Bacteria on stainless steel surfaces | experiment

The attachment of bacteria on food processing surfaces and in the environment can cause potential cross-contamination, which can lead to food spoilage, possible food safety concerns, and surface destruction. Food contact surfaces used for food handling, storage or processing are areas where microbial contamination commonly occurs. Even with proper cleaning and sanitation regimes or practices in place, bacteria can remain attached to the surfaces and this attachment can lead to biofilm formation. The purpose of this study was to identify the presence of pathogenic microorganism in a food processing area and to evaluate the effect of the cleaning procedure on the microbial load in the food processing area. Ten replicate food contact surfaces were tested: stainless steel, marble and wood, with adjacent areas being sampled before and after cleaning. The test surfaces were analyzed with a swab method before and after the cleaning stage. The results of these studies indicate that three of ten stainless steel surface were contaminated before cleaning and no surface was contaminated after cleaning. Furthermore, three out of ten marble surfaces were contaminated before cleaning and one surface was contaminated after cleaning. Six of ten wood surfaces were heavily contaminated before cleaning and three surfaces were contaminated after cleaning. The difficulty in cleaning was related to the amount of surface damage and it is best to avoid this type of surface. Hypochlorite solution that was used for cleaning the surfaces in this study was considered to be effective against the foodborne pathogens tested. This study has highlighted the fact that pathogens remain viable on dry stainless steel surfaces and present a contamination hazard for considerable periods of time, dependent on the contamination levels and type of pathogen.

Keywords: Microorganisms; Survival; Cross-contamination; Food contact surface

Introduction

Food contact surfaces are the chief denizen of biofilm that can host potentially harmful microorganisms. This, therefore, is a prominent phenomenon in food processing plants owing to dregs and residues of all sorts – chemical, biological, organic, and/or inorganic -which build up on the surfaces of equipments that may get in contact with food (Mafu et al. 2010). The presence of these undesirable microorganisms to the material surfaces is a source of concern, as this can result in food cross-contamination, leading to food poisoning. Under favourable circumstances (temperature, pH, relative humidity), pathogenic microorganisms are able to survive and/or replicate on a large scale within the biofilm. In domestic kitchens and food processing industries, foodborne illness can result from incorrect storage of foods, particularly with respect to temperature, contamination of raw or cooked foods before consumption, by contact with other foods or utensils (food contact surfaces) carrying pathogens, and inadequate cleaning procedures that may not see complete removal of microorganisms (Teixeira et al. 2007).

In food processing industries, food contact surfaces, such as stainless steel, marble and wood may create an enabling environment for the survival of the microorganism, leading to serious hygienic problems. Furthermore, dead ends, corners, joints, valves and any other hard-to-reach places are the most appropriate areas for the presence of bacteria. (Peng et al. 2001).

The value of maintenance and disinfection processes in food processing industries depends, to a large extent, on the design and maintenance programmes adopted by the company.

Lack of efficacy in cleaning procedures may allow persistence and survival of pathogens in foods owing to their consistent adherence to food contact surfaces. This may lead to transfer of microorganisms from people, objects or contaminated food to other food or material, hence leading to cross-contamination. People can, in many ways, be a source of cross-contamination to foods (Holah and Thorpe, 1990).

Food can be contaminated when it is handled, so it is very important that people who may be carrying or suffering from certain diseases do not handle food. Contamination can also be passed from equipment when contacting food. It specifically happens when utensils or equipment are not efficiently cleaned and sanitized between each use and may lead to development of biofilm, creating favourable conditions for the survival of the pathogens. Contamination from food to food occurs mainly when raw foods come into contact with cooked or prepared foods (Montville et al. 2001).

The persistent presence of microorganisms in food processing factories, specifically on food contact surfaces despite deliberate efforts to combat the phenomenon, poses great challenges to the company. It reduces the profit margins of the industries due to the increased cost incurred in the attempts to adopt advanced cleaning services and programmes. A potential effect of the presence of microorganisms on food surfaces is food poisoning. Occurrence of food poisoning will mean great damage to the image of the

company and persistent stress on the part of the management, thus derailing the progress of the company.

Cross contamination is also becoming a common problem both in the kitchen setting and in industry. Transfer of resistant pathogens and microorganisms across and around these food producers through various agents and factors that propagate and carry the pathogens is a health hazard. Studies show that the level of contamination varies depending on the duplication and the rate of material handling that occurs in the factory. In this context, therefore, workers′ hands, utensils and the broad extension of all food contact surfaces contribute to in cross contamination (Zhao et al. 1998).

