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(Research Article)

Biochemical profile and resistance phenotype of isolates responsible for bacteremia in 111 HIV-infected patients hospitalized at the National Reference University Hospital of N'Djamena from 2020 to 2023

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Abstract

Introduction: Surveillance of antibiotic resistant bacteria provides important information to optimize care for people living with HIV (PLHIV). The objective of this work was to determine the prevalence of bacteremia in PLHIV and to describe the biochemical profile and resistance of bacteria to antibiotics commonly prescribed for the care of patients and followed in the cohort in the infectious disease departments (SMI) of the National Reference University Hospital (CHU-RN) of N'Djamena.

Material and methods: This was a descriptive, analytical and etiological diagnostic study carried out on isolates responsible for bacteremia in PLHIV, from 01/01/2020 to 03/31/2023. The isolation, the identification of the bacteria and the antibiogram were carried out by standard methods of clinical microbiology.

Results: The study included 111 PLHIV, 51 of who were positive on blood culture (54%). The average age of PLHIV was 45.5 years with the extremes ranging from 15 and 76 years. The age group most affected by bacteremia was that of 55 years and over. A significant difference was observed in terms of the predominance of women over men (P = 0.02). The most identified bacteria were *Staphylococcus aureus* (56.86%) and *Escherichia coli* (16.68%). The most determined resistance phenotypes were multi-resistant bacteria (BMR) and extended-spectrum beta-lactamases (ESBL) with the proportions of 29.41% and 23.52% respectively.

Conclusion: The results of this study raise the need for continuous monitoring of antibacterial resistance and to apply the recommendations for the proper use of antibiotics among PLHIV in Chad.

Keywords: Biochemical profile; Resistance phenotype; Bacteremia; PLHIV; N'Djamena; Chad

1. Introduction

Bacterial blood infections in patients infected with the human immunodeficiency virus (HIV) are constantly increasing [1]. The characteristics of the management of these patients in Africa are the absence of bacteriological diagnostic tools in the vast majority of health care structures on the one hand and on the other hand the diagnosis at very advanced stages with profound immunosuppression, the absence or unavailability of specific treatments [2]. Since the advent of

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multi-therapy antiretroviral, bacterial infections represent an increasing share of morbidity and mortality in HIV-infected patients in developing countries and their incidence remains significantly higher than in the general population [19]. More than 20% of these infections consist of bacteremia, the prognosis of which is often guarded [4, 5]. In addition, the therapeutic options are reduced over the years by the emergence of bacteria resistant to the usual antibiotics. In the United States, methicillin-resistant *Staphylococcus aureus* (MRSA) infections are approximately 10 times more common in PLHIV than in the general population [6]. In countries with limited resources, co-trimoxazole prophylaxis, self-medication, diagnostic errors and poverty are the main factors contributing to this resistance [7, 8, 9].

In Chad, there is little data in the literature on these infections, particularly with regard to their bacteriological documentation and their biochemical and antibiotic resistance profile. The objective of this work was to determine the prevalence of bacterial strains responsible for severe bacteremia and to describe their biochemical profile and resistance to antibiotics commonly prescribed for the care of PLHIV and followed in the cohort of infectious disease departments of the Hospital Center National Reference University (CHU-RN) of N'Djamena.

2. Material and methods

2.1. Framework, period and type of study

This was an analytical, descriptive, cross-sectional and etiological diagnostic study conducted on isolates responsible for bacteremia in people living with HIV/AIDS, ranging from 01/01/202 to 31/03/2023, which was conducted and performed at the laboratory bacteriology unit of the National Reference Hospital Center (CHURN) in Ndjamena. Blood samples were taken at the infectious diseases department (SMI) of the CHU-RN. The SMI is one of the services of the CHURN responsible for receiving infected patients from other health institutions in N'Djamena and the provinces of Chad.

2.2. Inclusion and exclusion criteria

Included were all PLHIV patients on triple therapy for at least eight months, referred or admitted to the infectious diseases department of the CHURN for a blood culture.

Were excluded, PLHIV under triple therapy, referred or admitted to the SMI of the CHU-RN whose blood culture is not requested.

2.3. Method and condition of blood collection

- Take a vein from the elbow crease of the febrile patient.
- Disinfect the skin with iodized alcohol or other antiseptics.
- Withdraw with the 10 mL syringe.

Collecting blood through an intravascular device increases the risk of contamination. It is necessary to disinfect the lid of the blood culture bottles and the puncture point with an alcoholic antiseptic.

The volume of blood collected determines the sensitivity of the examination. In adults, it must be at least 20 mL, i.e. 10 mL per vial (aerobic and anaerobic hemoline vial). Whether the samples are multiple (spaced out over time, 2 to 3 samples from 2 bottles) or single (carried out simultaneously, a single sample from 4 to 6 bottles), the sensitivity is equivalent. On the other hand, the risk of contamination increases in the event of multiple samples and the interpretation is more delicate. Single sampling is not recommended for infective endocarditis (take 3 blood cultures over 24 hours) and infections related to an intravascular device. Blood culture samples are sent to the laboratory as soon as possible.

2.4. Incubation technique of blood culture broths in the BacAlert 3D

- Register the sample and assign it a code.
- Detach and paste the barcode from the vial in the register.
- Enter the name and password of the manipulator in the Bact Alert 3D.
- Click on the vial in the dialog box.
- Enter the vial barcode using the barcode/magnetic reader.
- Enter the patient number (register) twice using the keyboard.
- Successively enter the patient's first and last name.

- Insert a vial into compartment (B, C or D) depending on cell availability.
- Validate from the dialog box.
- Repeat the same operation for the second bottle

2.5. Bacteriological analysis

2.5.1. Direct examination and isolation of bacteria

- Remove the blood culture bottle when the screen shows a positive bottle in a cell (screen appears yellow).
- Disinfect the cap of the blood culture bottle.
- Syringe a quantity of blood culture broth to:
- Fresh examination and GRAM staining;
- The choice of culture media and biochemical identification galleries are made based on direct examination.

