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Restaurant Crockery and Cutlery: How Safe? A case study of University of Jos

*Grace O. Chris-Otubor and Christopher W. Joel
Department of Pharmaceutical Microbiology & Biotechnology,
Faculty of Pharmaceutical Sciences, University of Jos, Jos, Plateau State, Nigeria

ABSTRACT
Background: The safety of restaurant crockery and cutlery are often questionable alongside incidences of food borne diseases from eateries.
Method: Determination of bacteriological quality of crockery and cutlery of seven restaurants in the University was studied. Sixty-three (63) samples were collected, cultured in appropriate media and the Total Bacterial Count per ml (TBC/ml) of each sample was determined.
Results: TBC/ml range of 1.1×105 - 2.8×106 for plates, 1.2×105 – 2.2×107 for spoons and 1.6×105 – 8.6×107 for cups was obtained. Isolates were identified according to morphological and biochemical characteristics which revealed a profile of seven (7) different bacteria species including *Staphylococcus aureus, Proteus vulgaris, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa* and species of *Shigella* & *Klebsiella*. Antimicrobial screening revealed organisms sensitive to the antimicrobial agents (mostly penicillins) while others were not. The Multiple Antibiotic Resistance Index (MARI) calculated showed seven (7[19.44 %]) isolates with values less than 0.3(<0.3) while the remaining isolates (29[80.56 %]) (including isolates of *Escherichia coli, Klebsiella spp, Salmonella typhi, Shigella spp, Pseudomonas aeruginosa* and *Proteus vulgaris*) had values equal to 0.3 or greater than 0.3 (≥0.3) indicating that the latter (≥0.3) likely originated from an environment pre-exposed to antibiotics.
Conclusion: Such pre-exposure is a source of concern clinically when patients are infected with food borne diseases that are resistant to first-line treatment for infections. Such resistance therefore threatens the safety of individuals using crockery and cutlery. The need to regularly monitor the hygienic status of eateries generally by regulatory bodies to forestall epidemics will be highly beneficial to public health.
KEYWORDS: Crockery, cutlery, restaurants, TBC, MARI

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I. INTRODUCTION

The safety of restaurant crockery and cutlery has been a challenge and a source of concern. Garden-Robinson (2017) admitted that hazards can be introduced into food service operations in numerous ways including through equipment and cleaning supplies. Fawole and Oso (1998), presumed that food-borne diseases are sometimes acquired in hotels and restaurants through dishes, plates and other kitchen equipment. The reputation of many hotels and restaurants often rest on the quality of dishes, spoons, drinking cups and cutlery (Cracknel & Nobis 1989). Venderzant and Splittsbesser (1992) mentioned that contamination of food by specific types or specie of microorganisms is due to poor sanitation during handling and processing of food. Also food borne diseases are common in developing countries because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of education of food-handlers (WHO, 2004). Tebutt (1986) found out that 74 % of cloths used in cleaning dishes and cutting equipment surface were contaminated with one or more of the following organisms; *Escherichia coli, Staphylococcus aureus, Faecalis* and *Clostridium perfringens.* Zattola (1994) reported that microbial cells attached to equipment surface especially those that come in contact with the food, may not be easily killed by chemical sanitizers. The washing of hands, utensil and dishes is often done in buckets or bowls in such a way that disinfection is not carried out (WHO, 2002).

Microorganisms like *Pseudomonas* where found attached to stainless steel surface within twenty minutes contact while *Listeria monocylogenes* was found attached to stainless steel, glass and rubber surface within twenty minutes of contact and some microorganisms like *Staphylococcus aureus, Escherichia coli, Bacillus* spp *and Pseudomonas* spp (Zattola, 1994). Surface and equipment used in kitchen may look sparkling clean, yet bacteria may be present in large numbers (Garden-Robinson, 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 process until it is finally served. Therefore, one important task is to make sure dishes, spoons and cutlery are kept clean.

