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# Potential Risk for Spread Multidrug Resistant *Enterobacteriaceae* through *Lactuca sativa* (Lettuce) and *Allium fistulosum* L. (Welsh onion) from Infulene Valley, Maputo City, Mozambique

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**Abstract** Vegetables can be exposed to pathogenic microorganisms during production, transportation, handling and processing, constituting a health risk for the consumer. The aim of this study was to determine the risk for spread of antibiotic resistant *Enterobacteriaceae* through *Lactuca sativa* and *Allium fistulosum* L. from Infulene valley, Maputo city, Mozambique. Between September and October 2014, a total of 24 samples (12 *L. sativa* and 12 *A. fistulosum* L.) were collected from the production field of Infulene valley. The vegetables were washed thrice with sterile distilled water and the third washing product was inoculated into enrichment media to increase the chance of bacteria isolation on solid media. MacConkey plates were observed to select all suggestive colonies, and oxidase negative organisms were considered for further identification. An average of approximately three organisms were isolated from each sample, and identified using API 20E (Biomerieux, France). All isolates were tested to 14 antibiotics using Kirby-Bauer disc-diffusion method, and strains resistant to three or more antibiotics were classified as multidrug resistant (MDR). Gram negative bacteria were detected in all samples. In a total of 68 Gram negative bacteria, 57/68 (83.5%) were *Enterobacteriaceae*, being the most frequent members were *Klebsiella oxytoca* (21.1%), *Proteus vulgaris* (19.3%) and *Enterobacter cloacae* (12.3%). We observed high rates of resistance to Amoxicillin-calvulanate (98.2%) along with MDR profile (35.1%). This study indicates the potential risk for spread antibiotic resistant bacteria through *L. sativa* and *A. fistolosum* L. There is a need for take actions in both producers and consumers sides to prevent spread of pathogenic bacteria and reduce risk for diseases.

***Keywords:*** *vegetables, risk of infection, antibiotic resistance, Enterobacteriaceae*

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## 1. Introduction

During the production, transportation, handling and processing vegetables can be exposed and contaminated with pathogenic microorganisms, mainly bacteria [1,2]. Among the factors that contribute to the microbiological contamination of vegetables the use of polluted water discharges from human and veterinary medicine for irrigation is the most relevant [3].

The Infulene Valley serves as a source of agricultural products, mainly vegetables for various markets of the Maputo and Matola cities in Mozambique [4]. To our knowledge at the local the irrigation is based on surface water. Surface water from urban regions, mainly green zones of big cities like Maputo, presents with chemical, physical and biological contaminants [5,6].

It is known and recognized that the dissemination of bacteria is not exclusively related to medical facilities, but involves food and environment [7]. On the other hand food can be important source of pathogenic bacteria [8] through cross-contamination or consumption [9].

The consumption of fresh vegetables contaminated with pathogenic bacteria can put the consumer at risk for diseases, especially due to *Enterobacteriaceae* members [3]. These diseases are difficult to manage and treat when associated with antibiotic resistance bacteria. It is known that antibiotic resistance is common among *Enterobacteriaceae* members and can be rapidly spread by plasmids [10]. However, there is a limited description of the extent of antibiotic resistance among bacteria isolated from vegetables in Mozambique.

This study aims to describe the risk for spread diseases associated with antibiotic resistant bacteria through *Lactuca* sativa and *Allium fistulosum* L. produced at Infulene valley in Maputo city, Mozambique.

# 2. Material and Methods

## 2.1. Vegetables Sampling

Between September and October 2014, 24 samples (12 *L. sativa* and 12 *A. fistulosum* L. were obtained from different points of Infulene valley. All samples were collected between 6 am and 8 am, packed in sterile bags (2 – 7°C) and transported to the Microbiology Laboratory (Faculty of Medicine University Eduardo Mondlane) for isolation and identification of bacteria.

## 2.2. Preparation of the Inoculum

About 300g of *L. sativa* and *A. fistulosum* L. (whole plant) were washed three times by stirring with about 1000 mL of distilled water in sterile plastic, to remove sand and other impurities. The water in the third wash was placed in a 15 mL sterile tube, labelled with the specimen name.

## 2.3. Isolation of Bacteria

1.0 mL of the third pre-homogenized washing was pipetted into 5.0 mL of enrichment media, alkaline peptone water (APW), brain heart infusion (BHI) broth and selenite broth (SB), followed by incubation 37 °C for 6 hours.

