

Source Tracking and Antibiotic Resistance Patterns of Selected Pathogenic Bacteria Isolated from Street Vended Food in Kisumu County, Kenya

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Abstract

Microbial studies from many developing countries, carried out on street vended food have revealed a high count of multidrug resistant bacterial strains. Although, there has been an increase in diarrheal diseases that are resistant to antibiotics in Kisumu, the contribution of street-vended foods towards enteric infections is unknown. This aim of this study was to determine the prevalence, source tracking and antimicrobial resistance of selected pathogenic bacteria in street vended-foods. Across-sectional study design was adopted. A total of 186 swabs were collected from vendors' hands, soils around the vending environment and water samples used in food preparation within Kisumu city. Bacterial strains were isolated and identified based on phenotypic and biochemical properties. Antimicrobial susceptibility was determined by disk diffusion method. This study demonstrates that the prevalence of enteric pathogens in street vended foods comprised of 74% total *Coliforms*, 20% *Staphylococcus aureus* and 6% *Escherichia coli*. *Salmonella*, *Vibrio* and *Shigella* were isolated from the soils surrounding where the foods are vended but not from the street food. There was a significant correlation between *S. aureus* isolated from the vendors' hands and vended foods ($r^2 = 0.076$, $p = 0.03$). Antimicrobial resistance was observed in *Enterobacteriaceae* and *S. aureus* isolates. Resistance to erythromycin (82.7%) was the most frequent followed by sulfamethoxazole (43.2%), chloramphenicol (35.8%), amoxicillin and ampicillin (33.3%), tetracycline (22.2%), nalidixic acid (17.3%) and norfloxacin (16%). The observed patterns of street food contamination with antimicrobial resistant entero-pathogens points to the need to regulate street-vending foods to control food-borne diseases and spread of antimicrobial

Keywords: Source Tracking; Antimicrobial; Vendors; Pathogenic Bacteria

Introduction

Street-vended food are those foods prepared on the street and ready to eat or prepared at home and consumed on the street without further preparations [1]. In many developing countries, a large proportion of ready to eat foods are prepared and sold at public places [2]. Street food vendors benefit from a positive cash flow, often evade taxation and can determine their own working hours; in this regard, they are likely not to observe critical health issues concerning food handling. Unlike in developed countries where food preparation in terms of packaging, processing and handling is advanced and safety is paramount, in developing countries traditional methods of food processing and packaging, improper storage and poor personal hygiene of food handlers are widespread especially among street vendors [3]. Street food vending has been associated with food borne illnesses in many parts of the world [4], as Street-food vendors are often unlicensed, untrained in food safety, food hygiene and sanitation and work under crude unsanitary conditions [5]. Moreover, stalls are

often crude structures with no clean running tap water, toilet or adequate washing facilities [6]. Adequate washing facilities hence exposing food cooked on the streets to cross contamination from various sources [6]. Analysis of foods and vending environment is therefore critical for understanding the hazards associated with street foods as a form of bacteria source tracking. A more pressing concern is the recent spread of several ailments caused by bacterial pathogens that are resistant to wide array of antibiotics [7]. Some of the most common microorganisms associated with food contamination in such environment include *Staphylococcus aureus* and members of the family *Enterobacteriaceae* [8]. Reports suggest that *E. coli*, a major cause of gastroenteritis is present where there is fecal contamination [9]. *Salmonella* on the other hand causes a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia [10]. It is estimated that food-borne salmonellosis is responsible for 1.3 million illnesses annually, resulting in 16,000 hospitalization and 600 deaths [11]. In most developing countries, there is scanty documented evidence on food borne infections [12]. While gastroenteritis are a major cause of hospitalization in Kisumu County, Kenya [13], the prevalence of food borne infections remain undocumented and the association between the food vending environment and contamination is not well established. Water and food contaminated with antibiotic-resistant bacteria is a major threat to public health, as the antibiotic resistance determinants can be transferred to bacteria of human clinical significance [14]. In Kenya antimicrobial resistance surveillance has been limited [12,15] meaning that the actual scale of regional antimicrobial drug resistance is not well known.

Aim of the Study

The aims of this study were to determine the prevalence, source of contamination and antimicrobial resistance of selected pathogenic bacteria in street vended-foods in Kisumu County, Kenya.

