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A Bacteriological Assessment of the Cleaning and Disinfection Efficacy at the Midlands State University Canteen, Zimbabwe

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Abstract: A bacteriological assessment of the cleaning and disinfection efficacy at the Midlands State University Canteen was done. Ten random assay visits were carried out and the total counts from various environmental surfaces were determined using the Agar Syringe method. Food quality was also determined by testing for coliforms and *Salmonella*. Results of the study indicated that cutting boards, vegetable-section and meat-section tables were frequently found to be the most highly contaminated surfaces. Cooked food was found to be of satisfactory microbiological quality. The maximum efficacy of the cleaning and disinfection program at the Midlands State University canteen was found to be 62% but with a high potential for improvement.

Key words: Bacteriological assessment, sanitation, disinfection

INTRODUCTION

Aerobic colony counts and coliform enumeration are a useful and most often used means of assessing overall sanitation in the environments of food service establishments[1]. The presence of indicator organisms, pathogens or high bacterial counts in foodstuffs, food contact surfaces, equipment and utensils provides a direct and relevant measure of cleaning efficiency and hygiene[2]. Effective hygiene control through bacteriological testing is vital to ensure acceptable levels of contamination and avoid adverse human health consequences of food-borne illnesses.

The Midlands State University (MSU) canteen is a commercial catering establishment that serves the campus populace. Over the years, food safety concerns have been raised over the quality of the food served. The hygiene standards of the food preparation areas, utensils as well as the personal hygiene practices of some of the kitchen personnel have been questioned. Some students have reported to have suffered from diarrhoea and abdominal pain after eating at the canteen. Such cases are usually followed by assumptions that it was microbial 'food poisoning' even though no clinical or laboratory substantiated evidence is provided.

Microbial food poisoning is usually caused by the proliferation of undesirable microorganisms from cross contamination, inadequate temperature control during processing and/or storage of food and inadequate sanitation[3]. Canteens feature frequently as a venue of faulty food preparation, mishandling, and/or contamination[4]. In most cases insufficient regard is given to the practice of cleaning and disinfection.

Effective cleaning is of prime importance since it not only removes gross contamination but also any residues that could support the subsequent survival and growth of microorganisms[2]. As a result of inadequate cleaning, microorganisms may persist on utensils and work surfaces and build in numbers[5]. Good hygiene practices including cleaning and disinfection are a primary preventive measure and the assessment and monitoring of their effectiveness not only provides an early warning of potential problems but also evidence of due diligence.

This study undertook hygiene-monitory bacteriological testing to asses the degree and nature of bacterial contamination on utensils, work surfaces, walls and floors in the MSU kitchen. It also sought to assess the bacteriological quality of the food served. The testing aimed to verify the effectiveness of the cleaning and disinfection programme(s) in place at the canteen.

MATERIALS AND METHODS

Environmental surface sampling: Environmental sampling for the detection and enumeration of microorganisms was carried out in the MSU canteen using the Agar Syringe method. Plate Count Agar syringe swabs were used for determination of total viable counts. MacConkey Agar syringe swabs were used for the qualitative determination of indicator organisms and/or

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suspected pathogenic bacteria on predefined surfaces and for food-handler hand swabs.

The surface locations for testing on each predefined site were randomized as were the days and times of sampling. However, sampling was mostly done during the afternoon after the major cleaning and disinfection operations were completed at MSU canteen.

Sampling sites: Environmental surfaces sampled in the study included the following areas: - walls, floors, scullery sinks, handwash basins, meat-section table, vegetable-section table, serving counter, cutting board, clean plates and the coldroom door handle. Four areas for each predefined surface were randomly selected for sampling e.g. four random sites per cutting board, table etc. For the sampling of small utensils, four randomly selected individual items were sampled e.g. four plates. For the sampling of food handlers for assessment of personal hygiene, hand swabs were taken from any four randomly picked kitchen personnel.

Agar syringe swab preparation: The ends were cut from the needle-ends of hypordemic syringes to create hollow cylinders of approximately 3.5 mm in diameter. These were autoclaved at 121psi for 15 min. Sterile syringe cylinders were filled with agar medium incorporating a disinfectant quencher/neutralizer, Tween 80® and left to set.

Swabbing: The swabbing was carried out by first extruding a layer of media of about 15 mm beyond the end of the syringe barrel by means of the plunger. The exposed agar surface was then pressed onto the surface to be examined and contact maintained for about 2-3 sec.

