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Microbial levels on the food preparation areas of a typical district hospital in South Africa

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The role of hospital surfaces (including surfaces in food preparation areas) in the transmission of hospital-acquired infections (HAI) has been long recognized; however, evidence regarding this critical information is not well documented. This study was conducted at a typical district hospital in the Free State Province of South Africa. Using swabs, surface samples were collected from nine kitchen areas, quantified and identified using the MALDI-TOF MS and API. Fungal counts (1 x 10^3 to 2.3 x 10^5 cfu.cm^{-2}) were higher compared to bacterial counts (1.5 x 10^3 to 1.1 x 10^5 cfu.cm^{-2}). A total of 25 bacterial species and 14 fungal species were identified from hospital kitchen surfaces using the MALDI-TOF MS. *Candida* was the most common fungal genus identified represented by 11 species while *Bacillus* was the most common bacterial genus isolated represented by 7 species. The presence of species from the genera *Acinetobacter, Enterobacter*, *Pseudomonas* and *Candida* amongst others on kitchen surfaces could have serious consequences as they have been implicated in various studies as probable causes of hospital acquired infections. The study highlights the need to ensure proper cleaning of working surfaces in the kitchen as well as stringent surveillance and monitoring to ensure the minimal contamination of food products prepared for patients.

Key words: Hospital-acquired infection, kitchen, MALDI Biotyper, surface swabs, food safety.

INTRODUCTION

Hospital surfaces, including those in food preparation areas, are some of the major contributing factors to the spread of food-borne illnesses and hospital-acquired infections [HAI] (Mead et al., 1999; Kir et al., 2006). In the United States of America, it has been reported that 48 million food-related illnesses occurred annually, with 128 000 people being admitted to hospital and 3000 deaths occurring (CDC, 2011). In addition, evidence indicates that this is a worldwide problem affecting both developed and developing countries (CDC, 2011). Spain, for example, recorded the hospitalization of 4.8 million people in one year (Sala et al., 2005). In 2005, an outbreak in Spain caused by norovirus in a hospital cafeteria was reported to have originated from contaminated vegetable salads, sandwiches, cheese and cooked meat products prepared by food handlers (Sala et al., 2005).

Food handlers play an important role especially in hospitals as they could be sources of contamination. They could also cross-contaminate food during its preparation and distribution within the hospital (Sala et al., 2005). Contamination from food handlers usually results due to inadequately washed hands, improper food preparation techniques as well as incorrect cleaning procedures of food preparation surfaces such as chopping boards and tables. Bacteria have been reported to survive on chopping-boards for more than three hours,

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especially when boards are not properly cleaned (Zhao et al., 1998; Salo et al., 2000), and their presence may also lead to the development of biofilms.

Although contaminated surfaces can serve as possible reservoirs for pathogenic microorganisms, studies have suggested that surfaces are not directly associated with transmission of hospital- acquired food-borne infections to patients and the source of transmission from kitchen surfaces to patients is mainly via hand contact with the surface (Jarvis, 2007; Kampf and Kramer, 2004). Moreover, the Centers for Disease Control and Prevention (CDC) reports that approximately 20% of food-related illnesses is due to food handlers (Michaels et al., 2004). However, Favero and Bond (2001) suggest that structural defects could also be potential sources of food contamination, increasing the levels of pathogenic microorganisms in healthcare settings. The predominant mechanism through which these structural defects spread and become harmful especially to immuno-compromised patients is when the dust from air starts settling on kitchen surfaces leading to food contamination (Favero et al., 2001).

In addition, Salo and colleagues (2000) reported that wet items such as dishcloths, hand towels and sponges, as well as sink drain areas with leaking pipes might also serve as continuous reservoirs that harbor potentially harmful microorganisms, which may end up settling on kitchen surfaces (Zhao et al., 1998; Salo et al., 2000). Improper food hygiene practices and unclean surfaces have been associated with opportunistic pathogenic microorganisms such as *Staphylococcus aureus* (Andargie et al., 2008; Garcia, 2007). The presence of *S. aureus* is often perturbing due to the possibility of antibiotic resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) (McCaughey, 2007).

Currently, information concerning microbial contamination of working surfaces and equipment in hospital food preparation areas, particularly in South Africa, is still lacking. Improperly cleaned surfaces along with deficient food handling practices have led to an increase in microbiological hazards in food preparation areas (Nkhebenyane, 2010). Kitchens are important contamination points for food and should be kept free from possible contaminants. In order to implement food safety systems, the abovementioned aspects need to be considered: this is what led to the formulation of the aims of this study: which were to determine the level and distribution of microbial contamination on kitchen surfaces in hospitals and to identify microorganisms.

MATERIALS AND METHODS

Sampling sites

Surface samples were collected at a district hospital in the Free State Province of South Africa from the selected hospital kitchen surfaces, namely storage, basin, sink, cutter, meat cutter, cutter surface, food preparation area, meat preparation area, and water pipes. This was done twice over four rounds in duplicate, between the hours of 10:00 and 11:00 (during preparation of food) using sterile surface swabs. The samples were kept on ice during transportation to the laboratory and analysed without delay on arrival.

Microbial quantification

Serial dilutions were carried out. Following this, 0.1ml of the diluents were transferred to Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) for bacteria and fungi respectively. Plates were incubited aerobically for 48 to 72 h at 35°C for bacteria and 25°C for fungi. After the appropriate incubation period, the plates were examined for colonial growth, agar plates that contained between 30 – 300 colonies were counted and results recorded manually (Weissmann, 2006).

