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Bacteriological Profile and Antimicrobial Susceptibility Pattern of Isolates from Patients with Septicaemia in Butembo, Democratic Republic of the Congo

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Authors' contributions

This work was carried out in collaboration between all authors. Author GKB designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors ZTK and AKN managed the literature searches and the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Emergence of antimicrobial resistance has limited treatment options against bloodstream infections. This study aimed to determine bacterial isolates from blood culture of patients with bloodstream infection and their sensitivity to antimicrobials at patients attending the Central Laboratory of Research of the "Université Catholique du Graben" (UCG) in Butembo.
Methodology: This was a cross-sectional study adopting a descriptive approach, conducted from January, 2015 to December, 2016. Blood was collected from a peripheral vein using an aseptic technique. The culture and the antimicrobial susceptibility testing were done by using standard methods according to the Clinical Laboratory Standard Institute guidelines.

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Results: The most isolated bacteria from blood culture of patient with bloodstream infections were *Staphylococcus aureus* (39.8%), *Listeria spp.* (17.4%), *Moraxella spp.* (14.3%), *Bacillus spp.* (13.4%) and *Klebsiella rhinoscleromatis* (4.1%). All bacterial isolates tested for sensitivity were resistant to the group of β-lactamine antibiotics except *S. aureus* which was sensitive only to vancomycin. It was also observed that all the bacterial species have a Multiple Antibiotic Resistance Index greater than 0.2.

Conclusion: Bloodstream infections are life-threatening conditions, continuous and regular monitoring of antimicrobial susceptibility pattern of responsible bacteria is needed. From this study, we suggested gentamicin, doxycycline, ciprofloxacin, and levofloxacin as the first line antibiotics of choice for empiric treatment of bloodstream infections in Butembo.

Keywords: Antibiotics; resistance; blood culture; septicaemia; DRC.

1. INTRODUCTION

Bloodstream infection (BSI) is defined as any form of invasiveness of the blood circulatory system caused by bacteria or fungi [1]. BSI remains one of the most important causes of morbidity and mortality globally [2,3]. Though the problem is still common in developed countries [4], the burden is high in sub-Saharan countries with 53% of children mortality rate [5]. Many bacteria which cause bacteraemia have been reported with variation in distribution from place to place [2,4,6,7]. Treatment of bacteraemia is usually done by timely administration of appropriate antibiotics. However, since many bacterial pathogens have developed resistance to most of antibiotics, it has become a serious health problem with many economic and social inferences all over the world [4,8]. Research findings have reported that inappropriate treatment of BSI aggravates to increased mortality of patients and emerging of drug resistance strains [9].

Antimicrobial resistance (AMR) is a serious threat to public health in Europe, leading to mounting healthcare costs, treatment failure, and deaths. Previous analyses from the European Centre for Disease Prevention and Control (ECDC) have estimated that infections caused by a subset of drug-resistant bacteria are responsible for about 25 000 deaths in Europe annually. In addition to these avoidable deaths, this also translates into extra healthcare costs and productivity losses of at least EUR 1.5 billion [10].

At the turn of the century, the World Health Organization (WHO) estimated that infections accounted for 45% of deaths in Africa and South-East Asia and that these diseases were responsible for 48% of premature deaths worldwide [11,12]. Bacteria cause a significant proportion of infections in Africa. Unfortunately, in a remarkably short time, resistance to antibiotics has undermined the idealistic hope that bacterial infection would cease to be an important cause of death and disease. Indeed, antibiotic resistance increasingly compromises the outcome of many infections that were, until recently, treatable and remain the most common diseases in Africa [11,13].

Vlieghe et al. [14] did a systematic review of the published literature on bacterial resistance in Central Africa between 1955 and 2008. Alarming resistance rates were noted in nearly all pathogens. Of special concern were multidrug resistant (MDR) in *Shigella* and *Salmonella spp*. and the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), high-level penicillin-resistant *Streptococcus pneumoniae* and extended-spectrum ß-lactamases (ESBL) among Gram-negative pathogens. These findings make clear that the Central African region shares the worldwide trend of AMR increasing and is in urgent need of sound surveillance based on competent and affordable microbiology to provide clear data on AMR.

In Democratic Republic of the Congo (DRC), infectious diseases are the leading cause of death [3,15]. In fact, the DRC has a crude mortality rate well above the average for sub-Saharan countries [16] and the highest under-5 years mortality rate in Africa [17], with malaria, pneumonia and diarrhoea as the leading causes of death [18,19].

In North Kivu District, most healthcare facilities lack the capacity to identify causative agents of infectious diseases reliably, including invasive bacterial infections such as BSIs.

