IJCMAS
INTERNATIONAL JOURNAL OF CURRENT MICROBIOLOGY AND APPLIED SCIENCES

MDPI
Academic Open Access Publishing
since 1996

RESEARCH PAPER

Bacterial and Viral contamination of table forks, table spoons, dessert forks and teaspoons in restaurants, coffee shops, and university/hospital cafeteria.

Nadia Debuisson[1], Ronen Gurevich[2], Richard Even[3]

Prof. Nadia Debuisson PhD, Dr. Ronen Gurevich PhD, Dr. Richard Even RD PhD.
[1,2] Institute for Biological, Chemical and Environmental Sciences, Ness Ziona.
[3] Institute for Medical Research Israel-Canada, University of Jerusalem.

RESEARCH PAPER OF 12 MAY 2021 PRESENTED FOR PUBLICATION TO IJCMAS

ABSTRACT

Pathogens found on spoons and forks can cause acute and chronic health outcomes of very different durations, severity and mortality, resulting in high costs and burdens to society. The issues of food safety and food poisoning are being increasingly emphasized. Infection/contamination with many agents i.e. bacterial, parasitic and viral entities can result in illness. More than 40% of 550 foodborne disease outbreaks reported by the CDC were attributed to restaurants from 1993-1997. The Centers for Disease Control and Prevention (CDC) estimates that each year roughly 1 in 6 Americans (or 48 million people) gets sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases in 2011. Bacteria and viruses, commonly referred to as germs, can live on surfaces, such as tabletops, kitchen countertops, picnic tables, and desktops, and in turn, can spread from tabletops, utensils, and food upon contact to a person's hand and mouth. These same germs can cause colds, the flu, foodborne illnesses, and other infections. Among the variety of microorganisms that play a role in the creation of food borne illnesses, bacteria cause the highest rate of illnesses. Some of these bacteria include heterotrophic bacteria, coliform and Escherichia coli. Examples of germs are known to live on surfaces such as Viruses, Influenza A – survive up to 48 hours, Para influenza, Noroviruses (causes vomiting and diarrhea) –survive for up to 12 hours, Rhinoviruses (cause of common cold), Bacteria, E.Coli and Salmonella, Staphylococcus aureus- survive for one day on cotton, polyester, terry cotton, and plastic, Methicillin-

resistant Stapylococcus aureus (MRSA) – survive weeks to several months on most surfaces, Vanomycin-resistant enterococci (VRE) – survive for several days. This study will focus on bacterial and viral agents of infection found on spoons and forks in restaurants and coffee shops. A range of different bacteria and viruses can cause food poisoning infection, and infection can result in a myriad of symptoms. Within a day or two of exposure to bacteria or virus, people may experience inflammation in the lining of the gut, causing vomiting and diarrhea, nausea and abdominal pain, fever and flu-like discomfort or muscle aches. Due to the inherent differences between bacteria and viruses, namely the fact that viruses do not replicate in food, while bacteria do, viruses are frequently difficult to detect. This is compounded by the fact that many of the viruses associated with enteric disease do not replicate in cell culture. These factors can lead to a lag between reporting, detection and analysis of viruses versus bacterial agents. Despite these constraints, it is now evident that there are both well-established and emerging viruses and bacteria found on forks and spoons in restaurants and coffee shops implicated in infections, and the role of molecular detection and characterization is becoming increasingly important.

A second study was carried out using metal spoons and forks used in a university and hospital cafeteria. The cafeteria was designed to evaluate their rate of contamination with heterotrophic bacteria, coliform and Escherichia coli, in order to take a step toward more compliance with health principles and promote good health in students at universities and medical staff in hospitals. (figure 4a, 4b).

Approach: An investigation was undertaken to determine bacteriological and viral contamination on stainless steel forks and spoons. A total of 121 cutlery including table forks, tablespoons, small forks and teaspoons were collected from 4 restaurants and 3 coffee shops. Samples were collected and cultured by the routine methodology in use at laboratory. Plates were incubated aerobically at 37°C for 48 h. All samples were examined for the antimicrobial activity for commonly used antimicrobials using disc diffusion method. The virus/bacteria isolates were identified according to their morphological and biochemical characteristics. In this study, we also investigated the survivability of norovirus on desiccated food residue-attached stainless steel forks and spoons.
In the cafeteria study, a random sample of 50 metal spoons and forks from university and hospital cafeterias, and hospital patient trays were studied. The samples were cultured and tested using the standard method for contamination with heterotrophic bacteria, total coliform and Escherichia coli.
Results: The results revealed a profile of different bacterial species of Shigella, Klebsiella and Bacillus, and Norovirus. The total value of bacterial count (TBC per ml) of the samples were in the range of 2.1×10^4 - 1.6×10^5 for forks, 1.5×10^4 - 4.7×10^5 for

spoons cfu/ml. The results of the cafeteria study showed that the spoons and forks were not contaminated with Escherichia coli, but the total coliform heterotrophic bacteria were found on the ready to use spoons and forks in the cafeteria. The total coliform and heterotrophic bacterial contamination of dishes were observed (81% and 27%) for spoons and forks.

Conclusions/Recommendations: This study demonstrated that due to the high bacterial density found on table forks, spoons, dessert forks and tea spoons that we regularly bring to the mouth, they are necessary to prevent bacterial and viral infections in restaurants and coffee shops, disposable cutlery or even better the antimicrobial protections of the "Formì" system that we got to know and appreciate.

Regarding the cafeteria study, despite being free of contamination with E. coli, spoons and forks in the university and hospital cafeteria and on patient trays, have a relatively high contamination with heterotrophic bacteria and total coliform, which probably is due to the lack of health compliance by kitchen staffs or/and inadequate washing and disinfection.

Key

Keywords: Salmonella, Shigella, Escherichia coli, Bacillus, Staphylococcus, Proetus vulgaris, Klebsiella, Human norovirus, Acute viral gastroenteritis, Enteric viruses, cafeteria, university, hospital, patient trays.

