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Research Article

Evaluation of Food Contact Surface Contamination and the Presence of Pathogenic Bacteria in Seafood Retail Outlets in the Sultanate of Oman

Ponnerassery Sukumaran Sudheesh, Aliya Al-Ghabshi, Nasser Al-Aboudi,
Sami Al-Gharabi and Humaid Al-Khadhuri
Microbiology Laboratory, Ministry of Agriculture and Fisheries Wealth, Fishery Quality Control Center, P.O. Box 427, P.C. 100, Muscat, Sultanate of Oman

Abstract: This study was undertaken to find out the total microbiological load and the presence of pathogenic microorganisms on food contact surfaces in seafood retail markets in the Sultanate of Oman. Microbiological and sanitary conditions on food contact surfaces in four retail fish markets was studied by using Food Stamp Rodac™ (Replicate Organism Detection and Counting) plates and ATP sanitation monitoring system. High plate readings of Total Colony Count (TCC) and indicator organisms such as total coliforms, yeasts and molds and *Escherichia coli* were obtained from samples collected from most food contact surfaces. Similarly, significant numbers of pathogenic bacteria such as *Salmonella* sp., *Staphylococcus aureus* and *Clostridium perfringens* were observed in microbiological samples from all fish markets. Hygiene status of the food contact surfaces studied using the AccuPoint® Sanitation Monitoring System showed extremely high levels of Adenosine Triphosphate (ATP) on all food contact surfaces in all fish markets. Only water samples showed very low ATP levels. This study reveals the presence of contaminating and pathogenic bacteria in seafood retail outlets and the urgent need to improve the hygiene status of retail fish markets in the Sultanate of Oman.

Keywords: Food contact surfaces, HACCP, hygiene monitoring, microbiological quality and safety, retail fish markets

INTRODUCTION

In developing countries, improper sanitary conditions in the whole food production chain starting from primary production to the consumers and the occurrence of a wide range of food borne diseases create vulnerability in seafood safety. Hence, it is increasingly important to monitor and verify the seafood safety risks along the entire seafood production chain. The retail outlets are the final point in the seafood production chain before the products reach the consumers. Monitoring microbial food safety risks at this level of food chain and ensuring safety is extremely important as the producers do not have any control of the product quality and safety once it is sold out to consumers.

Many bacterial species are indigenous part of seafood, but they can also be found on the food processing surfaces, where they can subsequently contaminate the products (Vogel *et al.*, 2001). Attachment of pathogens and other bacteria to food contact surfaces can lead to product contamination, spoilages and surface deterioration. Research in the food industry has revealed that most bacteria are able to colonize surfaces in natural habitats (Wirtanen *et al*., 2000).

Sanitary monitoring of seafood contact surfaces is a powerful tool for the detection of risks associated with the production, manufacture and consumption of seafood. The types of microorganism's presents in products will depend on the way they have been elaborated, transported, stored, or prepared before eating. Prevention of seafood-associated infections requires an understand ingot only of the etiologic agents and seafood commodities associated with illness, but also of the routes of contamination that are amenable to control. Food contact surfaces are the major route of contamination in seafood processing plants and retail outlets. US Food and Drug Administration GMP, 21 CFR 110.3 regulation defines food contact surfaces as those surfaces that contact human food and those surfaces from which drainage on to the food or onto the surfaces that contact the food ordinarily occurs during the normal course of operations (US Food and Drug Administration, 2006). The food contact surfaces include all equipment, utensils and facilities used during processing and storage, as well as worker clothing, hands and packaging material.

Several *in situ* and *in vitro* methods are currently available that may be used to detect and/or quantify food soiling on surfaces (Verran *et al.*, 2002; Verran

