

Full Length Research Paper

Bacterial contamination of crockery and cutlery within the kiosks' restaurants of the Federal University of Technology, Yola

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An investigation was undertaken to determine bacteriological quality of crockery and cutlery of seven restaurants in Federal University of Technology, Yola (FUTY) kiosk. Samples (147) were collected and cultured in appropriate media and the bacterial isolates were identified according to their morphological and biochemical characteristics. The result revealed a profile of seven (7) different bacterial species including *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella typhi*, *Escherichia coli* and the species of *Shigella*, *Klebseilla* and *Bacillus*. The total values of bacterial count (TBC per 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 knives, 1.2×10^4 - 2.5×10^5 for plates and 1.5×10^4 - 4.7×10^5 for spoons cfu/ml. So, it may be concluded that in order to prevent food-borne infection, food inspectors should monitor the hygienic status of the restaurants within FUTY kiosk.

Key words: *Salmonella*, *Shigella*, *Escherichia coli*, *Bacillus*, *Staphylococcus*, *Proteus vulgaris*, *Klebseilla*.

INTRODUCTION

Presumably, food-borne diseases sometimes acquired in hotels and restaurants are through dishes, plates and other kitchen equipments (Fawole and Oso, 1988). The reputation of many hotels often rest on the quality of dishes, spoons, drinking cup and cutlery (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 during the handling and processing of the food. Tebutt (1986) found out that 74% cloths used in cleaning dishes and cutting equipment surfaces were contaminated with one or more of the following organisms *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Clostridium perfringes*. It is clear that wiping kitchen equipment with cloths may result in the contamination of equipment. Microbial attachment and biofilm formation to solid surface of crockery and cutlery provide some protection of the cells against physical removal of the cells by washing and cleaning of crockery (Scott and Bloomfield, 1981). These cells seem to have greater resistance against sanitizers and heat, thus spoilage and pathogenic microorganisms attached to food surface. Zattola (1994) reported that microbial cells attached to

equipment surfaces, especially those that come in contact with the food, may not be easily killed by chemical sanitizers or heat designed to be effective against unattached microbial cells; and thus they can contaminate food. The washing of hands, utensils and dishes is often done in buckets or bowls in such disinfection are not carried out (WHO, 2002). It also has been reported that several species and strains of *Pseudomonas* were found to attach to stainless steel surface within 30 min of contact. *Listeria monocytogenes* was found to attach to stainless steel surface, glass and rubber surfaces within 20 min of contact and some of the microorganisms found are *Staphylococcus* specie, *Escherichia coli*, *Bacillus* specie and *Pseudomonas* specie (Zattola, 1994).

Surface and equipment used in kitchen may look sparkling clean, yet bacteria may be present in large numbers (Julie, 2007). The intention of food safety is to prevent food poisoning, (the transmission of disease through food) and to maintain the wholesomeness of the food product through all stages of processing, until it is finally served. Therefore, one important task is to make sure dishes, spoons and cutlery are kept clean

Table 1. Distribution of samples per restaurant.

Restaurants	Cups	Forks	Plates	Spoons	Knives	Washing pot
A	3	4	5	2	5	2
B	4	3	2	4	3	5
C	2	4	4	6	2	3
D	4	5	3	3	4	2
E	5	2	1	6	3	4
F	4	7	3	4	1	2
G	2	6	4	3	2	4
Total	24	31	22	28	20	22

Key: A to G = Restaurants in kiosk.

(Duke, 2002).

This study determined the bacterial profile in crockery and cutlery used in restaurants and the densities of bacteria in plates, spoons, forks and cups used in restaurants in Federal University of Technology, Yola, Adamawa State.

MATERIALS AND METHODS

Sample area

Restaurants in the kiosk of Federal University of Technology, Yola were selected for this study. There are about 15 restaurants located in the kiosk and student center of the Federal University of Technology Yola. The restaurants were selected based on the availability and affordable cost of food and the samples were selected based on random selection.