INTRODUCTION

A *Consumer* survey interviewed over one thousand individuals, asking for the feedback on the cleanliness of the restaurants they visit most often. Dirty silverware were the number one issue that restaurant patrons complained about 76 percent of respondents said the forks, spoons, and knives were not up to acceptable standards. While this survey didn't touch on the specifics of the germs that appear on your silverware, it did prove that this is a real issue in the food industry. A study by Yepiz-Gomez looked at dishcloths used to wipe tables and cutlery in restaurants to determine the occurrence of bacteria. Coliforms (bacteria) were isolated from 89.2% of dishcloths and 70% of tabletops. E.coli Staphylococcus aureus, Streptococcus faecalis and Clostridium perfringes were isolated from both dishcloths and tabletops. E.coli is the same bacteria found in fecal matter. E.coli numbers on tabletops were found to be 19 times higher after wiping. Even if you're a germaphobe - you clean your hands all day at work, you use your knuckles (rather than your fingertips) to steady yourself on the subway, and you would never dream of getting on that cycle at the gym without wiping it down first - you probably don't think much about how dirty restaurant silverware is. You know, the cutlery you not only touch with your bare hands, but use to scoop up food and put directly into your mouth. The reputation of many restaurants and coffee shops often rests on the quality of forks and spoons (Cracknel and Nobis, 1989). Vanderzant and Splittstoesser (1992) mentioned that contamination of food by specific types or species

of microorganisms is due to poor sanitation. Tebutt (1986) found out that 74% cloths used in cleaning cutlery surfaces were contaminated with one or more of the following organisms Escherichia coli, Staphylococcus aureus, Streptococcus faecalis andn Clostridium perfringes.

RNA virus, Human Norovirus, is highly infectious and easy to pass around, but much less likely to be lethal than viruses such as influenza. Norovirus causes "stomach flu". Chances are you've had it, and you'll probably get it again; it is among the leading causes of food-borne disease and deaths. Noroviruses are a constantly changing group. That makes it hard for the human immune system to recognize and combat them. The sheer number affected, and particular risk to the young and the old, make Norovirus a big deal. It can lie in wait on tabletops, forks and spoons for weeks or longer. Although soap and running water can wash the virus down the drain, it's hard to destroy with disinfectants. The Centers for Disease Control and Prevention (CDC) estimates that every year, Human noroviruses (HuNoVs) have caused around 685 million cases of infection and cost the World economy about $65 billion in healthcare costs and lost worker productivity. Human noroviruses usually move from person to person but contaminated cutlery are a common transmission route, usually from restaurant forks and spoons, and coffee shop teaspoons and small forks. Despite their highly contagious nature, an effective vaccine for HuNoVs has yet to become commercially available. Therefore, rapid detection and sub-typing of noroviruses is crucial for preventing viral spread.

Table 1. Distribution of samples per restaurant/coffee shop.

Restaurant/ Coffee Shop	**Forks**	**Spoons**	**Teaspoons**	**Dessert Forks**
A	3	5	5	4
B	5	5	5	4
C	5	4	5	5
D	3	5	2	5
E	3	5	5	3
F	5	4	5	3
G	4	5	4	5
Total	**28**	**33**	**31**	**29**

Key: A to G = Restaurant/Coffee Shop

MATERIALS AND METHODS

Sample area

7 restaurants/coffee shops in Jerusalem were randomly selected for this study, based on their popularity, availability and affordable cost of food and the samples were selected based on random selection.

Sample size

A total of 60 samples were collected from table forks, tablespoons, dessert forks and teaspoons. Six (6) samples were collected from each of the five (5) restaurants and five (5) coffee shops and all the samples were labeled appropriately (Table 1). These five restaurants and five coffee shops were popular among students in terms of availability and affordable cost of food.

Sample collection

The items were sampled after the cleaning process was done for tablespoons, table forks, dessert forks and teaspoons. Samples were collected using the rinse method for cutlery and other accessories (Cheesbrough, M.2005). The study period was between April and June, 2021. Sterile distilled water was used to rinse tablespoons, table forks, dessert forks and teaspoons. For each item, about 5 ml of distilled water was used for rinsing purpose and then the water was collected in sterile conical flask. After that, 5 ml of water was used again for rinsing purpose and then was mixed with the first washing kept in the conical flask. This was done for all the other crockery and cutleries. The washings (10 ml) for all the items in sterile conical flasks were kept in a cooler packer with ice and were transported to the Microbiology Laboratory within 30 min for analysis purpose.

Isolation and enumeration of microorganisms

Dilutions up to 10-5 were made from the original suspension (washings) using the method described by (Cheesbrough, M. 2005). 1ml of each dilution (10-1 to 10-5) was introduced onto the dried agar medium (nutrient agar, chocolate agar, blood agar and MacConkey agar were used respectively, BDH Chemicals Ltd., Poole, England). Sterile glass spreader was used aseptically to spread the suspension on the surface of the agar medium. The inoculated plates were incubated at 37°C for 24 h and the total bacterial count was expressed in cfu/ml. Distinct colonies were isolated and re-inoculated onto appropriate agar media and kept at 4°C for identification purpose. The isolates were labeled as A1-A4, B1-B4, C1-C4, D1-D3, E1-E2, F1-F4 and G1-G4

Identification of Isolates

Gram staining

Gram staining was done according to method as described in (Cheesbrough,2005).

Biochemical tests
Biochemical tests were performed according to the methods as described in Cheesbrough,2005.

Citrate utilization test
For each isolate, 10 ml of citrate medium was dispensed into each of five tests tubes and sterilized by autoclaving at 121°C for 15 min. The test organism was then inoculated into citrate medium and incubated at 37°C for 48 h. A blue color indicated a positive result. One test tube containing only the citrate medium served as a control (Cheesbrough,2005).

