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ASSESSMENT OF BACTERIAL STATUS OF FOOD CONTACT SURFACES IN A HOSPITAL CENTRAL CATERING FACILITY

By

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ABSTRACT

Contaminated equipment and food contact surfaces. One of the top risk factors for foodborne disease outbreaks particularly for the immune compromised patients who are at risk of being affected and suffering from more serious complications as a result of infection. Moreover the single most important means to prevent spread of infection is hand washing and if poorly or improperly implemented, can lead to foodborne illness because workers may carry pathogens as Staph. Aureus and *E. coli* in their nails or their skin that led to contaminating cooked food with these pathogens. So, the aim of the present study is to assess the hygienic status of food contact surfaces (Cutting boards, serving dishes, knives, Meat mincer, Meat saw Sieve for chicken thawing, Tape surface and Presentation Plate) in addition to employee's hand by conventional and convenient methods through enumeration of total mesophilic aerobes which is one of the most common parameters used to assess the microbiological quality of food contact surfaces and workers' hands.A total of 55 swabs were collected from food contact surfaces and from worker's hands then examined for total aerobic mesophilic count.

The traditional hand swabs result before starting work was below the detectable limit (< 2 Log10 CFU) while during working process revealed an elevation in the total count. Results of rapid method (ATP) agreed with the traditional methods. For other food contact surfaces, the APC for clean (Washed) equipment swab samples are high indicating unsatisfactory conditions.

INTRODUCTION

Everyone is susceptible to food-borne diseases but the immune compromised patients are particularly at risk of being affected by food-borne diseases and suffering from more serious complications as a result of infection. Foodborne illnesses can be caused by microorganisms and/or their toxins, fungi and their related toxins physical and chemical contaminants so that

hospitals may impose dietary restrictions to limit exposure of patients to pathogens **(French *et al.*, 2001; Newell *et al.*, 2010; Petruzzelli *et al.*, 2010 and Khamis and Hafez, 2011)**. Microbiological contamination of foods can be caused by contaminated raw materials or cross-contamination by microorganisms originating from various sources like water, air, dust, hair, infected wounds, dirt **(Gorman *et al.*, 2002 and Osimani *et al.*, 2013).** Therefore, hospital catering must provide patients with foods that covering their nutritional requirements and must be microbiologically safe with mass production meal safety constitute a real challange. **(Hartwell and Edwards, 2001 and FEADRS, 2009).** So food safety quality management systems and high standard of hygiene in the work environment (Surfaces, equipment, and utensils) as a fundamental requisite for the prevention of microbial contaminations must be in place to ensure that such meals do not compromise public health **(Carrascosa *et al.*, 2012)**. Several pathogens, including *Staphylococcus aureus*, *Listeria monocytogenes Salmonella* spp., *Campylobacter jejuni*, *Yersinia enterocolitica* and enteropathogenic strains of *Escherichia coli* can survive on different surfaces for periods ranging from several hours to days **(Martinon *et al.*, 2012 and Simoes, *et al.*, 2010)** and even form biofilms. The latter are surface-associated microbial communities, consisting of micro-colonies entrapped in an exopolymeric matrix **(Davey and O'Toole, 2000).**

Microbial cells can persist and survive decontamination procedures representing a potential reservoir for food contamination. In food production plants, the formation of biofilms generally starts when cleaning and sanitation procedures are not performed correctly and the food residues that remain on. The improperly cleaned surfaces constitute a source of nutrients for the microorganisms which may be present **(Srey *et al.*, 2013)** which mean that poorly cleaned utensil and equipment surfaces harbour and promote the spread of microorganisms **(Byran,1990)**. One of the top five risk factors for foodborne disease outbreaks in food service operations is contaminated equipment and food contact surfaces due to inadequate cleaning or disinfection, because cleaning work surfaces, equipment and utensils is the key to preventing microorganism contamination that can subsequently multiply in prepared foods, reaching unacceptable levels.**(USDHHS -FDA-CFSAN, 2000;WHO, 2007 and Rodriguez-Caturla *et al.*, 2012).** Microbiological analysis of surfaces has been proven to be an effective tool for assessing the cleaning practices that are carried out in a kitchen and for improving hygienic behaviors in food handlers and making them more permanent. Therefore, regular monitoring of work surfaces by means of microbial counts can demonstrate the level of cleanliness more