After 6 hours subculture was performed as follows: 0.1 mL from APW on Thiosulfate citrate bile salts sucrose agar (TCBS); 0.1 mL from BHI on Blood agar; and 0.1 mL from SB on MacConkey agar and XLD. All plates were incubated at 35 ± 2 °C for 24 hours. MacConkey plates were observed to select all suggestive colonies (morphology and lactose fermentation reaction), and oxidase negative organisms were considered for further identification. An average of approximately three organisms were obtained from each sample and identified using API 20E (Biomerieux, France).

## 2.4. Antibiotic Sensitivity Test

All identified isolates were tested for 14 antibiotics [Ampicillin 10µg, Amoxicillin-calvulanate 30 µg, Ceftriaxone 30µg, Ceftazidime 30µg, Piperacicllin-Tazobactam 10µg, Ertapenem 10µg, Imipenem 10µg, Meropenem 10µg, Nalidixic acid 30 µg, Ciprofloxacin 5 µg, Gentamicin 10 µg, Chloramphenicol 30 µg, Tetracycline 30 µg, Co-trimoxazole 25 µg], using Kirby-Bauer disc-diffusion method to determine the resistance patterns according to Clinical and Laboratory Standard Institute [11]. Organisms resistant to three or more antibiotics were defined as MDR in this study.

# 3. Results

A total of 68 Gram negative bacteria were isolated from both *L. sativa* and *A. fistulosum* L. The majority 57/68 (83.8%) of the isolates belongs to *Enterobacteriaceae* family, being *Klebsiella oxytoca* (21.1%), *Proteus vulgaris* (19.3%) and *Enterobacter cloacae* (12.3%) the most frequent within this group as shown in Figure 1. We did not observe significant difference in regard of type and number of bacteria isolated by vegetable species.