Meanwhile, in South Kivu, Irenge et al. [3] found high rates of resistance to cotrimoxazole, erythromycin and ampicillin and moderate to high resistance to ciprofloxacin, ceftazidime,

ceftriaxone, cefuroxime and cefepime were observed among Gram negative bacteria. Furthermore, there were high rates of MDR and of ESBL production phenotype among Enterobacteriaceae. Butembo city is not so far from this reality.

Thus, this study aimed to determine isolates from blood culture of patients with bloodstream infection and their sensitivity to antimicrobials at patients attending the Central Laboratory of Research of the "Université Catholique du Graben" (UCG) in Butembo.

2. MATERIALS AND METHODS

2.1 Study Design

This was a cross-sectional study adopting a descriptive approach, carried out from January 2015 to December 2016 in the department of microbiology at the Central Laboratory of Research of the "Université Catholique du Graben" (UCG) in Butembo.

2.2 Population and Sample Size

We enrolled patients with suspicion of Bloodstream infection (BSI) attending the Central Laboratory of Research of the UCG for blood culture. Patients living out of Butembo, those who were taking antimicrobials, and those who did not consent to the study were excluded from this study.

The sample size was exhaustive including all patients who met the inclusion criteria. Convenience selection of blood culture reports was employed as a sampling method. Thus, 156 patients were retained for this study.

2.3 Specimen Collection

Blood was collected from a peripheral vein using an aseptic technique [20]. Approximately 5 millilitres of blood (1 to 3 millilitres for neonates) was inoculated directly into blood culture medium vials for cultivation and subsequent processing.

2.4 Specimen Processing

The blood culture was incubated aerobically at 37℃ and observed daily for the first 3 days for the presence of visible microbial growth by one of the following: turbidity of the broth and coagulation of broth. At the same time, subcultures were made during 3 successive days on enriched and selective media including blood, chocolate, MacConkey, and mannitol salt agar plates and examined for growth after 24-48 hours of incubation. The same protocol was repeated until the seventh day before blood culture was considered to be free of microorganism (negative). For the microorganism known as normal flora, the culture was repeated and if the same microorganism was isolated again, it was considered as pathogen and this in conjunction with other blood results exploring septicaemia. Isolates obtained were identified by standard microbiological techniques, namely, Gram staining, colony characteristics, and biochemical properties including catalase, coagulase (free and bound), DNase production, growth on mannitol salt agar, and haemolytic activity on blood agar plates for Gram-positive isolates, and Triple Sugar Iron (TSI), motility, indole, citrate utilization, ureases, oxidase and hydrogen sulphide production, Voges-Proskauer (VP) test, and growth of Gram-negative bacilli [21].

2.5 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of all bacterial isolates from bloodstream infection was performed by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Oxoid) according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2014) [22]. The various antimicrobials that were tested for susceptibility testing were as follows: amoxicillin-clavulanic acid (30 µg), oxacillin (1 µg), meropenem (10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), vancomycin (30 µg), gentamicin (10 µg), spectinomycin (100 µg), chloramphenicol (30 µg), doxycycline (30 µg), clindamycin (2 µg), erythromycin (15 µg), azithromycin (15 µg), clarithromycin (15 µg), ciprofloxacin (5 µg) and levofloxacin (5 µg). MDR bacteria were defined by its resistance to three or more antimicrobial classes and bacteria with intermediate susceptibility were considered as resistant for data analysis.

2.6 The Multiple Antibiotic Resistance Indices (MARI)

The MARI calculation was done by dividing the number of antibiotics to which a microorganism is resistant by the total number of antibiotics to which the organism was subjected to. Bacteria with a MARI greater than 0.20 implies that the resistance strains of such bacteria are from an environment where several antibiotics are used or misused.

2.7 Data Analysis

We used the software WHONET 5.6 for data analysis. Results were expressed as counts and percentages. Association of variables was analysed by using odds ratio (OR), with a confidence interval of 95% (95% CI). We considered associations as significant when OR were greater than 1 with a *P*-value of less than 0.05.

2.8 Ethical Consideration

Ethical clearance was obtained from the research ethics committee of the Faculty of Medicine at the "Université Catholique du Graben". Patients were explained and adequately informed on the purpose of the study and were assured of privacy and confidentiality. A consent form was signed by the patient who accepted participation to the study. For patients under 18 years, the consent was signed by their parents or guardians.

3. RESULTS

The blood culture proved to be positive in 59.2% of females and the age group of ≤ 5years and ≥46 years are the more touched, respectively in 58.2% (P=0.03, OR: 2.1) and 5.1% (P=0.06) (Table 1).