2.5.2. For information:

- Bacilles GRAM négatif, gélose : EMB, Hektoen, Mac Conkey ;
- Cocci GRAM positif, gélose : GSF, Chapman ;
- Suspicion d'*Haemophilus* : GC + Isovitalex (ou polyvitex) ;
- Entérobactéries et Pseudomonas : API20E (BioMérieux, 2017);
- Staphylocoques : la Galerie API® Staph (BioMérieux, 2017);
- Streptocoques : la galerie Strep (BioMérieux, 2017).

2.6. Culture

2.6.1. Bacteria isolation

The different media chosen were inoculated with blood culture broth.

NB: Bacteria requiring CO2 for their growth, the inoculated media were placed in the jar containing 5% CO_2.

- All of the inoculated media were incubated at 37 °Cin a bacteriological oven for 16 to 24 hours and carried out a first reading by noting the development of bacterial colonies on the various agar media.
- After every 24 hours, the jar was removed from the oven and removed and we proceeded to isolate the bacteria on different positive media.
- In the event of a negative culture, we reincubated for an additional 24 hours. Then take another reading. Then read every day for 2 weeks, inoculating the agar every 48 hours.

2.6.2. Biochemical identification of bacteria

McFarland's homogeneous bacterial suspension with opacity of 0.5 was prepared from a 24-hour fresh colony in a 5 mL bottle of medium water to inoculate the biochemical identification galleries.

After 18 to 24 hours of incubation, the reactions were observed and the galleries were read by adding one drop of each of the following additional biochemical reagents:

- VP test: VP1 and VP2; after 10 minutes, a clear pink or purple color is considered a positive reaction and a pale pink or light pink color is considered negative;
- NIT test: NIT1 and NIT2, after 10 minutes, a red color is a positive reaction;
- PAL test: ZYMA and ZYMB; after 10 minutes, a purple color is a positive reaction; a beige-pink or very pale violet color obtained after 10 minutes is considered negative.

Reading is done with the catalog of each gallery.

2.7. Catalase research

After isolation on Chapman medium for 24 hours, a very distinct colony was removed and placed on a slide, a drop of hydrogen peroxide H_2O_2 will be added to the colony. Catalase is an enzyme that catalyzes the degradation of hydrogen peroxide (H_2O_2) into H_2O and ½ O_2.

2.8. Coagulase research

Free coagulase is present in *S. aureus*, but can also be produced by *S. intermedius* or *S. hyicus.* This test consists in highlighting the coagulase released into the external environment. This coagulase is detected by adding 0.5 mL of human plasma and 0.5 mL of a 24-hour culture of staphylococci in broth to a hemolysis tube. The mixture is placed in an oven at 37 ° C and incubated for 24 hours. Strains of *S. aureus* cause plasma to clot most often in the first three hours. A positive test results in the formation of a clot.

2.9. Oxidase test

The oxidase test was performed on a fresh colony from Mueller-Hinton agar. In practice, with the cut of a Pasteur pipette or a platinum handle, a colony of germs to be studied is taken and crushed on a filter paper impregnated with a 1% solution of Tetramethyl-para- phenylene-diamine. Oxidase negative organisms will remain colorless or turn purple after 10 seconds. We do not take into account the colorations appearing beyond this time.

2.10. Study of the sensitivity of blood bacteria to antibiotics

2.10.1. Choice of antibiotics

The antibiotics were chosen according to their prescription for the treatment of opportunistic diseases in PLHIV in the different care structures.

Table 1 Antibiotics chosen for the susceptibility test

Category	Family	Antibiotic	Dose/disk
Antibiotic (Bio-Rad)	Aminosides	Gentamicin (GNN)	10 µg
		Amikacin (AKN)	30 µg
	Beta-lactams	Oxacillin (OXA)	5µg
		Méthicillin (MET)	5µg
		Amoxicillin (AMX)	25 µg
		Ampicillin (AMP)	10 µg
		Amoxicillin + clavulanic acid (AMC)	20/10 µg
		Aztreonam (ATM)	30 µg
		Ceftriaxone (CRO)	30 µg
		Céfoxitin (FOX)	30 µg
		Cefotaxim (CTX)	30 µg
		Ceftazidim (CAZ)	30 µg
		Imipenem (IMP)	10 µg
	Cyclins	Tetracyclin (TET)	30 µg
		Doxycyclin (DOX)	30 µg
	Fluroquinolone	Ciprofloxacin (CIP)	5 µg
		Norfloxacin (NXN)	5 µg
	Macrolides	Erythromycin (ERY)	15 µg
		Azithromycin (AZM)	15 µg
	Phenicoles	Chloramphenicol (CHL)	30 µg
	Quinolones	Nalidixic acid (NAL)	30 µg
	Sulfamides	Trimethoprim-sulfamethoxazole (SXT)	1.25 /23.75 µg
	8 families	22 antibiotics	

Quality control was performed using the reference strain *Escherichia coli* ATCC 25922.

2.11. Antibiogram

The antibiogram was carried out by the technique of Kirby and Bauer which is the method by diffusion of discs impregnated with antibiotics in MH agar medium flooded with the bacterial inoculum in suspension, as recommended by the Antibiogram Committee of the French Society of Microbiology (CA-SFM / EUCAST, 2016-2020).

2.12. Detection of extended-spectrum beta-lactamase (ESBL) production

The demonstration of the ESBL was made on Muller-Hinton agar by the double disc synergy test method according to the procedure of Jarlier et al. (1988). Discs of cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), and aztreonam (30 µg), were placed 30 mm (center to center) from an amoxicillin/acid disc clavulanic (20/10 µg) then incubated at 35 - 37°C. After 18 to 24 hours of incubation, the production of ESBL by the test organism was based on the partial inhibition of ESBL by acid clavulanic. The existence of even weak synergy between cefotaxime, ceftazidime, cefepime, aztreonam and clavulanic acid is characterized by a champagne cork-shaped image.

2.13. Reading

2.13.1. Double disc method

- C3G-AMC-C3G, 25mm distance;
- If formation of a champagne cork image, BLES+.

2.13.2. Combine disk method

- C3G-CLAV-CG3, distance 30 mm.

