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Antibiotic Resistance of Enterobacteria and Staphylococci Isolated from Hospital Soils and Patients' Biological Fluids in Brazzaville

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A B S T R A C T

The inappropriate use of antibiotics since their discovery has been the cause of the appearance of resistant or multi-resistant bacteria both in the environment and in hospitals. Although major organizations such as the WHO have set up several systems to combat bacterial resistance, it remains a major public health problem. In order to assess bacterial resistance in Brazzaville's soils and clinics, strains of *Staphylococcus* and *Enterobacteriaceae* were isolated and antibiogram. The results of this study show that a wide variety of potentially pathogenic bacteria can be found in soil. The distribution of species isolated according to their biological origin revealed that the majority of *Staphylococcus* came from vaginal swabs (75%), followed by urine (15%) and spermatic fluid (10%), while the majority of *Enterobacteriaceae* came from urine (55%), followed by vaginal swabs (35%) and spermatic fluid (10%). The majority of strains used were resistant to almost all the antibiotic families used for susceptibility testing. Hospital-derived *Staphylococcus* strains showed very high rates of resistance (55 to 100%) to all antibiotics tested, compared with soil-derived strains. Soil-derived strains also showed high levels of resistance to certain molecules. *Enterobacteriaceae* strains were resistant to quinolones. Strains from both environments were more resistant to beta-lactam antibiotics (FOX CXM, CEX, IMI, ATM), with the exception of Ertapenem, where the resistance rate was higher in soil strains (55%) and lower in hospital strains (30%). OFX, NA, MXF and NOR showed very high rates, as did other antibiotic molecules (colistin and kanamycin). This work has shown us an increase in resistance in clinical and environmental strains to the most widely used antibiotics. This is a cause for concern in the therapeutic management of patients, and can lead to high costs for prolonged hospitalization, increased morbidity and mortality.

Introduction

Nowadays, there is a correlation between bacteria and man, more precisely in hospital environments. Bacteria, which are responsible for many infections, are isolated from patients' biological fluids for various analyses and then disposed of in the soil, which is a habitat for a variety of living organisms, hosting a diversity of prokaryotic and eukaryotic species that live in a variety of environments (Prescott *et al.,* 2010). Prokaryotic species include bacteria, which are very abundant micro-organisms in soils (1 billion individuals per gram of soil on average, close to 2.5 t/ha in carbon equivalent) (Prescott *et al.,* 2010).

Once they have found their way into the organism, these soil-borne bacteria exert their pathogenic power, causing infections of various kinds. However, during their metabolism, they synthesize several molecules, some of which have harmful effects on the organism, while others, such as antibiotics, are essential to man (Ndelilionel, 2009). Antibiotics have been an important therapeutic discovery for human health. Their use has long reduced mortality and morbidity worldwide (Longtin Jean *et al.,* 2017). However, the misuse of these antimicrobial agents and their increased use have resulted in the emergence of certain forms of resistance in microbial strains, counterbalancing the effects of antibiotics (Longtin Jean *et al.,* 2017). Thanks to their genetic flexibility and plasticity, pathogenic bacteria are capable of setting up a specific resistance program against a particular antibiotic. Today, many strains are able to establish resistance to several families of antibiotics at once, giving rise to what are known as multi-resistant bacteria (MRB), which have become a major public health problem requiring worldwide attention (Longtin Jean *et al.,* 2017; Bouyahya *et al.,* 2017). The rate of mortality and morbidity due to infections caused by antibiotic-resistant bacteria continues to rise worldwide, despite the fact that many of the world's leading organizations have been conducting studies into bacterial resistance to antibiotics.

These studies have revealed the emergence of multi-resistant strains for which it has now become urgent to develop or research new treatments (Baloki Ngoulou Tarcisse *et al.,* 2019; Moyen *et al.,* 2012; Ahombo Gabriel *et al.,* 2019). In the Congo, several studies have been carried out on the resistance of bacterial strains isolated from the biological fluids of hospital patients (Baloki Ngoulou Tarcisse *et al.,* 2019; Moyen *et al.,* 2012; Ahombo Gabriel *et al.,* 2019; Mpelle Fils Landry *et al.,* 2019; Gangoue Léa Gwladys *et al.,* 2021), from household wastewater (Baloki Ngoulou Tarcisse *et al.,* 2019; Moyen *et al.,* 2012; Ahombo Gabriel *et al.,* 2019) and from fish to fish meal (Morabandza Cyr Jonas *et al.,* 2020). No studies have addressed the resistance of bacteria isolated from hospital soils or from biological fluids collected from outpatients. It is in this context that the present study evaluates the resistance of bacteria isolated from hospital soils and patients' biological fluids.

