

# Semiquantitative Analysis of Gaps in Microbiological Performance of Fish Processing Sector Implementing Current Food Safety Management Systems: A Case Study

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## ABSTRACT

Fish processing plants still face microbial food safety–related product rejections and the associated economic losses, although they implement legislation, with well-established quality assurance guidelines and standards. We assessed the microbial performance of core control and assurance activities of fish exporting processors to offer suggestions for improvement using a case study. A microbiological assessment scheme was used to systematically analyze microbial counts in six selected critical sampling locations (CSLs). Nine small-, medium- and large-sized companies implementing current food safety management systems (FSMS) were studied. Samples were collected three times on each occasion ( $n = 324$ ). Microbial indicators representing food safety, plant and personnel hygiene, and overall microbiological performance were analyzed. Microbiological distribution and safety profile levels for the CSLs were calculated. Performance of core control and assurance activities of the FSMS was also diagnosed using an FSMS diagnostic instrument. Final fish products from 67% of the companies were within the legally accepted microbiological limits. *Salmonella* was absent in all CSLs. Hands or gloves of workers from the majority of companies were highly contaminated with *Staphylococcus aureus* at levels above the recommended limits. Large-sized companies performed better in *Enterobacteriaceae*, *Escherichia coli*, and *S. aureus* than medium- and small-sized ones in a majority of the CSLs, including receipt of raw fish material, heading and gutting, and the condition of the fish processing tables and facilities before cleaning and sanitation. Fish products of 33% (3 of 9) of the companies and handling surfaces of 22% (2 of 9) of the companies showed high variability in *Enterobacteriaceae* counts. High variability in total viable counts and *Enterobacteriaceae* was noted on fish products and handling surfaces. Specific recommendations were made in core control and assurance activities associated with sampling locations showing poor performance.

Global trade in fishery commodities reached US\$58.2 billion in 2002 (2). A net trade surplus of US\$17.4 billion was registered by developing countries in 2002, which accounted for almost 50% by value and 55% of fish exports by volume (2). The total annual production of fish in Kenya is approximately 180,000 metric tons that earns the country about US\$50 million of foreign exchange through export (3, 31). About 10% (13 million metric tons) of the world's total fish production is lost due to spoilage (3). In 2001 and 2002, seafood was responsible for about 1/10 of the rejected food imports to the United States.

A quarter of the rejections can be attributed to detection of *Salmonella*, the second cause of rejection after presence of filth, which constitutes 50% of rejections (10). Consumption of unwholesome fish and fishery products accounts for as much as 30% of worldwide foodborne illnesses (3).

A major goal of food processing companies is to control microorganisms in order to provide safe, wholesome,

and acceptable food to consumers. However, this can be very challenging as contamination of products take place at all stages of the food chain (41). Cross-contamination may occur during food processing or preparation, when bacteria are transferred from raw fish or contaminated surfaces and from utensils to uncontaminated fish. The pathogens may proliferate and reach infective levels in the presence of ideal conditions, such as inadequate methods of handling, hygiene, sanitation, and distribution (43). Fish may be contaminated with pathogens such as *Salmonella* spp., *Staphylococcus aureus*, *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, and *Listeria monocytogenes*, which occurs at various stages in the value chain, including preharvest, during capture, processing, distribution, and/or storage (41).

There was a series of restrictions on fish exports from Kenya by the European Commission between 1997 and 1999 due to poor food safety management systems (FSMS) in the fish chain. In such circumstances, response by the government and the private sector is largely as a result of regulatory changes or demand from major customers (8). The most significant regulations for the Kenyan fisheries sector are

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TABLE 1. Characteristics of fish companies in which the effectiveness of existing FSMS was studied using the microbiological assessment scheme<sup>a</sup>

Company	Total employees (n)	QA standards implemented	QA department personnel (n)	Main product group	Installed capacity per day (metric tons)
A	50–249	HACCP, ISO 22000:2005	5	Nile perch	L
B	10–49	HACCP, PRP, ISO 9001	3	Frozen octopus	S
C	10–49	HACCP, PRP	4	Frozen octopus	M
D	50–249	HACCP, PRP, ISO 22000:2005	12	Nile perch	L
E	10–49	HACCP, PRP	6	Nile perch	S
F	50–249	ISO 22000:2005	8	Nile perch	M
G	50–249	HACCP, PRP, ISO 22000:2005	5	Nile perch	M
H	10–49	HACCP, PRP	3	Frozen octopus	L
I	50–249	HACCP	1	Frozen octopus, fin fish	S

<sup>a</sup> HACCP, hazard analysis and critical control points; PRP, prerequisite programs; S, small size (installed capacity of 0.5 to 10 metric tons/day); M, medium size (installed capacity of 10 to 30 metric tons/day); L, large size (installed capacity of 30 to 60 metric tons/day).

European Union (EU) directives 91/493/EEC and 98/83/EEC that establish the requirements for handling and marketing of fishery products. The regulations are enforced by the Fisheries Department, with periodic audits by EU inspectors (3). However, the sector still faces constraints in reducing postharvest losses, fish safety, and quality assurance (31).

Such safety and quality challenges still persist despite current FSMS, in the form of legislation, with well-established quality assurance guidelines and standards, being implemented efficiently. Adoption of improved scientific tools and novel flexible approaches to safety is, therefore, needed to ensure regulatory actions reflect the most current scientific evidence. Improved scientific tools for diagnosis of microbial performance of a FSMS and improvement of existing control and assurance systems have recently been described in the European Commission project Pathogen Combat (20, 21). A microbiological assessment scheme (MAS) is used for systematic analysis of microbial counts in the FSMS. The principle behind MAS is that an effective FSMS results in products with lower contamination levels and less variation in contamination loads (25). The target of the systematic evaluation of microbiological performance is on selected critical sampling locations (CSLs) and further links the information to a descriptive diagnosis of the FSMS. This quantitative risk profiling identifies the risk of a product or process with the aim of providing remedial measures. This is in contrast to the traditional practice of final product microbial testing aimed at acceptance or rejection of a batch (21). The analysis provides in-depth understanding of the contamination profiles, taking into account the distribution in microbial contamination and maximum level of microbial counts in an implemented FSMS. A descriptive FSMS diagnostic instrument enables assessment of core control and assurance activities to provide insight in the performance of crucial safety management activities, in view of the riskiness imposed by context factors in the environment wherein the company operates (29). The combined MAS and FSMS diagnostic instrument diagnosis provides directions for improvement toward more effective FSMS and reduced riskiness in the context environment independently from the implemented FSMS (29).

