



Antibiotic susceptibility patterns of microbial isolates from food and utilities in selected restaurants in Akoko communities of Ondo State, Nigeria

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Abstract

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The study shows the antibiotic resistance of microbial isolates encountered in foods, table surfaces, cleaning cloths and washing water at selected restaurants in some Akoko Communities, Nigeria. Food samples such as rice, beans, fufu, moin-moin, eba, amala and pounded yam were purchased from different restaurants at Oka, Ikare and Akungba-Akoko, Nigeria for microbiological analysis within 24 hours of collection. The antibiotic susceptibility profile of the isolates was determined in vitro using standard techniques.

*A total number of eighteen (18) bacteria and seventeen (17) fungal species were isolated from the samples. The bacterial species were identified as *Aeromonas aerogenes*, *Aeruginosa* spp, *Bacillus cereus*, *Bacillus marscecens*, *Bacillus megaterium*, *Bacillus subtilis*, *Clostridium freundii*, *Clostridium sporogenes*, *Enterobacter cloacae*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Pseudomonas putida*, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Staphylococcus epidermis* and *Staphylococcus faecalis*. The fungi isolates are *Aspergillus lutaceus*, *Aspergillus Rhizomucur pusillus*, *Fusarium poae*, *Cunninghamella elegans*, *erythrocephalus*, *Gelasinospora retispora*, *Heteroconium chaetospora*, *Hormiactis candida*, *Penicillium fellutanum*, *Rhizopus oryzae*, *Torulaherbarum*, *Scopulariopsis brevicaulis*, *Syncephalastrum racemosum*, *Saccharomyces*, *Gliocladium viride*, and *Scopulariopsis brumptii*. Most isolates obtained during the study showed multiple antibiotic resistance to Erythromycin (E) and Chloramphenicol (CH), Septrin (SXT), Zinnacef (Z), Gentamycin (CN) and Streptomycin (S).*

This study suggests the need to observe high level hygiene in eateries. Similarly, the nature of antibiotic resistance in this area can form a sort of database for health care management systems in case of infection from these sources.

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INTRODUCTION

Restaurants are business center where food and drinks are being sold to customers in exchange for money. The busy nature of people all over the world has increased the patronage of restaurants, cafeteria and fast food centers. In Nigeria, rice, beans, eba, amala, fufu, moin-moin, and pounded yam are the prevalent food in most restaurants. They are present in menu in various forms. Similarly, washing water, washing surfaces and cleaning cloths also found in restaurants all carry various level of microbial load because of their nutritional constituents which provide a rich medium for the growth of

microorganisms that might contaminate them (Bankole and Joda, 2004; Madukwe and Eneobong, 2007). The potential sources of contamination of these foods, washing surfaces, utensils, washing water and cleaning cloths are soil, water, air, plants feed or fertilizer, animals and vectors including flies that are sporadically landing on the foods, human beings, sewage, processing equipment, ingredients, product to product and packaging materials (Gorris, 2005).

Ready-to-eat foods are processed (peeled, squeezed, cut up and/or cooked) and readily available for purchase and consumption. However, restaurant

foods have been implicated in the transmission of food borne diseases (Gillespie *et al.*, 2000; Fang *et al.*, 2003). Food borne illness is an international health problem and an important cause of reduced economic growth (World Health Organization, 1983). Food borne illness of microbial origin is a major cause of death in developing Countries (WHO, 2002; Rehydration Project, 2004). The problems of food safety in the industrialized world differ considerably from those faced by developing Countries. In developing countries traditional methods of processing and packaging, improper holding temperature, poor personal hygiene of food handlers are still observed during food marketing and technology (Barro *et al.*, 2002). The use of antibiotic(s) after the intake of the organism(s) may not be effective as the organisms may be susceptible or resistant to it. Resistance to antibiotics in food borne pathogens may create problems for disease or illness treatment while antibiotic susceptibility leads to healing of the illness which the organism caused. Traveler's diarrhea is a major inconvenience to visitors arriving in developing Countries from more industrialized areas (Dupont *et al.*, 1982).