Materials and Methods

Biological Material

The biological material consisted of strains of *Enterobacteriaceae* and Staphylococci isolated from hospital soils (Bissita integrated health centre, Sino-Congolese Hospital in Mfilou) and biological fluids (Laboratoire National de SantéPublique and Clinique Pasteur).

Sampling

Two hospital sites were selected for soil sampling (Bissita integrated health centre, Hôpital Sino-Congolais de Mfilou). Strains from the National Public Health Laboratory and the Pasteur Clinic were isolated from ambulatory patients (urine, PV, spermactic fluid).

Isolation of Strains

Soils

A series of dilutions ranging from 10^{-1} to 10^{-4} were made in tubes containing 9ml of sterile distilled water from 1g of soil. A volume of 100µl was seeded onto previously cast Petri dishes (EMB (Bio Rad) and Mannitol Salt Agar (Bio Rad). The plates were then incubated in an oven (Thermosi SR1000) at 37°C for 24 hours. Colonies were purified and stored at 4°C.in an Eppendorf tube containing 800 µl of LB and 200 µl of glycerol (Soumaila Garba, 2012; Moutou *et al.,* 2018).

Biological Fluids from Outpatients

They were isolated at the the National Public Health Laboratory and the Pasteur Clinic from outpatients on EMB (Bio Rad) and Mannitol Salt Agar (Bio Rad) media.

Strain identification

Identification was based on cultural, morphological and biochemical characteristics.

Cultural characteristics: specific culture media were used (EMB for *Enterobacteriaceae* and Mannitol Salt Agar for *Staphylococcus*).

Morphological characteristics: a fresh state of the cells was determined using a microscope with an X40 objective (Nguimbi *et al.,* 2020).

Biochemical characteristics: tested using the catalase test and Gram stain.

Antibiotic Sensitivity test

The antibiotics tested on *Staphylococcus* were: Cefoxitim (FOX, 30µg), Cefuroxim (CEX, 30µg), Cefotaxim (CXM,30µg), Oxacilline (OX, 5µg), Aztreonam (ATM, 30µg), Kanamycine (K,30µg), fusidic Acid (FC,10µg), Ofloxacin (OFX,5µg), Rifampicin (RD, 5µg), Clindamycine (CD, 2µg), naldixic Acid (NA, 30µg), Moxifloxacin (MXF, 5µg), Norfloxacin (NOR, 10µg). For Enterobacterie :Cefoxitim (FOX, 30µg), Cefuroxim (CEX, 30µg), Cefotaxim (CXM,30µg), Ertapenem (ETP, 10 µg), Imipenèm (IMI, 10µg), Aztreonam (ATM, 30µg), Kanamycin (K,30µg), Fusidic Acid (FC,10µg), Ofloxacin (OFX,5µg), nalidixic Acid (NA,30µg), Moxifloxacine (MXF, 5µg), Norfloxacine (NOR, 10µg), Colistine (CL, 50 µg). The antibiotic resistance profile of the bacterial strains was evaluated by the standard Kirby-Bauer disc diffusion method (Durmaz *et al.,* 1997; Prats *et al.,* 2000).

The inoculum was prepared by suspending of a well-isolated colony of a young; pure bacterial culture (24 hours on agar medium) in 5 ml normal saline and the turbidity of the suspension was adjusted using spectrophotometer to 0.1 at 625 nm.

The optical density of 0.1 at a wavelength of 625 nm is equivalent to 0.5 Mac Farland (Boukhatem, 2013). The culture medium, Mueller-Hinton agar, was inoculated using the swab as recommended by CLSI (Clinical and Laboratory Standard Institute, 2010).

The antibiotic discs were then applied to the inoculated Mueller Hinton agar medium. The plates were incubated at 37˚C for 18 - 24 h. The diameter of bacterial growth inhibition area around the disc after incubation were measured and the antibiotics susceptibility was interpreted based on the breakpoint values published by the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2023). The strains were categorized as either: sensitive, intermediate or resistant against the antibiotics.

Results and Discussion

Enumeration

Soil

Table I shows the number of bacteria counted in sites 1 and 2. Bacteria of the *Staphylococcus* genus were more abundant than *Enterobacteriaceae*, with a predominance at site 1, with an average of 2.8.105 and 10.9.103 CFU/g for *Staphylococcus* and *Enterobacteriaceae* respectively.

Clinical media

Clinical strains were isolated at the National Public Health Laboratory and the Pasteur clinic in Brazzaville.

Identification

Soil

Staphylococcus and *Enterobacteriaceae*, which are potentially pathogenic micro-organisms causing various infections and isolated from hospital soils, were present at equal rates (50%) at the 2 sites (Figure1).