Materials and Methods

This was a cross-sectional study conducted in three open-air markets within Kisumu County: Kibuye, Kondele and Oile in Kisumu city, Kenya. These sites were purposively chosen because of the higher concentration of food vendors serving most residents within Kisumu city. From each vending site, the following samples were collected and analyzed for the presence of pathogenic bacteria: swab from the vendors hands, 100g of food, 100g of soil from the vending environment and 150 ml of water used for food preparation. 62 samples collected were analyzed within 12 hours of collection according to protocol by Gilbert RJ., *et al.* (2000) [16] briefly, 30g of test samples were weighed in a sterile Stoppard bottle of at least 500 ml capacity. 270 of maximum recovery diluents was then added for *Staphylococcus aureus* and similar quantities of buffered peptone water for *Salmonella*, *Shigella* broth containing novobiocin for *Shigella* and alkaline saline peptone water for *Vibrio cholera* and mixed thoroughly by shaking. The mixers were transferred into a sterile stomacher bags; and blended for 30 seconds. Using a fresh sterile pipette, 1 ml of the emulsified sample (10^{-1}) was transferred into 9 ml of sterile diluents to make (10^{-2}) dilution. This was repeated with further dilutions using fresh sterile pipette for each decimal dilution depending on the product. Soil samples were processed by transferring 30g into a pre-sterilized bottle. 100 mls of sterile buffered peptone water was added and mixed for 2 minutes and then filtered through a pre-sterilized 28 μm -pore size nylon filter. The filtrate was then used to recover *Salmonella*, *Shigella*, *Vibrio cholera* and *Enterobacteriaceae* by direct plating on selective media. For water and swabs 10mls of samples were added to 90ml of buffered peptone water for *Salmonella* and similar quantities for *Shigella* broth containing novobiocin for *Shigella* and alkaline saline peptone water for *Vibrio cholera* and mixed thoroughly by shaking. The mixture was then analyzed as per water methods (ISO6887-1, 1999). For enumeration of *Staphylococcus* in food and soils (ISO6579:2002), 0.1 ml of the prepared sample above and the serial dilutions (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6}) were added to the surface of prepared Baird parker agar plate in duplicate. Carefully the inoculums were spread starting from the lowest dilution to the highest dilution as quickly as possible over the surface of the agar plates using glass spreader for each plate to avoid as much as possible contact with the side of the Petri dishes. The plates were covered and left on the bench for 15 minutes for absorption of inoculums to take place. Also, one prepared and dried Baird parker plate was used a control for checking sterility. The inoculated plates were inverted and incubated at 37°C. Typical colonies appeared as black shining and convex surrounded by clear zone that is partially opaque, 1 mm to 1.5 mm in diameter after incubation for 24 hours and 1.5 mm to 2.5 mm in

diameter after incubation for 48 hours. A typical colonies appearance as grey colonies free of clear zones (ISO6579, 2002). Confirmation was done using coagulase test. Five typical and five a typical colony were transferred into a tube 5 ml-Brain-Heart Infusion broth and incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 hours. Aseptically, 0.1ml of each culture was added to 0.3 ml of rabbit plasma. The tube was examined after 4 - 6 hours for clotting of the plasma. Coagulase test was considered positive if the volume of the clot occupied more than half of the original volume of the liquid. The blank/control plasma showed no signs of clotting.

Detection of *Salmonella* species in food and soil (ISO 6579:2002): It was done by incubating pre-enriched food sample at 37°C for 16 to 20 hours. 10ml volumes of Rappaport-vassiliadis broth was inoculated with 0.1 ml of the pre-enrichment culture. Similarly, 10 ml of tetrathionate broth was inoculated with 0.1 ml of pre-enrichment culture. Rappaport-vassiliadis broth was incubated at 41.5°C for 18 - 24 hours, and the tetrathionate broth at 37°C for 20 - 24 hours. A loop was taken from each enrichment tube and streaked onto the surface of Brilliant Green agar and Xylose Lysine Decarboxylase agar. The plates were incubated at 37°C for 18 - 24 hours. The plates were examined for the presence of typical bacterial colonies and confirmation was done using biochemical tests. Typical *Salmonella* colonies were characterized as follows: on XLD agar typical colonies appeared black centers and a slightly transparent zone of reddish colour due to colour change of the indicator on BGA, pink colonies with bright red surrounding medium was observed. Confirmation was done by streaking selected colonies onto the surface of dried nutrient agar plates. Single colonies were used for biochemical and serological confirmation. Biochemical confirmation was done on the following Triple Sugar Iron agar, Urea agar, Lysin Decarboxylation Medium, Tryptone water for indole reaction and Voges Proskauer Medium. Serological confirmation was done by observing agglutination reaction of O-antigen, Vi-antigen and H-antigens. If agglutination occurred the reaction was considered positive (ISO 6579, 2002).

Detection of *Shigella* species in food and soil (ISO 21567: 2004): This was done using prepared food sample as there above by inoculating on surface agars by means of a loop to obtain well isolated colonies. MacConkey agar with low selectivity; XLD agar with moderate selectivity and Hektoen enteric agar with greater selectivity. The plates were incubated at 37°C for between 20 - 24 hours. Typical and suspect colonies were selected from each of the three selective agars using a sterile loop and streaked on nutrient agar, then confirmed using biochemical and serological tests. Biochemical confirmation was inoculated on the following specified media: TSI agar (TSI slopes), semi-solid nutrient agar for motility tests. On TSI typical *Shigella* cultures showed a yellow butt (acid formation) and no gas bubbles, no change in the colour of the slant (no utilization of lactose or sucrose) and no hydrogen sulphide production. All *Shigella* species were non- mobile. Serological confirmation was carried out using agglutination tests. The agglutination results were observed against a dark background and if necessary, with the aid of a magnifying lens. In cases where all tests were negative and biochemical tests were characteristics of *Shigella* a suspension of pure cultures was heated in a water bath at 100°C for 60 minutes and a repeat of the agglutination tests was done.

Detection of *Vibrio cholera* in food and soil (ISO/TS 21872-1:2007): In selective enrichment 1 ml of the prepared food sample was transferred into a tube containing 10ml of Alkaline Saline Peptone Water (ASPW) and incubated at 37°C for 18 ± 1 hour. From the culture obtained in the ASPW, a sampling loop was inoculated on the surface of a Thiosulphate Citrate Bile Salt (TCBS) agar plate. The plates were inverted and incubated at 37°C for 24 ± 3 hours. *Vibrio cholera* from yellow colonies on TCBS. The selected colonies were inoculated onto the surface of saline nutrient agar and incubated at 37°C for 24 ± 3 hours. The pure cultures were used for biochemical confirmations. Presumptive identification was done using oxidase test and microscopic examination. In oxidase test, a portion of pure culture was taken from the saline nutrient agar and streaked onto the filter paper moistened with oxidase reagent, in positive tests the color turned violet or deep purple within 10 seconds. In microscopic examination a film for Gram staining was prepared and examination of morphology and Gram reaction observed, similarly a drop of the culture was covered with a cover slip and examined for motility under the microscope. *Vibrio cholera* appeared as oxidase-positive, Gram negative and motility test was positive. Presumptive positive colonies were selected for biochemical confirmation using saline TSI agar and indole. Typical reaction showed an acid slant (yellow) and an acid butt (yellow) without formation of gas or hydrogen sulphide.