Catalase test
A drop of 3% hydrogen peroxide was placed on a glass slide. A bit of growth of each isolates was collected from the medium using a wire loop and the growth was emulsified in the drop. A positive test was indicated by bubbling and frothing, negative test did not show bubbling and frothing (Cheesbrough,2005).

Coagulase test
The slide method test was used for this study. A drop of saline on two separate spots was placed on the same grease free slide, speck of growth of the test organism was picked and emulsified in both spots, to one spot a drop of plasma was added and to the other a drop of saline was added, both mixtures were mixed thoroughly by rocking. A positive test indicates coagulation in the emulsion in the spot to which plasma was added (Cheesbrough,2005). The presence of clotting indicates positive test for *Staphylococcus aureus*.

Indole test
The test organism was grown in peptone water and incubated at 37°C for 24 h to give optimum accumulation of indole. A positive result of this test was indicated when a red coloration was observed in the uppermost layer of the tube, after adding 0.5 ml of kovac's reagent to 5 ml of peptone water culture (Cheesbrough,2005).

Kligler Iron Test (KIA)
In this method each isolate was grown in a medium containing (KIA), which contains 0.1% glucose and 0.1% lactose. The surface of the slant was exposed to ambient air, while the agar deeper (butt) in the tube portion provided an anaerobic environment for inoculation. KIA tubes were inoculated with a wire loop full of pure colony. The wire loop was stabbed into the deep (butt), the bottom of the tube while the slant surface was streaked with a back- and forth motion. Inoculated tubes were placed into an incubator at 350C for 18 to 24 h. Gas formation was determined by the appearance of one or several bubbles in the butt, vigorous gas formation resulted in cracks in the butt or the

butt may be pushed from the bottom. Glucose fermentation was indicated by the butt becoming yellow. Tubes showing slant red (alkaline) and deep (butt) yellow (acid) was positive for *Shigella* specie, that ferment glucose slant red (alkaline); yellow (acid) with black for hydrogen sulphide (H2S) was positive for *Proteus* species; with slow gas without hydrogen sulphide was positive for *Salmonella* species and ferment glucose. Slant yellow (acid) and deep (butt) yellow (acid) fermenting both lactose and glucose was positive for *Escherichia coli* and *Klebseilla* species with gas production (Cheesbrough,2005).

Motility

A single colony of each of the organisms was inoculated into labeled test tubes containing peptone water (5mls) and the tubes incubated at 370C over night. A drop of the well-mixed organism in peptone water incubated over night was placed on a cover slip and the edges surrounded with oil immersion. A microscope slide was then placed over the cover slip taking care that the slide those not touch the drop on the cover slip but suspended by the oil immersion. The slide was then turned quickly but gently. This preparation was then observed under the microscope for motile bacteria under x 100 objectives (Cheesbrough,2005).

Cell-based immuno-assay

Food residue attached stainless steel forks and spoons without any food component were incubated at 25°C over a 7-day experimental period. Error bars indicate standard deviations obtained from three independent experiments. (figure 1).

Vero cells were harvested from cell culture flasks using trypsin (~15 min). The cells were then suspended in 2% FBS-MEM and aliquoted to tubes (step 1). The viruses are then added to the cells and incubated at 4 °C with shaking to allow adsorption to the cells surface (1 h) (step 2). After attachment to the cells, a specific fluorescent antibody is added (step 3, ~40 min) and the cells are analyzed in FACS or fluorescence microscopy (figure 2).

In the cafeteria study, all samples of spoon and forks were taken using gloves to avoid contamination. To determine the presence or absence of heterotrophic contamination, HPC (heterotrophic plate count) was used. So that, the petri dishes containing Nutrient agar medium was put between two Flames and a line with Spiral moves was drawn on the medium. The cultured Medium was put in incubator with the temperature 35±0. 5 for 48 hours. After incubation, the petri dishes were taken out of the incubator. In case of Colony growth, the number of created bacteria were counted by using Colony Counter device and recorded in the table of microbial report. In laboratory, the taken sample was Diluted (Dilution =50) and 9-tube culture was done with Dilution 1, 0, 1, 10

inside Lactose broth medium and put inside the incubator for 24-48 hours in order to determine the presence of absence of total coliform and Escherichia coli. The creation of gas inside the Durham tube and Opaque Lactose broth means its contamination. From positive samples, 2 drops were collected by Loop inside the Brilliant Green medium and E. coli. After 48 hours of incubation, the creation of gas inside Durham tube in confirmation stage showed contamination with total coliform. Also, the samples cultured in E. coli medium were put in bain marie (water bath) for 24 hours with the temperature of 44 degrees. The creation of gas inside Durham tube showed contamination with Escherichia coli. The results were recorder in microbial contamination table. The obtained results were analyzed by using descriptive statistics methods in SPSS 16 software.

RESULTS

The isolates obtained from the different samples were labeled accordingly as A1-A4, B1-B4, C1-C4, D1-D3, E1-E2, F1-F4 and G1-G4. Results of gram staining and the cultural and morphological characteristics of isolates revealed that isolates A1-A4 were *S. aureus*, isolates B1-B4 were *Klebsiella* sp., C1-C4 were *E. coli*, D1-D3 were *Salmonella* sp. , E1- E2 were *Proteus* sp., F1-F4 were *Bacillus* sp., and G1-G4 were *Shigella* sp. The results of the biochemical tests were expressed in Table 2. These bacterial species were seen in almost all the restaurants and coffee shops but in different levels. The presence of these from cutleries could create health hazard when they are ingested, or they come in contact with the human skin. This exposed clients eating in these outlets to the risk of infection. Similar sources of outbreaks have been reported in the United States of America (Bryan et al.,1981). Although *E. coli* itself is not harmful, its presence in any numbers can be regarded as evidence that eating utensils were contaminated with fecal discharge, if not of human origin then at least is an important cause of food intoxication (Berdgoll, 1989). *Salmonella* species causes several diseases such as gastroenteritis, septicemia typhoid etc. which is transmitted via food or water (Michael et al., 2004). In fact, there are many reasons for concern when *S. aureus* is present in eating utensils. It survives for longer period in water than the coliform. The need for urgent improvement in the hygienic condition of the restaurant and coffee shops cannot be over emphasized. *E. coli, P.vulgaris, Klebseilla* sp. and *Shigella* sp. are bacteria that were most frequently isolated from the restaurants (Steward, 1976) with no or low hygiene, some of them like *Klebseilla* sp and *Proteus vulgaris* are frequent causes of urinary tract infections, though they are usually associated with some underlying predisposing factors in the urinary tract (Nester et al., 2004). The total values of bacterial count (TBC), cfu/ml of the samples were in the range of 1.1×10^4 - 3.0×10^5 for cups, 2.2×10^4 -1.6×10^5 for forks, 1.0×10^4 - 3.3×10^5 for dessert forks, 1.2×10^4 - 2.5×10^5 for Teaspoons and 1.5×10^4 - 4.7×10^5 for spoons cfu/ml (Table 3). According to Collins and Patricia (1979), standard for cutlery in