National Public Health Laboratory and the Pasteur Clinic

Figure 2 provides very detailed information on the distribution of the various hospital strains (*Staphylococcus* and *Enterobacteriaceae*) studied here. Although these strains all have the same origin (hospital environments), strains from the National Public Health Laboratory are more abundant, with a frequency of 60% for *Enterobacteriaceae* and 70% for *Staphylococcus*. 30% of *Staphylococcus* strains and 40% of enterobacteria came from the Pasteur clinc.

Table II shows the distribution of clinical strains isolated according to biological fluid.

Antibiotic susceptibility testing

Environmental Staphylococcus and Enterobacteriaceae

Soil *Staphylococcus*

Figure 3 shows that ofloxacin was most active on environmental strains from Mfilou, with a rate of 10%. Kanamycin and Norfloxacin were weakly active on all soil strains, with a resistance rate of 20%. High rates of resistance were observed with the other antibiotic molecules, with resistance rates ranging from 40% to 100%.

Enterobacteriaceae

Figure 4 shows the high rates of resistance, ranging from 40% to 100%, to the various families of antibiotics tested. *Enterobacteriaceae* isolated from Bissita showed higher resistance rates than those isolated from Mfilou.

Sensitivity of Staphylococcus and clinical Enterobacteriaceae

Staphylococcus

Figure 5 shows the antibiotic resistance profile of *Staphylococcus* strains isolated from hospital environments. *Staphylococcus* strains all showed resistance to the antibiotics tested, but at different frequencies. The figure below shows that cefoxitin was most active on *Staphylococcus* from Pasteur clinic, with a resistance rate of 16.66%. The other antibiotic molecules showed high resistance rates.

Enterobacteriaceae

Figure 6 shows the resistance profile of hospital-derived *Enterobacteriaceae* strains (Pasteur clinic and National Public Health Laboratory). Although the strains were resistant to the majority of antibiotics tested, strains from the LNSP were resistant more often than those from the clinic, although ertapenam was more active on enterobacteria strains from the Pasteur clinic.

Enumeration of bacteria of the genus *Staphylococcus* and *Enterobacteriaceae* showed that in both sites. Depending on the sampling sites, our study showed that the soil has a variety of bacterial species. Although the strains had the same origin (soil), there was a difference in the abundance of the different strains present on the 2 media. The presence of this microflora has already been confirmed by the work of Berge *et al.,* (2005). Colinon *et al.,* (2013) demonstrated that soil contains a wide variety of potentially pathogenic bacteria. The distribution of species isolated according to their biological origin reveals that the majority of *Staphylococcus* came from vaginal swabs (75%), followed by urine (15%) and spermactic fluid (10%), while the majority of *Enterobacteriaceae* came from urine (55%) and followed by vaginal swabs (35%) and spermactic fluid (10%). All these species are responsible for a variety of infections. However, it has been shown that enterobacteria (particularly *E.coli*) account for 80% of germs responsible for urinary tract infections (Christensen *et al.,* 1985). Basically, bacteria isolated in hospital environments are opportunistic germs responsible for severe nosocomial infections and epidemics, due to their resistance to a wide variety of antibiotics, leading to major difficulties in patient management and therapeutic impasse situations (Mukubwa *et al.,* 2023). The majority of strains came from female subjects, which is explained by the fact that vaginal samples were in the majority compared with other samples (semen and urine). Females were more exposed to infections (92.85%) caused by the 2 strains (*Staphylococcus* and *Enterobacteriaceae*) than males (7.15%). In fact, females are much more exposed to infections (urinary, vaginal, etc.) than males, due to a lack of hygiene that favors the inoculation of environmental bacteria (Sekhsokh *et al.,* 2008). This predominance is confirmed by several authors (Nouri and Ziadi, 2015; Zohoun *et al.,* 2012), with frequencies of 71.62%, 54% and 85% respectively. One of the main host factors is age: the majority of infections increase with age, and advanced age is a risk factor for infection. This is because the elderly are mostly prone to infections (urinary tract infections) (OuLd Baba Ali and Taibi, 2019). In this study, the age group most affected was 62 and 83 years old. For infections caused by strains of the enterobacteria genus, of which infections with strains of the *Staphylococcus* genus and enterobacteria were respectively 62 corroborate. With those reported by Munoz-Price *et al.,* (2013); Nedjai *et al.,* (2012). In the case of infections caused by strains of the *Staphylococcus* genus, the highest age group was 83 years; these results concur with those found by Nouria *et al.,* (2016). This can be explained by the fact that the elderly are the most vulnerable to infection, due to their fragile immune systems (Matute *et al.,* 2004). Antibiotics have brought about a revolution in antibiotic therapy, both socially and

