

Antibiotic Resistance of Enterobacteria Isolated from Medicinal Plant Powders Marketed in Abidjan, Côte d'Ivoire

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Abstract

Objective: Medicinal plant powders are widely used in Côte d'Ivoire for treating various ailments and are often marketed without rigorous health controls. This lack of regulation increases the risk of contamination by pathogenic bacteria, particularly antibiotic-resistant enterobacteria, which poses a significant challenge for infection control. **Methods:** This study aimed to evaluate the antibiotic susceptibility of enterobacteria isolated from 100 samples of medicinal powders sold in different markets in Abidjan. Bacterial identification was performed using biochemical and proteomic methods (MALDI-TOF). Antibiotic susceptibility testing was conducted using the agar diffusion method, following the EUCAST/CASFM 2024_V1.0 guidelines. **Results:** A total of 48 enterobacterial strains were isolated, including *Enterobacter hormaechei* (21), *Escherichia coli* (18), *Citrobacter freundii* (2), *Enterobacter asburiae* (2), *Klebsiella pneumoniae* (2), *Enterobacter cloacae* (1), *Enterobacter bugandensis* (1), and *Cronobacter sakazakii* (1). Antibiotic susceptibility testing revealed that 54.16% of the strains were resistant to at least one antibiotic, with the highest resistance rates observed for pefloxacin (25%) and ertapenem (12.5%). Resistance to ertapenem, a carbapenem frequently used as a last-resort treatment, is particularly concerning. **Conclusions:** The presence of enterobacteria in these samples suggests fecal contamination, reflecting poor hygienic conditions. These findings highlight the potential risk posed by medicinal plant powders as vectors of antibiotic-resistant bacterial strains. Implementing strict quality control measures and enhanced surveillance of antibiotic resistance is crucial to reducing their impact on public health.

Keywords

Medicinal Plants, Antibiotic Resistance, Enterobacteriaceae, Côte d'Ivoire

1. Introduction

Medicinal plants constitute a cornerstone of traditional healthcare systems and are extensively employed for the treatment of a wide range of ailments. Globally, the prevalence of herbal medicine (HM) use is estimated to range between 50% and 95%, with projections indicating a compound annual growth rate of 5.5% by 2027 [1] [2]. In sub-Saharan Africa, more than 60% of the population reportedly relies on herbal remedies [3], which are used to manage both acute medical emergencies such as snakebite envenomation and chronic conditions, including cancer, diabetes, HIV/AIDS-related symptoms, infertility, ulcers, and kidney diseases [3] [4]. However, numerous studies have documented bacterial contamination in herbal products, identifying pathogenic microorganisms as a significant health concern [5] [6]. In Europe, systematic reviews have reported frequent contamination of commercial herbal medicines with *Salmonella* spp., *Escherichia coli*, *Clostridium perfringens*, and *Listeria monocytogenes* [7] [8]. Cases of bacterial illnesses linked to the consumption of contaminated herbal products have also been observed in European populations [7], although the antimicrobial resistance profiles of these pathogens were not investigated. In North America, bacterial strains such as *Bacillus* spp., *Erwinia* spp., *Staphylococcus* spp., and *Enterobacter cloacae* exhibiting resistance to antibiotics have been isolated from commercial herbal preparations [9]. Similarly, in Asia, contaminants isolated from herbal products have shown resistance to multiple antibiotics, including penicillin, tetracycline, gentamicin, erythromycin, trimethoprim-sulfamethoxazole, and ciprofloxacin [10]. The situation is equally concerning in Africa, where high levels of antibiotic resistance have been reported among pathogens such as *E. coli*, *Klebsiella pneumoniae*, *Salmonella* spp., *Acinetobacter baumannii*, and *Staphylococcus aureus* [11] [12]. A study in Nigeria notably identified multidrug-resistant strains of *E. coli*, *Klebsiella* spp., and *Salmonella* spp. in commonly used herbal medicines [13]. These resistant bacteria may circulate between humans, animals, and the environment—via water, soil, and medicinal plants—thereby perpetuating transmission cycles aligned with the “One Health” framework [14].