Microbial sample preparation for API

For sample collection and preparation, the microorganisms to be identified were first isolated on a suitable culture medium according to standard microbiological techniques. After sample preparation, a single well-isolated colony was removed from an isolation plate and emulsified into the API Medium to achieve a homogeneous bacterial suspension of a 0.5 McFarland standard. The suspension was used immediately after preparation. For preparation of strips, an incubation box (tray and lid) was prepared and 5 ml (or 10 ml depending on the test used) of distilled water was distributed into the honeycombed wells of the tray to create a humid atmosphere. The strain reference was recorded on the elongated flap of the tray, following which; the strip was placed in the incubation box for inoculation. A sterile pipette was used to distribute the bacterial suspension into the tubes. For some tests, only the tubes and not the cupules were filled while others were overlaid with mineral oil to create anaerobiosis. To avoid the formation of bubbles, the tip of the pipette was placed against the side of the cupules. After inoculation of strips, the incubation box was immediately closed and incubated at 36°C ± 2°C for 18-24 h. The strips were read after the stipulated incubation period (24, 48 h and/or 72 h, depending on the microorganism and the type of reaction studied). For the interpretation of results, a numerical profile was used and for identifying bacterial species, a database (V4.0) was performed with the analytical profile index by looking up the numerical profile in the list of profiles or with the identification software by entering the 7-digit numerical profile manually via the keyboard (van Veen et al., 2010).

Microbial sample preparation for MALDI Biotyper

The MALDI Biotyper uses Matrix Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) for microbial identification. The MALDI-TOF MS Microflex mass spectrometer (Bruker Daltonik, Bremen, Germany) FlexControl software (version 3.0) measures highly abundant proteins that are found in all microorganisms. The characteristic patterns of these highly abundant proteins are used to reliably and accurately identify a particular microorganism by matching the respective pattern with an extensive open database to determine the identity of the microorganism down to the species level (Bruker). For identification of colonies using the MALDI-TOF MS; direct placing or placing on a steel target after extraction was performed according to the manufacturer's instructions. Briefly, single colony from each plate was picked up using a sterile pipette tip and smeared as a thin film directly on a MALDI steel target. Microorganisms that could not be

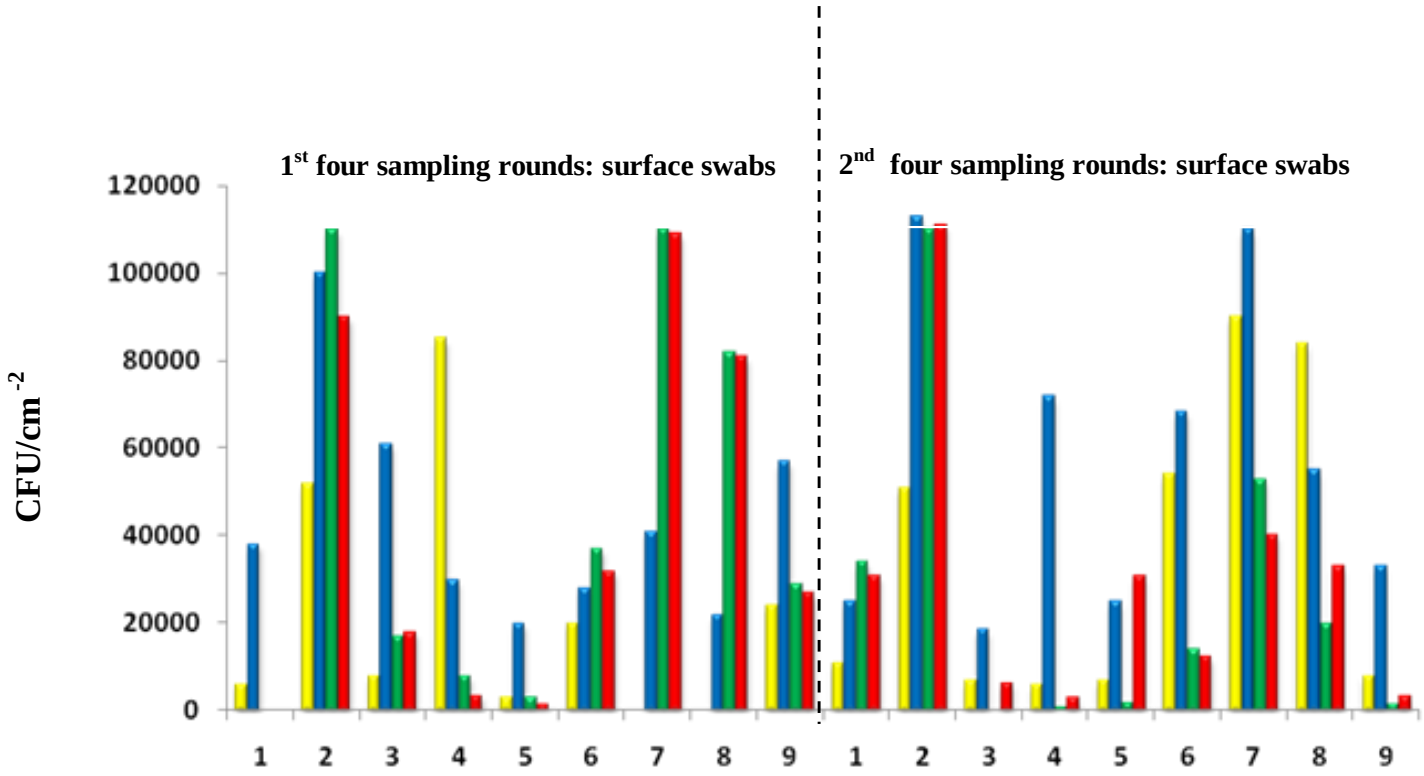

Figure 1. Total Viable Counts isolated from food preparations area using surface swabs (Storage (1), Basin (2), Sink (3), Cutter (4), Meat cutter (5), Cutter surface (6), Food preparation area (7), Meat preparation area (8), Water pipes (9)).

