Research Journal of Medical Sciences 7 (2): 35-39, 2013
ISSN: 1815-9346

Bacteriologic Profile and Antibiotics Susceptibility Pattern of Suspected Septicaemic Patients in Uyo, Nigeria

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Abstract: Septicaemia is a common potentially life-threatening condition with significantly high morbidity and mortality. Due to the serious threat it poses to lives of patients and the fact that results of blood cultures take a while to be ready, effective empirical therapy would require that clinicians should be armed with the knowledge of current bacteriologic profile of septicaemia and their susceptibility patterns. This study is aimed at determining the bacterial profile of septicaemia and their susceptibility pattern in University of Uyo Teaching, South-South region of Nigeria. A total of 689 blood samples were aseptically collected from patients of various ages with suspected septicaemia admitted at University of Uyo Teaching Hospital (UUTH) from January 2011 to December 2012. The samples were inoculated into blood culture bottles containing brain heart infusion broth and thyoglycolate broth and incubated at 37°C, aerobically and anaerobically respectively. Subcultures were made, isolates were identified and antibiotics susceptibility set up using standard methods. Data was analysed using SPSS Version 16. The study showed that 161 (23.4%) were culture positive. The commonest bacterial isolates were *Staphylococcus aureus* (55.3%), *Escherichia coli* (18.0%), *Klebsiella pneumoniae* (11.2%) and Coagulase Negative Staphylococci (CONS) (4.3%). Ampicillin and amoxicillin/clavulanic acid were the most resistant antibiotics while imipenem and vancomycin were the most sensitive. Regular bacteriologic profile and antibiotics susceptibility surveillance studies is advocated for effective management of septicaemia.

Key words: Bacteriologic profile, antibioic susceptibility, septicaemia, Uyo, CONS

INTRODUCTION

Septicaemia is a systemic disease associated with the presence of pathogens or their toxins in the blood (Odeh, 1996). It is a common potentially life-threatening condition in healthcare institutions worldwide with a resultant significantly high morbidity and mortality. For example in the United States, Bloodstream Infections (BSI) accounts for 10% of all nosocomial infections with mortality rate of 15%. It is also the eighth leading cause of death in the US (Wenzel and Edmond, 2001). Patients with septicaemia usually present with fever, chills, malaise, tachycardia, mental confusion, hyperventilation, toxicity, prostration and hypotension (Fauci *et al*., 2008). Complications like Disseminated Intravascular Coagulation (DIC) and acute renal failure may follow and indicate poor prognosis (Fauci *et al*., 2008; Mandell *et al*., 2010).

Though careful clinical assessment may provide a clue for making provisional diagnosis of septicaemia, blood culture remains the gold standard for diagnosis as it aids isolation of offending pathogen (Iregbu *et al*., 2006). Moreover isolated bacteria are tested against an array of antibiotics to help select the best for patients management. Blood culture involves aseptically collecting blood from the patient through venepuncture and inoculating same into culture medium with the aim of growing pathogens.

Due to the serious threat septicaemia poses to the lives of patients and the fact that the results of blood culture take a while to be ready, it is important that the clinicians be armed with the knowledge of the pattern and nature of bacteria commonly implicated and their susceptibility pattern for prompt and efficient management of such patients.

This study is aimed at determining the current bacterial profile and their susceptibility pattern of septicaemia in University of Uyo Teaching, South-South region of Nigeria.

MATERIALS AND METHODS

This is a prospective cross sectional study. A total of 689 blood samples were collected from patients of various ages with suspected septicaemia admitted in different

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wards at University of Uyo Teaching Hospital (UUTH) from January 2011 to December 2012. This hospital is a 500 bed tertiary care facility located at the South-South region of Nigeria. After obtaining verbal consent from the patients or care-givers, the proposed venepuncture sites were cleaned with 70% alcohol and providine iodine, 10 and 2-3 mL of blood samples were collected from adults and children/neonates, respectively and shared equally into blood culture bottles containing Brain Heart Infusion (BHI) broth and thyoglycolate broth labelled with patients' name, hospital number, ward, date and time of collection.

The culture bottles were incubated at 37°C aerobically and anaerobically and checked for signs of bacterial growth (haemolysis, turbidity, clot formation, gas production and cotton ball effect) daily. Sub-cultures were made on blood, chocolate and MacConkey agar plates from blood cultures with signs of bacterial growth. The plates were also incubated at 37°C aerobically and anaerobically for 24 h. Culture bottles with no signs of bacterial growth were similarly sub-cultured after 7 day and declared culture negative if there was no growth. Isolates were identified through standard microscopic and biochemical procedures (WHO/CDC, 2003). Antibiotics susceptibility tests against locally available antibiotics were done for isolated organisms using disc diffusion method according to Clinical Laboratory Standards Institute (CLSI) guideline (CLSI, 2011). Antibiotics tested were ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), cefuroxime (30 μg), ceftriaxone (30 μg), gentamicin (10 and 120 μg for high level aminoglycoside resistance), ciprofloxacin (5 μg), imipenem (10 μg), cefoxitin (30 μg) and vancomycin (30 μg). The antibiotics discs were obtained from Oxoid UK. *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used as control organisms. Data analysis was done using SPSS Version 16.

