

**Antimicrobial susceptibility patterns of pathogenic bacteria in cooked, raw meat, and vegetables from select markets of Entebbe municipality in Wakiso district, Uganda.  
A cross-sectional study.**

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**Abstract**

**Background:**

Consuming food contaminated with microorganisms may lead to a significant increase in morbidity and mortality rates. The aim of the study is to determine the antibacterial susceptibility patterns of the pathogenic bacteria isolated from the cooked meat, raw meat, and raw vegetables purchased from select markets.

**Methodology:**

A descriptive cross-sectional study was carried out in common food markets of Entebbe municipality, that is, Katabi, Abayita, Kawuku, Kasatiro, and Namulanda, between the months of November 2021 and January 2022. Susceptibility patterns were analysed using susceptible, resistant, and intermediate proportions of isolates per antibiotic used.

**Results:**

*E. coli*, *Salmonella*, and *Serratia* showed 100% resistance to all the antibiotics it was tested. *Enterococcus* was relatively resistant to all the antibiotics, though the highest resistance was observed with gentamycin (CN) (100%). However, the highest susceptibility was exhibited with Ampicillin (AM) (50.0%) as compared to Ciproflaxin (CIP) (20.0%) and linezolid (LZ-D) (20.0%), and inconclusive patterns (ATU) were seen with Ciproflaxin (CIP), Amoxicillin (AM), and linezolid (LZ-D). For *Neisseria*, the highest resistance patterns were displayed with Ampicillin (AM), while being highly susceptible to CRO. *Moraxella* isolates showed 100% resistance to amoxicillin-clavulanic acid (AMC), tetracycline (TE), and Trimethoprim/sulfamethoxazole (SXT), and showed susceptible patterns with only erythromycin (33.3%) and the only inconclusive percentage.

**Conclusion :**

AM (Ampicillin) and Cefoxitine (CRO) are still effective antibiotics in enterococcal and *Neisseria* infections. All the isolates were resistant to at least three of the antibiotics used

**Recommendations:**

Mass sensitization of the community regarding proper handling of meat, chicken, and vegetables may be adapted to minimize cross-contamination.

**Keywords:** Antimicrobial Susceptibility, Patterns of pathogenic bacteria, Cooked, Raw Meat, Vegetables.

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**Background**

Consuming food contaminated with microorganisms may lead to a significant increase in morbidity and mortality rates, which has negative socioeconomic and public health implications (Madoroba, 2021). When it comes to health, quite a number of deaths and illnesses have been reported around the world. WHO reports that approximately 1 in 10 people in the world fall ill and 420,000 die every year after eating contaminated food (Chileshe *et al* 2018). Unfortunately, very limited research has been done to draw attention to microbial hazards in food consumed, especially

in Uganda, as a Food and safety surveillance programme isn't as stringent as in European and American countries. WHO previously estimated that 31 food-borne diseases resulted in 600 million illnesses and 420,000 deaths worldwide (Madoroba, 2021). To add fuel to the flame, farming practices like the use of antibiotics in animal production have also raised concerns about transmission of multiple antimicrobial-resistant bacteria via the food chain (Mensah *et al* 2012).

Pathogenic bacteria are the causative agents of two-thirds of human food-borne diseases worldwide, with a high burden

in developing countries (Abebe, 2020). More than 200 different diseases may be transmitted through food contaminated with pathogenic bacteria. They are globally important because of their high incidence and the cost they impose on society. Their looms a potential threat of large outbreaks of foodborne diseases due to pathogenic bacteria in both developing and developed countries (Abakari, Cobbina, & Yeleliere, 2018). Despite all of this, the consumption of raw vegetables with undercooked meat has increasingly become common, and because of the weakness of bacteriological/diagnostic resources in most sub-Saharan African countries associated with the unawareness and non-enforcement of laws, the estimation of food-borne diseases, as well as resulting losses, is under-evaluated (Hippolyte *et al* 2020). The aim of the study is to determine the antibacterial susceptibility patterns of the pathogenic bacteria isolated from the cooked meat, raw meat, and raw vegetables purchased from select markets.

## Methodology

### Research Design

This was a descriptive cross-sectional study that was carried out in common food markets of Entebbe municipality, that is, Katabi, Abayita, Kawuku, Kasatiro, and Namulanda, between the months of November 2021 and January 2022.

### Study Area

The study was conducted in a few selected raw meat, vegetables (cabbage), and cooked (roasted) vending stalls of Katabi, Abayita, Kawuku, Kisubi, and Namulanda. It will be from such stalls that the respective meat and vegetable samples will be collected (purchased).

### Study setting

#### Katabi

Katabi Central market is in Wakiso district, Entebbe municipality, near the National Water and Sewage Corporation treatment plant. The final results of the 2002 population census put Entebbe Municipality at a total of 55,086 people, of whom 27,135 are males and 27,951 females (United Nations Human Settlements Program, 2003), and as of 2021-2022, the population of the city of Entebbe, Uganda, is 67,271 people (Allpopulations.com, 2022). The increase in population over the years suggests a corresponding increase in the number of people visiting the market. Katabi Central Market operates once a week, every Saturday.

