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PREVALENCE, ANTIBIOTIC RESISTANCE, AND RISK FACTORS OF BACTERIAL PATHOGENS IN "ROLEX FOOD" AT MAKERERE UNIVERSITY: A CROSS-SECTIONAL STUDY.

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ABSTRACT

Background
This was a baseline study aimed at determining bacterial load in Rolex food sold in selected points of Kikumi kikumi in MUK, establishing the antibiograms of *E. coli*, *Staphylococcus aureus*, and *Salmonella spp*, screening for mecA and Tet M genes in the isolates that code for resistance to penicillin and tetracycline, respectively.

Methods and tools
A total of 50 samples were collected, of which 25 of them had raw salads while the remaining had their salads cooked in omelettes. Isolation and identification were done, and antibacterial susceptibility was done using a disc diffusion test.

Results
The results showed that 11.50% of 50 samples collected were positive with common bacterial contaminants, and in these positive samples, E. *coli* and CNS were the most prevalent with 30.43% and 26.09%, respectively followed by *Klebsiella spp* and *Pseudomonas Spp* with a same prevalence of 15.22%, Bacillus spp 6.52%, Streptococcus Spp 4.35% and then *Staphylococcus aureus* with the lowest prevalence of 2.17%, while *Salmonella Spp* were not found completely in the samples. The susceptibility tests on E. coli showed that CXC had the highest resistance of 93.75%, followed by SXT and GM with resistance of 43.73% and 37.50%, respectively.

Conclusion
This study revealed that *Rolex* food, particularly when containing raw salads, harbors a significant bacterial load, with *E. coli* and coagulase-negative staphylococci (CNS) being the most prevalent contaminants. *E. coli* isolates exhibited high resistance to cefotaxime (93.75%), trimethoprim-sulfamethoxazole (43.73%), and gentamicin (37.50%), raising concerns about potential treatment challenges.

Recommendations
People should buy cooked Rolex food as opposed to raw one because of its low prevalence of bacterial contaminants. The Rolex makers should clean their clothes, acquire and put on aprons all the time while operating, and above all, wash their hands with clean water every time they serve customers.

Keywords: *Drug susceptibility, Isolates, Antibiotic resistance, Bacterial pathogens, Food poisoning, Street food, Uganda*
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INTRODUCTION

Globally, food is the single most important commodity in urban consumers' list of goods and services utilized, accounting for about 55% of the total household

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expenditure (Dawson and Cent 1991). Worldwide street vended food poses a big public health challenge caused by many agents, including bacterial pathogens. Bacterial pathogens such as *E. coli, Salmonella species, and Staphylococcus aureus* are a source of many foodborne illnesses, which present as vomiting, diarrhea, fever, and in worst cases, can be life-threatening if such conditions persist without immediate treatment. The transmission of human diseases through food, water, and wastewater is a global problem where gastrointestinal diseases are among the most important causes of morbidity and mortality (WHO, 1994). Studies in the developing world have shown that microbial contamination is the potential cause of serious food poisoning and the use of unpermitted additives with the presence of other adulterants (WHO, 1992). *Escherichia coli,* one of the coli forms, causes gastroenteritis, which is characterized by diarrhoea. Diarrhea is the most common cause of mortality in children in Asia and sub-Saharan Africa with inadequate health systems and limited access to clean food and drinking water (Ekici et al.,2019) The Ministry of Health of Uganda on 24th /2/ 2015 notified the World Health Organization of the outbreak of typhoid fever in Kampala city and it spread to all divisions and the neighboring districts. The most affected age groups were young males aged between 20 and 39 years (Kabwama et al., 2017). It was confirmed that contaminated food, juices, and poor hygiene standards were among the predisposing factors of the disease. Rolex food is an example of street-sold food sold along roads of many peri-urban areas across the entire country and more so around universities such as Makerere University because of its delicacy, cheapness in teams of cost, and less preparation time. Much as many people consume this food delicacy, there has been limited research done to establish the level of its safety, potential pathogens associated with it, and hygienic standards followed while preparing it, and this poses a great threat to the consumers of this food delicacy. "Rolex" is an easy-to-access, cheap food comprising a chapatti, containing fried chicken eggs, onions, raw tomatoes, and cabbage; some people can add sausages or even minced meat. Its delicacy and the fact that it takes the least time during preparation make "Rolex" be sold along the roadside of peri-urban areas of Kampala and other areas of the country. This food suits many Makerere students based on the characteristics associated with it, and many students buy it for their lunch and supper frequently. "Rolex" is so popular that visitors coming to Uganda often are surprised to see signposts advertising "Rolex" and wondering how the expensive Swiss Rolex watch can be sold in roadside stands! Just to get surprised that it is a Ugandan "Rolex" with the constituents already mentioned above. Rolex food got international attention as American CNN read the news, terming "Rolex" as the favorite fast food of Ugandans. It was recently launched in Uganda as a culinary tourist attraction and the first ever Kampala "Rolex" festival, which was held in August 2016 at the Uganda Museum and now occurs every year. According to Mackay (2019), the strategy of eating street food, including Rolex food, was more common among young, single males, and the reasons were their convenience, accessibility, and not wanting to cook.