objectively than visual inspection **(Food Safety and Hygiene Working Group, 1997; Kassa *et al.*, 2001 and Sagoo *et al.*, 2003)**. The role of hand washing in the presence and transfer of bacteria has been studied in a variety of settings, including hospitals **(Vollaard *et al.*, 2004**). The bad hand hygiene of workers who carry pathogens like *Staph. Aureus* and *E. coli* in their nails or their skin led to contaminating cooked food with these pathogens. **(Protocarrero *et al.*, 2002)**. Enumeration of total mesophilic aerobes is one of the most common parameters used to assess the microbiological quality of food contact surfaces **(Çetin *et al.*,2006 and Olgunoglu, 2010).** In recent decades, alternative more rapid methods have been developed for the real-time evaluation of the cleanliness of food contact surfaces. One of these methods relies on the measurement of the bioluminescence produced by the firefly (*Photinus pyralis*) luciferase through the oxidative decarboxylation of luciferin in the presence of adenosine triphosphate (ATP), a molecule occurring in either living organisms or food, as non-microbial ATP. The amount of light emitted, measured with a luminometer, which consists of a photomultiplier and an amplifier connected to a recorder, is strictly dependent on both surface abiotic and biotic contamination; it is expressed as relative light units (RLU) **(Hawronskyj and Holah,1997).** One of the major advantages of ATP bioluminescence technology is having potential for the real time monitoring of surface cleanliness, for the self-evaluation by the staff responsible for the cleanliness and sanitation and for verification of cleaning procedures **(Cooper *et al.*, 2007 Amodio and Dino 2014 and Osimani *et al.*, 2014).** In this study Aerobic plate counts (APC) were chosen as indicators of the effectiveness of cleaning and disinfection procedures where the traditional APC plating methods was assessed versus the resultsobtainedfrom rapid method of theHygiena EnSURE™ device(ATP bioluminescence measurements) to reveal the hygienic status of the food contact surfaces within a catering facility as well as to compare between the two methods of examination.

MATERIAL AND METHODS

Samples collection from worker's hands and equipment:

Total number of 55 swabs were collected as following: 18 swab samples from worker's hands (Before starting work and during working); 19 swab samples from cutting boards (Washed-washed and disinfected-during working); 9 swab samples from serving dishes; 4 swab samples from knives and 5 swab samples from other equipment (Meat mincer - Meat saw - Sieve for chicken thawing - Tape surface - Presentation Plate).

Samples preparation and examination:

Swab samples were taken using sterile cotton swabs (Each surface was swabbed in the area inside a sterile metal template (10 by 10 cm^2), where the swabs were transferred to tube containing 10 mL of peptone water, then samples were transported immediately to hospital laboratory. Over there the swabs in the peptone water tubes shook in a vortex for 1 min and serial dilution were done then one mL of the dilutions inoculated on surface of plate count agar media (Oxoid CM 463), then the plates were incubated at 35 °C for 24-48 hrs to determine the total mesophilic aerobic plate count (APC). Each individual colony was counted, and then the average readings of the two plates were reported **(Swanson *et al.*, 1992).** The results were expressed in CFU/ hand for hand swabs and CFU/cm^2 for food contact surfaces.

Investigations using Hygiena EnSURE™ device (ATP bioluminescence measurements):

Hygiene swabbing was performed on areas adjacent (100 cm^2) to those subjected to bioluminescence measurements and the instructions for examination was followed as shown.