Corresponding Author: Ponnerassery Sukumaran Sudheesh, Microbiology Laboratory, Ministry of Agriculture and Fisheries Wealth, Fishery Quality Control Center, P.O. Box 427, P.C. 100, Muscat, Sultanate of Oman

and Whitehead, 2006). The use of microbial ATP to generate bioluminescence is an assay that has been used for many years in a variety of applications to estimate microbial load and/or actual bacterial populations (Stanley, 1989). These systems deliver a rapid, direct, objective measurement of cleaning efficiency, hygienic status and risk, primarily by the measurement of ATP. This biochemical test uses an enzyme, luciferase that emits light in the presence of ATP. The light is measured quantitatively in an instrument called an illuminometer and results are available in 20 sec. Since almost all organic matter contains ATP, it is present in almost all foodstuffs in huge amounts. ATP is also present in viable microbes (although in much smaller amounts). So, the system measures ATP presents in both food residue and in microorganisms present on the food contact surfaces. Many reports over the past 20 years have shown a good correlation between surface cleanliness and plate counts, such that it is now a widely accepted method of hygiene monitoring by industry, retailer and regulatory agencies (Verran *et al*., 2002; Verran and Whitehead, 2006).

Food Stamp Rodac™ (Replicate Organism Detection and Counting) plates are a simple-to-use bacteriological method to check the microbiological hygiene of food contact surfaces. Food Stamps are designated for hygiene control of viable bacteria on foodstuffs and working areas. It is a simple-to-use bacteriological method, which indicates the presence of bacteria.

Microbiological quality and safety studies of fish markets in the Sultanate of Oman are completely lacking. In order to have an initial assessment of hygiene practices currently followed in the fish markets; it is highly desirable to have detailed scientific information on the microbiological quality of the food contact surfaces in seafood plants. The data generated will have tremendous benefits in planning and formulating specific Hazard Analysis and Critical Control Point (HACCP) based hygiene programs in retail fish markets in Sultanate of Oman.

The present study was carried out with the aim of ascertaining the food safety risks associated with food contact surfaces in four seafood retail outlets in Sultanate of Oman. The sanitation condition of food contact surfaces was studied using two rapid methods, ATP bioluminescence and Food Stamp Rodac™ (Replicate Organism Detection and Counting) plates. The application of novel technologies for the microbiological analysis of seafood and seafood environments allow us to show the microbial risk and also identify practices that compromise the safety of seafood, with the ultimate objective of reducing or eliminating health risk due the seafood consumption.

MATERIALS AND METHODS

Seafood retail outlets/fish markets: Four selected seafood retail outlets/fish markets were studied for microbiological sanitation. The fish markets were located in Muttrah, Quriyat, Seeb and Sohar. A variety of fish and shellfish species are sold in these fish markets depending on the season and availability. Locally available seafood products are transported in the ambient temperatures or chilled conditions, brought in and kept on crushed ice during sale. Imported seafood are brought in as frozen blocks, thawed at the outlets and kept on crushed ice during sale. These fish markets very often do not have adequate facilities for hygienic storage and handling of seafood.

Sanitation monitoring: Microbiological sanitation sampling in all four fish markets were done onsix specific food contact surface areas viz., fish cutting board, fish boxes, ice container, fish storage area, knives and hands of people handling fish in the market. The study sampling was carried out during January-May, 2011. Assessment of sanitary condition of food contact surfaces was done using two different methods. The first method employed Food Stamp Rodac™ (Replicate Organism Detection and Counting) plates (HyServe, Uffing, Germany). The second method was based on measuring the ATP bioluminescence using an AccuPoint 2 Instrument (Neogen, MI, USA).Sampling for ATP sanitation monitoring was also done from water and ice, in addition to the food contact surfaces mentioned above. Sampling from each point of analysis was replicated three times. Food contact surface sampling was done using sterile surface samplers with spongy sampling heads. Sampling was done on food contact surfaces in an area of 4”×4”, from edge to edge in crosshatch pattern, by applying slight pressure at the sampling head. While sampling, the sampling head moved in both directions to assure access to grain of surface and making sure that the tip of the head is not touched by hand. This kind of sampling is supposed to reduce user variability and provide consistent data for trending. The sampling head was placed back in the instrument for reading the results.

For sampling water samples, the liquid sampler was dipped into the water until the sampler head sponge is completely submerged in the water. The sampler head was activated by shaking the head twice gently and placed back in the instrument for reading the results. The instrument measures the total ATP present on the sampling head. The levels indicate the levels of both food residue and microbial matter present on a surface or in the water. The luciferin/luciferace substrate/enzyme complex binds with the ATP present

on the sampling head to produce light. Increase in microbes or food residue will increase the ATP levels on the sampler head, which result in increase in light. The increase in light emission is read as Relative Light Units (RLU) by the AccuPoint 2 instrument, which is a numerical representation of the cleanliness of the surface.