This food is prepared by the roadside at a stall that is crafted from simple materials like eucalyptus poles, plywood, and a charcoal stove with a metallic stand about 3 feet tall. The stall is exposed to dust from the traffic moving along the road adjacent to the stall. The individuals preparing the food do not use gloves when handling the food, and no hair net; however, some do invest in an apron. Furthermore, the handling and preparations of tomatoes, cabbages, onions, and other materials used to make this food delicacy are not certain, there is no water tap or water source seen near the stall where these vegetables can be washed before they are cut, and served, to make their safety status an uncertainty. Rolex food is being widely consumed by many people and yet there is limited documented information about environmental hygienic standards where the Rolex makers are operating from, potentially pathogenic microorganisms such *as Listeria, Salmonella, E. coli, Pseudomonas, Proteus*, and *Klebsiella* which Rolex food may harbor, and they are threats to human life. There is an unknown prevalence of these pathogens and their susceptibility patterns, and there is limited knowledge of the risk factors associated with Rolex contamination. This poses a public health risk concern to the consumers, and therefore, the intended study aims to determine the common contaminants of Rolex food, their susceptibility to antibiotics, and the possible risk factors associated with the contamination of this delicacy.

Objectives of the study

- To determine the bacteriological prevalence in "Rolex" food at selected road site points of Makerere University
- To determine the prevalence of common bacterial contaminants of "Rolex" food
- To establish antibiograms of Staphylococcus aureus, E. coli, and Salmonella species identified.
- To screen for the Mec A and Tet M genes in the isolates that code for resistance to Penicillin and tetracycline resistance respectively.
- To identify the risk factors that predispose to common bacterial contaminants of "Rolex"

Research questions

- What is the prevalence of common bacterial contaminants in "Rolex" food sold?
- What are the antibiograms of Staphylococcus aureus, E. coli, and Salmonella species identified in the roadside sold "Rolex" food?

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- Are Mec A and Tet M present in the isolated resistant bacteria to penicillin and tetracycline?
- What are the risk factors that predispose to common bacterial contaminants of "Rolex" food sold?

METHODS AND MATERIALS

Study area and design

The research samples were obtained in the area around Makerere University, that is Kikumi Kikumi, which is 0.33930 N, 32.56980 E, Muganzi Awongererwa Road, Kawempe division, one of the divisions of Kampala district. Kampala is the capital city of Uganda with a population of 1,353,189 (Geonames, 2016) and borders Lake Victoria and Wakiso district. Kawempe division is in the Northwest corner of the city, bordering.
The research was conducted at CDL, COVAB in Makerere University.
The study was a baseline cross-sectional study involving the assessment of the bacteriological load and antimicrobial susceptibility of organisms isolated from the Rolex food samples collected.

Sampling and transportation

Sampling was conveniently done, and stalls were purposely selected depending on Rolex attendants working every day. Rolex samples were collected twice every week on Monday and Wednesday from the selected consistent stalls between 10:00 am to 12:00 pm for 1 month. 5 sampling points will be selected and named A, B, C, D, and E. The samples were purchased from vendors and then wrapped in sterile polyethylene bags, labeled with a code assigned to each point, level of salad processing whether raw or cooked, and date of collection, and then transported to the Central Diagnostic Laboratory (CDL) at the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University and worked upon immediately within 24 hours of collection.