The agar surface was pressed and not wiped against the sampled surface to allow a duplicate image of the microbial flora to be developed on the incubated slice of medium. About 15 mm of media, from the inoculated end, was cut off with a sterile knife and placed in a sterile petri dish with the contact surface uppermost. The blade was flamed between successive cuts to avoid marginal infection of the edge of the media during slicing. Up to four slices of inoculated media from each predefined area were placed in each petri dish, which was then labeled with the sampled site, date and time. Sterility controls were made randomly during the sampling by cutting unexposed media slices into petri dishes. The samples were incubated for 18-24 h at 37°C. Resultant colonies on each agar slice were counted.

Food sample collection: Thick porridge (staple food of Zimbabwe), stew and vegetables served by the MSU canteen were tested to assess the nature and degree of bacterial contamination. Sampling was conducted by dividing each food container into four quadrants and a portion of each food from each quadrant was randomly collected. The samples from the quadrants for each food were collected into a sterile glass jar as aseptically as possible using a sterile steel spoon and covered.

Food homogenate preparation: Each food sample was analyzed separately. The samples were cut into small pieces with a sterile scalpel and/or ground using a sterile mortar in a sterile jar. About 10 g of sample representative of each food specimen was weighed out and suspended in 90 mL of Phosphate buffered saline and thoroughly mixed. This represented a dilution of 10^{-1}. A decimal dilution series was prepared volumetrically by pipetting 1 mL of the 10^{-1} dilution of each homogenate into a 9 mL dilution blank of Buffered Peptone water, constituting the 10^{-2} dilution. Subsequent dilutions of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were made volumetrically. To duplicate sets of pre-labeled petri dishes, 1 mL aliquots from each dilution were pipetted.

Standard plate counts: About 10-15 mL of melted Plate Count Agar tempered to 45°C was poured into each of the petri dishes. As a sterility check, two plates of medium only were also poured as controls. After the agar had solidified, the petri dishes were inverted and incubated at 35^0C for 24 h. All colonies on plates containing 25-250 colonies were counted .

Coliform determination: A loopful from each 10^{-1} food sample dilution was streaked onto MacConkey Agar and Blood Agar plates. The plates together with two control plates of each medium were incubated at 37°C for 18-24 h. The formation of pink colonies on MacConkey Agar plates was taken to confirm the presence of coliforms in the original food samples.

Presumptive *E. coli* determination: The streaked MacConkey agar and Blood Agar 24 h cultures of each food sample were examined for typical *E. coli* colonies. Suspected *E. coli* colonies were picked and suspended into Peptone Water, ONPG broth (O-nitrophenyl-P-D-galactopyranoside) and stabbed into Motility Agar. Colonies that tested positive for motility, Indole production after addition of Kovac's reagent into Peptone Water culture suspension (pink) and ONPG (yellow) were presumed to be *E. coli*.

Determination of *Staphylococcus aureus*: The streaked MacConkey agar and Blood Agar 24 h cultures of each food sample were examined for typical *Staphylococcus* colonies. Suspected *Staphylococcus* colonies were picked from the blood agar plates and streaked onto Mannitol

Table 1: Environmental hygiene grade scale for a 3.5 mm diameter agar syringe

Growth on media	Score
Negative	0
Slight growth (10< colonies)	1
Little growth (10-30 colonies)	2
Moderate growth (up to 80 colonies)	3
Heavy growth (over 80 colonies)	4
Complete colony coalescence (<200 colonies) (lawn)	5

Source: Thornton, H.[6]

Table 2: Food quality assessment criteria

Grade	Criteria
A (Good)	TVC <100, no coliforms, no pathogen
B (Satisfactory)	100<TVC<1000, no coliforms, no pathogen
C (Unsatisfactory)	a)TVC>1000, coliforms present/absent, no pathogen b)TVC low/high, coliforms present, no pathogen
D (Unacceptable)	TVC low/high, coliforms present/absent, Pathogen present

Salt Agar. The plates were incubated for 24 h at 37°C. Presumptive *S. aureus* colonies (surrounded by a yellow halo zone) were picked and a glass slide coagulase test carried out for the definitive identification of *S. aureus*. Coagulase negative colonies were reported as *Staphylococcus* spp.

Presumptive *Streptococci* determination: The streaked Blood Agar 24 h cultures of each food sample were examined for typical streptococci [either α haemolytic (green-zone) or β haemolytic (clear zone)]. Suspected colonies were picked and inoculated onto Aesculin agar to test for aesculin hydrolysis. The inoculated cultures on Aesculin agar were incubated for 24 h at 37°C.