2.13.3. Calculation of the difference of 2 diameters

- If < 5 mm ESBL-;
- If > 5 mm ESBL+.

2.14. Data collection and processing

An investigation data collection sheet for blood culture including: origin, sex, age and suspected origin of contamination (source of drinking water, food and lifestyle of each PLHIV admitted or referred to the Infectious Diseases Service (SMI) of the CHU-RN The data were entered and analyzed using Microsoft Word and Excel 2010. The chi-square test (χ^2) was used for the comparison of the variables qualitative with a significance level set at 5%.

2.15. Ethical consideration

This study received prior approval from the Dean of the Faculty of Human Health Sciences (FSSH), University of N'Djamena and the Director of the National Reference University Hospital Center of N'Djamena. The research was carried out on clinical samples received at the laboratory for blood culture. The results were given to the clinicians for the treatment of **the patients.**

3. Results

3.1. Study population, biological parameters of patients, overall prevalence of bacteremia

In our study, we collected 111 people living with HIV referred or admitted to the infectious diseases department of the CHU-RN for blood culture, of which 51 (46%) cases were confirmed positive bacteremia and 60 (54%) negative cultures (Chi2 = 0.253< Chi20<3.84; dof = 1; *P* = 0.50; difference not significant). There is a clear female predominance at 78 (70.27%) and 33 (29.73%) of the men (Chi2 = 5.303> Chi20>3.84; ddl = 1; *P* = 0.02; significant difference) with the male/female sex ratio equal to 0.42. The average age of PLHIV was 45.5 years with extremes of 15 and 76 years. The age group most affected by bacteremia was that of 55 years and over (Figure 1).

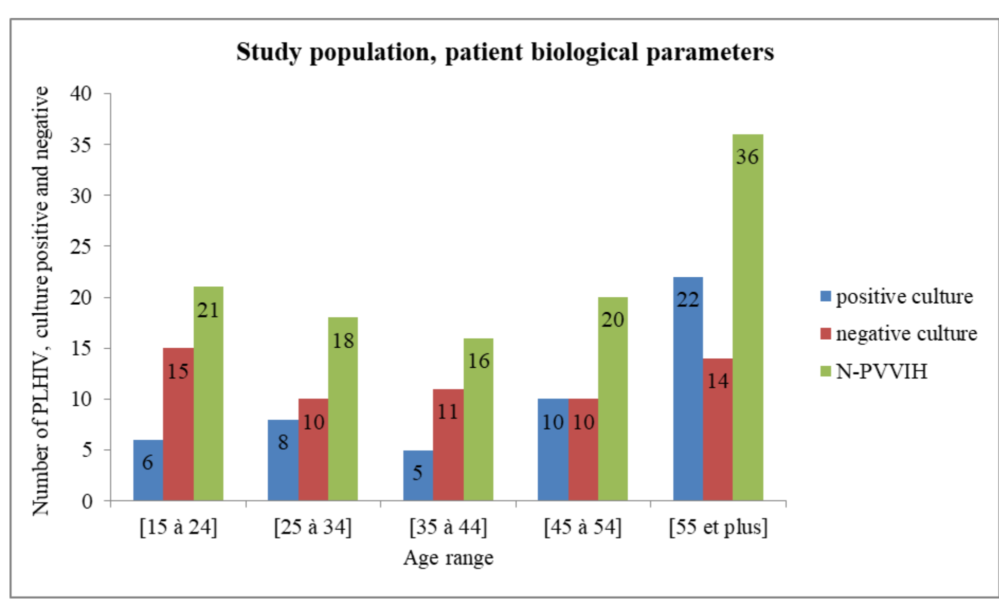


Figure 1 Sociodemographic and biological characteristics of patients

3.2. Frequency of bacteria isolated from the blood of PLHIV

Table 2 shows the frequency of bacteria isolated from the blood of PLHIV. These are: *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Salmonella* spp. *Staphylococcus aureus* (56.87%) were in the lead followed by *Escherichia coli* (16.68%).

Table 2 Frequency of bacteria isolated from the blood of PLHIV

A bacterial strain	Number	%
Staphylococcus aureus	29	56.86
Escherichia coli	8	16.68
Staphylococcus haemolyticus	6	11.76
Streptococcus pneumoniae	3	5.88
Pseudomonas aeruginosa	2	3.92
Klebsiella pneumoniae	1	1.96
Acinetobacter baumannii	1	1.96
Salmonella spp	1	1.96
Total	**51**	**100**

3.3. Distribution of bacterial strains isolated according to departments

Table 3 shows the distribution of isolates according to the origin of the PLHIV. The infectious diseases service (SMI) of the National Reference Hospital Center (CHURN) of N'Djamena, as its name suggests, is a sensible service to receive infected patients from other health institutions in N'Djamena and the provinces of Chad.

PLHIV hospitalized at the SMI-CHU-RN were in the lead (37.25%), followed by C-AlN-APMS (35.29%). It should be remembered that the Al Nadjma Center with its psycho-medical support service (APMS) is a national reference center for HIV testing and care for PLHIV based in N'Djamena.

Table 3 Distribution of bacterial strains isolated by department

A bacterial strain	Origin/Service				
	C-AlN-APMS	**CHU-BS**	**HNDA**	**SMI-CHU-RN**	**Total**
Escherichia coli	3	1	2	3	8
Staphylococcus aureus	9	5	4	11	29
Staphylococcus haemolyticus	2	0	2	2	6
Klebsiella pneumonieae	1	0	0	0	1
Acinetobacter baumannii	0	0	0	1	1
Salmonella spp	1	0	0	0	1
Streptococcus pneumoniae	1	1	0	1	3
Pseudomonas aeruginosa	1	0	0	1	2
Total (%)	18 (35.29)	7 (13.72)	8 (15.68)	19 (37.25)	51 (100)

C-AlN-APMS= Center al Nadjma-Support Psycho Medical; CHU-BS= Good Samaritan University Hospital Center; HNDA = Our Lady of the Apostles Hospital; SMI-CHU-RN= Infectious Diseases Department of the National Reference University Hospital Center of N'Djamena.

