**Biochemical identification and characterization of the antibiotic resistance profile of pathogenic germs isolated from plant-based medicines produced in Côte d'Ivoire**

ABSTRACT:

This study aimed to identify and characterize the antibiotic resistance profile of pathogenic germs isolated from plant-based medicines (MBP) produced in Côte d'Ivoire. It was carried out over a period of 15 months: August 2023 to October 2024.

The samples were collected from traditional medicine practitioners in different health regions and then transported to the laboratory within 24 hours of collection for processing.

The search and enumeration of germs were carried out according to standardized procedures (AFNOR, 1996), and the isolated colonies were identified by morphological and biochemical methods.

Antibiotic sensitivity was assessed by the diffusion method on Mueller-Hinton agar, in accordance with the recommendations of the French Society of Microbiology (CA-SFM, 2022).

The results show a predominance of non-fermentative Gram-negative bacteria (50.23%), such as *Pseudomonas sp* (19.35%), *Pseudomonas fluorescens* (10.13%), *Flavobacterium sp* (6.45%) and *Pseudomonas aeruginosa* (5.06%), as well as enterobacteria (35.02%), with species such as *Escherichia coli* (11.99%), *Enterobacter sp* (5.53%) and *Klebsiella sp* (4.60%).

The study revealed high resistance to the antibiotics used. Shigella sp and Brucella sp showed complete resistance (100%) to Tobramycin, Kanamycin and Amikacin. In addition, moderate resistance was observed in certain strains such as Micrococcus sp (25%) and Strenotrophomonas maltophilia (20%) to levofloxacin.

These results highlight the risks for public health. This study highlights the importance of regulating the production of herbal medicines and strengthening microbiological controls to prevent the spread of resistant bacteria and ensure the effectiveness of antimicrobial treatments.

Key words: Plant-based medicines, microbiological analyses, pathogenic germs, antibiotic resistance profile, Côte d'Ivoire.

**INTRODUCTION**

Antibiotic resistance poses one of the most serious threats to global public health today, jeopardizing advances in medicine, food security and the socio-economic development of nations (WHO, 2016; WHO, 2020 ).

In Côte d'Ivoire, bacterial resistance to antibiotics is a major concern for healthcare workers and patients. Several publications, notably those of (Guessennd *et al.,* 2004; Gbonon *et al.,* 2007; Guessennd *et al.,* 2008) bear witness to this. This problem is all the more alarming as the resistance rate for certain bacteria, such as enterobacteria and non-fermentative Gram-negative bacilli, increased from 9% in 2002 to 46% in 2018

This phenomenon seriously compromises the treatment of infectious diseases (Ouedraogo *et al.,* 2017; WHO, 2020). Although natural, bacterial resistance is exacerbated by the abusive and inappropriate use of antibiotics in humans and animals (ANSES, 2018; Inserm, 2019). Faced with this challenge, bacteria develop defense mechanisms such as genetic mutations or resistance gene transfers, rendering treatments ineffective (Inserm, 2019). This leads to prolonged hospitalizations, increased healthcare costs and increased mortality (ECDC, 2022; WHO, 2023).

Pathogens such as *E. coli,* *Klebsiella pneumoniae* and *P. aeruginosa* are among the most worrying, requiring surveillance and innovative therapeutic solutions (WHO, 2017; Ouédraogo *et al.,* 2017).

In Africa, particularly in Côte d'Ivoire, traditional medicine remains an essential pillar of care, particularly in rural areas where access to modern services is limited (WAHO, 2011). Rich in biodiversity, these practices use plants with proven antimicrobial, antioxidant and anti-inflammatory properties (Ouattara, 2006; N'Guessan, 2008; Kroa *et al.,* 2016; Béné *et al.,* 2016; MSLS, 2014; Serge- Roland, 2020).

However, the rise of herbal medicines, although aimed at increased standardization, raises concerns related to microbiological contaminations due to inadequate manufacturing practices or unfavorable environmental conditions and their potential role in the spread of bacteria resistant (Julie, 2006; WAHO, 2011; WHO, 2013).