identified directly by MALDI-TOF MS underwent extraction and were retested. Cells of a whole colony and pure colonies were transferred to a 1.5 ml tube (Eppendorf, Germany) mixed thoroughly in 300 µl of distilled water. Nine hundred micro liters (900 µl) of absolute ethanol were added, the mixture was centrifuged at 15,500 *g* for 2 min, and the supernatant was discarded. The pellet was air-dried at room temperature. Subsequently, 50 µl of formic acid (70% v/v) was added to the pellet and mixed thoroughly before the addition of 50 µl of acetonitrile. The mixture was centrifuged again at 15,500 *g* for 2 min. One microliter of the supernatant was placed onto a spot of the steel target and air-dried at room temperature. Following this, 1 µl of matrix solution (20 mg/ml 3,5-dimethoxy-4-hydroxycinnamic acid in acetonitrile (ACN): purified water: trifluoroacetic acid (TFA) (50:50:0.1)) was used to overlay the smeared colonies on the steel target. The steel target was air-dried for 10 min and placed in the MALDI Biotyper for analysis. Measurements were performed using a Microflex mass spectrometer (Bruker Daltonik, Bremen, Germany) by means of a FlexControl software (version 3.0). Spectra were recorded in the positive linear mode (laser frequency, 20 Hz; ion source 1voltage, 20 kV; ion source 2 voltage, 18.4 kV; lens voltage, 9.1 kV; mass range, 2,000 to 20,000 Da). For each spectrum 240 shots in 40-shot steps from different positions of the target spot (automatic mode) were collected and analyzed. All colonies reported were above 1.80 score value. Identification of unknown microbes found in the hospital was classified using modified score values proposed by the manufacturer: a score of ≥2 indicated species identification; a score between 1.7 and 1.9 indicated genus identification and a score of <1.7 indicated not reliable identification (Bizzini et al., 2010).

RESULTS AND DISCUSSION

Enumeration of bacterial colonies from swab samples

Figure 1 depicts bacterial counts collected using swabs from the surfaces at various areas in the hospital kitchen. In general, counts ranged from 1.5 x 10^3 to 1.1 x 10^5 cfu/cm^{-2}. Counts obtained during the second (2 x 10^4 to 1.1 x 10^5 cfu/cm^{-2}), third (2 x 10^3 to 1.1 x 10^5 cfu/cm^{-2}) and fourth round (1.7 x 10^3 to 1.1 x 10^5 cfu/cm^{-2}) of sampling showed higher microbial counts when compared to the first sampling round (1.5 x 10^3 to 8.9 x 10^4 cfu/cm^{-2}), however, the differences were not statistically significant (p= 0.9). The basin (5.5 x 10^4 to 1.1 x 10^5 cfu/cm^{-2}), food preparation area (4 x 10^4 to 1.1 x 10^5 cfu/cm^{-2}) and meat preparation area (2 x 10^4 to 8 x 10^4 cfu/cm^{-2}) were commonly found to have higher bacterial counts when compared to other areas in the kitchen (8 x 10^2 to 7.9 x 10^4 cfu/cm^{-2}). These results are alarming when compared to recent microbiological standards (Orion Diagnostics, 2010) and results of other studies such as those conducted by Nkhebenyane (2010), since microbial levels did not decrease after the first sampling visits and furthermore they serve as an indication that proper food, handling and preparation techniques are not practiced at

