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Microbiological Assessment of the Palms of Food Handlers in Restaurants at a University Setting (*Bukateria*), Nigeria

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Abstract

The study was carried out to investigate the level of hygiene of food handlers in the bukateria at the Commercial Centre in a University setting. Palms of food handlers from six different Bukateria were swabbed and a total number of 13 bacteria were isolated. Nine of which are coliforms and 11 fungal isolates were also obtained. The Colony Forming Unit for each sample A, B, C, D, E and F was 14, 17, 15, 13, 16, and 10 X 10^6 respectively. *Penicillium spp* occurred on three plates from three restaurants, *Aspergillus spp* occurred on five plates and five restaurants, *Candida spp* occurred on two plates from two restaurants, and *Rhizopus spp* which occurred on one plate from one restaurant. The bacteria count ranged from 12 x 10^6 to 18 x 10^6 while enteric count ranged from 4 x 10^6 to 9 x 10^6. *Escherichia spp* had the highest frequency with occurrence on eight on plates from five restaurants, *Bacillus spp* occurred on three plates and three restaurants, *Pseudomonas spp* occurred on two plates and in two restaurants, *Salmonella spp* occurred on two plates and from two restaurants, *Staphylococcus spp* occurred on three plates and two restaurants, *Streptococcus spp* occurred on two plates and two restaurants, *Proteus spp* occurred on one plates and one restaurant, *Klebsiella spp* occurred on one plate and one restaurant. The occurrence of the fungus, Candida and enteric organisms in all the six restaurants was an indication that the level of hygiene of the food handlers was poor and may be dangerous to the customers who are mostly students of the University. In view of the possible of outbreak of diseases due to hand to food contamination, it was recommended that a more strict supervision of the food handlers is needed in order to enforce the Health Regulations of the University to ensure compliance and forestall an outbreak.

Keywords: Food handlers, restaurants, *Penicillium spp*, *Streptococcus spp*, palms, food hygiene

INTRODUCTION

World Health Organization (WHO, 2003) defines food as any substance whether processed, semi-processed or raw, juice, fruit, and other materials considered safe which is intended for human consumption which provides the body with needed nourishment. Food is a basic human need in the society. Good and adequate nutrition promote good health thereby enhancing the social and psychological well-being of the people.

Food procurement among individuals and families in various communities entails many difficulties. Thus, in an effort to meet the body requirements, people often resort to buying cheap raw and cooked foods that may pose health hazards to them. Isara and Isah, (2009) opined that there is the need to consider certain qualities in the procurement of food people eat. They explained that such considerations should include the selling of food, its freshness, nature of its proportions, the environment under which it is prepared and the personal cleanliness of the providers. In other words, hygiene is an important concept to be given proper consideration in the process of food procurement, preparation and consumption by the individuals to ensure healthy living. This is to safeguard against food poisoning that may endanger the life of the people. Food poisoning is usually caused by organisms such as *Clostridium botulinum, Campylobacter cholerae, Staphylococcus aureus, Salmonella typhi* and *Shigella dysentriae* among others.

Bacteria may get into the food through different ways such as water that may contain animal or human waste, improper food handling or preparation, meat or poultry may come into contact with intestinal bacteria when being processed. Food poisoning also occurs from eating or drinking any prepared food item using unclean cooking utensils and other tools. Isara and Isah, (2009) noted that the food itself may contain food poisoning bacteria when it is brought into the kitchen or bacteria may enter the food due to faulty

handling during preparation. Therefore, the sources of food poisoning include the food handlers, the environment where the food is stored and the food itself. However, personnel involved in food preparation such as food vendors have been associated with the transmission of some food poisoning bacteria such as *Staphylococcus aureus, Salmonella,* and *Cholerae* etc. It is necessary that food vendors have the basic knowledge of food hygiene to prevent food poisoning in the community. Food handler are likely to be carriers of food pathogens and personnel suspected to be carriers of such should not be allowed to access food production areas (Okojie *et al.,* 2005).

The Center for Disease Control (CDC) (2018) noted that the principal sources of contamination by *Staphylococci* are the human mouth, throat, nose and nasal passages. The onset of illness is rapid because the toxin is present in the food before it is eaten. This is why it is important that food vendors should protect the foods they sell from coughs and sneezes. Food vendors have to use handkerchiefs and take their faces away. Illness resulting from the consumption of contaminated food has become one of the most widespread public health problems in contemporary society (Oranusi and Braide, 2012). Against this backdrop is the need to investigate the level of contamination of food handlers especially their palms. The aim of the study is to determine the microbiological quality of palms of food handlers in Achievers University bukateria, Owo.