If a population of microorganisms is exposed to a sufficiently high concentration of an antimicrobial compound, susceptible cells will be killed. However, some cells may possess a degree of natural resistance or they may acquire it later through mutation or genetic exchange and will, therefore, survive and grow (Bower and Daeschel, 1999). To fully understand antimicrobial resistance, one must understand the mechanisms of action and/or the specific targets of an antimicrobial within a microorganism. For example, antibiotics used for therapeutic purposes often have specific target sites in a microbial cell and the development of resistance to these compounds is the result of changes in these target sites.

The resistance responses of microorganisms to antimicrobials including disinfectants may be innate, apparent, or acquired. Mechanisms of innate resistance may include cellular barriers preventing entry of the antimicrobial (for example, the outer membrane of Gram-negative bacteria and teichoic acids contained within Gram-positive bacteria), cellular efflux (that is, mechanisms that pump compounds out of the cell), lack of a biochemical target for antimicrobial attachment or microbial inactivation, and inactivation of antimicrobials by microbial enzymes (Bower and Daeschel, 1999). Sorbic acid has been used as an antimicrobial in foods in the United States since the 1940s when it was patented for use in foods and on packaging to retard spoilage by molds (Sofos and Busta, 1993). Innate resistance to sorbate is demonstrated by bacteria, including catalase- negative lactic acid bacteria, *Sporolactobacillus*, some *Pseudomonas*, yeasts (including *Brettanomyces*, *Candida*, *Saccharomyces*, *Torulopsis*, and *Z. bailii*), and molds (including *Aspergillus*, *Fusarium*, *Geotrichum*, *Mucor*, and *Penicillium*) (Sofos and Busta, 1993). As with benzoic acid, some microorganisms can

metabolize sorbic acid. Molds isolated from cheese, including seven *Penicillium* species, exhibited growth in the presence of and degradation of 0.3 to 1.2% sorbate (Finol *et al.*, 1982).

The microbiological quality of these foods is influenced by a number of factors, such as cuisine type, cooking, raw materials, serving methods and management of food handling (FEHD, 1995). Both bacteria and fungi isolated by Obeta and Ariba (2004) in previous work done include *Bacillus* spp., *Klebsiella aerogenes*, *K. pneumoniae*, *Staphylococcus* spp, *Micrococcus*, *Rhizopus oryzae*, *Rhizomucor pusillus*, *Aspergillus* spp *etc.* Lu and Wang, (2015) and Temeche *et al.*, (2016) reported that some of these problems emanate from food handlers in ready- to- eat centers that had no formal training on food safety practices and prevention of food borne diseases coupled with lack of food safety policies.

Antibiotic resistance has been attributed to factors such as antibiotics abuse and misuse, use of some soap and deodorants, overuse of antibacterial cleaning agents at home, use of antibiotics in animal feeds (Prescott *et al.*, 2008). The main mechanisms of drug resistance are drug inactivation or modification, alteration of target site, alteration of metabolic pathway and reduced drug accumulation by decreasing drug permeability and/ or increasing active efflux of the drugs across the cell surface (Li and Nikado, 2009). Due to the increased consumption of rice, beans, fufu, amala, moin-moin, eba and pounded yam in restaurants, there is the need to determine the level of safety of these foods in cafeteria or restaurants.

This study therefore is aimed at isolating bacteria from cooked food, washing surfaces, washing water and cleaning cloths in restaurants, determining the susceptibility of the isolates to antibiotics and determining if the antibiotic resistance is plasmid or chromosomal mediated. Ideally, cooking or heating is expected to reduce the microbial load of cooked foods, but such foods can also become contaminated due to cross contamination, particularly from raw meat or poultry (Singleton, 1997) and environmental sources such as air and dust, food utensils and food handlers. Similarly, this study will help to create awareness to the public about the menace of microbial food contamination among eateries in Nigeria and to investigate the degree of resistance and susceptibility of the bacterial isolates to tested antibiotics.