3.4. Phenotypes of resistance and susceptibility of bacterial strains isolated from the blood of PLHIV

Table 3 shows the distribution of the bacterial strains isolated according to the phenotypes of resistance and sensitivity to the antibiotics tested. The most detected resistance phenotypes were ESBL (31.37%) followed by MDR (23.52%) and the phenotype of multi-sensitive bacteria (BMS) was (39.21%).

Table 4 Distribution of isolates according to resistance and susceptibility phenotypes

	Resistance and susceptibility phenotype				
A bacterial strain	**BMS**	**ESBL**	**MDR**	**XDR**	**Total**
Escherichia coli	2	3	2	1	8
Staphylococcus aureus	13	8	7	1	29
Staphylococcus haemolyticus	3	1	2	0	6
Klebsiella pneumoniae	0	1	0	0	1
Acinetobacter baumannii	0	0	0	1	1
Salmonella spp	1	0	0	0	1
Streptococcus pneumoniae	1	2	0	0	3
Pseudomonas aeruginosa	0	1	1	0	2
Total	20 (39.21)	16(31.37)	12 (23.52)	3 (5.88)	51 (100)

Legend: BMS= Multi Sensitive Bacteria (sensitivity of a bacterial agent to antibiotics from at least three antibiotic families); MDR=Multi-Drug-Resistance (resistance of a bacterial agent to antibiotics to at least two antibiotic families); XDR=Extensive Drug Resistance (resistance of a bacterial agent to antibiotics in all but two families); ESBL=Beta Lactamase Extended Spectrum

3.5. Distribution of bacterial strains isolated from the blood of PLHIV according to the biochemical profile

In addition to the biochemical characters in Table 5, *Staphylococcus aureus* were coagulase positive and *Staphylococcus haemolyticus* were coagulase negative and all were catalase positive, raffinose negative, xylose negative. All Staphylococcus identified reduced nitrate to nitrite and all positive (NAG (N-acetyl-glucosamine), PAL (a-naphthyl-phosphate), and VP (Voges-Proskauer). *Staphylococcus aureus* did not react with methyl D-glucoside (MDG).

On the other hand, *Staphylococcus haemolyticus* reacted positively with MDG.All identified Enterobacteriaceae were confirmed oxidase negative. *Streptococcus pneumoniae* identified was catalase negative. *Pseudomonas aeruginosa* was confirmed oxidase positive.

Table 5 Biochemical profiles of bacterial strains isolated from the blood of PLHIV with the carbohydrates tested

A bacterial strain	Carbohydrates																							
	ONPG	**ADH**	**LDC**	**ODC**	**CIT**	**H2S**	**URE**	**TDA**	**IND**	**VP**	**GEL**	**GLU**	**MAN**	**INO**	**SOR**	**RHA**	**SAC**	**MEL**	**AMY**	**ARA**	**LAC**	**TRE**	**MAL**	**FRU**	**XLT**
Escherichia coli	+	+	+	-	-	-	-	-	+	-	-	+	+	-	+	+	+	+	-	+	+				
Staphylococcus aureus		+					+					+	+				+	-			+	+	+	+	-
Staphylococcus haemolyticus		+					+						+/-				+	-			+	+	+	+	-
Klebsiella pneumoniae	-	+/-	+/-	-	+	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+					
Acinetobacter baumannii	--	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+/-	-	+	-	+					
Salmonella spp	-	+	+	+	+	+	-	-	-		-	+	+	-	+	+	-	+	-	+	-				
Streptococcus pneumoniae		+/-								+	+		+		+					-	+	+			
Pseudomonas aeruginosa	-	+	-	+	+	-	+/-	-	-	+	+	-	-	-	-	+/-	-	+	-	+					

Legend: + = positive (use of carbohydrate by the bacterial strain); - = negative (non-utilization of carbohydrate by the bacterial strain); +/- = sometimes positive or negative.

NB: empty boxes: these are biochemical tests not carried out for the bacterial strain.

ONPG = Ortho-Nitro-Phenyl-Galactopyranosidase; ADH = Arginine Dihydrolase; LDC = Lysine Decarboxylase, ODC = Ornithine Decarboxylase; TIC = Simmons Citrate; H2S = Dihydrogen Sulfide; Urea; TDA = Tryptophan Deaminase; IND = Indole; VP; Vogues-Proskauer; GEL = gelatin; GLU = Glucose; MAN=Mannitol; INO = Inositol; SOR=Sorbitol; RHA = Rhamnose; SAC = Sucrose; MEL = Meliosis; AMY = Amygdalin; ARA = Arabinose, Oxidase, LAC = Lactose, TRE = Trehalose; MAL = Maltose; FRU=Fructose; Xylitol.

3.6. Susceptibility and resistance profiles of bacterial strains isolated from the blood of PLHIV

Table 6 Sensitivity and resistance profiles of bacterial strains isolated from the blood of PLHIV to the antibiotics tested