MATERIALS AND METHODS

Sample collection

A total of six (6) samples were collected by 8 O'clock in the morning from the palms food handlers of restaurants at the Achievers University commercial centre. Each handler's palms were swabbed using new sterile swab sticks and labelled alphabetically. The samples were dissolved in 5ml peptone water to keep the microbes present viable. The samples were kept in a refrigerator at 4°C until use.

Microbiological analysis

Serial dilution*:* Six (6) folds serial dilution preparation for the microbiological analysis of the swab samples carried out. Each tube was filled with 9ml of sterile distilled water. The test tubes were plugged with cotton wool and were labelled 10^{-1} to 10^{-6} for each test sample respectively. 1ml of the culture sample (supernatant) was taken and added to the first test tube (10^{-1}) containing 9ml of distilled water. The mixture was shaken to ensure all round circulation of particles. 1ml of the aliquot (10^{-1}) was taken and transferred to 10^{-2} and the process was repeated for the remaining test tubes to get 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} respectively.

Total bacterial count: The total bacterial count was done using the pour plate method as described by Cheesebrough (2000). 1ml of test tube 10^{-6} was aseptically pipetted into sterile Petri dishes in duplicates. Media depending on the organism to be isolated (i.e. Nutrient agar, Potato dextrose agar and MacConkey agar) was poured on it and the Petri dishes were swirled to homogenize. The plates were left to solidify on the flat smooth surfaced workbench and incubated in an inverted position in an incubator at 37°C for 24 hours. After 24hours of incubation, the plates were examined for growth and the colonies formed were counted using a colony counter.

Preparation of pure cultures: Under aseptic condition, colonies that formed from the first culture were then sub-cultured on similar freshly prepared enrichment medium (Nutrient Agar, MacConkey Agar and Potato Dextrose Agar) using the streaking method.

***Characterization and Identification of Isolates from pure culture*:** Isolates were identified using Morphological, cultural and biochemical tests including, motility test, catalase test, citrate test, indole test, oxidase test, methyl-red test and sugar fermentation tests and the final identification was based on the criteria of Berger's Manual of Determinative Bacteriology. Fungi, however was identified based on their shape, spore, colour and hyphae development.

RESULTS

A total number of thirteen (13) bacteria, nine (9) coliforms and eleven (11) fungi were isolated from the samples. Each restaurant sample was labelled A, B, C, D, E and F.

Total Microbial Count

Total Bacterial Count: Table 1 presents the total bacteria count for coliform and non-coliform bacteria. Sample A, B, C, D, E and F had a total bacterium count of 12, 18, 14, 12, 10, and 8 X 10^6 respectively. 2, 3, 2, 2, 2 and 2 pure cultures were obtained accordingly. The total coliform count for each sample A, B, C, D, E and F was 6, 5, 7, 9, 6, and 4 X 10^6 respectively and 1, 2, 2, 2, 1 and 1 pure cultures were obtained accordingly.

Total Fungal Count

The fungal isolates exhibited radial growth showing mycelia. The characteristics of the isolates are presented in Table 6.

Identification and Characterization of Microbial Isolates

There were 33 isolates designated according to the sample source, i.e. isolates from sample and are named A1, A2, A (n). The results of the various morphological examination and biochemical tests are represented in the Tables 2, 3 and 4 below with the probable organisms.