A thorough examination of the whole concept of microbial survival and persistence on food contact surfaces despite typical cleaning procedures and revised designs of the food contact surfaces (such as textural properties, maintained solid surface hydrophobicity) will reveal that more detailed analysis and studies should be focused on the factors that create an enabling environment for the persistent replication and presence of the foodborne pathogens in the food processing industries and kitchen setting (Scott and Bloomfield, 1990). The study of various relevant properties for the microbial adhesion process has been another imperative goal of this study and the purpose behind it is to obtain a broader knowledge base of the mechanisms of bacterial adhesion to food contact surfaces so as to formulate strategies for its control.

The objective of this study is to identify the microorganisms that can survive in the food contact surface, such as stainless steel, marble and wood, even

after cleaning procedures, thus increasing the risk of food cross-contamination. The study will focus on microorganisms that survive in the food processing areas even after the cleaning procedure. Foodborne pathogenic bacteria adhere to inert surfaces; they may exhibit a greater scale of resistance to chemical or ordinary cleaning and fumigating agents (Barnes et al. 1999). The concept of cross contamination is of major concern in the food processing industries that constitute a threat to human health because they cause most food borne illness outbreaks. Food poisoning is one of the consequences of adherence of microorganisms to food contact surfaces (Sattar et al. 2001).

Materials and Methods

Premises

In order to assess the microbiological safety of a food processing area in Oman, three types of food contact surfaces were studied: Stainless steel, marble and wood. Ten surfaces of each of the three types were tested, with the adjacent areas of each one being sampled before and after cleaning. This study was performed randomly in nineteen selected Army camps kitchen.

Data analysis

Swabs were taken from the food processing area within the Royal Army camps kitchen and sent to the food microbiology laboratory of the environmental of health unit for analysis. The swabs were each tested for pathogenic bacteria linked with food and coliforms that can survive on the surface of food preparation areas before and after cleaning. The plates were read for the number of colonies of pathogenic bacteria and coliforms. A Phoenix machine was used to identify the bacteria and readings were taken

directly from the Phoenix machine. A Phoenix is automated microbiology system is intended to provide rapid identification results for most aerobic and facultative anaerobic Gram positive bacteria as well as most aerobic and facultative anaerobic Gram negative bacteria. The identification of the Phoeonix panal uses a series of conventional, chromogenic and fluorogenic biochemical tests to identify the organism. The growth-based and enzymatic substrates are employed to cover the different types of reactivity among the range of taxa. The tests are based on the use of bacteria and deterioration of specific substrates detected by different indicator systems. Acid production is indicated by a change in phenol red indicator when an isolate is able to utilize a carbohydrate substrate. A yellow colour is produce by Chromogenic substrates upon enzymatic hydrolysis and the enzymatic hydrolysis of fluorogenic substrates results in the release of a fluorescent coumarin derivation. Organisms that utilize a specific carbon source reduce the resazurine based indicator. These results were recorded and the log reduction was calculated for each plate at each dilution rate after and before cleaning of the surface (BD Phoenix, 2007).

Sampling methods and microbiological examination (Before Cleaning)

Tests using the swab method were carried out on surfaces contaminated with food borne pathogens in a food processing area. Tubes containing 10 ml of sterile buffered peptone saline solution were used to wet the swabs prior to sampling. Cotton swabs were removed from their sterile packaging and were held by the stick while they were moistened with buffered peptone saline solution, the excess broth was returned into the bottle. All surfaces were prepared in sizes of 20 x 20 cm2 for survival experiments. The swabs

were rotated while in contact with the food preparation surface. After the defined area was swabbed, the swab was returned to the test tube containing the buffered peptone saline solution to dislodge the bacteria. Serial dilutions of the swab solutions were prepared and duplicate pour plates were prepared for each dilution using nutrient agar, MacConkey agar and Blood agar. The plates were incubated for 24 hours at 37oC.

Sampling methods and microbiological examination (After Cleaning)

The surfaces were washed with hot water and chemical detergent and then rinsed with hot water. Then the surfaces (stainless steel, marble, and wood) were disinfected with 5. 25% of hypochlorite solution for 10 minutes. The surfaces were allowed to dry before sampling. The swabbing method used was as above. Duplicate pour plates were prepared for each dilution using nutrient agar, MacConkey agar and Blood agar. The plates were incubated for 24 hours at 37oC.