This study aimed at investigating the bacteriological content found on eating utensils in restaurants within University of Jos. The specific objectives include:

- Collecting samples from restaurants within University of Jos using rinsing method
- To determine the colony forming unit by counting the Total Bacteria Count (TBC).
- To identify the bacteria present in such samples using gram staining and other biochemical tests
- To determine whether the bacteria are sensitive to antimicrobials by using antimicrobial multi disk.

II. MATERIALS AND METHOD

Sample collection

Restaurants within the University of Jos main campus were selected for the study. Seven restaurants (tagged with letters A-G) within the school were used. The restaurants were chosen based on availability and affordable cost of food. Samples were taken randomly within the restaurants. Three samples each were collected for plates, cups, and spoons, making up to nine (9) samples per restaurant bringing the total number of samples collected to sixty-three (63). The items were sampled after the normal cleaning process was done for plates, spoons and drinking cups. Samples were collected using rinse method (Cheesbrough, 2005). Sterile distilled water was used to rinse plates, spoons and drinking cups. For each item, about 5 ml of sterile distilled water was used to rinse it and then the water collected into a sterile sample bottle. Another 5 ml of sterile distilled water was used to further rinse the item and then transferred to the initial one in the sample bottle making up to 10 ml per sample. The sample bottle was thereafter transferred into a cooler with ice packs and transported to the laboratory within 30 minutes for analysis. The same procedure was repeated for all samples taken from plates, cups or spoons.

Preparation and inoculation of sample

The original sample was diluted serially to 10^{-5} using the method described by (Cheesbrough, 2005). From each dilution (10^{-1} – 10^{-5}), 1 ml was introduced into different agar media- nutrient agar, chocolate agar, blood agar and MacConkey agar. Sterile glass spreader was used to aseptically spread the suspension on the surface of the agar medium. The inoculated plates were incubated at 37 ^{o}C for 24 hours and the total bacterial count was expressed in colony forming units/ml (cfu/ml). Distinct colonies were isolated and re-inoculated into agar slants and kept at 4 ^{o}C for identification. The isolates were labeled accordingly.

Identification of isolates

i. Gram Staining: The method of Cheesbrough (2005) was utilized.

ii. Biochemical tests: The methods of Cheesbrough (2005) were utilized for the following tests Citrate utilization, Catalase, Coagulase, Indole, Kingler iron and Motility test.

Sensitivity test: The method of Jorgen *et al., 1999* was utilized.

III. RESULTS

A. Total Bacterial Count (TBC)

TBC results were obtained from restaurants A to G and the ranges from least to the highest are shown in Table 1 below. Restaurant F had cups with TBC of $2.6 \times 10^7 – 8.6 \times 10^7$ cfu/ml representing the highest TBC value while the plates in the same restaurant with $1.1 \times 10^5 – 2.1 \times 10^5$ cfu/ml had the lowest. The restaurant with the lowest TBC for spoons was A with $3.1 \times 10^5 – 3.2 \times 10^5$cfu/ml.

Table 1: Total bacteria count (TBC) in cfu/ml on nutrient agar plates

Sample	A	B	C	D	E	F	G
Plates	2.3×10^5– 4.6×10^5	3.6×10^5– 5.1×10^5	1.1×10^6– 2.6×10^7	1.4×10^5– 1.0×10^7	7.2×10^6 – 2.8×10^6	1.1×10^5– 2.1×10^5	3.0×10^5– 2.5×10^6
Spoons	3.1×10^5– 3.2×10^5	4.2×10^5– 4.5×10^7	1.4×10^7– 2.2×10^7	1.2×10^5– 1.3×10^5	1.2×10^7– 6.0×10^6	4.0×10^6– 8.0×10^6	3.1×10^6 – 8.3×10^6
Cups	2.6×10^5– 2.8×10^5	1.6×10^5– 4.2×10^5	5.0×10^5– 1.8×10^6	2.1×10^6– 5.60×10^7	5.1×10^6– 6.0×10^7	2.6×10^7 – 8.6×10^7	3.7×10^6 – 9.0×10^6

