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Full length Research Paper

Multidrug resistance among bacteria isolated from some foods sold in Restaurants in Abraka, Nigeria

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Egusi soup and jollof rice samples were collected from various restaurants in Delta State, Nigeria. Isolation of the bacteria present was done by culturing, using spread plate technique on Nutrient agar, blood agar and MacConkey agar plates incubated at 37°C for 24 hours. The bacteria isolated were *Bacillus cereus*, *Klebsiella*, *pneumoniae, Escherichia coli*, *Lactobacillus acidophilus, Bacillus subtilis, Micrococcus luteus* and *Acinetobacter calcoaceticus*. The total colony count ranged from $8.0x10^3$ to $45.6x10^3$ cfu/g. *K.pneumoniae and E.coli* had the highest frequency of occurrence. The plasmid analysis revealed that of the isolates with resistance, the percentage resistance of the bacterial isolates before plasmid curing was highest (100%) in Cefixime, Augmentin, Ceftriazone and Cefuoxime while resistance to septrin was the lowest (60%). After plasmid curing, the percentage resistance of the bacterial isolates was highest in Cefixime and Cefuroxime(100%) while resistance to Septrin was still the lowest (60%). Agarose gel electrophoresis revealed that some of the *K.pneumoniae*, *E.coli* and *B. subtilis* isolates harboured multi-drug resistant plasmids with DNA sizes less than 23130bp while those that did not harbor multidrug resistant plasmids included *Lactobacillus acidophilus, B. subtilis*.

Key words: Resistance, multidrug resistance, bacteria, egusi soup, jollof rice.

INTRODUCTION

The busy nature of people all over the world has increased the patronage of restaurants, cafeteria and fast food centres. In Nigeria, rice and egusi soup are the prevalent food and soup respectively in most restaurants and are present in the menu in various forms. Egusi soup is a popular soup eaten in West Africa. It is thickened with ground melon, ground or squashed seeds (Madukwe and Eneobong, 2007). Melon seeds which are the major raw materials are important sources of proteins and oils but still constitute a major source of contamination by microorganisms into the soup (Bankole and Joda, 2004). Jollof rice which is a cooking variation of rice is a food appreciated in most countries. The nutritional value of jollof rice is provided by its content in carbohydrate, sugar, fibre, energy, fat, protein, water, iron, calcium, magnesium and zinc (Kaneko et al., 1995). The nutritional constituents of these foods provide a rich medium for the growth of microorganisms that might contaminate them. The potential sources of contamination of these foods are soil, water, air, plants feed or fertilizer, animals, human beings, sewage, processing equipment, ingredients, product to product and packaging materials (Banwart, 2004). The microbiological quality of these foods is influenced by a number of factors, such as cuisine type, cooking, raw materials, serving methods and management and food handling (FEHD, 1995). Obeta and Abriba (2004), isolated *Bacillus sp., Klebsiella aerogenes, K. pneumonia, Lactobacillus spp. and Micrococcus* from egusi soup. Bess et al. (2009), reported that food handlers in ready- to- eat centres had no formal training on food safety practices and prevention of food borne diseases. Due to minimal or no adherence to food safety practices and the indiscriminate establishment of restaurants in most developing and underdeveloped countries, there is need to examine the microbiological quality of some of the foods in the restaurants from time to time. Laws should be enacted on the establishment of restaurants and penalties should be enforced when such laws are violated. Unsafe practices in food processing can lead to foodborne diseases such as typhoid fever when contaminated food is consumed.

There is the current problem of emergence of strains of microorganisms implicated in foodborne diseases that

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are resistant to most of the present antibiotics which has been attributed to antibiotics being used both within medicine and veterinary medicine (Soulsby et al., 2005). 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 resistance can be plasmid or chromosomal mediated however many antibiotic resistance genes reside on plasmids facilitating their transfer. If a bacterium carries several resistance genes it is called multi-drug resistant organism or a "super bacterium" (Arians and Murray, 2008). *Staphylococcus spp, Enterococcus spp, Streptococcus pneumonia, Shigella dysentriae* were found to exhibit resistance to antibiotics (Ash et al., 2002; Abdalla et al., 2013). Molin and Nielsen (2003) found drug resistance in strains of bacteria, viruses, parasites and fungi. The main mechanism 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 jollof rice and egusi 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 pathogenic bacteria from jollof rice and egusi soup in restaurants, determining the susceptibility of the isolates to antibiotics and determining if the antibiotic resistance is plasmid or chromosomal mediated.

