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Studies on Microbial Contamination of Cut and Exposed Onions

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Authors' contributions

This work was carried out in collaboration among all authors. Author VAN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CPA and PCA managed the analyses of the study. Author PCA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Onions (*Allium cepa)*, a vegetable plant is consumed globally for its culinary and medicinal importance. Despite the various health benefits ascribed to onions, several rumours have been spread, especially on the online media as regards its ability to act as a sponge absorbing microorganisms from the atmosphere, once cut open and left exposed in the atmosphere.

This research work involved the exposure of the half portion of an onion at different sites, while culturing the other half immediately without exposure, to serve as a control. Thirty healthy and fresh onion samples were exposed, and the sites of exposure include: living rooms, public conveniences, kitchens, fridge, freezer, and bole joints (roasted plantain eatery).

After inoculation and identification of the cultured microorganisms, *Pseudomonas sp., Bacillus cereus, Escherichia coli, Staphylococcus sp., Klebsiella sp., Enterobacter sp., yeast and Aspergillus sp.,* were isolated with *Klebsiella sp., Pseudomonas sp.* and *Staphylococcus sp.* having the highest number of occurrence of 40% having been isolated from twelve test samples each. *B. cereus* had a percentage occurrence of 30%, having been isolated from nine test samples. *E. coli* had a 10% occurrence having been isolated from three test samples, while *Enterobacter sp.* had the lowest rate of occurrence having been isolated in just one test sample. For the fungi isolates, *Aspergillus sp.*

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had a percentage occurrence of 76.6% having been isolated in twenty three test samples, while yeast had a percentage occurrence of 66.6%, having been isolated in twenty test samples.
The isolated organisms were all pathogenic organisms, and some such as *Staphylococcus sp.* and *Bacillus sp.* have been implicated in causing food poisoning. Some other organisms isolated have also been implicated in the spoilage of onions. It is therefore recommended that users of onions should reduce to the barest minimum the tendency to consume raw cut onion sample.

Keywords: Onion; microorganisms; food poisoning; contaminate; agar.

1. INTRODUCTION

Onions, scientifically referred to as *Allium cepa* is a vegetable plant that possesses various culinary and medicinal importance. They originated from central Asia, but now have a worldwide distribution. They are consumed for the several nutritional and medicinal properties they contain. Depending on the variety, and growing conditions, the nutritional value may vary [1]. They are cholesterol free, very low in calories, fat, sodium and, a good source of vitamin B6, potassium and the flavonoid quercetin and the trace mineral chromium [1].

Despite the many benefits ascribed to onions, several rumours have spread concerning the ability of cut onions to serve as a sponge for microorganisms and other disease causing agents. One of such articles was written by Sarah McCann, who writes online about food related matters, the article was written under the pen name Zola Gorgon [2]. In the article, it was claimed that cut onions had a tremendous ability to serve as a bacterial sponge, and cause food poisoning, even if it was cut open for only a short period of time. Another of such account was conveyed by Dr Bartholomew Brai, a nutritionist at the Federal University, Oye-Ekiti in an interview with the National News Agency of Nigeria, Dr. Bartholomew claimed that the vegetable contained sugar which promotes bacterial growth thus exposed cut portions should be avoided as it could cause adverse stomach infections and food poisoning due to secretion of bile salts [3].

Another very prevalent anecdote that was spread on the internet which originated from the Epoch Times by Conan Milner is about the 1919 influenza epidemic claiming that in a community where everyone one else had fallen ill, a doctor found a family which didn't get sick because they left cut onion around the house, and on microscopic examination, the flu virus was found in the onions [4]. According to 17th century herbalist Nicholas Culpepper, onions have the ability "to draw any corruption to itself, for if you peel one, and lay it on a dunghill, you shall find it rotten in half a day, by drawing putrefaction to itself" [4].

Many of the above cited accounts and myths have existed for many years, including myths on their use in curing ailments such as vision problems, dog bites, and toothaches. Other modern researches have shown that onions can help strengthen the immune system, help in the clearing of cholesterol, help in respiratory problems, and a host of other health related benefits [1].

In this study, we are exposing and inoculating one half of freshly cut onions in various locations at varying time intervals while immediately culturing the other half as a control in order to isolate microorganisms absorbed by the onions if any. This will enable us know if onions has the ability to absorb microorganisms from the atmosphere even for a short period of time, the type of microorganisms and if they are implicated in the spoilage of cut onions.