The Fig. 1 shows that Gram positive bacteria are isolated in 73.5% and Gram negative bacteria in 26.5% (Fig. 1). Following their frequency, the most isolated bacteria from blood culture are *Staphylococcus aureus* (39.8%)*, Listeria spp.* (17.4%)*, Moraxella spp.* (14.3%), *Bacillus spp.* (13.4%) and *Klebsiella rhinoscleromatis* (4.1%) (Table 2). The Table 3 shows that all bacterial isolates tested for sensitivity were resistant to the group of β-lactamine antibiotics except *S. aureus* which was sensitive only to vancomycin. Most of bacterial isolates show to be sensitive to aminoglycoside and quinolone antibiotics group. The MARI of all bacterial isolates was greater than 0.20 (Table 4).

4. DISCUSSION

Blood cultures were positive in 59.2% of females and 40.8% of males. Our results are close to those found by Wasihum et al. [4] in 2015, in their study on bacteriological profile and antimicrobial sensibility pattern of isolates from blood culture among febrile patients hospitalized at Mekelle hospital, in Ethiopia. They had observed predominance of bloodstream infections in females in 52.3%. Nevertheless, Irenge et al. [3] observed a light male predominance (50.6%) in Bukavu.

The group age the more touched is the one lower or equal than 5 years (58.2%) (OR=2.1>1 and P=0.03<0.05). These results are similar to those found in most sub-Saharan countries where the infantile death rate due to septicemia is 53% [5]. In Bukavu, Irenge et al. [3] had found a rate of 29.4% among the patients whose age was lower than 17 years. In this study, the high rate of septicaemia in children under 5 years may be explained by the presence of co-morbid conditions (prematurity, congenital heart diseases, leukaemia, lung diseases, etc.) during this period. It may be also explained by the weakness of immune system in childhood.

The Gram positive bacteria were more isolated (73.5%) than Gram negative bacteria (26.5%). These results are different from those found by Irenge et al. [3] in Bukavu who found a predominance of the Gram negative bacteria.

Table 1. Socio-demographic characteristics of patients

Variables	Results of blood culture		OR	95% IC	*P*-value
	Positive, n (%)	Negative, n (%)			
Sex					
Female	58 (59.2)	32 (55.2)	1.2	0.6-2.4	0.6
Male	40 (40.8)	26 (44.8)	0.8	0.4-1.7	
Total	**98 (100)**	**58 (100)**			
Age (years)					
≤ 5	57 (58.2)	23 (39.7)	2.1	1.0-4.3	0.03
6-15	12 (12.2)	9 (15.5)	0.5	0.2-1.3	0.1
16-25	8 (8.2)	7 (12.1)	0.7	0.2-2.1	0.4
26-35	7 (7.1)	5 (8.6)	0.8	0.2-3.1	0.7
36-45	9 (9.2)	6 (10.3)	0.9	0.3-3.0	0.8
≥ 46	5 (5.1)	8 (13.8)	0.3	0.1-1.2	0.06
Total	**98 (100)**	**58 (100)**	**-**	**-**	**-**

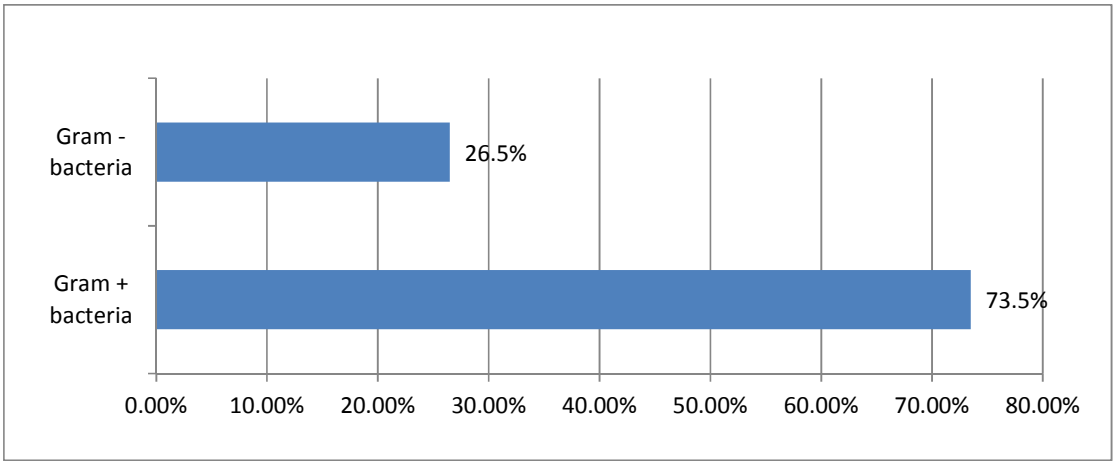

Fig. 1. Repartition of bacterial isolates according to their gram stain

This difference would be due to the fact that distribution of pathogens varies from a region to another and this variation can be observed in a same hospital [2,4,6,7].

