

Microbiological assessment of cutleries

Abstract

Microbiological assessment of cutleries was carried out with a view to ascertain the microbiological qualities (bacteria and fungi contamination) of cutleries—spoons, forks and knives. A total of 15 samples were collected from 3 hostels around Delta State Polytechnic, Ozoro. The samples were cultured and sub-cultured in appropriate media and the bacterial isolates were identified according to their morphological and biochemical characteristics. Two (2) different bacterial species were revealed and they are: *Staphylococcus aureus* and *Escherichia coli*. The total bacterial count (TBC) per ml of the samples ranges from 1.8×10^3 to 7.7×10^3 cfu/ml. *E. coli* has the highest result occurrence of 66.67% while *S. aureus* has the least occurrence of 33.33%. The result also revealed a profile of three fungi: *Aspergillus niger*, *Candida albicans* and *Penicillium spp* respectively based on their characteristics. *Candida albicans* a fungi isolate has the least occurrence of 20% while *Aspergillus niger* and *Penicillium spp* has the highest occurrence of 80%. In order to prevent food-borne infection, food inspectors should monitor the hygienic status of the hostels within and outside the school campus.

Keywords: microbiological, assessment, cutleries, samples

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Abbreviations: TBC, total bacterial count; TST, triple sugar iron agar test; WHO, world health organization

Introduction

Microbial contamination of food is a major public health problem since it affects millions of people worldwide. Unproved hygienic practices within the food processing environment can cause the contamination with pathogenic particles, turning food into a risk for the safety of the consumers. For the assessment of the hygienic condition related to food handling is essential to know the sanitary conditions of the establishment since that can reduce the risks of food contamination. That food contamination can cause severe problem to the consumer's health. Microbial attachment and biofilm formation to solid surface of crockery and cutlery provide some protection of the cell against physical removal of the cell by washing and cleaning crockery.¹ These cells seem to have greater resistance against sanitizers and heat, thus spoilage and pathogenic microorganisms attached to food surface. Zattola² 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 carried out.³ It also has been reported that several species and strains of *Pseudomonas* were found to attach to stainless steel surface within 30 minutes of contact. *Listeria monocytogenes* was found to attach to stainless steel surface, glass and rubber surface within 20 minutes of contact and some of the microorganisms found are *Staphylococcus species*, *Escherichia coli*, *Bacillus species* and *Pseudomonas species*.²

Surface and equipment used in kitchen may look sparkling clean, yet bacteria may be present in large numbers.⁴ 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 spoons and cutlery are kept clean.⁵ Microbial assessment of cutleries can be initiated for a variety of

reasons, including to identify microorganisms isolated from cutleries and to assess the potential for human risk associated with exposure to a known pathogen in cutleries.

Materials and methods

Study area

This study was conducted with 15 samples of cutleries in Ozoro, Delta State. These samples were collected from Hostels in various areas in the town. This town (Ozoro) is located in Delta State South-South Nigeria. Five samples each were collected from three (3) different hostels in Ozoro and were labelled appropriately. These three hostels were popular among students in terms of proximity to school.

Sample collection

The sample were displayed on the laboratory, desk. A swab stick was immersed into normal saline and was used to take a swab of the displayed samples (Cutleries). Normal saline was poured into the swab stick tube to the point where the wool is and it was properly covered and allowed to distribute its particles into the tube containing normal saline. Each sample was taken to the lab before taking swab of them for assessments.

Isolation and enumeration of microorganisms

The solution containing the normal saline and the collected swab was inoculated into each already prepared agar medium (Nutrient, MacConkey and SDA receptively). The swab stick was used to streak each Petri dish containing solidities agar medium. The inoculated plate was incubated at 37°C for 24hrs. Distinct colonies were isolated and re-inoculated unto appropriate agar medium (Nutrient Agar). The sub-cultured plate was incubated for another 24hrs at 40°C for the purpose of identification.

Identification of isolates

Gram staining: A Colony from the purified subculture was isolated and emulsified in sterile distilled water and a thin preparation was made on the slide. It was evenly spread to cover an approximately

area of about 15–20mm in diameter on the slide. The smear made was left to dry being protected from dust and sunlight. The smear was fixed using gentle heat by rapidly passing the slide with the smear uppermost three (3) times through the flue of a Bunsen burner. The slide was checked with the back of the hand just to make sure too much heat was not used which can affect or even kill the microorganisms. The smear was allowed to cool before staining was done.

The microscopic slide containing the smear was placed on the staining rack and covered with crystal violet and allowed for 30–60sec. wash off with distill water slowly and gently. A drop of Lugol's iodine was added and allowed for 30–60sec as well; then wash off slowly and gently with distill water. The water was then tipped off. A drop of Acetone (Alcohol) was added off immediately. Again, a drop of Neutral red was added and allowed for 2mins then washed off with distill water. The back of the slide was wiped clean and placed on a draining rack for the smear to air-dry, using a microscope. The smear was examined with 40x objective lens to check the staining pattern (Checked without oil immersion). Oil immersion was added and viewed fewer than 100x objectives lens to observe the shape colour and other characteristics.

