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Etiologic agents and antibiotic susceptibility of pathogens isolated from blood cultures of patients admitted to some hospitals in Kisangani city, Republic Democratic of Congo

Agents étiologiques et sensibilité aux antibiotiques des agents pathogènes isolés des hémocultures des patients admis dans quelques hôpitaux de la ville de Kisangani, République Démocratique du Congo

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Résumé

Contexte et objectif. Les bactériémies sont des causes importantes de morbidité et de mortalité chez les patients hospitalisés. Cependant, les données sur la bactériémie dans les pays d'Afrique subsaharienne sont presque inexistantes. L'objectif était d'identifier les agents étiologiques isolés dans la bactériémie des patients hospitalisés dans quelques formations hospitalières de Kisangani.

Méthodes. Il s'agissait d'une étude de série des cas suspects de bactériémies admis dans cinq hôpitaux de Kisangani (Cliniques Universitaires de Kisangani, Village pédiatrique, Hôpitaux généraux de références de Kabongo, Lubunga et de Makiso) entre 2017 et 2019. Les hémocultures étaient réalisées dans des flacons d'hémoculture de type BACT/Alert FA. L'identification des organismes a été effectuée à l'aide des techniques microbiologiques classiques. La sensibilité aux antibiotiques a été réalisée à l'aide de la méthode de diffusion en milieu gélosé de Mueller Hinton selon les recommandations du Comité européen sur les tests de sensibilité aux antimicrobiens (EUCAST).

Résultats. Sur 2825 échantillons d'hémocultures prélevés chez des patients, 338 (12,0 %) étaient revenus positifs. Les agents pathogènes isolés comprenaient respectivement, le *Staphylococcus aureus* (25,7 %, n = 87), le *Salmonella Typhi* (19,6 %, n = 66), le *Pseudomonas* sp (16,0 %, n = 54), l'*Entérobactérie sp* (9,2 %, n = 31), le *Klebsiella sp* et l *E. coli* (6,2 %, n = 21). A l'antibiogramme, presque toutes les bactéries étaient sensibles aux antibiotiques testés, à l'exception des Gram négatifs qui étaient résistants à l'ampicilline, le cotrimoxazole, la gentamicine et les céphalosporines de troisième génération.

Conclusion. Les souches d'Entérobactéries, *Staphylococcus aureus* ont été les principaux agents étiologiques isolés des bactériémies. Ces souches étaient très résistantes aux antibiotiques couramment prescrits en pratique routinière.

Summary

Context and objective. Bacteremia is a major cause of morbidity and mortality in hospitalized patients. However, data on bacteremia in sub-Saharan African countries are scarce. The aim of the study was to identify the etiological agents isolated in the bacteremia of patients admitted in several hospitals in Kisangani, Democratic Republic of Congo. *Methods.* This was a series of suspected cases of severe infections admitted to five Kisangani hospitals (Cliniques Universitaires de Kisangani, Village pédiatrique, Hôpitaux généraux de références de Kabongo, Lubunga et de Makiso) between 2017 and 2019. Blood cultures were performed in BACT/Alert FA blood culture bottles. Organisms were identified using standard microbiological techniques. Antibiotic sensitivity was determined using the Mueller Hinton agar diffusion method, as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). *Results.* Of 2825 blood culture samples taken from patients, 338 (12.0%) were positive. The pathogens isolated included, *Staphylococcus aureus* (25.7%, n = 87), *Salmonella Typhi* (19.6%, n = 66), *Pseudomonas sp* (16.0%, n = 54), *Enterobacter sp* (9.2%, n = 31), Klebsiella sp and E. coli (6.2%, n = 21), respectively. On sensitivity testing, almost all bacteria were sensitivity to the antibiotics tested, with the exception of Gram-negative bacteria, which were resistant to ampicillin, cotrimoxazole, gentamicin and third-generation cephalosporins. *Conclusion*. Strains of Enterobacteriaceae and *S. aureus* were the main etiological agents isolated from bacteremia. These strains were highly resistant to antibiotics commonly prescribed in routine practice.