MATERIALS AND METHODS

i. COLLECTION OF SAMPLES

Food sample were collected towards 12 noon and at the peak of business to ensure the sampling for analysis of appropriate food source. The Food samples were purchased from different restaurants at Oka, Ikare and Akungba Akoko in Ondo State. Samples include rice, beans, fufu, moin-moin, eba, amala, pounded yam and were set to be eaten. Other samples include washing

waters, cleaning cloths, washing surfaces. The cooked and ready to eat food samples collected, were transported aseptically to the laboratory in sterile sample bottles and then processed and within few hours of collection day.

ii. ISOLATION PROCEDURE

Nutrient agar, macConkey agar and potato dextrose agar were used for the isolation of bacteria and fungi respectively. Microbial isolation of the samples was carried out using pour plate method. The procedure involved pipetting 10ml from each samples and 1ml from sample was pipette into 9ml sterile distilled water to make a stock solution of 10 ml. The samples diluted aseptically were enumerated using a pour plate technique. The pure culture was then transferred on to agar slant (Horwitz, 2003).

iii. Standardization of inoculums

The growth method was used in the preparation of the inoculum. Organism was picked from fresh colonies on plates containing the test organism, inoculated into each of 5 ml freshly prepared nutrient broth. The tubes were incubated at 37°C for 24 hours and the density of the suspension in each tube matched with that of 0.5 McFarland turbidity standards prepared (Baron and Finegold, 1990).

iv. Test plates inoculation

This was done with the aid of sterile swab sticks within 15minutes after adjusting the turbidity of the inoculum suspensions. The swab in each case was rotated and pressed firmly on the inside wall of the tube containing the broth standardized inoculum suspension above the fluid level. The swab was used to inoculate the surface of a nutrient agar plate by streaking it over the entire agar surface.

v. Isolation of bacteria

Microbial isolation of samples was carried out by using pour plate method. A 1ml portion of dilution was aseptically transferred into a sterile Petri dish. About 15ml of nutrient agar was poured into separate sterile plate and aseptically swirled gently on the laboratory bench and were incubated at 37°C for 24hours. After incubation time the different culture plates were examined for microbial growth; subcultures were made to get discrete colonies, which were then stored at 40oC for further biochemical investigations in order to identify microorganisms.

vi. Isolation of fungi

1ml of each sample was inoculated on potato dextrose agar and incubated at room temperature for five days, after which the plate was examined to detect fungal growth. The pure cultures were then transferred to agar slant (Domsch et al., 2007).

vii. Antibiotic susceptibility tests

A modified in vitro antibiotic susceptibility testing methods of the CLSI, (2016) was used during the study. The antibiotics were tested with sterile Muller Hilton Agar, and was poured into

sterile petri dishes and allowed to solidify. A suspension of the isolated organisms was transferred into petri-dishes accordingly and swab over the entire plate, it was then incubated for 1 h at 37°C and a forcep was used to transfer each sensitivity disc on the plate and incubated for 24 h at 37°C. The antibiotics used included amoxicillin, streptomycin, chloramphenicol, septrin, sparfloxacin, pefloxacin, zinnacef, recephin, tarivid, gentamycin, augmentin, ciprofloxacin, ampiclox and erythromycin. Antibiotic discs were mounted aseptically, onto the surface of the inoculated agar plates. Each disc was firmly pressed to ensure complete contact with the agar surface. The discs were evenly distributed so that they are not too close to one another. In an inverted position, the plates were incubated at 37°C for 18 hours in an inverted position. The results were expressed as the zone of inhibition around the antibiotic disc as either sensitive or resistant (European Committee on Antimicrobial Susceptibility Testing, 2013).

viii. These discs were placed firmly on the agar with sterilized forceps to ensure complete contact with the agar. The plates were inverted and incubated for 18 hour at 37°C within 15 minutes after the discs were applied. After 18 hours of incubation, the plates were examined, the susceptibility of each isolate to each antibiotic were indicated by a clear zone of growth of inhibition around each disc.

