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PREVALENCE OF SEPTICAEMIA AND ANTIBIOTIC SENSITIVITY PATTERN OF BACTERIAL ISOLATES AT THE UNIVERSITY TEACHING HOSPITAL, YAOUNDÉ, CAMEROON

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Abstract

Bloodstream infections are important causes of mortality and morbidity. Rapid empiric antibiotic therapy is often needed. Knowledge of epidemiological data of common pathogens and their antibiotic sensitivity pattern is needed for rapid therapy. This study was aimed at determining the common causes of septicaemia and their antibiotic sensitivity pattern from the University Teaching Hospital, Yaoundé. Blood samples were collected and cultured aerobically. Isolates were identified using bacteriological and biochemical methods and antibiotic sensitivity was done using the Kirby-Bauer disc diffusion method. Results showed that of the 396 patients examined 112 (28.3%) had septicaemia. Children below the age of 15 years constituted the greatest percentage of infected subjects (63.4%) followed by patients aged between 16-30 years (10.7%) ($P < 0.05$). The highest incidence of septicaemia were from medicine (8.95%), followed by paediatrics (7.04%), surgery (6.46%), out-patients (5.79%), neonatology (5.12%), obstetrics and gynaecology (5.05%) and emergency (2.05%) wards. The overall incidence of septicaemia was 5.79 per 1000 admissions. Gram-positive bacteria were encountered more often than gram negative bacteria (56.2% versus 43.8%, $P < 0.05$). Among the gram-positive bacteria, 52 (82.5%) were *Staphylococci*; 6 (9.5%) were *Streptococcus species*; while 5 (7.9%) were unidentified gram-positive bacteria. Among gram-negative bacteria, *Enterobacteriaceae* 39 (79.6%) and non-fermenting bacteria 10 (20.1%) were more frequent. *Staphylococci* were generally sensitive to Minocyclin and Rifampin (90%) while *Enterobacteriaceae* were most sensitive to Cefoxitin (71%) and Aztreonam (74%). *Staphylococcus epidermidis*, *S. aureus* and *Salmonella typhi* are the leading causes of bacteraemia among patients attending the University Teaching Hospital, Yaoundé

Keywords: Septicaemia, antibiotic sensitivity, Cameroon

Introduction

Septicaemias are important causes of mortality and morbidity and are among the most common healthcare associated infections [1]. Illnesses associated with bloodstream infections range from self-limiting infections to life threatening sepsis that require rapid and aggressive antimicrobial treatment [2]. A wide spectrum of organisms has been described and this spectrum is subject to geographical alteration. Patients who are granulocytopenic or inappropriately treated may have a mortality rate that approaches 100% [1]. Moreover, fatalities among patients infected with Gram-negative bacilli are higher than those among patients who have Gram-positive cocci as causative agents of their bacteraemia [3]. Worldwide, emergence of antibiotic resistance in all kinds of pathogenic bacteria is a serious

public health issue. It is associated with greater hospital mortality and longer duration of hospital stay, thereby increasing health care costs [4]. Also, colonization and infection with antibiotic-resistant bacteria has made the therapeutic options for infection treatment extremely difficult or virtually impossible in some instances [5]. There are many reasons for this alarming phenomenon, including increasing antibiotic use and misuse in humans, animals and agriculture, clustering and overcrowding and poor infection control [6].

Due to the high mortality and morbidity associated with septicaemia, antimicrobial therapy in most cases is initiated empirically before the results of blood culture and antimicrobial susceptibility pattern of the isolates are available [1]. Knowledge of local antimicrobial resistance

patterns from accurate bacteriological records of blood culture results is needed to provide guidance towards an empirical therapy before sensitivity patterns are available. There is large excess mortality in Sub-Saharan Africa particularly in children. The mortality rate among five-year-old children is about 25-100 per 1000 compared with 10-30 per 1000 in developed countries [7]. Bacteraemia is usually caused by a wide spectrum of bacteria with varying antimicrobial susceptibility pattern. However, there is a paucity of information about the relative contribution of different bacteria to infections in Sub-Saharan Africa and how this varies across the full range age groups [7]. Bacteraemia often require prompt diagnosis and effective treatment to prevent death and complications from septicæmia. Physical signs and symptoms are usually useful in identifying patients with septicæmia and other non-localized infections but these have limited specificity [8]. Bacteriological culture to isolate the offending pathogen and determine its antimicrobial sensitivity pattern has remained the mainstay of definitive diagnosis of septicæmia [9]. In most cases of suspected All the subjects were patients suspected clinically for septicæmia and sent to the bacteriology laboratory for blood culture by physicians. There was no formal definition and recruitment was at the discretion of the attending physicians. However, prior to blood collection, patients were explained the purpose of the study and made to understand that it was not a hospital obligation to participate in the research and neither was it a prerequisite to accessing any hospital services publicly available. Written informed consent forms were got. 5-10 mls of blood was collected from adults and 1-2ml from children and inoculated into the biphasic culture medium (brain-heart infusion (BHI) agar and trypticase soya agar (TSA) with slopes. The blood culture bottles were immediately incubated aerobically at 35°C for 24 hrs.

