

## Microbiological Assessment and Antimicrobial Properties of Edible Clay (Nzu) Sold in Port Harcourt Metropolis

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### Abstract

This study was conducted to evaluate the microbiological quality and antimicrobial properties of microbes found in edible clay sold in Port Harcourt metropolis. Random purchase of Nzu was made, from various local markets: Creek-road, Mile 1, Mile 3, Navy and Ojoto markets in Port Harcourt. The samples were analyzed using standard microbiological techniques for microbiological assessment and antimicrobial properties. The bacterial counts obtained from the various locations ranged between  $8.0 \times 10^3$  to  $2.0 \times 10^4$  CFU/g. The fungal counts, ranged between 0 to  $3.0 \times 10^3$  CFU/g. Bacteria isolated include *Bacillus* sp., *Klebsiella* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Streptococcus* sp., *Escherichia coli* and fungi such as *Aspergillus* sp., *Mucor* sp., *Penicillium* sp., *Candida* sp. and *Fusarium* sp. Resistance to Ceftriaxone, cefuroxime, augmentin, and ceftazidime was found in *Staphylococcus aureus*. *Bacillus* sp. was resistant to Ceftriaxone, cefuroxime, ceftazidime, and augmentin. *Streptococcus* sp were resistant to Augmentin, ceftriaxone, cefuroxime, and ceftazidime. *Klebsiella* sp showed some level of resistance to augmentin, ceftazidime, and cefuroxime. *Escherichia coli* was resistant to cefixime, cefuroxime, and augmentin. *Pseudomonas* sp. was resistant to ceftazidime, cefuroxime, and augmentin. Fluconazole drug resistance was found in *Fusarium* sp.; however, fluconazole susceptibility was found in *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., and *Candida* sp. Except for *Fusarium*, which was intermediate, all of the fungal isolates were responsive to clotrimazole. Nystatin was resisted by *Aspergillus* sp., *Penicillium* sp., and *Mucor* sp., but susceptible by *Candida* and *Fusarium*. *Mucor* sp. and *Fusarium* sp. showed intermediate and resistant responses to ketoconazole, respectively: as other fungal isolates were susceptible. The level of resistance demonstrated by the microorganisms isolated from edible clay may pose a risk to the consumers and general public, with time.

**Keywords:** Antimicrobial Properties; Edible Clay; Bacteria; Fungi; Resistant; Susceptible

### Introduction

Kaolin Clay, also known as calabash chalk or calabash clay, is a water-soluble clay soil found in mining pits. It's one of Africa's most popular geophagic foods. This clay is commonly applied to the skin during festivities in many African tribes, and it is thought to have healing powers. Raw or processed kaolin clay is available for purchase. With the addition of additional components, it can be processed into powder, moulded blocks/pellets, or moulded balls (nzu). By combining salt and ash with kaolin clay, nzu is created

(edible clay). After that, the substance is formed into balls and heated to harden it [1]. The processed kaolin clay is popular among Africans and different tribes call it different names Nzu (igbo), Ndom (Efik/Ibibio), Eko (Bini). People consume clay for a variety of reasons, ranging from its appetite-suppressing properties to its weight-loss and anti-emetic capabilities. Clay consumption offers both benefits and drawbacks; nevertheless, the hazards exceed the benefits. Nzu provides nutrients to your body that may be obtained from other nutritious meals with fewer hazardous ingredients [2]. The rule is to consume moderate amounts to optimize benefits and

reduce risk: as little as a teaspoon or less is sufficient. Because it includes vital electrolytes like salt, magnesium, and potassium, as well as trace minerals like zinc and copper, edible clay has some nutritional value. It contains large amounts of trace metals and other hazardous chemicals that can destroy key cells in your body, in addition to these critical nutrients. Many individuals are concerned about edible clay's addictive properties. PICA is the term for the compulsive, indiscriminate consumption of non-nutritive material like clay and charcoal. Pica is a psychological disease and a symptom of iron insufficiency. Although it includes traces of iron, which is required for the development of red blood cells in the body, it also contains significant levels of zinc, which inhibits iron absorption. This means that compulsive clay eating is a symptom of an underlying condition, and that eating it worsens anemia. Pica and nzu have a two-way relationship as a result of this nzu causes iron deficiency anaemia which leads to compulsive eating of edible clay which worsens the anaemia and the cycle continues [2]. Regardless of the benefits of edible clay intake, due to differing viewpoints on geophagia, various microbes have been discovered to dwell the soil, performing either a beneficial or destructive role. While certain helpful roles for earth consumption have been identified, others continue to criticize it for its harmful and abnormal nature. As a result, edible clay is a process worth paying attention to [3]. This study, therefore, aimed to assess the microbial quality and antibiotic sensitivity of edible clay sold in some markets in Port-Harcourt.