In Côte d'Ivoire, medicinal plant powders, commonly sold in markets, serve as an accessible and cost-effective alternative for local populations [15]. However, these products, often prepared under inadequate sanitary conditions, can be contaminated by pathogenic bacteria, particularly antibiotic-resistant Enterobacteriaceae, posing a public health risk. The presence of resistant bacteria in these powders raises significant concerns, especially due to the potential transmission of antibiotic resistance genes [16]. Bacterial resistance is a global health issue, particularly alarming in developing countries where quality control of traditional medi-

nal products is often limited [17]. These resistant bacteria, frequently found in the environment, can contaminate the powders at various stages of production, including cultivation, harvesting, storage, and processing. Such contamination not only threatens consumer safety but may also reduce the effectiveness of traditional medical treatments [18]. Scientific interest in the microbiological quality of plant-based medicinal products has grown significantly in recent years. Several studies have reported the presence of pathogenic microorganisms in these products, including *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* [19] [20]. This contamination is primarily attributed to poor production, storage, and handling practices. Moreover, the emergence of antibiotic resistance represents a major challenge, even among bacteria isolated from traditional medicine products [21]. In Africa, where access to modern healthcare remains limited, the consumption of contaminated medicinal plants can lead to severe health complications, especially among immunocompromised individuals. Despite the critical nature of this issue, studies on the microbiological contamination and antibiotic resistance of medicinal powders in Côte d'Ivoire remain scarce. This research aims to address this gap by: Identifying Enterobacteriaceae present in these powders and Assessing their antibiotic susceptibility in the context of rising bacterial resistance. The findings of this study could provide valuable insights for microbiological control policies and contribute to enhancing the safety of traditional medicinal products.

2. Materials and Methods

2.1. Sampling

In November 2022, medicinal plant powder samples, already prepared and packaged, were collected from 60 vendors at market stalls in three municipalities of Abidjan: Abobo, Adjamé, and Yopougon. These locations were selected due to their strategic role as primary entry points and supply hubs for medicinal plants in the District of Abidjan. The selection of plant powders was based on two main criteria: Frequent use by urban populations to treat malaria, a common disease in Côte d'Ivoire and Availability on vendor stalls Sample collection for microbiological analysis was conducted under strict aseptic conditions. The collector used sterile latex gloves, and each sample was carefully collected and packaged in sterile zip-lock bags before being placed in portable refrigerated coolers (+4°C) to ensure secure transportation to the Environmental Microbiology Laboratory of the Institut Pasteur of Côte d'Ivoire. Upon arrival at the laboratory, samples were immediately subjected to analysis without prior storage or preservation. A total of 100 samples were collected for this study.

2.2. Microbiological Analysis

Each sample was inoculated onto MacConkey agar and incubated at 37°C for 24 hours. Following incubation, the agar plates were examined to assess bacterial growth. Distinct colonies of lactose-fermenting and non-lactose-fermenting bacteria were identified and selected. The selected colonies were further purified on

MacConkey agar, subjected to Gram staining for microscopic observation, and tested for catalase and cytochrome oxidase production. Gram-negative bacilli that were oxidase-negative and catalase-positive underwent further identification using MALDI-TOF mass spectrometry. The identified isolates were subsequently evaluated for antibiotic susceptibility. The antibiotic susceptibility test was conducted using the agar diffusion method, following the recommended standards [22]. The tested antibiotics included: Ciprofloxacin (5 µg), Ticarcillin-acid clavulanic (85 µg), Amikacin (30 µg), Cefepime (30 µg), Ertapenem (10 µg), Azithromycin (15 µg), Pefloxacin (5 µg), Ceftazidime (10 µg), Cefotaxime (5 µg), Gentamicin (10 µg). All plates were incubated at 37°C for 24 hours. After incubation, the inhibition zones around each antibiotic were measured only once using a caliper, and their diameters were interpreted into three categories susceptible, intermediate, or resistant according to the EUCAST/CASFM 2024_V1 recommendations.

2.3. Statistical Analysis

To carry out this analysis, we first performed a descriptive analysis. This involved calculating parameters and determining proportions. In the second part, we conducted a bivariate analysis. Our dependent variable was the number of phenotypic resistances in a bacterium. Through a comparison of proportions, we examined the relationship with other variables. The statistical test used was the Kruskal-Wallis test.

3. Results

3.1. Isolated Enterobacteriaceae

During this study, 48 bacterial isolates belonging to the Enterobacteriaceae family were identified from the analyzed medicinal plant powders. These isolates were distributed as follows (**Figure 1**): *Enterobacter hormaechei* (21), *Escherichia coli* (18), *Citrobacter freundii* (2), *Enterobacter asburiae* (2), *Klebsiella pneumoniae* (2), *Enterobacter cloacae* (1), *Enterobacter bugandensis* (1), *Cronobacter sakazakii* (1).