Sample size

A total of 147 samples were collected from plates, spoons, forks, knives and drinking cups as well as kitchen equipment, Twenty one (21) samples were collected from each of the seven (7) restaurants and all the samples were labeled appropriately (Table 1). These seven restaurants 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 dishes, spoons, drinking cups and cutlery. Samples were collected using the rinse method for crockery and cutlery and other accessories (Cheesbrough, 2005). The study period was between July and August, 2007. Sterile distilled water was used to rinse crockery, cutlery, knives, spoons, forks, plates and drinking cups. Sterile distilled water was used to rinse crockery, cutlery, knives, spoons, forks, plates and drinking cups. 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 cutlery. 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 (2005). 1 ml 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-A6, B1-B4, C1-C6, D1-D3, E1-E2, F1-F5 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 test tubes and sterilized by autoclaving at 121°C 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 or frothing (Cheesbrough, 2005).

Coagulase test

The slide method test was used for this study. A drop of saline on

Table 2. Results of biochemical tests.

Isolate No.	Biochemical tests									Isolate
	Citrate utilization	Motility	Indole	Catalase	Coagulase	KIA				
						Slope	Butt	H ₂ S	Gas	
A1-A6	-	-	-	+	+	-	-	-	-	<i>S. aureus</i>
B1-B4	+	-	-	-	-	Y	Y	-	+	<i>Klebsiella</i> Sp
C1-C6	-	-	+	+/-	-	Y	Y	-	+	<i>Shigella</i> sp
D1-D3	+	+	-	+/-	-	R	Y	-	+/-	<i>Salmonella typhi</i>
E1-E2	-	+	+	-	-	R	Y	+	-	<i>Bacillus</i> sp
F1-F5	-	+	-	-	-	-	-	-	-	<i>Proteus vulgaris</i>
G1-G4	-	-	-	-	-	R	Y	-	-	<i>E. coli</i>

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

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 37⁰C 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 (5 mls) 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).

RESULTS AND DISCUSSION

The isolates obtained from the different samples were labeled accordingly as A1-A6, B1-B4, C1-C6, D1-D3, E1-E2, F1- F5 and G1-G4. Results of gram staining and the cultural and morphological characteristics of isolates revealed that isolates A1-A6 were *S. aureus*, isolates B1-B4 were *Klebsiella* sp., C1-C6 were *E. coli*, D1-D3 were *Salmonella* sp. , E1- E2 were *Proteus* sp., F1-F5 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 FUTY restaurants but in different levels. The presence of these isolates from crockery and cutleries could create health hazard when they are ingested, or they come in contact with the human skin. This exposed clients (students) eating in these houses to the risk of food borne infection. Similar sources of food-borne 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 and cutlery 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 equipment. It survives for longer period in water than the coliform. The need for urgent

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

Equipments	A	B	C	D	E	F	G
Plates	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
Knives	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
Cups	1.1×10^5	1.0×10^4	2.5×10^5	3.0×10^5	1.0×10^5	4.1×10^4	1.1×10^4
Washing pots	1.5×10^4	1.4×10^5	3.0×10^5	1.0×10^5	4.7×10^4	4.5×10^4	1.5×10^4