RESULTS AND DISCUSSIONS

This study shows different types of microbes including bacterial and fungal species that are indication of contaminants that colonize food sources in the study area, that is, some Akoko communities, Nigeria. A total of eighteen (18) bacterial species and sixteen (16) fungi were isolated from the food samples, hand-washing water, cleaning surfaces and cleaning cloth in restaurants at Akungba, Ikare and Oka Akoko in Ondo State, Nigeria. Isolates from each site, distribution and the frequencies of occurrence are as shown in Tables 2 and Table 4 respectively.

Streptococcus feacalis, *Micrococcus luteus*, *Citrobacter freundii*, *Bacillus megaterium*, *Rhizomucor pusillus* and *Bacillus cereus* were found in Eba. *Pseudomonas aeroginosa*, *Pseudomonas fluorescens*, *Staphylococcus epidermis*, *Penicillium fellutanum*, *Syncephalastrum racemosun* were found in Beans. *Staphylococcus aureus*, *Pseudomonas fluorescens* and *Aspergillus erythrocephalus* were found in Amala. *Bacillus cereus*, *Proteus vulgaris*, *Fusarium poae*, *Cunninghamella elegans* and *Gelasinospora retispora* were found in Fufu. *Bacillus subtilis*, *Rhizopus oryzae* and *Hormiactis candida* were found in Pounded Yam. *Bacillus subtilis*, *Hormiactis candida* and *Rhizopus oryzae* were found in Moin-Moin. *Proteus rettgei*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Torula herbarum*, *Scopulariopsis brevicaulis* were isolated from Rice.

Similarly Table 2 and 3 shows *Staphylococcus aureus*, *Pseudomonas putida*, *Saccharomyces* and *Gliocladium viridae* were found in washing surfaces. *Streptococcus faecalis*, *Aerobacter aerogenes*, *Clostridium sporogenes*, *Bacillus marscecens*, *Heteroconium chaetospora*, *Scopulariopsis brumptii* and *hormiactus candida* were found in Hand-washing water. *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Staphylococcus epidermis*, *Enterobacter cloacae* and *Aspergillus alutaceus* were isolated from cleaning-cloth.

All the bacterial isolates showed multiple antibiotic resistance to erythromycin (E), chloramphenicol (CH) except *Aerobacter aerogenes* (22 S) and *Proteus vulgaris* (20 S). All the isolates were also resistant to Ampiclox (APX), zinnacef (Z), amoxicillin (AM), augmentin (AU), septrin (SXT), Streptomycin (S), Rocephin (R), gentamycin (GN). Many of the isolates were susceptible to ciprofloxacin (CPX), sparfloxacin (SP), Tarivid (OFX) and pefloxacin (PEF), except in few cases showing the effectiveness of some of this antibiotics tested on food microbial contaminants (Table 5).

Table 1: List of bacteria isolates from the various samples

Source	Bacterial isolates
Amala	<i>Staphylococcus aureus</i> , <i>Pseudomonas fluorescens</i> .
Beans	<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas fluorescens</i> , <i>Staphylococcus epidermis</i> .
Eba	<i>Streptococcus faecalis</i> , <i>Micrococcus luteus</i> , <i>Citrobacter freundii</i> , <i>Bacillus megaterium</i> , <i>Bacillus cereus</i>
Fufu	<i>Bacillus cereus</i> and <i>Proteus vulgaris</i> .
Pounded yam	<i>Bacillus subtilis</i>
Moin-Moin	<i>Bacillus subtilis</i>
Rice	<i>Proteus rettgei</i> , <i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> .
Washing surface	<i>Staphylococcus aureus</i> and <i>Pseudomonas putida</i> .
Hand-washing water	<i>Streptococcus faecalis</i> , <i>Aerobacter aerogenes</i> , <i>Clostridium sporogenes</i> and <i>Bacillus marscecens</i> . <i>Heteroconium chaetospora</i> , <i>Scopulariopsis brumptii</i> and <i>Hormiactus candida</i> .
Cleaning cloth	<i>Staphylococcus aureus</i> , <i>Pseudomonas fluorescens</i> , <i>Staphylococcus epidermis</i> and <i>Enterobacter cloacae</i> .

