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Microbiological Assessment and Antimicrobials' Use in an Infectious Diseases Department in Mali

Mikaila Kaboré[1*], Issa Konaté[1,2], Yacouba Cissoko[1,2], Ibrehima Guindo[3,4], Bintou Coulibaly[1], Meli Hermine[1], Aboubacar Alassane Oumar[2,5], Mariam Soumaré[1], Assetou Fofana[1], Abdoulaye Zaré[1], Mohamed Aly Cissé[1], Dramane Sogoba[1], Omar Magassouba[1], Hama Hamidou Issa[1], Fodé Kouyaté[1], Sounkalo Dao[1,2,6]

[1]Infectious Diseases Department, Point "G" University Teaching Hospital, Bamako, Mali
[2]Faculty of Medicine and Odontostomatology, University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako, Mali
[3]Laboratory and Biomedical Research Department, National Institute of Public Health, Bamako, Mali
[4]Faculty of Pharmacy, University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako, Mali
[5]Laboratory of Biomedical Analysis, University Teaching Hospital of Kati, Kati, Mali
[6]Serefo Program, University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako, Mali
Email: *mikailakab@gmail.com

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Abstract

The use of antimicrobials without microbiological proof is frequent and contributes to the emergence of resistance. The aim was to identify the organisms isolated during laboratory examinations and the type of antimicrobials consumed by patients hospitalized to Infectious Diseases' Clinic. This is a cross-sectional and analytical study, carried out from January 1 to December 31, 2017 in the Infectious Diseases Department of Point "G" Teaching Hospital in Mali. All biological specimens from patients during the study period were analyzed. In total, 322 patients benefited from microbiological test, with a mean age of 40.9 ± 12.2 years (range 15 and 74 years) with a sex ratio of 0.93. Only 5.6% of patients were HIV negative. In all participants, a total of 658 microbiological specimen were taken, 224 (34.0%) identified at least one pathogen including bacteria in more than a half of the cases (58.5%). On the report of bacteriological analysis, the most frequently identified organisms were *Mycobacterium tuberculosis* (42.2%) mainly in sputum; *Escherichia coli* (24.2%) and *Klebsiella pneumoniae* (7.8%) commonly in urine and *Gardnerella vaginalis* (7.0%) exclusively in vaginal secretions. *Candida albicans* (5/8) and *Cryptococcus neoformans* (3/8) were the most common fungi while *Plasmodium falciparum* (96.4%) represented the parasite frequently found in blood. From a therapeutic standpoint, 1143 antimicrobials were prescribed to

322 admitted patients, *i.e.* 3.55 anti-infectives per patient. Antibiotics (excluding tuberculosis drugs), antiparasitics and antifungals represented respectively 46.2%; 18.8% and 15.1% of anti-infectives. Antibiotic therapy was effective in 274 (85.1%) patients and among them, only 76 (27.7%) cases were based on microbiological evidence. Antibiotics are the most widely used antimicrobials in an infectious disease department. Empiric treatments are common but must be minimized by the search for microbiological evidence.

Keywords

Antimicrobial, Microbiology, Infectious Diseases, Mali

1. Introduction

Infectious Diseases remain the main causes of human morbidity and mortality, especially in developing countries. Antimicrobials are probably one of the most effective forms of chemotherapy in the history of medicine [1] [2]. Unfortunately, antimicrobial resistance is a worldwide concern [3] [4] [5]. It leads to a reduction in the molecular choice by prescribers, an increase in the length of patients' hospitalization period, an increase in healthcare costs, exposure to more toxic drugs and mortality [6] [7] [8].

According to the World Health Organization (WHO), antimicrobial resistance is a serious threat and currently affects all regions of the world and is likely to affect anyone of any age or country [5]. Every year, there are 700,000 deaths due to drug resistance and if nothing is done by 2050, mortality could reach 10 million in the world [9].