the U.S.A., Public Health Service requires counts of not more than 5.0 x 10^4 and 2.5 x 10^5 cfu/ml per container as fairly satisfactory and over 2.5 x 10^5 cfu/ml as unsatisfactory. This implies that count above 2.5 x 10^5 cfu/ml is a contamination. In case of restaurant no. 3, the TBC count of *E. coli* for Dessert forks was 3.3 x 10^5 and in case of restaurant no. 4 and no. 5, the TBC values for teaspoon and spoon were 4.3 x 10^5 and 4.7 x 10^5 respectively. These values were higher than the recommended values. These high bacterial densities in such restaurants suggested that cutlery kept on the table in the open air are prone to contamination with bacteria. Disease through contaminated cutleries are major causes of morbidity throughout the world (WHO, 1984). Microorganisms that contaminate these equipments damage the restaurants and coffee shop reputation, sometimes beyond repair and eventually ruin their business. It is in view of these findings tourist are advised to utilize restaurants that are hygienic. It is always safer and easier to prevent the contamination of these cutleries. It is more difficult to make the equipments safe again. Infection by food poisoning organisms is a threat requiring constant vigilance unless kitchen equipment that comes in contact with food are adequately cleaned and sanitized; it may still be an important source of contamination of food. Not only may organism persist on cutleries, but they may increase in numbers when treatment has been inadequate.

Results of the cafeteria study are as follows: On average 81% of spoons and forks were contaminated with heterotrophic bacteria colonies, and 78% of spoons and forks were contaminated with coliform in university and hospital cafeteria, and in trays for hospital patients. The spoons and forks used on hospital trays came from the same batch used in hospital cafeteria. (figure 4c).

DISCUSSION

Most bacteria can live on surfaces for at least a week, and in some cases, up to months. And most viruses can survive on surfaces for hours to days. Table forks, tablespoons, small forks and teaspoons are not clean enough at restaurants and coffee shops. We were looking for clean, sanitized, and sterile standards. The utensils at a restaurant, hotels and coffee shops must be washed at high temperature, and usually rinsed with a sanitizer of some sort. They must be kept clean, with tables regularly cleaned with both a cleaner and a sanitizing spray. This must be done both as part of the image of a restaurant and coffee shop, and because of health laws. The restaurant owner wants you to have a great experience, and return many times to enjoy their food, some places are better than others at this, and the health department wants to make sure that a restaurant does not cause health risks.

No matter how well you clean at home, you probably can't make it as safe as the table at a restaurant, so it should be safer and cleaner there, even if the utensils are left out a

few minutes while you are being seated and greeted. However looking at our results, they are not clean and are making us sick. Tables are almost never sanitized between customers (unless there is a table cloth that actually gets changed) so you're eating in whatever germs got coughed up by whoever sat there last. If you're giving the forks at your favorite cafe the side eye now, don't worry - you're not just being paranoid. There are plenty of reasons to be a bit cautious about what we put in our mouths.

Our study proved that the odds are high that bacteria and gastroenteritis-causing virus are present on public silverware, even after they've been washed and dried. We found that it didn't matter whether forks or spoons was hand-washed or run through a dishwasher; the murine norovirus (MNV-1) - which is the cause of 90 percent of severe childhood diarrhea and epidemic gastroenteritis cases worldwide - lived on. Although the cutlery put through the machine came out cleaner, they still held remnants of the nasty stuff because certain food like milk products, act as a barrier between cutlery and cleaning products, neutralizing the powers of soap and disinfectants. They cause the germs to stick around longer. Even if the restaurant is following cleanliness protocol to the highest standards, things like this simply can't be helped. Our study showed that even minute food residues left behind from improper cleaning may influence the survivability of human norovirus on stainless steel fork and spoon surfaces.

Within a day or two of exposure to norovirus, people may experience inflammation in the lining of the gut, causing vomiting and diarrhea, nausea and abdominal pain, fever and flu-like discomfort or muscle aches. It's generally a mild illness, and people get better within a few days; some have no symptoms at all. But norovirus can be serious in babies, older people and those with other conditions. There is no cure for norovirus, and infected people risk dehydration thanks to all that vomiting and diarrhea. So it's important to drink liquids; in severe cases, physicians can provide intravenous fluids.

The study claimed that norovirus, Escherichia coli (E. coli K12) and Listeria innocua were left on the table forks, tablespoons, dessert forks and teaspoons after washing, too. Those names look scarier than they actually are — not everyone who comes into contact with the norovirus instantly becomes ill - but they do pose a threat to your immune system. A little common sense goes a long way. Bacteria thrive on surfaces that aren't properly cleaned, and where bacteria live, viruses follow. You definitely don't want your silverware anywhere near that kind of tabletop. We just have to learn how to reduce the number of germs that are entering our system and one way is to use disposable cutlery, or even better, biodegradable antimicrobial protections for table forks, tablespoons, dessert forks and teaspoons which provide protection against pathogenic microorganisms and gastroenteritis-causing virus and therefore we recommend these to all restaurants and coffee shops.