2: FUNGAL SPECIES ISOLATED AND CULTURAL CHARACTERISTICS

Fungal species isolated were *Rhizopus* species, *Fusarium* species, *Aspergillus erythrocephalus*, *Aspergillus alutaceus*, *Penicillium chrysogenum*, *Heteroconium chaetospora*, *Scopulariopsis brumptii*, *Hormiactis candida*, *Syncephalastrum racemosum*, *Aspergillus erythrocephalus*, *Gliocladium viride*, *Cunninghamella elegans*, *Torula herbarum*, *Rhizomucor pusillus*, *Scopulariopsis brevicaulis* and *Gelasinospora retispora*. They were isolated from this food sources and using standard mycology literatures as compendium of fungi (Domsch *et al.*, 2007).

Table 3: Frequency of occurrence of each bacterial isolate

Isolates	Frequency	Cumulative frequency	% Occurrence
<i>Aerobacter aerogenes</i>	2	2	6.5
<i>Bacillus cereus</i>	2	4	6.5
<i>Bacillus marscecens</i>	1	5	3.2
<i>Bacillus megaterium</i>	1	6	3.2
<i>Bacillus subtilis</i>	2	8	6.5
<i>Citrobacter freundii</i>	1	9	3.2
<i>Clostridium sporogenes</i>	1	10	3.2
<i>Enterobacter cloacae</i>	1	11	3.2
<i>Klebsiella pneumonia</i>	1	12	3.2
<i>Micrococcus luteus</i>	1	13	3.2
<i>Proteus rettgei</i>	1	14	3.2
<i>Proteus vulgaris</i>	1	15	3.2
<i>Pseudo aeruginosa</i>	1	16	3.2
<i>Pseudomonas fluorescens</i>	4	20	12.9
<i>Pseudomonas putida</i>	1	21	3.2
<i>Staphylococcus aureus</i>	6	27	19.4
<i>Staphylococcus epidermis</i>	2	29	6.5
<i>Streptococcus feacalis</i>	2	31	6.5

Table 5: Antibiotic susceptibility profile of the bacterial isolates

Isolates	Zone of inhibition (mm)													
	SXT	CH	SP	CPX	AM	AU	GN	PEF	OFX	APX	Z	R	S	E
A. a	-	22 S	-	-	-	-	-	-	-	-	-	-	-	-
B. c	-	-	-	18 S	-	-	-	-	-	13 R	-	-	-	-
B. m	-	-	-	-	12 R	-	-	-	-	-	-	15 S	-	-
B. me	-	-	-	18 S	-	-	-	-	-	-	12 R	-	-	-
B. s	-	-	-	10 R	-	-	11 R	-	-	-	-	-	10 R	-
C. f	-	-	-	15 S	-	-	-	-	12 R	-	-	-	-	-
C. s	-	-	-	15 S	-	-	-	-	-	-	-	15 S	-	-
E. c	-	14 R	20 S	-	-	-	11 R	10 R	18 S	-	-	-	-	-
K. p	12 R	-	25 S	11 R	-	-	-	-	15 S	-	-	-	-	-
M. l	-	-	-	20 S	-	-	-	-	-	-	-	12 R	-	-
P. r	15 S	12 R	20 S	20 S	-	-	20 S	20 S	20 S	-	-	-	-	-
P. v	-	20 S	25 S	22 S	-	-	-	20 S	25 S	-	-	-	20 S	-
P. a	-	14 S	12 R	16 S	-	-	-	-	12 R	-	-	-	-	-
P. f	-	-	-	11 R	-	12 R	15 S	-	-	-	-	-	-	-
P. p	12 R	-	12 R	15 S	-	-	11 R	-	-	-	-	-	-	-
S. a	13 R	-	-	20 S	-	-	15 S	12 R	-	-	-	-	-	-
S. e	-	-	-	12 R	-	-	-	-	-	-	-	-	11 R	-
S. f	-	-	-	15 S	-	-	10 R	20 S	-	-	-	-	-	-