ATB	**A bacterial strain**															
	***E. coli* (n=8)**		***S. aureus* (n=29)**		***S. haemolyticus* (n=6)**		***K. .pneumoniae* (n=1)**		***A. baumannii* (n=1)**		***Salmonella spp* (n=1)**		***Str.pneumoniae* (n=3)**		***P. aeruginosa* (n=2)**	
	%(R+I)	%S	%(R+I)	%S	% (R+I)	%S	%(R+I)	%S	% (R+I)	%S	%(R+I)	%S	%(R+I)	%S	%(R+I)	%S
GNN	2 (25)	6 (75)	12 (41.37)	17 (58.62)	1 (16.66)	5 (83.33)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (33.33)	2 (66.66)	1 (50)	1 (50)
AKN	3 (37.5)	5 (62.5)	8 (27.58)	21 (72.41)	0 (0)	6 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	3 (100)	0 (0)	2 (100)
OXA	NR		19 (65,51)	10 (34,48)	5 (83.33)	1 (16.66)	NR		NR		NR		3 (100)	0 (0)	NR	
MET	NR		29 (100)	0 (0)	5 (83.33)	1 (16,66)	NR		NR		NR		NR		NR	
AMX	6 (75)	2 (25)	22 (75,86)	7 (24,13)	3 (50)	3 (50)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	3 (100)	0 (0)	2 (100)	0 (0)
AMP	7 (87,5)	1 (12,5)	17 (58,62)	12 (41,37)	4 (66,66)	2 (33,33)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	3 (100)	0 (0)	2 (100)	0 (0)
AMC	3 (37,5)	5 (62,5)	11 (37,93)	18 (62,06)	2 (33,33)	4 (66,66)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	3 (100)	2 (100)	0 (0)
ATM	2 (25)	6 (75)	NT		NT		NT		NT		0 (0)	1 (100)	0 (0)	3 (100)	2 (100)	0 (0)
CRO	4 (50)	4 (50)	13 (50)	16 (55,17)	4 (66,66)	2 (33,33)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	3 (100)	2 (100)	0 (0)
FOX	4 (50)	4 (50)	11 (37,93)	18 (62,06)	5 (83,33)	1 (16,66)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	3 (100)	2 (100)	0 (0)
CTX	3 (37,5)	5 (62,5)	10 (34,48)	19 (65;51)	3 (50)	3 (50)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	3 (100)	0 (0)	2 (100)	0 (0)
CAZ	4 (50)	4 (50)	NT		NT		0 (0)	1 (100)	1 (100)	0 (0)	NT		NT		0 (0)	2 (100)

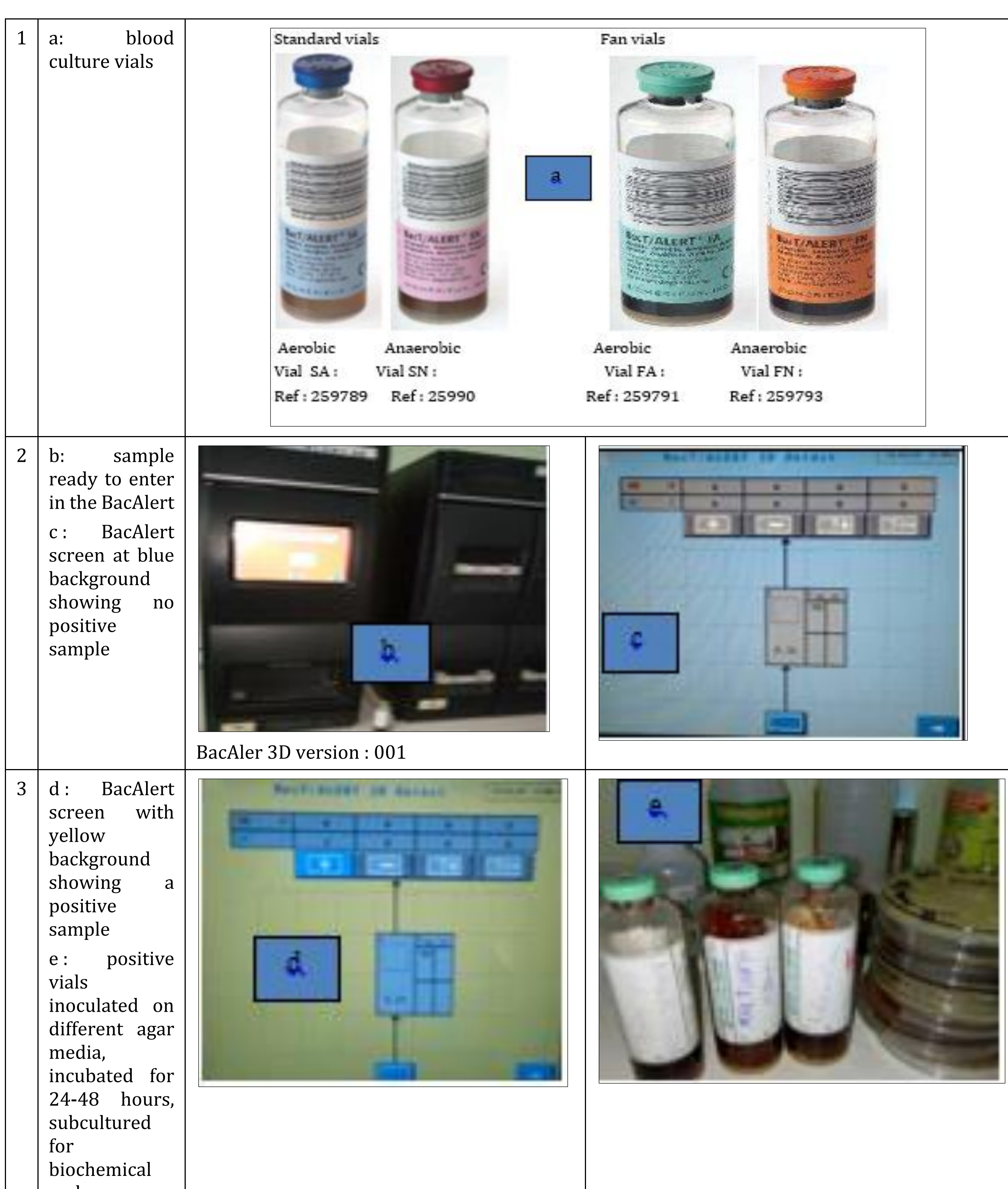
IMP	2 (25)	6 (75)	3 (10.34)	27 (93.10)	6 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	3 (100)	2 (100)	0 (0)
TET	5 (62.5)	3 (37.5)	15 (51.17)	14 (48.27)	4 (66.66)	2 (33.33)	0 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	3 (100)	0 (0)	0 (0)	02(100)
DOX	NT		17 (58.62)	12 (41.37)	3 (50)	3 (50)	NR		NR		NR		NR		NR	
CIP	5 (62.5)	3 (37.5)	9 (31.03)	20 (68.96)	0 (0)	6 (100)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)	3 (100)	0 (0)	2 (100)	0 (0)
NXN	4 (50)	4 (50)	6 (20.68)	23 (79.31)	0 (0)	6 (100)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	3 (100)	2 (100)	0 (0)
ERY	NR		16 (55.17)	13 (44.82)	2 (33.33)	4 (66.66)	NT		NR		NR		3 (100)	0 (0)	NR	
AZM	NR		11 (37.93)	18 (62.06)	0 (0)	6 (100)	NT		NR		NR		0 (0)	3 (100)	NR	
CHL	4 (50)	4 (50)	14 (48.27)	15 (52.72)	5 (83.33)	(16.66)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	3 (100)	2 (100)	0 (0)
NAL	5 (62.5)	3 (37.5)	NR		NR		1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	3 (100)	0 (0)	2 (100)	0 (0)
SXT	8 (100)	0 (0)	29 (100)	0 (0)	6 (100)	0 (0)	1 (100)	0 (100)	1 (100)	0 (0)	1 (100)	0 (0)	3 (100)	0 (0)	2 (100)	0 (0)