2. MATERIALS AND METHODS

2.1 Source of Onions

The 30 onion samples used in carrying out this work were randomly purchased from various vegetable shops in the Mile 1, Mile 3, and Rumuokoro open markets all in Port Harcourt. They were packaged in polythene bags, and transported to the laboratory.

2.2 Media Preparation.

The media used in the isolation and differentiation of the microorganisms include: Sabouraud dextrose agar, blood agar, nutrient agar, peptone water, and MacConkey agar. The media were weighed and prepared according to the manufacturer's instructions.

2.3 Material Sterilization

The materials used in carrying out this work were washed in sterile water and detergent, then sterilised with hot air oven. Other heat destructible materials were immersed in 90% alcohol or hypochlorite solution as deemed suitable.

2.4 Sample Preparation

The dried outer scale of the onions was peeled off, and the onions were rinsed in distilled water. They were cut into two halves. While a portion of each half was cultured immediately, the other half was transported in sterile zip lock bags to their various sites of exposure which are living rooms, public conveniences, kitchens, fridge, freezer, bole joints (roasted plantain eatery). After exposure, they were transported back to the lab for culturing.

2.5 Inoculation and Incubation

After exposure, one g was cut from each sample with the use of a sterile razor blade, and homogenised with peptone water with the use of a waring commercial blender. For the first batch of onion samples, which were exposed for one hour, a 10^3 dilution was performed on the samples, and with the use of an automatic pipette, one uL of the 10^3 diluted sample was inoculated into freshly prepared nutrient agar, macConkey agar, sabouraud dextrose agar, and blood agar and spread with a glass spreader. In batches 2 and 3 exposed for four hours and eight hours respectively, a direct streaking from the onion surface was done. The plates were incubated at 37°C for 24 hours. The same procedure was repeated for other samples. For fungi, the antibiotic chloramphenicol and tetracycline was added to the sabouraud dextrose agar to prevent the growth of bacterial contaminants. It was then incubated at room temperature and monitored for 5-7 days.

2.5 Microbial Colony Count and Identification

Colonial counts were carried out in all the plates with countable colonies, including the bacterial and fungal plates.

2.6 Identification of Bacteria and Fungi

Gram staining technique was used to differentiate the Gram positive organisms from the Gram positive ones [5]. This is done by dropping a drop of normal saline on a clean slide, and then emulsifying a colony of bacterial growth into it to make a smear. The smear was then passed through gentle heat to fix it. It is stained with crystal violet for 60 seconds, after which it washed with distilled water, and Lugol's iodine was added for one minute after which it was washed with distilled water. The slide is decolourised with alcohol for one minute, the slide was then counterstained with safranin for one minute, and washed with tap water then allowed to dry. Oil immersion was used, and the smear was examined microscopically using the 100X objective. Purple coloured organisms denoted gram positive, while red coloured organisms denoted gram negative. For fungi: Little portion of the fungal growth was placed on a clean glass slide, a drop of the Lactophenol blue was added, and the colony was emulsified on the slide and viewed at X10 and X40 objective lens [6].

2.7 `Characterization of Bacteria with Biochemical Tests

2.7.1 Catalase test

This test helps to differentiate Staphylococci from Streptococci. Microorganisms that produce the enzyme catalase are detected through the ability of the catalase they produce to breakdown hydrogen peroxide and give off oxygen, which is physically seen as bubbles. This test is done by emulsifying a few colonies of the test organism in distilled water on a glass slide, and adding one to two drops of hydrogen peroxide. Gas bubbles indicate a positive result, and *Staphylococci species* are positive, while *Streptococci sp.* are negative [7].

2.7.2 Oxidase test

The enzyme oxidase will oxidise a redox dye such as tetramethyl paraphenaline diamine dihydrochloride to deep purple colour [8]. The test is done by adding a few drops of oxidase reagent to a few drops of the test colonies on the culture plate, and observe as the colonies turn purple within 5-10 seconds. The test aids in the identification of *Pseudomonas, Neisseria, Vibrio* etc.

2.7.3 Citrate utilisation test

This is an important test used in the identification of *Enterobacteriaceae*. It is based on the

organism's ability to utilise citrate as its only source of carbon, and ammonium as its only source of nitrogen, with the citrate being metabolised to acetoin and CO_2. The organism is stab inoculated into Simmons agar. A blue colouration of the medium denotes positive result. The *Klebsiella pneumonia* is a positive control, while *E. coli* is negative control [7].