Table 2. Incidence of bacterial isolates from blood samples of the patients

Bacterial isolates	n (%)
Staphylococcus aureus	39 (39,8)
Listeria spp	17 (17,4)
Moraxella spp	14 (14,3)
Bacillus spp	13 (13,4)
Klebsiella rhinoscleromatis	4 (4,1)
Pseudomonas aeruginosa	2 (2,0)
Yersinia pseudotuberculosis	1 (1,0)
Streptococcus spp	1 (1,0)
Laetobacilles spp	1 (1,0)
Lactobacillus spp	1 (1,0)
Acinetobacter spp	1 (1,0)
Cedecea neteri	1 (1,0)
Salmonella spp	1 (1,0)
Enterobacter sakazaki	1 (1,0)
Klebsiella ozaenae	1 (1,0)
Total	**98 (100)**

Bacteria responsible of bloodstream infection in this study are *Staphyloccoccus aureus* (39.8%), *Listeria spp* (17.4%), Moraxella spp (14.3%), *Bacillus spp* (13.3%) and *Klebsiella rhinoscleromatis* (4.1%). This distribution of pathogens is not identical to the one reported in literature by other authors [2,4,6,7].

S. aureus is the most bacterial isolates find in this study. It would be explained by the fact that the *S. aureus* is ubiquitous germ finding in soil, air and water. It is also commensal germ of skin and the mucous membranes. It can be also found as normal flora in oropharynx and in stools. A third of individuals are carrier of *S. aureus* in their nasal pits. The carelessness of hygiene is a factor that favours the infections bound to this species [23,24].

As it is shown in Table 3, all bacterial isolates tested for sensitivity were resistant to the group of β-lactamine antibiotics except *S. aureus* which was sensitive only to vancomycin. Most of bacterial isolates are sensitive to aminoglycoside and quinolone antibiotics group.

These results are similar to those find in Ethiopia by Deyno et al. [25]. They observed high level of resistance of S. aureus to amoxicillin, penicillin, ampicillin, tetracycline, methicillin, cotrimoxaziole, doxycycline and erythromycin. Relatively low prevalence of resistance was observed with kanamycin and ciprofloxacin.

A majority of the isolates reported to European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2013 was resistant to at least one of the antimicrobial groups under surveillance, and many of these showed combined resistance to third-generation cephalosporins, fluoroquinolones and aminoglycosides [26].

In Central Africa, the alarming rate of resistance has been noted for nearly all pathogens. Of special concern were MDR in *Shigella* and *Salmonella spp*. as well as the emergence of meticillin-resistant *Staphylococcus aureus* (MRSA), high-level penicillin-resistant *Streptococcus pneumoniae* and extended-spectrum ß-lactamases (ESBL) among Gram-negative pathogens [14].

Table 3. Antimicrobial susceptibility pattern of the bacterial isolates from blood specimens