In Africa, where antimicrobials are much prescribed compared to other parts of the world [10], several publications address the issue of bacterial resistance to antibiotics. *Enterobacteriaceae* are cited as the most relevant group of pathogens and the production of extended-spectrum beta-lactamases (ESBL) is described as the most common type of resistance. This situation creates therapeutic impasses with ever-increasing hospital morbidity and mortality [11] [12] [13] [14]. Authors are unanimous on the factors that cause antimicrobial resistance, especially those that are common in resource-limited countries such as: self-medication, the sale of counterfeit medicines, over-the-counter antibiotics, use of antibiotics as a poultry and livestock growth promoter rather than to control infection, storage and conservations difficulties, the incompetence of prescribers, the insufficiency of the laboratories for the microbiological diagnosis [13] [15] [16]. In addition, the inappropriate prescription of antibiotics largely contributes to their emergence through the selection pressure [17] [18].

The financial consequences are enormous. In 2006, about 50,000 Americans died due to two common hospital acquired infections, namely pneumonia and sepsis, costing about 8 billion dollars to the Unities States economy [19]. Nosocomial infections of patients due to these bacteria, sometimes pose legal problems for hospitals in developed countries [20].

The link between antibiotic using and antibiotic resistance has even been proven by mathematical models [21]. Monitoring this consumption is one of the recommended strategies for preventing and controlling antimicrobial resistance. It is a reality in developed countries. Indeed, this surveillance has seen a decrease in antibiotic consumption of 11.4% between 2000 and 2015 in France [22].

World Health Organization (WHO) gave definition about the rational use of medicines. It's the patients who receive medications appropriate to their clinical needs, in doses that meet their own individual requirements for an adequate period of time, at the lowest cost to them and their community [23]. It appears that the correct and rational use of antimicrobials is the only way to fight against antibiotic resistance and this could be done through training of health professionals, in particular prescribers but also patients [8].

In Africa, some countries are developing lists of essential drugs including antimicrobials, along with guidelines for the management of health workers. This is the case in Ghana and Nigeria [24] [25]. However, studies still report unreasoned prescriptions of antibiotics by caregivers [15]. Most antimicrobial treatments are done without microbiological evidence with broad-spectrum antibiotics that destabilize local flora and increase the selection pressure of resistant organisms to these antibiotics.

In Mali, the prescription of antibiotics has reached high levels in hospitals. It would represent 18.5% of drug spending in Bamako [26]. A hospital study analyzing the local microbial ecology and antimicrobials' use hasn't yet been performed. It's in this context that our study was realized in a Clinical ward for the specific management of infectious diseases. The objective was to review the frequently isolated pathogen in pathological fluid of patients and quantitative antimicrobial prescribing.

2. Methods

2.1. Study Area and Period

The study was performed in the Department of Infectious Diseases at Point "G" Teaching Hospital. It's the national reference center of infectious diseases in Mali. The admission capacity is 30 beds and nearly 400 patients are managed there each year. This was a cross-sectional and analytical study. It was performed from January 1 to December 31, 2017.

2.2. Study Population

Have been concerned by study all the patients admitted for infectious diseases during the period. The not included patients were those observed for discontinuous care and those who died within 24 hours of admission.

2.3. Study Process

All specimens of patients' biological fluids were sent to the laboratory for microbiological test after eliminating duplicates and contaminants. Included laboratories were: Point "G" Hospital Laboratory, National Institute of Research and Public Health laboratory and two other private laboratories.

Bacteriological analysis: standard methods of bacteria isolation and identification have been used. Antibiotic susceptibility testing was performed according to the recommendations of French Microbiology Society [27]. To simplify the interpretation of susceptibility tests, intermediate categorized strains were considered resistant. Data were collected using a questionnaire.

2.4. Study Variables

The following variables were collected:

- sociodemographic: age, sex, occupation,
- clinical: main diagnosis,
- microbiological: type of biological analysis, isolated germs according to the infectious site,
- therapeutic: antiviral (antiretroviral, acyclovir), antiparasitic (antimalaria, anthelmintic, sulfamide), antibacterial (beta-lactam, quinolone, macrolide, aminoglycoside, imidazole, sulfamide, polymyxin, antituberculosis), antifungal (azole, polyene).

Has been considered like antimicrobial treatment, any prescription of anti-infectious whose purpose is to treat a suspected infection or microbiologically proven. Thus, anti-infectious prophylaxis was not taken into account.

2.5. Statistical Analysis

Data were collected and analyzed using SPSS (*Statistical Package for Social Science*) version 22. Quantitative variables were expressed in terms of mean (± standard deviation) or median [interquartile range (IQR)] according to the distribution curve of the values of these variables. Mean was calculated when the curve was symmetrical and median in the opposite case. Qualitative variables were expressed as percentages. Chi-square tests were used for statistical analysis. p-value at < 0.05 was considered significant.