Recent studies have revealed that some herbal medicines are contaminated with multidrug-resistant pathogenic bacteria, exposing consumers to opportunistic and nosocomial infections (Becila, 2009; Konan, 2012).

These observations highlight the need for in-depth studies to identify the pathogenic germs present in these products and analyze their resistance profiles.

Thus, this study aimed to identify by biochemical methods the pathogenic germs isolated from plant-based medicines produced in Côte d'Ivoire and to evaluate their resistance profile to certain essential antibiotics. The data obtained will help to strengthen the health safety of plant-based medicines and to better understand the challenges linked to antibiotic resistance.

**MATERIALS AND METHODS**

Biological material

Bacterial strains

It was composed of bacterial germs isolated from samples of herbal medicines produced in Côte d'Ivoire

Sampling

Sample size

217 potentially pathogenic germs were isolated from 1,585 herbal medicine samples collected in 14 health regions.

Choice of antibiotics

In this study, the antibiotics used were Amikacin AK30, Kanamycin K30, Tobramycin TOB10, Cefepime FEP30 and Levofloxacin LEV15

**Methods**

Location and duration of the study

This study took place over a period of 15 months (August 2023-October 2024). The microbiological analyzes were carried out at the Environmental Chemistry and Microbiology Unit of the Environment and Health Department of the Pasteur Institute of Côte d'Ivoire. The samples were collected from traditional medicine practitioners in the health regions of Côte d'Ivoire.

Sample collection

Herbal medicines were drawn at random from a batch of products manufactured under the same conditions and on the same day and then placed in a cooler containing cold accumulators.

Transportation

Samples are sent to the laboratory within 24 hours after collection and then processed upon receipt.

Analysis of samples

The search and enumeration of germs were carried out according to the method based on standardized procedures (AFNOR, 1996).

Isolation and purification

A well isolated colony characteristic of the bacteria sought on the selective medium was selected then subcultured by streaking on ordinary agar. The boxes were then incubated at 37°C for 24 hours.

-Identification of isolated germs

The identification of the isolated germs was carried out on the basis of morphological characters (fresh state, Gram staining) and biochemical characters following the method of Le Minor and Richard (1993), supplemented by MALDI-TOF mass spectrometry from young colonies subcultured on ordinary agar.

- Carrying out the antibiogram

The sensitivity of isolated bacteria to antibiotics was carried out by the solid medium diffusion method, on Mueller-Hinton (MH) agar in accordance with the recommendations of the French Society of Microbiology (CA-SFM, 2022)

Quality control

To evaluate the validity of the discs and the conformity of the Muller Hinton (MH) medium, reference strains were used, in particular *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and Salmonella A517.

Statistical analyzes

The results were interpreted according to CASFM standards.

**RESULTS**

Morphological characterization of potentially pathogenic germs isolated from herbal medicines produced by traditional medicine practitioners.

During this study, 217 potentially pathogenic germs were isolated in 1585 herbal medicines from 14 health regions. The majority of germs belong to non-fermentative Gram-negative bacteria (50.23 ± 9.08%). Enterobacteria are among the germs isolated with a rate of 35.02 ± 4.90%. Were also isolated, Gram-positive cocci (8.76 ± 2.24%), Gram-negative bacteria (5.53 ± 1.50%), and Gram-positive bacilli (0.46 ± 0. 00) (Table I).

Table I: Germs isolated from plant-based medicines according to their morphological characters

|  |  |  |
| --- | --- | --- |
| Germs isolated | Number of isolates | Percentage (%) |
| Enterobacteria | 76 | 35,02 ± 4,90 |
| Non-fermentative Gram-negative bacteria | 109 | 50,23 ± 9,08 |
| Gram-negative bacteria | 12 | 5,53 ± 1,50 |
| Gram-positive cocci | 19 | 8,76 ± 2,24 |
| Gram-positive bacilli | 1 | 0,46 ± 0,00 |
| **Total** | **217** | **100,00 ± 17,71** |

**Biochemical identification of germs isolated from herbal medicines**

Bacterial identification indicates that at the level of enterobacteria, there is a predominance of the E. coli species with a rate of 11.99% or 26 out of 76 enterobacteria, followed by *Enterobacter sp* and *Klebsiella sp* with respective rates. 5.53% and 4.61%. As for *Sarratia marcescens*, *Yersinia sp* and *Salmonella sp*, they had the lowest presence rates (0.46%) (Table II).