The MAS tool has been effectively validated and important insight gained into the performance of existing FSMS in food service establishments (25), pork processing plants (20), lamb processing chain (35), poultry processing (38), and dairy industry (34). The objective of this study was to evaluate the microbiological performance of a fish exporting sector, applying current FSMS to examine the effectiveness of control and assurance activities and suggest potential improvements.

## MATERIALS AND METHODS

**Description of fish processing plants.** Nine companies were grouped into three classes representing small-, medium-, and large-sized companies. This was based on varying installed and utilized capacities, the number of employees, and FSMS (e.g., hazard analysis and critical control points [HACCP], prerequisite programs [PRP], and International Organization for Standardization [ISO] standards, EU, and government regulation; Table 1). The companies handle different fish species, including Nile perch, prawns, lobsters, octopus, cuttlefish, and squids. The major fish type per company is shown in Table 1.

**CSLs.** CSLs are points in which loss of control may lead to unacceptable food safety problems due to contamination with or growth and/or survival of microorganisms. A total of six CSLs were selected. CSL 1 was receipt of raw fish materials (skin intact) that was expected to provide information on the potential safety risks posed by the fish raw materials. It was also selected to establish fitness of use and effectiveness of the raw material control measures, such as supplier procedures and fish specification. Sampling at this point helps to determine the initial contamination level with microorganisms and verifies appropriate supplier selection (20, 25).

CSL 2 was at the heading and gutting stage. This stage may be a potential source of cross-contamination, especially from fish contact surfaces, equipment, and fish handling operators. Analysis of samples from this intermediate product point was expected to indicate effectiveness of the implemented preventive measures, such as personal hygiene, sanitation programs, specific preventive measures for fish, and hygienic design of equipment and facilities (20).

CSL 3 was the final fish product after candling, trimming, and the final washing. The location was expected to indicate performance of the overall technological and managerial control activities implemented to reduce microbial contamination and/or

multiplication in the FSMS (20). In addition, the location is an important indicator for final product safety and quality (34).

CSL 4 consisted of fish contact surfaces, including processing tables and facilities before cleaning and sanitation. Working surfaces, such as packaging tables, are usually the most contaminated among food contact surfaces and are believed to allow cross-contamination because they are in direct contact with food (7). The surfaces are also frequently handled by workers. Microorganisms can, therefore, directly contaminate the fish, especially if the surfaces are moist. The location was therefore selected to indicate the actual status of hygienic design of the tables and facilities (20).

CSL 5 was processing tables and facilities after cleaning and disinfection. The location provided insight on the adequacy of cleaning and sanitation procedures present as a preventive measure in the FSMS (20). CSL 6 was the operator's hands or gloves. Personnel skin may harbor various microorganisms that can be transmitted to fish and environment through hands. Hands can also act as a source of *S. aureus* (1). The location was selected to provide insight into the performance of personal hygiene as a preventive measure in the FSMS (20).

**Selection of microbiological parameters.** Microorganisms indicative of fish safety problems were monitored. *Salmonella* spp. was selected as a fish safety indicator. High incidences of *Salmonella*-related foodborne outbreaks from fish and seafood consumption have been reported (7). In addition, *Salmonella* outbreaks have been linked to fish bans by importing countries (9). *E. coli* and *Enterobacteriaceae* were analyzed as hygiene indicators (12). Total viable counts (TVCs) were analyzed as indicators of overall microbiological performance (utility parameter) (12) and *S. aureus* as an indicator of personal hygiene (1).

**Sampling frequency.** Samples (324) were collected in three visits per company over a 4-month period. In each visit, fish raw materials, fish at the heading and gutting stage, and fish fillets after final washing were sampled once per day for three different days ( $n = 81$ ). Surfaces of facilities and working tables before and after cleaning and the operator's hands or gloves were sampled three times, including once in the morning, afternoon, and evening to capture the daily variations for three different days ( $n = 243$ ).

**Sampling and analytical methods.** Environmental sampling from surfaces was done by contact plates and swabs using horizontal method for collecting samples in accordance with ISO 18593:2004 (18). The method was also applied in detection and enumeration of viable microorganisms from food contact surfaces. A sampling area of 50 cm<sup>2</sup> was used for facilities, crates, and tables, while 10 cm<sup>2</sup> was used for knives due to limited surface area. Swabs covered 25 cm<sup>2</sup> (5 by 5 cm<sup>2</sup>) of hands or gloves for each food handler. The swabbing area was delineated by a sterilized steel template. The swabs were returned aseptically into their tube, stored, and transported in a cool box at  $\leq 4^{\circ}\text{C}$  to the laboratory for microbial analyses. ISO 6887-3:2003 (17) was used in the preparation of fish and fishery products for microbiological examination. This involved preparation of test samples, initial suspensions, and decimal dilutions for microbiological examination.

For enumeration purposes, ISO 4833:2003 (15) was used for total viable count (TVC), where colony counts were done on plate count agar. ISO 16649-2:2001 (13) was used for enumeration of *E. coli*, which involved plating on tryptone bile X-glucuronide agar (Oxoid, Basingstoke, Hampshire, UK) and subsequent calculation of the number of CFUs of presumptive *E. coli* per gram or per milliliter of sample. ISO 6579:2002 (14) was used for detection of *Salmonella*

spp. After selective enrichment, the culture was streaked onto solid selective media, where the presence of *Salmonella* spp. was checked after incubation. ISO 21528-2:2004 (19) was used for *Enterobacteriaceae* enumeration. Plating was done on solid selective culture medium (violet red bile glucose [Oxoid]) followed by colony counts of the number of confirmed typical colonies per plate. EN ISO 6888-1:1999 (16) was used for enumeration of *S. aureus*. Test samples were surface plated on Baird Parker agar (Oxoid) supplemented with egg yolk tellurite emulsion. The number of coagulase-positive staphylococci was then calculated and expressed as CFU per gram or milliliter sample after incubation.