2.6. Ethical Aspects

The study was performed in a healthcare setting and patients gave their informed consent. Confidentiality of information and anonymity of participants were respected during the study and data processing. The conduct of study required prior authorization from the Head of the Infectious diseases Ward.

3. Results

3.1. Characteristics of Study Population

A total of 387 patients were admitted, 322 of whom benefited microbiological testing from January 1 to December 31, 2017. The average age of the 322 patients was 40.9 ± 12.2 years (15 - 74 years). The majority of the patients was female at

167 (51.9%) and sex ratio was 0.93. The informal activity sectors were the most frequent found as occupation (46.0%) followed by housewives (28.6%). The majority of admitted patients were infected by human immunodeficiency virus (HIV) (85.7%) (Table 1).

Respiratory (60.3%), central nervous system (CNS) (39.7%) and digestive (32.4%) pathologies were the most frequently encountered in patients (Figure 1).

Table 1. General characteristics of study population.

Variables	Number (n)	Pourcent (%)
Average age (years old): 40.9 ± 12.2 (15 and 74)		
Gender		
Male	155	48.1
Female	167	51.9
Occupation		
Informal sector	148	46.0
Housewife	92	28.6
Salaried	43	13.4
Farmer	32	9.9
Student	7	2.2
HIV Status		
HIV-1	265	82.3
HIV-2	4	1.2
HIV-1,2	7	2.2
HIV negative	18	5.6
Unknown	28	8.7

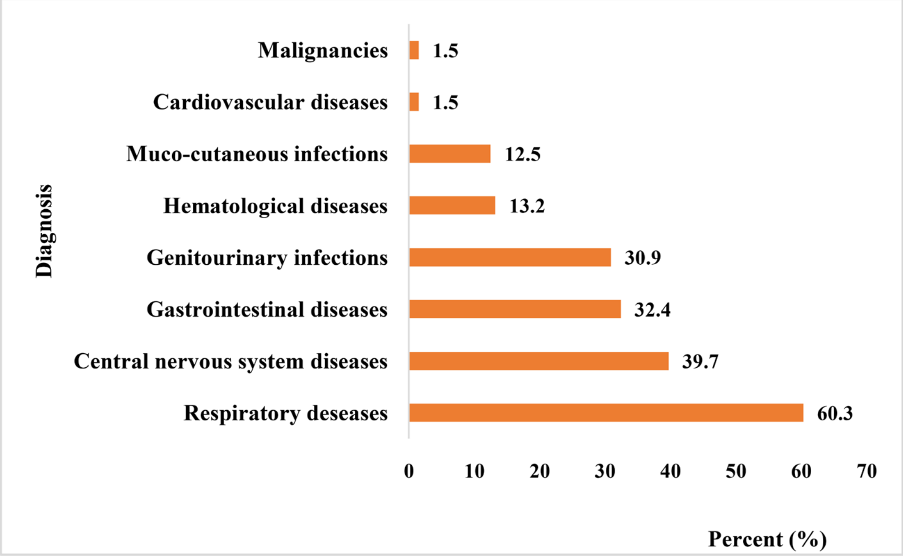

Figure 1. Distribution of patients' pathologies during their admission.

3.2. Microbiological Features

Out of 658 specimens taken and analyzed, 219 (33.3%) pathogenic organisms have been identified including 128 (58.5%) bacteria, 83 (37.8%) parasites and 8 (3.7%) fungi (**Figure 2**).

According to bacteriological analysis, most frequent germs found were: *Mycobacterium tuberculosis* (42.2%) in sputum; *Escherichia coli* (24.2%) and *Klebsiella pneumoniae* (7.8%) in urine samples; and *Gardnerella vaginalis* (7.0%) exclusively in vaginal secretions (**Table 2**). There was no anti-tuberculous drug resistance detected through GeneXpert.

Mycological analysis identified *Candida albicans* (5/8) and *Cryptococcus neoformans* (3/8) respectively in urine and cerebrospinal fluids. Among parasites, *Plasmodium falciparum* predominated at 96.4% (**Table 3**).