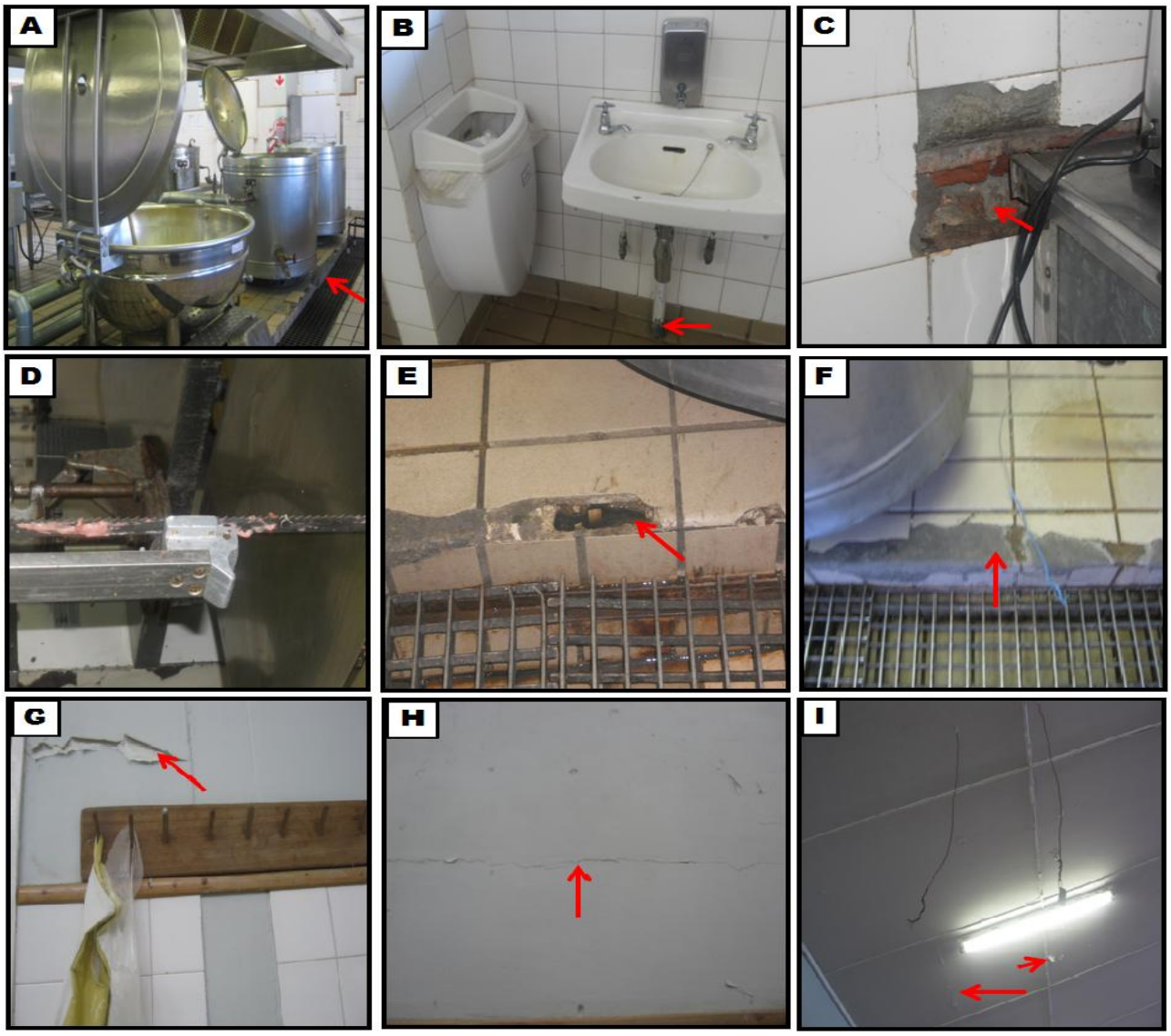

Figure 2. Figures A-H indicating structural defects and unhygienic conditions in the kitchen. A-Cracked floor tiles; B – Peeling paint from basin.

all times by food handlers. Lack of good hygiene practices by food handlers has been reported to influence microbial rates and could also place patients at risk of contracting food-borne illnesses, which may increase statistical rates of deaths (Askarian et al., 2004).

Apart from food handlers, other possible sources that might influence microbial loads on kitchen surfaces of the hospital are structural defects (Pastuszka et al., 2005). During sampling rounds, floors, walls and ceilings were found not to be free from visible dust, soot, holes and cracks (Figure 2). The presence of structural defects in the hospital kitchen might be attributable to the age of the building (the hospital was built in1892). According to hospital management, little or no maintenance was done to most of the areas within the hospital, thus making it a potentially hazardous area for food preparation as well as for occupational hygiene aspects. The challenge with structural defects is that they may serve as harboring places for microbial proliferation and may be difficult to clean, thus also increasing possible cross-contamination and the formation of biofilms.

Enumeration of fungal colonies from swab samples

Fungi were also isolated in the food preparation areas of the hospital where counts ranged from 1 x 10^3 to 2.3 x 10^5 cfu/cm^{-2}. Results obtained during the first, second and third rounds of sampling showed low microbial counts compared to the fourth rounds which was contrary to bacterial counts, nevertheless, the observed differences