Enumeration of other *Enterobacteriaceae* in food and soil (ISO 21528-2004). Using a sterile pipette, 1 ml of the serial dilutions (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6}) was transferred to 2 sterile Petri dishes. 15 mls of the Violet Red Bile Glucose medium was poured into each Petri dish. Immediately after pouring the plates were kept in a horizontal position taking care not to wet the lid, the media and inoculums was gently and thoroughly mixed. The media was then allowed to solidify with the Petri dishes standing on a cool horizontal surface. After

solidification a covering layer of about 5 ml of VRBG medium was added and allowed to cool. A control plate, with 15ml media was also prepared for checking sterility. Typical colonies appeared as pink to red or purple with precipitation haloes. Sub culturing of selected colonies was done by selecting five characteristic colonies at random and streaking each onto the already dried Nutrient agar plates. Since certain *Enterobacteriaceae* causes discolouration of the medium therefore, where no characteristic colonies was present five whitish colonies were chosen for confirmation and incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 hours \pm 2 hours. Well isolated colonies were subjected to biochemical tests using oxidase reaction and fermentation tests. Oxidase test was done by streaking onto commercially available oxidase filter paper; the test was considered negative when the colour of the filter paper failed to turn dark within 10 seconds. Fermentation tests were done using a wire where the selected colonies were stabbed into the tube indicated a positive reaction. Colonies that were oxidase negative and glucose positive were confirmed as *Enterobacteriaceae*. Similarly, *E. coli* was confirmed by indole test at 44°C , typical *E. coli* turned brick red on addition of kovac's reagent. Further biochemical tests were done using Indole, Methyl Red, Voges Proskauers and Citrate utilization tests, urease test, TSI tests and motility test to isolate other *Enterobacteriaceae* for antimicrobial sensitivity tests. Typical *E. coli* was oxidase (-ve), glucose (+ve), IMVIC test (+++), ureases (-ve), TSI (A/A/-) and motile, *Klebsiella* was oxidase (-ve), glucose (+ve), IMVIC test (-++), urease (+ve), TSI (A/A/-) and non-motile, *Citrobacter* was oxidase (-ve), glucose (+ve), IMVIC (+++), urease (-ve), TSI (A/A/-) and motile, *Enterobacter* was oxidase (-ve), glucose (+ve), IMVIC (-++), urease (+ve), TSI (A/A/-) and motile and *Proteus* appeared as oxidase (-ve), glucose (+ve), IMVIC (+++), urease (+ve), TSI (A/A/-) and highly motile.

Detection of *Salmonella* in water and swabs (ISO 6340:1995): Water and swab samples prepared there above were poured into a sterilized 500ml capacity funnel to the 100 ml mark and filtered through a sterile filter membrane $0.45\ \mu\text{M}$. The filter was then placed using a sterile forceps in 100 ml of sterile buffered peptone water and incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 16 - 20 hours. From the enrichment culture 10 ml of Rappaport Vassiliadis broth was inoculated with 0.1 ml of pre-enrichment culture. Similarly, 10 ml of Selenite Cystine broth was inoculated with 1.0 ml of pre-enrichment culture. RVS medium was incubated at $41.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 hours and SBB at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 hours. After incubation a loop from each enrichment tube was streaked onto the surface of Brilliant Green Lactose broth and Xylose Lactose Deoxychocolate agar to obtain well isolated colonies. The plates were then incubated a $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 18-24 hours. Typical *Salmonella* colonies appeared as red colonies without black centers on XLD and pink colonies with bright red surrounding on BGA. Further confirmatory tests on the biochemical and serological were done as per ISO6340-1, 1995 (see above).

Detection of *Vibrio cholera* in water and swabs (ISO/TS 21872). Water and swab samples were poured into a sterile 500ml capacity funnel to the 100 ml mark and filtered through a sterile filter membrane $0.45\ \mu\text{M}$. The filter was then placed using a sterile forceps in 100 ml of sterile Alkaline Saline Peptone Water (ASPW) and incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 16 - 20 hours. From the culture obtained in the ASPW, a sampling loop was inoculated on the surface of a Thiosulphate Citrate Bile Salt (TCBS) agar plate. The plates were inverted and incubated at 37°C for 24 ± 3 hours. *Vibrio cholera* form yellow colonies on TCBS. The selected colonies were inoculated onto the surface of saline nutrient agar and incubated at 37°C for 24 ± 3 hours. The pure cultures were used for biochemical confirmations. Further confirmatory tests were done as per ISO/TS21872, 2007.