An instrument reading of <150 RLU indicates clean surfaces and is considered to pass the quality test. A reading of >150 and <300 RLU indicates marginal cleanliness and requires caution and more cleaning during the next clean up. A value of >300 RLU indicates unclean surfaces and failure of the sanitation test, requiring re-cleaning and sanitation before next start up.

Food Stamp is a prepared agar medium, on which agar stands up slightly above the rim of special Petri dish of 10 cm^2. The samples were obtained by taking off the cap of Food Stamp and gently pressing the medium against the food contact surface to be tested. The cap was immediately replaced carefully by pressing firmly.

Standard method agar was used for counting Total bacterial Colony Counts (TCC) and incubated at 37°C for 24 h. Deoxycholate agar for Total Coli forms (TC), X-Gluc-Magenta-Gal (XM-G) agar for *Escherichia coli*, Tellurite Glycine Soya Egg yolk (TGSE) agar for *Staphylococcus aureus*, Mannitol Lysine Crystal violet Brilliant green (MLCB) agar for *Salmonella* sp. and cereus agar for *Bacillus cereus*, were incubated at 37°C for 24 h. Food borne Fungi (FF) and Environmental Fungi (EF) were counted by inoculating potato dextrose agar with chloramphenicol and sabauraud agar plates, respectively and incubating at 30°C for 72 h

RESULTS AND DISCUSSION

Good Hygienic Practices (GHP) is essential to ensure food safety. They are required by law under national and international food hygiene regulations and are frequently considered as pre-requisites to food safety systems based on Hazard Analysis and Critical Control Point (HACCP). Compromising good hygiene almost always results in establishment and proliferation of pathogenic as well as spoilage microorganisms on the processing and storage food contact surfaces. This leads to contamination of fishery products with hazardous microorganisms making them unsafe for human consumption. Good hygienic practices are a primary preventative measure and the monitoring of their effectiveness not only provides an early warning of potential problems but also evidence of due diligence.

Until the 1980's, the only method available to measure the hygienic status of food contact surfaces was the conventional cultural method based on agar plate counts. However, a true picture of the hygiene status of the food contact surfaces can only be ascertained by knowing the microorganism load as well as the amount of food residue remaining on the surfaces. A number of sanitation monitoring tests of processing plants and retail shops are used by the seafood industry to check that the microbiological status is satisfactory. In this study, we used a culture based agar plate count method to determine the microbial load and an ATP sanitation monitoring

Table 1: ATP sanitation monitoring of muttrah fish market

Food contact surface	Reading (RLU) ±S.D.*	Status**
Cutting board	36332±4299	Fail
Fish boxes	1543±441	Fail
Fish storage area	99999±0	Fail
Ice container	891±37	Fail
Ice	325±36	Fail
Water	94±16	Pass
Tools (knifes)	49429±438	Fail
People (hands)	65890±4317	Fail

*: Mean values of triplicate samples±standard deviation; **: <150 RLU = pass, >150 and <300 RLU marginal, >300 RLU fail

Table 2: ATP sanitation monitoring of quariyat fish market

Food contact surface	Reading (RLU)	Status
Cutting board	57804±696	Fail
Fish boxes	13339±1032	Fail
Fish storage area	74278±236	Fail
Ice container	99999±10345	Fail
Ice	676±43	Fail
Water	137±13	Pass
Tools (knifes)	5681±238	Fail
People (hands)	786±79	Fail

*: Mean values of triplicate samples±standard deviation; **: <150 RLU = pass; >150 and <300 RLU marginal; >300 RLU fail

Table 3: ATP sanitation monitoring of seeb fish market

Food contact surface	Reading (RLU)	Status
Cutting board	50132±673	Fail
Fish boxes	4236±129	Fail
Fish storage area	99999±1578	Fail
Ice container	46016±974	Fail
Ice	249±9	Marginal
Water	0±0	Pass
Tools (knifes)	3100±262	Fail
People (hands)	1396±182	Fail