Key: **A, B, C, D, E, F** and **G** indicate the various restaurants; **cfu/ml**- colony forming units per ml

B. TBC based on different media

The TBC results obtained from the different media used are as described in Table 2 below. The results show that cups have the highest level of bacterial contamination having a range of $2.1 \times 10^6 – 5.6 \times 10^7$ in the

chocolate agar while plates generally recorded the least growth $2.3 \times 10^5 - 4.6 \times 10^5$ as observed in the Blood agar.

Table 2: TBC in cfu/ml based on different media results

Sample	Blood Agar	Chocolate Agar	MacConkey Agar	Nurtrient Agar
Cups	$2.6 \times 10^5 - 2.8 \times 10^6$	$2.1 \times 10^6 - 5.6 \times 10^7$	$2.1 \times 10^6 - 4.5 \times 10^7$	$3.7 \times 10^6 - 8.6 \times 10^7$
Spoons	$4.0 \times 10^6 - 8.0 \times 10^6$	$2.6 \times 10^5 - 2.8 \times 10^6$	$1.2 \times 10^6 - 6.0 \times 10^7$	$3.1 \times 10^6 - 2.2 \times 10^7$
Plates	$2.3 \times 10^5 - 4.6 \times 10^5$	$1.6 \times 10^5 - 1.0 \times 10^7$	$7.2 \times 10^6 - 2.8 \times 10^6$	$3.6 \times 10^5 - 2.6 \times 10^7$

C. Gram staining

Table 3 shows the observations and inferences made from the gram staining procedure carried out on the various isolates from cups, plates and spoons. Both gram positive and gram negative organisms were suspected to be present

Table 3: Gram staining results

Sample	Observation	Inference
A. Spoon	Purple group of cocci	Gram positve organism, *Staphylococcus aureus* suspected
A. plate	Purple group of cocci	Gram positive organism, *Staphylococcus aureus* suspected
A. Plates	Purple cocci	Gram positive organism, *Staphylococcus* spp suspected
B. Plates	Red short rods	Gram negative organism, *E. coli* suspected
B. Spoon	Red long rods	Gram negative suspected
B. Cups	Purple group of cocci	Gram positive organism, *Staphylococcus aureus* suspected
C. Cups	Purple group of cocci	Gram positive organism, *Staphylococcus* spp suspected
C. Plate	Pink short rods	Gram negative organism, *Shigella* spp suspected
C. Spoons	Pink short rods	Gram negative organism, *Shigella* spp suspected
C. Plate	Purple cocci	Gram positive organism, *Staphylococcus* spp suspected
D. Cups	Red rods	Gram negative organism, *Klebsiella* spp suspected
E. Plates	Red long rods, dispersed	Gram negative organism, *Salmonella* spp suspected
E. Spoon	Pink short rods	Gram negative organism, *Escherichia coli* suspected
E. Cups	Red long rod, dispersed	Gram negative organism, *Salmonella* spp suspected
F. Cups	Pink rods	Gram negative organism suspected
F. Spoons	Purple cocci	Gram positive organism suspected
F. Plates	Pink long rods	Gram negative organism, *Salmonella* spp suspected
G. Plates	Pink long rods	Gram negative organism, *Pseudomonas* spp suspected
G. Cups	Scattered red rods	Gram negative organism, *Proteu*s organism suspected
G. Spoons	Purple cocci	Gram positive organism suspected

Key: A, B, C, D, E, F, G = Various restaurant

D. Biochemical tests

Biochemical tests employed for confirmation of the suspected organisms revealed to presence of certain bacteria in samples gotten from various restaurants.

