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MICROBIOLOGICAL QUALITY AND SAFETY OF *RASTRINEOBOLA ARGENTEA* RETAILED IN KISUMU TOWN MARKETS, KENYA
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ABSTRACT

Objective: **To investigate faecal contamination and safety of *Rastrineobola argentea* sold in retail markets in Kisumu town.**
Design: **This was a repeated cross sectional study and based on random sampling.**
Setting: **Kisumu city, targeting six markets; Oile, Jubilee, Kibuye, Kondele, Nyalenda and Manyatta.**
Results: **A total of 60 fish samples were analysed. All the fish were found to be contaminated with *E. coli*, and in addition 6.67% of the fish products tested positive for *Salmonella*. *Shigella* was absent in all samples analysed. 26.53% of *E. coli* isolates tested were resistant to two or more antimicrobial agents tested, with the highest level of resistance detected against cotrimoxazole at 38.76%. The *E. coli* multiple antibiotic resistance (MAR) index was 0.084 indicating that the contamination was not originating from a high – risk source. A plasmid of approximately 5.6 kb was commonly isolated from *E. coli* isolates that showed resistance to ampicillin. Plasmids isolated were not transferable by conjugation.**
Conclusion: **The presence of *Salmonella* spp and occurrence of MDR *E. coli* were identified as some of the possible health risks that may be associated with *R. argentea* displayed for sale in Kisumu city markets. This possess a real health risk through consumption or directly through contact with the fish products.**

INTRODUCTION

Rastrineobola argentea (*omena* or *dagaa*) is among the most important commercial fish species of Lake Victoria, it is a relatively cheap source of animal protein for man and livestock and its demand is substantially increasing, as other protein sources such as beef and poultry get expensive (1). The product is marketed at retail markets as whole sun dried fish. Although *R. argentea* plays an important role in the socio-economic development of rural areas and is of critical importance in meeting the food security and nutritional requirements of riparian communities, data on health risks associated with consumption of fish and fish products particularly *R. argentea* is lacking. Several studies have reported on incidences of pathogenic bacteria such as *Staphylococcus* spp, *Shigella* spp, *Salmonella* spp and *Vibrio* spp in fish and fishery products and these could pose major health risks to consumers (2, 3). *Rastrineobola argentea* fishing takes place at night using canoes that do not have any form of cold storage for the catch. For preservation drying is usually done on the ground at the landing beaches leading to contamination with sand, flies and possibly

microorganisms. The dried products are similarly exposed to further contamination during storage, transportation and sale at open-air markets (4).

Food products that show evidence of faecal contamination are generally regarded as a greater risk to human health, as they are more likely to contain human-specific enteric pathogens. Indicator microorganisms in food microbiology have been used to predict the presence of potential risks associated with pathogenic microbes. Among the enteric bacteria, *Escherichia coli* is always considered to be of faecal origin, and exists only transiently in other environments (5). *E. coli* is also found in abundance in almost any moist environment, notably soil, water and the domestic environment (6).

A high prevalence of antibiotic resistance is often encountered in *Enterobacteriaceae* of human origin (7,8). Irrational usage of antibiotics has been identified as an important factor that promotes the emergence, selection and dissemination of antibiotic – resistant microorganisms in both veterinary and human medicine (9, 10). Locally, few resistance data exist for populations outside of the clinical environment, though this is vitally important for understanding the dynamics of the spread of antimicrobial resistance. The aim of the present study was to document the level of faecal contamination of sundried *R. argentea* based on faecal coliform counts, and presence of enteric pathogens including *E. coli, Salmonella* and *Shigella spp*. The study also investigated presence of plasmids as mediators of resistance to antimicrobials among bacterial isolates.

MATERIALS AND METHODS

Collection of fish samples: Samples of *R. argentea* were collected from six fish markets in Kisumu town. A total of 60 fish samples were purchased in regular consumer packages (500g tins - approximately 100 - 150g of *R. argentea).* The samples were immediately transported aseptically to the Fish Quality Laboratory in Kisumu for analysis. The sample design was a repeated cross sectional study based on random sampling.