After 24hrs, bottles were checked for positive cultures (growth on the agar slope and/or turbidity in the broth). Negative cultures were re-incubated and checked daily for up to three weeks unless growth occurred. Before re-incubation, the slope was re-inoculated by tipping the bottle. Blind culture was done on all bottles without visible growth at the end of the three weeks

septicæmia antimicrobial therapy is always initiated empirically because bacteriological culture results take about a week to be available. Epidemiological data on common blood stream pathogens and their antimicrobial sensitivity pattern is thus very important to make the right choice of empiric therapy.

In Cameroon, such data are scarce in most healthcare settings due to dwindling resources [4]. Data used is usually from developed countries. This study was therefore carried out to determine the common causes of bacteraemia and their antibiotic susceptibility pattern in Yaoundé to help guide healthcare providers initiating empirical therapy on the choice of antibiotics to be used.

Materials and Methods

This study was conducted in the University Teaching Hospital of Yaoundé, Cameroon, a tertiary health care hospital providing a full range of surgical, medical and super speciality facilities. The study was carried out from January to June 2010.

Gram staining was done using both the broth and the colonies on the slope. The gram stain reaction of the bacteria guided on which medium to be used for subculture. Gram positive bacteria were inoculated onto chocolate agar supplemented with polyvitex and blood agar and incubated at 35°C in a candle jar for about 24hrs depending on the growth rate of the bacteria. Gram-negative bacilli were subcultured on Eosine Methylene Blue (EMB) aerobically at 35°C and gram positive cocci subcultured aerobically at 35°C on Chapman agar for 24 hrs.

Routine laboratory techniques were used to identify the bacteria [10]. SLIDEX Strepto PLUS® reagent was used for Lancefield grouping of Streptococci. Api20E® was used following the manufacturer's instructions for the *Enterobacteriaceae*. Non-fermenting bacilli were tested as described above but Api20nE® system was used instead of Api20E®.

Antibiotic susceptibility testing was done on MH using the Kirby-Bauer disc diffusion technique [11]. Antibigram for *Streptococcus* species was done on blood agar. Susceptibility testing of *Staphylococcus* species to oxacillin, vancomycin and teicoplanin was done on salted MH. Antibiotic discs used included penicillin, amoxicillin (25µg),

gentamycin(15µg), streptomycin(30µg),
 ceftazidime(30µg), fuscidicacid(30µg),
 cotrimoxazole(1.25/23.7µg), vancomycin(30µg),
 erythromycin(15µg), lincomycin(15µg),
 spiramycin, (100µg), rifampicin(30µg),
 tetracycline(30µg), perfloxacin(30µg),
 oxacillin(30µg), imipeneme(10µg),
 ceftazidime(30µg), aztreonam (30µg), Cefalotine
 (30µg), Cefixime (30µg) Cefuroxime (30µg),
 Cefotaxime (30µg), eftriaxone(30µg), Ceftazidime
 (30µg), Aztreonam (30µg), Amikacin
 (30µg), Netilmicin(30µg), Nalidixic acid (30µg),
 Norfloxacin (5µg), Ciprofloxacin (5µg) and
 Cefoxitine (30µg). Quality control for
 antibiogram was done weekly using *E. coli* ATCC

25922, *S. aureus* ATCC 25923 and *P.aeruginosa*
 ATCC 13048. Each batch of culture media
 prepared was controlled using *S. aureus*
 ATCC25923 (Chapmman), *S. pyogenase* (Trypticase
 soja) and *E. coli* ATCC 25922(EMB).

Results

Of the 396 patients examined for septicaemia,
 positive culture was found in 112 (28.3%). Age
 distribution of the patients is shown in Table 1.
 Children below the age of 15 years constituted the
 greatest percentage of infected subjects (63.4%),
 followed by patients aged between 16-30 years
 (10.7%)(P<0.05).