Sampling methods and microbiological examination (Control)

Some of the food borne pathogen strains used as a control for these experiments on the surfaces (stainless steel, marble, and wood), such as Staphylococcus aureus and Escherichia coli were obtained from the Armed Forces Hospital Laboratory. For their control strains a clean stainless steel table without tiny groove was prepared as the food contact surface because it can be fabricated with a smooth cleanable finish. The table also was disinfected with 5. 25 % of hypochlorite solution for 10 minutes. The surface was then washed with hot water, with chemical detergent and rinsed with hot water. The surface was allowed to dry before sampling. The test suspensions were prepared by making serial dilutions of the microorganisms

in peptone saline solution. Two different levels of contamination were prepared: high contamination (approximately 106 colony forming units (CFU)/100 cm2) and low contamination (approximately 103 CFU/100 cm2), obtained by spreading 1 ml of an appropriate solution on a surface of 20 x 20 cm2 over the grid reference table. The table was allowed to dry for 15 minutes to represent the environment of food preparation area. Selective agar media were used for the enumeration of pathogens: Blood agar for Staphylococcus aureus, incubated for 24 hours at 37oC and MacConkey agar for Escherichia coli incubated for 18 â€” 24 hours at 37oC. Furthermore, the effects of two different contamination levels on the survival of pathogens on dry stainless steel surfaces for 24 hours at room temperature were investigated.

Result

Microbial survival on food contact surface (stainless steel surface)

Table 1: The Colony descriptions of the microbial survival on stainless steel surface

Sample Location No	Nutrient Agar	MacConkey Agar	Blood Agar
1	No growth	No growth	No growth
2	No growt	Pink in colour,	No growt

	h	mucoid	h
3	No growth	No growth	No growth
4	No growth	No growth	No growth
5	No growth	No growth	No growth
6	Small colony with dry surface	No growth	Yellow colony
7	No growth	No growth	No growth

8	No growth	No growth	No growth
9	Circular in shape and small white patches	Pink in colour, mucoid	No Growth
10	No growth	No growth	No growth

Table 1 shows the Colony descriptions result of the microorganisms isolated from stainless steel surface. Three of ten stainless steel surface were contaminated with bacteria before cleaning.

Table 2: The colony count of the microbial survival on stainless steel

Sample No.

Serial ten-fold dilutions in deionised water diluents

colony count (CFU ml-1) before cleaning

colony count (CFU ml-1) After cleaning

2

3. 2 x 102

Bacteria Not Detected

6

2. 6 x 102

Bacteria Not Detected

9

4. 3 x 102

Bacteria Not Detected

Table 2 shows the result of the colony count obtained before and after cleaning of the stainless steel surface.

Table 3: Gram stain result of the microbial survival on stainless steel surface

Sample No.: 2

Gram stain result: Gram negative, rod shape

Sample No.: 6

Gram stain result: Gram positive cocci

Sample No.: 9

Gram stain result: Gram negative, rod shape

Table 3 show the result of the Gram stain of bacteria that were isolated from the stainless steel surface before and after the cleaning stage.

Sample No.: 2

Sample No. In phoenix machine: 344

Name of Bacteria detected before cleaning: Klebsiella aerogenes

Name of Bacteria detected After cleaning: Not detected

–

Sample No.: 6

Sample No. In phoenix machine: 367

Name of Bacteria detected before cleaning: Staphlococcus aureus

Name of Bacteria detected After cleaning: Not detected

–

Sample No.: 9

Sample No. In phoenix machine: 382

Name of Bacteria detected before cleaning: Klebsiella aerogenes

Name of Bacteria detected After cleaning: Not detected

Table 4: The Identification of bacteria by phoenix machine that survived on the stainless steel surface before the cleaning stage

Table 4 show the result of bacterial identification that obtained by phoenix machine which was isolated from stainless steel surface before and after the cleaning stage.

Microbial survival in food contact surface (Marble surface)

Table 5: The Colony descriptions of the microbial survival on marble surface

Sample of location No.: 1

Nutrient agar: No Growth

MacConkey agar: No Growth

Blood agar: No Growth

–

Sample of location No.: 2

Nutrient agar: No Growth

MacConkey agar: No Growth

Blood agar: No Growth

–

Sample of location No.: 3

Nutrient agar: No Growth

MacConkey agar: Pink in colour, mucoid

Blood agar: white, large and mucous colonies

–

Sample of location No.: 4

Nutrient agar: No Growth

MacConkey agar: No Growth

Blood agar: No Growth

–

Sample of location No.: 5

Nutrient agar: No Growth

MacConkey agar: No Growth

Blood agar: smooth, round, grayish-white colonies

–

Sample of location No.: 6

Nutrient agar: No Growth

MacConkey agar: No Growth

Blood agar: No Growth

–

Sample of location No.: 7

Nutrient agar: No Growth

MacConkey agar: No Growth

Blood agar: No Growth

–

Sample of location No.: 8

Nutrient agar: No Growth

MacConkey agar: No Growth

Blood agar: No Growth

–

Sample of location No.: 9

Nutrient agar: No Growth

MacConkey agar: No Growth

Blood agar: No Growth

–

Sample of location No.: 10

Nutrient agar: Small circular colonies, yellow in colour

MacConkey agar: No Growth

Blood agar: swarming motility

Table 5 shows the colony descriptions result of the microorganisms isolated from the marble surface. Three of ten marble surfaces remained contaminated with bacteria before and after cleaning.