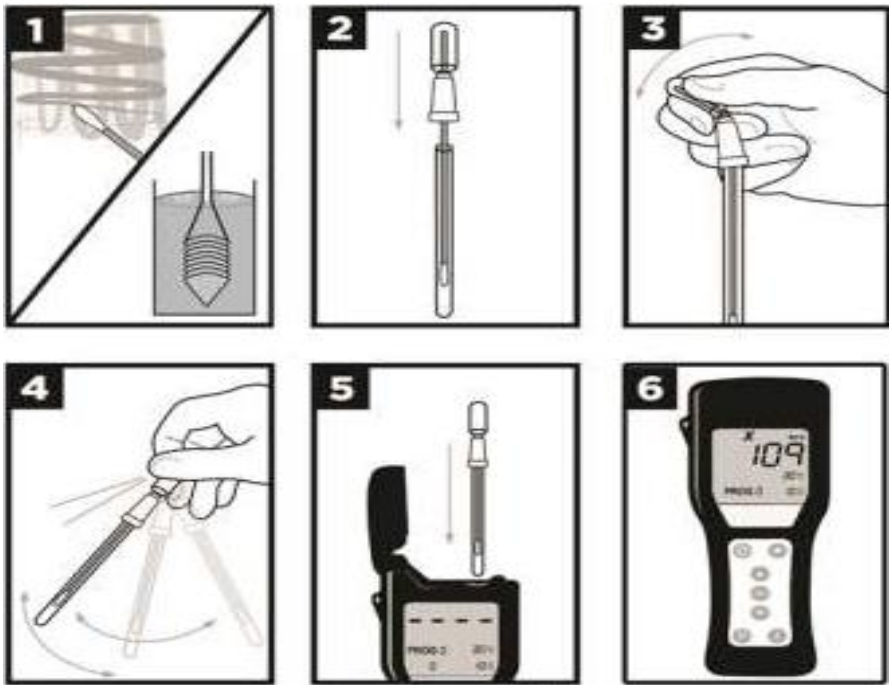

Fig. (1): The procedures of swabbing and sample examination using hygiena Ensure device (Reproduced from System SURE *Plus* and EnSURE™ Operator Manual V5.0, by Hygiena LLC, 2020).

Statistical analysis:

The mean values and comparing the results obtained from the traditional and rapid method was assessed using T-test of SPSS program for windows.

RESULTS AND DISCUSSION

Table (1): Mean APC values (± SE) t for hand swabs collected from workers using traditional swabbing and Hygiena EnSURE™ device.

	Before working		During working	
	Traditional APC count (Log_{10} CFU)	**Rapid count using ATP (RLU)**	**Traditional APC count (Log_{10} CFU)**	**Rapid count using ATP (RLU)**
Butchery preparation chef	**< 2**	**ND**	**3.3 ± 0.08***	**59.5 ± 13**
Hot food preparation chef	**< 2**	**4 ± 0.7**	**1.7 ± 0.57**	**68.2 ± 35**
Salad preparation chef	**< 2**	**4 ± 0.8**	**< 2**	**4.5 ± 0.28**
Pastry preparation chef	**< 2**	**5 ± 0.5**	**2.4 ± 0.03**	**ND**
Total count	**< 2**	**4.3 ± 0.6**	**1.85 ± 0.17**	**44 ± 0.28**

***Data represent mean ± standard error; ND= not detectable.**

From the obtained results all traditional swabs from worker's hands before starting work was below the detectable limit (< 2 Log_{10} CFU). Meanwhile the obtained results using rapid method (ATP) ranged from 4 to 5 RLU with mean value of 4.3 ± 0.6 which indicate a high workers sanitation level before starting the work. On the other hand, swabs collected during working process revealed an elevation in the total count using the traditional APC method, where the highest mean value was obtained from the butcher worker's hands (3.3 ± 0.08 Log_{10} CFU) while the lowest value of (< 2 Log_{10} CFU) was obtained from hands of salad preparation workers. Correspondingly to the elevation in the APC using the ATP method was well correlated to traditional swab method and showed a parallel elevation in the RLU where