Regarding non-fermentative Gram-negative bacteria, the identification results reveal a predominance of the genus Pseudomonas sp, which represents 19.36% of the isolates. It is followed by *P. fluorescens* (10.14%) and *Flavobacterium sp* (6.45%). *Acinetobacter baumanii* and *Burkholderia multivorans* recorded the lowest presence rates (0.92%) (Table II).

Regarding Gram-negative bacteria, the results reveal a predominance of the genus *Alcaligenes sp,* which represents 41.67%. This genus is followed by *Chryseobacterium indologene* and *Alcaligenes denitrificans* with respective rates of 33.33% and 16.67. In this group, Brucella sp had the lowest presence rate which was 8.33% (Table II).

For Gram-positive cocci bacteria, the identification results reveal a marked predominance of the genus *Micrococcus sp,* which constitutes 42.11% of the isolates. This genus is followed by *Micrococcus roseus* (26.32%) and *Plesiomonas sp* (21.05%), while *Salinicoccus roseus* displays the lowest presence rate with 5.26%.

For Gram-positive bacilli, only one bacterium has been identified: it is the genus Bacillus sp (Table II).

Table II: germs isolated from herbal medicines according to their biochemical profile

|  |  |  |  |
| --- | --- | --- | --- |
| **Germes** | Bacterial species | Number of strains | Percentage (%) |
| Enterobacteria | *Escherichia coli* | 26 | 11,981567 |
| *Klebsiella sp* | 10 | 4,6082949 |
| *Shigella sp* | 2 | 0,921659 |
| *Enterobacter sp* | 12 | 5,5299539 |
| *Proteus mirabilis* | 3 | 1,3824885 |
| *Salmonella sp* | 1 | 0,4608295 |
| *Citrobacter sp* | 6 | 2,764977 |
| *Yersinia sp* | 1 | 0,4608295 |
| *Providencia stuartii* | 7 | 3,2258065 |
| *Proteus sp* | 3 | 1,3824885 |
| *Morganella morganii* | 2 | 0,921659 |
| *Sarratia marcescens* | 1 | 0,4608295 |
| *Enterobacter cloacea* | 2 | 0,921659 |
| **Sub/Total** | **76** | **100** |
| Bacteria to  gram negative  non-fermentative | *Flavobacterium sp* | 14 | 6,451612903 |
| *Flavobacterium odoratum* | 5 | 2,304147465 |
| *Pseudomonas aeruginosa* | 11 | 5,069124424 |
| *Pseudomonas fluorescens* | 22 | 10,13824885 |
| *Pseudomonas sp* | 42 | 19,35483871 |
| *Acinetobacter baumanii* | 2 | 0,921658986 |
| *Strenotrophomonas maltophilia* | 7 | 3,225806452 |
| *Burkholderia multivorans* | 2 | 0,921658986 |
| *Burkholderia gladioli* | 2 | 0,921658986 |
| *Burkholderia sp* | 2 | 0,921658986 |
| **Sub/Total** | 109 | 100 |
| Bacteria to Gram negative fermenters | *Alcaligenes denitrificans* | 2 | 16,666667 |
| *Brucella sp* | 1 | 8,3333333 |
| *Alcaligenes sp* | 5 | 41,666667 |
| *Chryseobacterium indologene* | 4 | 33,333333 |
| **Sub/Total** | **12** | **100** |
| Cocci to gram positive | *Micrococcus roseus* | 5 | 26,315789 |
| *Micrococcus luteus* | 1 | 5,2631579 |
| *Micrococcus sp* | 8 | 42,105263 |
| *Salinicoccus roseus* | 1 | 5,2631579 |
| *Plesiomonas sp* | 4 | 21,052632 |
| **Sub/Total** | 19 | 100 |
| Gram bacilli positive | *Bacillus sp* | 1 | **100** |
|  | **Total** | **217** | **100** |