Presumptive *Salmonella* testing: For the detection of *Salmonella*, 0.1 mL of each food homogenate was transferred to 10 mL of Rappaport-Vassiliadis (RV) broth. The inoculated broth cultures were incubated for 24 h at 42°C. The incubated broth cultures were then streak plated onto Xylose-Lysine Desoxycholate agar (XLD). The plates were incubated for 24 h at 35°C. The XLD plates were then examined for typical *Salmonella* colonies i.e. pink colonies with /without black centres or as almost completely black colonies. Suspected *Salmonella* colonies were picked using a sterile inoculating needle and inoculated onto Kligler Iron Agar (KIA) by streaking the slant and stabbing the butt. Without flaming the inoculating needle, Lysine Iron Agar (LIA) slants were also inoculated by stabbing the butt twice and streaking the slant (*Carboxylase* test). The slants were incubated at 37°C for 24 h with loose caps to promote aerobic conditions. They were examined for typical *Salmonella* biochemical reactions of an alkaline (red) slant and acid (yellow) butt with/without H_2S production (blackening) in KIA and an alkaline (purple) butt in LIA. Slants with distinct acid (yellow) butt in LIA were considered negative.

Personnel hand swab microflora analysis: MacConkey Agar hand swab growths were examined for the presence of indicator organisms i.e. Coliforms (pink colonies on MacConkey agar due to lactose fermentation). Suspected *E. coli*, *Enterobacter* and non-lactose fermenting bacteria colonies (pale straw) suspected to be of public health significance were subcultured for their presumptive identification in the same manner as the food sample cultures were tested above.

Data analysis: Average bacterial counts from Plate Count Agar slices for each sampled area were used to calculate the overall hygiene quality indices indicative of the cleaning and disinfection efficacy for the MSU canteen. The environmental grade scale in Table 1 was used to calculate the scores.

The overall hygiene indices were calculated using the following formula:

$$\text{Hygiene quality index (\%)} = \frac{\text{1 - sum of score grades for n observations}}{\text{5 x n observations}} \times 100$$

Food-handler hand swab average bacterial counts were used quantitatively to calculate a hygiene quality index for the kitchen personnel for the entire assessment period. The results of food testing i.e. Total Viable Counts (TVC), coliforms, *Staphylococci*, *Streptococci* and *Salmonella* were used to determine the food quality according to a graded scale based on criteria in Table 2.

RESULTS

Environmental hygiene: The calculated overall hygiene quality indices for the MSU canteen for each assessment visit ranged between 48 and 62% (Fig. 1). The quality indices show an initial decrease in the hygiene quality followed by a small increase after the third visit. The quality indices for visits 4, 6 and 9 were the same at 62% and for visits 7, 8 and 10 were the same at 60%.

The overall hygiene quality indices for each individual sampled area at the MSU canteen for the entire assessment period (Fig. 2) show that the walls, clean plates and cold room door hand have high quality indices of 92, 82 and 80%, respectively. These observed values are generally higher than those for other areas. The quality indices for the meat-section and vegetable-section tables, personnel hands and cutting boards are generally very low i.e. 38, 44, 46 and 36%, respectively.

Table 3: Major bacterial isolates from environmental surfaces

Area	Bacterial isolate
Floors	*Pseudomonas*, Coliforms
Scullery sink	Coliforms
Handwash basin	*Pseudomonas*
Vegetable section table	Coliforms
Meat section table	*E. coli*, *Pseudomonas*, *Staphylococcus*, streptococci
Cutting board	*Staphylococcus*, *E. coli*, streptococci, *Pseudomonas*
Personnel hygiene	

Table 4: Nature of bacterial hand contamination in food-handlers

Bacterial isolate	Number tested	%Percentage positive
Coliforms	40	92
E. coli	40	70
Streptococci	40	25
Staphylococcus spp	40	40
Enterobacter spp	40	80

Table 5: Food quality at the MSU canteen

Food sample	No. tested	Good	Satisfactory
Thick porridge	10	100	0
Stew	10	80	20
Vegetables	10	90	10