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

improvement in the hygienic condition of the restaurant cannot be over emphasized. Towels provide an ideal environment for bacteria to grow and harbour. Wet towels can harbour potentially harmful organisms and become breeding grounds for bacteria (www.foodsafetymatters.gov.au, 2004). The use of towels in a kitchen can cause the spread of bacteria to hands, equipment, crockery and cutlery (www.foodsafetymatters.gov.au, 2004). Harmful organisms can not only survive, but continue to grow in contaminated towels which remain damp. *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 knives, 1.2×10^4 - 2.5×10^5 for plates and 1.5×10^4 - 4.7×10^5 for spoons cfu/ml (Table 3a). The TBC values are significantly different as the items (spoons, forks, Knives, cups, plates, washings of pot) vary in each restaurant (Table 3b). The TBC values are also significantly different for the items among the restaurants (Tables 3c, d, e and f). According to Collins and Patricia (1979), standard for crockery and utensils in the U.S.A., Public Health Service requires counts of not more than 5.0×10^4 and 2.5×10^5 cfu/ml per container as fairly satisfactory and over 2.5×10^5 cfu/ml as unsatisfactory. This implies that count above 2.5×10^5 cfu/ml is a contamination. In case of restaurant no. 3, the TBC count of *E. coli* for knives was 3.3×10^5 and in case of restaurant no. 4 and no. 5, the TBC values for plate and spoon were 4.3×10^5 and 4.7×10^5 respectively. These values were higher than the recommended values. These high bacterial densities in such restaurants suggested that kitchen equipment kept in open basket or trays in the open air are prone to contamination with bacteria. Food-borne disease through

contaminated crockery and cutleries are major causes of morbidity throughout the world (WHO, 1984).

Microorganisms that contaminate these equipments damage the caterer's reputation, sometimes beyond repair and eventually ruin his 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 kitchen equipment. 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 kitchen equipment, but they may increase in numbers when treatment has been inadequate.

In conclusion the study has shown that the higher the bacterial densities were found in plates, spoons, drinking cups, forks and cutting instrument used 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. As a result of these seven (7) organisms were isolated. 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 best way to protect public health is to enhance sanitation control. It is also good for chefs and hotels waiters never to use any kitchen equipment without 'sterilizing'. Thelma

Table 3b. Biostatistics (Analysis of variance) showing the relationship of TBC values among the items in each restaurant (A, B, C, D, E, F and G).

Values	A	B	C	D	E	F	G
Count	6	6	6	6	6	6	6
Mean	37,000	95,833.33	207,666.66	225,833.33	96,166.66	70,333.33	84,666.66
Standard deviation	38,157.56	90,687.19	138,236.99	200,683.24	53,704.43	68,806.00	95,807.44
Standard error of mean	15,577.76	37,022.89	56,435.01	81,928.59	21,924.74	28,089.93	39,113.22
Minimum	10,000	10,000	14,000	22,000	20,000	11,000	11,000
Maximum	110,000	250,000	330,000	470,000	160,000	180,000	250,000
Range	100,000	240,000	316,000	448,000	140,000	169,000	239,000
Sum	222,000	575,000	1,246,000	448,000	577,000	422,000	508,000
Sum standard error	93,466.57	222,137.34	338,610.10	491,571.56	131,548.47	168,539.60	234,679.35
Total sum squares	15,494,000,000	96,225,000,000	354,300,000,000	507,373,000,000	69,909,000,000	53,352,000,000	88,906,000,000
Adjusted sum squares	7,280,000,000	41,120,833,333.33	95,547,333,333.33	201,368,833,333.33	14,420,833,333.33	23,671,333,333.33	45,895,333,333.33
Geometric mean	25,380.47	53,433.66	132,476.23	128,017.89	78,366.07	43,864.78	46,043.95
Harmonic mean	19,069.02	25,428.58	57,495.32	64,140.83	57,825.55	27,594.31	26,868.79
Mode	#N/A	10,000	300,000	#N/A	#N/A	#N/A	#N/A
Variance	1,456,000,000 Significant level	8,224,166,666.66 Significant level	19,109,466,666.66 Significant level	40,273,766,666.66 Significant level	2,884,166,666.66 Significant level	4,734,266,666.66 Significant level	9,179,066,666.66 Significant level

TBC values used for analysis of variance were transformed using log transformation.

Table 3c. Biostatistics (Analysis of variance) showing the relationship of TBC values among restaurants.