#### Abayita

Abayita Ababiri is located in Kyaddondo South Constituency, Wakiso District, Central Uganda. Abayita Ababiri's main market is a local communal marketplace. The market is situated between Kasenyi Road and

Abayita Ababiri bus stop along Entebbe express highway, opposite Abayita Ababiri Police Station (Google Maps, 2022). As of 2014 and to date, the population is unknown (Wikiwand, 2021), but the marketplace is busiest once a week, every Friday.

#### Kawuku

Kawuku market is in Kawuku, Wakiso District (Central Region), Uganda, about 12 miles (or 20 km) south of Kampala, the country's capital city. It is situated along Kampala-Entebbe Road, about 3.8km from the main road next to Mt. Zion Church, Kawuku (Google Maps, 2022). The Kawuku communal market operates seven days a week but attracts bigger numbers on Saturdays.

#### Kisubi

Kisubi local market, indigenously known as "Kasatiro," is located in Kisubi, the Central region in Uganda, along Kampala-Entebbe Road. It is located just after the University of Kisubi, formerly known as Kisubi Brothers University College, coming from Kampala.

Kasatiro market operates seven days a week, attracting numbers from other businesses and travelers around.

#### Namulanda

Namulanda is a village in Central Uganda, and it is situated east of Buzzi and south of Kasali. Namulanda market, next to Kibungo Fishing village, just like Kisubi and Kawuku, operates on a daily basis but attracts bigger numbers on Thursdays. It is a local communal market with a variety of goods.

### Study Materials.

Autoclaves and hot air oven were used for the sterilisation of all materials used during analysis, e.g., sample collection containers and bags, tubes, loops, plates, etc

Sterile normal saline was prepared in sterile bottles in which the cut sample pieces were immersed on reception.

Selective media, i.e., MSA, XLD, MAC, and BEA agar, were prepared for primary culture growth.

Nutrient agar base for sub-culturing from the peptone culture broth, rabbit plasma (coagulase test), TSI, Simmons' citrate agar, oxidase strips, and hydrogen peroxide. SIM media was prepared for all the necessary biochemical tests.

MHA, antibiotic discs, and AST international guideline booklet.

Microcentrifuge tubes, microcentrifuge, TENT buffer solution, cold ethanol, and cryo-vials for the extraction and banking of genomic DNA of the isolates.

Electrophoresis unit for the visualization of extracted DNA.

Spectrophotometer for purity testing and determination of the DNA concentration.

### Sampling Strategy

Different samples from randomly selected vending stalls totaling n=50 were collected in the sterilised sample collection bags. The sample size was then calculated using Fisher's method (Charan *et al* 2013).

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where; n= Sample size  
Z= level of confidence P= Expected prevalence d= precision  
P was determined from a pilot study with a smaller sample from the population to determine the Prevalence of contaminated food (beef, chicken, and vegetables) from all the 5 sampling areas.

### Determination of n

Z= 0.95 (CI), P= 0.8333, d= 0.05

$$n = \frac{0.95^2(0.8333)(1-0.8333)}{0.05^2}$$

n=50.140

A sample space of 50 samples was handled.

### Isolation, Identification, Antibiotic Susceptibility Testing (AST), and extraction of DNA of identified Bacterial Pathogens from the collected food samples.

These were aggregated into 4 stages, i.e., sample collection, preparation, culture, sensitivity, and extraction.

### Collection of Samples from Sampling Sites.

- Sample collection bags were sterilised using an autoclave prior to sample collection.
- The sample bags were triple packaged in more sterile bags, ensuring minimal or no contamination of the samples.
- On sample collection, proper disinfection of hands was done to minimize contamination of the samples.
- The sample was then placed in a disinfected cool box with ice packs and immediately transported to the laboratory for examination.

### Preparation of collected food samples.

- In the laboratory, the samples were cut into small pieces and immersed in sterile normal saline in sterile containers.
- Covering the containers with loosely tight lids to prevent contamination, these were incubated in a shaking incubator overnight (24hrs) at 37°C.

### Antimicrobial Susceptibility Testing of isolates obtained.

- The samples were then inoculated on selective media the following day to get primary cultures.

The primary cultures were sub-cultured on NA recovering colonies on which biochemical tests were done to confirm the different isolates.

Using Kirby-Bauer's disc diffusion method, AST with selected antibiotic classes was done on all the identified bacteria (Cheesbrough, 2005).

The isolates were stored in 70%BHI and 30% glycerol for future reference.

### Extraction and banking of DNA from the isolates.

The boiling method was used to release the bacterial DNA into the surroundings of the TENT buffer. The buffer had been prepared prior to extraction the previous day (Hassanzadeh *et al.*, 2016).