Table 6: The colony count of the microbial survival on marble surface

Serial dilutions in deionised water diluents

colony count (CFU ml-1) before cleaning

colony count (CFU ml-1) After cleaning

Sample No.: 3

*TFTC

Bacteria Not Detected

Sample No.: 5

5. 1 x 102

Bacteria Not Detected

Sample No.: 10

#TMTC

TMTC

*TFTC: Too Few To Count #TMTC: Too Many To Count

Table 6 shows the result of the colony count obtained before and after cleaning stage of marble surface.

Table 7: Gram stain result of the microbial survival on marble surface

Sample No.: 3

Gram stain result: Gram negative, rod shape

Sample No.: 5

Gram stain result: Gram negative, rod shape

Sample No.: 10

Gram stain result: Gram negative, rod shape

Table 7 show the result of the Gram stain of bacteria that was isolated from the marble surface before and after the cleaning stage.

Table 8: The Identification of bacteria by phoenix machine that survived on the marble surface before the cleaning stage

Sample No.: 3

Sample No. In phoenix machine: 301 Marble

Name of Bacteria detected before cleaning: Klebsiella pneumonia

Name of Bacteria detected After cleaning: Not Detected

–

Sample No.: 5

Sample No. In phoenix machine: 326 Marble

Name of Bacteria detected before cleaning: Yersinia enterocolitica

Name of Bacteria detected After cleaning: Not Detected

–

Sample No.: 10

Sample No. In phoenix machine: 381 Marble

Name of Bacteria detected before cleaning: Proteus vulgaris

Name of Bacteria detected After cleaning: Proteus vulgaris

Table 8 show the result of bacterial identification that obtained by phoenix machine which was isolated from marble surface before and after the cleaning stage.

Microbial survival in food contact surface (Wood surface)

Table 9: The Colony descriptions of the microbial survival on wood surface

Sample location No.: 1

Nutrient agar: No Growth

MacConkey agar: Non-lactose fermenters colonies

Blood agar: White, non haemolytic colonies

–

Sample location No.: 2

Nutrient agar: No Growth

MacConkey agar: No Growth

Blood agar: No Growth

–

Sample location No.: 3

Nutrient agar: smooth, translucent large colonies , greenish blue growth and pigment diffuses into medium

MacConkey agar: No Growth

Blood agar: large brownish colonies

–

Sample location No.: 4

Nutrient agar: White, smooth, round colonies

MacConkey agar: No Growth

Blood agar: No Growth

–

Sample location No.: 5

Nutrient agar: No Growth

MacConkey agar: No Growth

Blood agar: No Growth

–

Sample location No.: 6

Nutrient agar: Circular, smooth, opaque colonies

MacConkey agar: No Growth

Blood agar: swarming motility

–

Sample location No.: 7

Nutrient agar: No Growth

MacConkey agar: No Growth

Blood agar: No Growth

–

Sample location No.: 8

Nutrient agar: smooth, translucent large colonies , greenish blue growth and pigment diffuses into medium

MacConkey agar: slight pink colonies

Blood agar: large brownish colonies

–

Sample location No.: 9

Nutrient agar: smooth, translucent large colonies , greenish blue growth and pigment diffuses into medium

MacConkey agar: slight pink colonies

Blood agar: No Growth

–

Sample location No.: 10

Nutrient agar: No Growth

MacConkey agar: No Growth

Blood agar: No Growth

Table 9 shows the colony descriptions result of the microorganisms isolated from the wood surface. Six of ten wood surfaces remained contaminated with bacteria before and after cleaning.

Table 10: The colony count of the microbial survival on wood surface

Sample No.:

Serial ten-fold dilutions in deionised water diluents

colony count (CFU ml-1) before cleaning

colony count (CFU ml-1) After cleaning

Sample No.: 1

6. 4 x 102

Bacteria Not Detected

Sample No.: 3

5. 3 x 102

Bacteria Not Detected

Sample No.: 4

2. 7 x 102

Bacteria Not Detected

Sample No.: 6

TMTC

TMTC

Sample No.: 8

1. 67 x 103

2. 9 x 102

Sample No.: 9

9. 3 x 102

3. 6 x 102

Table 10 shows the result of the colony count obtained before and after cleaning stage of wood surface.