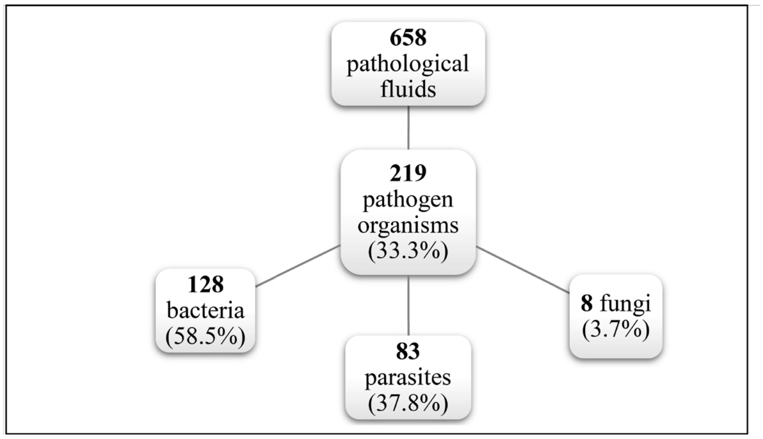

Figure 2. Distribution of different types of organisms identified in pathological fluids.

Table 2. Distribution of bacteria according to pathological fluids.

	Pathological fluids						
Bacteria	**Sputum**	**Urine**	**Vaginal secretion**	**Blood**	**Pus**	**CSF***	**Total (%)**
Mycobacterium tuberculosis	48	0	0	0	3	3	54 (42.2)
Escherichia coli	3	23	5	0	0	0	31 (24.2)
Klebsiella pneumoniae	3	4	0	3	0	0	10 (7.8)
Gardnerella vaginalis	0	0	9	0	0	0	9 (7.0)
Enterobacter spp.	1	4	0	2	1	0	8 (6.3)
Acinetobacter spp.	6	2	0	0	0	0	8 (6.3)
Staphylococcus aureus	0	3	0	0	0	0	3 (2.3)
Enterococcus spp.	0	3	0	0	0	0	3 (2.3)
Citrobacter spp.	1	0	0	0	0	0	1 (0.8)
Streptococcus spp.	0	1	0	0	0	0	1 (0.8)
Total (%)	62 (48.4)	40 (31.3)	14 (10.9)	5 (3.9)	4 (3.1)	3 (2.3)	128

*CSF: cerebrospinal fluid.

Antibiotic susceptibility profile of *E. coli* was 100% for colistin and imipenem. Its susceptibility to beta-lactams was 52.0% for ceftriaxone, 43.5% for ceftazidime and 16.7% for amoxicillin/clavulanic acid. Amikacin and gentamycin were active with respectively 96.0% and 34.6%. Among quinolones tested, ofloxacin, ciprofloxacin and nalidixic acid were active on this microorganism respectively in 53.3%; 41.7% and 20.8% of cases (Table 4).

Table 3. Distribution of fungi and parasites according to pathological fluids.

Pathological fluids					
Pathogens	**Blood**	**Stool**	**Urine**	**CSF**	**Total**
Fungi					
Candida albicans	0	0	5	0	5
Cryptococcus neoformans	0	0	0	3	3
Total	0	0	5	3	8
Parasites					
Plasmodium falciparum	80	0	0	0	80 (96.4)
Entamoeba histolitica	0	1	0	0	1 (1.2)
Ancylostoma duodenale	0	1	0	0	1 (1.2)
Cystoisospora belli	0	1	0	0	1 (1.2)
Total	80 (96.4)	3 (3.6)	0 (0)	0 (0)	83 (100)

Table 4. Antibiotics susceptibility profile of *E. coli.*

Antibiotics	**Test results**	
	Susceptibility n (%)	**Resistance n**
Amoxicillin/clavulanic acid (n = 30)	5 (16.7)	25
Ticarcilline (n = 18)	0 (0)	18
Imipenem (n = 25)	25 (100)	0
Cephalothin (n = 17)	1 (5.9)	16
Ceftriaxone (n = 25)	13 (52.0)	12
Ceftazidime (n = 23)	10 (43.5)	13
Gentamycin (n = 26)	9 (34.6)	17
Amikacin (n = 25)	24 (96.0)	1
Kanamycin (n = 8)	5 (62.5)	3
Nalidixic acid (n = 24)	5 (20.8)	19
Ciprofloxacin (n = 24)	10 (41.7)	14
Ofloxacin (n = 15)	8 (53.3)	7
Colistin (n = 20)	20 (100)	0
Sulfamide (n = 20)	2 (10.0)	18

3.3. Antimicrobial Drugs Used

Global data: There were 1143 antimicrobials drug used in 322 hospitalized patients treated for infectious diseases whether an average of 3.55 antimicrobials per patient. Non-tuberculosis antibiotics (46.2%) followed by antiparasitics (18.8%) and antifungals (15.1%) were the most commonly used anti-infective agents (**Table 5**).