RESULTS AND DISCUSSION

A total of 689 samples from patients suspected to have septicaemia were studied comprising of 357 (51.8%) neonates, 199 (28.9%) children and 133 (19.3%) adults. Also, 349 (50.7%) of the patients studied were males while 340 (49.3%) were females as shown in Table 1, giving a sex distribution ratio of 1:1. Out of 689 samples collected during the study period, 161 (23.4%) were culture positive (Table 2), comprising of 101 (62.7%) Gram positive cocci and 60 (37.3%) Gram negative bacilli. Table 2 also shows that the rate of culture positivity decreased as age increased as 25.5% of neonates, 23.1% of children and 18.0% of adults were culture positive. This observed trend, however, is not statistically significant ($p>0.05$). With respect to gender, culture positive rate was slightly higher in male (24.1%) than female (22.6%) as shown in Table 3 ($p>0.05$).

The commonest bacterial isolates were *Staphylococcus aureus* (55.3%), *Escherichia coli* (18.0%), *Klebsiella pneumoniae* (11.2%) and Coaggulase Negative Staphylococci (CONS) (4.3%). Other less common isolates include *Enterobacter* sp. (3.1%), *Pseudomonas aeroginosa* (1.9%), *Proteus mirabilis* (1.9%), *Salmonella typhi* (2.1%), *Enterococcus faecalis* (1.2%), *Streptococcus pneumoniae* (1.2%) and *Streptococcus pyogenes* (0.6%) (Table 4). The most prevalent isolate in the three groups was *Staphylococcus aureus* with a rate of 42.6% in neonates, 39.3% in children and 16.9% in adults. This is followed by *Escherichia coli* with the rates of 79.3, 17.2 and 3.4% in neonates, children and adults, respectively.

Table 1: Age groups and gender distribution of patients

	Gender		
Age groups	Male (%)	Female (%)	Total (%)
Neonates (0-28 days)	169 (47.3)	188 (52.6)	357 (51.8)
Children (>28 days to <18 years)	114 (57.4)	85 (42.7)	199 (28.9)
Adults (≥18 years)	66 (49.8)	67 (50.2)	133 (19.3)
Total	349 (50.7)	340 (49.3)	689 (100.0)

Table 2: Distribution of blood culture positive cases by age groups

	Blood culture results		
Age groups	Culture positive (%)	Culture negative (%)	Total (%)
Neonates (0-28 days)	91 (25.5)	266 (74.5)	357 (51.8)
Children (>28 days to <18 years)	46 (23.1)	153 (76.9)	199 (28.9)
Adults (≥18 years)	24 (18.0)	109 (82.0)	133 (19.3)
Total	161 (23.4)	528 (76.6)	689(100.0)

Table 3: Distribution of blood culture positive cases by gender

	Blood culture results		
Gender	Culture positive	Culture negative	Total
Male	84 (24.1)	265 (75.9)	349 (50.7)
Female	77 (22.6)	263 (77.4)	340 (49.3)
Total	161 (23.4)	528 (76.6)	689 (100.0)

Table 4: Distribution of bacterial isolates among the different age groups

	Age groups			
Bacteria	Neonates	Children	Adults	Total (%)
S. aureus	39 (43.8)	35 (39.3)	15 (16.9)	89 (55.3)
E. coli	23 (79.3)	5 (17.2)	1 (3.4)	29 (18.0)
K. pneumonia	15 (83.3)	2 (11.1)	1 (5.6)	18 (11.2)
CONS	4 (57.1)	1 (14.3)	2 (28.6)	7 (4.3)
Enterobacter sp.	5 (100.0)	-	-	5 (3.1)
Pseudomonas sp.	-	1 (33.3)	2 (66.7)	3 (1.9)
Proteus mirabilis	3 (100.0)	-	-	3 (1.9)
S. typhi	-	-	2 (100.0)	2 (1.2)
E. faecalis	2 (100.0)	-	-	2 (1.2)
S. pneumonia	-	2 (100.0)	-	2 (1.2)
S. pyogenes	-	-	1 (100.0)	1 (0.6)
Total	91 (56.5)	46 (28.6)	24 (13.0)	161 (100.0)