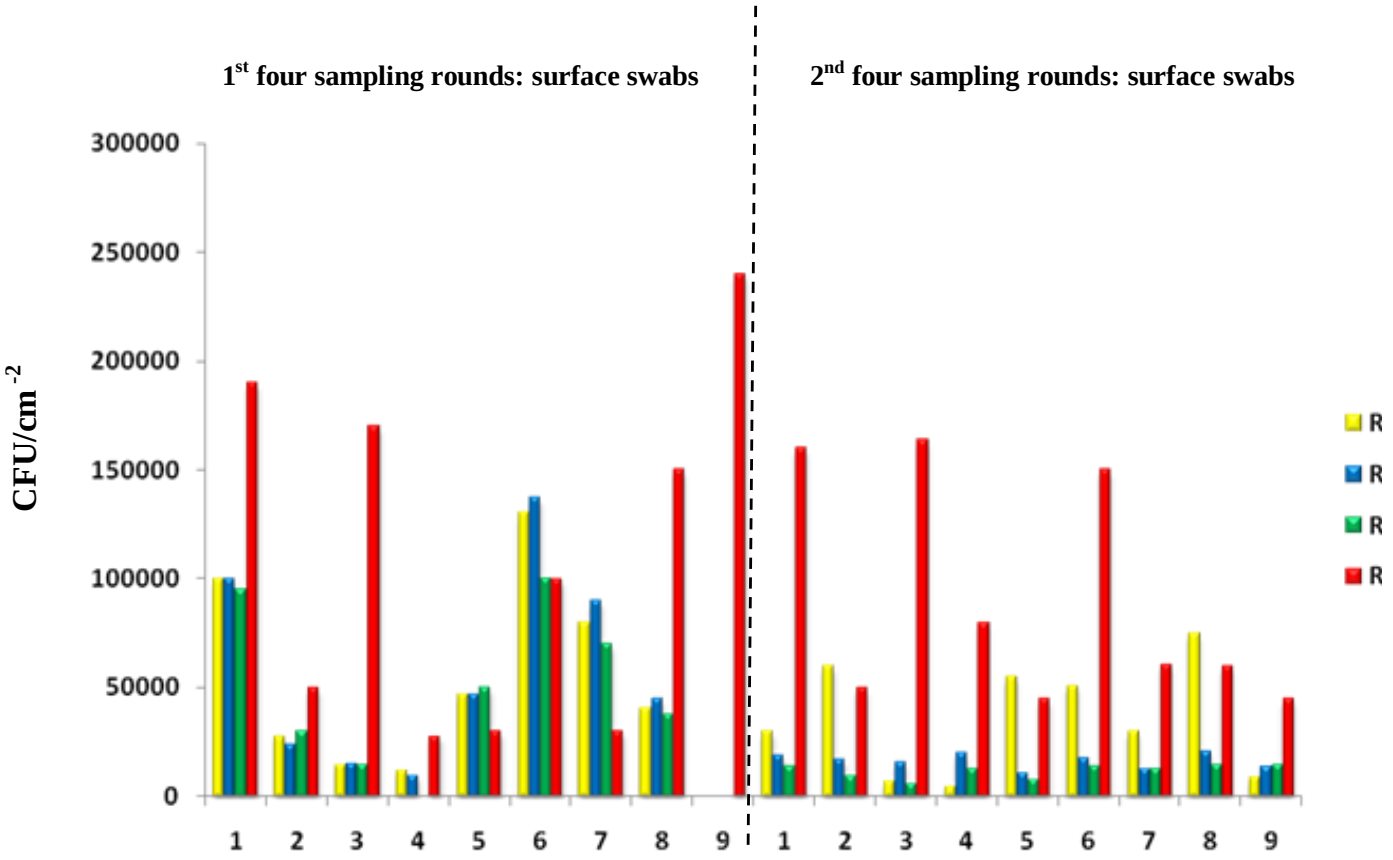

Figure 3. Average culturable fungal microorganisms isolated from food preparations area using surface swabs (Sink (1), Storage (2), Basin (3), Cutter (4), Meat cutter (5), Cutter surface (6), Food preparation area (7), Meat preparation area (8), Water pipes (9)).

for fungi were not statistically significant (p = 0.1). In order to determine the exact relationships amongst various microbiota, Spearman's correlation coefficient and F-Test (two-tailed probability) was used to construct a correlation matrix and significant differences. Microbial counts in kitchen surfaces showed a correlation coefficient between bacteria and fungi to be r^2= -0.396 (first sampling rounds), r^2= -0.387 (second sampling rounds), r^2= 0.023 (third sampling rounds) and r^2= 0.684 (fourth sampling rounds) respectively. The first and second sampling rounds in the food preparation areas showed a negative correlation. Whereas a positive correlation was observed for the third and fourth sampling rounds.

The basin (6 x 10^3 to 1.5 x 10^4 cfu/cm^{-2}), cutter (5 x 10^3 to 1 x 10^4 cfu/cm^{-2}) and water pipes (1.2 x 10^2 to 1.4 x 10^4 cfu/cm^{-2}) had the lowest microbial counts whereas the fourth rounds of the specified areas had the highest counts that ranged as follows: basin (1.6 x 10^5 cfu/cm^{-2}), cutter (2.8 x 10^4 to 8 x 10^4 cfu/cm^{-2}) and water pipes (4.5 x 10^4 to 2.4 x 10^5 cfu/cm^{-2}).

Increased microbial loads during the fourth sampling rounds might have been influenced by the unhygienic status of the kitchen during these sampling rounds (Figure 3).

In comparison to other sampling rounds, working surfaces in the fourth sampling rounds were dirtier, water pipes were leaking on the floors and the kitchen sink had drainage problems. Fungal counts together with bacterial counts obtained in the present study were high (≤1.5 x 10^5 cfu/cm^{-2}) when compared to microbial counts in other studies: ≤ 2 x 10^2 cfu/cm^{-2} were recorded by Abu-Elteen et al. (2009) in healthcare settings; and ≤ 10 cfu/cm^{-2} were recorded by Nkhebenyane (2010) in hospices. Nkhebenyane (2010) also reports that personnel, kitchen tables, utensils, dishcloths and sponges may serve as possible sources of possible contaminants especially in a health-care setting.

Bacterial and fungal characterization

The growth of unknown bacterial colonies from the kitchen surfaces were identified using API and the MALDI-TOF MS. In general, using the MALDI-TOF MS more bacterial species (25 species) could be identified than when the API technique (7 species) was used (Tables 1 and 2). Bacteria found on several kitchen surfaces example storage, basin, sink and water pipes were identi-

Table 1. Gram-positive bacterial characterization: food preparation surfaces.

Origin	Species identified using MALDI Biotyper	Species identification (Gram (+) bacteria) using API	Source	Health effects	Reference
Storage	*Bacillus pumilus* DSM 1794 DSM *Bacillus endophyticus* DSM 13796T DSM *Arthrobacter oxydans* DSM 20119T DSM	*Bacillus pumilus*	Water, soil and air	Central venous catheter infection Causes severe irritation to humans	Eschbach et al., 2003; Salzman and Rubin, 1995
Basin	*Bacillus megaterium* DSM 32T DSM *Bacillus sonorensis* DSM 13779T DSM	*Bacillus megaterium*	Soil	Considered non-pathogenic Food-borne illness	Ryan and Ray 2004; Kotiranta et al., 2000
Meat cutter	*Kocuria varians* DSM 20033T DSM *Kocuria rhizophila* DSM 11926T DSM *Kocuria rhizophila* DSM 348 DSM	*Micrococcus* spp.	Soil, dust, water and air	Brain abscess Fever, acute pancreatitis, blood stream infection	Stackebrandt et al., 1995; Breitkopf et al., 2005; Madigan and Martinko 2005
Cutter surface	*Arthrobacter nasiphocae* DSM 13988T DSM *Kocuria rhizophila* DSM 46222 DSM *Kocuria rhizophila* DSM 348 DSM		Soil, dust, water and air	Causes severe irritation to humans Fever, acute pancreatitis, blood stream infection	Stackebrandt et al., 1995; Madigan and Martinko 2005
Sink	*Bacillus mojavensis* DSM 9205T DSM *Bacillus subtilis* DSM 5552 DSM *Bacillus subtilis* ssp *subtilis* DSM 5660 DSM	*Bacillus subtilis*	Soil	Food poisoning	Ryan and Ray, 2004
Water pipes	*Bacillus cereus* DSM 31T DSM	*Bacillus cereus*	Soil	Food-borne illness causing severe nausea, vomiting and diarrhea	Kotiranta et al., 2000
Meat preparation area	*Micrococcus caseolyticus* DSM 20597T DSM *Staphylococcus aureus* DSM 20609 DSM	*Micrococcus* spp.	Soil Exterior of a human ear and animals	Has not been seen in human or animal infections Causes human skin infections.	Madigan and Martinko 2005