Key:

A.a = Aerobacter aeruginosa, B. c= Bacillus cereus, B. m= Bacillus marscecens, B. me= Bacillus megaterium, B. s= Bacillus subtilis, C. f= Citrobacter freundii, C. s= Citrobacter sporogenes, E. c= Enterobacter cloacae, K. p= Klebsiella pneumonia, M. l= Micrococcus luteus, P. r= Proteus rettgeri, P. v= Proteus vulgaris, P. a= Pseudomonas aeruginosa, P. f= Pseudomonas fluorescens, P. p= Pseudomonas putida, S. a= Staphylococcus aureus, S. e= Staphylococcus epidermis, S. f= Streptococcus feacalis

Antimicrobial sensitivity: S – Sensitive; R – Resistant

SXT= Seprin, CH= Chloramphenicol, SP= Sparfloxacin, CPX= Ciprofloxacin, AM= Amoxicillin, AU= Augmentin, GN= Gentamycin, PEF= Pefloxacin, OFX= Tarivid, APX= Ampiclox, Z= Zinnacef, R= Rocephin, S= Streptomycin, E= Erythromycin

Findings of this study have shown that the food samples vended for human consumptions were contaminated by pathogenic bacteria which if ingested may be deleterious to consumers' health and may lead to food borne illness or disease. The prevalence of antimicrobial resistant food borne pathogens has increased during recent decades (Boonmar *et al.*, 1998a, b; Chui *et al.*, 2002). Similarly, Nyabe *et al.*, (2017) also described the implications of this resistance strains in food sources that are capable of transduction and replications that may be epidemiologically significant. However, the discovery of various plant sources with medicinal values gives the hope of overcoming this antibiotic resistant surge (Wyk and Wink, 2017).

The total resistance (100%) shown by all the isolates to erythromycin (Table 3) a broad-spectrum antibiotic which acts by inhibiting cell wall synthesis in growing or dividing cells (Kathleen and Arthur, 2000) shows the resistance nature of this organism, this mechanism inhibits antibiotics like Amoxicillin. Resistance under this context can also be associated with the production of enzymes that modify and inactivate antibiotics (Levy and Marshall, 2004). Antibiotic resistant microorganisms may be associated with reduced penetration of the antibiotic into the cell, or can result from active processes such as changes in the transport of those compounds onto or from the bacterial cells (Hermansson *et al.*, 1997).

Microorganisms such as *Bacillus* spp., *Pseudomonas* spp. and members of the enterobacteriaceae group isolated in this study, have being reported to adapt to environmental stress (Lu and Wang, 2015). Nevertheless, this study illustrated the extent of antibiotic resistance in all the isolated organisms found. It is necessary to pay more attention to food hygiene practices to reduce or eliminate the risk from food borne pathogens; especially those that are originated from restaurant. Also, strict implementation of food sanitation code and license for restaurant is needed to make the consumers save. If possible, public health authorities should intensify efforts to monitor conditions of sanitation and hygiene in establishment serving foods and drinks to the public.

Most of the food samples used in this study did not meet Microbiological quality standards. Most of the organisms isolated are multidrug resistant so in case of any food borne illness due to these organisms, appropriate antibiotics should be given especially in a combined therapy regimen. This issue of antibiotic resistance cannot be divulge from the information given by American Chemical Society, (2017) which shows the detection of antibiotic resistant genes in unexpected places in various environments including marine sources. This study clarifies multiple antibiotic resistance to most of the antibiotics tested except in few cases

(Table 5). However, the concentration at which antibiotics were used is a factor that plays a significant role to bacteria response to antibiotics. This study recommends that food safety personnel should enforce the adherence to rules and regulations on food safety by restaurants and cafeteria. Similarly, it is very important that proper food hygiene is ensured in food eateries to minimize the risk of microbial cross contamination in restaurant foods.

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