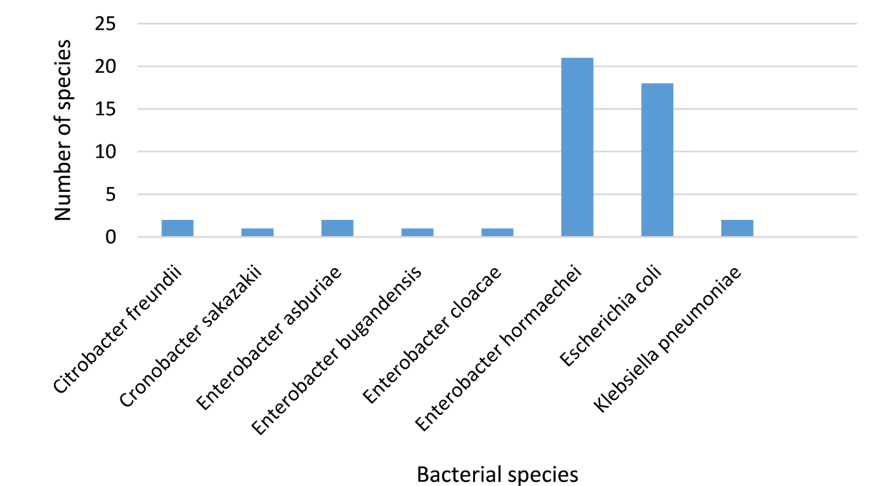


Figure 1. Distribution of the isolated bacteria by species.

3.2. Antimicrobial Susceptibility of Bacteria

Table 1 presents the antibiotic resistance profile of the studied bacteria and the identified resistance phenotypes. Among the 48 bacterial strains, 54.16% exhibited antibiotic resistance. The antibiotic-specific resistance rates are shown in **Figure 2**. The resistance phenotype analysis identified: Low-level fluoroquinolone resistance in 7 strains (14.6%). Carbapenemase production in 4 strains (8.3%), Cephalosporinase activity in 1 strain (2.1%). Antibiotic resistance was primarily observed in *Enterobacter hormaechei*, *Enterobacter asburiae*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Escherichia coli*.

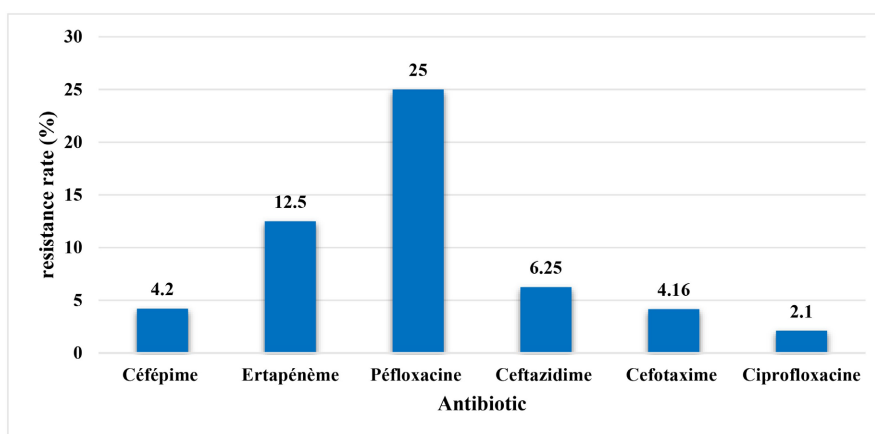


Figure 2. Antibiotic resistance rates of the isolated strains.

Table 1. Antibiotic resistance profile and identified resistance phenotypes.

Germes	Origin	Antibiotic resistances	Identified phenotypes
<i>Enterobacter hormaechei</i> , <i>Enterobacter cloacae</i> , <i>Citrobacter freundii</i> , <i>Escherichia coli</i>	Medicinal plant	Pefloxacin Cefepime, Ciprofloxacin, Cefotaxime	FQPSO3: Low-level fluoroquinolone resistance
<i>Enterobacter hormaechei</i> , <i>Enterobacter cloacae</i> , <i>Enterobacter asburiae</i> , <i>Escherichia coli</i>	Medicinal plant	Ertapenem	Carbapenemase
<i>Enterobacter hormaechei</i>	Medicinal plant	Ceftazidime	C ₃ G-R: Probable hyperproduced cephalosporinase

3.3. Statistical Analysis

There is no statistically significant association between the bacterial genus and the number of resistances (**Table 2**).

Table 2. Number of resistances according to bacterial genus.

Genus	Mean	Standard deviation	Median	P-value
<i>Citrobacter</i>	1	0	1	0.2724

Continued

<i>Cronobacter</i>	0	NA	0	0.2724
<i>Enterobacter</i>	0.44	0.9165	0	
<i>Escherichia</i>	0.33	0.5941	0	
<i>Klebsiella</i>	0	0	0	

There is no statistical association between the number of resistances and the origin (**Table 3**)

Table 3. Number of resistances according to origin.