2.7.4 Motility test

A colony of the isolate was inoculated into sterile peptone water and cultured overnight. A drop of the cultured organism is dropped on a clean grease free glass slide, and viewed for motility [9].

2.7.5 Indole test

This is an important test for identifying *Enterobacteriaceae*. The test was carried out as described by Cheesbrough [5]. A colony of each isolate was inoculated onto 10ml of sterile peptone water which was the source of tryptophan and incubated at 37°C for 24 hours. To the culture, 0.5ml of Kovac's reagent was added and gently stirred. A red colour indicated positive result while a yellow colour indicated negative test result of bacteria.

3. RESULTS

The results in the culture plate yeilded bacterial and fungal growths. Colonial characteristics and biochemical tests were used in identifying the bacteria and fungi observed in the culture plates. Table 1 represents the various organisms which were isolated from the first batch of each test onion sample, and the control as well. A 10^3 serial dilutions were carried out on the first batch of the sample, and visible growth was seen in only three of the control samples. There were 4 growths in macConkey, three in the blood agar, four in nutrient and *Aspergillus sp.* was the only fungal growth in four sabouraud dextrose agar plates. The remaining media plates showed no growth. However, the organisms isolated in the first batch samples include: *Pseudomonas sp., Bacillus cereus, Escherichia coli, Staphylococcus sp., Klebsiella sp. and Aspergillus sp.*

In the batch 2 and 3 of the study samples, direct streaking of the onion surface was carried out. Despite the development of colonies which could not be counted, there was 100% growth in all culture plates, with some plates showing mixed growths. Sabouraud dextrose agar plate yielded visible growths of two organisms (*yeast* and *Aspergillus*) in all the plates of batch 2 and 3 respectively.

The distinct individual bacterial colonies were subcultured in peptone water, from which samples were used to carry out motility test and indole test. Other biochemical tests such as catalase, oxidase, citrate utilization test, as well as gram staining were also carried out to identify the isolates. Table 4 summarises how the different organisms reacted in the course of the biochemical test to which all the test samples and controls were reacted with.

For the fungal isolates, wet preparations of the isolates were prepared with lactophenol cotton blue, and viewed under the microscope. The microscopic and macroscopic features of the fungal isolates are recorded in Table 4.

Tables 1, 2 and 3 summarises the various organisms isolated from the test samples exposed at various sites of exposure, as well as their controls. The organisms isolated includes: *Pseudomonas sp., B. cereus, E. coli, Staphylococcus sp., Klebsiella sp., Enterobacter sp., yeast and Aspergillus sp.*

3.1 Statistical Analysis

Simple percentage method of analysis was employed in determining the statistics of the microorganisms isolated in the various onion samples and their rate of occurrence. Table 6 shows the different bacterial isolates, the number of test samples from which they were isolated, their rate of occurrence, as well as the sites from which they were isolated.

Klebsiella sp., Pseudomonas sp. and *Staphylococcus sp.* had the highest number of occurrence which is 40%, with each of them having been isolated from twelve test samples each. The *B.cereus* had a general percentage occurrence of 30%, having been isolated from nine test samples. *E. coli* had a 10% occurrence having been isolated from three test samples, while *Enterobacter sp.* had the lowest rate of occurrence having been isolated in just one test sample.

For the fungi isolates, *Aspergillus sp.* had a percentage occurrence of 76.6% having been isolated in twenty-three test samples, while yeast had a percentage occurrence of 66.6%, having been isolated in twenty test samples.

Table 1. Control and test onion samples for those exposed for 1 hour and isolated organisms

Control	Organism Isolated	Test	Organism Isolated	Place Of Exposure
C1	No growth	T1	No growth	Hostel room F50
C2	No growth	T2	$6.5x10^7$ *Escherichia coli*	Hostel F public toilet
C3	$1.4x10^7$ *Pseudomonas sp.*	T3	*Pseudomonas sp.* *Bacillus cereus* *Aspergillus sp.*	Medical Microbiology lab
C4	No growth	T4	No growth	Kitchen
C5	No growth	T5	*Klebsiella sp.*	Bole joint I
C6	No growth	T6	*Staphylococcus sp.* *Klebsiella sp.*	Bole joint II
C7	$8.0x10^7$ *Bacillus cereus*	T7	*Bacillus sp.* *Klebsiella sp.* *Aspergillus sp.*	Kitchen
C8	No growth	T8	No growth	Freezer
C9	No growth	T9	No growth	Fridge
C10	$3.8x10^7$ *Bacillus cereus*	T10	*Bacillus cereus* *Aspergillus sp*	Room at Billy close (private living room)