## Materials and Methods

### The study area

The study areas were Navy, Creek-road, Ojoto, Mile1 and Mile 3, markets in Port-Harcourt Local Government Area, Rivers State. The geographical coordinates are as follows: 4°44'37.12795" North, 7°2'27.35829" East for sample collected on Navy market and 4°45'30.83919"North, 7°1'23.77807" East for Creek road market and 4°47'46.12436" North, 6°59'15.43447" East for Ojoto market and 4°47'46.51950" North, 6°59'15.56482" East for Mile1 market and 4°48'14.45191" North, 6°59'23.50051" East for Mile 3 market.

### Sample collection

In each market, three replicate samples of each edible clay were purchased and used for the study. Samples from two study areas (Navy and Mile 1 Market) were readily packaged in a polyene before purchase, while the other samples were found exposed before

purchase (Creek-road, Ojoto and Mile 3 market). The samples from the five different markets were obtained by hand picking and then placed into a sterile transparent bag and were transported to the microbiology laboratory of the Rivers State University for analysis.

## Preparation of media

### Nutrient agar (NA)

It was prepared according to the manufacturer's standard; 28g of the (NA) powder was weighed using electronic weighing balance and dissolved in 1000ml of distilled water in a conical flask which was stoppered with cotton wool and aluminum foil. The medium was sterilized by autoclaving at 121°C for 15 minutes. The media was allowed to cool down to 45°C and 20 ml of the medium was aseptically dispensed into petri-dishes and allowed to solidify. After solidification it was dried in the hot air oven at 160°C for 1 hour.

### Sabourand dextrose agar (SDA)

This is a selective media used for the cultivation of fungal organisms. The SDA was prepared according to the manufacturer's standard. 6.5g of the powder was weighed and dissolved in 1000 ml distilled water in a conical flask which was corked with a cotton wool and aluminum foil. The medium was sterilized by autoclaving at 121°C for 15 minutes. The media was cooled to 45°C and it was aseptically dispensed into sterile petri-dishes and allowed to solidify and it was dried in the hot air oven. Before pouring into the plates, chloramphenicol was added to the medium in order to inhibit the growth bacteria.

## Inoculation of and isolation of bacteria and fungi

1g of the edible clay (nzu) sample was weighed and put into test tube containing sterile 9ml normal saline (diluent) and was shaken. The suspension was subsequently serially diluted using ten-fold serial dilution up to 10<sup>-4</sup> dilution. Aliquot of 0.1 ml of the appropriate dilution was plated and spread in the already prepared nutrient agar plate for isolation of bacteria and Sabourand dextrose agar plate for isolation of fungi. The nutrients agar (NA) plates were incubated at 37°C for 24 hours while the Sabourand dextrose agar (SDA) plates were incubated at 37°C for 72-120 hours. After incubation, the numbers of discrete colonies were counted in terms of colony forming units (cfu). The viable count was obtained and the bacterial and fungal counts was used to enumerate the total viable counts of the isolates in colony forming unit per gram (cfu/g).

## Maintenance of pure microbial cultures

### Isolation and identification of pure cultures

Discrete colonies were counted, purified by subculturing into freshly prepared (NA) and (SDA) plates by streak plate technique, to obtain pure isolates/colonies. This was done by flaming wire loop until it's red-hot and then allowed to cool in air, and by streaking a loopful of a particular isolate on an already prepared plates for bacterial and fungi growth. The sub-cultured (NA) and (SDA) plates were incubated at 37°C for 24 hours and 72-120 hours respectively. The pure cultures were stored accordingly in (NA) slant at 40°C and (SDA) slant in the refrigerator for further studies.

### Characterization and identification of bacteria isolates

Cultural characterization of bacteria isolates were observed on the plates. The colonial characteristics are grouped under form/shape, elevation, margin, surface texture, size and colour/pigment.