Keywords: Bacteremia, Etiologic agents, antibiotic resistance

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Introduction

Bacteremia are responsible for considerable mortality and morbidity in community and hospitalized patients, mostly in children under 5 years, in low- and middle-income countries in general, and particularly in sub-Sahara countries (1-2). This could be linked to the lack of the improvements in infection control and prevention procedures regarding the management and the treatment of this potentially lethal infection applying appropriate antimicrobial therapy. The related mortality rate could increase when the initiation of effective and appropriate antibiotic therapy with regard to the agent, dose, and duration is delayed. Blood cultures remain the only one diagnostic means to identify the microorganisms responsible for bacteremia and are also an important antibiotic stewardship tool which directly influences patient management (3). Nevertheless, a great improvement has been recorded in recent years with a reduction in the time required to identify germs from blood cultures with the development of new methods, including Matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrometry (4-5) and Polymerase Chain Reaction (PCR) (6). However, patients in the no industrialized world do not have easy access to blood culture, so that the epidemiological characteristics of bacteremia are not well documented. In the Democratic Republic of Congo (DRC), data on blood cultures are quite rare and mainly concentrated in the Western of the country, notably Kinshasa, Bamwanda and Kisantu. These data concern especially *Salmonella* species in most cases, and do not take into account the wide range of microorganisms potentially responsible for bacteremia (7). Indeed, the majority of general referral hospitals, especially in urban, peri-urban and rural regions do not have bacteriological diagnostic laboratories. Furthermore, the Laboratory staff are not sufficiently trained in sampling techniques, blood cultures and in carrying out antimicrobial susceptibility tests. Thus, clinicians' resort to probabilistic antibiotic therapy based on their own clinical experiences and/or epidemiological data, themselves unreliable. In consequence, the causes of a large number of deaths are not fully investigated, thereby reducing knowledge of the real impact of bacteraemia. The aim of this study was to identify the etiologic agents involved in bacteremia from hospitalized patients in the five major hospitals of Kisangani region from 2017 to 2019.

Methods

Design, setting and period of the study

This was a case series of suspected severe infection attending five healthcare structures in Kisangani, the main town of the Province of Tshopo, in the North-East of the DRC. Blood samples were collected in the following hospitals: Kisangani University Hospital (CUKIS), the Pediatric Village (VDP), the Kabondo General Referral Hospital (HGRK), the Makiso General Referral Hospital (HGRM), and the General Referral Hospital of Lubunga (HGRL).

Criteria selections

All individuals (children and adults) admitted to hospital suspected of presenting a severe infection (meningitis, pneumonia, pyelonephritis, typhoid fever, etc.) were included in the study. A total of 2825 blood samples were collected in vials (5 ml for adults and children, 1- 2 ml for

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newborns) of the Bact/ALERT FA type (bioMérieux, France). All samples were transported to microbiology pediatric laboratory of CUKIS within 2 hours of collection for incubation at 35-37°C during a maximum of 7 days. Only positive blood cultures (cloudy, haemolyzed or coagulated vials) were taken into account.

All positive blood cultures were sub-cultured onto blood agar, MacConkey agar, and Sabouraud plus chloramphenicol plates for microbial isolation. Inoculated plates were incubated at 37°C for 48 hours. All isolates were identified and processed according to standard techniques summarized in Table 1.

Table 1. Laboratory methods for bacteria identification

Organisms	Tests for identification
Enterobacterales and other Gram-negative bacilli	Gram staining, Oxidase test, Indole and Urease production, Methyl red test, Voges-Proskauer test, Citrate utilization test, Urease production, hydrogen sulphide, gas production and fermentation of sugars, phenylalanine deaminase, lysine decarboxylase (L.D.C.), Ornithine decarboxylase (O.D.C.), Arginine dihydrolase (A.D.H.) tests.
Staphylococci species	Gram staining, Catalase, Coagulase and DNase tests.
Yeast species	Morphological and biochemical characteristics: Verification of germinative tube and chlamydoconidium production and filamentation, followed by tests of carbon and hydrogen assimilation and carbohydrates fermentation.

The antibiotic sensitivity profile of gram-negative isolates was determined by the disc diffusion method according to EUCAST guidelines and interpreted by the S-I-R system. The following antibiotic discs were used: ampicillin, amikacin, gentamicin, ciprofloxacin, co-trimoxazole, cefotaxime, ceftazidime and meropenem. For the quality control we used *Escherichia coli* ATCC 25922.