**FSMS diagnosis.** A previously described FSMS diagnostic tool (29) was used to diagnose core control and core assurance activities and riskiness in context wherein the food safety management of the firms operate. Indicators of context risk, core control, and core assurance activities and food safety management output are shown in Supplementary Table 1 (available at: [https://www.researchgate.net/publication/259969613\\_Semi-quantitative\\_analysis\\_of\\_gaps\\_in\\_microbiological\\_performance\\_of\\_fish\\_processing\\_sector\\_implementing\\_current\\_food\\_safety\\_management\\_systems\\_case\\_study/file/ef31752ebb59e8a57b.pdf?origin=publication\\_detail](https://www.researchgate.net/publication/259969613_Semi-quantitative_analysis_of_gaps_in_microbiological_performance_of_fish_processing_sector_implementing_current_food_safety_management_systems_case_study/file/ef31752ebb59e8a57b.pdf?origin=publication_detail)). This enabled assessment independent of the implemented company-specific FSMS. The FSMS diagnosis involved an interview with the responsible quality assurance (QA) personnel of the respective companies. The QA managers responded to and chose the FSMS activity level and context risk level for each indicator. This was followed by onsite confirmatory visits (29).

**Data processing and interpretation of the results.** Microbial safety level profiles (MSLP) were calculated according to Jacxsens et al. (20), Lahou et al. (25), and Opiyo et al. (34). Figures were made using Microsoft Office Excel 2007 (Microsoft, Redmond, WA) to visualize the levels and distribution of microbial contamination at the CSLs. Counts from each analyzed microbiological parameter in a specific CSL were compared with legal requirements or guideline values shown in Table 2. If the legal requirements or the guide values were exceeded for a specific microorganism in a CSL, it indicated that the specific control activity in the FSMS dedicated toward the defined CSLs was not effective.

A score attribution system was used to evaluate the results (Table 3). A score of 0, which indicated poor performance, was assigned when the legal criteria or the guideline values were exceeded for a particular microorganism at a specific CSL or when *Salmonella* was present. This meant that specific hygiene practices of control activities in the FSMS at that location were inadequate; therefore, corrective action(s) was required to address the situation toward improved FSMS. A score of 1 was given when the microbial counts were equal to the maximum level considered marginally acceptable, indicating average to poor performance. An average performance score of 2 was given whenever the counts were less than the maximum level considered marginally acceptable but more than the maximum level considered acceptable. A score of 3, indicating good performance, was awarded when bacterial counts were below the minimum acceptable value for a specific microorganism at a specific CSL or when *Salmonella* was absent. The results for each analyzed parameter were then summed up for the six CSLs to create the MSLP. The maximum MSLP score was 15 because five microbiological parameters were analyzed per CSL with a maximum assigned score of 3 each.

## RESULTS AND DISCUSSION

We assessed the microbiological quality of fish independent of FSMS implemented by the fish processors

TABLE 2. Legal requirements or guideline values and analytical methods for microbial parameters at critical sampling locations selected for microbial assessment in nine fish industries<sup>a</sup>

Microbial parameters	Analytical method	Criteria (log CFU/cm <sup>2</sup> ) <sup>b</sup>			
		CSL 1	CSL 2 and 3	CSL 4 and 5	CSL 6
Total viable bacteria	ISO 4833:2003	$m = 5.0^c$ ; $M = 7.0^c$	$M = 6.0^d$	Good, $\leq 1$ ; average, $\leq 1.8$ ; bad, $\leq 2.5$ ; intolerable, $> 2.5$	Good, $\leq 1$ ; average, $\leq 1.8$ ; bad, $\leq 2.5$ ; intolerable, $> 2.5$
<i>Enterobacteriaceae</i>	ISO 21528-2:2004	$M = 2.0^d$	$M = 2.0^d$	Good, $\leq 1$ ; average, $\leq 1.8$ ; bad, $\leq 2.5$ ; intolerable, $> 2.5$	Good, $\leq 1$ ; average, $\leq 1.8$ ; bad, $\leq 2.5$ ; intolerable, $> 2.5$
<i>E. coli</i>	ISO 16649-2:2001	$m = 1.0^c$ ; $M = 2.5^c$	$M = 1.0^d$	A	A
<i>Salmonella</i>	ISO 6579:2002	Absent in 25-g sample <sup>d</sup>	Absent in 25-g sample <sup>d</sup>	A	A
<i>S. aureus</i>	EN ISO 6888-1:1999	$m = 3.0^c$ ; $M = 4.0^c$	$m = 3.0^c$ ; $M = 4.0^c$	A	A

<sup>a</sup> CSL 1, fish at arrival with intact skin (log CFU per square centimeter); CSL 2, fish fillets at heading and gutting (log CFU per gram); CSL 3, fish fillets after washing (log CFU per gram); CSL 4, working tables and facilities surfaces before cleaning and disinfection (log CFU per square centimeter); CSL 5, working tables and facilities surfaces after cleaning and disinfection (log CFU per square centimeter); CSL 6, hands or gloves of operators (log CFU per square centimeter);  $m$ , maximum level of bacteria per test volume considered acceptable;  $M$ , maximum level of bacteria per test volume considered marginally acceptable (values at or above  $M$  are unacceptable); A, absent on 10, 25, or 50 cm<sup>2</sup>.

<sup>b</sup> According to microbiological guide values of LFMFP-U Ghent for food service operation (40).

<sup>c</sup> According to International Commission on Microbiological Specifications for Foods (11).