The Table 7 represents the bacterial occurrence in each of the batches. Batch 1 had only four isolates which were *Klebsiella sp.* 30%, *Bacillus cereus* 30%, *Aspergillus sp.* and *E. coli* 10%. Batch 2 had seven isolates which were *Staphylococcus sp.* 60%*, Pseudomonas sp. 50%, Klebsiella sp.* 40%*, Bacillus cereus 30%, E. coli* 10%, *Enterobacter sp.* 10%. *Yeast and Aspergillus sp.* had 100% isolation. Batch 3 had seven isolates as well *Pseudomonas sp.* 60%*, Staphylococcus sp.* 50%*, Klebsiella sp.* 40%*, Bacillus cereus* 30%*, E. coli* 10%*, Enterobacter sp.* 0%. *Yeast and Aspergillus sp.* had 100% isolation.

4. DISCUSSION

Samples obtained from open markets were cut and exposed in 3 batches. Each batch signified various time frames in which the onion samples were exposed for. Batches 1, 2, and 3 were exposed for one hour, four hours and eight hours respectively and were cultured in nutrient agar, macConkey agar, blood agar and sabouraud dextrose agar (for fungal growth) , with the unexposed half of each sample serving as the control, as it was cultured immediately in only nutrient agar.

This study was conducted on healthy fresh looking onion samples without any form of spoilage or mechanical injuries in order to limit already existing microbial contaminants. The onion samples however yielded certain microbial isolates after open air exposure at different sites, such as living rooms, public conveniences, kitchens, fridge, freezer, and bole joints (local barbeque joints, which deal in barbequed fish, plantain, yam, potato etc with a sauce produced with sufficient amounts of onions). The purpose for the exposure was to ascertain if cut and exposed onion has the ability to absorb microorganisms in the environment, and if these microorganisms are can cause spoilage in the onion cuts.

After exposure, culture and isolation of microorganisms, some isolates derived from the exposed onions were not isolated from their corresponding control samples. However, a good number of organisms were isolated from the controls. This shows that the microorganisms isolated from the test onions were likely acquired from the environments in which they were exposed. The organisms isolated includes: *Pseudomonas sp, B. cereus, E. coli, Staphylococcus sp., Klebsiella sp., Enterobacter sp., yeast and Aspergillus sp.* These organisms agree with those isolated by Orpin et al. [6] which were implicated in the spoilage of onion. The fungi isolated, also agrees with those isolated by Shehu and Muhammad [10], who reported a high frequency of occurrence for *Aspergillus sp.* in the onion bulbs they studied and Baiyewu et al. [11], who isolated these fungi from garlic, pawpaw fruits in Nigeria. Even though onion spoilage is not the primary purpose for the study, organisms from which a high growth of certain organisms such as those exposed at sites such as bole joints, public toilets and kitchen with heavy growths of *Klebsiella sp., Enterobacter sp.* and *E. coli* showed very rapid putrefaction in the test samples from which they were isolated. There was rapid putrefaction in other test samples and

control samples; however the signs of putrefaction were more obvious and rapid in the exposed test samples. This tells that there is the presence of more organisms which were acquired by the onion by virtue of its exposure, which had assisted in speeding up the putrefaction of the test onion samples.

One of the major purposes for this study was to determine the microorganisms that cause putrefaction of the onion cuts. Some of these organisms, for example some *Staphylococcus sp*. have been greatly implicated in food poisoning, and a wide range of other diseases.

The *Klebsiella sp*. occur worldwide, particularly in tropical and subtropical regions, and are ubiquitous, including forest environments, vegetation soil, water, and mucosal membranes of host species [12]. *Klebsiella sp*. can cause bacteraemia and hepatic infections, and have been isolated from a number of unusual infection, including endocarditis, primary gas-containing mediastinal abscess, peritonitis, acute cholecystitis, crepitant myonecrosis, pyomyositis, necrotizing fasciitis, psoas muscle abscess, fascial space infections of the head and neck, and septic arthritis, they are also important opportunistic pathogens, particularly among the

Table 2. Control and test onion samples for those exposed for 4 hours and isolated organisms