the lowest value also recorded from hands of salad preparation workers as in traditional method. Microbial transfer by hands is a potential method of cross-contamination **(Pe´rez-Rodrı´guez *et al.*, 2008)**, where contact surfaces are more likely to be contaminated than food contact surfaces **(DeVita *et al.*, 2007).** The use of microbiological testing should not be underestimated as a part of hygiene training. The impact of seeing agar plates covered in colonies that have been isolated from swabs taken from hands pre-washing or surfaces pre-cleaning, and the reduction achieved following washing or sanitation, can be significant. The rapid results achievable by ATP bioluminescence can be particularly useful for the motivation and training of sanitation and production staff by providing a means for them to judge their own performance and by demonstrating the importance of their work. Regular swabbing of hands can also help to reinforce hygiene procedures **(Blackburn, 2006)**. Acceptability limits based on ATP bioluminescence were defined through a series of preliminary analysis carried out on the same surfaces known as control point (CP) subjected to routine analysis. In more detail, for each CP, reference values for the maximum levels of dirt and cleanliness were defined by measuring RLU values before and immediately after vigorous cleaning and sanitation, respectively; hence, 20 measurements at each surface, carried out before (10 measurements) and after vigorous cleaning and sanitation (10 measurements) were taken over the course of 10 days using the Clean-Trace ATP surface test (3M) and the bioluminescence reader Clean-Trace NG Luminometer (3M); at the end of this step, the appropriateness of the cleaning and sanitation procedures was verified through the calculation of RLU percentage reduction before and after cleaning **(Osimani *et al.*, 2014).** A few internationally accepted standards have been published to define acceptable levels of microbial contamination on surfaces (**Commission Decision 2001/471/EC**). Meanwhile, **Henroid *et al.*, (2004); Sneed *et al.*, (2004) and Marzano and Balzaretti (2013),** suggested the following total bacterial count as standards for cleaned and sanitized food-contact surfaces and hands which is count <1.3 $\log_{10}$ CFU/cm^2. According this standard 100% of the collected hand swabs results before working are accepted, while **Marzano and Balzaretti (2013)** found that the total aerobic bacterial count exceeded the reference standards in 18.1% of cases. It is necessary to improve food handlers' implementation of hand drying as residual moisture can considerably enhance the transfer of any remaining micro-organisms present on the hands to other surfaces.

The importance of the use of soap and other hand sanitizers as part of an effective hand wash

to remove organic debris and microbial load, especially the potential pathogens are well documented **(Snyder 1998 and Santana *et al.*, 2009)**. That is why food safety measures have been focused on training of food handlers in appropriate hygiene practices and on improving the sanitary quality of meals **(Veiros *et al.*, 2009 and Buccheri *et al.*, 2010).**

Table (2): Mean APC values (± SE) for swabs collected from food contact surfaces using traditional swabbing and plating method represented by Log_{10} CFU.

Cutting boards	Before working		During working
	Not disinfected	disinfected	-
Salad cutting board	HUC	1 ± 0.7*	3.6 ± 0.31
Preparation cutting board	HUC	NE	3.85 ± 0.02
Hot area cutting board	HUC	< 2	2.8 ± 0.05
Pastry cutting board	HUC	1 ± 0.7	NE
Chicken cutting board	HUC	< 2	NE
Meat cutting board	HUC	NE	NE
Fish cutting board	HUC	NE	NE
Other food contact surfaces			
Knives (Garde manger)	2.15 ± 0.11		2.9 ± 0.21
Meat mincer	4.8± 0.31		NE
Meat saw	2.85 ± 0.14		NE
Seive for chicken thawing	3.79 ± 0.5		NE
Tape surface	2.6 ± 0.33		NE
Presentation Plate	2.7 ± 0. 14		NE
Serving dishes	Cleaned only	Cleaned and disinfected	-
Serving dish (salad)	3.3 ± 0.1	< 2	NE
Serving dish (hot area)	3.8 ± 0.4	2 ± 0.1	NE
Serving dish (butcher)	3.9 ± 0.21	< 2	NE
Shaving dish (pastry)	3.3 ± 0.33	<2	NE

***Data represent mean ± standard error; HUC= high uncountable results; NE= not examined.**

In the present study,food contact surfaces with the highest microbial loads were obtained from only washed boards and before disinfection (Uncountable), which may be refer to