Enumeration of *Staphylococcus aureus* in water and swabs (ISO 5944:2001): Water and swab samples prepared were poured into a sterilized 500 ml capacity funnel to the 100 ml mark and filtered through a sterile filter membrane $0.45\ \mu\text{M}$. The filter was then placed using a sterile forceps on the surface of prepared Baird agar plate and left on the bench for 15 minutes for absorption of inoculums to take place. One prepared and dried Baird parker plate were used as a control for checking sterility. The inoculated plates were inverted and incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 16 - 20 hours. Typical colonies appeared as black shiny and convex surrounded by a clear zone that is partially opaque, 1 mm to 1.5 mm in diameter after incubation for 24 hours and 1.5 mm to 2.5 mm in diameter after incubation for 48 hours. Typical colonies appeared as grey colonies free of clear zones.

Detection of *Shigella* species in water and swabs (ISO 21567-1:2004): Water and swab samples were poured into a sterilized 500 ml capacity funnel to the 100 ml mark and filtered through a sterile membrane $0.45\ \mu\text{M}$. The filter was the placed using a sterile forceps

in 100 ml of sterile *Shigella* broth containing Novobiocin and incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 16 - 20 hours. The pre-enriched samples were inoculated by means of a loop on the surface of selective agar plates to obtain well isolated colonies: MacConkey agar with low selectivity; XLD agar with moderate selectivity and Hektoen enteric agar with greater selectivity. The plates were incubated at 37°C for between 20 and 24 hours. Typical and suspect colonies were selected from each of the three selective agars using a sterile loop and streaked on nutrient agar, then confirmed using biochemical and serological tests as per ISO21567:2004 there above.

Enumeration of Coliforms and *E. coli* in water and swabs (ISO 9308-1:2000): Water and swab samples were poured into a sterilized 500 ml capacity funnel to the 100 ml mark and filtered through a sterile filter membrane $0.45 \mu\text{M}$. The filter was then placed using a sterile forceps on the surface of prepared Violet Red Glucose agar and left on the bench for 15 minutes for absorption of inoculums to take place. One prepared and dried VRBG plate was used as control for checking sterility. The inoculated plates were inverted and incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 16 - 20 hours. Typical colonies appeared as pink to red or purple (with or without precipitation haloes). *E. coli* colonies appeared as pink to red or purple with precipitation haloes. Sub culturing of selected colonies was done by selecting five characteristic colonies at random and streaking each onto the already dried Nutrient agar plates. Since certain *Enterobacteriaceae* causes discoloration of the medium therefore, where no characteristic colonies were present five whitish colonies were chosen for confirmation and incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 hours ± 2 hours. Further biochemical tests and calculations were done as per (ISO 9308-1, 2001).

Quality control checks: Fresh reference cultures/working stocks from NCTC batch number NCTC 04840 *S. poona*, NCTC 10885 *Shigella sonnei*, NCTC 9001 *E. coli*, NCTC 06571 *S. aureus*, NCTC 10006 *Enterobacter aerogenes*, NCTC 10975 *Proteus mirabilis*, NCTC 9528 *Klebsiella aerogenes*, NCTC 9750, *Citrobacter fremundii* and NCTC 1134 *Vibrio cholera* were subjected to the same procedure as the test samples. The reference culture showed appropriate/characteristic features and reactions as earlier described. A test was regarded as invalid if the results were not the same as the reference culture.

Antimicrobial susceptibility testing: The isolates from *Enterobacteriaceae* family were differentiated based on their cultural and cellular morphological studies, after which they were subjected to various biochemical tests. Further biochemical tests were done using Indole, Methyl Red, Voges Proskauer Citrate utilization tests, urease tests, TSI tests and motility test to isolate other *Enterobacteriaceae* for antimicrobial sensitivity tests. Typical *E. coli* was oxidase (-ve), glucose (+ve), IMVIC test (++--), urease (-ve), TSI(A/A/-) and non-motile, *Klebsiella* was oxidase (-ve), glucose (+ve), IMVIC test (---+), urease (+ve), TSI (A/A/-) and non-motile, *Citrobacter* was oxidase (-ve), glucose (+ve), IMVIC (++++), urease (-ve), TSI (A/A/-) and motile, *Enterobacter* was oxidase (-ve), glucose (+ve), IMVIC (---+), urease (+ve), TSI (A/A/-) and highly motile. The isolates from different foods, swabs, soils and water namely: *Salmonella*, *Vibrio cholera*, *Shigella*, *Proteus*, *Enterobacter*, *Citrobacter* and *Klebsiella* were subjected for susceptibility test using eight antibiotics namely ampicillin, amoxicillin, tetracycline, sulphamethoxazole, norfloxacin, nalidixic acid, erythromycin and chloramphenicol was performed using standard Kirby-Bauer disk diffusion method on Muller Hinton agar (Oxoid UK). Using a sterile swab the broth dilution containing the bacteria was spread on the surface of Muller Hinton agar plates, the disks were then pressed onto the inoculated plate surfaces and incubated at 37°C for 18 to 20 hours. The diameters in millimeters of clear zones of inhibition around the antimicrobial agent disk, including the 6 mm disk diameter was measured using precision calipers (Clinical and Laboratory Standard Institute (CLSI), 2002). A standard reference strain of *E. coli* (ATCC 25922) was used as a control. The breakpoint used to categorize the isolates as resistant, susceptible or intermediate to each antimicrobial agent were those recommended by Clinical Laboratory Standards Institute (CLSI) (CLSI, 2009) [12].

Results

Prevalence of selected pathogenic bacteria in street vended foods

A total of 62 foods were collected from Kondele, Oile and Kibuye markets in Kisumu over a period of four months. Results of this study shows that foods sampled from the three markets had prevalence of 74%, 20% and 6% of total *Coliforms* *S. aureus* (20%) and *E. coli* re-

spectively.