Antibiotics: Antibiotic therapy carried out 528 drugs were prescribed in 274 patients whether an average of 1.93 antibiotics per patient. Beta-lactams (48.1%), imidazoles (22.7%) and aminoglycosides (19.3%) were the most frequently used. More than half of the patients on antibiotics (56.2%) were on dual therapy (**Table 6**). Talking about active ingredients, ceftriaxone (25.0%), metronidazole (22.7%), amoxicillin/clavulanic acid (21.0%) and gentamycin (15.7%) were the most prescribed (**Figure 3**).

In 76 patients (27.7%) whether 94 samples (27.8%), antibiotic therapy was based on microbiological evidence. This represented 31.2% of HIV-infected patients as compared to 9.3% of non-HIV patients ($p = 0.003$).

Antiparasitics. The most common used antiparasitic drugs were trimethoprim/sulfamethoxazole (58.6%), albendazole (17.1%) and artemether (11.6%) (**Table 6**).

Antifungals. Fluconazole was the predominant antifungal (83.8%) (**Table 6**).

Antivirals. It was represented by antiretrovirals at 91.3% (**Table 6**).

4. Discussion

Our study showed the profile of microbiological organisms commonly found in patients admitted to an Infectious diseases department in resource limited setting in Africa and has revealed a high antimicrobial use.

Respiratory, CNS and digestive infections were the most common causes of admission of patients in our survey. Other studies evaluating the antibiotics' prescriptions in one or several hospitals found a higher frequency of respiratory [10] [15] [28] [29] and then urinary [10] [15] [28] infections. Our work was performed in an Infectious Diseases Department where most patients were infected with HIV (85.7%) and admitted for opportunistic infections. In this context,

Table 5. Distribution of anti-infectious drugs by pharmacotherapeutic group.

Pharmacotherapeutic group	Number (n)	Percent (%)
Non-tuberculosis antibiotics	528	46.2
Antiparasitics	215	18.8
Antifungals	173	15.1
Antiretrovirals	158	13.8
Antituberculous	54	4.7
Antivirals excluding antiretrovirals	15	1.3
Total	1,143	100

Table 6. Distribution of antimicrobial prescribed to patients.

Variables	Number (n)	Percent (%)
Antibiotic associations (n = 274)		
Monotherapy	68	24.8
Dual therapy	154	56.2
Triple therapy	48	17.5
Quadruple therapy	4	1.5
Antibiotic groups (n = 528)		
Betalactams	254	48.1
Imidazoles	120	22.7
Aminoglycosides	102	19.3
Macrolides	25	4.7
Quinolones	18	3.4
Others*	9	1.7
Antivirals (n = 173)		
Antiretrovirals	158	91.3
Aciclovir	15	8.7
Antiparasitics (n = 215)		
Trimethoprim/sulfamethoxazole	101	47.0
Albendazole	47	21.9
Artemether	32	14.9
Artesunate	24	11.2
Clindamycin	8	3.7
Quinine	3	1.4
Antifungals (n = 173)		
Fluconazole	145	83.8
Miconazole	24	13.9
Nystatin	4	2.3

*Others: nitrofurantoin (n = 2), vancomycin (n = 2), doxycycline (n = 2), chloramphenicol (n = 2), lincomycine (n = 1).

CNS opportunistic infections are very often encountered [30] which explains the high frequency of anti-infective drug used.