Bacteriological Laboratory Analysis of the samples

The bacteriological examination was done according to the Abrahamsen et al. (2014)

Preparation of Buffered Peptone Water

Buffered Peptone water is composed of 10g/L Peptone, 3.5g/L Sodium chloride, 3.5g/L Disodium phosphate, and 1.5g/L Potassium dihydrogen phosphate (Laboratorios Conda, South Africa).
Exactly 15 grams of the medium was suspended in one litre of distilled water. The culture medium was mixed well, and each 9mls was suspended in capped test tubes. Then, the test tubes were sterilized by autoclaving at 121°C (International PBI Italy) for 15 minutes and cooled to 25°C before use.

Preparation of MacConkey Agar

50 grams of the medium were suspended in one liter of distilled water, mixed well, and boiled for one minute for complete dissolution. The mixture was then sterilized by autoclaving at 121°C for 15 minutes, then cooled to (45-50) °C, mixed well, and dispensed onto plates. The plates were allowed to solidify and were placed upside down to avoid excessive moisture in the medium.

Preparation of Blood Agar

Blood Agar: 15g blood agar base, 380 ml distilled water, and 20 sheep blood. Initially, 15 grams of the agar base was suspended in 380 mL of distilled water and mixed thoroughly, then sterilized by autoclaving at 121°C for 15 minutes. The sterile medium was placed in a water bath set at 50°C for one hour and after cooling, then 20mls of defibrinated sheep blood was added using a sterilized plastic syringe to a final concentration of 5%., mixed well and poured into sterilized plastic plates using aliquots of 20ml for each (100x15) mm plate. The plates will be stored inverted in a refrigerator at 6°C until needed for use. Just before use, the surface of the 5% Sheep blood agar plate is dried for 30 minutes in an incubator.

Preparation of Plate Count Agar. (PCA)

Twenty-three point five (23.5) grams of the medium were suspended in one liter of distilled water, boiled for one minute or agitated to mix very well, autoclaved at 121°C for 15 minutes to sterilize, and cooled to 50°C.
Preparation of Mueller-Hinton Agar (MHA)
Initially, 38 grams of the medium will be dissolved in one liter of distilled water, stirred to complete dissolution, then autoclaved at 121°C for 15 minutes. The medium will be cooled at 50°C in a water bath, and a volume of 25- 30 mL will be poured onto sterile plates (100x15) mm.

Culturing Procedures and Isolation

All samples were cultured and sub-cultured on Blood Agar and MacConkey at 37°C for 18 hours. Isolated colonies will be aseptically picked from mixed cultures and streaked onto the new sterile media to obtain pure cultures after inoculation. (Cheesbrough, 2009)

Biochemical tests

The bacterial colonies were subjected to biochemical tests, and these are Indole, Methyl Red, motility, Citrate

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utilization, coagulase test, triple sugar iron test, gram stain, and Catalase test reaction.

Indole test

Four (4) MLS of Tryptophan broth were added to a sterilized tube, then an inoculum was added and incubated at 37°C for 24 hours. After 0.5 mL of Kavoc's reagent was added and the color change was noted. The red coloration of the surface layer indicates a positive Indole test (Cheesbrough, 2008).

Methyl Red test

Each of the isolates was inoculated in 5mls sterile Glucose Phosphate broth and incubated at 37°C overnight. One drop of Methyl Red solution was added and observed. Bright Red colour formation will be considered positive (Cheesbrough, 2008).

Citrate utilization

The slopes of the Simmons citrate agar will be prepared in tubes. Isolate culture will be streaked first and then stubbed in the butt using a straight wire. Then the tubes will be inoculated at 35°C for 48 hours. A change in color of the indicator from Light green to Blue due to the alkaline reaction following citrate utilization indicates a positive test.

Motility test

As isolate was suspended in a small drop of Peptone water placed on the slide and covered with a cover slip. It will then be observed on the microscope under X10 and X40 for motility (Carter et al., 1995)

Catalase test

A slide method was used where a drop of 3% Hydrogen peroxide was put on a glass slide and mixed with an isolated colony of the test culture. Bubble formation caused by gas production will indicate a positive reaction.

Coagulase test

A slide method was done where a drop of blood plasma was put onto a glass slide and then followed by the isolated colony, and then mixed. White solid small particles suspended in the plasma drop are indicative of a positive test.