TABLE 1: AGE DISTRIBUTION OF PATIENTS WITH SEPTICAEMIA

Age ranges (years)	No clinically examined	No (%) of positive culture
0-15	224	71 (63.4)
16-30	76	12 (10.7)
31-45	32	9(8.0)
46-60	36	11(9.8)
61-75	18	5 (4.5)
>76	10	4(3.6)
Total	396	112 (100)

Throughout the study period the hospital
 admitted a total of 19348 patients. Table 2 shows
 septicaemia cases/1000 admissions in the various
 wards and clinics. The highest incidence of
 septicaemia were from medicine (8.95‰),
 followed by paediatrics (7.04‰), surgery (6.46‰),
 out-patients (5.79‰), neonatology (5.12‰),
 obstetrics and gynaecology (5.05‰) and

emergency (2.05‰) wards. The overall incidence
 of septicaemia was 5.79 per 1000 admissions.
 Septicaemia was very high among children less
 than one month old (34.9%). The overall rate of
 isolation reduced with increasing age but the type
 of bacteria isolated did not vary with age except
 for *Staphylococci*.

TABLE 2: SEPTICAEMIA CASES/1000 ADMISSIONS IN THE VARIOUS WARDS AND CLINICS

Ward/Clinic	No of admissions During study period =(a)	Cases of septicaemia during the period= (b)	Cases per 1000 admissions= b/a x 1000
Surgery	2785	18	6.46
Emergency	1466	3	2.05
Neonatology	3909	20	5.12
Paediatrics	3127	22	7.04
Medicine	782	7	8.95
Obstetrics and Gynaecology	198	1	5.05
Out-Patients	7082	41	5.79
Total	19348	112	5.79

The type and pattern of bacteria isolates in the various age groups is shown in Table 3. Gram-positive bacteria were encountered more often than gram negative bacteria (56.2% versus. 43.8%, P<0.05). Among the gram- positive bacteria, *Staphylococci* constituted 52 (82.5 %), *Streptococci* species 6 (9.5%) and unidentified gram-positive bacteria 5 (7.9%). Among gram-negative bacteria, enterobacteriaceae 39 (79.6 %) and non-fermenting bacteria 10 (20.1 %) were more frequent

Table 4 shows the *in-vitro* sensitivity pattern of Staphylococci to selected antibiotics. *Staphylococcus epidermidis* was most sensitive to minocycline (91%) followed by rifampin (88%). *S. aureus* was mostly sensitive to rifampin (95%) and *S. saprophyticus* to perfloracin (100%). The sensitivity pattern of gram-negative bacilli to selected antibiotics is shown in Table 5 Among the enterobacteriaceae *Salmonella typhi* showed high sensitivity to ceftioxin (71%) and Aztreonam (74%).

TABLE 3: THE TYPE AND DISTRIBUTION OF BACTERIA ISOLATES ACCORDING TO AGE

Bacteria Isolates	Age ranges (years)						Total (%)
	0-15 n(%)	16 -30 n(%)	31- 45 n(%)	46-60 n (%)	61-75 n (%)	>76 n (%)	
<i>S.aureus</i>	12 (10.9)	2 (1.8)	4 (3.6)	3 (2.7)	1 (0.9)	1 (0.9)	23 (20.9)
<i>S. epidermidis</i>	18 (16.4)	1 (0.9)	2 (1.8)	3 (2.7)	1 (0.9)	0	25 (22.7)
<i>S. saprophyticus</i>	3 (2.7)	0	0	1 (0.9)	0	0	4 (3.6)
<i>Streptococcus sp.</i>	5 (4.5)	0	0	1 (0.9)	0	0	6 (5.5)
<i>S. typhi</i>	3 (2.7)	6 (5.4)	0	1 (0.9)	0	0	10 (9.1)
<i>Salmonella spcies</i>	3 (2.7)	1 (0.9)	1 (0.9)	2 (1.8)	0	0	7 (6.4)
<i>K. pneumonia</i>	3 (2.7)	1 (0.9)	1 (0.9)	2 (1.8)	0	0	7 (6.4)
<i>E. coli</i>	3 (2.7)	0	2 (1.8)	0	1 (0.9)	0	6 (5.5)
<i>Enterobacter sp.</i>	6 (5.5)	1 (0.9)	0	0	0	0	7 (6.4)
<i>Pseudomonas sp.</i>	2 (1.8)	1 (0.9)	0	0	0	0	3 (2.7)
<i>Acinetobacter sp.</i>	2 (1.8)	0	0	0	0	0	2 (1.8)
<i>Proteus mirabilis</i>	1 (0.9)	0	0	0	0	0	1 (0.9)
Non enterobacteriaceae	2 (1.8)	0	0	0	1 (0.9)	0	3 (2.7)
Gram positive bacilli	3 (2.7)	1 (0.9)	1 (0.9)	0	0	0	5 (4.5)
<i>Klebseilla oxytoca</i>	1 (0.9)	0	0	0	0	0	1 (0.9)
Total	67 (60.9)	14 (12.7)	11(10.0)	13 (11. 8)	4 (3.6)	1 (0.9)	110(100)