Origin	Mean	Standard deviation	Median	P-value
Abobo	0.65	0.9881	0	0.2724
Adjamé	0.24	0.5623	0	
Yopougon	0.1818	0.4045	0	

4. Discussion

The results of this study reveal the presence of Enterobacteriaceae in medicinal plant powders sold in the markets of the Abidjan district. Their presence is generally an indicator of fecal contamination, poor hygiene practices, or insufficient thermal treatment [23] [24]. Bacteria such as *Escherichia coli*, *Citrobacter freundii*, and *Klebsiella pneumoniae* are involved in food poisoning causing severe diarrhea, which can be bloody (*E. coli* O157:H7) [25], fever and abdominal pain (*Citrobacter freundii*, *Enterobacter asburiae*) [26], vomiting, and severe dehydration, which are particularly dangerous for children and immunocompromised individuals [27]. Some species, like *Cronobacter sakazakii*, often found in infant formulas, can cause fatal neonatal meningitis [27]. Additionally, *Enterobacter bugandensis* and *Klebsiella pneumoniae* are involved in severe nosocomial infections, including septicemia and pneumonia [28]. The presence of bacteria such as *Enterobacter hormaechei*, *Escherichia coli*, *Citrobacter freundii*, *Klebsiella pneumoniae*, and *Cronobacter sakazakii* in food poses a major public health risk. An integrated approach combining prevention, surveillance, and awareness is essential to mitigate these risks and improve food safety. A high prevalence of antibiotic resistance was noted in the bacteria isolated from the medicinal plant powders sold in Côte d'Ivoire, which are used to treat common diseases. Of the 48 strains of enterobacteria isolated, an overall resistance rate of 54.16% was recorded, confirming a growing trend of antibiotic resistance observed globally. These data align with recent reports highlighting antibiotic resistance as a major threat to global public health [3] [29]. The results reveal varying resistance rates depending on the antibiotics tested. The highest resistance rates were noted for pefloxacin (25%) and ertapenem (12.5%). Resistance to ertapenem, a carbapenem, is particularly concerning because this type of antibiotic is often used as a last resort for severe infections, and the emergence of resistance to these drugs could signifi-

cantly limit available therapeutic options and even lead to a therapeutic deadlock [30]. These results corroborate the work of Bush and Bradford [18], who attribute carbapenem resistance to the production of carbapenemases by certain bacteria such as *Enterobacter cloacae* and *Escherichia coli*. Resistance to third- and fourth-generation cephalosporins (Ceftazidime, Cefepime, Cefotaxime) ranges from 4.16% to 6.25%, Could indicate the potential presence of extended-spectrum beta-lactamases (ESBLs) among strains of *Enterobacter* spp. and *Citrobacter freundii*. These findings are consistent with recent studies conducted in West Africa, which report a growing prevalence of ESBLs in hospital and community settings [31]. Indeed, several studies have highlighted the often inappropriate and unregulated use of antibiotics in the country, both in human and veterinary medicine. Self-medication, over-the-counter sales of antibiotics without medical prescriptions, and the intensive use of antibiotics in livestock particularly for prophylaxis or as growth promoters contribute to the selection and dissemination of antibiotic-resistant bacterial strains in the environment [32] [33]. These practices can lead to cross-contamination of medicinal plants during their cultivation, handling, or marketing in environments exposed to animal or human waste containing multi-drug-resistant bacteria. Integrating these epidemiological dimensions would strengthen the risk analysis for public health and underscore the need for a “One Health” approach to effectively combat antibiotic resistance.

5. Conclusion

This study highlights the urgent need to address the contamination of medicinal plants by antibiotic-resistant Enterobacteriaceae in Côte d’Ivoire. The presence of *Enterobacter hormaechei*, *Escherichia coli*, *Citrobacter freundii*, and *Klebsiella pneumoniae* in these powders poses a major public health risk, particularly for immunocompromised individuals and patients with chronic illnesses. The detection of multidrug-resistant strains, especially those exhibiting high resistance to critically important antibiotics such as carbapenems and fluoroquinolones, is particularly alarming. This situation heightens the risk of antibiotic resistance gene transmission, threatening the effectiveness of antimicrobial treatments and exposing consumers to difficult-to-treat infections. To address this issue, preventive and corrective measures must be urgently implemented. Enhanced microbiological surveillance, combined with strict regulations on the quality of medicinal plants, is essential to limit the spread of resistant bacteria. Furthermore, close collaboration between researchers, public health authorities, and traditional medicine practitioners is crucial to curbing antibiotic resistance and ensuring consumer safety.

Declaration of Competing Interest

None declared.

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Ethical Statement

The study does not involve the use of human or animal subjects.

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Conflicts of Interest

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

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