Legend: NR= not required; NT= not tested; ATB= antibiotic; n= number; % (R+I)= resistance rate; %S = sensitivity rate; *E.coli= Escherichia coli*; S.aureus= *Staphylococcus aureus; S.haemolyticus=Staphylococcus haemolyticus; K. pneumoniae= Klebsiella pneumoniae; A. baumanii= Acinetobacter baumannii; Salmonella spp; Str pneumoniae = Streptococcus pneumoniae; P. aeruginosa = Pseudomonas aeruginosa.*

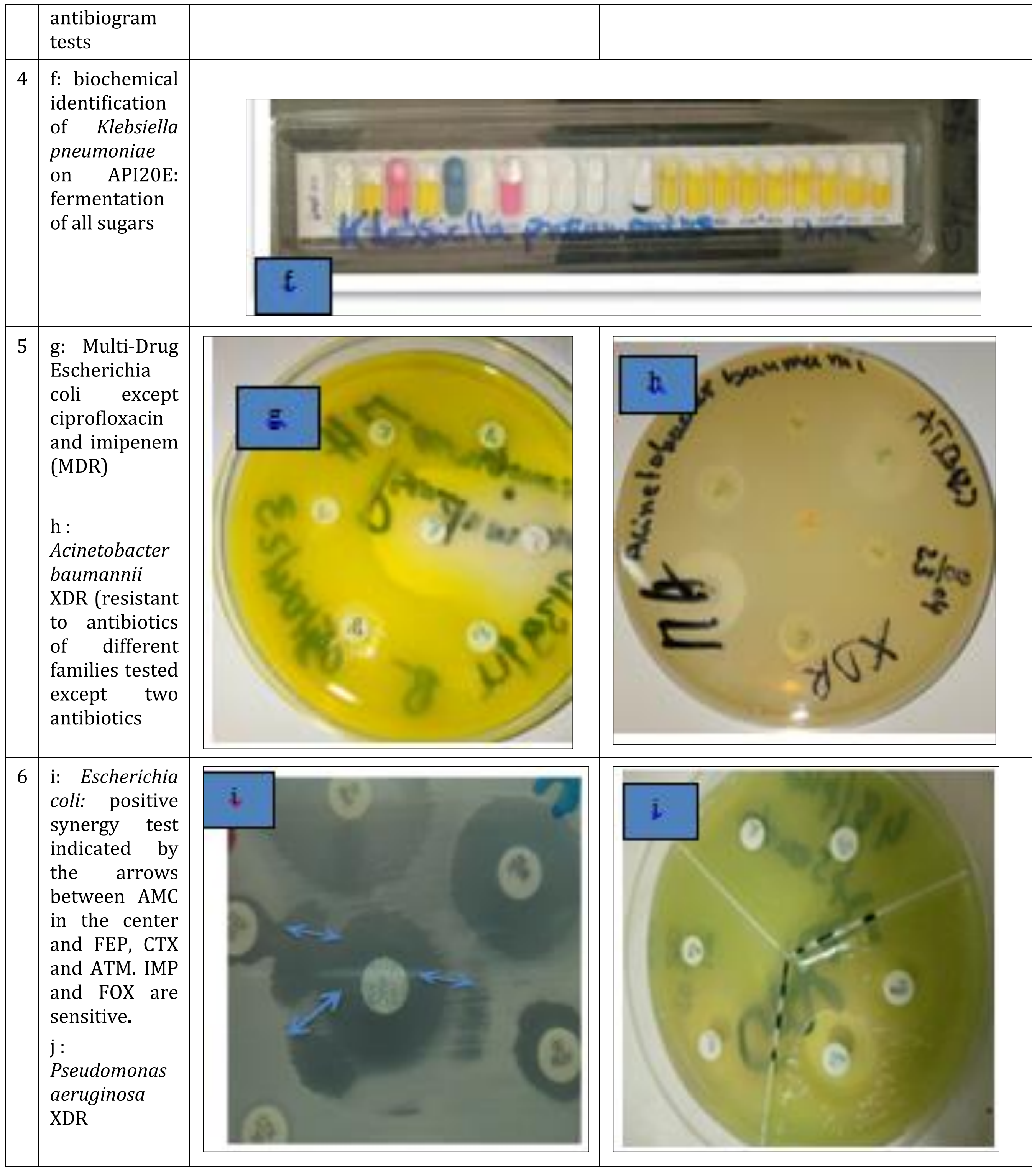
Gentamicin (GNN); Amikacin (AKN); Oxacillin (OXA); Methicillin (MET); Amoxicillin (AMX); Ampicillin (AMP); Amoxicillin + clavulanic acid + (AMC); Aztreonam (ATM); Ceftriaxone (CRO); Cefoxitin (FOX); Cefotaxim (CTX); Ceftazidim (CAZ); Imipenem (IMP); Tetracyclin (TET); Doxycyclin (DOX); Ciprofloxacin (CIP); Norfloxacin (NXN); Erythromycin (ERY); Azithromycin (AZM); Chloramphenicol (CHL); Nalidixic acid (NAL); Trimethoprim-sulfamethoxazole (SXT).