Biochemical tests

Citrate utilization test: For each bacterial isolate, 10ml of citrate medium was dispensed into each of four test tubes and sterilized by the use of gas cylinder and pressure pot for 30mins. The organism was then inoculated into citrate medium and incubated at 57°C for 24–48hrs. A change in colour from green to blue indicated containing only the citrate medium served as a control.⁶

Catalase test: A drop of 3% hydrogen peroxide was placed on a glass slide. A bit of growth of each isolated 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 frothing.⁶

Oxidase test: A piece of filter paper was placed on a clean sterile Petri dish and 3 drops of oxidized reagent was added. The bacteria isolated were sheared on the filter paper by means of sterilize rod. Organism indicates positive when it retains the purple colouration within five to ten seconds of the analysis.

Indole test: Bacteria isolates were inoculated into peptone water medium contained in sterilized test tubes then incubated at 37°C for 24hrs to give optimum accumulation of Indole. After the incubation period, about 0.5ml of Kovac's reagent was added to 5ml of peptone water culture. The bottles were shaken thoroughly and allowed to stand and observed for colour development. A red colouration in the uppermost layer of the tube indicated a positive result. And if the isolate is negative, the reagent layer will remain yellow or slightly cloudy.⁶

Triple sugar iron agar test (TST): Bacterial Isolate were stabbed into TSI slant media and also streaked on the surface of the slant after which the medium was incubated at optimal temperature of 37°C for 24hrs. The TST slant medium was used to check for the presence of the following.

- a. **Gas:** If bubble is present in the media (gas positive).
- b. **H₂S:** If black is present in the media (H₂S positive).
- c. **Lactose:** If the top of the media turns from pink to yellow (Lactose positive).
- d. **Glucose:** If the bottom of the media turns from pink to yellow (glucose positive).

Motility test: A single Colony of each of the organism was inoculated into labelled test tube containing peptone water (5ml) and the tube incubated at 37°C over-night. A drop of the well-mixed organisms is peptone water incubated over-night, was placed on a cover slip and the edges surrounded with oil immersion. A microscopic slide was then placed over the cover slip taking care that the slide does not touch the drop on the cover slip but suspended by the oil immersion. The slide was then observed under the microscope for motile bacteria under x100 objective.⁶

Result and discussion

Results

The bacteria isolated from the cutleries were *Escherichia coli* and *Staphylococcus aureus* (Table 1) while the fungi isolated from the cutleries were *Aspergillus niger*, *Candida albican* and *Penicillium spp.* (Table 2). Table 1 shows bacterial isolates that have ability to utilized sugar as their substrate. Table 3 shows the heterotrophic plate count for each plate. *Escherichia coli* have the highest occurrence while *Staphylococcus aureus* has the least occurrence. Table 4 shows the number of bacterial isolates and their percentage of occurrence. Table 2 shows the characteristics of fungi isolates. Table 5 shows the occurrence of fungi isolates and their percentage of occurrence.

Discussion

Result of the gram staining, the cultural and morphological characteristics of isolates revealed that *Staphylococcus aureus* and *Escherichia coli* are the bacterial species present in the samples assessed (Table 1). The biochemical test results were expressed in Table 1. From the table, it shows that the samples had two bacterial species—*S. aureus* and *E. coli* (Table 1). The presence of these isolates from cutleries could create health hazard when they are ingested or when they come in contact with the human skin. This exposed students eating in these hostels to the risk of food-borne infection. Towel provides an ideal environment for bacteria to grow and labour. Wet towels can harbour potentially harmful organisms and become breeding grounds for bacteria.³ The use of towels in kitchen can cause the spread of bacteria to hands, equipment, cookery, and cutlery.³

Harmful organism can not only survive, but continue to grow in contaminated towels which remain damp. The number of food-borne disease outbreaks due to bacteria has increased in recent years. Several potential causes of these outbreaks include storage temperature, inadequate thermal treatment, cross contamination, poor hygiene conditions of processing facilities and contaminated food contact surfaces.^{7,8} The total bacterial count (TBC) cfu/ml of the samples were in the range of 1.8×10^3 – 6.1×10^3 for spoons, 2.0×10^3 – 5.4×10^3 for knives and 4.0×10^3 – 7.7×10^3 cfu/ml for fork (Table 2). The TBC values are significantly different as the items vary in each hostel. According to Collins and Lyne (1979), standard for cookery and utensils in the USA, 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.⁹ In case of the spoons, knives and fork of flourish, penile and peace lodge, the TBC range of the bacterial species are below the recommended values (Table 2). These low bacterial densities in such hostels suggested that cutleries were kept in closed baskets or trays that are not openly prone to contamination with bacteria in the open air.