Statistical analysis

Statistical analysis was done using Excel (Microsoft office, USA). Data were presented as tables to calculate frequencies, prevalence and distribution of isolates.

Results

Study population

The majority of the 2825 patients included in the present study were children (67%), among them 16% (*n*=452) in early neonatology (≤ 7 days), 22% (*n*=622) from 1 month to 1 year, preschool children were 29% (*n*=819), and 19% (*n*=537) were school-aged children. Only 14% (*n*=395) of patients were adults (≥ 18 years old) (Figure 1).

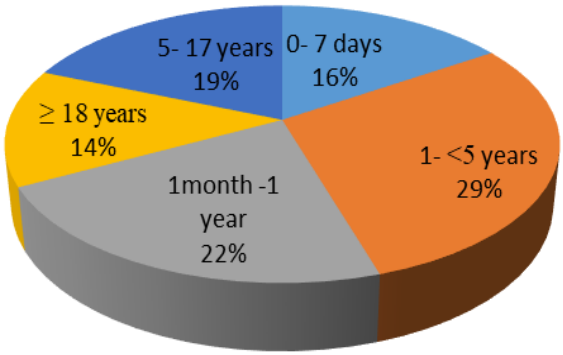

Figure 1. Distribution of patients according to age Distribution of samples and positive blood cultures

From a total number of 2825 blood samples collected in this study, 338 blood cultures (12 %) were positive (Table 2).

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Table 2. Distribution of positive blood cultures according to the origin of the samples

Hospitals	Number (%) of samples	Number (%) positive blood cultures	Number (%) positive blood cultures / hospital
GRK	1246 (44)	150 (44)	150 (12.0)
VDP	1118 (40)	121 (36)	121 (10.8)
HGRM	155 (5)	12 (4)	12 (7.7)
HGRB	216 (8)	40 (12)	40 (18.5)
CUKIS	90 (3)	15 (4)	15 (16.7)
Total	**2825 (100)**	**338 (100)**	**338 (12.0)**

Legends: CUKIS: Kisangani University Clinics; VDP: Pediatric Village; HGRK: Kabondo General Referral Hospital; HGRM: Makiso General Referral Hospital; HGRL: General Referral Hospital of Lubunga.

As shown in Table 3, the most microorganisms isolated in the blood cultures were *S. aureus* with 87 strains (25.7%), followed by *Salmonella* Typhi with 66 strains (19.6%), *Pseudomonas* sp with 54 strains (16.0%), *Enterobacter* sp with 31 strains (9.2%), *Klebsiella* sp and *E. coli* with 21 strains (6.2%), respectively. Only 13 strains of *Candida* sp. (3.9%) were isolated in the blood samples.

Table 3. Distribution of microbial strains by hospital

Organisms	**HGRK**	**CUKIS**	**VDP**	**HGRM**	**HGRL**	**Total**
	N	*n*	*n*	*n*	*n*	*n (%)*
Candida sp	3	0	6	0	4	13 (3.9)
Citrobacter sp	6	0	7	0	4	17 (5.0)
E. coli	11	3	4	2	1	21 (6.2)
Enterobacter sp	17	2	9	0	3	31 (9.2)
Klebsiella pneumoniae	10	0	8	1	2	21 (6.2)
Proteus sp.	1	0	3	0	1	5 (1.5)
Providencia sp.	1	0	1	0	0	2 (0.5)
Pseudomonas spp.	2	0	43	1	8	54 (16.0)
Salmonella Paratyphi	4	0	2	2	0	8 (2.3)
Salmonella Typhi	43	3	10	3	7	66 (19.6)
Serratia spp.	5	0	5	0	3	13 (3.9)
S. aureus	47	7	23	3	7	87 (25.7)
Total	**150/1246**	**15/90**	**121/1118**	**12/155**	**40/216**	**338 (100%)**

Legends: CUKIS: Kisangani University Hospital; VDP: Pediatric Village; HGRK: Kabondo General Referral Hospital; HGRM: Makiso General Referral Hospital; HGRL: General Referral Hospital of Lubunga.

S. aureus (n= 12) was the predominant pathogen isolated from patients of all age combined. *Salmonella* Typhi were isolated particularly to children of 1-5 years old (n=33). *Pseudomonas* species were found to patients of 1-15 years old. *E. coli* (*n*= 11), *Citrobacter* species (*n*= 7), and *Candida* species (*n*= 6) were isolated from patients of > 18 years old (Table 4).