*: Mean values of triplicate samples±standard deviation; **: <150 RLU = pass; >150 and <300 RLU marginal; >300 RLU fail

Table 4: ATP sanitation monitoring of sohar fish market

Food contact surface	Reading (RLU)	Status
Cutting board	80066±12750	Fail
Fish boxes	97779±17534	Fail
Fish storage area	99999±10876	Fail
Ice container	28231±1264	Fail
Ice	651±24	Fail
Water	138±18	Pass
Tools (knifes)	58405±1051	Fail
People (hands)	10936±547	Fail

*: Mean values of triplicate samples±standard deviation; **: <150 RLU = pass; >150 and <300 RLU marginal; >300 RLU fail

system to determine the bacterial load as well as the food residue remaining on the food contact surfaces.

The results of the ATP sanitation monitoring experiments are summarized in Table 1 to 4. The results show that all food contact surfaces tested in all four fish markets failed the sanitation quality test with really high RLU readings, sometimes reaching as high as 100,000 RLU. Only water samples from all fish markets passed the test with very low RLU values. The ice samples from Seeb market showed marginal values. ATP bioluminescence has been widely used for the detection of microbial contamination and food residues in the food industry (Griffith *et al*., 1994; Davidson *et al*., 1997), providing a real time estimate of total surface cleanliness including the presence of organic debris and microbial contamination (Davidson *et al*., 1999). It has been successfully used for determining cell numbers in fish processing factories (Miettinen *et al*., 2001) and the dairy industry (Oulahal-Lagsir *et al*., 2000). The findings of this study show that all the five fish markets have very unhygienic food contact surfaces. It is probable that the daily cleaning of food contact surfaces is not done employing standard procedures. There was significant amount of seafood residues left on these surfaces. These surfaces are only subjected to routine washing before and after processing, that removes only the upper detaching layers of the firmly attached soil.

The results of Food Stamp plate counts are presented in Table 5 to 8. All indicator bacteria and pathogens tested were present on most food contact surfaces in all four fish markets. High colony counts were obtained for TCC, coli forms and for some pathogenic bacteria.

The TVC of bacteria on all the six food contact surfaces tested in Muttrah, Seeb and Soharfish markets showed counts above 100. Surface stamp samples from Quariyatfish market showed that the TVC on peoples' hands were 63 and on all other food contact surfaces exceeded 100.

Total coli forms were detected in all four fish markets. It exceeded 100 in samples from cutting board in Muttrahfish market. Coli forms were moderately present on all six food contact surface samples from Seeb and Soharfish markets. Coli forms were absent on the food contact surfaces in Quariyatfish market, except the food storage area and knives, where the samples showed values of 16 and 1, respectively.

Food Stamp sampling in all four fish markets detected the presence of *E. coli* on food contact surfaces. High numbers of *E. coli* were detected on peoples' hands in Muttrahfish market. *E. coli* were also detected on cutting board and knives in this fish market. In Quariyatfish market *E. coli* were detected on cutting board and fish storage area. In Seebfish market *E. coli* were detected on all food contact surfaces except fish

Table 5: Results of food stamp RODAC™ plate count of mattrah fish market

	Colony counts/ plate (cfu)†					
Organisms	Cutting board	Fish boxes	Ice container	Fish storage area	Knives	People (hands)
TCC	>100	>100	>100	>100	>100	>100
Coliforms	>100	22	29	14	1	36
E. coli	10	0	0	0	1	32
S. aureus	0	0	0	0	0	0
Salmonella	0	3	0	0	1	8
B. cereus	5	0	0	0	0	0
Food-borne fungi	32	1	>100	>100	15	0
Environmental fungi	>100	>100	>100	>100	44	13

†: Degree of contamination for TCC; Environmental fungi and Food-borne fungi: 0-not contaminated, 1-9 ± barely contaminated, 10-29 + slightly contaminated, 30-99 ++ moderately contaminated, >100 +++ heavily contaminated; Degree of contamination for all other tests: 0-not contaminated, >1 contaminated criteria based on Verran *et al*. (2002)