### Macroscopic and microscopic identification of isolates

A physical morphological analysis was done on the isolates to determine their morphological features. Microscopic examination was also done on the isolates by subjecting the isolates into gram staining so as to determine their staining reaction in a bid to group them into gram positive or gram-negative organisms. The microscopic view of the fungal isolates was done using lacto-phenol cotton blue (LPCB) stain.

### Anti-microbial susceptibility profile

Antimicrobial susceptibility profile of the bacterial isolates was carried out using kirby-Bauer disc diffusion method and readings interpreted by adopting the break points of clinical and laboratory standard institute (CLSI). Purified isolates were inoculated on 5ml nutrient broth and incubated over-night. The optical density (OD) of the turbidity of the broth was determined to conform with the optical density (OD) of the McFarland turbidity standards (i.e, 0.5 McFarland standard) where the bacterial suspension or cells are equivalent to  $1.5 \times 10^8$  colony forming units (cfu/ml). Using a sterile swab stick, inoculum from the broth (i.e., respective standards) were aseptically swabbed on Mueller Hinton Agar. A total of 8 antibiotics disc which includes; Gentamicin (10 ug), Erythromycin (5 ug), Ofloxacin (5 ug), Cloxacillin (5 ug), Ceftriaxone (30 ug), Cefuroxime (30 ug), Ceftazidime (30 ug) and Augmentin (30 ug) were employed. The respective discs were also aseptically

impregnated on the agar plates using a sterile forceps. Plates were allowed to stand at room temperature for 5minutes to allow the media to absorb effectively and incubated at 37°C for 18-24hours. Characterization of the resistance, intermediate and susceptibility profile of the isolates were determined by measuring the zone of inhibition and then compared with the interpretative chart to determine the sensitivity, intermediate and resistant nature of the isolates to the antibiotics used using the CLSI [4] interpretative chart.

### Preparation of peptone broth for antibiotic sensitivity testing

Peptone broth was prepared and dispensed into test tubes (6 tubes). It was sterilized by autoclaving at 121°C for 15minutes. After autoclaving, it was allowed to cool down before inoculating a loopful of the bacteria isolate into the test tube containing the Peptone broth.

The Peptone broth was compared with 0.5 McFarland standard for turbidity. After inoculation, it was incubated at 37°C for 6-24hours. After incubation, sterile swab stick was used to rub/swab the surface of already prepared nutrient agar (NA) plates and antibiotic sensitivity disc was gently placed on top surface of the agar plates using sterile forceps and incubated uprightly at 37°C for 24-hours.

### Note

The sensitivity disc was coded positive and negative. The gram-positive isolates were placed with sensitivity disc bearing positive while the Gram-negative bacteria were placed with sensitivity disc bearing negative with different antibiotics impregnated with the disc.

After 24hours of incubation, the zone of inhibition was measured in 'mm' using meter rule and recorded as sensitive, intermediate and resistant respectively.

### Preparation of anti-fungal drugs and SDA broth

SDA broth was prepared and poured into 5test tubes and autoclaved at 121°C for 15 minutes. After autoclaving, it was allowed to cool to room temperature before inoculating the different fungal isolates into the test tubes containing the SDA broth. Each of the fungi isolate were inoculated into different SDA broth, it was then incubated for 3-5 days. After incubation, a sterile swab stick was used to rub/swab the surface of already prepared SDA plates.

Anti-fungal drugs that were found to be soluble were used for agar well, while the insoluble one such as Nystatin, Ketoconazole were flooded at the surface of the SDA plates. After dipping the agar well, anti-fungal drugs such as clotrimazole and fluconazole were aseptically poured into the well. Both the agar well plates and the flooding plates were all incubated uprightly for 3-5days before observing for total clearing or resistant. Different concentration of anti-fungal drugs was prepared by grinding the drugs using sterile pistol and mortal. After grinding, it was dissolved in 100 ml of distilled water before using it for sensitivity testing.

**Results**

The bacterial and fungal counts obtained in the study are presented in table 1. The sampling locations were labeled as follows; Navy market, Creek-road market, Ojoto market, Mile 1 market, and Mile 3 market. The bacterial counts obtained from the various location ranged between  $8.0 \times 10^3$  to  $2.0 \times 10^4$ . Samples from Ojoto and mile 1 market recorded the least bacterial count while the highest was obtained from mile 3. The fungal counts, ranged between 0 to  $3.0 \times 10^3$ . There was no fungal count recorded from creek road market. The highest fungal count was observed in Ojoto market.