The results of the cafeteria study that was done to examine contamination with heterotrophic bacteria, total coliform and Escherichia coli on reusable spoons and forks of university and hospital cafeteria and hospital patient trays showed that despite the lack of Escherichia coli contamination, heterotrophic bacteria and total coliform could be seen in all spoons and forks which had a high rate of heterotrophic contamination. One of the factors that causes high rate of heterotrophic contamination in reusable spoons and forks is the handling of cutlery by kitchen staff without gloves and washing them with low accuracy.

CONCLUSION

The study has shown that the higher the bacterial densities were found in spoons and forks at the dining table, most especially in restaurant D and F were high (shown in Table 3a) compare to the standard set by the USA Public Health Services (Collins and Lyne, 1979). These high bacterial densities in such restaurants suggested that the sources of contamination included water and food sources that were inadequately removed during routine cleaning. Six organisms were identified by their appearance on medium of which three bacteria species were gram negative rods namely; *E. coli, P.vulgaris, and Klebseilla sp*. The gram positive rod identified was *Bacillus sp* while *Staphylococcus aureus* was the only gram positive cocci. Biochemical tests were carried out to identify the organisms on MacConkey agar. The tests included citrate, coagulase, indole, Catalase, motility and KIA. These tests indicated that the following isolates were present; *S. aureus, Klebseilla* sp, *E. coli, Shigella* sp*, Salmonella typhi, P. vulgaris and Bacillus* sp. The results of the second study showed that contamination rate with heterotrophic and total coliform bacteria on washed and ready-to-use spoons and forks was high in university and hospital cafeteria and increases the probability of contamination with Pathogenic bacteria. This issue indicates that more accuracy should be applied in washing, disinfection and handling of cutlery in order to reduce the rate of microbial load and improve the status of cafeteria. The best way to protect public health is to enhance sanitation control. It is also good for kitchen staff never to use any cutlery without 'sterilizing' them first. It is important for them to wear gloves so not to contaminate further. For the final consumer (the customers), on the other hand, it is advisable to use disposable cutlery, in particular the antimicrobial protections of the "Formì" system that we tested and used in the same sample taken into consideration.

ACKNOWLEDGEMENT

This study was conducted at the request of the Institute for Medical Research to show the actual risks of hygiene in the world of collective catering and outside the home.

Table 2. Results of biochemical tests.

Isolate No.	Biochemical tests									Isolate
	Citrate utilization	Motility	Indole	Catalase	Coagulase	KIA				
						Slope	Butt	H2S	Gas	
A1-A4	-	-	-	+	+	-	-	-	-	*S. aureus*
B1-B4	+	-	-	-	-	Y	Y	-	+	*Klebsiella* sp
C1-C4	-	-	+	+/-	-	Y	Y	-	+	*Shigella* sp
D1-D4	+	+	-	+/-	-	R	Y	-	+/-	*Salmonella typhi*
E1-E4	-	+	+	-	-	R	Y	+	-	*Bacillus* sp
F1-F4	-	+	-	-	-	-	-	-	-	*Proteus vulgaris*
G1-G4	-	-	-	-	-	R	Y	-	-	*E. coli*

Key: **-** = Negative, **+** = Positive, **R** = Red, **Y**= Yellow.

Table 3. Determination of TBC (cfu/ml) of different samples.

Equipments	A	B	C	D	E	F	G
Sm.Spoons	1.2×10^4	2.5×10^6	1.4×10^4	4.3×10^5	1.1×10^5	1.1×10^4	1.4×10^4
Spoons	4.5×10^4	1.0×10^4	3.0×10^5	4.7×10^5	1.4×10^5	1.5×10^4	3.3×10^4
Forks	3.0×10^4	7.0×10^4	5.2×10^4	2.2×10^4	1.6×10^5	1.8×10^5	5.0×10^4
Sm. Forks	1.0×10^4	9.5×10^4	3.3×10^5	3.3×10^4	2.0×10^4	1.3×10^5	2.5×10^5

Key:-A – Restaurant 1; B – Restaurant 2; C – Restaurant 3; D – Restaurant 4; E – Restaurant 5; F – Coffee Shop 6; G – Coffee Shop 7.

Figure 1
A schematic representation of the cell-based immuno-assay.