Table 4: Biochemical test results

Isolates	CU	M	I	C	CO	KINGLER IRON TEST				Confirmed Organisms
						S	B	H	G	
$A_1 - A_4$	–	–	+	–	–	Y	Y	–	+	*Escherichia coli*
$B_1 - B_5$	+	+	–	+	–	R	R	–	–	*Pseudomonas aeruginosa*
$C_1 - C_{13}$	–	–	–	±	–	R	Y	–	–	*Shigella spp*
$D_1 - D_5$	±	–	–	–	–	Y	Y	–	–	*Klebsiella spp*
$E_1 - E_5$	–	–	–	+	+	R	Y	–	–	*Staphylococcus aureus*
$F_1 - F_5$	–	+	–	–	–	R	Y	–	–	*Salmonella typhi*
$G_1 - G_4$	±	+	+	–	–	R	Y	+	–	*Proteus vulgaris*

Key: $A_1 - A_4$; $B_1 - B_4$; $C_1 - C_4$ etc. represents isolates with similar reactions grouped together

+ means Positive test result;
- means Negative test result;
Y means yellow;
R means red
± means some isolates are positive while others are negative
Abbrevations for biochemical tests: CU- Citrate utilization test; M- Motility test; I- Indole test; C- Catalase test; CO- coagulase test;
Reactions at various sites of Kingler iron test: S- Slope; B- Butt; H- Hydrogen Sulphide (H_2S); G- Gas

E. Bacterial Sensitivity Test

i. Gram-Positive result: The only gram positive organism isolated from the research was *Staphylococcus aureus* which showed resistance to amoxicillin, ciprofloxacin and erythromycin while it was susceptible to all other antimicrobial drugs used as shown below.

Table 5: Sensitivity test results for *Staphylococcus aureus* - Gram positive bacteria

Antimicrobial agent	Isolate E_1	Isolate E_2
Erythromycin	+	-
Amoxycillin	-	+
Ofloxacin	+	+
Streptomycin	+	+
Chloramphenicol	+	+
Ceftriaxone	+	+
Gentamycin	+	+
Pefloxacin	+	+
Cotrimoxazole	+	+
Ciprofloxacin	-	+

Key: - = resistant; + = sensitive

ii. Gram negative test result

Some gram negative bacteria were identified, out of which some showed greater resistance to tetracycline and amoxicillin while others were susceptible to gentamycin, perfloxacin, ciprofloxacin and ofloxacin**.**

Table 6: Gram negative bacteria sensitivity test results

Organism	I	A	P	T	C	A2	O	C2	G	N	C3
E. Coli	A_1	_	+	+	+	_	+	_	+	_	_
	A_2	_	+	_	+	_	+	_	+	+	+
	A_3	_	+	_	+	_	+	_	+	+	+
	A_4	_	+	_	+	_	+	+	+	+	_
Klebsiella spp	D_1	_	+	_	+	_	+	_	+	_	+
	D_2	_	+	_	+	_	+	_	+	_	_
	D_3	_	+	_	+	_	+	_	+	+	_
	D_4	_	+	_	+	_	+	_	+	_	+
	C_1	_	+	_	+	_	+	_	+	_	+
	C_2	_	+	+	+	_	+	+	+	+	+
	C_3	_	+	_	+	_	+	_	+	+	_
	C_4	_	+	_	+	_	+	_	+	_	+
	C_5	_	+	_	+	_	+	_	+	_	+
	C_6	_	+	_	+	_	+	+	+	_	_
	C_7	_	+	_	+	_	+	_	+	_	+