Discussion

This study is a record of septicaemia in patients attending the University Teaching Hospital in Yaoundé, Cameroon. We included patients of all age groups. Results showed that septicaemia was

present in 28.3% of patients examined. Gram positive bacteria were encountered more than gram-negative bacteria, and the most frequent invasive bacteria were *Staphylococcus epiderimidis*, *S. aureus*, *Salmonella typhi* and *Klebseilla* species.

These results are similar to those obtained in some previous studies [9]: Bacteriamia was identified in 552 (45.9%) of 1201 children in Nigeria; 53.4% of the infections were due to gram positive bacteria and 46.6% due to gram negative bacteria. The most frequent isolate was *S. aureus* (47.7%) followed by coliforms (23.4%), unidentified gram negative rods (8.0%), *Pseudomonas aeruginosa* (5.8%), Streptococcal species (4.7%) and Chromobacteria species (4.5%). Hill et al [7] also reported an incidence of 34% (297) out of 871 patients studied. The isolates were dominated by gram-positive bacteria. *Streptococcus pneumoniae* study. The high rate of isolation from children may be due to their weak immune system as compared to adults, and most children often take in medications by means of intravenous devices that may easily introduce bacteria into their bloodstream when proper hygiene is not ensured.

The *in-vitro* susceptibility test of most common isolates showed very high resistance to commonly used antibiotics as penicillin, cotrimoxazole, amoxicillin, and amoxicillin/clavulanic acid. Staphylococci were generally sensitive to minocycline and rifampin (90%). Also noted was high rate of resistance to glycopeptides (Teicoplanin-29% and vancomycin-32%) by Staphylococci. Cotrimoxazole, amoxiciline and

accounted for 45.2%, *S. aureus* -18.3%, *E. coli*-9.7% and non-typhoidal *Salmonella* (8.6%) of the isolates. Results have also shown a very high incidence of septicaemia among children below the age of 15 years. This is similar to the results obtained from Nigeria [9] where 44.4% suspected cases were neonates and the rate of isolation among newborn was 22.6% out of the overall 45.9% incidence cases. It is also in accordance with results from Laos in which 69.2% of Staphylococci were from infants [12]. The rate of isolation also reduced with increasing age as seen in this

penicillin are extensively used in Africa due to their spectrum of activities [9]. This may account for the high resistance observed. Among the enterobacteriaceae, there was high rate of resistance to cephalosporins and other classes of antibiotics except for few *Salmonella typhi* that showed 100% sensitivity to cefotaxim, ceftriaxone and aztreonam. There was a high rate of resistance among the few *Klebsiella* species to all antibiotics used. This is probably due to production of penicillinase and extended spectrum beta lactamases [13]. Among the antibiotics used for all isolates, Staphylococci had a sensitivity of 70% and 66% to cefoxitin and gentamycin. Gram negative bacilli had sensitivity of 67% and 65% to cefoxitin and gentamycin respectively.

TABLE 4: SENSITIVITY PATTERN OF STAPHYLOCOCCI TO SELECTED ANTIBIOTICS

Bacteria Isolates	P n(%)	AMC n(%)	GN n(%)	S n(%)	FA n(%)	SXT n(%)	VA n(%)	E n(%)	L n(%)	SP n(%)	RA n(%)	TE n(%)	MI n(%)	PEF n(%)	OX n(%)	TEC n(%)	FOX n(%)
<i>S. Epidermidis</i>	5 (20)	20 (80)	15 (60)	15 (75)	18 (78)	5 (22)	18 (72)	15 (60)	18 (72)	13 (65)	22 (88)	13 (52)	21 (91)	17 (68)	14 (70)	17 (68)	14 (56)
<i>S. aureus</i>	5 (23)	17 (77)	16 (80)	15 (68)	17 (85)	5 (25)	15 (68)	13 (62)	11 (50)	16 (84)	21 (95)	11 (52)	19 (90)	15 (68)	18 (82)	17 (77)	21 (95)
<i>S. Saprophiticus</i>	2 (50)	2 (66)	2 (50)	2 (50)	2 (50)	2 (50)	1 (33)	1 (25)	1 (25)	2 (66)	3 (75)	2 (50)	1 (33)	4 (100)	1 (50)	1 (25)	1 (50)