MORPHOLOGICAL CHARACTERISTICS								BIOCHEMICAL CHARACTERISTICS								
Colour	Shape	Edge	Colony Surface	Transparency	Gram Shape	Gram Reaction	Motility	Calalase Test	Citrate Test	Indole Test	Oxidase Test	Sucrose	Glucose	Lactose	Maltose	Methyl Red	Probable Organism
Creamy Mucoid	All Circular	All Round	Elevated	Opaque	Long Rod	-	Motile	+	-	+	-	-	+	+	+	+	Escherichia spp
Milky Red	Irregular	Round and Separated	Raised	Dull	Rods	+	Motile	+	+	-	+	+	+	+	+	-	Bacillus spp
creamy	Irregular	Round	Elevated	Opaque	Rods	-	Motile	+	+	-	+	-	-	-	-	-	Pseudomonas spp
Creamy	Round	Round	Raised	Opaque	Rods	-	Motile	+	-	-	-	-	+	-	+	+	Salmonellas spp
Creamy Mucoid	All Circular	All Round	Elevated	Opaque	Long Rod	-	Motile	+	-	+	-	-	+	+	+	+	Escherichia spp
Milky White	All Circular	Round	Elevated	Shining	Clustered Cocci	+	Non Motile	+	+	-	-	+	+	+	+	+	Staphylococcus spp
Milky Red	Irregular	Round and Separated	Raised	Dull	Rods	+	Motile	+	+	-	+	+	+	+	+	-	Bacillus spp
Creamy	All Circular	Round	Elevated	Opaque	Paired Cocci	+	Non Motile	-	-	-	-	+	+	+	+	+	Streptococcus spp
Creamy Mucoid	All Circular	All Round	Elevated	Opaque	Long Rod	-	Motile	+	-	+	-	-	+	+	+	+	Escherichia spp
Creamy	Irregular	Round	Elevated	Opaque	Rods	-	Motile	+	+	-	+	-	-	-	-	-	Pseudomonas spp
Milky Red	Irregular	Round And Separated	Raised	Dull	Rods	+	Motile	+	+	-	+	+	+	+	+	-	Bacillus spp
Creamy Mucoid	All Circular	All Round	Elevated	Opaque	Long Rod	-	Motile	+	-	+	-	-	+	+	+	+	Escherichia spp
Creamy	Round	Round	Raised	Opaque	Rods	-	Motile	+	-	-	-	-	+	-	+	+	Salmonellas spp

Identification and Characterization of the Fungal Isolates (Potato Dextrose Agar): The fungal isolates were described based on their shape, colour and hyphae type. The various probable fungi are present in Table 4 below.

Table 1: Total Bacterial Count (Coliform and non-coliform).

Samples	Non coliform (cfu/ml)	Pure cultures	Coliform	Pure cultures isolated
A	12 X 10^6	2	6 X 10^6	1
B	18 X 10^6	3	5 X 10^6	2
C	14 X 10^6	2	7 X 10^6	2
D	12 X 10^6	2	9 X 10^6	2
E	10 X 10^6	2	6 X 10^6	1
F	8 X 10^6	2	4 X 106	1

Table 4: Identification and Characterization of the Fungal Isolates

Isolates	Shape	Colony Appearance	Hyphae	Probable Fungi
A1	Fruity	Green	Septate	*Penicillium spp*
A2	Irregular	Black Cloudy Mycelia	Septate	*Aspergillus spp*
B	Irregular	Black Hairy Mycelium	Septate	*Aspergillus spp*
C1	Irregular	Blackish Cottony Mycelium	Septate	*Aspergillus spp*
C2	Circular	Greenish Mycelium	Septate	*Penicillium spp*
D1	Irregular	Darkish Cottony Mycelium	Septate	*Aspergillus spp*
D2	Fan Like	Creamy Coloured	Septate	*Penicillium*
D3	Round	Whitish Mucoid	Pseudohyphae	*Candida spp*
E1	Irregular	White Cottony Velvet Mycelium	Septate	*Rhizopus spp*
E2	Irregular	Blackish Cottony Mycelium	Septate	*Aspergillus spp*
F	Round	Yellowish Mucoid	Pseudohyphae	*Candida spp*

DATA ANALYSIS

From a total of thirty-three (33) isolates from six (6) samples, twelve (12) microorganisms were identified and characterized; eight (8) of which are bacteria and (4) of which are fungi. The microbial distribution chart (Figure 1) below shows the occurrence of the 33 isolates and their distribution in the sampled restaurants. *Escherichia spp* (highest occurring organism) occurred in five restaurants (83%), *Bacillus spp* occurred in three restaurants (50%), *Pseudomonas spp* occurred on two plates and in two restaurants, *Salmonella spp* occurred on two plates and two restaurants,

Staphylococcus spp occurred on three plates and two restaurants, *Streptococcus spp* occurred on two plates and two restaurants, *Proteus spp* occurred on one plates and one restaurant, *Klebsiella spp* occurred on one plates and one restaurant, *Penicillium spp* occurred on three plates and three restaurants, *Aspergillus spp* occurred on five plates and five restaurants, *Candida spp* occurred on two plates and two restaurants, and *Rhizopus spp* which occurred on one plate and one restaurant

