofloxacin is not routinely recommended for pediatric use except in special cases where the benefits out ï weigh the short term risk of joint toxicity, such as in cystic fibrosis (Adolf, 2000).

The present study revealed that Ceftriaxone is less effective for septicaemia treatment in Abeokuta (46.1%) this result is not in agreement with a previous work (Adeleke and Belonwu, 2006) done with ceftriaxone in Kano. In the study conducted in Kano community ceftriaxone recorded about 96.0% effectiveness across all tested bacterial isolates.

5.0. CONCLUSION

The present study revealed that Ceftazidime a third generation cephalosporin can be considered as a drug of choice for empirical treatment of septicaemia in Abeokuta. Ceftazidime is generally very well tolerated in children and was 76.9% effective across all the bacterial isolates tested *in vitro* in this study, therefore before the blood culture antibiotic susceptibility report, Ceftazidime should be considered as a first choice of reliable antibiotics for

empirical treatment of septicaemia in Abeokuta community and environs.

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