Among the sampled foods from the markets, ice cream had the highest number of counts with mean of (4,949 c.f.u). Oile market had the highest mean colony counts for coliforms (1,546 c.f.u) followed by Kibuye market (1,221 c.f.u) and Kondele market (941 c.f.u). Simi-

Vending site (s)	Population size	Sample size	Total samples collected
Kondele	63	9	36
Oile	157	23	92
Kibuye	202	30	120
Total	422	62	248

Table 1: Sample size per study market (as per Kisumu municipal council records).

larly, Oile recorded the highest mean count for *S. aureus* (396 c.f.u), followed by Kondele (310 c.f.u) and Kibuye (277 c.f.u). Oile had the highest counts in *E. coli* (152 c. f.u), followed by Kibuye (74 c.f.u) and Kondele (32 c.f.u) (Table 1).

Possible sources of contamination of street vended foods

Swabs collected from hands of vendors were contaminated with *E. coli*, total coliforms and *S. aureus*. Isolation rate of *E. coli* from the swabs was 30% from Kibuye, 21% Oile and 0% Kondele. Total coliforms in Kibuye were 68%, Kondele 67% and Oile 52%. *S. aureus* contaminations in swabs were; Kibuye 57%, Kondele 57% and Oile (39%).

The prevalence of *E. coli* in water was Kibuye 30%, Kondele 22%, and Oile 30%. Coliforms were Kibuye 66%, Kondele 67% and Oile 61%. *Staphylococcus aureus* contaminations in water were Kibuye 53%, Kondele (44%) and Oile (39%).

The results further indicate that soil sampled around the vending environment were highly contaminated with pathogenic bacteria with results from Kibuye showing *E. coli* 63%, Kondele 78% and Oile 78%. Total coliforms results were Kibuye 83%, Kondele 78% and

Market	Sample type	N	<i>E. coli</i>	Coliforms	<i>S. aureus</i>	Shigella	Salmonella	Vibrio
Kibuye	Swabs	30	9 (30%)	20 (67%)	17 (57%)	nd	nd	nd
	Soil	30	19 (63%)	19 (63%)	27 (90%)	7 (23%)	17 (57%)	1 (3%)
	Water	30	9 (30%)	20 (67%)	17 (57%)	nd	nd	nd
Kondele	Swabs	9	nd	6 (67%)	4 (44%)	nd	nd	nd
	Soil	9	7 (78%)	7 (78%)	9 (100%)	2 (22%)	4 (44%)	nd
	Water	9	nd	6 (67%)	4 (44%)	nd	nd	nd
Oile	Swabs	23	5 (21%)	12 (52%)	9 (39%)	nd	nd	nd
	Soil	23	18 (78%)	20 (87%)	23 (100%)	4 (17%)	11 (48%)	3 (13%)
	Water	23	5 (21%)	12 (52%)	8 (35%)	nd	nd	nd

Table 2: Pathogens isolated from soil, swab and water by market.

Key: nd = Not Done.

Oile 100%. Other pathogenic *Enterobacteriaceae* isolated in soil were *Shigella*, *Vibrio* and *Salmonella*, which were not isolated in foods, water and swabs. The prevalence of *Shigella* in Kibuye was 23%, Kondele 33% and Oile 22%. *Salmonella* was 53% in Kibuye, Kondele 56% and Oile 48%. *Vibrio* in soil was found to be 3% in Kibuye, 0% in Kondele and 13% in Oile (Table 2).

There was no correlation between *E. coli* isolates from hand swabs and *E. coli* in food ($r^2 = -0.009$, $P = 0.947$), however, *S. aureus* isolated in food and in swabs were significantly correlated ($r^2 = 0.276$, $p = 0.03$). There was no correlation between coliforms in food and in hand swabs ($r^2 = 0.079$, $p = 0.54$). *E. coli* isolated in food and in water showed a negative correlation ($r^2 = -0.073$, $p = 0.575$). *E. coli* in food and water, *S. aureus* in food and in water all showed a negative correlation. There was no correlation between coliforms and food, water or soil.

There was no association between *E. coli* isolated from food and those other swabs ($X^2 = 6.000$, $p = 0.199$), soil ($X^2 = 3.000$, $p = 0.223$)

Pathogen	Source of Food Contamination	Chi-Square Value	Significance (P value)
<i>E. coli</i>	Hand Swabs	6.000	0.199
	Soil	3.000	0.223
	Water	6.000	0.199
Total Coliforms	Hand Swabs	3.000	0.223
	Soil	6.000	0.199
	Water	3.000	0.223
<i>S. aureus</i>	Hand Swabs	3.000	0.223
	Soil	3.000	0.083
	Water	3.000	0.223

Table 3: Association between the possible source and the food contamination.

or water ($X^2 = 6.000$, $p = 0.199$). Similarly, Total coliform isolated from food had no association to those isolated from: hand swabs ($X^2 = 3.00$, $p = 0.223$, soil ($X^2 = 6.000$, $p = 0.199$) and water ($X^2 = 3.000$, $p = 0.223$). Also, analysis of *S. aureus* isolates in food and from the hand swabs, soil and water used in preparation of food showed a significant levels of association ($X^2 = 3.000$, $p = 0.223$, $X^2 = 3.000$, $p = 0.083$ and $X^2 = 3.000$, $p = 0.0223$ respectively (Table 3).