fied as *Bacillus* (Table 1). *Bacillus* is a spore-forming, Gram-positive, facultative anaerobic bacterium commonly associated with food poisoning in humans (Madigan and Martinko, 2005). In kitchen areas of the hospital studied, paper towels were recognized as possible source of *Bacillus* because of the presence of paper towels that were mainly used for drying hands and sometimes covering food, and the use of possibly contaminated towels could lead to contamination of surfaces and food. Recent studies have implicated paper towels as possible sources of *Bacillus* (Gendron et al., 2012).

The presence of *Bacillus* strains in food preparation areas is of concern as these bacteria produce toxins that cause food poisoning (Gendron et al., 2012). At one point

Table 2. Gram-negative bacteria characterization: Food preparation surfaces.

Origin	Species identification (Gram (−) bacteria) using MALDI-TOF MS	Species identification (Gram (−) bacteria) using API	Source	Health effects	Reference
Cutter	*Enterobacter cloacae* MB_8779_05 THL *Enterobacter cloacae* MB11506_1 CHB *Enterobacter cloacae* MB_5277_05 THL		Internal yellowing of Hawaii-grown papayas	Urinary tract and respiratory tract infections	Nishijima, 1987; Fraser, 2008
Water pipes	*Pseudomonas marginalis* DSM 13124T HAM *Pseudomonas koreensis* 2_2 TUB		Soil, water, tomato plants and animals	Causes disrupted physical barriers to bacterial invasion (e.g. burn injuries, intravenous lines, urinary catheters, dialysis catheters), neutropenia, etc.	Smith et al., 1988
Food preparation area	*Acinetobacter baumannii* B389 UFL *Acinetobacter baumannii* DSM30007T HAM *Stenotrophomonas maltophilia* DSM 50170T HAM	*Acinetobacter baumannii*	Soil and plants	Causes nosocomial pneumonia, skin and wound infections, bacteremia, and meningitis Cause of pneumonia, urinary tract infection, or blood stream infection	Antunes et al., 2011; Gerischer, 2008; Berg et al., 1999; Waters et al, 2007

during observations, food handlers used paper towels to cover and wrap food which was a worrying practice as this could lead to food contamination by possibly contaminated towels.

However, future studies will have to be conducted to determine if paper towels are the source of *Bacillus* at this hospital. *Bacillus cereus* one of the species identified in the current study has been shown to be more harmful to people with weakened immune systems, like children and the elderly, and for patients who take medications that suppress their immune function (Gendron et al., 2012). Infection with the identified bacteria usually leads to symptoms such as diarrhea, vomiting, nausea, abdominal pains, etc., and additionally *Bacillus cereus* is also known to cause infections of the lungs, blood, eyes and nervous system (Klietmann and Ruoff, 2002; Gendron et al., 2012).

Supported by the literature, results from this study indicate that the most commonly isolated bacteria in the kitchen area could be distributed by the use of paper towels coming into contact with food handlers either by washing hands, covering and wrapping food. *Bacillus cereus* has been reported in food-borne illness cases that were acquired in hospitals (Al-Abri et al., 2011). Based on the results in this study, urgent steps have to be taken to eliminate the presence of *Bacillus* from food preparation areas in this hospital. Other studies have shown that the transmission of *B. cereus* may also result from contaminated foods as well as from improper handling or storage and improper cooling of cooked foodstuffs since *Bacillus* spores are resistant to heat and can survive even recommended cooking temperatures (Klietmann and Ruoff, 2002).

When samples were collected in the kitchen storage area, the genus positively identified using the MALDI-TOF MS other than *Bacillus* was *Arthrobacter*. When the same colonies where subjected to analysis using the API technique, the technique generated inconclusive results (Table 1). *Arthrobacter* is a common genus of soil bacteria with all species being non-sporulating, Gram-positive and obligate aerobes (Shen et al., 2009; Su et al., 2011).

These bacteria usually grow at the surface of smeared ripened cheese and cause skin infections as well as urinary tract infections. Even though *Arthrobacter* species have been isolated a few times from patients with immuno-deficiencies, their health implications on patients are reported not as severe as other microbes because most of their strains do not appear to be pathogenic (Mounier et al., 2006; Jerke et al., 2008).

On the kitchen meat cutter, cutter surface and meat preparation area, *Kocuria* a Gram-positive bacteria aerobic bacteria which are part of the human skin flora (Stackebrandt et al., 1995; Cocconcelli and Fontana 2008; Tsai et al. 2010), was the most predominant genus identified when using the MALDI-TOF MS. When the API technique was used, the same colonies where identified as *Micrococcus* spp. (Table 1). *Kocuria* spp. were previously

classified under the genus *Micrococcus* however, reclassification based on phylogenetic and chemotaxonomic techniques have placed them in the genus *Kocuria* (Stackebrandt et al., 1995; Cocconcelli and Fontana, 2008; Tsai et al. 2010).