Bacteriological analysis: The analysis of omena products for faecal coliforms (FC) and *E. coli* were performed according to FDA bacteriological analytical manual (11) using violet red bile agar (VRBA) (Oxoid, Basingstoke, UK) and incubated at 44.5°C for 48 hours. Characteristic *E. coli* colonies were selected for further identification and confirmation by morphological and biochemical tests (12). Presence of *Salmonella* and *Shigella* was detected using the FAO food and nutrition paper 14/4 rev.1 (13). Briefly, selenite cystine broth (Oxoid) and tetrathionate broth (Oxoid) were used for enrichment of fish samples. Xylose Lysine Desoxycholate (XLD) agar (Oxoid,) Mac Conkey (Oxoid) and Bismuth sulfite (BS) agar (Oxoid) were used for selection of all presumptive positives cultures and colonies were then identified by biochemical and serological tests (12).

Antibiotic susceptibility testing: One *E. coli* colony was chosen randomly from each sample. The standard Kirby-Bauer disk diffusion method was used to determine the susceptibility to ten antimicrobial agents (ampicillin 10μg, tetracycline 30μg, cotrimoxazole 25μg, augmentin 30μg, gentamicin 10μg, kanamycin 30μg, cefuroxime 30μg, chloramphenicol 30μg, nalidixic acid 30μg and norfloxacin 10μg) using the protocol in the NCCLS manual (14). The plates were incubated at 37°C for 18 hours. A standard reference strain of *E. coli* (ATCC 25922) was used as control for growth of bacteria and potency of antibiotic disks.

Plasmid analysis: Plasmids were isolated using the method of Kado and Liu (15). Samples were analysed by electrophoresis on horizontal 1% agarose gel in 0.5 X TBE buffer at 125V for 2 hours. Plasmid sizes were determined by co-electrophoresis with plasmids of known sizes for *E. coli* strains V517 (53.7, 7.2, 5.6, 3.9, 3.0, 2.7, 2.1 kb) and 39R861 (147, 63, 43.5, 6.9 kb). DNA bands were visualised with an ultraviolet transilluminator (UVP inc.) after staining with 0.05% ethidium bromide.

In-vitro conjugative experiments: To determine mobility of antibiotic-resistance genes, *in-vitro* conjugation was carried out as described previously by Yamamoto and Yokota (16) using *E. coli* K-12 (nalidixic acid resistant) as recipient. Transconjugants were then selected on MacConkey agar supplemented with nalidixic acid (30µg/ml) and ampicillin (30µg/ml). To determine the transferable antibiotic resistance, transconjugants were tested for susceptibility to the battery of antibiotics previously used for isolates.

Statistical analysis: The data collected was entered in Microsoft Excel (windows XP Professional) spreadsheet and analysed and confidence levels of 95% were considered significant. The means (µ) FC and antimicrobial agents for the *R. argentea* were estimated for each market. Analysis of variance was used to determine differences between and within markets and antimicrobial agents tested respectively. The MAR index was calculated based on the formula described by Krumperman (17); a / (b x c), where a; is the aggregate antibiotic resistance score of all isolates from the sample, b; is the number of antibiotics, and c is the number of isolates.

RESULTS

All the markets surveyed showed FC means above those recommended by the Kenya Bureau of Standards zero (0) faecal coliforms (Table 1). Oile market

demonstrated the highest levels of contamination (log_{10} 4.33 cfu/g) whereas Kibuye market registered the lowest level at log_{10} 3.62 cfu/g. The study did not find any significant difference among the sampled markets relating to the faecal coliform load (FC) [P >0.05]. *E. coli* were isolated from all the 60 fish products examined, while *Salmonella* was present in four samples (6.67%). Two isolates were identified as *serovar enteritidis;* one each as *serovar typhimurium* and *serovar paratyphi* B. No *Shigella* spp was isolated from the samples.