Control	Organism isolated	Test	Organism isolated	Place of exposure
C21	*Staphylococcus sp.* *Bacillus cereus*	T21	*Enterobacter sp* *Aspergillus sp* *Yeast*	Hostel F public toilet
C22	*Staphylococcus sp.*	T22	*Staphylococcus sp,* *Bacilluscereus,* *Pseudomonas sp.* *Aspergillus sp.* *Yeast*	Room at Billy close (private living room)
C23	*Klebsiella sp.*	T23	*Klebsiella sp.* *Aspergillus sp.* *Yeast*	Fridge
C24	*Staphylococcus sp.*	T24	*Klebsiella sp.* *Aspergillus sp.* *Yeast*	Bole joint I
C25	*Staphylococcus sp.*	T25	*Staphylococcus sp.* *Pseudomonas sp.* *Aspergillus sp.* *Yeast*	Medical Microbiology lab
C26	*Staphylococcus sp.*	T26	*Staphylococcus sp.* *Klebsiella sp.* *Aspergillus sp.* *Yeast*	Bole joint II
C27	*Bacillus cereus*	T27	*Bacillus cereus,* *Staphylococcus sp.* *Pseudomonas sp.* *Aspergillus sp.* *Yeast*	Hostel room F50
C28	*Pseudomonas sp.*	T28	*Klebsiella sp.* *Pseudomonas sp.* *Aspergillus sp.*	Freezer
C29	No growth	T29	*Pseudomonas sp.* *Escherichia coli* *Aspergillus sp.* *Yeast*	Fridge
C30	*Staphylococcus sp.* *Pseudomonas sp.*	T30	*Klebsiella sp.* *Pseudomonas sp.* *Aspergillus sp.* *Yeast*	Kitchen

Table 3. Control and test onion samples for those exposed for 8 hours and isolated organisms

Control	Organism isolated	Test	Organism isolated	Place of exposure
C31	*Staphylococcus sp.*	T31	*Staphylococcus sp.* *Klebsiella sp.* *Aspergillus sp.* *Yeast*	Kitchen
C32	*Bacillus cereus.*	T32	*Klebsiella sp.* *Bacillus cereus.* *Aspergillus sp.* *Yeast*	Bole joint I
C33	*Klebsiella sp.*	T33	*Klebsiella sp.* *Aspergillus sp* *Yeast*	Hostel room F50
C34	*Pseudomonas sp*	T34	*Staphylococcus sp* *Escherichia coli* *Aspergillus sp.* *Yeast*	Hostel F public toilet
C35	*Bacillus cereus*	T35	*Bacillus cereus* *Pseudomonas sp.* *Aspergillus sp.* *Yeast*	Medical Microbiology lab
C36	*Staphylococcus sp.* *Pseudomonas sp.*	T36	*Pseudomonas sp.* *Aspergillus sp.* *Yeast*	Fridge
C37	*Staphylococcus sp.*	T37	*Staphylococcus sp.* *Pseudomonas sp.* *Aspergillus sp.* *Yeast*	Room at Billy close (private living room)
C38	*Staphylococcus sp.*	T38	*Klebsiella sp.* *Staphylococcus sp.* *Aspergillus sp.* *Yeast*	Bole joint II
C39	*Staphylococcus sp.* *Pseudomonas sp.*	T39	*Pseudomonas sp.* *Staphylococcus sp.* *Aspergillus sp.* *Yeast*	Kitchen
C40	*Staphylococcus sp.* *Bacillus cereus*	T40	*Klebsiella sp.* *Bacillus cereus* *Aspergillus sp.* *Yeast*	Freezer

Table 4. Biochemical tests used in identifying the various bacteria

Bacteria	Gram	Cat	Oxi	Cit	Ind	Mot	Ba	Mac
Klebsiella sp.	GNR	+	-	+	-	-	NH	LF
Escherichia coli	GNR	+	-	-	+	+	NH	LF
Bacillus cereus	GPR	+	+	+	-	+	AH	NIL
Staphylococcus sp.	GPC	+	-	+	-	-	NH	NIL
Pseudomonas sp.	GNR	+	+	+	-	+	NH	NLF
Enterobacter sp.	GNR	+	-	+	-	+	NH	LF

Gram, Gram stain: GNR, Gram Negative Rods; GPR, Gram Positive Rods; GNR, Gram Negative Rods; Cat, Catalase test; Oxi,Oxidase; Cit, Citrate utilisation test; Ind, Indole; Ba, Blood agar; NH, Non Haemolytic; AH, Alpha Haemolytic; BH, Beta Hemolytic; Mac, MacConkey agar; LF, Lactose Fermenter; NLF, Non Lactose Fermenter

immuno-compromised [12]. A 10^8 *Klebsiella* organisms per gram of faeces are required to produce damage [12]. However, in this work, microbial colony count could not be determined

for *Klebsiella sp.*, as to know if one g of onion contained enough *Klebsiella sp.* to cause disease on consumption.