**Sensitivity of germs isolated from enterobacteria to antibiotics**

Susceptibility testing of Enterobacteriaceae to cefepime revealed significant variability in resistance rates across genera. Among the strains studied, *Shigella sp* stands out for the highest resistance rate, reaching 50%, which indicates a worrying adaptation to this antibiotic. *P.* *mirabilis* and *Citrobacter sp* showed identical resistance rates of 33.33%, while *P. stuartii* recorded resistance of 28.57%. On the other hand, *Enterobacter sp* presents a significantly lower resistance, with a rate of 8.33% reflecting a relative sensitivity to cefepime (Table VI).

Among the Enterobacteriaceae tested with trobamycin, *Shigella sp* had the highest resistance rate, reaching 100%. The other strains in this group showed complete or intermediate sensitivity, with no resistance detected (Table VI).

Kanamycin susceptibility testing revealed marked variability in resistance rates among bacterial species studied. *Shigella sp* is distinguished by total resistance (100%) demonstrating complete adaptation to this antibiotic. Conversely, the other Enterobacteriaceae tested showed varied susceptibility profiles, including cases of complete and intermediate susceptibility (Table VI).

As for susceptibility to amikacin, significant variability in resistance rates among bacterial species is noted. *Shigella sp* still stands out with total resistance (100%), followed by Proteus sp, which displays a resistance rate of 66.67%. *Klebsiella sp* and E. cloacae strains each have a resistance rate of 50%, while *Citrobacter sp* and Enterobacter sp show lower resistances, 33.33% and 25%, respectively. Conversely, the other enterobacteria tested presented complete or intermediate sensitivity profiles (Table VI).

Susceptibility to levofloxacin shows that only the species *Micrococcus sp* and *M. roseus* presented resistance, with respective rates of 25% and 20% (Table VI). These figures highlight a limited but worrying capacity of these bacteria to develop resistance mechanisms to this antibiotic.

Table III: Resistance profile of enterobacteria strains to antibiotics

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Test  Bacteria | SUST | CEF | TBR | KN | AM | LEV |
| *Escherichia coli* | R  S  I | 0  96,15  3,85 | 0  100  0 | 0  100  0 | 0  100  0 | 0  100  0 |
| *Klebsiella sp* | R  S  I | 10  20  70 | 0  50  50 | 0  60  40 | 50  50  0 | 0  100  0 |
| *Shigella sp* | R  S  I | 50  0  50 | 100  0  0 | 100  0  0 | 100  0  0 | 0  50  50 |
| *Enterobacter sp* | R  S  I | 8,33  66,67  25 | 0  58,33  41,67 | 0  91,67  8,33 | 25  41,67  33,33 | 0  100  0 |
| *Proteus mirabilis* | R  S  I | 33,33  66,67  0 | 0  100  0 | 0  100  0 | 0  100  0 | 0  100  0 |
| *Salmonella sp* | R  S  I | 0  100  0 | 0  100  0 | 0  100  0 | 0  100  0 | 0  100  0 |
| *Citrobacter sp* | R  S  I | 33,33  66,67  0 | 0  16,67  83,33 | 0  83,33  16,67 | 33,33  16,67  50 | 0  100  0 |
| *Yersinia sp* | R  S  I | 0  100  0 | 0  100  0 | 0  100  0 | 0  100  0 | 0  100  0 |
| *Providencia stuartii* | R  S  I | 28,57  57,14  14,29 | 0  85,71  14,29 | 0  100  0 | 0  85,71  14,29 | 0  100  0 |
| *Proteus sp* | R  S  I | 0  100  0 | 0  33,33  86,67 | 0  0  100 | 66,67  0  33,33 | 0  100  0 |
| *Morganella morganii* | R  S  I | 0  100  0 | 0  100  0 | 0  100  0 | 0  50  50 | 0  100  0 |
| *Sarratia marcescens* | R  S  I | 0  100  0 | 0  0  100 | 0  100  0 | 0  0  100 | 0  100  0 |
| *Enterobacter cloacea* | R  S  I | 0  100  0 | 0  50  50 | 0  100  0 | 50  0  50 | 0  50  50 |