Antimicrobial susceptibility patterns

Further analysis shows that all pathogens isolated from food, soil, water and hand swabs were resistant to erythromycin except *S. aureus*. All the isolates showed some level of resistance to the selected antibiotics with *S. aureus* showing 100% resistance to nalidixic acid

Antimicrobial agent	% Resistance Total Coliform	% Resistance E. coli	% Resistance Salmonella species	% Resistance Shigella species	% Resistance S. aureus
Erythromycin	100	100	100	100	0.0
Chloramphenicol	44.4	100	0.0	33.3	0.0
Amoxycillin	63.9	0.0	0.0	33.3	0.0
Sulfamethoxazole	64.0	59.9	0.0	0.0	21.8
Tetracycline	45.0	10.0	0.0	0.0	0.0
Ampicillin	52.0	0.0	0.0	0.0	57.6
Norfloxacin	22.2	0.0	0.0	0.0	0.0
Nalidixic acid	0.0	0.0	0.0	0.0	100

Table 4: Resistance pattern of total Coliforms, *Escherichia coli*, *Salmonella species*, *Shigella species* and *Staphylococcus aureus*. Key: % = Percentage resistance.

and total coliforms 22.2% to norfloxacin, *E. coli* showed 100% resistance to chloramphenicol, total coliform 44.4% and *Shigella* 33.3%. Total coliforms and *Shigella* showed 63.9% and 33.3% resistance to amoxicillin, *E. coli*, *Salmonella* and *S. aureus* were susceptible to amoxicillin. Resistance to tetracycline was total coliforms 44.4% and *E. coli* (10%). *Salmonella*, *Shigella* and *S. aureus* showed no resistance to tetracycline. *E. coli*, *Salmonella* and *Shigella* were susceptible to ampicillin whereas total coliforms and *S. aureus* showed resistance level of 52.2% and 55.6% respectively to ampicillin (Table 4).

Most of the isolates from the three markets demonstrated similar resistance pattern to erythromycin; Kondele and Kibuye (85%) and Oile 85.7%. The isolates from the three markets showed least resistance to nalidixic acid with Kondele showing 15% resistance, Kibuye 15% and Oile 14.3% and norfloxacin; Kondele (10%), Kibuye (10%) and Oile 19%.

Discussion

The study findings indicate that street vended foods in Kisumu were contaminated with coliforms, *S. aureus* and *E. coli*. This is comparable to a study which reported high prevalence of total coliforms, faecal coliforms and *S. aureus* in street vended foods sold in India [17]. Total coliforms were the dominant bacteria in the sampled street foods in our study. The presence of high counts of total coliforms can be linked to the fact that this bacteria is capable of growing at an environment with a high biological activity such as decomposing litter at 35°C and also in poor hygienic conditions of the premises as in the case of vending environment. The contamination could be introduced during handling and vending environment where by street vended foods are displaced and sold in the open air and handled by vendors with bare hands. The study findings are also comparable to that of Murray who reported that majority of food borne pathogens are *Enterobacteriaceae* including pathogens such as coliforms [18]. However, a study conducted in industrial area Nairobi, Kenya reported that total coliforms isolated from street vended foods was much higher 94% [19] in all the food sampled. In our study, total coliform isolated from the three study markets was 74% in all the food sampled.

The presence of *S. aureus* in the sampled foods might be explained by the fact this organisms form part of the normal micro flora present on and in several parts of human body including the nose, skin or mouth of some carriers. This can be introduced into the street foods during handling, processing and vending [6,20]. This is supported by the fact that cooked foods usually are subjected to cross-contamination and contamination from various sources e.g. utensils, knives, raw food stuffs, flies that sporadically land on the foods, by vendors bare hands serving and occasionally food handing by consumers. The study finding is comparable to a study done in India, which reported high prevalence of *S. aureus* in street vended foods [17]. It should be noted that pathogenic *Enterobacteriaceae* can survive on finger tips and other surfaces for varying periods of time and in some cases even after washing, supports the reports of contamination of street vended food with toxigenic *S. aureus*, the major being suppurative lesions of human beings and the environment [21,22]. Lomei [23] reported that in Darkar Senegal and India, faecal coliforms and *Staphylococcus* spp and *E. coli* were found to contaminate fruit salads and preparation waters. In this study *Salmonella*, *Shigella* and *Vibrio cholerae* were not detected in all the food sampled. This may be because their numbers were too low to be detected by the conventional methods or the organisms were absent in the food samples. A study done in Nigeria on the safety and quality evaluation of street foods sold in Zaria also reported no *Salmonella*, *Shigella* and *Vibrio cholerae* in food samples collected from mobile food sellers [3]. Similarly, a study done in industrial area, Nairobi Kenya on microbial quality of selected food borne pathogens in street vended foods did not report the presence of *Salmonella* [19].

Only 6% of the sampled street vended foods were contaminated with *E. coli*. This is lower than findings from a study in Amravati city, Ethiopia where the prevalence of *E. coli* was 41% of all the food sampled [20]. The fact that faecal coliforms were detected in 44% of the foods is an indication of potential presence of enteric pathogenic bacteria. This could be introduced during food handling by the vendors.