The extracted DNA was visualised by electrophoresis and the purity determined by the spectrophotometric method (Optic density) (Hassanzadeh *et al.*, 2016).

The purest DNA was stored at -20°C for future use.

### Data Analysis

Ms-Word and Ms-Excel were used to organize the collected data into tables. The prevalence of all the isolated bacterial pathogens by nature of food (cooked and raw), area (Katabi, Kasatiro, Abayita, Kawuku, and Namulanda) was determined. This data was then presented in bar graphs. Susceptibility patterns were also analysed using susceptible, resistant, and intermediate proportions of isolates per antibiotic used.

### QA and QC Pre-analysis

Sterilisation of all equipment, including sample bottles, collection bags, normal saline, and plates, was done *e.t.c* to prevent bacterial contamination of the samples.

Samples were immediately transported to the laboratory to maintain the viability of the pathogens while also minimising contamination.

Proper labeling of the samples with specific identification codes of collection site, nature of sample (raw or cooked), and sample type (beef, chicken, or vegetables) was done.

### Analysis

The samples were immediately prepared on arrival at the laboratory, minimizing contamination and maintaining viability of possible pathogens.

Careful sample verification procedures were followed.

While analysing the samples, all microbial aseptic techniques were meticulously observed.

- Frequently used equipment, like the incubators, was maintained on a daily, weekly, and monthly basis, e.g., careful temperature monitoring using the daily temperature maintenance logs.
- Adherence to procedural SOPs.

Results were recorded in both hard and soft copies for proper data management.

• Reports were written for proper documentation of result discussions.

Page | 4 **Post-analysis**

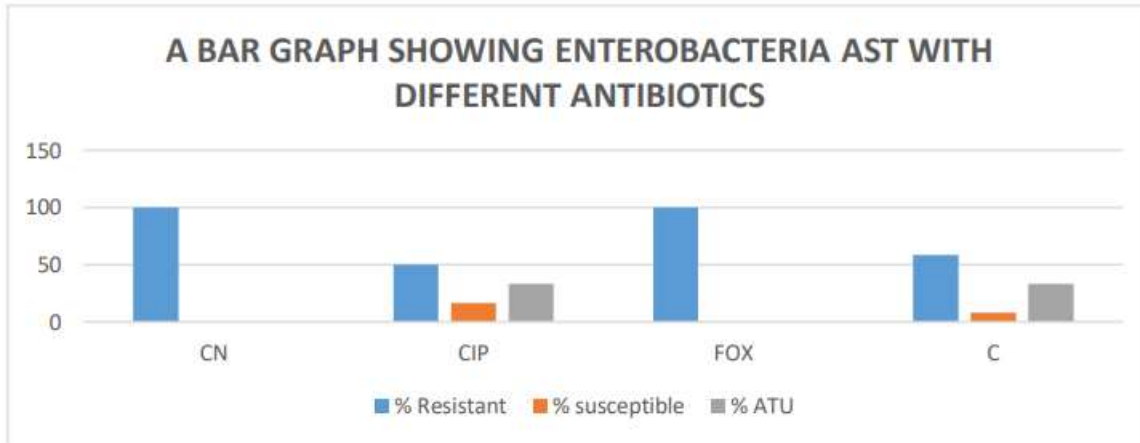
- Inconclusive or unclear results were troubleshoot prior to recording.
- Test results were double-verified prior to recording.

**RESULTS**

**Enterobacteria susceptibility patterns**

Generally, the enterobacteria were showing significant resistance patterns to all the antibiotics used. With Gentamicin (CN) and cefoxitin (FOX), they showed 100% resistance.