Antimicrobial susceptibility test

Following the National Committee for Clinical Laboratory Studies (NCCLS, 2001) Standardized test of Bauer et al., 1966. The isolates were recovered from Peptone water medium, then streaked onto MacConkey agar and incubated at 37°C for 24 hours. Subculturing was done by swabbing onto Mueller-Hinton Agar using a sterile swab. The swab was streaked over the surface of the medium 3 times, rotating the plate at an angle of 60 degrees on each application. Using sterile forceps, antibiotic discs containing Gentamicin, Tetracycline, Ampicillin, Trimethoprim, Nalidixic acid, Sulphamethoxazole, Streptomycin, and Clavulanic were placed on the inoculated plate at an equal distance from each other. The plates were incubated for 24 hours at 37°C while inverted so that the agar faces upwards.
The diameter of zones of inhibition was recorded using a ruler, recorded in mm, and was characterized as Susceptible(S), Intermediate (I), and Resistant(R) according to the Standard interpretive chart of the Bioanalyse® limited (Table 1)

Table 1: Different drugs with their corresponding sensitivities based on the zone of clearance

Drug initials	*Resistant (R) (mm)*	*Intermediate (I) (mm)*	*Susceptible(S) (mm)*
SXT (Trimethoprim sulfamethoxazole)	≤ 10	11-15	≥ *16*
CXM (Cefuroxime sodium)	≤ 14	15-17	≥ *18*
TE (Tetracycline)	≤ 14	15-18	≥ *19*
AMC (Amoxicillin with clavulanic)	≤ 13	14-17	≥ *18*
NA (Nalidixic acid)	≤ 13	14-18	≥ *19*
CN (Gentamicin)	≤ 12	13-14	≥ *15*
N (Neomycin)	≤ 12	13-16	≥ *17*
AK (Amikacin)	≤ 14	15-16	≥ *17*
CRO (Ceftriaxone)	≤ 13	14-20	≥ *21*
AM (Ampicillin)	≤ 13	14-16	≥ *17*
S (Streptomycin)	≤ 11	12-14	≥ *17*
C (Chloramphenicol)	≤ 12	13-17	≥ *18*

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CIP (Ciprofloxacin)	≤ 15	16-20	≥ *21*
OFX (Enrofloxacin)	≤ 16	17- 19	≥ *20*
XNL(Ceftiofur)	≤ *17*	*18-20*	≥ *21*

Molecular characterization

DNA extraction

The suspected methicillin-resistant Staphylococcus isolates were processed to obtain DNA. 3 colonies were picked from the agar plate and emulsified in 100µl of sterile deionized water. This was followed by boiling in a water bath at 970C °C for 5-10 minutes, centrifugation of the cell lysate at 15000 x g for 5-15 minutes, and finally removing the supernatant that contained DNA, which was re-suspended in nuclease-free water (Moore, Arnscheidt, & Mau, 2004).

PCR for detection of the mecA gene

A crude DNA template was attained from a 1 ml culture after centrifugation of the latter using a tabletop eppendorf® centrifuge 5424R at 15000 rpm in a 1.5 ml Eppendorf tube for 10 minutes. The supernatant was decanted, the pellet resuspended in 100µl of distilled water, and boiled in a water bath for 10 minutes. The PCR reaction conditions to be used are; 4 µl of the crude DNA as template in 25 µl reaction volume, 15mls of a 2X DreamTaq Green PCR Master Mix (which includes DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM MgCl2- Thermoscientific®), 0.75µl forward primer (5`-TCCAGGAATGCAGAAAGACCAAAGC -3`) and 0.75µl reverse primer (5`-GACACGATAGCCATCTTCATGTTGG -3`), all primers were a 10 picomole/µl concentration. This reaction was carried out in a GeneAmp® PCR system 9700 (Applied Biosciences) thermal cycler at 35 cycles, each consisting of 30 seconds at 94°C, 30 seconds at 45°C, and 1 minute at 72°C besides the initial denaturation step of 4 minutes at 94°C and the final extension at 72°C for 5 minutes. The expected band for this PCR is a 499-bp fragment detected after agarose gel electrophoresis. A crude DNA template was attained from a 1 ml culture after centrifugation of the latter using a tabletop eppendorf® centrifuge 5424R at 15000 rpm in a 1.5 ml Eppendorf tube for 10 minutes. The supernatant was decanted, the pellet resuspended in 100µl of distilled water, and boiled in a water bath for 10 minutes. The PCR reaction conditions used were; 4 µl of the crude DNA as template in 25 µl reaction volume, 15mls of a 2X DreamTaq Green PCR Master Mix (which included DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM MgCl2- Thermoscientific®), 0.75µl forward primer (5`-TCCAGGAATGCAGAAAGACCAAAGC -3`) and 0.75µl reverse primer (5`-GACACGATAGCCATCTTCATGTTGG -3`), all primers were a 10 picomole/µl concentration. This reaction was carried out in a GeneAmp® PCR system 9700 (Applied Biosciences) thermal cycler at 35 cycles, each consisting of 30 seconds at 94°C, 30 seconds at 45°C, and 1 minute at 72°C besides the initial denaturation step of 4 minutes at 94°C and the final extension at 72°C for 5 minutes. The expected band for this PCR is a 499-bp fragment detected after agarose gel electrophoresis.