demographically, drastically reducing infectious mortality (Nouria *et al.,* 2016). Antibiotic resistance is now at the heart of research worldwide, as several major organizations (WHO) are seeking a solution to limit the increasing growth of resistant strains. With this in mind, a study of resistance to enterobacteria and *Staphylococcus* was carried out at various sites. Once the strains had been identified, they were subjected to antibiotic susceptibility testing. The results showed that the strains were highly resistant to beta-lactam antibiotics, particularly oxacillin (100%), in line with the findings of several authors including Baloki *et al.,* (2019); Nordmann and Carrer (2008) and Ahombo *et al.,* (2019). In addition, significant resistance to cefoxitime 40% has been observed. This sensitivity varies according to the beta-lactam molecules used, and is due to the synthesis of penicillinase, a beta-lactamase that hydrolyzes the beta-lactam cycle of penicillins, rendering them inactive. This protein is encoded by the mec A gene (Rebiahi *et al.,* 2011). Strains of the genus *Staphylococcus* isolated in both environments were all resistant to oxacillin (100%). These results are clearly similar to those found by Baloki *et al.,* (2019) in Brazzaville. In this study, *Staphylococcus* strains showed a very high rate of resistance to MLS, more precisely to Clindamycin (80%). This result is significantly higher than that reported by Baloki *et al.,* (2019), who reported resistance rates of 12.2%. Kanamycin, a member of the aminoglycoside family, showed a very low resistance rate (20%), close to that of Ahombo *et al.,* (2019) (14.24%). The rifampicin molecule showed a very high rate of resistance (100% and 70%) in strains isolated at both sites. This result differs from those obtained by Dia *et al.,* (2014), who obtained very high sensitivity rates (96%) to this molecule. A very high resistance rate was observed against fusidic acid (60%), this result is higher than that found by Morabandza *et al.,* (2020) (20%), this translates into the fact that *Staphylococcus* are able to develop severe forms of resistance.

Next to Nalidic acid, Norfloxacin presented a different level of resistance depending on the origins. It was more active on strains isolated in the environment, i.e. a rate of 20%, and strains isolated in hospital environments presented a resistance rate of 100%. These results are similar to those found by Morabandza *et al.,* (2020). Furthermore, they are similar to those found by Fahimed *et al.,* (2016) with a rate of 83.3% resistance on strains of the *Staphylococcus* genus. The rate of resistance observed against moxifloxacin differed depending on the origins of the strains. The strains isolated from the soil presented a high rate of resistance, however the hospital strains presented a very high rate of resistance. This is explained by the increase in the prescription of quinolones which today constitutes an important factor in the increase in resistance (Kayode *et al.,* 2020). Enterobacteria constitute a very important family in human pathology and are among the bacteria most frequently isolated in infections (meningitis, urinary infections and gastroenteritis) as described by Parterson (Paterson, 2000). Cephalosporins (cefotaxime, cefoxime, Cephalexime) which have been tested on enterobacteria showed resistance rates ranging from (65 to 100%). These results are significantly higher than those found by Ouedraogo *et al.,* (2017) who reported resistance rates of 41.6%. This could result in the production of beta-lactamases by enterobacteria strains. Alongside cephalosporins, carbapenems (imipenem and ertapenem) which have presented different resistance rates depending on the origin of the strains. The strains isolated in the two environments presented high levels of resistance, particularly to imipenem, i.e. 100% and 70%. These results are higher than those reported by Moyen *et al.,* (2022) (20%) and Sire *et al.,* (2007) 0.68%. Furthermore, ertapenem, although considered a major antibiotic of the carbapenem family in the treatment of certain enterobacterial infections, has presented fairly high resistance rates of 30 to 55%. These results are different from those found respectively by Storberg (2014); Sire *et al.,* (2007). Kanamycin presented resistance rates of 80% in strains isolated from both sites. This result differs from that found by Tansarli *et al.,* (2014). Colistin, which was once considered an antibiotic capable of containing infections of strains of the *Enterobacteriaceae* genus, presented very high resistance rates (90 and 65%), which differs from the results reported by Touati *et al.,* (2012) who obtained resistance rate of 1.3%. Furthermore, Nouri and Ziadi (2015) demonstrated that enterobacteria strains had become increasingly resistant to colistin. Among the quinolone molecules which are inhibitors of nucleic acid synthesis, Nalidixic acid presented different resistance rates depending on the origins of the strains which were respectively 35% and 70%. These results are different from those found by Moyen *et al.,* (2021) who obtained rates of 100%. Moxifloxacin showed resistance rates of 85%; these results are comparable to those found by superior to those reported by Paterson *et al.,* (2005) who found resistance of 53%. Norfloxacin presented different resistance rates depending on the origins of the strains, respectively 50% and 85%. These results are different from those found by Morabandza *et al.,* (2020) in Brazzaville who obtained rates of 100%.