Key: SUST= Susceptibility, R= Resistance, I=intermediate, S =sensitive, CEF=Cefepime, TBR=Tobramycin, KN=Kanamycin AM=Amikacin LEV= Levofloxacin

**Sensitivity of non-fermentative gram-negative bacterial strains to antibiotics**

Cefepime susceptibility testing results show overall low resistance. Among the strains tested, only *Pseudomonas sp* presented resistance, with a rate of 2.38%. Although moderate, this figure requires increased vigilance, given the intrinsic capacity of this bacteria to develop resistance mechanisms (Table IV).

Regarding sensitivity to tobramycin, bacteria showed variability in resistance rates. *S.* *maltophilia* displays a resistance of 42.86%, highlighting a notable adaptation to this antibiotic. Conversely, *Pseudomonas sp* shows a much lower resistance rate of 2.38%. Other non-fermentative Gram-negative bacteria included in this study showed no resistance, indicating persistent effectiveness of tobramycin against these strains (Table IV).

Susceptibility to kanamycin has shown significant variability in resistance rates. *P. aeruginosa* displays the highest resistance rate (54.55%), followed by S. maltophilia (42.86%), Conversely, *Pseudomonas sp* shows a significantly lower resistance rate (7.14%). The other bacteria in this group showed no resistance (Table IV).

As for amikacin, notable variability was also observed with these strains. *S. maltophilia* displays the highest resistance rate (28.57%). Conversely, *Pseudomonas sp* has a very low resistance rate (2.38%). The other bacteria in this group demonstrated a total absence of resistance (Table IV).

In contrast, all bacteria in this group displayed complete or intermediate sensitivity, with no resistance detected against levofloxacin (Table IV).

Table IV: Resistance profile of non-fermentative gram-negative bacterial strains

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Test  Bacteria | SUST | CEF | TBR | KN | AM | LEV |
| *Flavobacterium sp* | R  S  I | 0  92,86  7,14 | 0  28,57  71,43 | 0  100  0 | 0  85,71  14,29 | 0  100  0 |
| *Flavobacterium odoratum* | R  S  I | 0  100  0 | 0  60  40 | 0  60  40 | 0  60  40 | 0  100  0 |
| *Pseudomonas aeruginosa* | R  S  I | 0  100  0 | 0  100  0 | 54,55  36,36  9,09 | 0  100  0 | 0  100  0 |
| *Pseudomonas fluorescens* | R  S  I | 0  86,36  13,64 | 0  90,91  9,09 | 0  95,46  4,54 | 0  95,45  4,55 | 0  100  0 |
| *Pseudomonas sp* | R  S  I | 2,38  83,33  14,29 | 2,38  54,76  42,86 | 7,14  85,71  7,14 | 2,38  76,19  21,43 | 0  100  0 |
| *Acinetobacter baumanii* | R  S  I | 0  50  50 | 0  100  0 | 0  100  0 | 0  100  0 | 0  100  0 |
| *Strenotrophomonas maltophilia* | R  S  I | 0  100  0 | 42,86  14,29  42,86 | 42,86  42,86  14,29 | 28,67  28,57  42,86 | 0  100  0 |
| *Burkholderia multivorans* | R  S  I | 0  100  0 | 0  0  100 | 0  0  100 | 0  0  100 | 0  100  0 |
| *Burkholderia gladioli* | R  S  I | 0  100  0 | 0  0  100 | 0  50  50 | 0  100  0 | 0  100  0 |
| *Burkholderia spp* | R  S  I | 0  100  0 | 0  0  100 | 0  100  0 | 0  100  0 | 0  100  0 |

Key: SUST= Susceptibility, R= Resistance, I=intermediate, S =sensitive, CEF=Cefepime, TBR=Tobramycin, KN=Kanamycin AM=Amikacin LEV= Levofloxacin

**Sensitivity of gram-negative and gram-positive bacterial strains**

Bacteria in this group were found to be sensitive and of intermediate sensitivity to cefepime, confirming the effectiveness of cefepime against these pathogens. Only the *Micrococcus sp* species presented resistance, with a rate of 12.5% ​​(Table V).