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Table 4. Distribution of microorganisms according to age

Organisms	**0 - 7 days**	**1-59 moths**	**1 - <5 years**	**5 - 17 years**	**Adults**	Total
	N	*n*	*n*	*n*	*n*	*n (%)*
Candida sp	0	3	2	2	6	13 (3.9)
Citrobacter sp	1	2	2	5	7	17 (5.0)
E. coli	2	2	4	2	11	21(6.2)
Enterobacter sp	9	6	5	6	5	31 (9.2)
Klebsiella pneumoniae	4	8	3	2	4	21 (6.2)
Proteus sp.	0	2	2	1	0	5 (1.5)
Providencia sp.	0	0	2	0	0	2 (0.5)
Pseudomonas spp.	6	16	15	16	1	54 (16.0)
Salmonella Paratyphi	1	4	3	0	0	8 (2.3)
Salmonella Typhi	1	11	33	11	10	66 (19.6)
Serratia spp.	0	3	7	2	1	13 (3.9)
S. aureus	12	25	19	9	22	87 (25.7)
Total	**36**	**82**	**97**	**56**	**67**	**338 (100%)**

Antibiotic susceptibility

The resistance rate of all gram-negative bacteria to ampicillin were ≥ 90%. *Salmonella* Typhi isolates showed increased resistance to co-trimoxazole (92%), meropenem (90 %), amikacin (72%), gentamicin (64%), cefotaxime (22%), ceftazidime (20%) and ciprofloxacin (4%). *Klebsiella pneumoniae* strains also showed resistance to meropenem (75 %), and co-trimoxazole (69 %), gentamicin (50%), amikacin (31%), cefotaxime and ceftazidime (25%) respectively and ciprofloxacin (19%). *Escherichia coli* were resistant to cotrimoxazole (86%), gentamicin (57%), amikacin (43%), ciprofloxacin (36%), cefotaxime, ceftazidime and meropenem (21%) respectively.

Pseudomonas aeruginosa were resistant to cefotaxime (79%), ceftazidime (63%), meropenem (26%), gentamicin (21%), ciprofloxacin (16%) and amikacin (11%). *Enterobacter* species were resistant to co-trimoxazole, amikacin and gentamicin (78%) respectively, ceftazidime (56%), cefotaxime (33%), ciprofloxacin (28%), meropenem (6%). *Citrobacter* species demonstrated resistance against co-trimoxazole (67%), ciprofloxacin (47%), gentamicin and ceftazidime (40%), respectively, cefotaxime (27%) and amikacin (20%). All *Citrobacter* species were sensitive to meropenem (Table 5). The antibiotic susceptibility of *S. aureus* strains was not tested.

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Table 5. Antimicrobial susceptibility of Gram-negative isolates

Isolate		Ampicillin			Cefotaxime			Ceftazidime			Gentamicin			Meropenem			Amikacin			Co-trimoxazole			Ciprofloxacin		
	N°	R(%)	I(%)	S(%)	R(%)	I(%)	S(%)	R(%)	I(%)	S(%)	R(%)	S(%)	I(%)	R(%)	I(%)	S(%)	R(%)	I(%)	S(%)	R(%)	I(%)	S(%)	R(%)	I(%)	S(%)
Salmonella typhi	50	94	0	6	22	0	78	20	0	80	64	4	32	90	2	8	72	0	28	92	0	8	4	10	86
K. pneumoniae	16	88	0	12	25	12	63	25	25	50	50	6	44	75	25	0	31	0	69	69	0	31	19	25	56
E. coli	14	100	0	0	21	0	79	21	0	79	57	14	29	21	0	79	43	7	50	86	7	7	36	14	50
Enterobacter species	18	94	0	6	33	17	50	56	11	33	78	5	17	6	6	88	78	5	17	78	5	17	28	11	61
Citrobacter species	15	80	0	20	27	20	53	40	13	47	40	13	47	0	0	0	20	7	73	67	20	13	47	6	47
P. aeruginosa	19	NA	NA	NA	78	11	11	63	0	37	21	11	68	26	0	74	11	21	68	NA	NA	NA	16	16	68