improper washing or storage of washed boards in unclean area. Disinfection of these boards carried on immediately before using it leading to reduction of the APC to 0.5 $\log_{10}$ CFU/Cm^2 which is satisfactory. Moreover, the APC mean result of swabs collected from boards during working is 2.9 $\log_{10}$ CFU/cm^2. The highest results of aerobic plate count for other equipment that doesn't disinfected before using it; like Serving dish, tape surface, plate, sieve, meat saw and meat mincer don't exceed 4.2 $\log_{10}$ CFU which is lower than results observed by **Pinto *et al.*, (2015)** which was up to 10^5 cfu/utensil and all of these results don't meet any of used standard. Microbial limits for food contact surfaces have been proposed at 10 to 20 CFU/cm^2 (**Solberg *et al.*, 2004)**. In another study, **Sneed *et al.* (2004**) proposed a standard for food contact surfaces of less than 20 CFU/cm^2 for APC. These authors reported high levels of APC on durable resin cutting boards (>20 CFU/cm^2); while **Montville and Schaffner (2004)** reported lower average levels for mesophilic aerobic bacteria in cutting boards (10.16 CFU/4 cm^2), but 6.7% of samples analyzed had levels above 50 CFU/4 cm^2. Their obtained results could be explained by lodging of microorganisms in cracks and crevices of cutting boards that are not properly sanitize. **(Todd *et al.*,2009)** and the humidity of cutting boards may favor detachment of bacteria from these food contact surfaces when they are swab sampled, enhancing bacterial recovery **(Marples and Towers, 1979)**.

The Canadian government establishes benchmarks for the evaluation of the cleanliness of work surface areas, being more restrictive for utensils and tableware (Maximum 1 CFU/cm^2) than for the actual work surfaces, equipment and apparatus in contact with food, allowing maximum levels of aerobic plate count of 100 CFU/cm^2 **(MAPAQ, 2009).** From the obtained results in current study it is obvious that, the APC for clean (washed) equipment swab samples are high and it is higher than results obtained from swabs collected during working which indicated unsatisfactory results. It's clear that application of good hygienic practices (GHP), good manufacturing practices (GMP) and food safety system (HACCP, ISO 22000) is mandatory for maintaining a safe environment for food preparation **(Attala and Kassem, 2011).**

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تقييم الحالة البكتيرية للأسطح الملامسة للأغذية في منشأة تقديم الطعام بالمستشفى

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تعتبر المعدات الملوثة والأسطح الملامسة للأغذية من أهم عوامل الخطر لتفشي الأمراض المنقولة عن طريق الأغذية، وخاصة بالنسبة للمرضى الذين يعانون من ضعف المناعة والذين يتعرضون لخطر الإصابة ويعانون من مضاعفات أكثر خطورة نتيجة للعدوى. علاوة على ذلك، فإن الوسيلة الأكثر أهمية لمنع انتشار العدوى هي غسل اليدين، وإذا تم تنفيذها بشكل سيئ أو غير صحيح، يمكن أن يؤدي ذلك إلى تفشي الأمراض المنقولة عن طريق الغذاء لأن العمال قد يحملون العديد من مسببات الأمراض مثل ميكروبات العنقوديات الذهبية والإشريكية القولونية في أظافرهم أو جلدهم مما يؤدي إلى تلويث الطعام المطبوخ بهذه العوامل الممرضة. لذلك، فإن الهدف من هذه الدراسة هو تقييم الحالة الصحية للأسطح الملامسة للأغذية بالإضافة إلى أيدي العمال بالطرق التقليدية والطرق الحديثة من خلال تقييم العد الكلي البكتيري الذي يعد أحد المعايير الأكثر شيوعًا المستخدمة لتقييم الجودة المكروبيولوجية الأسطح التي تلامس الطعام وأيدي العمال. في هذه الدراسة تم جمع 53 مسحة من السطوح الملامسة للغذاء ومن أيدي العمال ثم فحصت لمعرفة العدد الكلي للبكتيريا الهوائية. كانت نتيجة مسحات اليد التقليدية قبل بدء العمل أقل من الحد القابل للاكتشاف (Log10 CFU 2>) بينما كشفت أثناء عملية العمل ارتفاعًا في العدد الإجمالي. نتائج الطريقة السريعة (ATP) جاءت متفقة مع الطرق التقليدية. بالنسبة للأسطح الأخرى التي تلامس الطعام، فإن العدد الكلي للبكتيريا الهوائية لعينات مسحة المعدات النظيفة (المغسولة) كانت مرتفعة مما يشير إلى ظروف صحية غير مرضية.