Among Gram-negative bacteria, *Brucella sp* is distinguished by a 100% resistance rate to tobramycin. The other strains in this group demonstrated complete or intermediate sensitivity. Among Gram-positive cocci, *M. roseus*, *S. roseus* and *Plesiomonas sp* exhibit 100% resistance. *Micrococcus sp* showed 37.50% resistance, while *M. luteus* showed intermediate susceptibility. On the other hand, tests carried out on Gram-positive bacilli, particularly *Bacillus sp*, indicate a total absence of resistance demonstrating high effectiveness of tobramycin against this bacteria (Table V).

Gram-positive cocci show varied resistance profiles to kanamycin. *M. roseus* and *S. roseus* show full resistance (100%), while Plesiomonas sp shows a high resistance rate of 75%. On the other hand, *Micrococcus sp* shows a moderate resistance of 37.50%. In contrast, *M. luteus* is distinguished by complete sensitivity, confirming the effectiveness of kanamycin against this species. In this group, *Brucella sp* presents total resistance (100%). *Alcaligenes sp* displays a resistance rate of 40%, while other strains in this group exhibit complete or intermediate susceptibility profiles (Table V).

Among Gram-positive bacilli, *the Bacillus sp* strain showed intermediate sensitivity with no resistance detected (Table V).

Regarding amikacin, tests on Gram-positive cocci reveal varied results. *M. roseus* and *Plesiomonas sp* show total resistance (100%), while *Micrococcus sp* shows moderate resistance, 37.50%. On the other hand, the other strains tested proved to be sensitive. Among Gram-positive bacilli, *Bacillus sp* showed total resistance (100%), highlighting a worrying adaptation of this strain to amikacin. *Brucella sp* shows total resistance (100%), indicating complete ineffectiveness of amikacin for this genus. The other species in this group showed complete or intermediate sensitivity profiles (Table V).

In contrast, all bacteria in these study groups displayed complete or intermediate susceptibility, with no resistance detected to levofloxacin (Table V).

Table V: Resistance profile of gram-negative and gram-positive bacterial strains to antibiotics

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| TEST  BACTERIA | SUST | CEF | TBR | KN | AM | LEV |
| *Alcaligenes denitrificans* | R  S  I | 0  100  0 | 0  100  0 | 0  100  0 | 0  0  100 | 0  100  0 |
| *Brucella sp* | R  S  I | 0  100  0 | 100  0  0 | 100  0  0 | 100  0  0 | 100  0  0 |
| *Alcaligenes spp* | R  S  I | 0  100  0 | 0  100  0 | 0  40  60 | 0  100  0 | 0  100  0 |
| *Chryseobacterium indologene* | R  S  I | 0  100  0 | 0  25  75 | 0  100  0 | 0  100  0 | 0  100  0 |
| *Micrococcus roseus* | R  S  I | 0  100  0 | 100  0  0 | 100  0  0 | 100  0  0 | 20  80  0 |
| *Micrococcus luteus* | R  S  I | 0  100  0 | 0  100  0 | 0  100  0 | 0  100  0 | 0  100  0 |
| *Micrococcus sp* | R  S  I | 12,5  75  12,5 | 37,5  62,5  12,5 | 37,5  62,5  0 | 37,5  62,5  0 | 25  75  0 |
| *Salinicoccus roseus* | R  S  I | 0  100  0 | 100  0  0 | 100  0  0 | 0  0  100 | 0  100  0 |
| *Plesiomonas sp* | R  S  I | 0  100  0 | 100  0  0 | 75  25  0 | 100  0  0 | 0  100  0 |
| *Bacillus sp* | R  S  I | 0  100  0 | 0  0  100 | 0  0  100 | 100  0  0 | 0  0  100 |

