

A microbial survey of food served to oncohematology patients at a university hospital

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doi: 10.3396/ijic.V7i1.005.11

Abstract

Although everyone is susceptible to foodborne diseases, the immunocompromised patients are particularly at risk of contracting foodborne illnesses and suffer more serious sequelae as a result of infection. The aim of the present work was to determine the microbial quality of different food stuffs, served to immunocompromised patients admitted at the Oncohematology unit in our hospital. Several types of foods were selected from those served to the oncohematology patients in our hospital. Samples of each food were taken at random from the central kitchen, the wards' pantry as well as from the patients. Food included processed food stuffs as well as non-processed food stuffs. Samples were processed according to the protocol obtained from the Centre Hospitalier Universitaire Bicêtre (2001). The total bacterial count, the total coliform count, the presence of *Staphylococcus aureus*, as well as the presence of fungal food contamination were determined and values obtained were compared to the accepted values cited in the protocol provided by the CHU Bicêtre (2001). Twenty one out of 28 examined processed food samples (75%) were free from any microorganisms. The remaining 7 samples (25%) yielded positive results on culture, yet the counts were considered satisfactory. On the other hand, all of the non-processed food samples contained high microbial counts (100%). Identified organisms were *Pseudomonas*, *Aeromonas*, enterococci, *Diphtheroid* and *Anthracoïd*. All examined samples were positive for fungal contamination with *Aspergillus niger* being the most commonly isolated mold. We concluded that non-processed food represents a potential risk of microbial exposure to the immunocompromised patients.

Key words

Foodborne diseases; foodborne illnesses; food microbiology; food contamination

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Introduction

Despite the progress seen in recent times in medical care and food technology, foodborne diseases are still, and increasingly, of major concern for human health, both in developing and developed countries.¹

Food-related disease threats are numerous and varied, involving biological and non-biological agents. Foodborne illnesses can be caused by microorganisms and/or their toxins, marine organisms and their toxins, fungi and their related toxins, and chemical contaminants. During the last 20 years, some foods that have been linked to outbreaks include: milk (*Campylobacter*); shellfish (Norwalk-like viruses); unpasteurized apple cider (*Escherichia coli* O157:H7), eggs (*Salmonella*); fish (ciguatera poisoning); raspberries (*Cyclospora*); strawberries (Hepatitis A virus); and ready-to-eat meats (*Listeria*).²

Although everyone is susceptible to foodborne diseases, certain segments of the population are particularly at risk of contracting a foodborne illness, namely the young children, the elderly, pregnant women, the immunocompromised and travelers.³

The immunocompromised are not only more susceptible to infection, but suffer more serious sequelae as a result of infection. Infections of healthy adults with foodborne pathogens usually result in self-limiting gastroenteritis that does not require antibiotic therapy. However, the immunocompromised persons are at increased risk of complications (septicemia, arthritis, meningitis, pneumonia) and death, even if the infecting dose is low.⁴

The aim of the present work was to determine the microbial quality of different food stuffs, served to immunocompromised patients admitted at the Oncohematology unit in our hospital, in reference to accepted microbial counts.

Methods

The present study is a cross-sectional, descriptive study that was conducted at a tertiary referral and teaching hospital in Cairo, Egypt during the period from September 2008 to November 2008.

Sampling

Several types of foods were selected from those served to the oncohematology patients in our hospital. Samples of each food were taken at random from the central kitchen, the wards' pantry (where food is stored prior to being served to patients) as well as from the patients. Food included processed food stuffs (rice, poultry, vegetables, and cheese) as well as non-processed food stuffs (raw fruits, green salad, sugar and regular tea).

Different foods were collected in sterile containers labelled with the type of sample, date, time and site of sampling and transported immediately to the microbiology laboratory. Unless immediately processed, foods were kept at 2-4°C for maximum 24 hours.