Table 6: Results of food stamp RODAC™ plate count of quariyat fish market

	Colony counts/plate (cfu)					
Organisms	Cutting board	Fish boxes	Ice container	Fish storage area	Knives	People (hands)
TCC	>100	>100	>100	>100	>100	63
Coliforms	0	0	0	16	1	0
E. coli	4	0	0	12	0	0
S. aureus	0	0	0	100	0	0
Salmonella	9	9	9	9	4	0
B. cereus	5	1	100	9	2	0
Food-borne fungi	>100	13	>100	>100	>100	12
Environmental fungi	>100	4	89	62	>100	>100

†: Degree of contamination for TCC; Environmental fungi and Food-borne fungi: 0-not contaminated, 1-9 ± barely contaminated, 10-29 + slightly contaminated, 30-99 ++ moderately contaminated, >100 +++ heavily contaminated; Degree of contamination for all other tests: 0 - not contaminated, >1 contaminated criteria based on Verran *et al*. (2002)

Table 7: Results of food stamp RODAC™ plate count of seeb fish market

	Colony counts/ plate (cfu)					
Organisms	Cutting board	Fish boxes	Ice container	Fish storage area	Knives	People (hands)
TCC	>100	>100	>100	>100	>100	>100
Coliforms	60	11	32	11	3	37
E. coli	5	0	5	2	4	2
S. aureus	100	34	>100	>100	>100	>100
Salmonella	4	0	0	2	1	0
B. cereus	11	3	6	4	0	0
Food-borne fungi	24	6	>100	>100	12	>100
Environmental fungi	>100	8	>100	>100	86	>100

†: Degree of contamination for TCC; Environmental fungi and Food-borne fungi: 0-not contaminated, 1-9 ± barely contaminated, 10-29 + slightly contaminated, 30-99 ++ moderately contaminated, >100 +++ heavily contaminated; Degree of contamination for all other tests: 0-not contaminated, >1 contaminated criteria based on Verran *et al*. (2002)

Table 8: Results of food stamp RODAC™ plate count of sohar fish market

	Colony counts/ plate (cfu)					
Organisms	Cutting board	Fish boxes	Ice container	Fish storage area	Knives	People (hands)
TCC	>100	>100	>100	>100	>100	>100
Coliforms	4	3	16	13	31	14
E. coli	1	1	0	0	0	16
S. aureus	12	>100	15	24	11	13
Salmonella	11	0	9	6	0	0
B. cereus	1	2	0	3	5	4
Food-borne fungi	58	3	13	16	25	19
Environmental fungi	>100	13	74	17	42	>100

†: Degree of contamination for TCC; Environmental fungi and Food-borne fungi: 0-not contaminated, 1-9 ± barely contaminated, 10-29 + slightly; contaminated, 30-99 ++ moderately contaminated, >100 +++ heavily contaminated; Degree of contamination for all other tests: 0-not contaminated, >1 contaminated (criteria based on Ten CATE, 1965)

boxes. In Soharfish market, bacteria were detected on peoples' hands appreciably and were present on cutting board and fish boxes.

S. aureus was not detected in any of the food contact surfaces tested in Muttrahfish market. In Quariyatfish market, the presence of the bacteria was found only in fish storage area, where the count exceeded 100. In Seebfish market, all food contact surfaces showed the count of the bacteria exceeding 100, except fish boxes, where it showed a count of 34. In Soharfish market, all six food contact surfaces tested showed the presence of *S. aureus* and in samples from fish boxes the counts were above 100.

Salmonella was detected in all four fish markets. It was detected on the peoples' hands, fish boxes and knives in Muttrahfish market. In Quariyatfish market, *Salmonella* was detected in significant numbers on all food contact surfaces except the hands of people. The bacteria were detected on cutting board, fish storage area and knives in Seebfish market. In Soharfish market, the bacteria were detected on cutting board, ice container and fish storage area.

B. cereus was detected on all food contact surfaces except the hands of people in Quariyatfish market; it was absent on most food contact surfaces except the cutting board in Muttrahfish market. Except on knives and hands of people, *B. cereus* was present on all food contact surfaces in Seebfish market. In Soharfish market, the bacteria were detected on all food contact surfaces except the ice container.