Table 11: Gram stain result of the microbial survival on wood surface

Sample No.: 1

Gram stain result: Gram negative, rod shape

Sample No.: 3

Gram stain result: Gram negative, rod shape

Sample No.: 4

Gram stain result: Gram negative, rod shape

Sample No.: 6

Gram stain result: Gram negative, rod shape

Sample No.: 8

Gram stain result: Gram negative, rod shape

Sample No.: 9

Gram stain result: Gram negative, rod shape

Table 11 show the result of the Gram stain of bacteria that was isolated from the wood surface before and after the cleaning stage.

Table 12: The Identification of bacteria by phoenix machine that survived on wood surface before the cleaning stage

Sample No.: 1

Sample No. In phoenix machine: 86 wood

Name of Bacteria detected before cleaning: Acinetobacter baumannii

Name of Bacteria detected after cleaning: Not Detected

Sample No.: 3

Sample No. In phoenix machine: 301 wood

Name of Bacteria detected before cleaning: Pseudomonas spp

Name of Bacteria detected after cleaning: Not Detected

Sample No.: 4

Sample No. In phoenix machine: 326 wood

Name of Bacteria detected before cleaning: Enterobacter hafinae alvei

Name of Bacteria detected after cleaning: Not Detected

Sample No.: 6

Sample No. In phoenix machine: 342 wood

Name of Bacteria detected before cleaning: Proteus vulgaris

Name of Bacteria detected after cleaning: Proteus vulgaris

Sample No.: 8

Sample No. In phoenix machine: 369 wood

Name of Bacteria detected before cleaning: Pseudomonas aeruginosa

Name of Bacteria detected after cleaning: Pseudomonas aeruginosa

Sample No.: 9

Sample No. In phoenix machine: 385 wood

Name of Bacteria detected before cleaning: Pseudomonas aeruginosa

Name of Bacteria detected after cleaning: Pseudomonas aeruginosa

Table 12 shows the result of bacterial identification that obtained by phoenix machine which was isolated from wood surface before and after the cleaning stage.

Control

Table 13: Survival of Staph aureus and E. coli on stainless steel surfaces

Staphylococcus aureus

Escherichia coli

Time of swab process after contamination

High contamination level (106 colony)

CFU/100 cm2

Low contamination level (103 colony)

CFU/100 cm2

High contamination level (106 colony)

CFU/100 cm2

Low contamination level (103 colony)

CFU/100 cm2

After 15 minute

2. 0 x 107

1. 0 x 104

1. 6 x 107

5. 2 x 103

After 2 Hours

1. 73 x 107

9. 1 x 103

8. 3 x 106

1. 8 x 103

After 6 Hours

1. 3 x 107

3. 8 x 103

2. 1 x 106

No growth

After 12 Hours

5. 8 x 106

No Growth

No Growth

No growth

After 24 Hours

No growth

No Growth

No Growth

No growth

Table 13 shows the survival of Staphylococcus aureus and Escherichia coli on stainless steel surfaces at room temperature (25oC) for 24 hours at two contamination level; high contamination level of (106 colony CFU/100 cm2) and Low contamination level (103 colony CFU/100 cm2).

Discussion

Sampling food contact surfaces is a complex problem, and the results depend on many factors, including the type of surface, the cleaning solution, the sources of contamination, and the temperature. The accuracy and reproducibility of all sampling methods are reduced when the numbers of bacteria on the surface are low. Some differences between methods are probably due to an uneven distribution of bacteria on the surface. The type of surface markedly influenced the cleaning results. For this study, nineteen selected premises were tested/studied (Ten replicate surfaces were tested; stainless steel, marble and wood, with adjacent areas being sampled before and after cleaning). The results of these studies indicate that three of ten stainless steel surfaces were contaminated before cleaning the surfaces and no surface was contaminated after cleaning, which means that stainless steel surfaces were more easily cleaned. Furthermore, three out of ten marble surfaces were contaminated before cleaning and one surface was contaminated after cleaning the surfaces, which means marble surfaces were easily cleaned but using the wrong cleaning products and the wrong cleaning techniques can damage the marble because marble is a calcium-based natural stone which is highly sensitive to acidic materials (Marble Institute of America, 2012). Stainless steel resists impact damage but is vulnerable to corrosion, while marble surfaces are prone to deterioration and

may develop surface cracks where bacteria can accumulate (Leclercq and Lalande, 1994). Wood surfaces were particularly diffi