

Research Article

## Antibiotic Resistance of Enterobacteriaceae in Wastewater Samples from the Mindoube Municipal Landfill, Libreville, Gabon

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### ABSTRACT

The objective of this study was to characterize antibiotic resistant Enterobacteriaceae present in wastewater discharged into the Mindoube Municipal Landfill.

Sampling was conducted on September 29, 2022, with 12 samples collected from the stream point of tanker truck in the landfill to the river. Bacteriological analysis at the IRET Microbiology Laboratory revealed the isolation of one bacterial colony per sample, with identified species including *Escherichia coli* (58.3%), *Serratia Ficara/Pantoea* spp (47.1%), *Klebsiella oxytoca* (33.3%), and *Klebsiella pneumoniae* (25%). Antibiotic susceptibility testing indicated high resistance rates to Ceftazidime and Cefepime (100%), moderate resistance to Cefotaxime (54%), and significant resistance to Amoxicillin and Clavulanic Acid (75%). Resistance to sulphonamides, quinolones, and aminoglycosides was observed at lower levels. These findings suggest that the wastewater from the Mindoube landfill is a significant source of contamination for the nearby stream. Consequently, the proximity of the landfill and the polluted river poses health risks to residents who rely on the river water for daily household activities. The presence of multi-resistant bacteria in the water raises concerns about potential therapeutic failures in treating infectious diseases in individuals exposed to this environment.

Keywords: Mindoube Municipal Landfill; Wastewater; Enterobacteriaceae; Antibiotic Resistance.

### INTRODUCTION

The rapid urban population growth and escalating consumer demands have led to a surge in waste generation per capita, posing a grave threat to the urban environment [1,2]. Public landfills like the MINDOUBE municipal landfill in Libreville

serve as repositories for community waste, creating a melting pot of cultures and a significant public health concern [3]. The MINDOUBE landfill receives diverse waste materials, including solid waste, liquid waste (wastewater), and pollutants from households, hospitals, and businesses,

presenting a tangible risk of human exposure to pathogenic bacteria [4]. Rainwater runoff can carry wastewater into the soil, contaminating groundwater, or contaminate nearby water bodies used by local populations for various activities [4]. Such wastewater often contains an array of pathogenic microorganisms, including viruses, bacteria, and antibiotic-resistant genes, contributing to the escalating threat of death caused by resistant bacteria [5,6]. Therefore, our study aims to characterize antibiotic resistance in Enterobacteriaceae isolated from wastewater originating from the MINDOUBE landfill.

## MATERIALS AND METHODS

Sampling was conducted at the MINDOUBE municipal landfill situated in the MINDOUBE district, within the 5th arrondissement of Libreville. On September 29th, 2022, sampling took place between 7 am and 9 am. Wastewater samples were collected at various points along the wastewater discharge route, starting from the tanker exit and continuing every 30 meters towards the river.

Sterilized plastic PolyEthylene (PET) bottles of 0.5 liters were used to collect the wastewater samples. A total of 12 samples were collected and distributed as follows:

- Sample E0: Collected at the tanker exit.
- Sample E1: First discharge point at the landfill; subsequent samples (E2, E3) were collected every 30 meters.
- Sample E4: Junction point between wastewater from the landfill and the river.
- Samples E5 to E11: Taken 30 meters downstream of E4.

Following collection, the samples were stored at 4°C in isothermal boxes and transported to the IRET microbiology laboratory for analysis.

### Culture, Isolation, and Identification of Bacterial Colonies

Each wastewater sample underwent centrifugation at 270 rpm for 2 minutes at 25°C. The pellet obtained after centrifugation was cultured on Eosin Methylene Blue (EMB) agar medium (Biomérieux, France) and incubated at 37.0±0.5°C for 24 hours. Bacterial colonies displaying distinct morphology and color were isolated on Trypticase soja agar medium (BioMérieux, France). Identification of these colonies was performed using Api 20E galleries (BioMérieux, France) and ApiWeb software.

### Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was conducted using the Kirby

Bauer Mueller-Hinton agar (MH) diffusion disc method. The presence of extended-spectrum  $\beta$ -lactamase was determined using the double disc diffusion method. An inoculum suspension was prepared from a pure culture, and the following antibiotics, recommended by the Clinical Laboratory Standard Institute (CLSI), were tested:

- Amoxicillin + Clavulanic Acid (AMC)
- Aztreonam (ATM)
- Cefepime (FEP)
- Cefotaxime (CTX)
- Ceftazidime (CAZ)
- Ticarcillin + Clavulanic Acid (TTC)
- Imipenem (IMP)
- Tobramycin (TOB)
- Gentamycin (GEN)
- Tetracycline (TET)
- Ciprofloxacin (CIP)
- Nalidixic acid (NAL)
- Trimethoprim + Sulfamethoxazole (SXT)
- Streptomycin (STR)

The diameter of the zone of inhibition around each disc was measured using a Vernier calliper, and the results were interpreted qualitatively as Sensitive (S), Intermediate (I), or Resistant (R).