	C_8	_	+	_	+	_	+	_	+	_	_
	C_9	_	+	_	+	_	+	_	+	_	_
	C_{10}	_	+	_	+	_	+	_	_	_	_
Shigella spp	C_{11}	_	+	_	+	_	+	+	+	+	_
	C_{12}	+	+	_	+	_	+	+	+	+	+
	B_1	_	+	+	+	+	+	+	+	+	_
	B_2	_	+	+	+	+	+	+	+	+	_
Pseudomonas	B_3	_	+	_	+	_	+	+	+	+	+
aeruginosa	B_4	_	+	_	+	_	+	+	+	+	+
	F_1	_	+	_	+	_	+	_	+	_	+
	F_2	_	+	_	+	_	+	_	+	_	_
	F_3	_	+	_	+	_	+	_	+	_	_
	F_4	_	+	_	+	_	+	_	+	_	+
Salmonella typhyi	F_5	_	+	_	+	_	+	+	+	+	_
	G_1	_	+	_	+	_	+	+	+	+	+
	G_2	_	+	_	+	_	+	_	+	_	_
	G_3	_	+	_	+	_	+	+	+	+	+
	G_4	_	+	_	+	_	+	+	+	+	+
Proteus vulgaris	G_5	+	+	_	+	+	+	+	+	+	_

Key + = Sensitive; - = Resistant; I- Isolates

Antimicrobial agent

A = Augmentin; P = Pefloxacin; T = Tetracycline; C = Ciprofloxacin;
A_2 = Amoxycillin; O = Ofloxacin; C_2 = Cotrimoxazole;
G = Gentamycin; N = Nitrofurantoin; C_3 = Ceftriaxone

TABLE 7: Multiple antibiotic resistance index (MARI)

Organism	Isolate number	MARI
Staphylococcus aureus	E_1	0.2
	E_2	0.1
Escherichia coli	A_1	0.5
	A_2, A_3, A_4	0.4
Klebsiella spp	D_1, D_3 ,D_4	0.5
	D_2	0.6
Shigella spp	C_1, C_3 ,C_4 ,C_5 ,C_6 ,C_7	0.5
	C_8, C_9	0.6
	C_{11}	0.3
	C_{12}	0.2
Salmonella typhi	B_1, B_2	0.2
	B_3 , B_4	0.3
Pseudomonas aeruginosa	F_1, F_4	0.5
	F_2, F_3	0.6
	F_5	0.4
Proteus vulgaris	G_1, G_3, G_4	0.3
	G_2	0.6
	G_5	0.2

Key: Letters with subscripts: Different Isolates

IV. DISCUSSION

From the study and results obtained, the total bacteria count (TBC), cfu/ml were in a range of 1.1×10^5 – 2.6×10^7 for plates (Table 1). These values were significantly different from one restaurant to another. This could be due to different levels of hygiene. According to Collins and Lyne (1979), standard for crockery and utensils in U.S.A, Public Health Service requires counts of not more than 5.0×10^4 and 2.5×10^5 cfu/ml as fairly satisfactory and over 2.5×10^5 cfu/ml as unsatisfactory (Orogu, Ehiwario & Adebisi, 2017) . This implies that all restaurants were contaminated except restaurant D (spoons) and F (plates). This could be due to personal hygiene and site of the restaurants (close to refuse dumping site and where some people defecate openly).

The study identified the presence of different bacteria such as gram positive and gram negative organisms. The positive organism observed was *Staphylococcus aureus* while the gram negative organisms were *Escherichia coli, Salmonella typhi, Shigella spp, Klebsiella spp, Proteus vulgaris* and *Pseudomonas aeruginosa.* This study's findings is similar to that of (Orogu, Ehiwario & Adebisi, (2017) who also identified *Escherichia coli and Staphylococcus aureus* in the samples they worked on. The presence of *Escherichia coli* in cooking utensils is an indication of recent feacal contamination. Restaurant D (Cups) had high feacal contamination since it contained a higher range of *E.coli.* The presence of *Salmonella typhi* is an indication that one can contact food borne disease like typhoid fever. This organism is often associated with unhygienic environments especially poor sources of water. *Pseudomonas aeruginosa* might have been obtained from the soil. The presence of *Staphylococcus aureus* is an indication of contamination due to poor personnel hygiene because *Staphylococcus aureus* can survive on varied parts of the body. The presence of these organisms have also proven that food borne disease can be acquired in eateries through cutleries, plates and other kitchen equipment as reported by Fawole and Oso, 1988. Zattola (1994) who also reported that bacteria like *Pseudomonas* were found in cooking utensils. This study has confirmed the presence of pathogenic organisms capable of causing food borne diseases to the human populace.