Table 5: Antibiotics resistant pattern of Gram positive bacterial isolates

	Gram positive bacterial isolates				
Antibiotics	*S. aureus* (n = 89)	CONS (n = 7)	*E. faecalis* (n = 2)	*S. pneumoniae* (n = 2)	*S. pyogenes* (n = 1)
Ampicillin	55 (61.8)	4 (57.1)	0 (0)	1 (50.0)	0 (0)
Cefuroxime	48 (53.9)	4 (57.1)	NT	0 (0)	0 (0)
Ceftriaxone	37 (41.6)	3 (42.9)	NT	0 (0)	0 (0)
Gentamycin	15 (16.9)	2 (28.6)	0 (0) HLAR	0 (0)	0 (0)
Ciprofloxacin	23 (25.8)	4 (57.1)	NT	0 (0)	0 (0)
Amoxicillin/Clavulanic acid	49 (55.0)	4 (57.1)	0 (0)	1 (50.0)	0 (0)
Cefoxitin	22 (24.7)	2 (28.6)	NT	NT	NT
Vancomycin	0 (0)	0 (0)	0 (0)	0 (0)	NT

CONS = Coagulase Negative Staphylococcus; HLAR = High Level Aminoglycoside Resistance; NT = Not Tested

Table 6: Antibiotics resistant pattern of Gram negative bacterial isolates

	Gram negative bacterial isolates					
Antibiotics	*E. coli* (n = 29)	*K. pneumoniae* (n = 18)	*Enterobacter* sp. (n = 5)	*P. aeroginosa* (n = 3)	*P. mirabilis* (n = 3)	*S. Typhi* (n = 2)
Ampicillin	19 (65.5)	15 (83.3)	5 (100.0)	3 (100.0)	3 (100.0)	0 (0)
Cefuroxime	11 (37.9)	13 (72.2)	5 (100.0)	2 (66.7)	2 (66.7)	0 (0)
Ceftriaxone	7 (24.1)	12 (66.7)	4 (80.0)	2 (66.7)	2(66.7)	0 (0)
Gentamycin	6 (20.7)	11 (61.1)	2 (40.0)	0 (0.0)	1 (33.3)	0 (0)
Ciprofloxacin	11 (37.9)	8 (44.4)	2 (40.0)	0 (0.0)	3 (100.0)	1 (50.0)
Amoxicillin/Clavulanic acid	16 (55.2)	13 (72.2)	5 (100.0)	2 (66.7)	3 (100.0)	0 (0)
Imipenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0)
Piperacillin/Tazobactam	4 (13.8)	2 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	NT

NT = Not tested

Table 5 and 6 show the resistance patterns of Gram positive and Gram negative organisms, respectively. *Staphylococcus aureus* was found to be most resistant to Ampicillin (61.8%) followed by Amoxicillin/Clavulanic acid (55.0%), cefuroxime (53.9%) and ceftriaxone (41.6%). *Staphylococcus aureus* resistance was relatively low against gentamycin (16.9%) and ciprofloxacin (25.8%); a quarter (24.7%) was Methicillin Resistant (MRSA) (Cefoxitin disc used) while no vancomycin resistance (VISA) was recorded. Coaggulase Negative Staphylococci (CONS) resistance against tested antibiotics ranged from 0-57.1%.

Among the Gram negative isolates, imipenem was shown to be the most effective as no resistance (0.0%) was recorded against it followed by piperacillin/ tazobactam against which 0-13.8% resistance was recorded. *Salmonella typhi* was the least resistant gram negative isolates, demonstrating 50.0% resistance against ciprofloxacin only from among all the antibiotics tested, while the resistance of *Escherichia coli*, *Klebsiella pneumoniae* and *Enterococcus* sp. to ceftriaxone was 24.1, 66.7 and 80.0%, respectively.

This survey aimed at determining the bacteriologic profile of blood culture isolates and their susceptibility pattern shows that isolation rate within the study period was 23.4%, a figure relatively higher when compared with 16.6% reported in Pakinstan (Qureshi and Aziz, 2011) and 17.6% in Malawi (Archibald *et al*., 2000) but lower than 31.4 and 44.9% reported in Ilorin (Komolafe and Adegoke, 2008) and Calabar (Meremikwu *et al*., 2005) respectively, both in Nigeria and 34.0% in Gambia (Hill *et al*., 2007).

This figure however agrees with 23.1 and 21.4% reported from Nepal (Amatya *et al*., 2007) and Ethiopia (Asrat and Amanuel, 2001), respectively. Gram positive organisms (62.7%) were more than the Gram negative organisms (37.3%), a finding comparable to earlier studies in Nigeria (Ako-Nai *et al*., 1990; Angyo *et al*., 2011; Adeleke and Belonwu, 2006). A study in Pakistan reported a preponderance of Gram negative organisms, 60% for Gram negative bacilli against 40% for Gram positive cocci (Qureshi and Aziz, 2011). The differences observed may be due to variation in geographical locations, methodologies and epidemiological differences in etiologic agents.