Tetracycline resistance gene PCR

A crude DNA template was attained from a 1 ml culture after centrifugation of the latter using a tabletop eppendorf® centrifuge 5424R at 15000 rpm in a 1.5 ml Eppendorf tube for 10 minutes. The supernatant was decanted, the pellet resuspended in 100µl of distilled water, and boiled in a water bath for 10 minutes. The PCR reaction conditions used were; 4 µl of the crude DNA as template in 25 µl reaction volume, 15mls of a 2X DreamTaq Green PCR Master Mix (which included DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM MgCl2- Thermoscientific®), 0.75µl forward primer (5`-GTGGAGTACTACATTTACGAG-3`) and 0.75µl reverse primer (5`-GAAGCGGATCACTATCTGAG-3`), all primers were a 10 picomole/µl concentration. This reaction was carried out in a GeneAmp® PCR system 9700 (Applied Biosciences) thermal cycler at 35 cycles, each consisting of 30 seconds at 94°C, 30 seconds at 45°C, and 1 minute at 72°C besides the initial denaturation step of 4 minutes at 94°C and the final extension at 72°C for 5 minutes. The expected band for this PCR is a 309-bp fragment detected after agarose gel electrophoresis.

Data Analysis

The data obtained from the Laboratory for Rolex analysis was entered into a Microsoft Excel Sheet and then SPSS for further statistical analysis. Means were compared and analyzed using Independent samples and One-way ANOVA (Analysis of Variance) with Post Hoc Tests and Turkey's HSD test for homogeneous subsets. Data was analyzed using STATA to generate descriptive statistics and exported to Microsoft Word.

Quality control and assurance

Rolex samples were collected in clean polyethylene bags, transported to CDL, and worked upon immediately within 24 hours of arrival. All the standard operating procedures of CDL were followed, and the experiment was carried out along with experienced personnel. All the instruments and media were sterilized before their

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usage to avoid contamination, which may lead to incorrect results.

Ethical approval statement

Ethical approval was granted by Makerere University College of Veterinary Medicine, Animal Resources, and Biosecurity Research Ethics Committee. Informed consent from vendors and all study protocols that processed and stored food samples were reviewed and approved to be ethical for food safety research that involves human subjects. No information was gathered from the vendors and data privacy was well observed. In its execution, all procedures complied with the policies of Makerere University and other regulations regarding research on food-borne pathogens and public health.

RESULTS

Isolation and identification of common bacterial contaminants of Rolex food

Macroscopic examination of cultures on MacConkey agar showed bright pink with or without white precipitate in the agar growth plate, indicating lactose fermenting colonies, and the colorless colonies indicate non-lactose fermenters. Colonies that showed such characteristic features were subjected to biochemical tests: methyl red, indole, urease, citrate utilization, hydrogen sulfide gas production, and motility tests. 14 isolates were presumptively identified as *Escherichia coli* based on morphological characteristics, and both indole and methyl red were positive, while citrate and motility tests were negative. 7 isolates were presumptively identified as *Klebsiella species*. Of all the samples, no single isolate of *Salmonella species* was identified. Coagulase-negative *Staphylococcus*, *Staphylococcus aureus*, *Bacilli* species, *Streptococcus* species, and *Pseudomonas* Species were all identified based on morphological characteristics and biochemical tests.