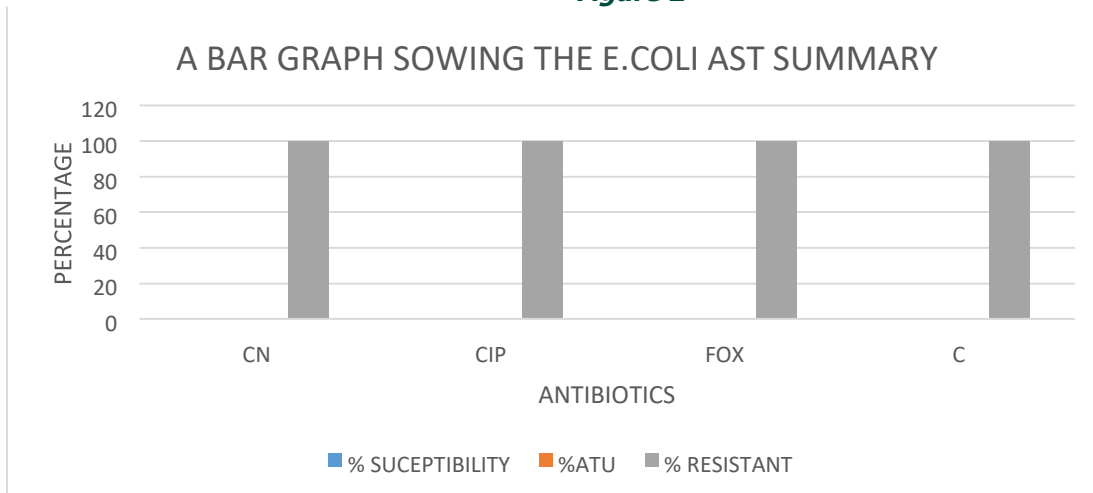
**Figure 1**



***E. coli.***

Figure 2, *E. coli* showed 100% resistance to all the antibiotics it was tested with.

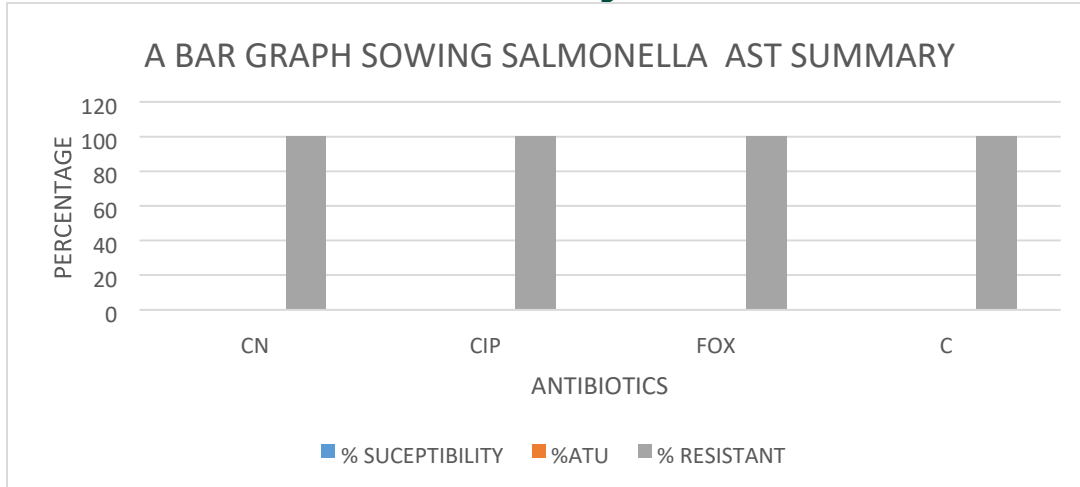
**Figure 2**



**Salmonella**

Figure 3, *Salmonella* showed 100% resistance to all the antibiotics it was tested with.

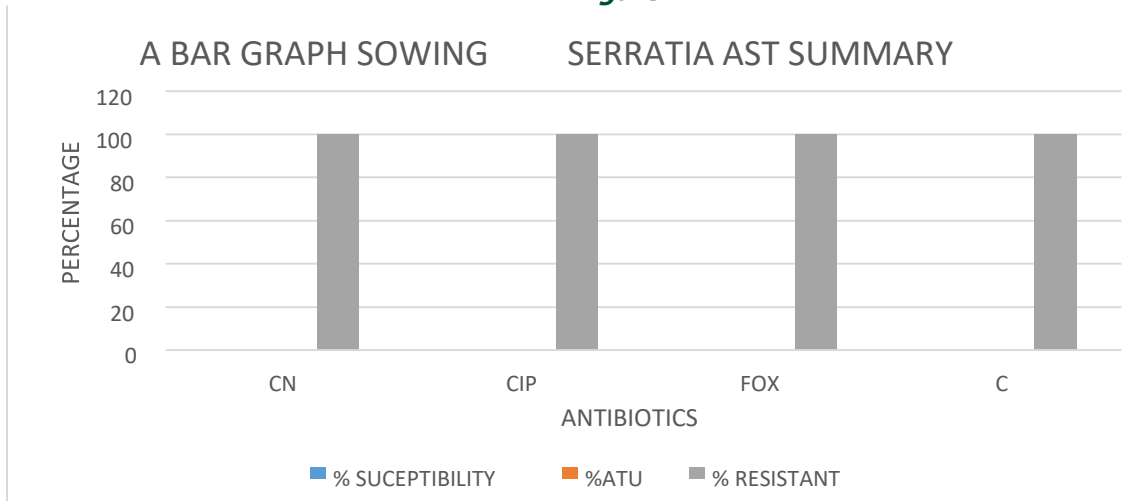
**Figure 3**



**Serratia**

Figure 4, *Serratia* was 100% resistant to all the antibiotics it was tested with.