E. coli has the highest occurrence of 66.67% (Table 4) in the three hostels. 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,¹⁰ *S. aureus* has the least occurrence of 33.33% (Table 4) in only two hostels. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as food poisoning and sinusitis.¹¹

The fungi isolates were *Aspergillus niger* with a percentage occurrence of 40%, *Candida albican* with 20% occurrence and

Penicillin spp with 40% occurrence (Tables 2 & 5). The presence of these fungi isolates from cutleries also creates health hazard to the human body. It is always safer and easier to prevent the contamination of these cutleries. It is more difficult to make the cutleries safe again. Infection by food poisoning organisms is a threat requiring constant vigilance unless cutleries that come 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 (kitchen equipment), but they may increase in numbers when treatment has been inadequate.

Table 1 Cultural, morphological and biochemical characteristics of bacteria isolates

Morphology	Mobility	Catalase	Oxidase	Citruse	Glucose	Lactose	H ₂ S	Indoles	Organisms
Gram -ve rod	+	-	+	-	+	-	+	+	<i>E. coli</i>
Gram +ve cocci	+	-	-	+	+	+	+	+	<i>S. aureus</i>
Gram +ve cocci	+	-	-	+	+	+	+	+	<i>S. aureus</i>
Gram -ve rod	-	-	+	-	+	+	+	+	<i>E. coli</i>
Gram -ve rod	+	-	+	-	+	-	+	+	<i>E. coli</i>
Gram -ve rod	+	-	+	-	+	-	+	+	<i>E. coli</i>

Key: +, Indicate positive reaction; -, Indicate negative reaction

Table 2 Characteristics of fungi isolates

Organisms	<i>Aspergillus niger</i>	<i>Candida albican</i>	<i>Penicillin spp</i>
Cultural Characteristics	Black, Wooly with Profuse Growth	Creamy without Profuse Growth	White, Wooly with Profuse Growth
Colour of Isolate	Dark	Creamy	White
Hyphae	Septate	No Septate	Septate
Conidiospore	Septate Upright	Non-septate Upright	Septate Upright
Conidia	Present, One Cell Globase in Dry Basipetal	Absent	Absent
Stolon	Present	Absent	Absent
Rhizoid	Absent	Absent	Absent
Spore Colour	Dark	Small Gossy Consent and Smooth	White
Spore Attachment	Bear Phiatides at the Apex with Conidia at the Top	Yeast Pseudolyphae	Bear Phiatides at the apex with Conidia at the Top

Table 3 Heterotrophic plate count

Samples	Bacteria count
Flourish 1	1.8x10 ³
Flourish 2	1.9x10 ³
Flourish 3	1.8x10 ³
Flourish 4	2.0x10 ³
Flourish 5	1.9x10 ³
Penile 1	2.0x10 ³
Penile 2	3.4x10 ³
Penile 3	5.4x10 ³
Penile 4	3.9x10 ³
Penile 5	2.6x10 ³
Peace 1	4.0x10 ³
Peace 2	5.8x10 ³
Peace 3	4.0x10 ³
Peace 4	7.7x10 ³
Peace 5	4.9x10 ³

Table 4 Number of bacterial isolates and % of occurrence

Isolate	% of occurrence
<i>Escherichia coli</i>	66.67
<i>Staphylococcus aureus</i>	33.33
Total	100

Table 5 Occurrence of fungi isolates and % of occurrence

Isolate	% of Occurrence
<i>Aspergillus niger</i>	40
<i>Candida albican</i>	20
<i>Penicillin spp</i>	40
Total	100

Conclusion

The study has shown that the ranges of the bacterial densities found in spoons, knives and forks used in Flourish, Penile and Peace lodge were low compared to the standard set by the U.S.A Public Health Service.⁹ These low bacterial densities in such hostels suggested that the sources that were inadequately removed during routine cleaning. Again, sources of contamination included contaminated hands and towels in the kitchen. The study has also shown that the percentages of occurrence of the fungi species found in spoons, knives and forks used in the three hostels made the cutleries contaminated and very unsafe for use. This is because if the fungi are parasitic, they will live in or on the tissues of their hostels (infected hostellers) and drain the nutrients of the host.

Recommendations

The following should be taken into consideration to help stop microbial contamination of cutlery use in homes, hostels, or restaurants:

- a. The best way to protect public health is to enhance sanitation control.
- b. It is also good for chiefs, hostellers, and hotel waiters never to use any cutlery (Kitchen equipment) without 'sterilization'. Utensils should undergo a sterilizing rinse for at least 1–2 mins. One of the methods used in sanitizing kitchen equipment is the dishwasher. The modern and advance dishwashers start functioning by spreading a mixture of hot water and detergent to remove the dirt from 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.

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None.

Conflict of interest

The author declares no conflict of interest.

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