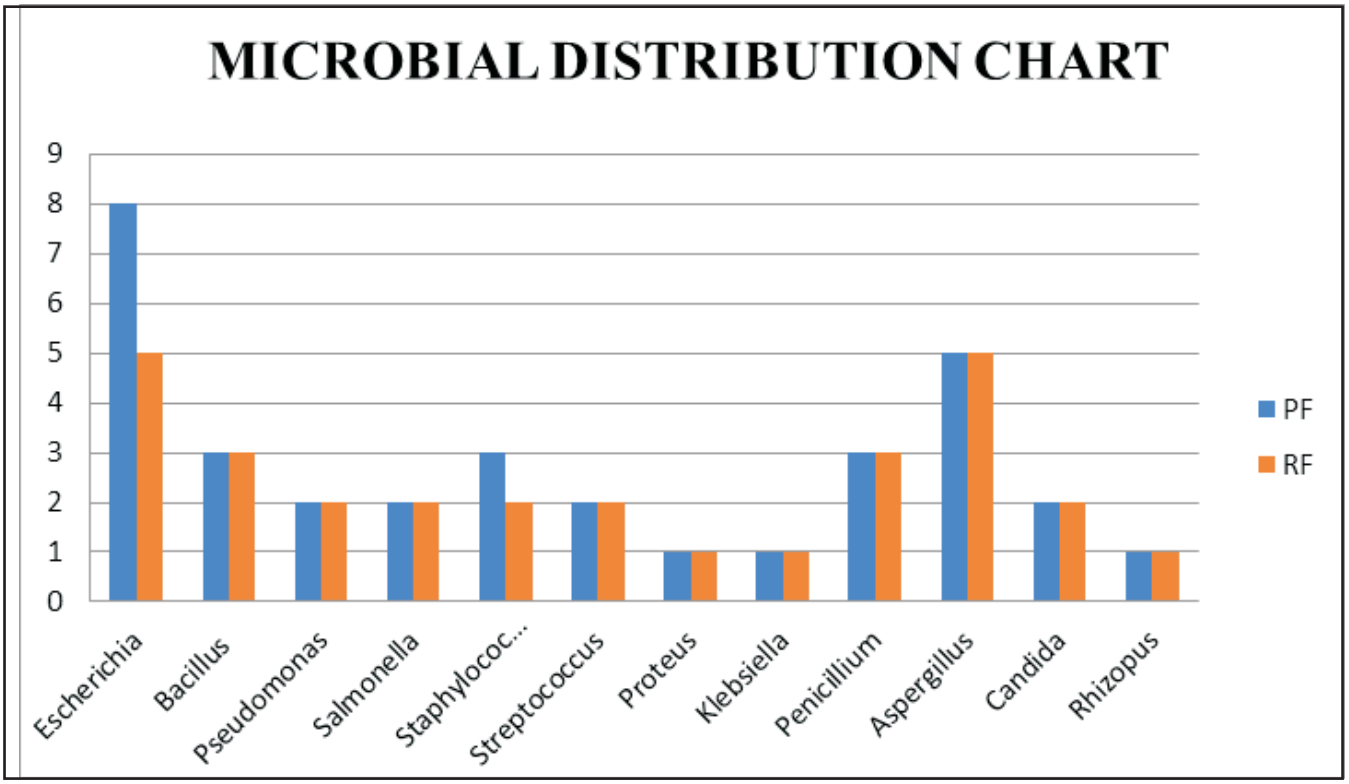

Figure 1: Microbial Distribution
LEGEND: PF = Plates Frequency**; RF =** Restaurants Frequency

Table 5 and Figure 2 show the restaurants from which the samples were taken from and the organisms isolated from them. Restaurant A, B, C, and E had five organisms each, D, which had the highest number of organisms, had six organisms while F had the lowest frequency of three organisms.

Table 5: Microbial Distribution in Restaurants

Restaurant	Organisms Found	Number Of Organisms Found
A	*Escherichia spp, Bacillus spp, Proteus spp, Penicillium spp, Aspergillus spp.*	5
B	*Escherichia spp, Pseudomonas spp, Salmonella spp, Klebsiella spp, Aspergillus spp.*	5
C	*Escherichia spp, Bacillus spp, Staphylococcus spp, Penicillium spp, Aspergillus spp.*	5
D	*Escherichia spp, Staphylococcus spp, Streptococcus spp, Penicillium spp, Aspergillus spp, Candida spp.*	6
E	*Bacillus spp, Pseudomonas spp, Streptococcus spp, Aspergillus spp, Rhizopus spp.*	5
F	*Escherichia spp, Salmonella spp, Candida spp.*	3