All *E. coli* isolates were fully susceptible to chloromphenicol, nalidixic acid and norfloxacin (Table 2). A total of 12 distinct antibiogram patterns were observed (Table 3). Fourty seven percent isolates showed resistance to at least one or more antimicrobial agent tested. Isolates were most frequently resistant to cotrimoxazole (38.8%), tetracycline (20.4%) and ampicillin (12.2%). 26.5% of the isolates tested showed resistance to two or more antimicrobial agents. Multiple antibiotic resistance (MAR) index of 0.084 was obtained for the *E. coli* contamination.

No plasmid was transferable by conjugation. A total of six different plasmid profiles were seen among the *E. coli* isolates. All the isolates that showed resistance to ampicillin had a common plasmid of approximately 5.6kb. The most frequent plasmids observed among the isolates were those of approximately 5.6kb or less; plasmids of molecular weights greater than 5.6 kb were observed in only two isolates.

Table 1

Levels of faecal contamination of R. argentea by various markets sampled in Kisumu city (n = 10 for each market)

Markets	Range	Mean	Mean Log_{10} cfu/g
Oile	$2.5 \times 10^2 - 6.4 \times 10^5$	1.34×10^5	4.33 ± 1.07
Jubilee	$1.0 \times 10^1 - 5.0 \times 10^5$	1.87×10^5	4.27 ± 1.54
Kibuye	$2.3 \times 10^2 - 8.8 \times 10^5$	1.03×10^5	3.62 ± 1.23
Kondele	$2.0 \times 10^1 - 7.2 \times 10^5$	1.80×10^5	4.33 ± 1.48
Nyalenda	$2.8 \times 10^2 - 8.4 \times 10^5$	1.60×10^5	4.32 ± 1.13
Manyatta	$1.8 \times 10^2 - 4.0 \times 10^5$	7.92×10^4	3.91 ± 1.08

Table 2

Antimicrobial susceptibility of E. coli isolates by antimicrobial agent

Antimicrobial agent	Resistant		Intermediate		Susceptible	
	No.	(%)	No.	(%)	No.	(%)
Ampicillin	6	12.24	0	0	43	87.76
Tetracycline	10	20.41	11	22.45	28	57.14
Cotrimoxazole	19	38.76	2	4.08	28	57.14
Augmentin	2	4.08	5	10.2	42	85.71
Kanamycin	2	4.08	25	51.02	22	44.90
Gentamicin	1	2.04	3	6.12	45	91.84
Cefuroxime	3	6.12	43	87.76	3	6.12
Chloromphenicol	0	0	0	0	49	100
Nalidixic acid	0	0	0	0	49	100
Norfloxacin	0	0	0	0	49	100

Table 3

Antibiogram patterns of E. coli isolates from R. argentea

Resistance pattern	No. of strains	Resistance pattern	No. of strains
Cot	9	Amp + Cot	2
Crx	1	Tet + Cot	3
Tet	1	Amp + Tet + Cot	2
Cot+Kan	1	Amp + Tet + Aug	1
Tet+Aug	1	Tet + Crx	1
Amp+Tet+Cot+Gen	1	Cot +Kan + Crx	1

Cot = Cotrimoxazole, Crx = Cefuroxime, Tet = Tetracycline, Kan = Kanamycin, Aug = Augmentin, Gen = Gentamicin, Amp= Ampicillin

DISCUSSION

Faecal coliform contamination were observed in all the 60 *R. argentea* samples tested. This would imply that the fish being sold in Kisumu city markets is of low microbial quality and does not comply with the locally laid down standards for sundried *R. argentea* (18). The source of contamination of the fish products was common among the markets. This could be attributed to the operations through which the fish undergoes during harvesting, processing and handling (19, 20). Good hygiene practices (GHP) and hazard analysis critical control point (HACCP) are not operational along the production, processing and distribution line and these may explain such findings. GHP and HACCP are important tools for managing and ensuring that food products are produced under hygienic conditions and that food safety measures are in place to address all possible risks that may be associated with the food product.