<sup>d</sup> According to Kenya legal standards KS 1399-1:2012 (24) and KS 1399-2:2012 (23).

using MAS for raw materials, fish at the heading and gutting stage, fillets after washing, fish contact surfaces before and after cleaning and sanitation, and hands or gloves of fish handlers. Table 4 presents detailed results of microbial performance of the nine fish companies at these CSLs. The FSMS diagnostic tool further described context factors, core control and assurance activities, and food safety output, which has an influence on the microbial performance. Detailed results for FSMS diagnosis of contextual factors, core control and assurance activities and food safety output in fish companies are presented in Supplementary Table 1.

*Salmonella* was absent in all the CSLs at all the companies; therefore, they had good performance (score 3) for this safety indicator. Additionally, at receipt of raw fish materials (CSL 1), all the companies had good performance (score 3) in *S. aureus* counts. At this CSL, 67% of companies had good performance (score 3) in *E. coli* and *Enterobacteriaceae* counts. The rest (33%) had average performance (score 2) and poor performance (score 0) in terms of *E. coli* and *Enterobacteriaceae*, respectively. For TVCs, 89% of the companies had good performance (score 3), with 11% performing averagely (score 2). Absence of *Salmonella*, which is a pathogen in all the CSLs, might have

indicated adequate raw material control measures. Cooling facilities were effective in all the processing plants. This was coupled with effective translation of external requirements, such as the EU hygiene directive (91/493/EEC and 98/83/EEC) into internal company FSMS. The pathogen has previously been reported as one of the causes of bans in fish exports from Kenya to the EU (9). The majority of the companies used clearly defined sampling frequency, location, analysis, and rejection criteria based on actual historical data of suppliers. Clearly defined sampling frequency based on actual historical data of suppliers results in more predictable food safety outcomes due to less ambiguity and uncertainty (27). In addition to EU 91/493/EEC and 98/83/EEC, the companies effectively applied various QA requirements, such as Kenya Fisheries Act Cap 378, HACCP, PRPs, and ISO standards 9000, 9001, and 22000:2005.

At the heading and gutting stage (CSL 2), 89% of companies performed well (score 3), but 11% of the companies performed poorly (score 0) in their *E. coli* and *Enterobacteriaceae* counts (Table 4). All companies showed good performance (score 3) in TVCs at this CSL. There was stable hygienic performance of equipment and facilities at CSL 2, and hygiene performance tests were conducted regularly according to Kenya Bureau of Standards. Stable

TABLE 3. Score attribution system for assignment of microbial food safety level profile scores<sup>a</sup>

Score	Benchmark	Performance level	Food contact surfaces <sup>b</sup>
0	$R > M$ , organism present in $x$ grams or on the surface	Poor	Present on surface
1	$R = M$	Poor to average	
2	$m < R < M$	Average	
3	$R < m$ , organism absent in $x$ grams and on the surface	Good	Absent on surface

<sup>a</sup>  $R$ , results obtained from analysis;  $m$ , maximum level of bacteria per test volume considered acceptable;  $M$ , maximum level of bacteria per test volume considered marginally acceptable (values at or above  $M$  are unacceptable).

<sup>b</sup> Specifically for *E. coli*, *S. aureus*, and *Salmonella*.

TABLE 4. Detailed results of microbial performance of nine fish companies at CSLs

CSL <sup>a</sup>	n	Food safety indicator <sup>b</sup>	Hygiene indicators <sup>c</sup>			Overall indicator <sup>e</sup>
		<i>Salmonella</i>	<i>E. coli</i>	<i>Enterobacteriaceae</i>	<i>S. aureus</i>	TVC
Company A						
CSL 1	3 × 1	A	<1.0	62-TNTC	<1.0	5.8 × 10 <sup>4</sup> –6.3 × 10 <sup>4</sup>
CSL 2	3 × 1	A	A	A	A	<1.0 × 10 <sup>1</sup>
CSL 3	3 × 1	A	13–54	A	A	2.1 × 10 <sup>4</sup> –6.2 × 10 <sup>4</sup>
CSL 4	3 × 3	A	A	A	<1.0	<1.0 × 10 <sup>1</sup>
CSL 5	3 × 3	A	A	A	A	1.0 × 10 <sup>1</sup> –2.0 × 10 <sup>1</sup>
CSL 6	3 × 3	A	NIL–3	NIL–15	<1.0	1.8 × 10 <sup>3</sup> –7.4 × 10 <sup>3</sup>
Company B						
CSL 1	3 × 1	A	5–87	NIL–108	A	5.4 × 10 <sup>4</sup> –6.7 × 10 <sup>7</sup>
CSL 2	3 × 1	A	23–67	A	<1.0	4.5 × 10 <sup>3</sup> –4.8 × 10 <sup>4</sup>
CSL 3	3 × 1	A	2–8	A	A	2.3 × 10 <sup>2</sup> –4.0 × 10 <sup>2</sup>
CSL 4	3 × 3	A	NIL–4	NIL–122	<1.0	A
CSL 5	3 × 3	A	A	A	A	A
CSL 6	3 × 3	A	NIL–3	NIL–3	A	2.0 × 10 <sup>1</sup> –3.1 × 10 <sup>2</sup>
Company C						
CSL 1	3 × 1	A	A	<3	2.4 × 10 <sup>1</sup> –4.6 × 10 <sup>1</sup>	4.5 × 10 <sup>3</sup> –5.6 × 10 <sup>3</sup>
CSL 2	3 × 1	A	A	<3	1.9 × 10 <sup>1</sup> –3.6 × 10 <sup>1</sup>	4.1 × 10 <sup>3</sup> –5.2 × 10 <sup>3</sup>
CSL 3	3 × 1	A	A	NIL–2	A	<2.1 × 10 <sup>3</sup>
CSL 4	3 × 3	A	NIL–4	A	A	2.3 × 10 <sup>1</sup> –3.6 × 10 <sup>1</sup>
CSL 5	3 × 3	A	A	A	A	<2.5 × 10 <sup>1</sup>
CSL 6	3 × 3	A	A	A	<1.0	1.8 × 10 <sup>1</sup> –3.7 × 10 <sup>1</sup>
Company D						
CSL 1	3 × 1	A	NIL–6	80–112	NIL–5	4.2 × 10 <sup>4</sup> –7.5 × 10 <sup>5</sup>
CSL 2	3 × 1	A	A	NIL–4	A	5.3 × 10 <sup>3</sup> –7.2 × 10 <sup>3</sup>
CSL 3	3 × 1	A	A	A	A	<2.3 × 10 <sup>2</sup>
CSL 4	3 × 3	A	A	A	A	7.2–7.5 × 10 <sup>7</sup> (3/9)
CSL 5	3 × 3	A	A	A	A	20–80
CSL 6	3 × 3	A	NIL–1	NIL–5	NIL–4	24–240
Company E						
CSL 1	3 × 1	A	NIL–12	A	A	2.4–1.43 × 10 <sup>2</sup>
CSL 2	3 × 1	A	A	A	A	NIL–3.2
CSL 3	3 × 1	A	1–3	20–28	19–32	2.5 × 10 <sup>4</sup> –3.0 × 10 <sup>4</sup>
CSL 4	3 × 3	A	A	NIL–34	<1.0	18–37
CSL 5	3 × 3	A	A	A	A	20–37
CSL 6	3 × 3	A	A	NIL–1	A	5–29
Company F						
CSL 1	3 × 1	A	1.0 × 10 <sup>1</sup> –1.3 × 10 <sup>1</sup>	TNTC	26–80	23–103
CSL 2	3 × 1	A	A	<10	10–42	<10
CSL 3	3 × 1	A	A	4.0 × 10 <sup>4</sup> –8.2 × 10 <sup>4</sup>	A	1–23
CSL 4	3 × 3	A	A	<10	A	7–41
CSL 5	3 × 3	A	A	<10	A	NIL–4
CSL 6	3 × 3	A	A	1–4	<1.0	<10
Company G						
CSL 1	3 × 1	A	A	NIL–56	<1.0	2.5 × 10 <sup>3</sup> –3.0 × 10 <sup>4</sup>
CSL 2	3 × 1	A	NIL–2	<10	A	NIL–2
CSL 3	3 × 1	A	A	A	<10	A
CSL 4	3 × 3	A	A	<10	A	NIL–4
CSL 5	3 × 3	A	A	A	A	NIL–9
CSL 6	3 × 3	A	A	2–43	<1.0	NIL–4
Company H						
CSL 1	3 × 1	A	A	NIL–43	A	1.3 × 10 <sup>2</sup> –2.2 × 10 <sup>3</sup>
CSL 2	3 × 1	A	NIL–2	NIL–35	A	1.1 × 10 <sup>2</sup> –2.0 × 10 <sup>3</sup>