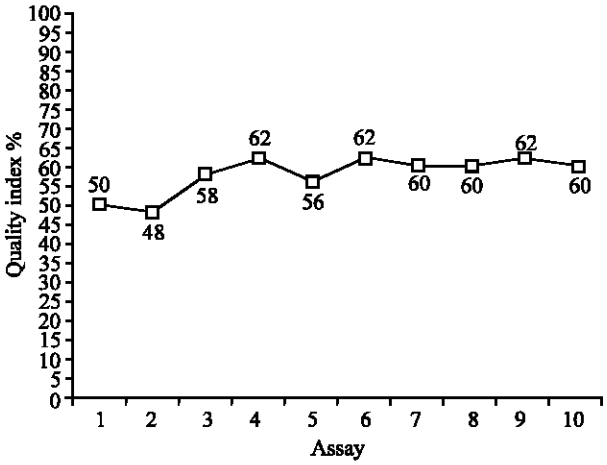

Fig. 1: Overall hygiene quality indices for the MSU canteen for each assay visit

Environmental microbial contamination: Bacterial isolates from the meat-section table and the cutting boards were similar (Table 3). The most common bacterial isolates from canteen environmental surfaces were *Pseudomonas* spp and coliforms.

Personnel hygiene: Qualititative analysis of peronnel hand swabs shows that 92% of tested food-handlers' hand were contaminated by coliforms (Table 4). It is also shown that *E. coli*, *Enterobacter*, *Staphylococcus* and Streptococci hand contamination incidences were 70, 80, 40 and 25%, respectively.

Food tests: All the tested thick porridge samples were of good quality as shown in Table 5. Eighty percent of the

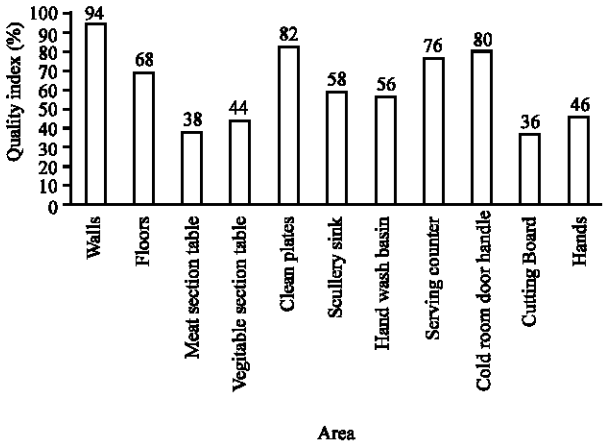

Fig. 2: Overall hygiene quality indices for individual surfaces and personnel hands for the entire sampling period

stew samples tested were of grade A quality and 20% were grade B i.e. satisfactory. 90% of the tested cooked vegetables were of good quality and 10% were satisfactory.

DISCUSSION

The results for the environmental testing of food contact surfaces, utensils and equipment indicate that the cleaning and disinfection effort at the MSU canteen is generally satisfactory. Effective disinfection does not always kill all microorganisms present but reduces their numbers to a level at which they can be reasonably assumed to present no health risks[6]. The fact that similar quality indices were obtained for three assays at two quality index levels 60 and 62% (Fig. 1) could be inferred to mean consistence of the cleaning and disinfection execution.

The initial quality index, 50%, decreased to 48% on the second assay but increased slightly from the third visit onwards. This could have been due to increased and improved sanitisation effort by the cleaning staff driven by suspicion that the researchers were from the City Health Department. The highest obtained quality index of 62% can be assumed to be the maximum attainable level of effectiveness of the cleaning and disinfection programme currently in place at the canteen.

From visual inspection and observation of the cleaning and disinfection activities at the canteen, the hygiene quality indices could not rise beyond the maximum observed, 62% due to a number of possible reasons. It was observed that in some instances visible food residues and food liquid droplets were sometimes left to dry out on food contact surfaces and floors before

being cleaned off. For disinfection to be more effective there must be adequate removal of visible gross food residues[7]. The 'clean as you go' policy was thereby not strictly adhered to. The food residues could then have constituted culture media for bacteria to grow and build up in numbers[5].

The application of detergent solution during cleaning, to loosen soil and bacterial films, was observed to be erratic. This could have contributed to the quality indices not rising to levels higher than those observed since detergent is the most efficient cleaning system component[8].

The hygiene quality indices for the various areas sampled at the MSU canteen indicate that the cleaning and disinfection effort has differing effectiveness on different surfaces, utensils and equipment. The walls had the highest quality index, 92%, indicative of the least contamination. This could be attributed to the fact that the walls do not come into contact with food and hence are less favorable microbial niches.