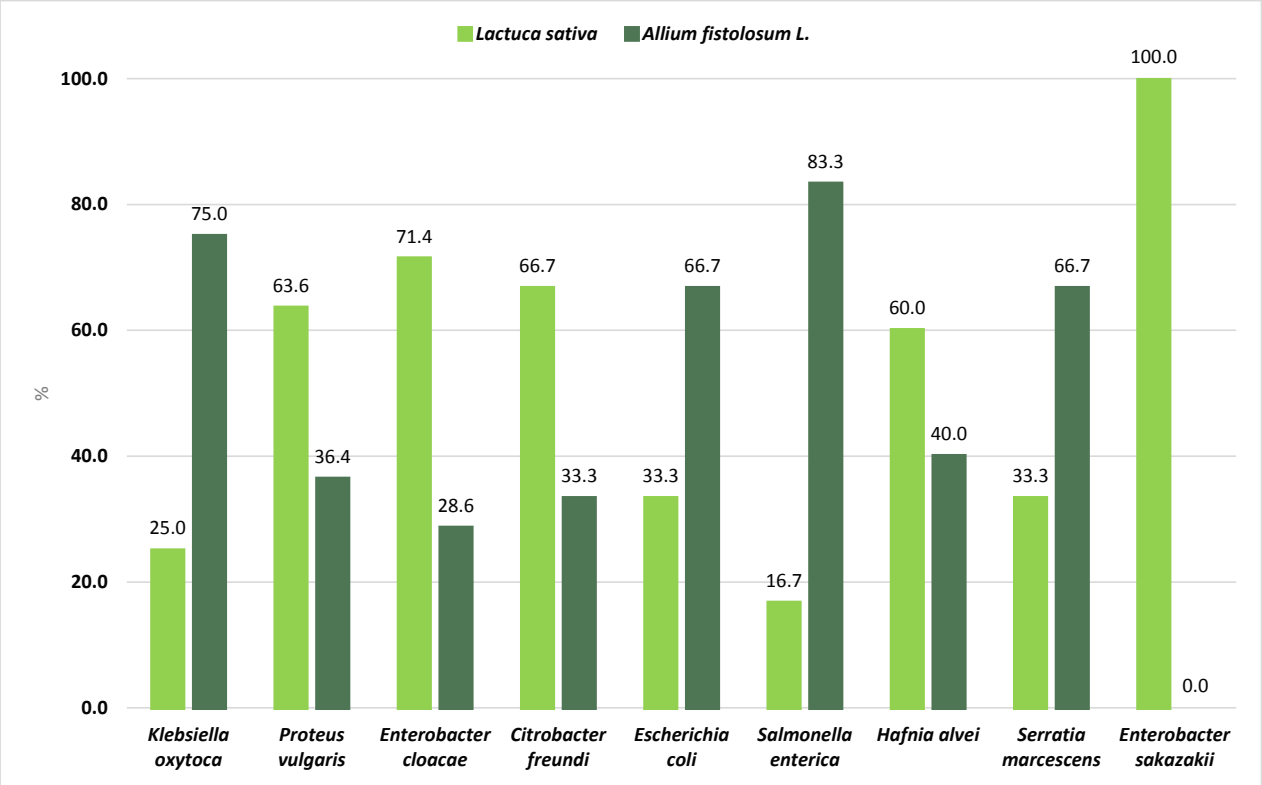


**Figure 1.** *Enterobacteriaceae* isolated from *Lactuca sativa* and *Allium fistulosum L.* cultivated in Infulene valley, Maputo, Mozambique

The majority of *Enterobacteriaceae* isolates showed resistance to two antibiotics, and 35.1% presented with multidrug resistant profile. Table 1 summarizes the antibiotic resistance profile of *Enterobacteriaceae* isolates.

**Table 1. Antibiotic resistance profile among *Enterobacteriaceae* isolates**

| Organism | Antibiotic resistance profile | | |
|---|---|---|---|
| | Resistant to one (%) | Resistant to two (%) | Resistant to three or more (%)* |
| ***Klebsiella oxytoca* (n=12)** | 4 (33.3) | 6 (50.0) | 2 (16.7) |
| ***Proteus vulgaris* (n=11)** | 4 (36.4) | 5 (45.5) | 2 (18.2) |
| ***Enterobacter cloacae* (n=7)** | 2 (28.6) | 3 (42.8) | 2 (28.6) |
| ***Citrobacter freundi* (n=6)** | 1 (16.7) | 0 (0.0) | 5 (83.3) |
| ***Escherichia coli* (n=6)** | 1 (16.7) | 1 (16.7) | 4 (66.6) |
| ***Salmonella enterica* (n=6)** | 1 (16.7) | 3 (50.0) | 2 (33.3) |
| ***Hafnia alvei* (n=5)** | 1 (20.0) | 3 (60.0) | 1 (20.0) |
| ***Serratia marcescens* (n=3)** | 0 (0.0) | 1 (33.3) | 2 (66.7) |
| ***Enterobacter sakazakii* (n=1)** | 0 (0.0) | 1 (100.0) | 0 (0.0) |
| **Total** | **14 (24.5)** | **23 (40.4)** | **20 (35.1)** |

* Multidrug resistant (excluding Ampicillin resistant *K. oxytoca*, *P. vulgaris* and *E. cloacae* due to intrinsic resistance)

The isolates showed high rates of resistance for Amoxicillin-calvulanate (98.2%). Another important finding was that related with MDR profile and uncommon susceptibility pattern for carbapenems. Table 2 presents the percentage of resistance for each isolate.

**Table 2. Percentage of resistance for each isolate**

| % of resistance | *Klebsiella oxytoca* (n=12) | *Proteus vulgaris* (n=11) | *Enterbacter cloacae* (n=7) | *Escherichia coli* (n=6) | *Salmonella enterica* (n=6) | *Citrobacter freundi* (n=5) | *Hafnia alvei* (n=5) | *Serratia marcescens* (n=3) | *Enterobacter sakazakii* (n=1) |
|---|---|---|---|---|---|---|---|---|---|
| **Amoxicillin-clavulanate** | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 83.3 | 100.0 | 100.0 | 100.0 |
| **Ampicillin** | 91.7 | 90.9 | 71.4 | 83.3 | 66.7 | 83.3 | 80.0 | 100.0 | 0.0 |
| **Piperacillin-Tazobactam** | 8.3 | 0.0 | 0.0 | 0.0 | 0.0 | 16.7 | 0.0 | 33.3 | 0.0 |
| **Ceftriaxone** | 8.3 | 0.0 | 14.3 | 0.0 | 0.0 | 16.7 | 0.0 | 0.0 | 0.0 |
| **Ceftazidime** | 0.0 | 0.0 | 14.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Meropenem** | 50.0 | 45.5 | 71.4 | 16.7 | 33.3 | 66.7 | 20.0 | 0.0 | 0.0 |
| **Ertapenem** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 16.7 | 0.0 | 0.0 | 100.0 |
| **Imipenem** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Ciprofloxacin** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Nalidixic acid** | 8.3 | 0.0 | 14.3 | 0.0 | 0.0 | 16.7 | 0.0 | 0.0 | 0.0 |
| **Gentamicin** | 8.3 | 0.0 | 14.3 | 16.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Tetracycline** | 8.3 | 0.0 | 0.0 | 16.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Chloramphenicol** | 8.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Co-trimoxazole** | 0.0 | 0.0 | 0.0 | 33.3 | 16.7 | 0.0 | 0.0 | 33.3 | 0.0 |