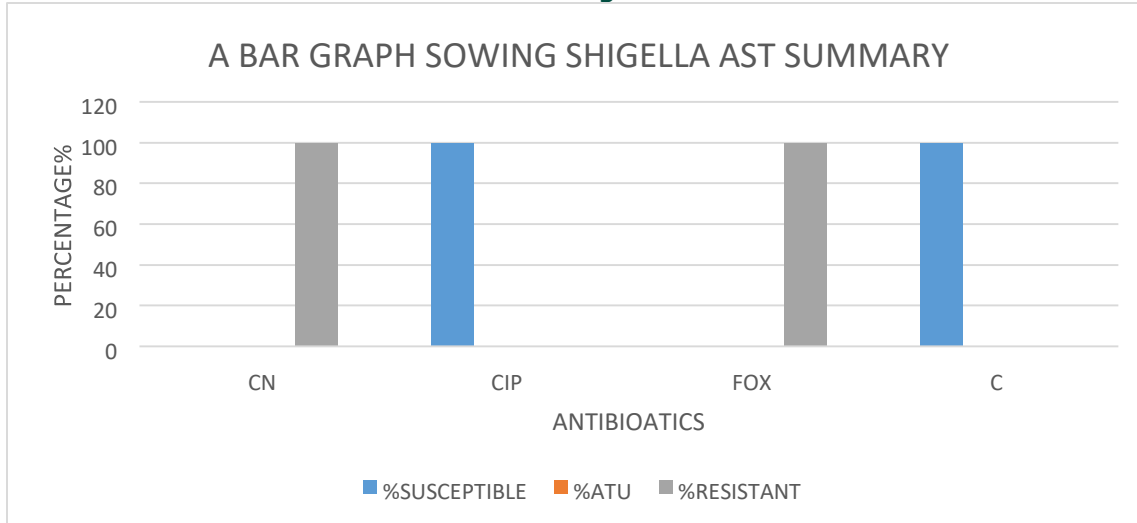
**Figure 4**



**Shigella.**

Figure 5, Unlike other *E. coli* and *Salmonella*, *Shigella* showed 100% resistance only to gentamicin (CN) and cefoxitin (FOX) while being 100% susceptible to ciproflaxin (CIP) and chloramphenicol (C).

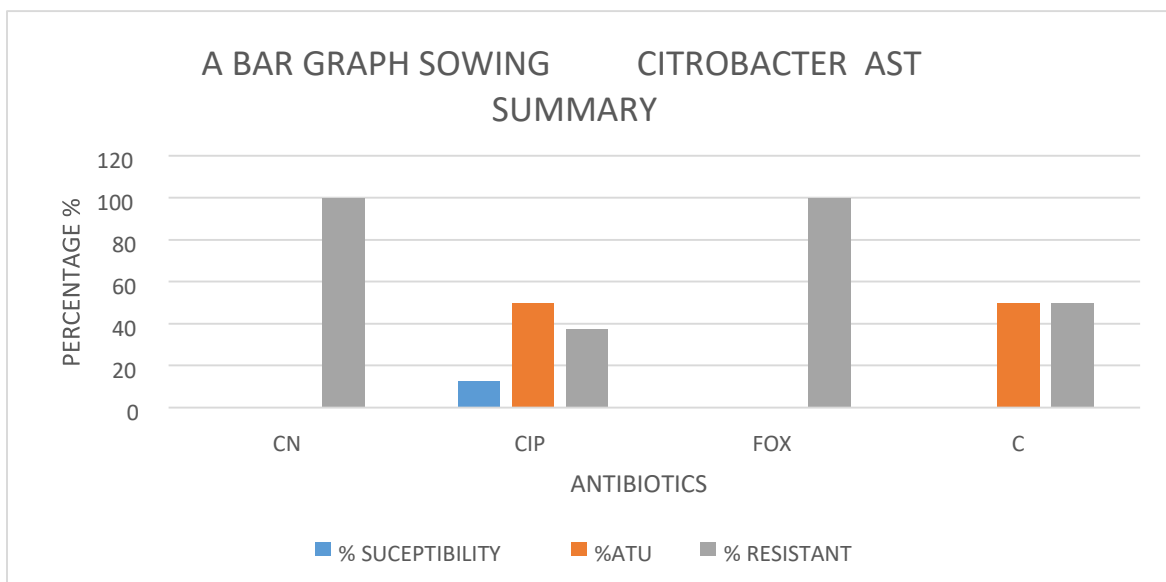
**Figure 5**



**Citrobacter**

Figure 6, *Citrobacter* isolates exhibited resistant patterns to all the antibiotics, but greatly with gentamycin (CN) and cefoxitin (FOX), 100%. In addition, it displayed inconclusive results (Area of technical uncertainty-ATU) with ciproflaxin (CIP) and chloramphenicol(C). However, *Citrobacter* showed susceptibility to only CIP.