Restaurants	Sample size	Sum	Mean	Variance
A	6	222,000	37,000	15,494,000,000
B	6	575,000	95,833.33	96,225,000,000
C	6	1,246,000	207,666.67	354,300,000,000
D	6	1,355,000	225,833.33	507,373,000,000
E	6	577,000	96,166.66	69,909,000,000
F	6	422,000	70,333.33	53,352,000,000
G	6	508,000	84,666.66	88,906,000,000
Total	42		116,785.71	14,944,513,937.28

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

Table 3d. ANOVA

Source of variation	d.f	SS	MS	F
Between restaurants	6	183,420,571,428.57	30,570,095,238.09	2.4923
Within restaurants	35	429,304,500,000.00	12,265,842,857.14	
Total	41	612,725,071,428.57		

Table 3e. Comparison among restaurants.

Restaurants vs restaurants	Difference	Test statistics	Critical value	Accepted?
1 vs. 2	-58,833.33	0.92	3.759	Rejected
1 vs. 3	-170,666.66	2.66	3.759	"
1 vs. 4	-188,833.33	2.95	3.759	"
1 vs 5	-59,166.66	0.92	3.759	"
1 vs 6	-33,333.33	0.52	3.759	"
1 vs 7	-47,666.66	0.74	3.759	"
2 vs 3	-111,833.33	1.74	3.759	"
2 vs 4	-130,000	2.03	3.759	"
2 vs 5	-333.33	0.005	3.759	"
2 vs 6	25,500	0.39	3.759	"
2 vs 7	11,166.66	0.17	3.759	"
3 vs 4	-18,166.66	0.28	3.759	"
3 vs 5	111,500	1.74	3.759	"
3 vs 6	137,333.33	2.14	3.759	"
3 vs 7	123,000	1.92	3.759	"
4 vs 5	129,666.66	2.02	3.759	"
4 vs 6	155,500	2.43	3.759	"
4 vs 5	141,166.66	2.20	3.759	"
5 vs 6	25,833.33	0.40	3.759	"
5 vs 7	11,500	0.17	3.759	"
6 vs 7	-14,333.33	0.22	3.759	"

Scheffe contrasts among pairs of means.

Table 3f. Turkey HSD test for differences between means.

Restaurants vs restaurants	Difference	Test statistics	p-level	Accepted?
1 vs 2	-58,833.33	1.30	0.96	Rejected
1 vs 3	-170,666.66	3.77	0.13	rejected
1 vs 4	-188,833.33	4.17	0.07	rejected
1 vs 5	-59,166.66	1.30	0.96	rejected
1 vs 6	-33,333.33	0.73	0.99	rejected
1 vs 7	-47,666.66	1.05	0.98	rejected
2 vs 3	-111,833.33	2.47	0.58	rejected
2 vs 4	-130,000	2.87	0.41	rejected
2 vs 5	-333.33	0.007	1	rejected
2 vs 6	25,500	0.56	0.99	rejected
2 vs 7	11,166.66	0.24	1	rejected
3 vs 4	-18,166.66	0.40	1	rejected
3 vs 5	111,500	2.46	0.59	rejected
3 vs 6	137,333.33	3.03	0.34	rejected
3 vs 7	123,000	2.72	0.47	rejected
4 vs 5	129,666.66	2.86	0.41	rejected

Table 3. Continues.

4 vs 6	155,500	3.34	0.21	rejected
4 vs 5	141,166.66	3.12	0.31	rejected
5 vs 6	25,833.33	0.57	0.99	rejected
5 vs 7	11,500	0.25	1	rejected
6 vs 7	-14,333.33	0.31	1	rejected

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

and Pawsey (1992) noted that eating utensils should undergo a sterilizing rinse for at least 1 - 2 min. One of the methods used in sanitizing kitchen equipment is the use of dishwasher. The modern and advanced dishwashers start functioning by spreading a mixture of hot water and detergent to remove the dirt form the messy items. This is followed by rinsing which is obviously done by clean water. Some of the branded models are enhanced with a heating stage which efficiently dries the wet plates and utensils efficiently (Jerry, 2008).

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