## RESULTS

### Bacterial Identification

After culture and isolation, between one and three colonies were obtained per sample, totaling 24 colonies from the 12 wastewater samples. All isolated bacteria were identified as belonging to the Enterobacteriaceae family. *Escherichia coli* (58.3%, 7/24) was the predominant bacterium, followed by *Serratia ficaria* and *Pantoea* spp (41.7%, 5/24), *Klebsiella oxytoca* (33.3%, 4/24), and *Klebsiella pneumoniae* (25%, 3/24) (Figure 1).

### Antibiotic Susceptibility Testing

The isolated bacteria displayed notable resistance to beta-lactam antibiotics, particularly third generation cephalosporins (CAZ 100%, 24/24; CTX 62.5%, 15/24) and fourth-generation cephalosporins (FEP 91.7%, 22/24), as well as amoxicillin/clavulanic acid (AMC 70.8%, 17/24).

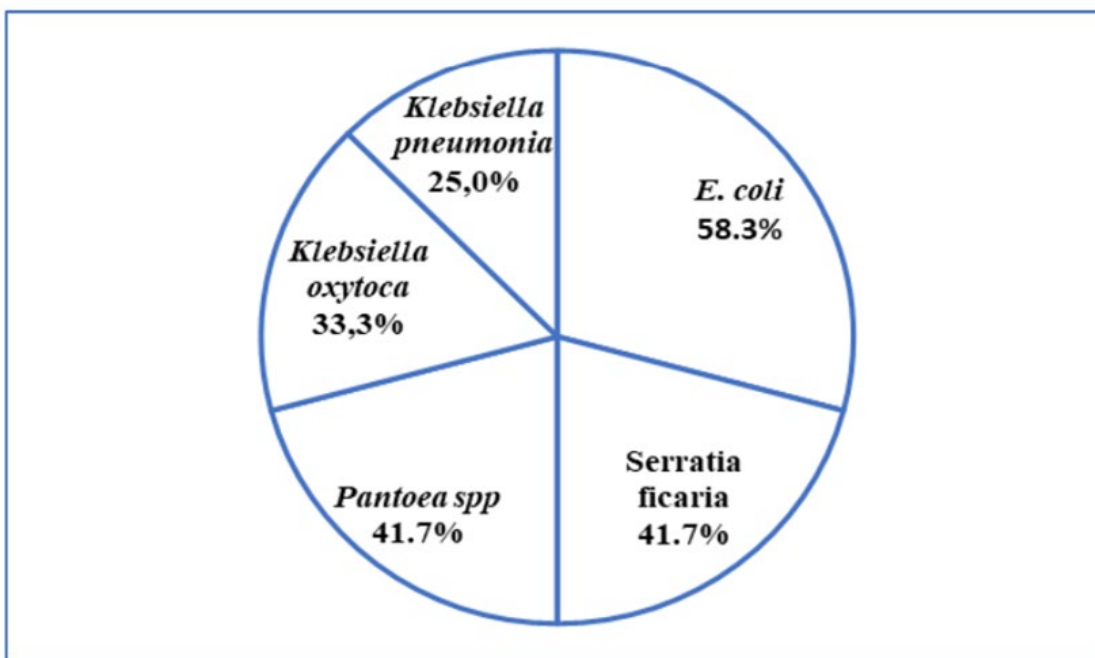


Figure 1: Types de detected bacteria.