The detection rate of microorganisms in patients' pathological fluids was 33.3%. Bacteriological analysis showed that *M. tuberculosis*, essentially isolated in sputum, was the most predominant germ. This finding would be justified by the immune deficiency of our patients exposing them to opportunistic infections whose tuberculosis remains the most important during HIV [31] [32]. There was no anti-tuberculous drug resistance detected with Gene-Xpert. Apart from this organism, *E. coli* and *K. pneumoniae* were the most frequently identified pathogens

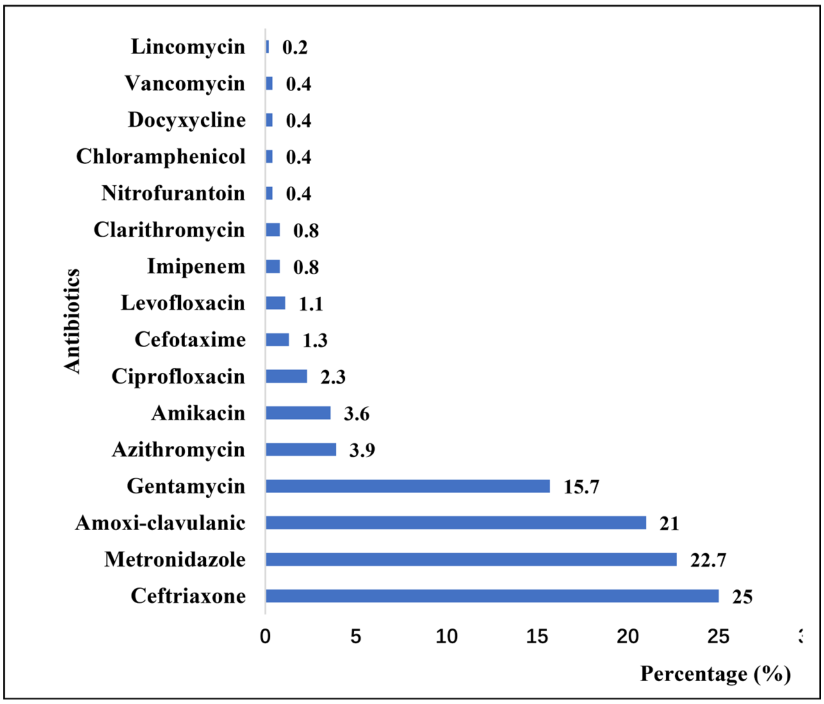

Figure 3. Antibiotics used by patients during their admission.

in the urine samples of patients. The same finding has been made in several other studies concerning the isolating frequency of these two enterobacteria in urinary tract infections [11] [12] [14] [33]. The morphological characteristics of these isolates, the extended hospitalization of patients, the presence of an intra-bladder and/or intra-vascular catheter, the immunodeficiency, are all factors that could explain the risk of urinary infection due to these organisms [33] [34].

Considering the antibiotic susceptibility pattern of isolated *E. coli* strains, beta-lactams had a very low inhibitory action. In fact, the susceptibility of the organism to amoxicillin/clavulanic acid and ceftriaxone was 16.7% and 52%, respectively. Our results were proximate to those found by Lo in Saint Louis [12] and Kouegnigan Rerambiah in Libreville in their studies [4]. The existence of an increasing number of hospital strains of Extended-spectrum beta-lactamase (ESBL) producing enterobacteria may explain this finding [11] [12] [14] [34]. Concerning aminoglycosides, gentamycin susceptibility was low (34.6%) but rather satisfying for amikacin (96.0%). These results were similar to those of Kouegnigan Rerambiah in Libreville [4], Hailaji in Nouakchott [14] but better than Dia's findings in Dakar [11] which were 76.4% susceptibility to amikacin. Quinolones had a medium inhibitory action (41.7% for ciprofloxacin) on *E. coli* strains. The same observation made by Dia [11] noted a susceptibility to ciprofloxacin of 51.2%. ESBL resistance is often associated with resistance to aminoglycosides and quinolones [34]. In addition, the frequency of beta-lactams and quinolones using in enterobacterial infections in general [4] and quinolones as first-line therapy in the empirical treatment of urinary tract infections [14] may explain the current level of resistance emergence to these molecules. All isolates of *E. coli* in our study were entirely susceptible to imipenem and colistin. On the other hand, their susceptibility to trimethoprim-sulfamethoxazole was very low at 10% compared to other studies [12] [14]. In our case, the molecule is regularly and constantly used by HIV patients as part of the primary prophylaxis against opportunistic infections and this permanent exposure could explain the decrease of susceptibility encountered in our survey.