Sampling Location	Total bacterial count CFU/g	Total fungal count CFU/g
Navy (A)	$1.3 \times 10^4$	$2.0 \times 10^3$
Creek road market (B)	$1.9 \times 10^4$	-
Ojoto market (C)	$8.0 \times 10^3$	$3.0 \times 10^3$
Mile 1 market (D)	$8.0 \times 10^3$	$2.0 \times 10^3$
Mile 3 market (E)	$2.0 \times 10^4$	$2.0 \times 10^3$

**Table 1:** Total Bacterial and Fungal Counts.

Table 2 shows the bacteria isolated from the sample and their occurrence. A total number of seventy-seven organisms were isolated. Samples from Ojoto market had the least number (11) of organisms isolated while samples from mile 3 recorded the highest number (22) of organisms isolated. The organisms isolated were, *Bacillus* sp, *Klebsiella* sp, *Pseudomonas* sp, *Staphylococcus aureus*, *Streptococcus* sp and *E. coli*. *Bacillus* sp occurred least in sample A and the highest was *Staphylococcus aureus*. For samples from Creek- road market, *E. coli* was not recorded, while *Bacillus* sp recorded the highest. Samples from Ojoto market recorded zero *Streptococcus* sp, while the highest occurrence was *Klebsiella* sp. The least that occurred in samples from mile 1 market was *Pseudomonas* sp and *Streptococcus* sp while the highest was *Bacillus* sp, *Klebsiella*, *Staphylococcus* and *E. coli*. Samples from mile 3 market recorded *Pseudomonas* sp and *E. coli* as the least occurred while the highest was *Klebsiella* sp.

Sampling Location	Total No of isolates	<i>Bacillus</i> sp	<i>Klebsiella</i> sp	<i>Pseudomonas</i> sp	<i>Staphylococcus aureus</i>	<i>Streptococcus</i> sp	<i>E. coli</i>
Navy market	15	1	3	2	5	2	2
Creek road market	19	5	3	3	4	4	0
Ojoto market	11	2	4	1	1	0	3
Mile 1 market	10	2	2	1	2	1	2
Mile 3 market	22	4	5	2	6	3	2
Total	77	14	17	9	18	10	9

**Table 2:** Bacteria Isolated and their Occurrence.

The fungal isolated and their occurrence are shown in table 3. A total number of nineteen (19) fungi were isolated. Samples from Ojoto and mile 1 market had the least number (2) of fungi isolated while samples from Creek-road market recorded the highest number (8) of fungi isolated. The fungi isolated were, *Aspergillus* sp, *Mucor* sp, *Penicillium* sp, *Candida* sp *Fusarium* sp. *Aspergillus* sp

was not isolated from Creek-road and mile 1 market. *Mucor* sp was not isolated from creek-road, Ojoto and mile 3 market. *Penicillium* sp was not isolated from Navy market. *Candida* was not isolated from Navy, Ojoto, mile 1 and mile 3 market. *Fusarium* sp was not isolated from creek road, Ojoto and mile 1 market.

Sampling location	Total No of fungal isolates	<i>Aspergillus</i> sp	<i>Mucor</i> sp	<i>Penicillium</i> sp	<i>Candida</i> sp	<i>Fusarium</i> sp
Navy market	4	2	1	0	0	1
Creek road market	8	0	0	1	7	0
Ojoto market	2	1	0	1	0	0
Mile 1 market	2	0	1	1	0	0
Mile 3 market	3	1	0	1	0	1
Total	19	4	2	4	7	2

**Table 3:** Fungal Isolated and their Occurrence.

Tables 4 - 9 showed the anti-microbial properties of different bacterial isolates from edible clay (Nzu). *Staphylococcus aureus* was resistant to ceftriaxone, cefuroxime, augmentin, and ceftazidime, but was susceptible to ofloxacin, gentamicin and erythromycin as shown in table 4.

*Bacillus subtilis* was susceptible to gentamicin, erythromycin and ofloxacin. The organism was resistant to ceftriaxone, cefuroxime, ceftazidime and Augmentin (Table 5).