Key: SUST= Susceptibility, R= Resistance, I=intermediate, S =sensitive, CEF=Cefepime, TBR=Tobramycin, KN=Kanamycin AM=Amikacin LEV= Levofloxacin

**DISCUSSION**

The microbiological quality of herbal medicines represents a crucial issue, raising questions relating to their safety and effectiveness. These drugs are widely used by the Ivorian population. However, the production of these drugs takes place in a relatively informal setting, with manufacturing practices that do not always respect health standards. This study was conducted to better understand the health risks associated with the use of these products.

The results of this study reveal notable bacterial diversity in plant-based medicines produced in Côte d'Ivoire. The predominance of non-fermentative Gram-negative bacteria (50.23%), such as *Pseudomonas sp* (19.35%), *P. fluorescens* (10.13%), *Flavobacterium sp* (6.45%) and *P. aeruginosa* ( 5.06%), which raise major concerns due to their natural resistance to many antibiotics and their role in serious nosocomial infections. Enterobacteriaceae with 35.02% of the isolates were found, with dominant species such as E. coli (11.99%), Enterobacter sp. (5.53%) and *Klebsiella sp.* (4.61%). This observation is explained by their ability to adapt to environmental conditions and their survival in environments rich in organic matter.

Other genera, such as *Shigella sp* (0.92%) and Salmonella sp (0.46%) were also found, although in lower proportions, thus reinforcing the need for rigorous microbiological control of medicines based on of plants. These results are similar to those obtained by Jérôme *et al.* (2018) in their study on the profile and antibiosensitivity of pathogenic bacteria associated with diarrhea in patients consulting at the Kousseri Regional Annex Hospital, located in the Far North region of Cameroon. In this study, a range of pathogenic bacteria was identified, the predominance of which was recorded for *E. coli* (66.6%), followed by *Serratia sp.* (30.9%), *Klebsiella sp.* (27.3%), *Enterobacter sp.* (18.18%), *Salmonella sp.* (15.5%), *P. mirabilis* (12.7%), *Citrobacter sp.* (7.3%) and *P. aeruginosa* (1.8%).

Furthermore, these results also corroborate with those of Coulibaly *et al.* (2018) on the assessment of the risk of contamination of milk preparations in the neonatology department of the Treichville University Hospital and antibiotic resistance of the bacterial flora. They showed that among the 59 samples collected, 8 strains of *E. coli* were isolated with a prevalence of 13.56%, 3 strains of *P.* *aeruginosa* with a prevalence of 5.08% and 16 strains of S. aureus (27.11%).

Antibiotic resistance testing has revealed alarming rates of resistance, particularly among isolated bacteria. Regarding resistance to β-lactams (Céfepime), although the latter has shown some effectiveness against certain enterobacteria, several strains, including *Shigella sp* (50%), *P.* *mirabilis* (33.33%), *Citrobacter sp* (33, 33%), *P. stuartii* (28.57%), and *Klebsiella sp* (10%), presented high resistance. This ineffectiveness with respect to β-lactams demonstrates the emergence of worrying resistance. These results further corroborate those obtained by Coulibaly *et al.* (2018). They showed that following sensitivity tests on *E. coli* to antibiotics, the highest rates of resistance were recorded with antibiotic molecules belonging to the beta-lactam family, in particular C3G (Cefotaxime , Ceftazidime, Céfixime and Céfépime), with a resistance rate of 87.5%.