Significant numbers of food-borne fungi and environmental fungi were detected on almost all food contact surfaces in all fish markets studied. The food-borne fungi were not detected on the hands of people in the Muttrahfish market. The fungi count on most food contact surfaces exceeded 100 in Muttrah, Quariyat and Seebfish markets. In Soharfish market, most fungi detected were below the level of 100. Only environmental fungi on cutting board and hands of people exceeded 100 numbers.

There may be two major routes of contamination of the food contact surfaces by indicator organisms such as coli forms and *E. coli* and pathogenic organisms such as *Salmonella* sp. and *S. aureus.* It may be that contamination took place before the raw material was brought into the receiving and subsequently contaminating food contact surfaces, especially those that tested positive for coli forms. On the other hand, the source of the contamination could be from the fish market itself, including the transfer of microorganisms from the people working in or visiting the fish market.

According to Venugopal (2002) contamination of fish particularly by pathogens such as *Salmonella* sp., *S. aureus*, *Campylobacter jejuni*, *E. coli* 0157:H7, *Vibrio parahaemolyticus*, *Yersinia enterocolitica* and

Listeria monocytogenes, may occur prior to harvest, during capture, processing, distribution and/or storage. Huss *et al*. (2000) have pointed out that some pathogenic bacteria are naturally present in the aquatic (*Clostridium botulinum*type E, pathogenic *Vibrio* sp., *Aeromonas*) and the general environment (*C. botulinum*, type A and B, *L. monocytogenes*) and may therefore be found on live or raw fish. Studies done by Vogel *et al*. (2001) on *L. monocytogenes*, indicated that contamination occurred along the processing line. Other studies dealing with different processing operations have similarly concluded that the plant and processing environment is the source of product contamination rather than the raw material. However, this does not exclude the possibility that the raw fish material is an important initial source for contaminating processing equipment and environment (Vogel *et al*., 2001). Also, water, like food, is a vehicle for the transmission of many agents of diseases (Kirby *et al*., 2003). However, the ATP sanitation monitoring of the water used in these fish markets passed the test. Therefore it is less likely that water may have acted as a vehicle of transmission of microorganisms.

Many studies such as the one done by Montville *et al*. (2002) have concluded that, during handling and preparation, bacteria may be transferred from contaminated hands of food workers to food and subsequently to other surfaces (including food contact surfaces). Snyder (1998) also found that low infectious doses from organisms such as *Shigella*and the pathogen *E.coli*were linked to hands as a source of contamination. Other studies such as done by Reij and Den Aantrekker (2004) attributed poor hygiene, particularly deficient or absence of hand washing as the causative mode of transmission. Containers, cutting boards, knives, pumps or tanks have very often been used in fish markets for processed products without any cleaning and disinfection. There are always bacteria on the hands of seafood handlers, these bacteria may be transferred onto fish and subsequently to food contact surfaces wile handling fish and touching surfaces. Very high RLU readings in ATP measurement was obtained for most food contact surfaces. This indicates the high amount of leftover seafood particles on these surfaces, in addition to the high load of microorganisms. It is desirable that specially made cutting boards with smooth polished even surfaces must be used for cutting fish, as normal household cutting boards, which have small hills on them, can collect minute flesh particles. It is essential to clean and sanitize the food contact surfaces properly, especially those that in contact with the seafood for a long period of time. Even the food processing grade cutting boards should be replaced at regular intervals as scratches and cuts on the surface of the boards can promote formation of biofilms. Biofilm formation by spoilage bacteria such as *Pseudomonas* sp., as well as pathogenic bacteria such as *Listeria* and *Salmonella* has been well documented (Hood and Zottola, 1997). Effective hygienic protocols in fish markets are essential to minimize the formation of biofilms and to prevent contamination of the products (Carballo, 2000).

CONCLUSION

There is absolutely no scientific information on the sanitary condition on the food contact surfaces in seafood processing plants in Oman. The present study revealed the level of contamination of food contact surfaces in seafood processing plants in Oman. There is heavy contamination of the food contact surfaces by indicator organisms as well as by pathogenic bacteria. Simple and easy to use ATP sanitation monitoring systems and RODAC contact plates could be a feasible and practical option to monitor sanitary conditions in Omani seafood processing plants in order to check Critical Control Points (CCP) in processing and to ensure compliance to Hazard Analysis and Critical Control Point (HACCP) regulations.

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