Table 6 shows the sensitivity and resistance profiles of bacterial strains isolated from the blood of PLHIV to the antibiotics tested. The resistance of the isolated strains was more noticeable with the aminopenicillins and almost 100% with the methicillin, and the third generation cephosporins. All bacterial strains were 100% resistant to sulfonamides (Trimethoprim-sulfamethoxazole). On the other hand, the sensitivity of the isolates was varied to (Aminosides, Macrolides, Fluoroquinolones, Carbapenems (Betalactams), Cyclins**).**

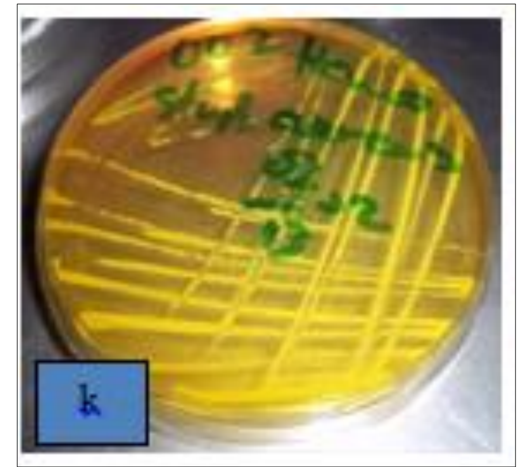
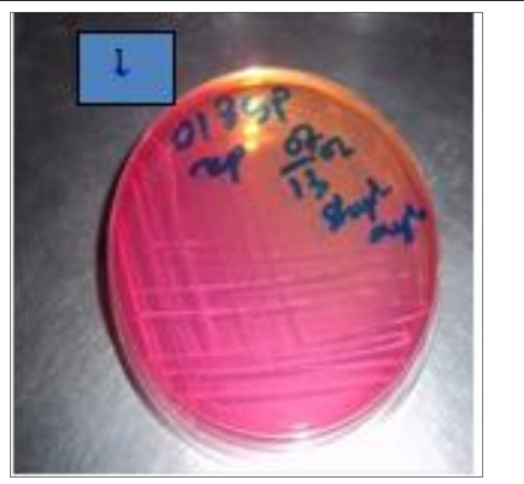
Table 7 Macroscopic and microscopic characteristics of the biotechnological steps of blood culture



1	a: blood culture vials		
2	b: sample ready to enter in the BacAlert c : BacAlert screen at blue background showing no positive sample	BacAler 3D version : 001	
3	d : BacAlert screen with yellow background showing a positive sample e : positive vials inoculated on different agar media, incubated for 24-48 hours, subcultured for biochemical and		



	antibiogram tests		
4	f: biochemical identification of *Klebsiella pneumoniae* on API20E: fermentation of all sugars	f	
5	g: Multi-Drug Escherichia coli except ciprofloxacin and imipenem (MDR) h : *Acinetobacter baumannii* XDR (resistant to antibiotics of different families tested except two antibiotics	g	h
6	i: *Escherichia coli:* positive synergy test indicated by the arrows between AMC in the center and FEP, CTX and ATM. IMP and FOX are sensitive. j : *Pseudomonas aeruginosa* XDR	i	j

7	k : *Staphylococcus aureus* colonies on Chapman agar l : *Staphylococcus haemolyticus* colonies on Chapman agar		

4. Discussion

Of the 111 blood cultures performed, 51 were positive for infection, a prevalence of 46%. This result is slightly higher than that found by Vandenhende et al [10] at the Bordeaux University Hospital in 2021 which was 33%. It is lower than that of Shinga Wembulua et al at Fann University Hospital in Dakar, Senegal in 2021, who obtain a prevalence of 51.4% among women living with HIV [4]. The occurrence of bacteremia in PLHIV is often linked to impaired anti-infective defenses [11]. This prevalence's reported in these studies show the susceptibility of PLHIV to opportunistic bacterial infections.

The study revealed a predominance of the female gender, which represented 70.27% of HIV-infected patients, i.e. a sex ratio of 0.45. This result can be superimposed on those obtained by Shinga Wembulua et al in 2021 [4] and are consistent with the general profile of HIV/AIDS in Africa [12]. This female predominance in the study could be explained by the large number of women in the study population, due to their exposure to the occurrence of HIV and opportunistic diseases [13].

Speaking of age, the age group most affected by bacteremia was 55 and over. The average age of PLHIV was 45.5 years with extremes of 15 and 76 years (Figure 1).

However, people aged 50 and over are increasingly affected [12, 14]. We observe an aging of PLHIV linked to bacterial contamination beyond the age of 50, but also to ARV treatment which keeps patients alive and healthy for a long time (Figure 1). A loss of HIV virulence has been described due to its adaptation to HLA and to ARV treatment which operates a selection of less virulent mutants explaining a longer survival of PLHIV [15]. In the series by Mbopi et al. [16] the most vulnerable age was between 15 and 24 years old. However, people over the age of 50 are increasingly affected by bacterial infection [14, 15].

The profile of blood culture isolates in PLHIV is very heterogeneous. This varies by study type, geographic location, and local bacterial ecology. In our series, the most isolated bacteria were *Staphylococcus aureus* (29), *Escherichia coli* (8), *Staphylococcus haemolyticus* (6), *Streptococcus pneumoniae* (3), *Pseudomonas aeruginosa* (2), *Klebsiella pneumoniae* (1), *Acinetobacter baumannii* (1) and *Salmonella* spp (1)

A retrospective study previously conducted elsewhere on the etiologies of bacteremia in PLHIV over a period of eleven years had reported a predominance of *Salmonella* sp (n = 3), *Escherichia coli* (n = 13), *Streptococcus pneumoniae* (n = 9) and *Staphylococcus aureus* (n = 8) [17]. In the series by Marwa et al [18], *Escherichia coli* and *Klebsiella s*p were the most common bacteria among Gram-negative bacteria and *S. aureus* the main Gram-positive bacteria. Coagulase negative staphylococci (SCoN), *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas* sp were the most isolated in our study.

(SCoN) are increasingly reported as the main cause of bacteremia, especially in HIV-infected patients [5, 11, 12]. Mostly belonging to the normal flora of the skin, their clinical responsibility remains unclear. Is this a notion of opportunism, given the immunosuppression that characterizes HIV infection? Are these cases of contamination? Further studies are therefore needed to shed more light on these issues. In addition, the plea for strict compliance with asepsis measures should be without appeal.