The overall prevalence of bacterial load found in Rolex food

From Table 2, the overall prevalence of bacterial load in the Rolex sample was 11.50% with 95% CI 8.71-15.03

Table 2: Overall prevalence of common bacterial contaminants of Rolex food

variable	n (%)	95% CI
Prevalence of bacterial pathogens		
Negative	354(88.50)	84.96-91.28
Positive	46(11.50)	8.71-15.03

Prevalence of the respective common bacterial organisms found in Rolex food

In Table 3, seven bacterial pathogens were found in the Rolex in the present study. The most common bacterial pathogens were *E. coli* and CNS, with a prevalence of 30.43% [95%CI (18.55-45.650] and 26.09% [95%CI (15.12-41.15)] respectively. Salmonella species was not found at all in the present study, and it had zero prevalence. Klebsiella species and the Pseudomonas species were also isolated in the Rolex samples in the study, with a prevalence of 15.22% [95%CI (7.21-29.290] each. Bacillus and Streptococcus were the least common bacterial organisms found in the samples, with a prevalence of 6.52% [95%CI (2.02-19.05) and 4.35% [95%CI (1.03-16.53)].

Table 3: The prevalence of different common bacteria in Rolex food

Variable	n (%)	95% CI
Bacterial Isolates		
E. coli	14(30.43)	18.55-45.65
Staphylococcus Aureus	1(2.17)	0.28-14.83
Salmonella Spp	0(0)	-
Klebsiella Spp	7(15.22)	7.21-29.28
Pseudomonas Spp	7(15.22)	7.21-29.28
CNS	12(26.09)	15.12-41.15
Bacillus Spp	3(6.52)	2.02-19.05
Streptococcus	2(4.35)	1.03-16.53

Prevalence of the common bacterial contaminants of Rolex food based on the selected sample type

Table 4 shows that raw Rolex contained the highest number of bacterial pathogens with a bacterial prevalence of 71.43% [95%CI (39.89-90.40)], unlike the cooked Rolex, which had a low level of bacterial contaminants of 28.57% .

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Table 4: The prevalence of common bacterial contaminants of Rolex food based on the selected sample type

Variable	n (%)	95% CI
Sample type		
Raw	10(71.43)	39.89-90.40
Cooked	4(28.57)	9.59-60.10

Antibiotic susceptibility test

In table 5, Cetaxim was the most resistant antibiotic drug to the isolates from the Rolex with 93.75% [95%CI (60.69-99.31). Sulphamethoxazole and the Gentamycin drugs were the second and third most resistant drugs to the isolates from Rolex food, with 43.75% [95%CI (20.41-70.22)] and 37.50% [95%CI (16.14-65.15)] resistance, respectively. The difference in the resistance of the drugs to the isolates was statistically significant at chi-square 43.23 and p-value <0.001.

Ampicillin was the most sensitive drug with 93.75% [95CI% (60.81-99.31)]. This was followed by chloramphenicol, which had 87.50% [95%CI (56.99-97.36)] resistance. Furthermore, Nalidixic acid and Tetracycline were among the drugs that showed high sensitivity with 85.71% [95%CI (51.98-97.08)] and 81.25% [95%CI (51.40-94.66)], respectively. The sensitivity of the antimicrobial drugs was statistically significant at the Chi-square of 48.23 and p-value <0.001.

Table 5: The susceptibility of E. coli to different drugs

	Drugs								
Susceptibility pattern	Nalidixic acid	Chloramphenicol	S & T	Gentamicin	Ampicillin	Tetracycline	Cetaxim	Chi^2	P-value
Sensitive n (%)	12(85.71)	14(87.50)	9(56.25)	10(62.50)	15(93.75)	13(81.25)	1(6.25)	48.23	<0.001
95% CI	51.98-97.08	56.99-97.36	29.77-97.36	34.84-83.85	60.81-99.31	51.40-94.66	0.68-39.30		
Resistance n (%)	2(14.29)	2(12.50)	7(43.75)	6(37.50)	1(6.25)	3(18.75)	15(93.75)		
95%CI	2.91-48.01	2.63-43.00	20.41-70.22	16.14-65.15	68.16-39.30	5.33-48.59	60.69-99.31		

DISCUSSION

The overall prevalence of bacteria in Rolex food is 11.50% of the samples collected and this shows that this food delicacy is contaminated. These results are similar to the work done in street vended foods where Rolex food is inclusive (Mugampoza, Byarugaba, Nyonyintono, & Nakitto, 2013b). However, the percentage of bacteria is relatively low because of heat treatment gone through during the preparation of chapatti and eggs.