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Discussion

In this study we performed blood culture and identified pathogens involved in bacteremia in 5 major hospitals of Kisangani region. Similar studies have been conducted in many African countries. In the present study we observed that the pathogens were cultured from the blood of 338 (12.0 %) patients, with the predominance of *S. aureus* (87 [25.7 %]), followed by *Salmonella* Typhi 66 isolates (19.6%), *Pseudomonas* sp (54 [16.0%]), *Enterobacter* sp (31 [9.2%]), *Klebsiella* sp and *E. coli* [21 (6.2%]), respectively. This positivity rate is low than which were obtained in a study conducted in Nigeria by Popoola, *et al*. They found that bacterial pathogen was cultured from 117 (17.2%) patients, with *S. aureus* (69 [59.0%]) and *Salmonella enterica* (34 [29.1%]) as prevalent species recovered (8). In Uganda, of 250 samples cultured, 45 (19.1%) had bacteremia. *Staphylococcus aureus* (42%), non-typhoidal *Salmonella* (24%), *Pseudomonas aeruginosa* (11%), and *Streptococcus pneumoniae* (9%) were the most common bacterial isolates (9). The results of studies conducted in Zanzibar demonstrated also that, pathogenic microbes were isolated from 14.0 % (66 patients /470) of the blood cultures. Etiologic agent profile was different with this study. The most frequent pathogens isolated by of Onke et al. were *K. pneumoniae*, *E. coli*, *Acinetobacter* spp. and *S. aureus* (10). The prevalence observed in this study is similar to study done in Gambia, which documented a prevalence of 12%. Interestingly, *Staphylococcus aureus* accounted for 54% (26/48) of the isolates followed by non-typhoidal *Salmonella*, 10% (5/48), *Streptococcus pneumoniae*, 8% (4/48), and *Salmonella* typhi, 6% (3/48)(11). By contrast, the study undertaken by Christopher et al. reported a low prevalence of 6.6%. Contrary to the present findings, the common bacteria isolated were *Escherichia coli and Klebsiella pneumoniae*, which accounted for 7 (33.3%) and 6 (28.6%) of total isolates respectively. Others Gram-negative isolated were *Citrobacter* spp 2 (9.5%), *Enterobacter* spp 1 (4.25%), *Pseudomonas aeruginosa* 2 (9.5%), *Proteus spp* 1 (4.25%) and *Salmonella spp* 1 (4.25%). Only one isolate was gram-positive. bacteria and was identified as *Staphylococcus aureus*(12). This is not in the same line with the current results. The etiology of bacteremia is similar with the results obtained in other studies (13–18). The Gram-negative bacteria studied had a resistance rate greater than or equal to 80% to ampicillin. These pathogens were highly resistant to commonly prescribed antibiotics such as co-trimoxazole, amikacin and gentamicin. The majority of strains were multidrug resistant. Indeed, *Salmonella* Typhi strains were particularly resistant to meropenem. *E. coli*, *Klebsiella pneumoniae* and *Salmonella Typhi* strains showed resistance rates between 20-25% to cefotaxime and ceftazidime. A non-negligible proportion of strains with intermediate sensitivity were observed. *Enterobacter* and *Citrobacter* species strains showed a resistance rate of between 27 and 47% to third-generation cephalosporins. *Pseudomonas aeruginosa* was highly resistant to cefotaxime (79%) and ceftazidime (63%). The results of this study confirm the emergence of MDR strains in the DRC as around the word. Several studies have shown that Enterobacterales and Gram-negative bacteria such as *Pseudomonas* are resistant to antibiotics commonly used in cases of bacteremia and these strains produce extended-spectrum beta-lactamases and carbapenamase enzymes (7,19–22).

Conclusion

The results of this study showed that Enterobacterales and *Staphylococcus aureus* isolates were the main etiological agents isolated from bacteraemia. Increased resistance to commonly prescribed antibiotics is evidence of the emergence of multidrug resistant strains, and prompts to take adequate measures to implement antimicrobial resistance surveillance in DRC.

Conflict of interest

Authors declare no conflict of interest.

Author's contribution

Abibi collected and process of samples. Ngbonda and Falay contributed to the design of the study. Liesse and Takaisi contributed to the treatment of data and to the conception of the manuscript.

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