The current MALDI-TOF MS results obtained are in agreement with the results observed by Stackebrandt and co-workers (1995), an indication that the MALDI-TOF MS is a rapid sensitive technique for microbial identification when compared to API. One of the species identified in the current study was *K. varians* which is known to occur in meat; these species are mainly used as starter cultures in meat fermentations for the conversion of nitrate into nitrite and frequently added to develop a stronger red color of meats (Cocconcelli and Fontana 2008). However, its presence on kitchen surfaces at hospitals is worrying because *K. varians* is an opportunistic pathogen especially in those with compromised immune systems (Tsai et al., 2010).

By means of the MALDI-TOF MS, *Staphylococcus aureus* and *Micrococcus caseolyticus* were identified on the kitchen meat preparation area (Table 1). When the API technique was used, a *Micrococcus* species could be identified from colonies isolated on this surface (Table 1). The presence of both bacterial species especially *S. aureus* found in the kitchen area highlights the need for providing food handlers with educational training in respect to proper hygiene practices since humans are possible sources of both bacteria. Improper food hygiene practices are worrying as *S. aureus* causes food poisoning with common severe symptoms such as headaches, muscle cramping, and transient changes in blood pressure and pulse rate (Madigan and Martinko, 2005; Crago et al., 2012). Furthermore, the presence of *S. aureus* in healthcare settings is a cause for concern due to the existence of antibiotic resistant strains that have been reported in other studies (Crago et al., 2012). On the kitchen food preparation area, *Acinetobacter baumannii* was identified using both identification techniques, this species originates from the soil (Table 2). Additionally, *Stenotrophomonas maltophilia* could be identified on this surface when using only the MALDI Biotyper (Table 2). *Acinetobacter baumannii* is mostly recognized as a major source of severe, life-threatening infections in compromised patients in the health care settings (Charnot-Katsikas et al., 2009). The survival of *Acinetobacter baumannii* in hospital environments is due to their reported ability to survive on various surfaces (both dry and moist). In previous studies, *Acinetobacter* strains were isolated from foodstuffs, medical equipment and healthy human skin. Moreover, *Acinetobacter* colonies were reported to be found on an everyday kitchen sponge also used in the hospital kitchen (Gerischer, 2008; Antunes et al., 2011). The presence of this bacterium on the sponge could have serious implications in health care settings since *Acinetobacter* has been characterized as a life - threatening bacterium that causes meningitis, bacteremia, skin and wound infections to patients (Gerischer, 2008; Antunes et al., 2011).

Gram negative bacteria isolated from the surface of the kitchen cutter and identified using the MALDI Biotyper included *Enterobacter cloacae*, when the API test was conducted on the same colonies inconclusive results were obtained (Table 2). *Enterobacter cloacae* are known as hospital acquired opportunistic pathogens found on human skin, fruits, vegetables and some dairy products (van Karin and Nelson, 2000; Fraser, 2008). The bacterium can also be contracted as a result of cross-contamination, for example, bacterial transfer from one food item to another food item by means of unwashed cutting boards (example kitchen cutter) or even unwashed hands of food handlers. Several symptoms of *Enterobacter* infections include bacteremia, lower respiratory tract infections, skin infections, soft tissue infections, urinary tract infections, UTI, endocarditis, etc. (van Karin and Nelson, 2000; Fraser, 2008). Results from this study indicate that an unwashed kitchen cutter (Figure 2d) was used or food handlers did not practice proper food hygiene during food preparation.

In the kitchen preparation area and water pipes, the bacteria most identified by means of the MALDI Biotyper were species from the genus *Pseudomonas*, with species *P. koreensis* and *P. marginalis*. The presence of *Pseudomonas* identified in the kitchen water pipes could be from splashes of contaminated water, since studies (Doring et al., 1996; Bendiak and Ratjen, 2009) have reported that *Pseudomonas* species form reservoirs of contamination in sink waste pipes which can be sources of nosocomial infection when splashes of contaminated water come into contact with human hands (Doring et al., 1996; Bendiak and Ratjen, 2009). Food preparation areas play a significant role in the health of patients because health-acquired infectious diseases can be caused either by food handlers or from microbial surface contaminants.

Results from the current study have indicated the vital role food preparation areas play in patient safety, and the study has shown that insufficiently cleaned surfaces and the presence of opportunist microorganisms can place patients at high risk of contracting a food-borne illness. More effort has to be put in by hospital management to control surface contamination: training for proper cleaning must be introduced and the levels of airborne contaminants which could settle on surfaces must be monitored and reduced.

Fungi were also present on kitchen surfaces at the studied hospital. The presence of fungi in the food preparation area is of concern since hospitals accommodate people with compromised immune systems who are particularly susceptible to diseases caused by fungi such as *Candida albicans* (Hidalgo, 2008). During the course of this study, the most common fungi identified using both identification techniques were *Candida* species (Table 3) except the kitchen food preparation area and kitchen

Table 3. Fungal characterization: kitchen.