Neonates, in this study were more susceptible to septicaemia than older children and adults, corroborating earlier reports of similar studies in Nigeria where neonates accounted for majority of blood culture isolates (Komolafe and Adegoke, 2008; Nwadioha *et al*., 2010; Motayo *et al*., 2011). This higher prevalence of septicaemia in neonates is generally attributed to immature immune system. Also in this study, the rate of culture positivity was found to be more in males (24.1%) than females (22.6%). Similar finding has been reported both in and outside Nigeria (Meremikwu *et al*., 2005; Karki *et al*., 2010). It is not exactly clear why there is male predominance but some researchers have adduced genetic factor, exposure factor and behavioural pattern of males (Komolafe and Adegoke, 2008). There are however some other Nigerian studies that reported female predominance (Ukaga *et al*., 2006; Omoregie *et al*., 2009).

The most common isolate in this study was *Staphylococcus aureus* (55.3%) followed by *Escherichia coli* (18.0%) and this same observation was made in other studies (Meremikwu *et al*., 2005; Japoni *et al*., 2008; Karki *et al*., 2010). The isolation rate of *Staphylococcus aureus* of 55.3% is comparatively >36.48 and 48.7% reported in Benin (Omoregie *et al*., 2009) and Calabar (Meremikwu *et al*., 2005) both in Nigeria and 16.7 and 27.4% reported in Iran (Mamishi *et al*., 2005) and USA (Daniel *et al*., 2004), respectively. In this study *Escherichia coli* was responsible for 18.0% of cases of bacteraemia which is in agreement with the 16.0 and 19.0% reported in Pakistan (Qureshi and Aziz, 2011) and Australia (Arora and Devi, 2007), respectively. *Klebsiella pneumoniae* accounted for 11.2% of the isolates which is similar to finding from other Nigerian studies (Nwadioha *et al*., 2010; Ayobola *et al*., 2011).

There have been several reports of emergence of isolates of blood culture exhibiting multi-drug resistance against commonly used antibiotics. In this study *Staphylococcus aureus* resistance against ampicillin (61.8%), amoxicillin/clavulanic acid (55.0%), cefuroxime (53.9%) and ceftriaxone (41.6%) were relatively high when compared with gentamycin (16.9%) and ciprofloxacin (25.8%). Similar pattern of resistance had been observed by some researchers (Komolafe and Adegoke, 2008; Zenebe *et al*., 2011). The high resistance of *Staphylococcus aureus* against these antibiotics as observed in this study may be due to frequent use of these drugs, some of them being the first line drug used in suspected cases of septicaemia in the centre. There is therefore need for reviewing the drug currently used for empirical therapies for suspected septicaemia in the study area. Moreover, the ease with which these drugs are obtained over the counter without prescription in Nigeria makes them very susceptible to abuse. It is heart-warming, however, to note that resistance against gentamycin and ciprofloxacin are still relatively low while resistance to vancomycin is 0%. Concerted efforts should therefore be made by doctors and other relevant stakeholders in the study area to ensure that these relatively sensitive antibiotics are well regulated to avoid emergence of resistance against them. Such efforts should include ensuring that antibiotics are not sold without prescription that correct dosing and duration are given and that rotation of antibiotics be practiced. Another important observation in this study is that a quarter (24.7%) of all *Staphyococcus aureus* isolates were Methicillin Resistant (MRSA). This is <49.3% reported in the United States (Karlowsky *et al*., 2004) and further supports the ever increasing number of MRSA isolates from blood culture, an observation made by other researchers (Diekema *et al*., 2000). The resistance of *Escherichia coli*, *Klebsiella pneumoniae* and *Enterococcus* sp. to ceftriaxone was 24.1, 66.7 and 80.0%, respectively. These figures are comparably lower than 16.0, 53.0 and 33.0%, respectively recorded against ceftriaxone in Iran (Mamishi *et al*., 2005).

CONCLUSION

This study has revealed that isolation rate from blood culture during the study period was 23.4%. The commonest isolated organisms were *Staphylococcus aureus* (55.3%) and *Escherichia coli* (18.0%). The rate at which multi-drug resistant isolates of blood culture are emerging is alarming. Resistance was high against ampicillin and amoxicillin/clavulanic acid. It has therefore become extremely important to keep constant antibiotics sensitivity surveillance on blood culture isolates. This will help clinicians to make rational prescriptions and provide effective empirical therapies for cases of suspected septicaemia.

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