Table.1 Colony counts on Petri dishes in CFU/g

Sites Genus	**Site 1**	**Site 2**
Enterobacteriaceae	$10,9.10^3$	$4,6.10^3$
Staphylococcus	$2,8.10^5$	6.10^5

Table.2 Distribution of clinical *Staphylococcus* and *Enterobacteriaceae*

	Staphylococcus			***Enterobacteriaceae***		
	Spermactic fluid	Urine	vaginal swabs	Spermactic fluid	Urine	PV
Pasteur clinic	1	3	2	0	4	4
LNSP	1	0	13	2	7	3
Percent (%)	10%	15%	75%	10%	55%	35%

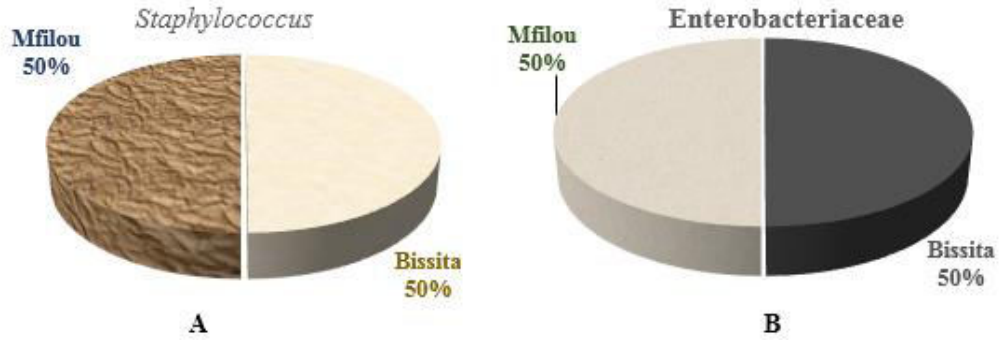


Figure.1 Distribution of (A) *Staphylococcus* and (B) *Enterobacteriaceae* in soils