Processing

Samples were processed according to the protocol obtained from the Centre Hospitalier Universitaire (CHU) Bicêtre⁵ as follows:

a) Sample preparation:

Liquid samples were diluted, 1 in 10, by adding 25ml of the sample onto 225ml of sterile peptone water. For solid foods, 25gm were aseptically weighed and added to 225 ml peptone water and aseptically blended using sterile electric blender (dilution 1:10).

b) Dilution of sample:

Serial 10 fold dilutions were then prepared by transferring one ml of the original, well mixed/blended, 1:10 dilution to a tube containing 9ml of sterile physiological saline and so forth until we obtained 1:10000 dilutions. Each test tube was labelled with the type of sample and number of the dilution.

c) Sample inoculation:

- For the determination of the total bacterial count, 1ml from each sample dilution was inoculated onto a sterile Petri dish which was then flooded with 15ml of Nutrient Agar (40-45°C). Plates were left to cool at room temperature for 15 minutes. Each plate was properly labelled with the type of sample, date, dilution and then incubated at 35°C for 48 hours.

- For the determination of the total coliform count, 1ml from sample dilution was inoculated onto a sterile Petri dish which was then flooded with 15ml of the Mac Conkey agar medium (40-45°C). Plates were left to cool at room temperature for 15 minutes. Each plate was properly labelled with the type of sample, date, dilution and then incubated at 35°C for 48 hours.
- For the determination of *Staphylococcus aureus*, 100µl of the original 1:10 dilution was streaked over a plate of Baird Parker (BP) medium. The plate was then incubated at 35°C for 48 hours.
- For the determination of the fungal contamination, 200µl of the original 1:10 dilution was inoculated onto 2 Sabouraud Dextrose agar (SDA) plates (100µl each). One plate was incubated at 35°C and the other at 25°C for two weeks.

d) Interpretation of results:

- Determination of the total bacterial count: plates were examined after 48 hours, each individual colony was counted, and the total number of colonies was multiplied by the dilution shown on the plate to get the total number of bacteria per gram product.
- Determination of the total Coliform count: plates were examined after 48 hours, each individual colony was counted, and the total number of colonies was multiplied by the dilution shown on the plate to get the total number of Coliforms per gram product. Coliforms were further divided according to the lactose fermentation of MacConkey agar to lactose-fermenting and non-lactose fermenting colonies. Then, colonies were further identified to the species level using in house biochemical tests (oxidase, citrate, urease, motility, indole, ornithine decarboxylase, bile esculin hydrolysis).
- Black colonies surrounded by clear halo on the BP medium were further tested by the tube coagulase test to identify the *Staphylococcus aureus* colonies.
- Any growth on SDA was considered significant and was further identified by culture morphology and methylene blue preparation.
- Values obtained were compared to the values cited in the protocol provided by the CHU Bicêtre.⁵

Results

In the present study, 55 samples from the different foods served to the immunocompromised patients, at the Oncohematology unit, were collected during the period between September 2008 and November 2008. Twenty eight processed food samples were examined including 8 from cooked rice (5 from the central kitchen and 3 from the ward's pantry), 7 from cooked poultry (5 from the central kitchen and 2 from the ward's pantry), 8 from cooked vegetables (5 from the central kitchen and 3 from the ward's pantry), in addition to 5 processed cheese samples. Regarding the non-processed food samples, 27 samples were examined including 5 from raw fruits, 12 from green salad (5 from the central kitchen and 7 from the ward's pantry), 5 sugar samples (from the patients) as well as 5 samples from regular tea (obtained from the patients).

As shown in Table I, 21 out of 28 processed food samples examined (75%) were free from any microorganisms. Although the remaining 7 samples (25%) yielded positive results on culture, yet the counts were considered satisfactory when compared to the accepted counts provided by the CHU Bicêtre.⁵ Apart from the five processed cheese samples, the cooked rice and vegetable samples yielded growth of *Bacillus* spp. on Nutrient Agar and counts were 1×10^3 CFU/gm and 2×10^2 CFU/gm, respectively. Regarding the processed cheese samples, the mean total bacterial count was 2.2×10^4 CFU/gm and the mean total coliform count was 2.6×10^3 CFU/gm. Organisms identified were of the *Lactobacillus* and *Enterococcus* spp.