Staphylococcus sp. is an opportunistic pathogen that can cause a variety of self-limiting to life-threatening diseases in humans [13]. The bacteria are a leading cause of food poisoning, resulting from the consumption of food contaminated with enterotoxins [14]. Staphylococcal food intoxication involves rapid onset of nausea, vomiting, abdominal pain, cramps, and diarrhoea [13,14]. Symptoms usually resolve after 24 hours [14]. The infectious dose of *Staphylococcus sp.* is at least 100,000 organisms in humans [15].

B. cereus causes self-limiting (24-48 hours) food-poisoning syndromes (a diarrhoeal type and an emetic type), opportunistic infections and is associated with clinical infections such as endophthalmitis and other ocular infections [16, 17,18]. The diarrhoeal form of *B. cereus* food poisoning is characterized by abdominal cramps, profuse watery diarrhoea, and rectal tenesmus, and, occasionally, fever and vomiting. The emetic form of *B. cereus* food poisoning is characterized by nausea, vomiting, and malaise, occasionally with diarrhoea [18]. In diarrhoeal illness, the toxin responsible is produced by organisms in the small intestine and infective dose is 10^4-10^9 cells per gram of food. The emetic toxin is preformed and indigested in food

Table 5. Identification of fungal isolates

Fungi	Macroscopic	Microscopic
Aspergillus sp.	Black colonies having white edges, with white reverse.	Thick septate hyphae with smooth colourless conidiophores.
Yeast	Creamy colonies	Round shaped budding cells.

Table 6. Percentage occurrence of bacteria isolated and places of exposure

Bacteria	General %	No of samples of occurrence	Place of exposure where bacteria were isolated
Klebsiella sp.	40%	12	Bole joints, kitchen, fridge, freezer.
Escherichia coli	10%	3	Public toilet, fridge
Bacillus cereus	30%	9	Medical microbiology lab, Room at Billy close (private living room), kitchen
Staphylococcus sp.	40%	12	Bole joint, Room at Billy close (private living room), Hostel room F50, freezer, fridge, kitchen.
Pseudomonas sp.	40%	12	Medical microbiology lab, Room at Billy close (private living room), kitchen, Hostel room F50, freezer, fridge.
Enterobacter sp	3.33%	1	Public toilet.

Table 7. Percentage occurrence of bacteria isolation by batches

Bacteria	1st Batch test % (1 Hour)	1st Batch Control %	2nd Batch Test % (4 Hours)	2nd Batch Control %	3rd Batch test % (8 Hours)	3rd Control %
Klebsiella sp.	30%	0%	50%	10%	40%	0%
Escherichia coli	10%	0%	10%	0%	10%	0%
Bacillus cereus	30%	20%	30%	20%	30%	30%
Staphylococcus sp.	0%	10%	50%	70%	60%	70%
Pseudomonas sp.	10%	10%	60%	20%	50%	30%
Enterobacter sp.	0%	0%	10%	0%	0%	0%

Table 8. Percentage occurrence of fungi isolated and places of exposure

Fungi	1st Batch% (1 Hour)	2nd Batch (4 Hours)	3rd Batch (8 Hours)
Yeast	0%	100%	100%
Aspergillus sp.	40%	100%	100%

(about 10^5-10^8 cells per gram in order to produce sufficient toxin) [18].

As opportunistic pathogens, *Pseudomonas sp.* often invades the host tissue and cause infection and bacteraemia in immunocompromised hosts (e.g., HIV/AIDS, cystic fibrosis, bronchiectasis, and severe chronic obstructive pulmonary disease, burns, malignancy, or diabetes mellitus) [19,20]. The infectious dose is not known for humans [21]. Enterotoxigenic *E. coli (ETEC)* usually have an abrupt onset of watery diarrhoea that does not contain blood, pus, or mucus (non dysenteric) [22]. The diarrhoea is usually mild to moderate in severity, but some patients may have severe fluid loss [22]. Low-grade fever, nausea, and abdominal pain may also be present [23].