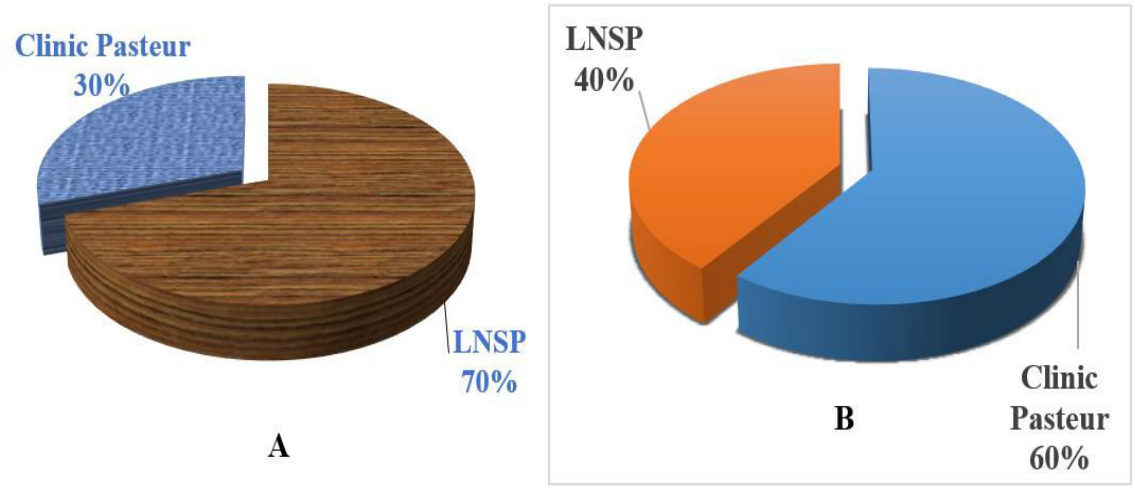
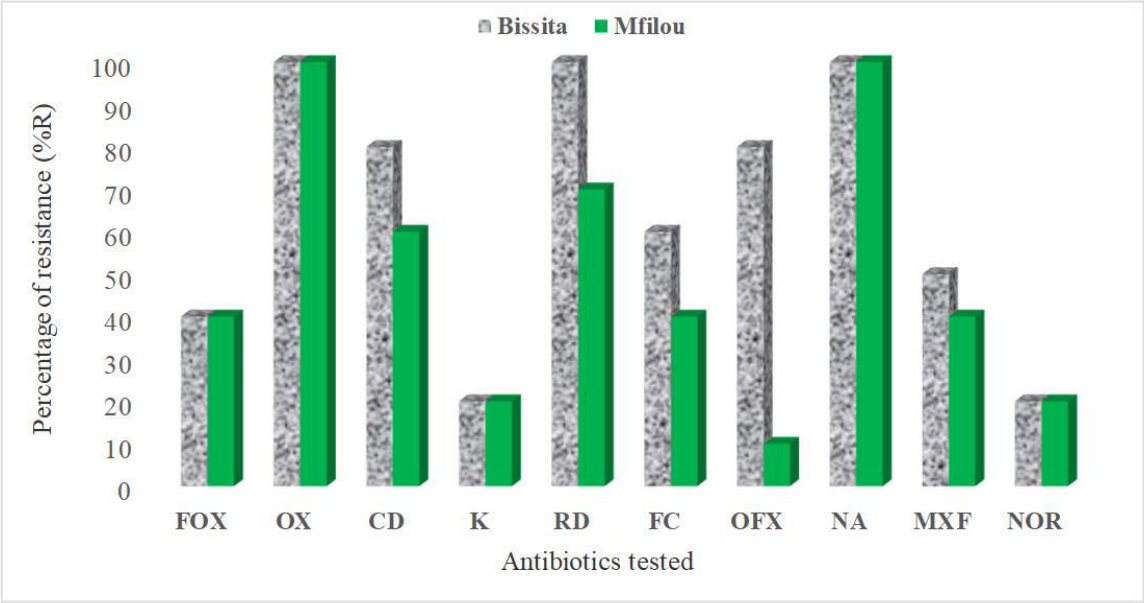
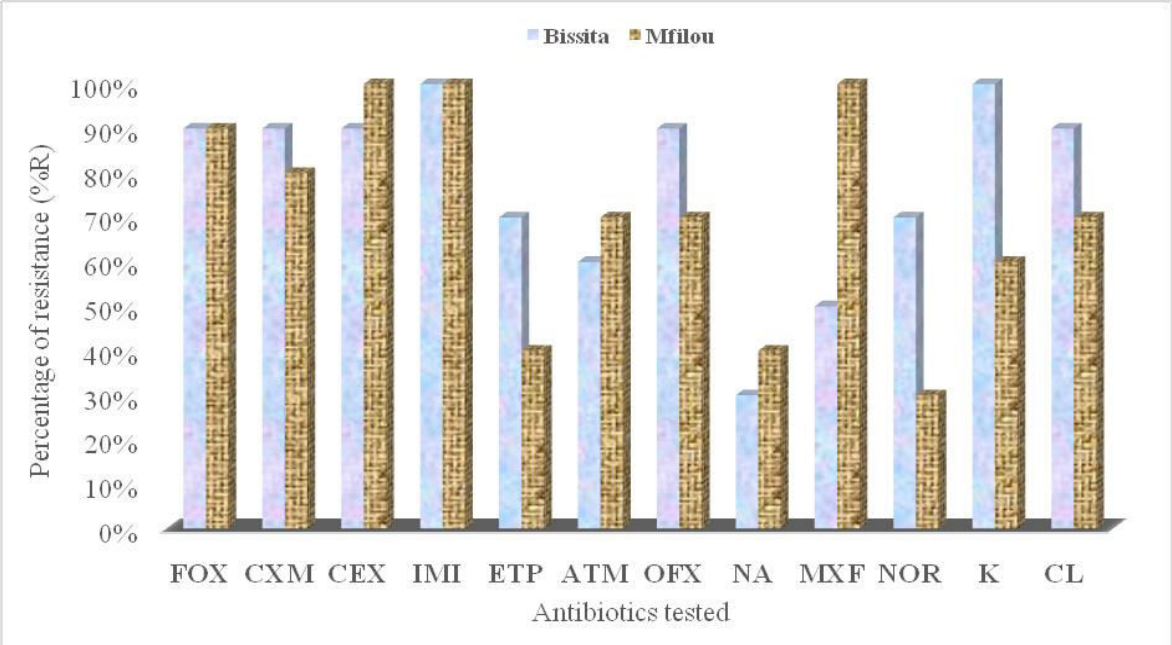


Figure.2 Distribution of (A) *Staphylococcus* and (B) *Enterobacteriaceae* strains isolated from biological fluids.