Table II shows that all of the non-processed food samples contained high microbial counts (100%). Yet, the total bacterial and total coliform counts were considered acceptable when compared to the norms of the Bicêtre.⁵ Identified organisms were *Pseudomonas*, *Aeromonas*, enterococci, *Diphtheroid* and *Anthracoïd*. All examined samples were positive for fungal growth, *Aspergillus niger* was the most commonly isolated mould followed by *Aspergillus fumigatus* and *Mucor* (Table III).

At the initial inspection of the central kitchen, a number of obvious faults were identified and included: areas of peeling paintwork, or cracked/missing tiles; large amounts of garbage was allowed to collect in

Table I: Results of the microbial examination of the processed food samples

Food Item	Site of collection (No.)	Number of positive samples (%)	Total Bacterial Count	Total Coliform Count	<i>S. aureus</i>	Fungal contamination
Cooked Rice	Central kitchen (5)	0 (0%)	Nil	Nil	Nil	Nil
	Ward pantry (3)	1 (33.30%)	1 x 10 ³	Nil	Nil	Nil
Cooked Poultry	Central kitchen (5)	Nil	Nil	Nil	Nil	Nil
	Ward pantry (2)	Nil	Nil	Nil	Nil	Nil
Cooked Vegetables	Central kitchen (5)	Nil	Nil	Nil	Nil	Nil
	Ward pantry (3)	1 (33.30%)	2 x 10 ²	Nil	Nil	Nil
Processed cheese	Central kitchen (5)	5 (100%)	2.20 x 10 ⁴	2.60 x 10 ³	Nil	Nil

Table II: Results of the microbial examination of the non-processed food samples

Food Item	Site of collection (No.)	Number of positive samples (%)	Total Bacterial Count	Total Coliform Count	<i>S. aureus</i>	Fungal contamination
Raw Fruits	Central kitchen (5)	5 (100%)	> 3x10 ⁶	Nil	Nil	Positive
Green Salad	Central kitchen (5)	5 (100%)	1.8 x 10 ⁶	3 x 10 ⁵	Nil	Positive
	Ward's pantry (7)	7 (100%)	>3 x 10 ⁶	> 1x10 ⁶	Nil	Positive
Sugar	Patient (5)	5 (100%)	2 x 10 ⁵	Nil	Nil	Nil
Regular Tea	Patient (5)	5 (100%)	Nil	Nil	Nil	Positive

Table III: Organisms identified in non-processed food samples

Food Item	Organism Identified
Raw fruits	Anthracooid (5/5, 100%)
	Diphtheroid (2/5, 40%)
	<i>Aspergillus niger</i> (2/5, 40%)
	<i>Aspergillus fumigatus</i> (3/5, 60%)
Green salad	<i>Pseudomonas spp</i> (7/7, 100%)
	<i>Aeromonas spp</i> (7/7, 100%)
	Enterococci (7/7, 100%)
	<i>Aspergillus niger</i> (4/7, 57.1%)
Regular tea	<i>Aspergillus niger</i> (3/5, 60%)
	Mucor (3/5, 40%)
Sugar	Anthracooid (5/5, 100%)

the kitchen; no soap or towels for the hand wash sinks; inadequate number of wash sinks resulting into poor separation of meat, poultry and vegetables during wash; lack of personal protective equipments (aprons, masks); and poor temperature records for the refrigerators.