High counts in total coliforms, *E. coli* and *S. aureus* were obtained from ice-cream, samosas, fried *R. argenteae*, across the markets, while low counts were reported in porridge, mandazi, home baked cakes, maize and beans. The counts obtained in these foods were above the

acceptable limits compared to the Kenyan standards specification limits (KS. No. 36:1999- Shall be absent) for microbiological requirements. The high counts were probably attributed to poor personal hygiene, poor handling and cross contamination from the vendors' hands and soils from the surrounding environment. The presence of high counts in ice cream could probably be attributed to insufficient heat treatment, unhygienic materials used for packaging, water being contaminated and lack of good processing practices not being followed during the production. It could also be due to ice cream, samosas and fish being high protein foods, which form a rich source of culture media favoring bacterial growth. The presence of high counts of *S. aureus* may have resulted from insufficient pasteurization of milk or improper handling. This is in agreement with a study done in Mumbai, India [24]. The findings of this study indicated that ice-cream sold in small portions from bulk containers, exposed to the open air, had a high microbial load, indicating low hygienic quality of this product in many countries.

All the markets sampled reported higher counts of total coliforms, followed by *S. aureus* then lastly *E. coli*. Oile market reported the highest number of counts followed by Kibuye then Kondele. The reason for the high numbers of counts in Oile, Kibuye and Kondele was not determined by this study but it could be attributed to poor sanitation and lack of basic hygiene in the markets. Food stalls often lack the necessary storage, refrigerators, and cooking facilities to prevent contamination with bacteria such as *Salmonella* spp, *E. coli* which in warm, moist conditions can duplicate into many disease-bearing organisms. Due to the shortage of clean potable water, many vendors tend to re-use the water, especially for cleaning utensils [25] and used dishes and hence Kisumu municipality authority therefore needs to enforce higher standards of hygiene in the markets.

Results from this study reported that high counts were obtained from both soils, water and hand swabs samples. Higher counts in soils could be attributed to bacteria being the smallest and most numerous microorganisms in soil and are most critical in decomposing of organic residues and recycling soils nutrients. Pathogenic bacteria like *Salmonella* and *Shigella* were only recovered from the soils and not the other sources. From the results reported there was a significant correlation between *S. aureus* isolated from hand swabs and from food samples suggesting that hands may be a major source of contamination. Hand washing policy should be enforced among the food vendors. Vendors are often of poor level of education, lack licenses from public health department, are untrained in food hygiene, and practice obsolete cooking technologies under crude unsanitary conditions that needs to be followed up using Food Safety Objective (FSO) concept developed by FAO and WHO for safe street food production [26]. It has been reported that, about 85% of the vendors prepared their foods in unhygienic conditions given that garbage and dirty wastes were close to the vending stalls [27]. Similar findings were reported in this study as higher counts were reported in soil from the vending environment, however this did not lead to contamination of vended foods despite the foods offered for sale being spread on the ground over a sack. It should be noted that holding cooked and uncooked foods at ambient temperatures for 6 hours or longer without any appropriate holding temperature constitute a major critical control point of street-vending operations surveyed and these should be discouraged.

The correlation between the isolates in the foods and the isolates from the hand swabs can be attributed to unhygienic handling of the foods by the vendors resulting in cross contamination of foods. This study is in agreement with previous studies which reported that enteric bacteria isolated on the selective agar can arise from a number of sources of contamination throughout the food production process [8].

Resistance to erythromycin occurred most frequently, followed by sulfamethoxazole, chloramphenicol, ampicillin, amoxicillin, tetracycline, nalidixic acid and lastly norfloxacin. This is in line with other studies that have reported an increase in prevalence of antimicrobial resistance among enteric pathogens in Kenya [12,15]. *S. aureus* isolated from foods, soils hand swabs and water were highly susceptible to erythromycin and the results of this study indicated erythromycin will be more effective in the treatment of *S. aureus* infections. This study identified no resistance to nalidixic acid and norfloxacin among the *Enterobacteriaceae* and minimal resistance to chloramphenicol, amoxicillin, ampicillin and tetracycline. All pathogens identified from the three markets showed similar resistance patterns an indication that the source of origin could be similar. Resistance of bacteria may possibly result from inappropriate or uncontrolled use of antibiot-

ics in humans and farming practices, so it is necessary to pay more attention to food hygiene practices to eliminate the risk of spreading antibiotic resistant pathogenic bacteria through street vended food.

Food vending is wide spread in almost every street within Kisumu city, but due to limited finances and time, only selected areas were studied. In addition, different antibiotics are currently used to treat infections but due to limited resources, only a few selected antibiotics were subjected for susceptibility tests [27-34].

Conclusion

Street vended foods sampled from the three markets in Kisumu city were contaminated with coliforms, *E. coli* and *S. aureus* but not *Salmonella*, *Shigella* and *Vibrio*. Antimicrobial resistance to the commonly used antibiotics occurred in all the pathogens isolated from street foods within the target markets. Hence, there is need to determine the health status of those preparing street vended foods and issuance of trade license to only those who have been proved to be of good public health status should be encouraged by the county government since this is a source of employment in the informal sector. The present study was not able to establish source of contamination, further investigations may be necessary to discern this.

Declaration of Conflict of Interest

There is no conflict of interest to declare.