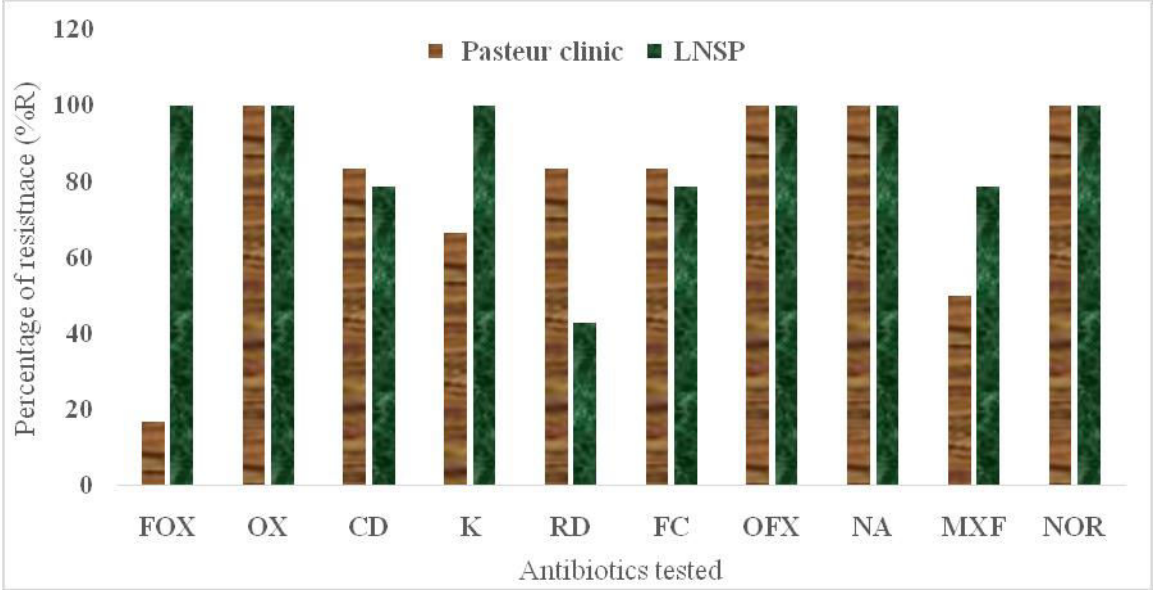
Legend: Cefoxitim (FOX, 30µg), Cefuroxim (CEX, 30µg), Cefotaxim (CXM,30µg), Oxacilline (OX, 5µg), Aztreonam (ATM, 30µg), Kanamycine (K,30µg), fusidic Acid (FC,10µg), Ofloxacin (OFX,5 µg), Rifampicin (RD, 5µg) Clindamycine (CD, 2µg), naldixic Acid (NA, 30µg), Moxifloxacin (MXF, 5µg), Norfloxacin (NOR, 10µg)

Figure.3 Antibiotic resistance profile of soil *Staphylococcus* strains.



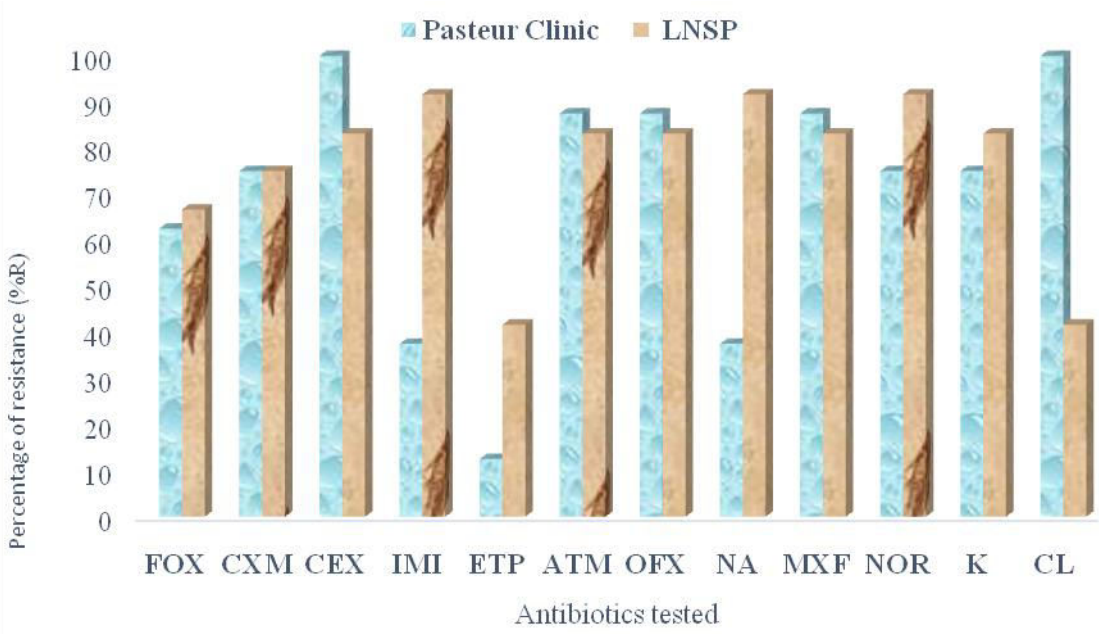
Legend: Legend: Cefoxitim (FOX, 30µg), Cefuroxim (CEX, 30µg), Cefotaxim (CXM,30µg), Ertapenem (ETP, 10 µg), Imipenèm (IMI, 10µg), Aztreonam (ATM, 30µg), Kanamycin (K,30µg), Fusidic Acid (FC,10µg), Ofloxacin (OFX,5 µg), nalidixic Acid (NA,30µg), Moxifloxacine (MXF, 5µg), Norfloxacine (NOR, 10µg) Colistine (CL, 50 µg)