Regarding resistance to aminoglycosides (Tobramycin, Kanamycin, Amikacin), bacteria such as *Shigella sp* and *Brucella sp* showed complete resistance (100%) to Tobramycin, Kanamycin and Amikacin. Similarly, the genera *Bacillus sp,* *M. roseus* and *Plesiomonas sp* also showed a 100% resistance rate to Amikacin, while Klebsiella sp showed 50% resistance to this antibiotic. Other bacteria, such as *S. roseus*, *P. aeruginosa*, *S. maltophilia* and *Pseudomonas sp*, showed respective resistance rates of 100%, 54.55%, 42.86% and 7.14% to Kanamycin. Additionally, *S. roseus* and *M. roseus* have developed complete resistance to Tobramycin. These results remain in agreement with those obtained by Coulibaly *et al.* (2018)

They showed that the study of the sensitivity of *P. aeruginosa*, isolated from milk, reveals a resistance rate of 100% for Tigecycline and a resistance rate of 66% for Cefepime, Ticarciline, Ticarciline + Clavulanic acid and Piperacillin, with a lower rate of 33.3% for Ceftazidime.

Furthermore, these results are in accordance with those of Jérôme *et al.* (2018), who showed in their profile and antibiosensitivity study that Aeromonas, *E. coli*, *Salmonella spp*, *Shigella* and *Vibrio spp* bacteria were 100% resistant to amoxicilin and amoxicilin + clavulanic acid .

Similarly, *E. coli* had high resistance rates to several antibiotics, such as ceftriaxone (53.3%), chloramphenicol (50%), tetracycline (83.3%), erythromycin (100%), nalidixic acid (69.5%), ciprofloxacin (43.3%), cotrimoxazole (83.33%) and colistin (100 %).

These results are comparable to those of Islam *et al.* (2008) who found in their work bacteria multi-resistant to 5 families of antibiotics with a resistance rate of 100% to ciprofloxacin, tetracycline, penicillin and erythromycin.

On the other hand, the rates were 50% and 90% respectively for gentamycin and chloramphenicol. This resistance demonstrates that these strains are multi-resistant. This is explained by the fact that antibiotics are among the most prescribed molecules in Africa, with beta-lactams in the lead Dosso *et al.* (2000). According to Philippon & Lagrange (1994), bacteria producing ESBLs (extended spectrum beta-lactamases), due to their genetic determinism, are often resistant to several other antibiotics.

These findings are particularly concerning because these multidrug-resistant bacteria can persist in environments with low resistance, such as herbal medicines, making their spread easier.

Additionally, although levofloxacin (a fluoroquinolone) has shown some effectiveness against isolated pathogens, moderate resistance has been observed in some strains such as Micrococcus sp. (25%) and *S. maltophilia* (20%). Although this resistance is less alarming than that observed for aminoglycosides and β-lactams, it nevertheless remains a concern given the importance of this drug in the treatment of serious infections. These results are comparable to those Jérôme *et al.* (2018), who showed in their study resistance rates of 16.7% and 56.7% of *E. coli*, respectively cotrimoxazole and ciprofloxacin, which are the most prescribed antibiotics in cases of suspected bacterial diarrhea.

Similarly, resistance of non-Enterobacteria to many antibiotics has been observed in studies, with resistance rates ranging from 90% to 95% for aminoglycosides and 90% for fluoroquinolones (Berche *et al.,* 1988). .

Consequently, the increase in the consumption of these traditional remedies, coupled with the absence of appropriate microbiological controls, constitutes a factor in the spread of bacteria resistant to standard treatments, with serious implications for public health.

CONCLUSION

Bacteria such as non-fermentative Gram-negative bacteria (*Pseudomonas sp* (19.35%), *P.* *fluorescens* (10.13%), *Flavobacterium sp* (6.45%) and *P. aeruginosa* (5.06%)), and enterobacteria (*E. coli* (11.99%), *Enterobacter sp* (5.53%) and *Klebsiella sp* (4.61%)) responsible infections dominate the microbial flora of herbal medicines.

Analysis of the resistance profile of tested antibiotics shows a high prevalence of bacterial resistance in herbal medicines raising major concerns about their safety for consumers.

It is crucial to establish strict microbiological quality criteria, including regular microbiological analyzes to detect any contamination.

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