Antibi-otics	Diameter (mm)	Susceptible (S)	Intermediate (I)	Resistant (R)
OFL	27	S		
GEN	25	S		
CTR	0			R
CRX	7			R
CXC	12		I	
ERY	30	S		
AUG	0			R
CAZ	0			R

**Table 4:** Sensitivity disc for gram positive (*Staphylococcus aureus*).

KEYS: CAZ = Ceftazidime; CRX = Cefuroxime; GEN = Gentamicin; CTR = Ceftriaxone; ERY = Erythromycin; CXC = Cloxacillin; OFL = Ofloxacin; AUG = Augmentin.

Antibiotics	Diameter (mm)	Susceptible (S)	Intermediate (I)	Resistant (R)
GEN	23	S		
ERY	25	S		
OFL	15	S		
CXC	12		I	
CTR	8			R
CRX	0			R
CAZ	0			R
AUG	0			R

**Table 5:** Sensitivity disc for gram positive (*Bacillus subtilis*).

KEYS: GEN = Gentamicin (10 ug); ERY = Erythromycin (5 ug); OFL = Ofloxacin (5 ug); CXC = Cloxacillin (5 ug); CTR = Ceftriaxone (30 ug); CRX = Cefuroxime (30 ug); CAZ = Ceftazidime (30 ug); AUG = Augmentin (30 ug).

*Streptococcus* sp was susceptible to erythromycin, gentamycin, ofloxacin but was resistant to augmentin, ceftriaxone, cefuroxime and ceftazidime (Table 6).

Antibiotics	Diameter (mm)	Susceptible (S)	Intermediate (I)	Resistant (R)
ERY	23	S		
CXC	14		I	
GEN	22	S		
OLF	20	S		
AUG	0			R
CTR	0			R
CRX	2			R
CAZ	0			R

**Table 6:** Sensitivity disc for gram positive (*Streptococcus* sp).

KEYS: GEN = Gentamicin (10 ug); ERY = Erythromycin (5 ug); OLF = Ofloxacin (5 ug); CXC = Cloxacillin (5 ug); CTR = Ceftriaxone (30 ug); CRX = Cefuroxime (30 ug); CAZ = Ceftazidime (30 ug); AUG = Augmentin (30 ug).

*Klebsiella* sp was susceptible to nitrofurantoin, gentamycin and ciprofloxacin but was resistant to augmentin, ceftazidime and cefuroxime (Table 7).

Antibiotics	Diameter (mm)	Susceptible (S)	Intermediate (I)	Resistant (R)
NIT	32	S		
GEN	30	S		
CPR	18	S		
OFL	14		I	
AUG	0			R
CAZ	0			R
CRX	0			R
CXM	12		I	

**Table 7:** Sensitivity disc for gram negative (*Klebsiella* sp).

Total clearance in Nitrofurantoin (NIT) and Gentamicin (GEN).

KEYS: NIT = Nitrofurantoin (300 ug); GEN = Gentamicin (10 ug); CPR = Ciprofloxacin (5 ug); OFL = Ofloxacin (5 ug); AUG = Augmentin (30 ug); CAZ = Ceftazidime (30 ug); CRX = Cefuroxime (30 ug); CXM = Cefixime (5 ug).

*Escherichia coli* was susceptible to ciprofloxacin, nitrofurantoin, gentamycin and ofloxacin but was resistant to cefixime, cefuroxime and augmentin (Table 8).

Antibiotics	Diameter (mm)	Susceptible (S)	Intermediate (I)	Resistant
CPR	28	S		
NIT	31	S		
GEN	29	S		
CXM	14		I	
OFL	18	S		
CRM	0			R
CRX	4			R
AUG	0			R

**Table 8:** Sensitivity disc for gram negative organism (*Escherichia coli*).

KEYS: CPR = Ciprofloxacin (5 ug); NIT = Nitrofurantoin (300 ug); GEN = Gentamicin (10 ug); CXM = Cefixime (5 ug); OFL = Ofloxacin (5 ug); CRX = Cefuroxime (30 ug); CAZ = Ceftazidime (30 ug); AUG = Augmentin (30 ug).

*Pseudomonas* sp was susceptible to gentamycin, nitrofurantoin and ciprofloxacin but showed resistant to ceftazidime, cefuroxime and augmentin (Table 9).

Antibiotics	Diameter (mm)	Susceptible (S)	Intermediate (I)	Resistant (R)
OFL	12		I	
GEN	22	S		
NIT	28	S		
CPR	19	S		
CAZ	0			R
CRX	0			R
AUG	0			R
CXM	13		I	

**Table 9:** Sensitivity disc for gram negative organism (*Pseudomonas* sp).

In table 10, *Fusarium* sp showed resistant to fluconazole while *Aspergillus* sp, *Penicillium*, *Mucor* and *Candida* sp were susceptible to fluconazole. All the fungal isolates in table 11 were susceptible to clotrimazole except *Fusarium* which was intermediate.