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Bibliography

1. Martins JM and Angelica LE. "Socio-economic features of street vending hygiene and microbiological status of street foods in Gauteng: Technical cooperation program project on improving street foods in South Africa". *South African Journal of Clinical Nutrition* 19 (2006): 18-25.
2. Muzaffar AT, et al. "Entrepreneurship of the streets. An analytical work of the street food vendors of Dhaka city". *International Journal of Business and Management* 4.2 (2009): 80.
3. Nicolas B., et al. "Street Vended Foods Improvement: Contamination Mechanisms and Application of Food Safety Objective strategy: Critical Review". *Pakistan Journal of Nutrition* 6.1 (2007): 1-10.
4. WHO. "The role of food safety in health development: Report of the joint FAO/WHO expert committee in food safety". Geneva (1983).
5. Canet C and N'diaye C. "Street food in Africa". *Food Nutrition and Agriculture* (1996): 17-18.
6. Bryan FL. "Risk associated with practices, procedures and processes that lead to outbreak of foodborne diseases". *Journal of Food Protection* 51.8 (1988): 663-673.
7. Singer RS., et al. "Antibiotic resistance-the interplay between antibiotic use in animals and human beings". *The Lancet Infectious Diseases* 3.1 (2003): 47-51.
8. Bryan FL., et al. "Hazards associated with holding and reheating foods at vending sites in a small town in Zambia". *Journal of Food Protection* 60.4 (1997): 391-396.

9. Chao KK and Chao CC. "Suitability of the traditional microbial indicators and their enumerating methods in the assessment of fecal pollution of subtropical fresh water environments". *Journal of Microbiology and Immunological Infections* 36.4 (2003): 288-293.
10. Gaikwad SS and Parekh YY. "Salmonellosis in the systematic form due to multiple drug resistance *Salmonella typhimurium*". *Journal of Postgraduate Medicine* 30.3 (1984): 159-162.
11. CDC. "Epidemiology and surveillance capacity development, Annual report". Atlanta (2006).
12. Steyn D., et al. "Factors which influences the consumption of street foods and fast foods in S.A- a national survey". *Nutritional Journal* 10 (2011): 104.
13. Kisumu East District Development Plan. "Kisumu East District Development plan: Effective management for Sustainable Economic Growth and Poverty Reduction" (2008-2012).
14. McComrmick BA., et al. "*Salmonella typhimurium* attached to human intestinal epithelial monolayers: Trans-cellular signaling to sub-epithelial neutrophile". *Journal of Cell Biology* 123.4 (1993): 895-907.
15. Sang WK., et al. "Multidrug-resistant enteroaggregative *Escherichia coli* associated with persistent diarrhea in Kenya children". *Journal of Emerging Infectious Diseases* 3.3 (1997): 373-374.
16. Gilbert RJ. et al. «Guidelines for the microbiological quality of some ready-to-eat foods samples at the point of sale". *Communicable Disease and Public Health* 3.3 (2000): 163-167.
17. Kumar M., et al. "Microbiological safety of street vended fruits in Patiala City". *Indian Journal of Medical Microbiology* 24.1 (2006): 75-79.
18. Murray RJ. "Recognition and management of *Staphylococcus aureus* toxin mediated disease". *International Medicine Journal* 35.2 (2005): s106-s119.
19. Gitahi MG., et al. "Microbial safety of street foods in industrial area, Nairobi". *Research Journal of Microbiology* 7.6 (2012): 297-308.
20. Tembekar DH., et al. "Bacteriological quality of street vended food punipuri: A case study of Amravita City (MS) India". *Bioscience Discovery* 2.3 (2011): 350-354.
21. International Commission on Microbiological, Specifications for foods (ICMSF). "Microorganisms in Foods5-Microbial ecology of food commodities". Blackie Academic & Professional, London (1998).
22. Mohapatra AD., et al. "Microbiological evaluation of street foods in Bhubaneswar". *Journal of Food Science and Technology* 39.1 (2002): 59-61.
23. Lomeli A. "The serious risk of street vending". Mexican Association of Studies for consumers defense (press bulletin) (2001).
24. Sandel MK and Mckilly JL. "Virulence recovery of *Staphylococcus aureus* relevant to the food industry using improvements on traditional approaches". *Journal of Food Control* 15.1 (2004): 5-10.
25. Warke K., et al. "Incidence of pathogenic psychrotrophs in ice creams sold in some retail outlets in Mumbai, India". *Food Control* 11.2 (2000): 77-83.
26. Muinde OK and Kuria E. "Hygiene and sanitary practice of vendors of street food in Nairobi Kenya". *African Journal of Food Agriculture and Nutrition Development* 5.1 (2005): 51-14.

27. Mensah P, *et al.* "Street food in Accra, Ghana; How safe they are?" *Bulletin of the World Health Organization* 80.7 (2002): 546-554.
28. Blake DP, *et al.* "Transfer of antibiotic resistance between commensal and pathogen members of the *Enterobacteriaceae* under ideal conditions". *Journal of Applied Microbiology* 95.3 (2003): 428-436.
29. Kipkorir CK, *et al.* "Epidemiology of Antimicrobial Resistance among *Escherichia coli* Strains in Trans-Nzoia County, Kenya". *Journal of Microbiology and Infectious Diseases* 6.3 (2016): 107-112.
30. Arslan S and Ozdemir F. "Prevalence and antimicrobial resistance of *Listeria species* in homemade white cheese". *Food Control* 19.4 (2008): 360-363.
31. Fischer R. "The design of experiments". Edinburgh: Oliver and Bod (1935).
32. CLSI. "Performance standards for antimicrobial disks susceptibility tests: Approved standards M2-A9". Wyne: CLSI (2009).
33. ISO6887-1. "Microbiology of food and animal feeding stuff-preparation of test samples, initial suspension and decimal dilution for microbiological examination". International Standards. 4th Edition (1999).
34. Umoh VJ and Adoba MB. "Safety and quality evaluation of street foods sold in Zaria, Nigeria". *Journal of Food Control* 10.1 (1999): 9-14.

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