E. coli was the most prevalent Rolex contaminant with a prevalence percentage of 30.43% and this is because of faecal contamination attributed to contaminated water they use from the taps which is used in washing cabbages, tomatoes, and onions which are constituents of this food delicacy, the bare hands which they use while making this food delicacy and the general poor sanitation of the area characterized by the sewage channels on the streets. The second most prevalent bacteria was CNS with a prevalence percentage of 26.09% because it is a normal flora on the skin especially in nosocomial passages (Becker, Heilmann, & Peters, 2014) and since they are always bare-handed while preparing the Rolex food, contamination from them is inevitable and also their aprons and their clothes which they put on were found dirty can still add contamination to the food. The study done by Al Mamun, Rahman, & Turin (2013) in Bangladesh had similar results. Both *Klebsiella species* and *Pseudomonas species* had a prevalence percentage of 15.22% because both Klebsiella and *Pseudomonas species* are normal flora on the skin, and this leads to contamination from the body of the Rolex maker, unhygienic conditions are exercised, such as touching dirty clothes while preparing the food. Also, *Pseudomonas species* are found in soils, water, on vegetation cover, and the Rolex stalls are found in open places where dust particles can easily fall onto the Rolex

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food from the surroundings and eventually cause contamination. *Bacillus* and *Streptococcus species* were

The least common bacterial organisms found in the sample, with a prevalence of 6.52% and 4.35%, and this is because to contamination from the dust particles from the open places they operate from. *Bacillus* presence in a food sample is a clear indication of soil contamination. *Streptococcus species* are also normal flora in the nasal cavity and trachea, and therefore, sneezing and touching the nostrils could lead to contamination by these microorganisms. Its percentage prevalence (15.22%) is lower than that of other bacteria, which are normal flora, because it is less widely spread on the body. *Salmonella species* was not determined at all in the present study, and it had zero percent prevalence. This is because *Salmonella species* are rare in the environment, and if a single bacterium were isolated could just indicate infection. Washing of tomatoes, cabbages, onions, and other materials used in Rolex preparation also contributes to the zero-percentage prevalence of *Salmonella species* in the food sample. Raw Rolex contained the highest number of bacteria pathogens with a bacterial prevalence of 71.43%, unlike the cooked Rolex which had a low level of bacteria contaminants of 28.57% this is because raw Rolex food have tomatoes, cabbages, and onions which are not fried together with egg and therefore no heat treatment and on the other hand cooked Rolex foods contain salads which are fried together with egg. Frying is a form of heat treatment that kills most of the bacteria on the salads and therefore presents less bacterial prevalence. But should be noted that before this study, I conducted a mini study to find out which constituent of Rolex food contains the most microorganisms and I found out that tomatoes and cabbages contain the most and the numbers were; for tomatoes, it had total coliform counts as 4.8x10^3 cfu/100ml, total E.coli count as 4.0x10^3 cfu/100ml, total plate count as 2.92x10^4 cfu/100ml and cabbages had total coliform count as 4x10^6 cfu/100ml, total *E.coli* count as 4x10^6 cfu/100ml and total plate count as 14x10^6 cfu/100ml. Both counts from the chapati and the egg were insignificant according to this mini study.
Seven drugs were used for susceptibility tests, and out of them, Cetaxim was the most resistant antibiotic drug to the isolate from the Rolex with 93.75%. (Trimethoprim and Sulphamethoxazole) and the gentamycin drugs were the second and third most resistant drugs to the isolates from Rolex, with 43.75% and 37.50%. Ampicillin was the most sensitive drug, with 93.75%. This was followed by chloramphenicol, which had 87.50% sensitivity. Furthermore, Nalidixic acid and Tetracycline were among the drugs that showed high percentage sensitivity with 85.71% and 81.25%, respectively. Cetaxim is a third-generation cephalosporin, and it is extensively used in the treatment of gram-negative bacteria in humans. Because of this use in human medicine, it is evident that *E. coli* has become resistant to this antibiotic. The resistant *E. coli* is passed to water through faecal deposition in water streams and then obtained in food through the usage of water in the preparation and handling of Rolex food. (Trimethoprim and Sulphamethoxazole) and the gentamycin drugs were the second and third most resistant drugs to the isolates from Rolex, with 43.75% and 37.50%, respectively. For Trimethoprim and Sulphamethoxazole, this high resistance is attributed to extensive use in food animals such as chicken, cows, pigs, goats, and sheep, and through the treatment, the *E. coli* acquires the resistance towards this antibiotic and through faecal contamination the resultant resistant strains of *E. coli* are dispersed to the environment and humans where humans through unhygienic conditions during Rolex preparations handling can find its way to food. Gentamycin is an example of an aminoglycoside, which are widely used in human medicine and veterinary medicine in the treatment of both gram-negative and gram-positive bacteria. Through this usage, resistance is developed, which is then spread through fecal contamination to water and food. Ampicillin was the most sensitive drug with 93.75%. This is because Ampicillin is rarely used in both plants and animals as the antibiotic of choice, as was chloramphenicol, which had 87.50% sensitivity, and this makes them highly responsive to microorganisms like *E. coli* once introduced to them. Nalidixic acid and Tetracycline were among the drugs that showed high percentage sensitivity of 85.71% and 81.25%, respectively. Nalidixic acid is very effective against *E. coli* and does not possess a beta-lactam ring, which is always worked upon by beta-lactamase enzymes, and therefore, high sensitivity from the isolates. The high sensitivity of Tetracycline is surprising because this antibiotic is extensively used in the treatment of bacterial diseases in plants, animals, and humans, and its susceptibility to *E. coli* is hard to explain scientifically and logically.