Figure.4 Antibiotic resistance profile of soil-borne *Enterobacteriaceae* strains**.**



Legend: Cefoxitim (FOX, 30µg), Cefuroxim (CEX, 30µg), Cefotaxim (CXM,30µg), Oxacilline (OX, 5µg), Aztreonam (ATM, 30µg), Kanamycine (K,30µg), fusidic Acid (FC,10µg), Ofloxacin (OFX,5 µg), Rifampicin (RD, 5µg) Clindamycine (CD, 2µg), naldixic Acid (NA, 30µg), Moxifloxacin (MXF, 5µg), Norfloxacin (NOR, 10µg)

Figure.5 Antibiotic resistance profile of *Staphylococcus* strains isolated in hospitals



Legend: Cefoxitim (FOX, 30µg), Cefuroxim (CEX, 30µg), Cefotaxim (CXM,30µg), Ertapenem (ETP, 10 µg), Imipenèm (IMI, 10µg), Aztreonam (ATM, 30µg), Kanamycin (K,30µg), Fusidic Acid (FC,10µg), Ofloxacin (OFX,5 µg), nalidixic Acid (NA,30µg), Moxifloxacine (MXF, 5µg), Norfloxacine (NOR, 10µg) Colistine (CL, 50 µg)

Figure.6 Resistance profile of hospital-isolated strains of *Enterobacteriaceae*

Ofloxacin presented a very high resistance rate of 85%, these results are different from those found by Mukubwa *et al.,* (2023) in the Democratic Republic of Congo. All these results concerning quinolones agree with those of Freney *et al.,* (2007) who demonstrated that resistance to Naldixic acid leads to resistance to other quinolone molecules. The results of this study show that, despite the different origins of the strains isolated, most strains showed increased levels of resistance to the various antibiotics. In this study, the distribution of species isolated according to their biological origin revealed that the majority of *Staphylococcus* came from vaginal swabs (75%), followed by urine (15%) and finally semen (10%), while the majority of Enterobacteriaceae came from urine (55%), followed by vaginal swabs (35%) and finally semen (10%). Susceptibility testing revealed that strains from both media were more resistant to beta-lactam antibiotics (FOX CXM, CEX, IMI, ATM), with the exception of ertapenem, where a high rate of resistance was observed in strains isolated from soil and hospital strains.

Staphylococcus strains of hospital origin showed very high rates of resistance (55 to 100%) to all the antibiotics tested, compared with strains isolated from soil. Bacteria isolated from the environment as well as from hospital environments are opportunistic germs, and due to their resistance to a wide variety of antibiotics, they are responsible for many severe nosocomial infections and epidemics, causing great difficulties for antibiotic therapy.

This work has shown an increase in resistance in clinical and environmental (soil) strains to the most widely used antibiotics. Stains showed increased rates of resistance to different antibiotics.

Howerver, norfloxacine and kanamycin were more active against *Staphylococcus* and naladixic acid for enterobacteria isolated form soil. For clinical enterobacteria, ertapenem was more active.

These increased resistance rates constitute a concern in therapeuctic management of patients, and can lead to high costs for prolonged hospitalization, increased morbidity and mortality.

Author Contributions

Tarcisse Baloki Ngoulou: Investigation, formal analysis, writing—original draft. Irène Marie Cécile Mboukou Kimbatsa: Validation, methodology, writing—reviewing. Prisca Nicole Nieko Dangui Makaya:—Formal analysis, writing—review and editing. Varlait Tizi Ewari Osso-Ciel: Investigation, writing—reviewing. Elgie Viennechie Gatse: Resources, investigation writing—reviewing. Armel Faly Soloka Mabika: Validation, formal analysis, writing—reviewing. Etienne Nguimbi: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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