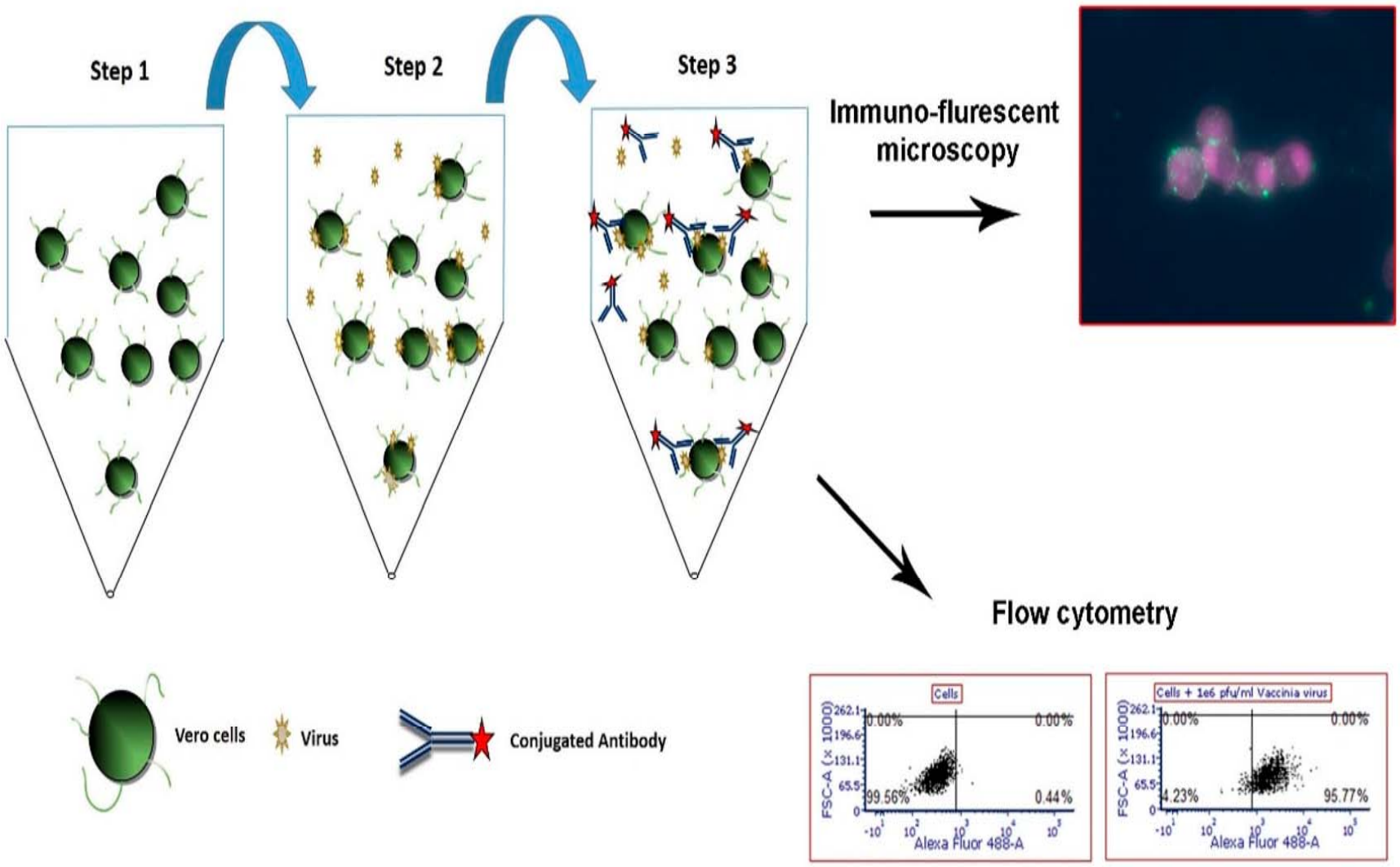


Figure 2

Food residue-attached stainless steel forks and spoons without any food component were incubated at 25°C over a 7-day experimental period. Error bars indicate standard deviations obtained from three independent experiments.

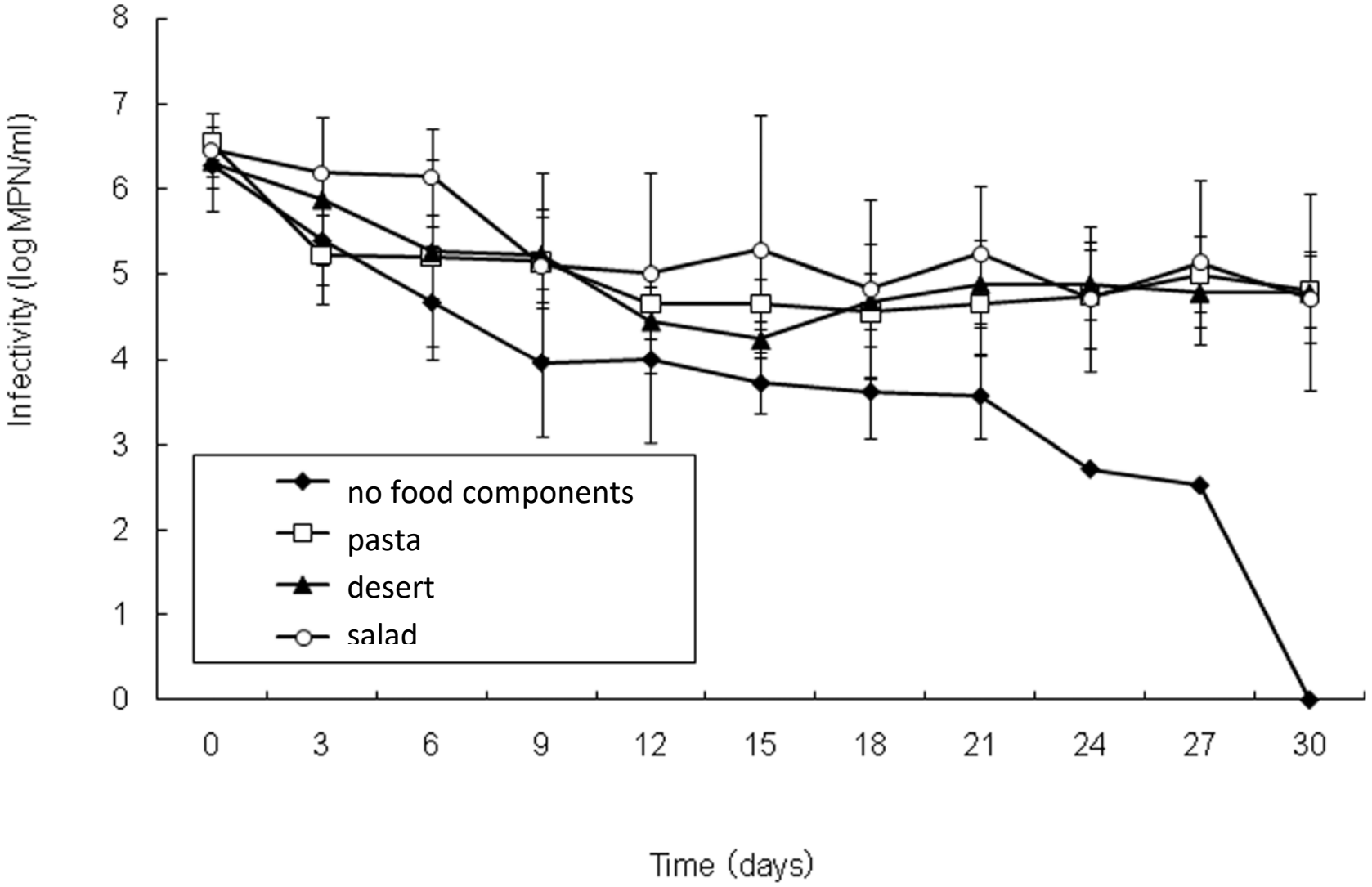
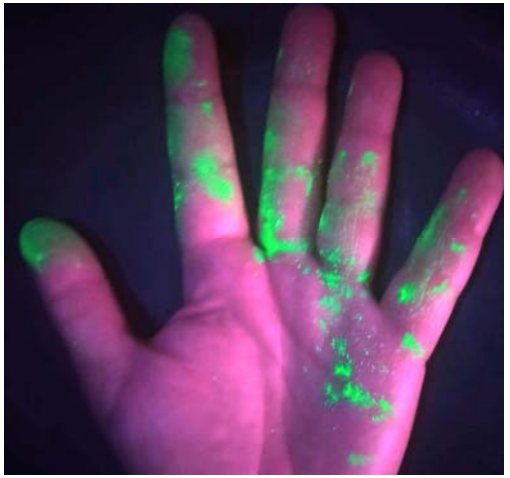