The infectious dose of Enterotoxigenic *E. coli* (ETEC) in adult is estimated to be at least 10^8 organisms, but the young, the elderly and the infirm may be susceptible to lower numbers [24].

There is no experimental or epidemiologic evidence of infectious dose of *Enterobacter sp*. available; however, approximately 1000 cells have been considered infectious [25]. Diseases caused by *Aspergillus sp*. include clinical allergies (allergic bronchopulmonary aspergillosis, rhinitis, farmers's lung), superficial and local infections (cutaneous infections, otomycosis, tracheobronchitis), infections associated with damaged tissue (aspergilloma, osteomyelitis), and invasive pulmonary and extra pulmonary infections [26]. The infective dose is unknown [25].

Candida albicans is of worldwide prevalence. It has been isolated from soil, animals, hospitals, inanimate objects and food [27,28]. Although mucocutanaeous infections caused by *Candida albicans* can occur in both immunocompetent and immunosuppressed individuals, invasive candidiasis such as candidemia or systemic disease are seen only in severely immunocompromised individuals [27]. The infective dose is unknown [25]. Most of the organisms isolated in this work, has been previously shown to be involved with the spoilage of onions. In the work carried out by Bishop and Davis [29], *Enterobacter cloacae* were implicated in the internal decay of onion bulbs. And the isolated organism when inoculated into healthy onion samples showed the disease symptoms present in the onions from which the organisms were isolated. Yurgel et al. [30] isolated *Enterobacteriaceae* and *Pseudomonas sp.* from both healthy and diseased onion samples. They also isolated yeasts from diseased onion samples. Dean and Timberlake [31] isolated *Aspergillus nidulans* from onions, and established that its mode of pathogenesis involves the production of cell wall degrading enzymes.

It is therefore established that isolates from the exposed test onion samples are organisms which are capable of causing disease. What is however not determined by this work is whether the amount obtained has the ability to cause diseases in humans.

5. CONCLUSION

Bacterial and fungal organisms were isolated from the exposed onion samples more than compared to their corresponding control samples which were not exposed, but cultured immediately on exposure. The isolated organisms were organisms which showed a high pathogenic ability, and some possess the ability to cause food poisoning if ingested. It is recommended that users of onions, should try as much as possible to desist from the habit of storing cut onions which are meant to be used later. This is because certain microorganisms, which are pathogenic contaminate the onions. Consumers of onions are advised to subject the onions to heat. This is necessary because even the control samples which were not exposed, but cultured immediately also showed the presence of spoilage microorganisms. This establishes onions as a reservoir of microorganism. Further studies on this subject matter, should employ the use of appropriate dilution factors, which could help to yield countable colonies, in order to be able to relate the number of isolated colonies to the infective dose of the organisms, so as to know if exposed onions has the right amount of microorganisms sufficient to cause food poisoning and other diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. White K, Zellner J. In College seminar 235 food for thought: The Science, Culture, & Politics of Food; 2008.
2. Snopes. Cut onion contamination warning; 2009.
Available:www.snopes.com
[Retrieved 14 July 2019].