The study however observed that the contamination based on the MAR indexing of *E. coli* was lower than 0.200, suggesting that the contamination may have arisen from a low risk source (17). The occurrence of *Salmonella* spp in some samples collected from the market and absence of *Shigella* spp strongly supports the existence of other high-risk sources within the geographical limits of the study that are not exposed directly to antimicrobials. *Salmonella* have a wide range of warm-blooded animals, including human beings as hosts whereas *Shigella* spp are restricted to higher primates, including humans and usually spread among humans by food handlers with poor personal hygiene (11). Based on our findings we would like to suggest that MAR indexing of *E. coli* alone may not be a good indicator of risk assessment of food products and especially those sourced from the wild, as there are other risk sources that MAR indexing of *E. coli* may not be able to detect since the intensive rearing of livestock is least practiced in the region.

In this study *E. coli* isolates showed relatively low level of resistance to antimicrobial agents tested compared to clinical isolates in other studies conducted locally (21). Our study agrees with Österblad *et al.*, (22) who found a very low frequency of antimicrobial resistance in *Enterobacteriaceae* isolated from vegetables although very few *E. coli* were isolated suggesting that faecal contamination was rare. The prominence of co-trimoxazole, tetracycline and ampicillin resistance among *E. coli* isolates in our study demonstrates the similarities in the development of resistance among the isolates and the *E. coli* of clinical importance. We therefore postulate that this exposure to antibiotics might be of a human origin. This is further supported by the presence of resistance to co-trimoxazole, a synthetic antibiotic generally used in humans only. The differences in levels of resistance and resistance patterns could be due to levels of exposure to the agents or other factors that may have increased or decreased the likelihood of the development and conservation of resistant bacteria. *R. argentea* is not exposed directly to antibiotics but may be contaminated with antibiotic resistant bacteria through personnel, polluted fishing grounds or animal droppings.

The study also found that isolates recovered from Kondele market showed the smallest mean diffusion zones for all the antimicrobial agents except for cefuroxime. This may indicate that there were other sources or practices that may have contributed to contamination of products or exposure to resistant bacteria within this market, which were not sampled in this study. Rysz and Alvarez, (23) demonstrated that bacteria in the soil can acquire resistance to tetracycline from environmental exposure, possibly creating a reservoir of resistance factors generated outside host animals. General observations indicated that the market is not designated and is situated on a road reserve; no sanitary facilities and solid waste management measures were available. The study also found significant differences in rates of *E. coli* resistance to tetracycline within the markets ($P < 0.05$). Tetracycline has for along time been used as a first-line antibiotic for many different species of domestic animals. Resistance to tetracycline is plasmid mediated, with a wide variety of genetic determinants, making it more prone for susceptible bacteria to acquire these resistance factors (24).

A plasmid of approximately 5.6 kb was more frequent among the isolates. Sherley *et al*., (25) observed that the majority of *E. coli* strains wild-type carried one or more plasmids, which varied in size, but 45% of the isolates from the plasmid bearing strains had plasmids smaller than 16 kb in size. In contrast, similar studies on clinical isolates involving the *Enterobacteriaceae* family have found plasmids of 100kb – 110 kb responsible for conjugative transfer of ampicillin, tetracycline, cotrimoxazole, and chloromphenicol resistance (21, 26, 27). Since the isolates were obtained from *R. argentea* sourced from markets, and based on the fact that the bacterial contamination may be from rural sources (based on MAR index of *E. coli*) such as the villages along the lake shores where the fish was processed, it is possible that the bacterial isolates may not favourably maintain large conjugative plasmids encoding for resistance to antimicrobial agents. However, in other studies small plasmids of < 10kbp have been reported to mediate single cross-resistance to ampicillin, tetracycline, sulfonamide and streptomycin (28).

In conclusion, our data provides evidence for significant faecal contamination and the presence of *Salmonella*, and antimicrobial resistant *E. coli* in *R. argentea* sold on markets in Kisumu, posses a real health risk through consumption or directly through contact with the fish products including livestock that may feed on contaminated animal feeds produced from *R. argentea*. It will be important for public health workers to create awareness for the need to institute GHP and HACCP as tools for ensuring that fish

products are produced under hygienic conditions and that food safety measures are in place. Long term prospective studies to examine isolates from *R. argentea*, including microbial source tracking, are required to give more accurate, temporal and spatial information on the levels and characteristics of faecal contamination.

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