TABLE 4. Continued

CSL <sup>a</sup>	n	Food safety indicator <sup>b</sup>	Hygiene indicators <sup>c</sup>			Overall indicator <sup>c</sup>	
		<i>Salmonella</i>	<i>E. coli</i>	<i>Enterobacteriaceae</i>	<i>S. aureus</i>	TVC	
CSL 3	3 × 1	A	A	1.2 × 10 <sup>3</sup> –4.2 × 10 <sup>3</sup>	A	A	
CSL 4	3 × 3	A	A	NIL–69	A	NIL–35	
CSL 5	3 × 3	A	A	A	A	A	
CSL 6	3 × 3	A	A	NIL–4.8 × 10 <sup>4</sup>	NIL–4	A	
Company I							
CSL 1	3 × 1	A	A	1.0 × 10 <sup>2</sup> –2.2 × 10 <sup>3</sup>	<1.0	1.7 × 10 <sup>2</sup> –2.6 × 10 <sup>3</sup>	
CSL 2	3 × 1	A	A	1.0 × 10 <sup>1</sup> –2.0 × 10 <sup>3</sup>	A	<1.0	
CSL 3	3 × 1	A	A	A	<1.0	<1.0	
CSL 4	3 × 3	A	A	A	A	1.0 × 10 <sup>1</sup> –7.6 × 10 <sup>4</sup>	
CSL 5	3 × 3	A	A	A	A	<1.0	
CSL 6	3 × 3	A	A	A	<1.0	<1.0	

<sup>a</sup> CSL 1, fish at arrival with intact skin; CSL 2, fish fillets at heading and gutting; CSL 3, fish fillets after washing; CSL 4, working tables and facilities surfaces before cleaning and disinfection; CSL 5, working tables and facilities surfaces after cleaning and disinfection; CSL 6, hands or gloves of operators.

<sup>b</sup> A, absent in 25-g sample or on 50 or 10 cm<sup>2</sup>.

<sup>c</sup> Results represent range in log CFU per gram for fish products, log CFU per 50 cm<sup>2</sup> for environment samples, and log CFU per 25 cm<sup>2</sup> for hands and/or gloves of workers. TVC, total viable bacteria count; TNTC, too numerous to count; A, below detection limit; NIL, zero count or below the level of detection in the bacteriological assay(s).

hygienic performance of equipment and facilities results in better control of cross contamination (27).

All companies performed well (score 3) in TVCs at final product after candling, trimming, and final washing (CSL 3). There was good performance (score 3) in 89 and 78% of the companies in *E. coli* and *Enterobacteriaceae*, respectively. However, the rest of the companies performed poorly in their *E. coli* and *Enterobacteriaceae* (score 0; Table 4). *E. coli* detection above the set limits (Table 4) in fish fillets of one company indicated poor hygienic handling of the fillets. Cross-contamination could have occurred from hands or gloves of the operator or from wash water whose quality was not adequately monitored. Operators in majority of the companies were aware of the existence and content of procedures and consciously followed them. Furthermore, safety tasks were internalized, and employees exercised self-control on compliance to candling, trimming, and washing procedures. Internalized procedures support the appropriate decision-making process, reduces variation, and helps to achieve safety and quality objectives (26). Standards and tolerances for critical process and fish fillet parameters were clearly specified in majority of the companies. Assessment of critical process and final fillet standards and tolerances was derived from process parameters, legal requirements, hygiene codes, and literature. Standards and tolerances were also tested and tailored for own process system. Such a scenario results in more accurate critical control points, which contribute positively to food safety (27).