Origin	Species identification using MALDI-TOF MS	Species identification using API	Source	Health effects	Reference
Storage	*Candida krusei* [anamorph] (*Issatchenkia orientalis* [teleomorph]) ATCC 14243 THL *Candida kefyr* [anamorph] (*Kluyveromyces marxianus* ssp. *marxianus* [teleomorph]) CBS 834 CBS	*Candida* spp.	Plants and warm moist body areas. Isolated from cheese and dairy products. Clinically from nails and lung infections.	Candidiasis yeast infection, vaginal yeast infections, thrush, skin and diaper rash, and nail bed infections. Diseases are diabetes, etc	Kreger-Van Rij, 1984; Rippon, 1988
Basin	*Aureobasidium pullulans* 15346 CBS *Candida* spp. DSM 1247 DSM	*Candida* spp.	Plant debris, soil, wood, textiles, and indoor air environment	Causes pneumonia, keratomycosis, peritonitis,etc	Pritchard and Muir, 1987
Meat cutter	*Candida valida* [anamorph] (*Pichia membranifaciens*[teleomorph]) 10 LBK *Candida krusei* [anamorph] (*Issatchenkia orientalis* [teleomorph]) ATCC 14243 THL *Candida lambica* [anamorph] (*Pichia fermentans* ssp. *fermentans* [teleomorph]) CBS 603 CBS	*Candida* spp.	Soil Cheese and dairy products. Clinically from nails and lung infections	Candidiasis Candidiasis Bloodstream infections, etc	Kreger-Van Rij, 1984; Rippon, 1988
Cutter	*Candida utilis* [anamorph] (*Pichia jandinii* [teleomorph]) DSM 2361 DSM *Candida utilis* [anamorph] (*Pichia jandinii* [teleomorph]) DSM 70167 DSM	*Candida* spp.	Wood, paper pulp, leaf litter and substrates that are rich in cellulose	Causes fungemia, pancreatitis, fever and bleeding problems	Alsina et al., 1988
Cutter surface	*Candida utilis* [anamorph] (*Pichia jandinii* [teleomorph]) DSM 70167 DSM	*Candida* spp.	Wood, paper pulp, leaf litter and substrates that are rich in cellulose	Causes fungemia, pancreatitis	Alsina et al., 1988
Sink	*Candida kefyr* [anamorph] (*Kluyveromyces marxianus* ssp. *marxianus* [teleomorph]) CBS 834 CBS		Isolated from cheese and dairy products. Clinically from nails and lung infections	Causes candidiasis	Kreger-Van Rij, 1984; Rippon, 1988
Water pipes	*Candida kefyr* [anamorph] (*Kluyveromyces marxianus* ssp. *marxianus* [teleomorph]) CBS 834 CBS		Isolated from cheese and dairy products.	Causes candidiasis	Kreger-Van Rij, 1984; Rippon, 1988
Food preparation area	*Aureobasidium pullulans* 12235 CBS		Plant debris, soil, wood, textiles, and indoor air environment	Causes pneumonia, keratomycosis, etc.	Chi et al., 2009
Meat preparation area	*Aureobasidium pullulans* 12235 CBS		Plant debris, soil, wood, textiles, and indoor air environment	Causes pneumonia, keratomycosis, etc.	Pritchard and Muir,1987

Spp. – Species; Anamorph – the asexual reproductive stage of a fungus; Teleomorph – the sexual reproductive stage of a fungus.

meat preparation area where *Aureobasidium pullulans* was isolated and identified using the MALDI Biotyper (Table 3).

C. albicans is commensal in humans and forms part of the human gut flora. It has the ability to live in the environment outside the human body as well as on inanimate objects such as on food, clothing, countertops, floors, and in air-conditioning vents by forming biofilms (Hidalgo, 2008). Isolation of antifungal resistant strains such as *C. krusei* (Achkar and Fries, 2010) was one of the noted concerns in the current study.

Aureobasidium pullulans is a yeast-like fungus found mostly in the soil, water, air and on limestone causing infectious diseases such as pneumonia, keratomycosis, pulmonary mycosis with sepsis eumycotic dermatitis and peritonitis to patients (Chi et al., 2009). From the results, it could be concluded that *Candida* species identified at the studied hospital kitchen may be transmitted through contaminated surfaces by food handlers. Other sources of contamination could be clothing, the floors and air-conditioning vents.

Therefore, air quality and proper hygiene practices are two areas that need constant monitoring and should not to be overlooked in order to prevent settling of micro-organisms on food preparation surfaces in the hospital kitchen.

CONCLUSIONS

The findings of this study indicate that the MALDI-TOF MS can be used as a rapid and reliable method for the identification and characterization of surface microorganisms when compared to the API technique. In other instances the MALDI-TOF MS produced results that were comparable with results obtained using molecular methods as reported in other studies (Stackebrandt et al., 1995). Even though known HAI associated microorganisms were not isolated in the current study, the isolation and identification of other opportunistic pathogens from kitchen surfaces was a matter of concern.

Furthermore, the current results indicate the possible role food preparation surfaces could play in the transmission of health-care associated foodborne pathogens. On the kitchen meat preparation area, *Staphylococcus aureus* was identified and numerous studies strongly report that surface contamination plays a key role in the transmission of methicillin-resistant *Staphylococcus aureus* (Weber et al., 2010).

Moreover, in the kitchen sink and kitchen food preparation area, *Acinetobacter* species were found. Recent evidence suggests that again surface contamination could be the source of nosocomial transmission of *Acinetobacter* spp. since this pathogen has the ability to survive on both dry surfaces and in water (kitchen sinks) for prolonged periods of time (Gerischer, 2008; Weber et al., 2010; Antunes et al., 2011).

Observations made during sampling rounds also caused concern, since many kitchen surfaces were not adequately cleaned and paper towels that have been reported as possible sources of *Bacillus* were used to cover leftover food.

Therefore, in order to reduce surface contamination, improved cleaning and disinfection of all kitchen surfaces is recommended in order to ensure proper food hygiene and safety.

In addition, stringent surveillance and monitoring of the kitchen especially surfaces is also recommended to ensure the minimal contamination of food products prepared for patients. Educational training of food handlers is proposed to limit possible health-care-associated foodborne disease outbreaks.

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