*Aspergillus*, *Penicillium* and *Mucor* sp were resistant to Nystatin, while *Candida* and *Fusarium* were susceptible to the drug (Table 12). Other fungal isolates were susceptible to ketoconazole except *Mucor* and *Fusarium* sp which showed intermediate and resistant, respectively.

Fungi isolates	Zone of inhibition (mm)	Susceptible (S)	Intermediate (I)	Resistant
<i>Aspergillus</i> sp	18 mm	S		
<i>Penicillium</i> sp	30 mm	S		
<i>Fusarium</i> sp	7 mm			R
<i>Mucor</i> sp	25 mm	S		
<i>Candida</i> sp	28 mm	S		

**Table 10:** The Sensitivity (S), Intermediate (I), And Resistant (R) of fluconazole to different fungi are shown below.

Fungi isolates	Zone of inhibition (mm)	Susceptible (S)	Intermediate (I)	Resistant (R)
<i>Mucor</i> sp	20 mm	S		
<i>Candida</i> sp	31 mm	S		
<i>Aspergillus</i> sp	23 mm	S		
<i>Fusarium</i> sp	12 mm		I	
<i>Penicillium</i> sp	16 mm	S		

**Table 11:** The Sensitivity (S), Intermediate (I), Resistant (R) of clotrimazole to different fungi isolates are shown below.

Fungi isolates	Zone of inhibition (mm)	Susceptible (S)	Intermediate (I)	Resistant (R)
<i>Candida</i> sp	15 mm	S		
<i>Aspergillus</i> sp	0			R
<i>Penicillium</i> sp	0			R
<i>Fusarium</i> sp	24 mm	S		
<i>Mucor</i> sp	5 mm			R

**Table 12:** The Sensitivity (S), Intermediate (I), and Resistant (R) of nystatin to different fungi isolates are shown below.

Fungi isolates	Zone of inhibition (mm)	Susceptible (S)	Intermediate (I)	Resistant (R)
<i>Aspergillus</i> sp	45 mm	S		
<i>Mucor</i> sp	14 mm		I	
<i>Candida</i> sp	17 mm	S		
<i>Penicillium</i> sp	21 mm	S		
<i>Fusarium</i> sp	3 mm			R

**Table 13:** The Sensitivity (S), Intermediate (I), and Resistant (R) of ketoconazole to different fungi isolates are shown below.

## Discussion

Bacterial counts recorded in the study was highest in samples obtained from mile 3 market while the least was recorded from Ojoto and mile 1 market. For the fungal counts, the highest was recorded in samples from Ojoto market while there was no fungal count from creek road market sample. The level of contamination recorded could be attributed to the handling processes. The bacterial isolated from various sampling location include *Bacillus* sp, *Klebsiella* sp, *Pseudomonas* sp, *Staphylococcus aureus*, *Streptococcus* sp and *Escherichia coli*. For the fungal isolates, *Aspergillus*, *Mucor*, *Penicillium*, *Candida* and *Fusarium* sp were isolated from the different locations. Some *Bacillus* sp and *Pseudomonas* sp which were among the organisms identified have pathogenic effects on humans [5]. The detection of *Staphylococcus* sp and *Candida* spp. also pointed to the unhygienic handling practices. The presence of *Klebsiella*, *Escherichia coli* and *Candida* in the samples could pose some risks to consumers. As enteric microorganisms, their presence may also suggest the presence of other faecal coliforms which may be pathogenic. Species of *Staphylococcus* are known food intoxicants. Their presence could therefore implicate the clays in cases of cross-contamination. Fungi have less effects on human but they can produce toxins which may be harmful to the host. *Aspergillus* spp produce mycotoxins. *Mucor* spp is a common fungus found in the soil and could affect immunocompromised individual [5]. *Candida* spp. are known to occur in the small intestine, respiratory tract, vaginal area and mouth as harmless commensals. They do not cause diseases in healthy individuals because their growth is suppressed by other microbiota, but if the normal microbiota is upset, their numbers increase rapidly to cause candidiasis [5]. In recent times, *Candida* spp. have become important nosocomial pathogens. Most of the organisms isolated from the samples have been isolated by other researchers [5,6]. Ceptriaxone, cefuroxime, augmentin, and ceftazidime resistance was found in *Staphylococcus aureus*. Ofloxacin, gentamicin, and erythromycin were found to be effective against *Staphylococcus aureus*. Gentamicin, erythromycin, and ofloxacin were all found to be effective against *Bacillus subtilis*. Ceftriaxone, cefuroxime, ceftazidime, and augmentin were all resistant to the bacterium. Erythromycin, gentamycin, and ofloxacin were all effective against *Streptococcus* sp., while augmentin, ceftriaxone, cefuroxime, and ceftazidime were not. Nitrofurantoin, gentamycin, and ciprofloxacin were all effective against *Klebsiella* sp., however