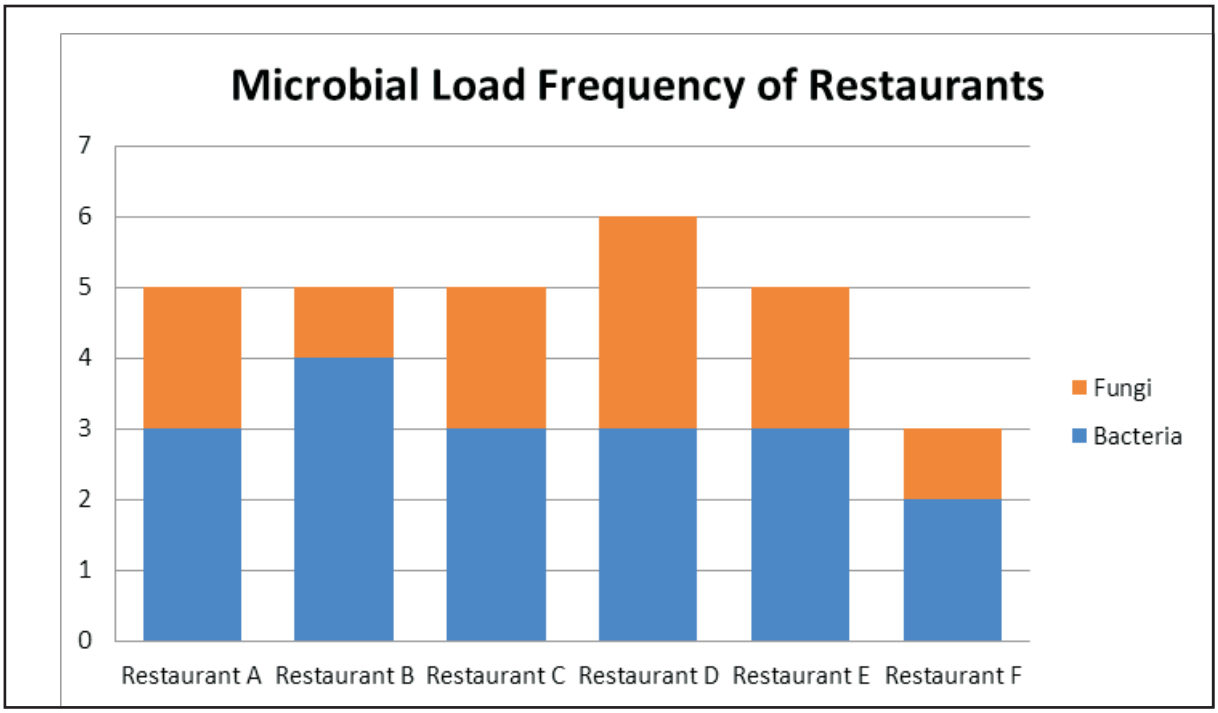

Figure 2: **Microbial Load Frequency of Restaurants**

DISCUSSION

This study has shown varieties and diversities of microorganisms that can/may be transferred into food products through handling. Some of these organisms are enteric organisms and can be pathogenic, hence, causing food borne diseases such as salmonellosis, diarrhoea, dysentery and other life threatening diseases. *Salmonella* and *E. coli* which are parts of the organisms isolated from this study are two of the three most common food borne infection agents (William *et al*., 2017). Handlers might have come in contact with these organisms using contaminated water, raw material or unhealthy hygiene practices. This may include unhealthy hygiene practices like improper washing of utensils and hand while handling food or serving customers.

These organisms can be transferred into food products by cross contamination during; holding of plates, handling of sachet water, dropping serving spoons in storage containers, and picking of cutleries. Cross contamination can also occur if sold or served food item already infested is returned to the storage, hence, contaminating the whole food product. In this study, the bacterial isolates from the handlers' palm included *Escherichia spp , Bacillus spp, Pseudomonas spp, Salmonella spp, Staphylococcus spp, Streptococcus spp, Proteus spp,* and *Klebsiella spp,* while fungal isolate included *Penicillium spp, Aspergillus spp, Candida spp,* and *Rhizopus spp.* Some of these organisms have been implicated in food poisoning or food infection outbreaks (Agbonlahor *et al*., 2000; Martyn *et al*., 2004).