Figure 3

UV photograph of cutlery from Restaurant B using UV fluorescence visual tool to show bacteria and viruses on surface of fork, spoon, coffee spoon and dessert fork after two weeks after sample was collected.

a. UV photograph of my left hand after handling the fork in Restaurant B.



b. UV photograph of the right hand of the waitress in the restaurant to show contamination before the fork was put on the table.

Figure 4a
Cutlery as served in university student cafeteria and hospital cafeteria

Figure 4b
Cutlery as served in hospital patient trays.

Figure 4c
UV fluorescent photograph of forks and spoons contaminated by kitchen staff in university and hospital cafeteria, and in hospital patient trays.

University cafeteria

Hospital cafeteria

Hospital patient trays

REFERENCES

1. Jerry A (2008). Dishwashers - Cook Without Any Tension Rupiz Compare Ltd. Parade House 135. The Parade High Street Watford, Hertfordshire WD17 1NS United Kingdom.
2. Berdgoll MS (1989). *Staphylococcus aureus,* Food Borne Bacteria Pathogen. Int. J. Food Microbiol. 26: 465-524.
3. Bryan FL, Bartleson CA, Christopher N (1995). Harzard analysis in refrence to Bacillus species in cantonese-style restaurants. J. Food Protect. p. 44.
4. Cheesbrough M (2005). District Laboratory Practice in Tropical Countries, Cambridge University Press 2: 62-70, 382-407.
5. Collins CH, Lyne MP (1979). Microbiological Methods, 4th Edition, Butterworth and Co. Limited, London pp. 75-314.
6. Cracknel W, Nobis CF (1989). Food Microbiology, 4th Edition Tata McGraw-Hill Publishing Company limited, New Delhi p. 56.
7. Duke V (2002). Chronicles Hotels and Restaurants Business. LVO Practical information.
8. Eugene W, Nester Denise G, Anderson C, Evans Roberts JR, Nancy N, Pearsall, Martha T, Nester (2004). Microbiology. A Human Perspective. Fourth edition, McGraw-Hill Company pp. 635-636.
9. Fawole MO, Oso BA (1988). Laboratory Manual of Microbiology Ibadan. Spectrum Books Limited p. 127.
10. Julie G (2007). Food Safety Basic. A Reference Guide For Food Service Operations.
11. Michael J, Pelczar JR, Chan ECS, Noel R, Krieg (2004). Microbiology 5th edition. Tata McGraw-Hill Publishing Company Limited p. 798.
12. Steward GJ (1976). Basic Food Microbiology, 1st edition, CBS publishers and distributors, 485 Jain Bhawan, Bhoha Nath Negar shahdra (India) p. 158.
13. Tebutt GM (1986). An evaluation of various working practices in shops selling raw and cooked meats. J. Hyg. 3: 81-90.
14. Thelma JP, Pawsey R (1992). Principles of Microbiology for Students of Food Technology 2nd edition publishers Stanley Thomas Limited (Thailands) pp. 156-158.
15. Vanderzant C, Splittstoesser DF (1992). Compendium of methods for the micro biological examination of foods, 3rd edition American public Health Association
16. WHO (1984). The Role of Safety in Health Development. WHO Tech Report Series No 705. Geneva p. 160.
17. WHO (2002). Bulletin of the World Health Organization 7: 80. Food Safety Matters.
18. Zattola EA (1994). Microbial attachment and biofilm formation: A new problem for the food industry, scientific summary Food Technol. 7: 107.
19. Hall A.J. Noroviruses: The perfect human pathogens? *J. Infect. Dis*. 2012;205:1622–1624. doi: 10.1093/infdis/jis251. [PMC free article] [PubMed] [CrossRef]
20. Scharff R.L. Economic burden from health losses due to foodborne illness in the United States. *J.Food Prot.* 2012;75:123–131 doi:10.4315/0362-028X.JFP-11-058.