**Figure 6**



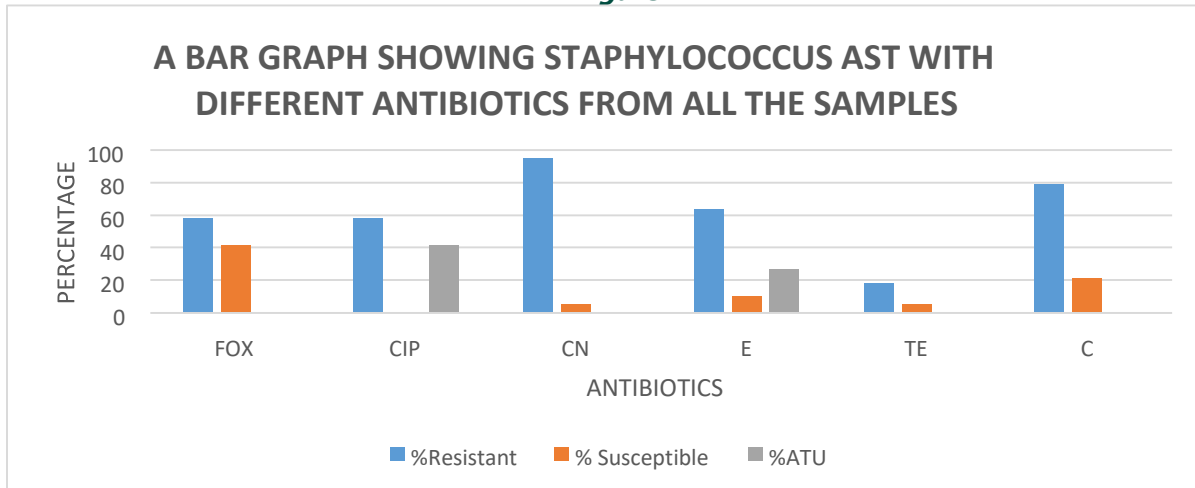
**Staphylococcus aureus.**

Figure 7, *Staphylococcus aureus* isolates showed different patterns with the antibiotics. It was resistant to all the

antibiotics to a greater extent in all, with the highest resistance patterns displayed with Gentamycin (CN) (94.7%), and the least with Tetracycline (TE) (18.0%).

There were some relatively susceptible patterns displayed the highest with Cefoxitin (FOX) (57.9%) and the least with gentamycin (CN) (5.3%) and Tetracycline (TE) (5.3%).

**Figure 7**

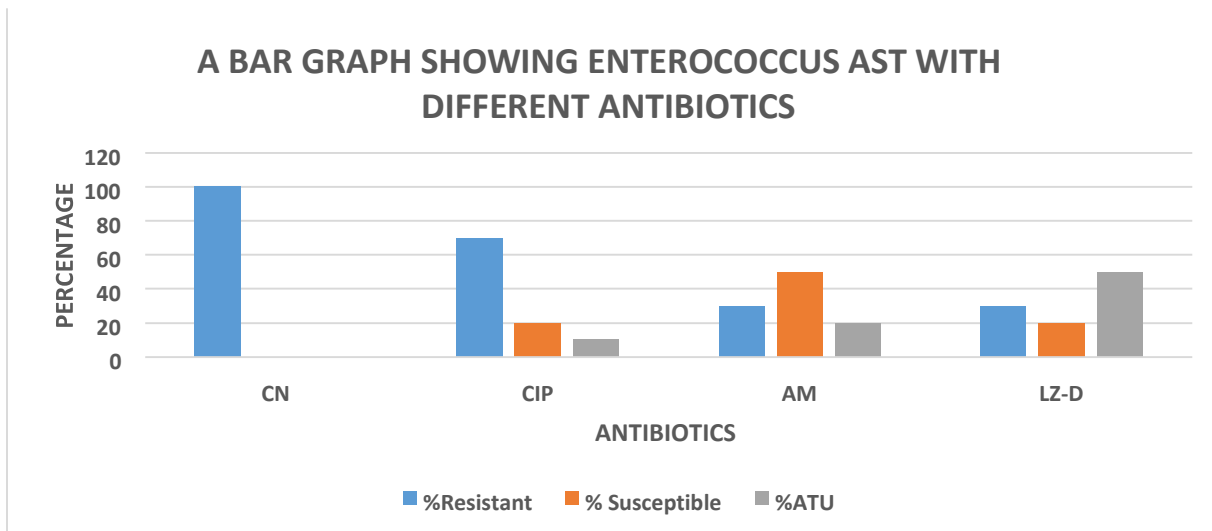


**Enterococcus**

Figure 8, *Enterococcus* was relatively resistant to all the antibiotics, though the highest resistance was observed with gentamycin (CN) (100%). However, the highest

susceptibility was exhibited with Ampicillin (AM) (50.0%) as compared to Ciproflaxin (CIP) (20.0%) and linezolid (LZ-D) (20.0%), and inconclusive patterns (ATU) were seen with Ciproflaxin (CIP), Amoxillin (AM), and linezolid (LZ-D).