augmentin, ceftrazidime, and cefuroxime were not. Ciprofloxacin, nitrofurantoin, gentamycin, and ofloxacin were all effective against *Escherichia coli*, however cefixime, cefuroxime, and augmentin were not. *Pseudomonas* sp. was susceptible to gentamycin, nitrofurantoin, and ciprofloxacin, whereas ceftazidime, cefuroxime, and augmentin were resistant. Fluconazole resistance was found in *Fusarium* sp, however fluconazole susceptibility was found in *Aspergillus* sp, *Penicillium*, *Mucor*, and *Candida* sp. Except for *Fusarium*, which was intermediate, all of the fungal isolates were responsive to clotrimazole. Nystatin was resistant to *Aspergillus*, *Penicillium*, and *Mucor* sp, but vulnerable to *Candida* and *Fusarium*. Except for *Mucor* and *Fusarium* sp, which showed intermediate and resistant responses to ketoconazole, all other fungal isolates were susceptible. Some of the organisms obtained demonstrated resistance to the medications utilized, which are well-known and widely used in the study region; yet, some of the organisms showed some susceptibility to some of the treatments. The emergence of resistance among the most significant bacterial infections is widely acknowledged as a major public health concern that affects people all over the world [7]. Antibiotic resistance has been designated as one of the top three public health problems of the twenty-first century by the World Health Organization [8]. Because the majority of the organisms discovered are pathogenic, the reported level of treatment resistance could constitute a concern to the consumers of these edible clays. The presence of resistance determinants on plasmids with similar selective markers in this study could be attributed to the presence of resistance determinants on plasmids with similar selective markers, or it could be the result of independent, simultaneous development of resistance to different agents, implying that bacteria have the unique ability to transfer resistance genes from one bacterium to another of a different population [9]. Self-medication, which is frequent in the study area, may have boosted resistance as well. Because of the lack of access to medical treatments, this practice is likely to persist.

## Conclusion

Some of the microorganisms isolated from the edible clay sold in markets in port Harcourt metropolis, are pathogenic in nature and resistant to available medications. Thus, individuals consuming this clay may be at risk, which could result to public health threat with time.

## Bibliography

1. Mills ME. "Craving more than food: the implications of pica in pregnancy". *Nursing for Women's Health* 11.3 (2007): 266-273.
2. Abrahams PW, *et al.* "Human geophagia, calabar chalk and undongo: mineral element nutritional implication". *PLoS One* 8.1 (2013): 1-5.
3. Price M. "Cosmetics, Styles and Beauty Concepts in Iran" (2012).
4. Clinical and laboratory standards institute (CLSI). "Performance standards for antimicrobial susceptibility testing; Twenty-seventh informational supplement". CLSI document M1005. Wayne, PA (2007).
5. Prescott L., *et al.* "Microbiology". William C. Brown, London. 5<sup>th</sup> edition (1996).
6. Tano-Debrah K and Bruce-Baiden G. "Microbiological characterization of dry white clay, a pica element in Ghana". *Nature Science Report and Opinion* 2.6 (2010): 77-81.
7. Munita JM and Arias CA. "Mechanisms of antibiotic resistance microbiology spectrum". 4 (2016): 1-24.
8. Prestinaci F, *et al.* "Antimicrobial resistance: a global multifaceted phenomenon". *Pathogens and Global Health* 109.7 (2015): 309-318.
9. Oluwe OA., *et al.* "Antimicrobial resistant pattern of *E. coli* from human clinical samples in Osogbo, South western Nigeria". *African Journal of Microbiology Research* 2 (2008): 8-11.