Discussion

In the present work we confirmed the presence of high microbial counts in 100% of the non-processed food samples. The most commonly isolated bacteria from green salad samples were *P. aeruginosa*, *Aeromonas* and enterococci. Similar findings were reported by Mensah and coworkers⁶ who found that samples obtained from salad and raw vegetables had high levels of microorganisms as part of their normal flora. Also, Remington and Schimpff⁷ and Marchetti *et al.*⁸ stated that raw produce salads may be an important route by which immunocompromised patients acquire *Pseudomonas*. Moreover, during microbiological examination of minimally processed lettuce, Szabo and associates isolated *A. hydrophila* and *A. caviae* from 55% of the samples examined.⁹ The authors were also able to isolate *Y. enterocolitica* and *L. monocytogenes* from 59% and 2.5% of the lettuce samples, respectively. Lettuce and tomato were

also found to be contaminated with faecal bacteria, *Salmonella* and *Shigella*.⁶

Salmonellosis has been linked to tomatoes, seed sprouts, cantaloupe, mamey, apple juice, and orange juice. *Escherichia coli* O157:H7 infection has been associated with lettuce, sprouts, and apple juice, and enterotoxigenic *E. coli* has been linked to carrots. Documented associations of shigellosis with lettuce, scallions, and parsley; cholera with strawberries; parasitic diseases with raspberries, basil, and apple cider; Hepatitis A virus with lettuce, raspberries, and frozen strawberries; and Norwalk/Norwalk-like virus with melon, salad, and celery have been made.¹⁰

The recovery of enterococci from green salads was not reported previously, however, this could be explained by the use of animal waste as manure in culture. Enterococci are known to reside in the intestinal tract of animals and humans, and are excreted in large amounts in animal faeces.

The current work confirms the previous findings of Bouakline *et al.* who detected fungal contamination in different types of non-processed foods.¹¹ The authors found that at least one sample of all the fruit types examined, except melons, was contaminated by moulds. The rate of contamination of fruits with downy skin, such as apricots, kiwis, and peaches was >50% compared to 12.5% to 33.3% for smooth skinned fruits such as apples, bananas, lemons and orange. Moreover, and in accordance to our findings, Bouakline and colleagues demonstrated the consistent contamination of regular tea as well as herbal teas with moulds.¹¹

In the present study, *Aspergillus niger* was the most frequently isolated mould from the non-processed food samples. This could be explained by the fact that *Aspergillus* spores are widely distributed in the environment. These spores can contaminate food and thus can be indirect source of airway or digestive tract colonization.

Regarding the processed food samples examined, the majority (75%) were considered satisfactory. The remaining 25%, although yielded growth of less-pathogenic microorganisms, yet the counts were

considered acceptable for the healthy individuals.

On the basis of these results, we conclude that non-processed food stuffs carry high levels of microorganisms as part of their normal flora. Thus, it represents a potential risk of microbial exposure to the immunocompromised patients. Lack of the basic measures for food hygiene in the central kitchen, the presence of untrained workers, the increase workload in the hospital kitchen, serving food at greater distances with inadequate temperature control, together with the increasing number of immunocompromised patients in the hospital, all of which increase the risk of illness associated with consumption of food.

Recommendations

First, we recommend that all foods for which there is no appropriate sterilization process should no longer be served to immunocompromised patients. Regular tea may be unacceptable for those patients who are confined to their rooms for long periods; tea should be prepared with boiling water in the ward's pantry and not by the patient. Serving downy fruits should also be prohibited.

Second, certain measures are recommended while handling food for immuno-compromised patients:

1. Wash hands before and after handling food.
2. Keep area where food is prepared clean.
3. After cutting up raw meat, soak the cutting board and all utensils for 30 minutes in a mixture of one part bleach to nine parts water.
4. When preparing foods, wearing gloves, head caps, and aprons is necessary. If possibly, jewellery (especially rings) should be removed, because they can hold germs.
5. Eliminate all contact between cooked and raw foods.
6. Use separate cutting boards and wash sinks for meat and raw vegetables.
7. Clean inside refrigerator regularly with soap and water to control molds. Throw away food that is more than 3-4 days old, especially salad dressings, sauces, milk and egg products, and processed meat.
8. Maintain adequate temperature control.
9. Food should be served hot with adequate temperature control during transport.
10. Food should be consumed immediately, avoid storage and reheating whenever possible.

Third, studies assessing the risk associated with the consumption of different types of foods are required.

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