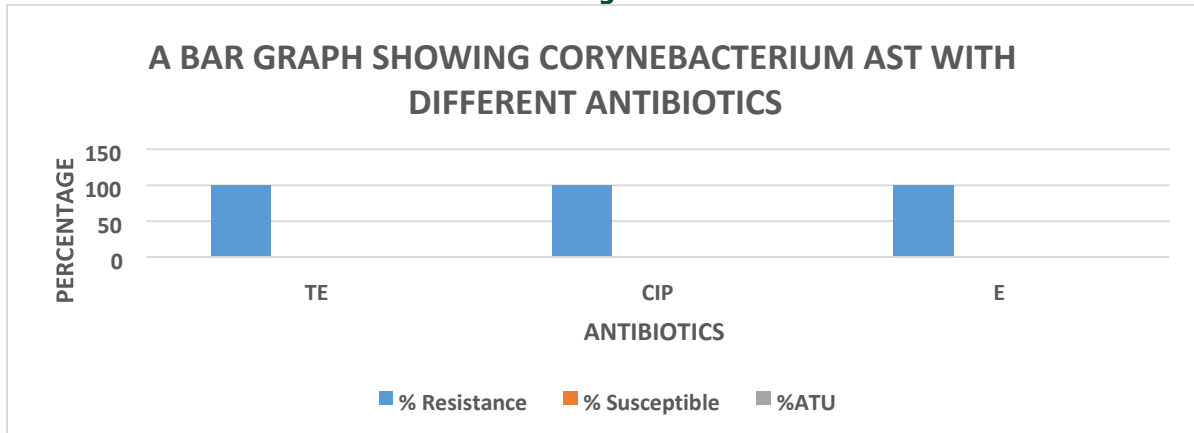
**Figure 8**



**Corynebacterium**

Figure 9: The isolate was resistant to all the antibiotics to which it was exposed.

**Figure 9**



**Neisseria**

Figure 10, Highest resistance patterns were displayed with Ampicillin (AM), while being highly susceptible to CRO.

**Figure 10**

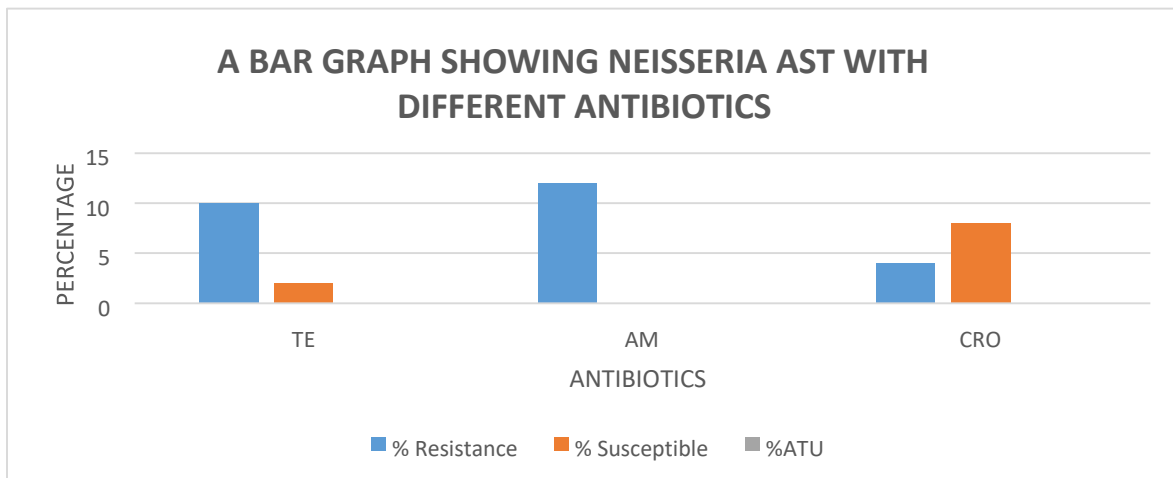
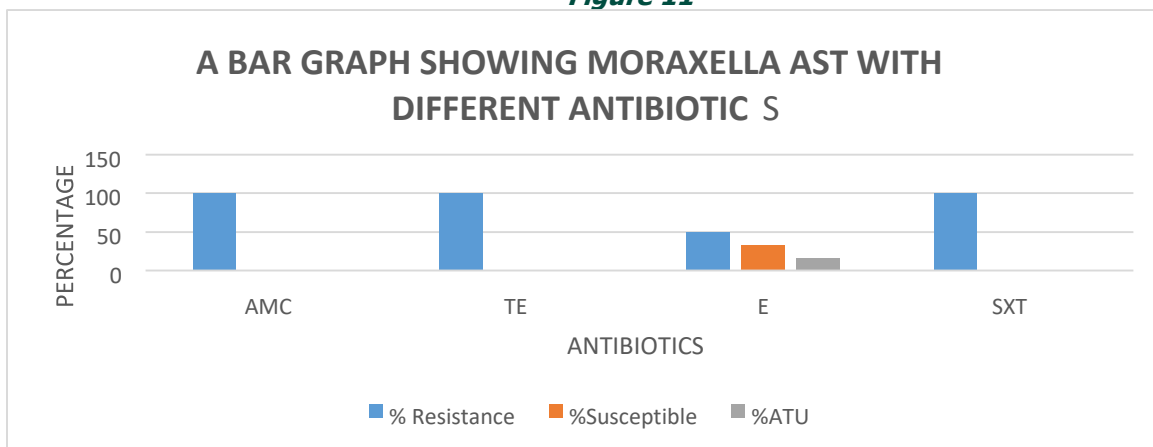