Antibiotics	*S. aureus*		*Listeria*		*Moraxella spp.*		*Bacillus spp.*		*Klebsiella rhinoscleromatis*	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Augmentin	5 (19.2)	21 (80.8)	1 (10)	9 (90)	2 (20)	8 (80)	0 (0)	5 (100)	0 (0)	3 (100)
Oxacilline	0 (0)	8 (100)	0 (0)	1 (100)	ND	ND	ND	ND	ND	ND
Meropenem	0 (0)	2 (100)	0 (0)	2 (100)	ND	ND	ND	ND	ND	ND
Cefuroxime	1 (7.1)	13 (92.9)	1 (16.7)	5 (83.3)	0 (0)	3 (100)	ND	ND	ND	ND
Ceftriaxone	2 (6.5)	29 (93.5)	0 (0)	12 (100)	1 (16.7)	5 (83.3)	0 (0)	8 (80)	0 (0)	3 (100)
Cefotaxime	1 (20)	4 (80)	ND	ND	0 (0)	2 (100)	1 (33.3)	2 (66.7)	0 (0)	1 (100)
Vancomycin	7 (87.5)	1 (12.5)	0 (0)	1 (100)	ND	ND	ND	ND	ND	ND
Gentamicin	7 (22.6)	24 (77.4)	9 (64.3)	5 (35.7)	5 (55.6)	4 (44.4)	9 (90)	1 (10)	1 (50)	1 (50)
Spectinomycin	0 (0)	9 (100)	1 (50)	1 (50)	ND	ND	ND	ND	ND	ND
Chloramphenicol	11 (28.9)	27 (71.1)	3 (33.3)	6 (66.7)	3 (37.5)	5 (62.5)	6 (66.7)	3 (33.3)	2 (66.7)	1 (33.3)
Doxycycline	11 (32.4)	23 (67.6)	14 (93.3)	1 (6.7)	8 (72.7)	3 (27.3)	12 (100)	0 (0)	4 (100)	0 (0)
Clindamycin	21 (50)	21 (50)	4 (26.7)	11 (73.3)	1 (10)	9 (90)	4 (40)	6 (60)	1 (33.3)	2 (66.7)
Erythromycin	3 (9.1)	30 (90.9)	5 (35.7)	9 (64.3)	4 (40)	6 (60)	5 (45.5)	6 (54.5)	4 (100)	0 (0)
Azithromycin	2 (22.2)	7 (77.9)	0 (0)	2 (100)	ND	ND	ND	ND	ND	ND
Clarithromycin	0 (0)	7 (100)	0 (0)	2 (100)	1 (100)	0 (0)	ND	ND	ND	ND
Ciprofloxacin	20 (51.3)	19 (48.7)	14 (87.5)	2 (12.5)	8 (88.9)	1 (11.1)	12 (92.3)	1 (7.7)	4 (100)	0 (0)
Levofloxacin	5 (55.6)	4 (44.4)	2 (100)	0 (0)	1 (100)	0 (0)	ND	ND	ND	ND

S: n (%) Sensible, R: n (%) Resistant, ND: Not done

Table 4. Multiple antibiotic resistance indices (MARI) of the bacterial isolates

Isolates	MARI	Total number of antibiotic tested	Antibiotics to which the isolates are resistant
S. aureus	0.76	17	AMC, OXA, MEM, CXM, CRO, CTX, GEN, SPT, CHL, DOX, ERY, AZM and CLR
Listeria spp.	0.69	16	AMC, OXA, MEM, CXM, CRO, VAN, CHL, CLI, ERY, AZM and CLR
Moraxella spp	0.58	12	AMC, CXM, CRO, CTX, CHL, CLI and ERY
Bacillus spp	0.56	9	AMC, CRO, CTX, CLI and ERY
Klebsiela rhinoscleromatis	0.44	9	AMC, CRO, CTX and CLI

AMC: Augmentin, OXA: Oxacilline, CRO: Ceftriaxone, CTX: Cefotaxime, CXM: Cefuroxime, GEN: Gentamicin, SPT: Spectinomycin, CHL: Chloramphenicol, DOX: Doxycycline, CLI: Clindamycin, ERY: Erythromycin, AZM: Azithromycin, CLR: Clarithromycin, CIP: Ciprofloxacin, LVX: Levofloxacin, MEM: Meropeneme, VAN: Vancomycin

According to the Multiple Antibiotics Resistance Indices, all bacterial isolates show a MARI greater than 0.20 (Table 4). This implies that a very large proportion of the bacterial isolates have been exposed to several antibiotics and thus have developed resistance to these antibiotics. Bunduki et al. [27] had already shown the irrational use of antibiotics among student in this area. Detection of resistance and monitoring its spread requires appropriate laboratory based surveillance. Thus, to maintain the useful life of antimicrobial agents, there needs to be improved access to diagnostic laboratories, improved surveillance of the emergence of resistance, better regulation and better education of the public, clinicians/prescribers and veterinarians in the appropriate use of antibiotics.

5. CONCLUSION

The most prevalent bacterial isolates from bloodstream infection were *S. aureus, Listeria spp,*, *Moraxella spp*, *Bacillus spp* and *Klebsiella rhinoscleromatis.* And most of them are MDR. Rational utilization of antibiotics may help in decrease the trend of bacterial resistance. As BSIs are life-threatening conditions, continuous and regular monitoring of antimicrobial susceptibility pattern of bacterial isolates responsible of BSIs is needed. This should provide guidelines for initial empirical treatment. From this study, we suggest gentamicin, doxycycline, ciprofloxacin, and levofloxacin as the first line antibiotics of choice for empiric treatment of BSIs in Butembo. Further studies should be done for determining the phenotypic and genotypic resistance characterization of the MDR microorganisms.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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