Some of the organisms isolated from this study are indicator organisms that are pathogenic and have been implicated in food borne infections Agbonlahor *et al*., 2000. These organisms are considered potential threats to the human health and hence always targeted during food and water microbiological assessments. These organisms include *Escherichia spp, Salmonella spp, Staphylococcus spp, Proteus spp, Klebsiella spp* and *Candida spp*.

Escherichia spp, the most frequently occurring isolate is an indicator organism that can be responsible for illnesses that can be severe like bloody diarrhoea and painful abdominal cramps, without much fever. Some strains of *Escherichia coli* have been implicated in food borne outbreaks due to consumption of meat-based snacks which were undercooked (Hayes; 1998, Dalton *et al*., 2004). Some strains produce toxins which lead to diarrhoea and vomiting (Pelczar *et al*., 1993).

Salmonella spp is a pathogen that can be deleterious to the human health. They can contaminate food product if mishandled, undercooked, or improperly stored. *Salmonella* is responsible for 40,000 cases of salmonellosis yearly and 12.5 million typhoid fever cases in the developing world (WHO, 2004). Symptoms of *Salmonella* infection include; fever, abdominal pain, diarrhoea, vomiting, weakness, headaches, appetite loss and in some cases patients may have rash of flat and rose-coloured spots. Staphylococcal food poisoning is a gastrointestinal illness caused by eating foods contaminated with toxins produced by the bacterium *Staphylococcus aureus.* About 25% of people and animals have *Staphylococcus* on their skin and in their nose. It usually does not cause illness in healthy people, but *Staphylococcus* has the ability to make toxins that can cause food poisoning. People who carry it

can contaminate food if they don't wash their hands before touching it. Foods that are not cooked after handling, such as sliced meats, puddings, pastries, and sandwiches, are especially risky if contaminated with Staph. Food contaminated with Staph toxin may not smell bad or look spoiled. Staph food poisoning is characterized by a sudden start of nausea, vomiting, and stomach cramps (CDC, 2018).

Proteus spp spread mainly through contact with infected persons or contaminated objects and surfaces (Yong, *et al*., 2010). The pathogens can also be ingested via the intestinal tract, for example, when it is present in contaminated food. The germs spread quickly because they are very agile. The bacteria can enter the human urogenital system from the intestine, or following a smear infection. Excessive bacterial colonization can lead to urinary tract infections and, as a late consequence, to kidney stones. Infections of other organs, such as peritonitis, infections of the bile ducts, or of the gastrointestinal tract are significantly less common. Severe cases can also lead to sepsis (blood poisoning). Yong, *et al*., (2010) associated an outbreak of food poisoning in Beinjing in 2008 with eating stewed pork balls in brown sauce contaminated with *Proteus mirabilis.*

Klebsiella spp is an opportunistic pathogen that can be responsible for nosocomial infections that initially colonize the intestinal tract of patients. It is an enteroinvasive food borne pathogen. The occurrence of this organism shows that the hygiene level of the food handler is considerably low as *Klebsiella spp* are common to sewers and hospitals. *Klebsiella* are a type of bacteria that cause healthcare-associated infections, which can take the form of pneumonia, sepsis, wound infections and urinary tract infections. Healthcare-associated infections numbered more than 700,000 in the US in 2011. In fact infections caused by multidrug-resistant *K. pneumonia* have resulted in up to 50 percent fatality in some studies. In the last two decades, antibiotic-resistant *Klebsiella* infections have been on the rise around the world. (ASM, 2017). These organisms are also important in food spoilage as they are associated with specific foods e.g *Bacillus spp* is associated with rice while *Candida spp* is a heterogeneous group of yeasts, some which also cause human infections and are involved in spoilage of fruits, some vegetables and dairy products (Rawat, 2015).

CONCLUSION AND RECOMMENDATIONS

Considering the kinds of organisms found and their frequency of occurrence, it is safe to say that the foods sold at the Achievers University bukateria, there is urgent need for the appropriate authority to monitor and enforce strict hygienic environment since there is possibility of food contamination. The health of the university community which consists mostly of students is at risk of food contamination if more practical monitoring practices are not adopted.

This research has shown the possibility of potential food threats. The following suggestions are proposed for consideration. The institution should set a standard of sanitation in order to ensure and maintain Good Hygiene Practices (GHP). Assessments of the microbiological qualities of the utensils as well as other materials used in the preparation of food need to be carried out.

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