Figure 11, *Moraxella* isolates showed 100% resistance with amoxicillin-clavulanic acid (AMC), tetracycline (TE), and Trimethoprim/sulfamethoxazole (SXT), and showed susceptible patterns with only erythromycin (33.3%) and the only inconclusive percentage.

**Figure 11**



## DISCUSSION

### Antimicrobial Susceptibility patterns of isolates in cooked, raw (chicken and meat), and vegetables (cabbage) from select markets of Wakiso district, Uganda (Namulanda, Kawuku, Kisubi, Abayita, and Katabi)

AST patterns of all isolates tested with gentamicin showed the highest resistance to the antibiotic, like Rajae et al (2021) findings. Almost all the antibiotics used showed more resistance patterns than susceptibility patterns.

*Staphylococcus aureus*, one of the most prevalent isolates, showed resistance to all the antibiotics it was tested with. The fact that *Staphylococcus aureus* was resistant to all the antibiotics used suggests a possibility of an MDR status of the isolate.

All enterobacteria and *corynebacterium spp* were resistant to all the drugs they were tested with, except *Citrobacter*, which showed some susceptibility with only one of the four drugs, even though it was still lower than the resistance. These results were still in line with Rajae's findings in 2021 since he reported that all isolates were at least 60-100% resistant to the antibiotics used.

*Enterococcus'* resistance was higher with almost all the antibiotics used, except for AM, which showed 50% susceptibility and 30% resistance.

*Neisseria* and *Moraxella spp* AST also showed resistance to all the drugs used except for CRO, with *neisseria* that showed a higher susceptibility than resistance. These results, as mentioned above, are contrary to most studies, as vegetables have not been associated with both pathogens in the past.

## Conclusion

AM (Ampicillin) and Cefoxitine (CRO) are still effective antibiotics in enterococcal and *Neisseria* infections, implying that they can be used in the management of an FBDs caused by *enterococcus spp* and *neisseria*, respectively. All the isolates were resistant to at least three of the antibiotics used. This threatens therapy and management of FBDs in the long run as a result of infection from any of these pathogens.

## Recommendations

Much emphasis and encouragement may be directed towards the government establishing a stringent regional microbiological hazard surveillance system (and later

national) to assess the safety of food staff, especially the street foods, prior to selling.

Mass sensitization of the community regarding proper handling of meat, chicken, and vegetables may be adapted to minimize cross-contamination.

Arafat Sooma, are students from the faculty of health sciences at the University of Kisubi.

Fortunate Lujjimbirwa is a lecturer at the faculty of health sciences at the University of Kisubi.

## Page | 10 **Study limitations**

The main limitation of the study was the lack of molecular characterisation techniques for identifying the pathogenic bacteria to a strain level, a procedure that requires a qualitative PCR and specific primers.

Some important antibiotics under certain drug classes were also not available, which narrowed AST to fewer drugs/antibiotics, hence making it difficult to determine multi-drug resistance.

## **Acknowledgement**

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## **List of abbreviations**

AST: Antimicrobial Susceptibility Testing  
BEA: Bile Esculin Agar  
DNA: Deoxyribonucleic Acid  
MAC: MacConkey  
MHA : Muller Hinton Agar  
MSA : Mannitol Salt Agar  
Ms-Word Excel: Microsoft-Word Excel  
NA: Nutrient Agar  
*P. aeruginosa* : Pseudomonas aeruginosa  
QA: Quality Assurance  
QC: Quality Control  
QTY: Quantity  
*S. aureus*: Staphylococcus aureus  
SIM: Sulphur Indole Motility  
SOPs: Standard Operating Procedures  
TSI: Triple Sugar Iron  
WHO: World Health Organisation  
XLD: Xylose Lysine Deoxycholate

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The study was not funded.

## **Conflict of interest**

The author did not declare any conflict of interest.

## **Author Biography**

Joel Philip Mwesigwa, Patricia Namubiru, Bruno Magobamaingi, Patricia Nagingo, Adrine Tusuubira, Abdallah Rutenta, Vicent Joseph Kasule, Hilda Tushabe,

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