Regarding resistance phenotype, ESBL accounted for 31.37% of cases. Our results were superimposed on those of Bessimbaye et al in 2015 [20] in seven departments of this same health structure in all-comers patients and Fissou et al in 2014 and 2019 at the N'Djamena Mother and Child University Hospital Center (Chad) who report on 60 strains of *E. coli* and 23 strains of *K. pneumoniae* isolated, 18 strains of *E. coli* and 13 strains *K. pneumonia*e had produced ESBLs with proportions of 30% and 56.52% respectively [21, 22].

Compared to the biochemical profile of bacteria isolated from blood, *Staphylococcus aureus* was coagulase positive and *Staphylococcus haemolyticus* coagulase negative and all catalase positive. The identified *Streptococcus pneumoniae* was catalase negative. All Enterobacteriaceae were identified as oxidase negative and *Pseudomonas aeruginosa* was confirmed as oxidase positive. Previously, the work of Nadlaou et al in 2020 reported the same biochemical profiles of Enterobacteriaceae identified in the diarrhea of PLHIV [24].

Regarding bacterial resistance profiles to antibiotics, co-trimoxazole prophylaxis and misuse of antibiotics promote antibacterial resistance.

Our isolates were all resistant to trimethoprim-sulfamethoxazole (Table 6). Only a few studies have examined this problem in PLHIV [3, 4, 23, 24].

Staphylococcal strains showed high resistance to ciprofloxacin (75%), norfloxacin (87.5%), oxacillin (65.51%), amoxicillin (75.86%), ampicillin (58.62%), ceftriaxone (50%), cefoxitin (37.93%), cefotaxime (34.48%), imipenem (10.34%) and amoxicillin-clavulanic acid (30.7%). In addition, Shinga et al in 2021 in Dakar, Senegal report that staphylococcal strains showed high resistance to cotrimoxazole (66.7%), pefloxacin (57.1%) and amoxicillin-clavulanic acid (37.5%). Methicillin-resistant coagulase-negative staphylococci accounted for 35.7% (5/14) and MRSA (22.2%) [4].Such observed bacterial resistance could be explained by the production of the type enzymes (penicillinases, cephalosporinases and cabapenimases) in question.

The mechanism of non-enzymatic resistance to beta-lactams has been observed since 1961, when methicillin was introduced into therapy. The strains which possess it are said to be heterogeneous resistant to methicillin or "méti R". In a méti R strain, only a small proportion of the bacteria is capable of expressing resistance and growing in the presence of methicillin. Métis R strains should always be considered resistant to all beta-lactams, including 3rd generation cephalosporins and imipenem. They are also producers of penicillinases. They are usually resistant to other antibiotics: aminoglycosides, tetracyclines and macrolides. From 10 to 40% of hospital strains of *S. aureus* isolated in France are mixed R. Their frequency among strains of extra-hospital origin is low [25].

Our strains of *Escherichia coli* isolated from the blood of PLHIV showed varied resistance to ampicillin (87.5%), amoxicillin (75%), aztronam (25%), cefoxitin, ceftriaxone and ceftacidime (50%), imipenem (25%), tetracycline (62.5%), ciprofloxacin (62.5%), norfloxacin (50%) and chloramphenicol (50%). Previously, Vandenhende et al in 2021 report that Escherichia coli resistance increased markedly for penicillins between 2000-2004 (18%) resistance to Amoxicillin/Clavulanic Acid) and from 2005-2008 (43%) before stabilize (40%) in 2014-2017. Resistance to 3rd generation cephalosporins increased gradually between 2000-2004 (0%) and 2014-2017 (12%). Resistance to fluoroquinolones increased from 4% in 2000-2004 to 24% in 2005-2008, with a decrease since (16% in 2014-2017). Finally, a gradual increase in the percentage of ESBL Enterobacteriaceae has been observed (from 4% in 2000-2004 to 14% in 2014-2017) [10].

Our results are also comparable to those of Tellevik et al (2016) and Fissou et al (2014, 2019) who report that such bacterial resistance is due to the production of extended-spectrum beta-lactamases [21, 23, 26].

Our strains of *Pseudomonas aeruginosa* were resistant to all antibiotics tested except ceftazidime, ciprofloxacin, norfloxacin, amikacin and imipenem. On the other hand, the strain of *Acinetobacter baumanii* was resistant to all the antibiotics tested except gentamicin and amikacin. The strains of *Klebsiella pneumoniae*, *Salmonella* spp and *Streptococcus pneumoniae* isolated in our series showed varied resistance and susceptibility to the antibiotics tested (Table 6). In addition, *Escherichia coli* 50% (5/10), *Klebsiella pneumoniae* 40% (4/10) and *Enterobacter* sp 25% (2/8) were the main germs producing ESBL. *Pseudomonas* strains isolated showed high resistance to piperacillin (30.8%), ceftazidime (66.6%) and gentamicin (66.6%) and carbapenems (22.2%) [27, 28, 29, 30].

5. Conclusion

The emergence of strains of bacteria responsible for bacteremia in PLHIV requires clinicians to rationally prescribe antibiotics. The establishment of a committee to fight against bacterial resistance to antibiotics in each health structure is essential to better monitor the circulation of its multi-resistant bacteria. Chadian health authorities in charge of human and animal health must develop communication strategies through the media on the danger of self-medication.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

In order to carry out this study we obtained:

- The authorization of the Dean of the Faculty of Human Health Sciences, University of N'Djamena, Chad;
- The authorization of the Director General of the Ministry of Public Health of Chad;
- The authorization of the Director of Research and Innovation, University of N'Djamena, Chad.

Statement of informed consent

- The verbal consent of each patient or his beneficiary to whom we have explained the procedures and the importance of the study;
- In compliance with medical ethics and in order to preserve the moral integrity and confidentiality of patients, we have taken anonymity into account;
- The results of our research work have been returned to clinicians for patient management.

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