The quality indices for the meat-section and vegetable-section tables and cutting boards were very low. The low quality indices could have being due to the fact that the raw food materials i.e. meat and vegetables handled on these surfaces are both characterized by high numbers and many kinds of primary contaminant bacteria[5]. The handling of these foods on these surfaces requires that they be given special added attention of thorough and regular cleaning as soon as possible after use, which evidently was not being given by the cleaning staff. The *E. coli* and coliforms isolated from these surfaces could most probably have come from the meat. The cutting boards had the lowest quality index, 36%. Wooden cutting boards develop cuts/grooves from their use which are inaccessible to the casual cleaning observed at the MSU canteen hence leading to possible microbial number build ups. Cutting boards were sometimes left uncleaned for long periods of time after use.

The low quality index for the food-handlers' hands is indicative of poor levels of personal hygiene on the part of kitchen personnel. Qualitative hand swab results showed that a high fraction of the personnel's hands were contaminated by coliforms, *E. coli* and *Enterobacter* . Even though the source of these contaminants was not determined, they are highly indicative of inadequate hand sanitation[9]. The *Staphylococcus* spp and Streptococci isolated, although normal commensals on humans, reflect improper hygiene practices such as pocking fingers into the anterior nares of the nose[4]. It was observed that there was no hand sanitizer (soap or otherwise) at each of the hand wash basins for personnel to wash their hands with, after using the toilet or handling raw food such as meat. A common practice observed among the canteen staff was the habit of using their aprons to wipe their hands after handling food and/or to dry their hands after washing them. These garments could then probably serve as sources of further contamination.

Visually, handwash basins at the canteen were noted to be in deplorable hygiene status. *Pseudomonas* spp was the most commonly isolated organism from environmental surfaces and this could probably be due to their ability to colonize any surface and utilize a wide range of non-carbohydrate compounds for energy, lipolytic and proteolytic activity and their resistance to many disinfectants and sanitizers used in the food industry[5]. They are however killed by heat. Some strains of Staphylococci and streptococci, also found in the canteen environment, tend to be resistant to drying and are also highly resistant to detergents and disnfectants[8] which could be a possible reason for their high incidence.

All the food samples tested from the MSU canteen had good or satisfactory microbiological quality. No pathogens (*Salmonella* or *S. aureus*) or coliforms were isolated from all the food samples tested. Sufficient measurement of the sanitary quality of foods is accomplished by means of the determination of coliforms[10] and these were not detected. The results could have been due to adequate cooking and/or correct time-temperature control[4] since all the foods are served hot at the canteen. However, the foods cannot be assumed to be completely microbiologically safe. This is because the possibility exists that the foods may have had acceptable total counts, no coliforms and pathogens but still contain toxins that remain stable under the conditions that may not have supported continued survival of viable cells[8].

Conclusions and recommendations: The cleaning and disinfection programme at the MSU canteen, although above average and consistent, is capable of improvement. Key areas noted to require additional attention include, the use of detergents, disinfection application (mixing to recommended concentrations) and frequency of cleaning. Staff education and training programmes on basic food and personal hygiene, food safety issues, fundamental food microbiology and the underlying fundamental principles of cleaning and disinfection processes covering choice and application of detergents and sanitizers should be organized for all the canteen staff. These might serve to enhance and reinforce the canteen staff's understanding and appreciation of the above mentioned disciplines. Strict adherence to the "Clean as you go" kitchen practice should be enforced at the canteen.

REFERENCES

1. Collins, C.H., 1964. Microbiological Methods. Butterworth and Company, London.
2. Clark, D.S., 1965. Method of estimating the bacterial population of surfaces. Canadian J. Microbiol., 11: 407-13.
3. Buttiaux, R. and D.A.A. Mossel, 1961. The significance of various organisms of faecal origin in foods and drinking water. J. Applied Bacteriol., 24:353-64.
4. Eley, A.R. (Ed.), 1992. Microbial Food Poisoning. Chapman and Hall, London.
5. Frazier, W.C. and D.C. Westhoff, 1988. Food Microbiology, 4th Edn. McGraw-Hill, Singapore.
6. Thornton, H., 1974. Meat hygiene practice, 6th Edn. Tindall, London.
7. Food and Agricultural Organisation (FAO), 1991. Manuals of food quality control 5: Food Inspection. Food and nutrition paper. 14/5, Rome.
8. Dunsmore, D.G. and M.A. Thomson, 1981. Bacteriological control of food equipment surfaces by cleaning systems. J. Food Protection, 44: 21-24.
9. Thatcher, F.S. and D.S. Clark, 1973. Microorganisms in foods. Their significance and methods of enumeration. University of Toronto, Toronto.
10. Jay, M.J., 1978. Modern Food Microbiology. Van Nostrand Company, NewYork.