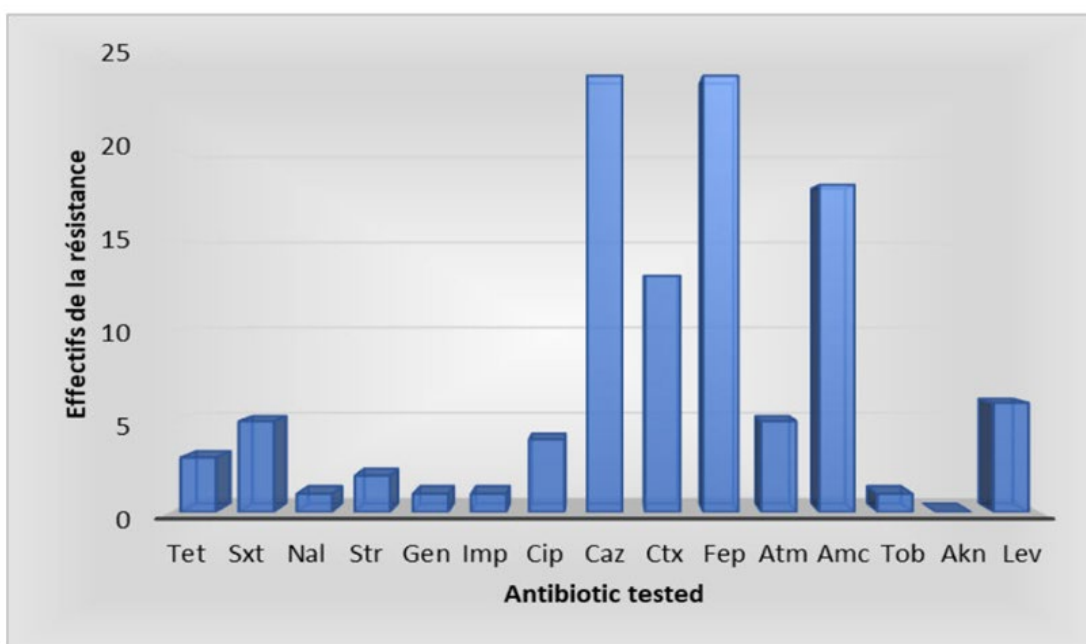


Figure 2: Susceptibility to antibiotics.

However, testing for extended-spectrum  $\beta$ -lactamase (ESBL) was negative. In addition to beta-lactams, resistance was observed against sulphonamides (SXT 20.8%, 5/24), quinolones/fluoroquinolones (LEV 25%, 6/24; CIP 16.7%, 4/24; NAL 4.2%, 1/24), and aminoglycosides (STR 8.3%, 2/24; GEN 4.2%, 1/24; TOB 4.2%, 1/24) (Figure 2).

Each enterobacterium exhibited resistance to at least two antibiotics. Table 1 illustrates that resistance was detected

against antibiotics from one, two, or three different antibiotic families. Notably, multidrug resistance occurred when bacteria demonstrated resistance to at least two antibiotics from distinct families. These findings reveal that 54.17% (13/24) of bacteria exhibited multidrug resistance.

Further analysis revealed that as the distance from the landfill increased (points E0, E1, E2, E3, and E4), multidrug resistance decreased to between two and three antibiotics. At

point E6, where multidrug resistance involved 12 antibiotics, it corresponded to the convergence point of wastewater from the MINDOUBE landfill and neighborhood wastewater discharged into the river.

## DISCUSSION

### Prevalence of Enterobacteria in Wastewater

Our study identified various species of enterobacteria, with *Escherichia coli* being the most prevalent (58.3%). This predominance of *E. coli* is consistent with its ubiquitous presence in the environment, as previous research has highlighted its prevalence in water sources [9]. *E. coli* is commonly associated with diarrheal and urinary tract infections in humans. Other identified bacteria, such as *Pantoea* spp. and *Serratia ficaria*, can also pose health risks, including necrosis, respiratory diseases, and skin infections [10-13]. *Klebsiella* spp., another prevalent species, is known for causing urinary tract infections, pulmonary infections, and bacteremia [14,15]. While these bacteria have been previously detected in wastewater studies, our findings indicate higher prevalence rates compared to some prior research [16,17]. The abundance of Enterobacteriaceae in wastewater underscores the potential health risks associated with exposure to contaminated water sources [18].

### Antibiotic Susceptibility Testing

Our antibiotic susceptibility testing revealed high levels of resistance among the isolated bacteria, particularly against beta-lactam antibiotics such as third and fourth-generation cephalosporins (C3G and C4G) and penicillins, as well as fluoroquinolones and tetracyclines. These antibiotics are commonly used to treat various infections, including gastrointestinal, respiratory, and urinary tract infections [19,20,21,22]. The observed resistance to these antibiotics suggests a concerning trend that could complicate the management of infectious diseases in clinical settings. Similar findings have been reported in other studies, indicating a widespread issue of antibiotic resistance in environmental bacteria [24]. The multi-drug resistance observed in over half of the isolated bacteria further exacerbates the challenge of combating bacterial infections [25,26].

### Environmental Implications

Our results suggest that the MINDOUBE landfill may serve as a significant source of environmental contamination with antibiotic-resistant bacteria. Wastewater from the landfill, along with hospital effluents and other sources, could contribute to the dissemination of antibiotic resistance genes in the environment, potentially impacting public health

[27,28]. The observed decrease in bacterial resistance further away from the discharge point underscores the landfill's role in environmental pollution and highlights the importance of effective waste management practices [29]. The emergence of multi-drug-resistant bacteria poses a serious threat to public health, leading to more complex and persistent infections with potentially higher mortality rates [11,31]. Addressing antibiotic resistance in environmental bacteria requires comprehensive strategies to mitigate pollution and promote responsible antibiotic use to safeguard human health. Ultimately, wastewater from the MINDOUBE landfill represents a potential reservoir of antibiotic-resistant strains in the Gabonese capital and its surrounding areas.

## CONCLUSION

The findings of this study underscore the significant health risks associated with wastewater from the MINDOUBE landfill in Libreville, Gabon. This wastewater, originating from various sources including hospitals, households, and industries, contains multiresistant bacteria that pose a serious threat to public health. The presence of these bacteria in the downstream river indicates widespread pollution and highlights the urgent need for effective waste management practices in the area.

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