21. Norozi J. Study on bacterial contamination of environment kitchen in some hospitals. Journal of Health Administration. 2005; 8(19):50-54.
22. Rönnqvist M., Maunula L. Noroviruses on surfaces: Detection, persistence, disinfection and role in environmental transmission. *Future Virol.* 2016;11:207–217. doi: 10.2217
23. Atmar R.L. Noroviruses: State of the art. *Food Environ. Virol.* 2010;2:117–126. doi: 10.1007/s12560-010-9038-1. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
24. Lawley R., Curtis L., Davis J. *The Food Safety Hazard Guidebook*. 2nd ed. Royal Society of Chemistry; Cambridge, UK: 2012. pp. 148–149. [Google Scholar]
25. D'Souza D.H., Sair A., Williams K., Papafragkou E., Jean J., Moore C., Jaykus L. Persistence of caliciviruses on environmental surfaces and their transfer to food. *Int. J. Food Microbiol.* 2006;108:84–91. doi: 10.1016/j.ijfoodmicro.2005.10.024. [PubMed]
26. FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization) *Microbiological Risk Assessment Series No. 14*. FAO/WHO; Rome, Italy: 2008. Viruses in food: Scientific advice to support risk management activities; p.151
27. Vinjé J. Advances in laboratory methods for detection and typing of norovirus. *J. Clin. Microbiol.* 2015;53:373–381. doi: 10.1128/JCM.01535-14. [PMC free article] [PubMed]
28. Moore M.D., Goulter R.M., Jaykus L.-A. Human norovirus as a foodborne pathogen: Challenges and developments. *Annu. Rev. Food Sci. Technol.* 2015;6:411–433. doi: 10.1146/annurev-food-022814.
29. Karst S.M. Pathogenesis of noroviruses, emerging RNA viruses. *Viruses*. 2010;2:748–781. doi: 10.3390/v2030748. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
30. Debbink K., Lindesmith L.C., Baric R.S. The state of norovirus vaccines. *Clin. Infect. Dis.* 2014;58:1746–1752. doi: 10.1093/cid/ciu120. [PMC free article] [PubMed].
31. Kou B., Crawford S.E., Ajami N.J., Czako R., Neill F.H., Tanaka T.N., Kitamoto N., Palzkill T.G., Estes M.K., Characterization of cross-reactive norovirus-specific monoclonal antibodies. *Clin. Vaccine Immunol.* 2015;22:160–167. doi: 10.1128/CVI.00519-14.
32. Chames P., Van Regenmortel M., Weiss E., Baty D. Therapeutic antibodies: Successes, limitations and hopes for the future. *Br. J. Pharmacol.* 2009;157:220–233. doi: 10.1111/j.1476-5381.2009.00190.x. [PMC free article] [PubMed] [CrossRef]
33. Lakhin A., Tarantul V., Gening L. Aptamers: Problems, solutions and prospects. *Acta Nat.* 2013;5:34–43. doi: 10.32607/20758251-2013-5-4-34-43. [PMC free article]
34. Shirato H. Norovirus and histo-blood group antigens. *Jpn. J. Infect. Dis.* 2011;64:95–103. [PubMed] [Google Scholar].
35. Harrington P.R., Vinjé J., Moe C.L., Baric R.S. Norovirus capture with histo-blood group antigens reveals novel virus-ligand interactions. *J. Virol.* 2004;78:3035–3045. doi: 10.1128/JVI.78.6.3035 2004.
36. Tan M., Xia M., Chen Y., Bu W., Hegde R.S., Meller J., Li X., Jiang X. Conservation of carbohydrate binding interfaces—Evidence of human HBGA selection in norovirus evolution. *PLoS ONE*. 2009;4:e5058. doi: 10.1371/journal.pone.0005058.

37. Tan M., Jiang X. Norovirus and its histo-blood group antigen receptors: An answer to a historical puzzle. *Trends Microbiol.* 2005;13:285–293. doi: 10.1016/j.tim.2005.04.004.
38. Singh B.K., Leuthold M.M., Hansman G.S. Human noroviruses' fondness for histo-blood group antigens. *J. Virol.* 2015;89:2024–2040. doi: 10.1128/JVI.02968-14.
39. Miura T., Sano D., Suenaga A., Yoshimura T., Fuzawa M., Nakagomi Histo-blood group antigen-like substances of human enteric bacteria as specific adsorbents for human noroviruses. *J. Virol.* 2013;87:9441–9451. doi: 10.1128/JVI.01060-13. [PMC free article]
40. Almand E.A., Moore M.D., Jaykus L.-A. Characterization of human norovirus binding to gut-associated bacterial ligands. *BMC Res. Notes.* 2019;12:1–6. doi: 10.1186/s13104-019-4669-2. [PMC free article].
41. Nasir W. *Ph.D. Thesis*. University of Gothenburg; Gothenburg, Sweden: 2014. Studies on Interactions of Norovirus Capsid Protein with Fucosylated Glycans and Galactosylceramide as Soluble and Membrane Bound Ligands. [Google Scholar]
42. Rydell G.E., Dahlin A.B., Hook F., Larson G. QCM-D studies of human norovirus VLPs binding to glycosphingolipids in supported lipid bilayers reveal strain-specific characteristics. *Glycobiology*. 2009;19:1176–1184. doi: 10.1093/glycob/cwp103.
43. Nilsson J., Rydell G.E., Le Pendu J., Larson G. Norwalk virus-like particles bind specifically to A, H and difucosylated Lewis but not to B histo-blood group active glycosphingolipids. *Glycoconj. J.* 2009;26:1171–1180. doi: 10.1007/s10719-009-9237-x
44. Lindesmith L.C., Debbink K., Swanstrom J., Vinjé J., Costantini V., Baric R.S., Donaldson E.F. Monoclonal antibody-based antigenic mapping of norovirus GII. 4-2002. *J. Virol.* 2012;86:873–883. doi: 10.1128/JVI.06200-11. [PMC free article] [PubMed]
45. Tan M., Jiang X. Norovirus gastroenteritis, carbohydrate receptors, and animal models. *PLoS Pathog.* 2010;6:e1000983. doi: 10.1371/journal.ppat.1000983.
46. Wang Y., Ye Z., Ying Y. New trends in impedimetric biosensors for the detection of foodborne pathogenic bacteria. *Sensors.* 2012;12:3449–3471. doi: 10.3390/s12030344
47. Byrne B., Stack E., Gilmartin N., O'Kennedy R. Antibody-based sensors: Principles, problems and potential for detection of pathogens and associated toxins. *Sensors.* 2009;9:4407–4445. doi: 10.3390/s90604407.