After mycological analysis, *Candida albicans* and *Cryptococcus neoformans* were the opportunistic fungi identified respectively in urine and cerebrospinal fluid given the immune failure of the majority of patients. The clinical expression of *C. neoformans* infection like cryptococcal meningitis occurs when the immunity of patients is severely compromised [35]. Associated to cerebral toxoplasmosis and tuberculous meningitis, they are a major cause of morbidity and mortality in HIV-positive individuals [30].

P. falciparum malaria was the most important parasitic infection. The malaria endemic context in Mali, in which more than 90% of cases are transmitted from June to December, may explain this situation [36].

Overall, there was a large antimicrobial using for infectious diseases at 3.55 antimicrobials per patient during their stay. The nature of the infections taken care of in the ward could explain that. Indeed, all patients admitted during the study period had at least one infection indicating the prescription of antimicrobials. In addition, the immune deficiency of these patients (85.7% of HIV-infected patients), favorable to opportunistic infections, bacterial, fungal, parasitic and viral, could explain this high antimicrobial consumption [29].

Antibiotics were the most commonly prescribed antimicrobials before antiparasitics and antifungals in our survey. This denotes the importance of bacterial infections among infectious diseases in our context. Among antibiotics, beta-lactams, followed by imidazoles and aminoglycosides were the most used in our patients. The predominance of beta-lactams use has been found in the literature [10] [16] [28] [29] [26]. This class of antibiotics is one of the most widely prescribed in general practice with a broad-spectrum of activity and a variety of molecules [37].

Among patients with antibiotic, three-quarters (75.2%) were at least dual therapy with an average of 1.93 antibiotics per patient. Randriatsarafara in Antananarivo [16] found in their study, 49.6% of patients undergoing dual therapy with at least 1.73 antibiotics per patient. Anand in Botswana [29] found a ratio of 1.76 antibiotics per patient. This difference could be explained that our patients were mostly immunocompromised by HIV with bacterial infections that very often require synergistic combinations of antibiotics in addition to antiretroviral therapy (ART). Only 27.7% of patients took antibiotics based on microbiological evidence. Thus, the majority of patients were under broad-spectrum empirical treatment to reach the suspected organisms, which increases the risk of resistance. In Botswana, where the study was conducted in several hospitals across the country, bacterial infections were recorded in 70.6% of cases [29]. This research for microbiological evidence during antibacterial treatment was even low in other studies. This finding could be explained by the laboratory setting of the health facilities in which the studies were performed, the patients' financial inability to pay the biological analyses and also by the profile and prescribing habits of caregivers [15] [16].

About antiparasitics, trimethoprim-sulfamethoxazole was the most used in patients. This molecule was primarily prescribed for the management of cerebral toxoplasmosis and cystoisosporiasis in HIV-infected patients with profound immunosuppression. Clindamycin has also been used as an antiparasitic agent in the cerebral toxoplasmosis' treatment in second intention. Malaria was mainly managed by artemether and artesunate.

Fluconazole predominated among the antifungals used in our patients. It is one of the molecules indicated for the treatment of opportunistic fungal infections, the most frequent in our study were digestive candidiasis and cryptococcal meningitis [38].

The majority of our patients were immunocompromised by HIV, which justified the ART using.

Our study had a number of limitations. Specimens from patients have been analyzed in several laboratories with sometimes different settings in the identification of organisms and antibiotic sensitivity tests. Antibiotic susceptibility profile was limited to *E. coli* taking into account the reduced number of other isolated strains. Finally, during data collection, we didn't consider antimicrobial forms, routes of administration and number of doses administered during the hospitalization.

5. Conclusion

We performed an inventory of the organisms usually identified in pathological fluids and antimicrobials prescribed to patients in an Infectious Diseases' Clinic. Bacteria such as *M. tuberculosis* in the sputum of HIV-infected patients, *E. coli* and *K. pneumoniae* in the urine were the most isolated in pathological fluids. *C. albicans* among fungals and *P. falciparum* among parasitics were mostly identified. The sensitivity profile of *E. coli* with antibiotics was analyzed. Concerning antimicrobial use in the service, antibiotics and more especially beta-lactams were predominant. The average number of antimicrobials per patient was 3.55. Antibiotic therapy without bacteriological evidence was frequently found. Due to the emergence of antimicrobial resistance, it's important for clinicians to optimize their prescription by microbiological analysis.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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