In fish contact processing tables and facilities before cleaning and sanitation (CSL 4), 78% of fish companies performed well (score 3) in *E. coli* counts, while 22% performed poorly (score 0). With respect to *Enterobacteriaceae* counts, 44% of the companies performed well (score 3), 33% had average to poor performance (score 1), while

22% performed poorly (score 0). When *S. aureus* was considered, 67% of companies performed well (score 3), while 33% performed poorly (score 0). For TVCs, 22% of the companies had average performance (score 2), 22% had average to poor performance (score 1), while the remaining 56% performed poorly (score 0; Table 4). Sanitation was not differentiated for specific equipment or facilities at the companies that performed poorly in the parameters. Common cleaning agents were also not specific for fish processing. Instructions were only derived from information on labels or company experience. Hygienic design of equipment and facilities for a majority of the companies was average. Suppliers were involved in the hygienic design of equipment and facilities according to the Kenya Bureau of Standards requirements. However, they lacked adaptation and testing according to the individual company specific fish processing circumstances. The hygiene design of company equipment and facilities should be modified for specific fish processing characteristics in collaboration with equipment and cleaning suppliers. The high prevalence of *S. aureus* in the food contact surfaces of three companies before cleaning and sanitation could have been due to contamination from hands of operators where *S. aureus* was detected from hand swabs. *S. aureus* counts exceeded limits in processing surfaces of three companies, and the organism was detected in higher numbers on hands or gloves of the operators, except one company (Fig. 1F). Transfer of fecal coliforms by handlers to fish and fish contact surfaces in processing environment has been reported (37).

Processing tables and facilities after cleaning and disinfection (CSL 5) performed well (score 3) in *E. coli*, *Enterobacteriaceae*, and *S. aureus* counts. However, 22% of the companies had average performance (score 2) in TVCs, 56% had average to poor performance (score 1),

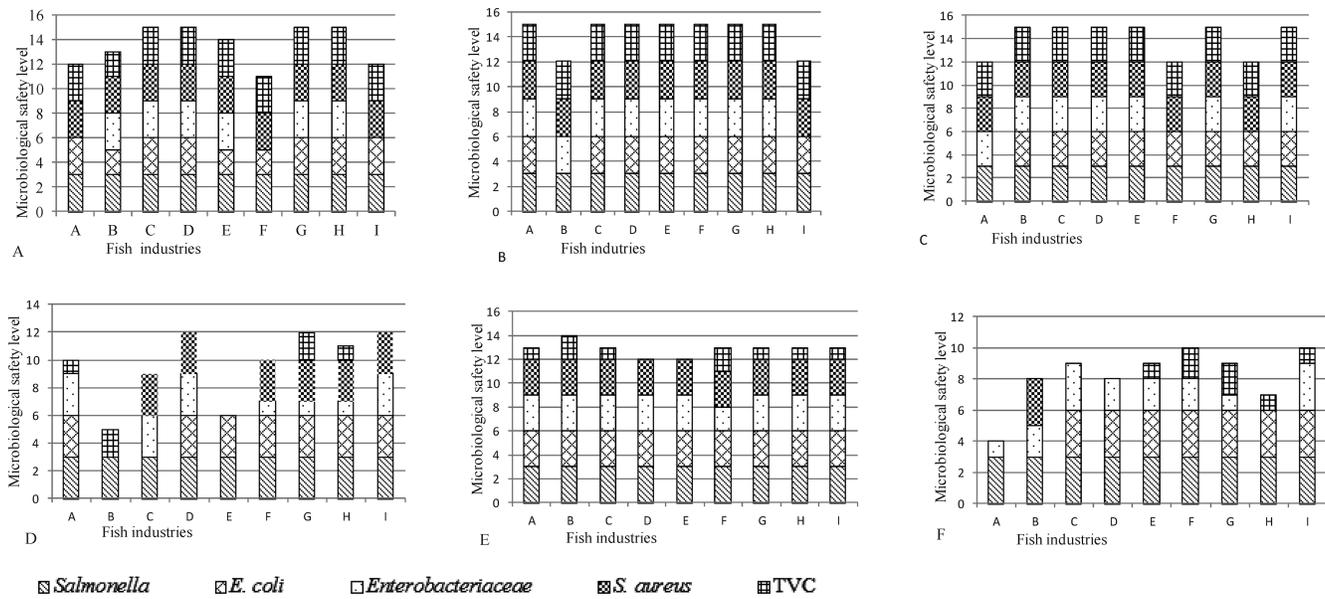


FIGURE 1. Microbial safety level profiles at six CSLs for nine fish processing plants. (A) CSL 1: raw fish with intact skin; (B) CSL 2: fish at heading and gutting; (C) CSL 3: final fish fillet product after candling, trimming, and final washing; (D) CSL 4: fish contact surfaces—processing tables and facilities before cleaning and sanitation; (E) CSL 5: processing tables and facilities after cleaning and disinfection; (F) CSL 6: hands or gloves of operator.

while 22% performed poorly (score 0; Table 4). Poor TVC scores indicated overall poor microbiological performance in the cleaning and sanitation procedures. The food contact surfaces with the lowest TVC scores were manually cleaned. Cleaning activities in one company were not effective against the organism because it was still present at higher levels (score 2) on surfaces after cleaning. Some surfaces, such as tables and fish transfer crates, were also rough and dented, which makes them difficult to clean effectively and could, therefore, harbor microorganisms. Poor performance may also be attributed to lack of well-defined and/or documented procedures and guidelines for cleaning of such equipment in the companies. Verification of the adequacy of cleaning operations was also conducted irregularly. Effective cleaning of food contact surfaces is an important component of an FSMS (22). Sanitation programs can be improved by tailoring them for different equipment and facilities (27). Cleaning agents should also be specifically modified and their effectiveness tested specifically for fish processing systems. Instructions on use of cleaning agents and frequency should be based on test results to more consistently prevent contamination (27).