CONCLUSION

The overall prevalence of bacterial load in the Rolex sample was 11.50% out of 50 samples and the most common bacteria contaminants in Rolex food were E. coli and CNS with prevalence of 30.43% and 26.09% respectively.
Raw Rolex contained the highest number of bacterial pathogens, with a bacterial prevalence of 71.43%, unlike the cooked Rolex, which had a low level of bacterial contaminants of 28.57%.
The isolated *E. coli* was most resistant to Cetaxim (Trimethoprim and Sulphamethoxazole) and Gentamycin, while the most sensitive drugs were Ampicillin, Chloramphenicol, Nalidixic acid, and Tetracycline. More studies should be done focusing on particular constituents of Rolex food, such as tomatoes, eggs, onions, chapatti, and cabbage, to determine their specific contaminants, which contribute to overall contamination of Rolex food, and also carry out the same research in different areas of the country to compare their significance. Also, studies should be done on the bacteria found on the shell surface of eggs versus what is found

in prepared ones to visualize the significance of handling the eggs during transportation, handling during preparation.

RECOMMENDATIONS

People should buy cooked Rolex food as opposed to raw one because of its low prevalence of bacterial contaminants. The Rolex makers should clean their clothes, acquire and put on aprons all the time while operating, and above all, wash their hands with clean water every time they serve customers.

LIST OF ABBREVIATIONS

0C: Degrees Celsius
AMP: Ampicillin
AMR: Antimicrobial Resistance
BA: Blood agar
CNS: Coagulase-negative Staphylococcus
COVAB: College of Veterinary Medicine, Animal Resources and Biosecurity
CP: Chloramphenicol
CXC: Cetaxim and clavulanic acid
E. coli: Escherichia coli
e.g., for example
GM: Gentamicin
I: Intermediate
MAC: MacConkey agar
mm: Millimetres
MUK: Makerere University
NA: Nalidixic acid
NA: Nutrient Agar
R: Resistance
S: Susceptible
SOP: Standard Operating Procedures
Spp: Species
SPSS: Statistical Program for Social Scientists
SXT: Trimethoprim and Sulfamethoxazole
TE: Tetracycline
WHO: World Health Organization

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION

I conceptualized the study, wrote the first draft of the paper, wrote the discussion, analyzed the data, and wrote the final draft of the manuscript.

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