Based on sensitivity to antimicrobial, some bacteria were sensitive while others were not, that is they were resistant to antimicrobial agents used. Most organisms like *E. coli, Klebsiella, Shigella, Pseudomonas aeruginosa, S. typhi* were resistant to the penicillins (augmentin and amoxicillin) . But other species were sensitive to them, example *Proteus vulgaris.* The resistance could be due enzymatic hydrolysis of the β-Lactam bond by β-Lactamases, alteration in binding site and alteration in the outer membrane. Ceftriaxone and oflaxacin showed high activity against both Gram positive and Gram negative organism. This is due to the ability of the drug to bind to one or more of the penicillin binding protein on bacteria which inhibit transpeptidation step of peptidoglycan synthesis of bacteria cell wall thereby leading to death of the organism. Streptomycin and gentamycin displayed activity against many of the Gram negative and Gram positive organisms. The sensitivity shown by the organisms maybe connected to the binding of the agents to the organism in order to inhibit peptidoglycan component of the cell wall since the drugs are known to be able to bind to protein 30S and 50S ribosomal subunits to disrupt synthesis of the bacteria. Only one isolate from S*higella spp* was resistant and this could be due to target modification by the bacteria or mutation. For ciprofloxacin and perfloxacin, most of the bacteria were also sensitive to them because they donot target cell wall, they inhibit DNA gyrase of the bacteria leading to death. *E coli , Klebsiella spp, Pseudomonas aeruginosa , Salmonella typhi and Proteus vulgaris* were resistant to tetracycline. This is due to decrease plasmid infux transport and also transposon leading to resistance. Some isolate were fairly sensitive to erythromycin, they were not fully sensitive due to resistance by plasmid mediated methylation of the RNA adenosine of the ribosome and plasmid mediated inactive erythromycin by an esterase. Other organisms like the Gram positive organisms were sensitive to choramphenicol because it binds to protein of the bacteria leading to death. Similar work was done based on contamination of water, food, and environment, *E coli, Klebsiella, Proteus, Shigella, Salmonella, Enterobacter, Citrobacter, Pseudomonas* species were isolated and their antimicrobial sensitivity tested. Generally most bacteria isolates except Salmonella and *Shigella* species were found to be resistant (Shubra *et al*., 2014).

Multiple Antibiotic Resistance Index (MARI) as found in Table 7 was determined using the formular below:

MARI = a/b

where 'a' represents the number of antibiotics to which the test isolate depicted resistance and

'b' represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility (Sandhu, Dahiya, & Sayal, 2016).

The results showed that seven (7[19.44 %]) isolates; C_2,C_{12},B_1,B_2,G_5,E_1 and E_2 were the only isolates with MARI values less than 0.3 while the rest (29[80.56 %]) were either greater than 0.3 or equal to 0.3. The isolates with MARI values greater than or equal to 0.3 indicates that the organisms probably originated from an environment where antibiotics are often used. This is an indication of pre-exposure of the isolates to antibiotics.

V. CONCLUSION

Restaurants within University of Jos main campus have been studied and it has been observed that there was some level of contamination of restaurants, both Gram negative and Gram positive bacteria, the level of contamination was high in some restaurants capable of causing food borne diseases. When antibiotics were used on the bacteria, some were sensitive while others were resistant to the antibiotics, which indicates pre-exposure of bacteria to antibiotic in the environment.

VI. CONFLICT OF INTERESTS

The authors declared no conflict of interest.

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