Samples from hands or gloves of operators (CSL 6) revealed that 78% of the companies performed well (score 3) in *E. coli* counts, while 22% performed poorly (score 0; Table 4). Only 22% of the companies performed well (score 3) in *Enterobacteriaceae*, 44% had average performance (score 2), 22% had average to poor performance (score 1), while 11% performed poorly (score 0). Additionally, 89% of the companies performed poorly (score 0) in *S. aureus* counts and only 11% performed well (score 3; Table 4). In terms of the TVCs, 44% of the companies performed poorly (score 0), 33% had average to poor performance (score 1), while 22% had average performance (score 2) at CSL 6 (Table 4). The presence of *E. coli* on the hands of operators

in two companies posed cross-contamination risks for fish products because all the companies engaged in manual packing of fish (using styrofoam, cartons, and polybags). Cross-contamination of fish products and packaging material by the food handlers could result in *E. coli* in the final product. Sanitation facilities in the companies that performed well in all the parameters were tailored to support personal hygiene. They also had specific requirements for handling and storage of operator clothing. Specific requirements were in place for storage, cleaning procedures, and schedules for protective clothing and separate and specific storage areas for used and clean clothing. Specific training on hygiene matters was also conducted. For instance, cleaning and disinfection procedures, hand washing and drying, personal hygiene, hygienic handling of ice, and hygiene during storage and transportation were clearly specified for their fish production situations. These practices have been found to assist in reducing the possibility of product contamination (27).

Differences in microbial performance of FSMS in the fish sector were also related to the production scale of the individual fish company. Large- and medium-sized companies D, H, C, and G attained maximum MSLP (score 15) at raw material reception (CSL 1). Large-sized company A and medium-sized company F scored 12 and 11, respectively, for raw material at reception. None of the small-sized companies attained maximum MSLP at CSL 1 (Fig. 1A). Large- and medium-sized companies had adequate supply chain coupled with properly cleaned and sanitized delivery vans. High initial contamination of raw materials can be carried over to other CSLs throughout the processing line due to lack of a subsequent intervention step (30). The companies might, therefore, reduce the risks of insufficient raw material quality by critically evaluating their specifications and by systematically inspecting FSMS of their suppliers (28).

The microbiological performance of both large-sized companies (A, D, and H) and medium-sized companies (C, F, and G) was good at the heading and gutting stage, and all attained maximum MSLP (score 15; Fig. 1B). However, the small-sized companies scored the MSLP of 12, with the exception of company E, which scored a maximum MSLP (score 15; Fig. 1B). The two small companies B and I performed poorly (score 0) in *E. coli* and *Enterobacteriaceae* counts, respectively, at this CSL (Tables 2 and 3). They have paper-based procedures for heading and gutting at various locations, and the procedures are only updated when need arises. Procedures should be easily available (e.g., digitized), designed for specific users, and updated on a regular basis to enhance personnel decision-making behavior (27). The two companies were also involved in intermittent heading and gutting of small quantities of fish products in batches rather than continuous flow and automated process, which prevents cross-contamination (28). The high degree of automation in product movement also restricts personnel interference.

All small-sized companies attained a maximum MSLP (score 15) for the final fish product after candling, trimming, and final washing (CSL 3; Fig. 1C). Medium-sized companies also attained a maximum MSLP (score 15), with the exception of company F, which had an MSLP score of 12. Two large-sized companies (A and H) scored an MSLP of 12, with the exception of company D, which scored a maximum MSLP (score 15; Fig. 1C). Companies A and F performed poorly (score 0) in terms of *Enterobacteriaceae* counts (Table 4). Company A also performed poorly (score 0) as a result of the *E. coli* counts (Table 4). The bacterial counts exceeded generally accepted microbial guidelines for fish (Table 4). The hands and gloves of operators in the companies were highly contaminated (Fig. 1F), which increases the possibility of cross-contamination of the final fish fillets. In addition, hygiene design for their equipment and facilities lacked integration, adaptation, and testing according to the individual company-specific fish production circumstances, which may decrease chance of cross-contamination (27). Companies A and F also had average performance in their raw fish material control (i.e., no statistically underpinned acceptance sampling procedures). Corrective actions for all small-sized companies were efficiently implemented. The actions were based on systematic causal analysis of their own product and process deviations. There were complete descriptions of process adjustments and handling of noncompliant products. Structured analysis of causes of deviations and their corrective actions was also done.

All companies had an MSLP of 12 and below for fish processing tables and facilities before cleaning and sanitation (CSL 4). Large-sized companies performed better (MSLP 10, 12, and 11 for A, D, and H, respectively) compared with medium-sized companies (C, F, and G). However, small-sized companies B, E, and I performed least well, scoring MSLP of 5, 6, and 12, respectively (Fig. 1D). All companies still scored less than the maximum in tables and facilities after cleaning and disinfection, with the highest score of 14 in a small-sized company B (Fig. 1E).

This was mainly attributed to *E. coli* detection in companies B and C and *Enterobacteriaceae* in companies B, E, F, G, and H. This is an indication of poor hygiene standards in working tables and processing surfaces (Fig. 1E). This may result from fecal contamination, due to poorly done gutting, cross-contamination during production, and inadequate personnel hygiene. Similar observations were previously made on microbiological performance of broiler processing industries (4). Poor performance could be attributed to lack of well-defined and/or documented procedures and guidelines for cleaning of the fish contact surfaces in the companies. It was observed that cleaning was only done at the end of the shift. Effective cleaning of food contact surfaces (cleaning-as-you-go policy) is, therefore, recommended (22), which additionally reduces the probability of cross-contamination and biofilm formation (33). The data also indicated understaffing in critical quality-based areas in all the companies. The large-, medium-, and small-sized companies averaged seven, six, and three QA personnel, respectively (Table 1). Adequate decision making is an important factor in meeting microbiological safety criteria and is negatively affected by lack of enough QA personnel. This might have contributed to underperformance due to lack of expertise (29).