P= penicillin, AMC amoxicillin, GN=gentamycin S=streptomycin, FA=fuscidicacid, SXT= ceftriaxone, A= vancomycin, E= erythromycin, L=lincomycin, SP=spiramycin, RA=rifampicin, TE=tetracycline, PEF=perfloxacin, OX=oxacillin, TEC=ceftazidime, FOX= cefoxime

Our results agree with the work of Meremikwu et al [9] who found that *S. aureus* and coliforms were highly resistant to amoxicillin, penicillin and cotrimoxazole. Sensitivity of *S. aureus* to gentamycin was 86.6% and 61.6% for coliforms. In India, Atul et al [1] found that 80% of *S. aureus*

strains were penicillin resistant. Resistance to erythromycin ciprofloxacin and gentamycin were above 45%. No strains showed resistance to vancomycin. Among enterobacteriaceae ceftriaxone was very effective and amikacin was very effective for gram-negative non-fermenters

like *Pseudomonas* and *Acinetobacter* species. This work also agrees with previous findings [13] in negative bacilli to amoxicillin (87%), piperacillin (74%) and cotrimoxazole (73%). Imipenem (98%) was the most active antibiotic followed by

which there were high rates of resistance among gram-ofloxacin (88%). Susceptibility to all isolates was 67%.

TABLE 5: SENSITIVITY PATTERN OF GRAM-NEGATIVE BACILLI TO SELECTED ANTIBIOTICS

Bacteria Isolates	AMX n (%)	AMC n (%)	CF n (%)	CFM n (%)	CTX n (%)	CRO n (%)	CAZ n (%)	AZN n (%)	AN n (%)	CIP n (%)	SXT n (%)	FOX n (%)	NET n (%)	GN n (%)	NO n (%)
<i>S. typhi</i>	1 (10)	7 (70)	6 (60)	9 (100)	7 (100)	9 (100)	9 (90)	10 (100)	9 (90)	9 (90)	2 (20)	8 (80)	6 (60)	8 (89)	9 (90)
<i>Salmonella</i> species	2 (28)	3 (42)	5 (71)	6 (85)	5 (7)	4 (80)	7 (100)	6 (85)	5 (83)	6 (85)	3 (42)	5 (71)	6 (85)	5 (71)	5 (83)
<i>Klebsiella pneumonia</i>	0	0	3 (37)	4 (50)	4 (50)	1 (14)	4 (50)	3 (37)	3 (37)	1 (12)	4 (50)	2 (40)	2 (40)	2 (50)	3 (37)
<i>E.coli</i>	2 (40)	2 (40)	2 (40)	2 (80)	4 (100)	4 (100)	3/5	5 (100)	3/5	4 (80)	3 (75)	6 (100)	2 (50)	1 (33)	3 (60)
<i>Enterobacter</i> species	1 (16)	1 (16)	2 (33)	2 (33)	2/5	1 (16)	2 (33)	2 (33)	3 (50)	1 (25)	1 (16)	6 (100)	2 (50)	1 (33)	3 (50)
<i>K. okytoa</i>	0	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)	1 (100)	0	0	0
Overall Enterobacteria ceae	6 (16)	13 (34)	18 (47)	25 (68)	23 (66)	21 (68)	25 (66)	28 (74)	23 (62)	25 (65)	11 (32)	27 (71)	17 (53)	25 (65)	23 (62)

AMX=amoxicillin, AMC=amikacin, CF= Cefuroxime CFM=, CTX= cotrimoxazole CRO=Cefuroxime CAZ= Ceftazidine AZN= aztreonam SXT=cotrimoxazole, AN= Nalidixic acid, CIP= Ciprofloxacin, SXT=ceftriaxone, FOX= Cefoxitime, NET=Netilmicin, GN=gentamycin, NO= Norfloxacin

However, while this study represents real life clinical practice in the hospital in which it was conducted, our approach had some limitations. The primary reason for requesting the blood culture from patients is still not clear.

Conclusion

This study shows that *Staphylococcus epidermidis*, *S. aureus* and *Salmonella typhi* are the living cause of bacteraemia among patients in the Yaoundé locality. In general Staphylococci were most sensitive to minocycline and rifampin while gram-

negative bacilli were more sensitive to cefoxitime. Observed decline in susceptibility of these common pathogens (especially gram-negative bacilli) to common antibiotics calls for increase effort to ensure more rational use of drugs. None of the antibiotics used singly showed high sensitivity to all the gram-negative bacteria, so a combination of two or more drugs (such as gentamicin, cefoxitime and ciprofloxacin) is needed to cover the broad range of gram-negative bacilli.

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