3. News Agency of Nigeria. Nutritionist warns against consumption of exposed sliced onions; 2014.
Available:www.nan.ng
[Retrieved 14 July 2019].
4. Milner C. Are leftover onions poisonous? Viral warning calls it a sponge for bacteria; 2019.
Available:https://www.theepochtimes.com/are-leftover-onions-poisonous_2002018.html
[Retrieved 14 July 2019]
5. Cheesbrough M. District laboratory practice in tropical countries. Cambridge, United Kingdom: Cambridge University Press; 2006.
6. Orpin JB, Yusuf Z, Mzungu I, Orpin CA. Investigation of microorganisms associated with the spoilage of onions around Dutsinma metropolis. Medcrave Online Journal of Biology and Medicine. 2017;*2*(4):00057.
7. Ochei JO, Kolhatkar AA. Medical Laboratory Science: Theory and Practice. New York, United States of America: McGraw Hill Education; 2000.
8. Steel KJ. The oxidase reaction as a taxonomic tool. Microbiology. 1961;25(2): 297-306.
9. Ederer GM, Clark M. Motility-indole-ornithine medium. Appl. Environ. Microbiol. 1970;20(5):849-850.
10. Shehu K, Muhammad S. Fungi associated with the storage rots of onion bulb in Sokoto Nigeria. International Journal of Modern Botany. 2011;1(1):1–3.
11. Baiyewu RA, Amusa NA, Ayoola OA, Babalola OO. Survey of the postharvest diseases and aflatoxin contamination of marketed pawpaw fruit (*Carica papaya* L.) in South Western Nigeria. African. Journal of Agricultural. Research. 2007;*2*(4):178-181.
12. Janda JM, Abbott SL. The Genera Klebsiella and Raoultella. The Enterobacteria. 2006;115-129.
13. Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, Yolken RH. (Eds.). Manual of Clinical Microbiology (8th ed.). Herdon VA., United States of America: American Society for Microbiology; 2003.
14. Le Loir Y, Baron F, Gautier M. Staphylococcus aureus and food poisoning. Genetics and Molecular Research. 2003;*2*(1):63-76.
15. Schmid-Hempel P, Frank SA. Pathogenesis, virulence, and infective dose. Public Library of Science Pathogens. 2007;3(10):147.
16. Collier LB, A Sussman M. Topley and Wilson's Microbiology and Microbial Infections, Vol 3-Bacterial infections, New Jersey: John Wiley; 1998.
17. Le Scanff J, Mohammedi I, Thiebaut A, Martin O, Argaud L, Robert D. Necrotizing gastritis due to *Bacillus cereus* in an immunocompromised patient. Infection. 2006;34(2):98-99.
18. Logan NA, Rodrigez-Diaz M. *Bacillus spp.* and Related Genera. In: Gillespie, S.H., Hawkey, P.M. Eds. Principles and practice of clinical bacteriology. 2nd ed., West Sussex, England, UK: John Wiley and Sons Ltd. 2006;139-158.
19. Liu PV, Mercer CB. Growth, toxigenicity and virulence of *Pseudomonas aeruginosa.* Epidemiology and Infection. 1963;61(4):485-491.
20. Feldman M, Bryan R, Rajan S, Scheffler L, Brunnert S, Tang H, Prince A. Role of flagella in pathogenesis of *Pseudomonas aeruginosa* pulmonary infection. Infection and Immunity. 1998;66(1):43-51.
21. Banerjee A, Dangar TK. *Pseudomonas aeruginosa,* a facultative pathogen of red palm weevil, *Rhynchophorus ferrugineus*. World. Journal of Microbiology and Biotechnology. 1995;11(6):618-620.
22. Sande M, Drew WL. Current diagnosis and treatment in infectious diseases. New York, United States of America: McGraw-Hill Professional; 2001.
23. Chao HC, Chen CC, Chen SY, Chiu CH. Bacterial enteric infections in children: Etiology, clinical manifestations and antimicrobial therapy. Expert Review of Anti-Infective Therapy. 2006;4(4):629-638.
24. Todar K. Pathogenic *E. coli* In Todar's Online Textbook of Bacteriology; 2008. Available:http://www.textbookofbacteriology.net/e.coli_4.html
[Retrieved 14 August 2019]
25. Public Health Agency of Canada. In Best M, Graham ML, Leitner R, Ouellette M, Ugwu K. (Eds.), Laboratory biosafety guidelines (3rd ed.). Canada: Public Health Agency of Canada; 2004.
26. Verweij PE, Brandt ME. *Aspergillus, Fusarium,* and other opportunistic

moniliaceous fungi. Manual of Clinical Microbiology. 2007;2:1802-1838.
27. Ruhnke M. Epidemiology of *Candida albicans* infections and role of non-*Candidaalbicans* yeasts. Current Drug Targets. *2006;*7(4):495-504.
28. Edwards JE. *Candida species*. In Mandell, GL, Bennett JE, Dolin R. (Eds.), Mandell, Douglas, and Bennett's Principles and Practices of Infectious Diseases (7th ed.). New York, United States of America: Churchill Livingston; 2009.
29. Bishop AL, Davis RM. Internal decay of onions caused by *Enterobacter cloacae*. Plant Diseases. 1990;74:692-694.
30. Yurgel SN, Abbey L, Loomer N, Gillis-Madden R, Mammoliti M. Microbial communities associated with storage onion. Phytobiomes. 2008;2(1):35-41.
31. Dean RA, Timberlake WE. Production of cell wall-degrading enzymes by *Aspergillus nidulans*: a model system for fungal pathogenesis of plants. The Plant Cell. 1989;1(3):265-273.

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