Large-sized companies performed least well (A, D, and H scoring MSLP of 4, 8, and 7, respectively) on hands or gloves of operators (CSL 6) compared with small- and medium-sized companies, the majority of which scored a MSLP of 9 or 10 (Fig. 1F). The majority of large-sized fish industries whose personnel had the poorest hand hygiene were those in which most operations were done manually, and food handlers were not well trained on technical and hygiene matters. The situation could have caused cross-contamination with *S. aureus* from the hands of operators to the food contact surfaces, particularly in companies A and E (Fig. 1D). The companies should consider more strict personal hygiene requirements, such as special hand washing facilities and provision of basic information (32), coupled with a provision of clearly understandable procedures for hygiene-related activities. Such specific training on hygiene matters assist in the reduction of contamination possibilities (27). Although basic personal hygiene was practiced, it required optimization and regular checking for effectiveness. Inadequate compliance to procedures and instructions and/or their misinterpretation may contribute to safety problems even when the design of hygiene practices is good (5). Food operators should be trained in good personal hygiene and practices and they should wear gloves and change them on a regular basis (39).

The microbial assessment revealed the possibility of cross-contamination, carryover, microbial build up, or in some instances, reduction across the CSLs. In company A, fish raw materials were highly contaminated by *Enterobacteriaceae* (MSLP 12). Heading and gutting were properly conducted (MSLP 15), but contamination, particularly with *E. coli*, increased (MSLP 12) in final fillets after washing. This trend was almost similar in company F, where *Enterobacteriaceae* was the major problem in raw fish (MSLP 11) and fillets after washing (MSLP 12; Fig. 1A through 1C). Other studies have shown that the plant and

processing environment, rather than the raw material, contributes highly to product contamination (42). Inadequately cleaned processing equipment surfaces have also been identified as sources of bacterial contamination in processed seafood (36). However, this does not exclude the possibility that the raw fish material is an important initial source of contamination for processing equipment and environment (42). The cleaning and sanitation procedure for company A enabled an increase in MSLP from 10 to 13. However, the hands and gloves of operators were highly contaminated (MSLP 4), particularly by *E. coli*, *S. aureus*, and TVCs (Fig. 1D through 1F). The trend was similar for company H, where cleaning and sanitation enabled an increase in MSLP from 11 to 13, while the hands and gloves of operators scored MSLP of 7 (Fig. 1D through 1F). Microbiological analysis of fish products between CSLs 1 and 3 revealed good performance (MSLP 15) in companies D and C (Fig. 1A through 1C). However, whereas cleaning and sanitation of surfaces resulted in reduction of contamination in company C (MSLP 9 to 13), this was not the case for company D, where an MSLP of 12 was maintained (Fig. 1D and 1E). Personal hygiene of the operators in the two companies (D and C) was poor (MSLP 8 and 9, respectively; Fig. 1F) because TVCs and *S. aureus* counts were found at higher levels (score 0; Table 4) on their hands or gloves. There was an increase in contamination of fish fillets (MSLP 15 to 12) in company H (Fig. 1A through 1C) mainly due to *Enterobacteriaceae* (score 0; Table 4). This might be due to cross-contamination from its operators, as shown by the poor *Enterobacteriaceae* score (score 0; Table 4). Even though small-sized companies B and I had the highest contamination levels at heading and gutting (MSLP 12), the final fillets had good microbiological quality (MSLP 15) after washing (Fig. 1B and 1C). Surfaces of companies B and E performed poorly before cleaning and sanitation at MSLP of 5 and 6, respectively. However, their cleaning and sanitation procedures enabled them to improve their MSLP to 14 and 12 for companies B and E, respectively (Fig. 1D and 1E). Utensils, particularly filleting knives and crates, were not replaced or sterilized regularly in companies B and E during the processing period. Improvement in core control activities, including cleaning and disinfection of food contact surfaces and proper cleaning of equipment in the fish processing plants, is, therefore, needed.

High variability in the *Enterobacteriaceae* count was noted in fish products of companies A (0 to 4.1 log CFU/g), F (0.6 to 4.53 log CFU/g), and I (0 to 3.41 log CFU/g) (Table 4). Surfaces of companies B and H showed the highest variability of 0 to 2.21 log CFU/cm<sup>2</sup> and 0 to 4.27 log CFU/cm<sup>2</sup>, respectively, in *Enterobacteriaceae* (Table 4). The high variability in *Enterobacteriaceae* in fish products indicated weaknesses of the implemented FSMS to effectively address this family of related enteric bacteria. High variability in TVCs was also noted in fish product from companies A (0.6 to 4.63 log CFU/g), B (2.45 to 6.71 log CFU/g), D (2.23 to 5.75 log CFU/g), E (2.43 to 4.27 log CFU/g), G (0 to 4.28 log CFU/g), H (0 to 3.21 log CFU/g), and I (0.2 to 3.26). Additionally, high

variability in TVCs from surfaces in companies A (0.7 to 3.74 log CFU/cm<sup>2</sup>), B (0.2 to 2.16 log CFU/cm<sup>2</sup>), D (1.7 to 8.17 log CFU/cm<sup>2</sup>), and I (0.1 to 4.34 log CFU/cm<sup>2</sup>) was noted (Table 4). Low variability was noted for *E. coli* and *S. aureus* (Table 4).

In conclusion, the absence of *Salmonella* spp. in all sampling locations indicated that the FSMS implemented by the fish exporting industries were effective against the pathogen. The hands or gloves of operators from the majority of the companies were contaminated by *S. aureus*, above the generally accepted microbial guidelines for fish, indicating inadequate personal hygiene. High initial levels of *Enterobacteriaceae* in raw fish and high TVCs on surfaces and subsequent cross-contamination from operators, on contact materials and equipment coupled with higher variability, was revealed. Companies should implement fish process tailored control and assurance activities, particularly in raw material control, personal hygiene, and cleaning and sanitation programs.

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