## 4. Discussion

This study indicates the potential risk for spread of diseases associated with multidrug resistant *Enterobacteriaceae* through *L. sativa* and *A. fistolosum L.* Therefore awareness of the presence of antibiotic resistant bacteria in vegetables has to be made to reduce the risk of diseases.

We isolated most frequently *Proteus vulgaris*, *Klebsiella oxytoca* and *Enterobacter cloacae* in vegetables from Infulene valley. Possible explanations for the occurrence of these bacteria in vegetables include the fertilization practices, use of untreated water from wells or lagoon for irrigation [12]. In Infulene valley fertilization consists of manure based on organic matter and animal faeces, along with use of surface water from a stream for irrigation. This therefore suggests faecal contamination of vegetables.

Most of *Enterobacteriaceae* members are opportunistic bacteria emerging as nosocomial pathogen, and can be associated with different diseases. *Klebsiella oxytoca* has been implicated with bacteraemia due to chronic alcoholism [13]. *Enterobacter cloacae* has also clinical significance and is frequently isolated from intensive care units [14]. Due to its ability to cause different diseases *Proteus vulgaris* can be isolated from various clinical specimens urine, blood, wounds, pus, etc. [15]. Other members of *Enterobacteriaceae* isolated in this study also have clinical significance [14]. These findings show that *L. sativa* and *A. fistolosum L.* produced at Infulene valley represents a risk for moderate to severe diseases among the consumers and the producers.

Our results show that Amoxicillin-clavulanate and Ampicillin are less effective against *Enterobacteriaceae* isolated from lettuce and welsh onion at Infulene valley. It was also detected strains with multidrug resistant (MDR) profile. Together with the clinical features discussed above, the antibiotic resistance are responsible for the

therapy failures, representing costs for both patients and health systems.

The occurrence of antibiotic resistant bacteria, including MDR bacteria in the environment can be explained by its survival and adaptation to a wide range of niches, as well as capability to exchange both virulence and resistance traits [16]. On the other hand it is described that medical, veterinary and community wastes contaminate the environment with both bacteria and drugs [17]. To our knowledge the Infulene valley is influenced by sewage, medical and veterinary wastes, and open defecation. This profile is consistent with the presence of several microorganisms and the possibility of exchange of virulence and resistance traits. Further analysis have to be done to understand the ecology and epidemiology of MDR bacteria circulation in Infulene valley.

We also observed uncommon susceptibility pattern to carbapenems, mainly for *Klebsiella oxytoca*, *Proteus vulgaris*, *Enterobacter cloacae*, *Citrobacter freundi and Salmonella enterica*. These strains showed resistance to Meropenem and sensitivity to Imipenem. Similar results were found by Shigemoto *et al.* [18] in *Klebsiella pneumoniae* and attributed this trait to the double production of metallo-β-lactamase and the extended-spectrum-β-lactamase. We strongly believe that this is an emergent trait in Maputo city and that it has been exchanged between *Enterobacteriaceae* members. Molecular experiments have to be performed to better characterize these bacteria.

With this study there is a clear evidence that immediate strategies need to be established to control environmental contamination and spread of antibiotic resistant *Enterobacteriaceae* through vegetables. The use of clean water for irrigation, and well wash ready-to-eat vegetables can reduce the risk of spread the bacteria. On the other hand it is important to control wastes from medicine, veterinary and community.

## 5. Conclusion

This study indicates the potential risk for spread multidrug resistant *Enterobacteriaceae* through *L. sativa* and *A. fistulosum L.* from Infulene valley. Therefore there is a need for take actions to avoid environmental contamination with bacteria and drugs and reduce the risk for diseases.

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