MATERIAL AND METHODS

Collection of Samples

Egusi and Jollof rice were purchased from five different restaurants within Abraka. The foods were collected in sterilized containers with covers and taken immediately to the laboratory for analyses. Samples were collected from five different sampling restaurants (A-E) from which j:jollof rice and e:egusi soup were collected.

Microbiological Analysis

Total Viable Count

From serially diluted samples 1ml inoculum was seeded on Nutrient agar plates using spread plate method, and the plates incubated at 37^0C for 18- 24 hours. Plates having distinct colonies of not more than 30-300 were counted and reported as colony forming units.

Isolation of Bacteria

Jollof rice samples of about one gram each from various restaurants were introduced into 9ml sterile water in five test tubes while 1ml of each of the egusi soup samples was introduced with the aid of a sterile syringe into 9ml sterile water. The tubes were left for about 15 minutes after which they were shaken vigorously and serially diluted. 0.1ml inoculum was taken from each tube and introduced on MacConkey agar and blood agar plates. The plates were incubated at 37^0C for 18- 24 hours.

Identification of Isolates

Each diluted sample of about 0.1ml was spread on nutrient agar, blood agar and MacConkey agar plates using a sterile glass rod. The plates were incubated at 37^0C for **24 hours**. Distinct colonies were picked and identified using morphological and biochemical characterization. The organisms were identified to the species level using Bergeys Manual of Determinative Bacteriology.

Antimicrobial Susceptibility Testing

This was done using Kirby- Bauer National Committee for Clinical Laboratory Standard (NCCLS) modified disc diffusion techniques (Cheeseborough, 2000). A loopful of each isolated organism was inoculated uniformly on freshly prepared Nutrient agar. Using a clamp, the antibiotic disc was placed on the seeded agar plates at equal spaces under aseptic conditions. The plates were incubated at 37^0C for 24hours. The zones of inhibition around each disc were measured in millimeters.

Plasmid Analysis

Plasmid DNA isolation was done using standard alkaline phosphate method of Birnboin and Doly while the electrophoresis was done using agar gel electrophoresis.

Plasmid DNA isolation

Buffer A(400mM Tris, 200mM NaEDTA, Acetic acid to adjust pH to 8.0) of about 200µl was added to a cell pellet and it was vortexed after which 400µl of lysing solution (4% Sodium dodecyl Sulphate, 100mM tris) was added and the tubes were inverted 20 times at room temperature. Ice cold buffer B (3M Na Acetate and pH adjusted to 5.5 with acetic acid) of about 300µl was then added, vortexed and the tubes kept on ice for 30 minutes. The tubes were centrifuged at 3000×g for 15 minutes after which 700µof chloroform was added to the supernatant and vortexed. The tubes were further centrifuged at 3000×g for 10 minutes. 1ml of absolute ethanol was added to 500µl aqueous layer and was kept

Table 1. Total viable count (CFU/g) of food samples.

Sample	Counts($x10^3$cfu/g)
Aj	32.61±1.6
Ae	41.0±3.0
Bj	20.0±6.0
Be	29.3±8.3
Cj	35.7±11.9
Ce	8.0±2.0
Dj	26.6±8.2
De	43.0±9.0
Ej	29.6±6.4
Ee	45.6±12.6
	j:jollof rice e:egusi soup

Table 2. Percentage occurrence of each isolate in the food samples.

Organism	Occurrence %	
Klebsiella pneumonia	6	**40**
E. coli	4	**26.67**
A. calcoaceticus	1	**6.7**
B. subtilis	1	**6.7**
L. acidophilus	1	**6.7**
B. cereus	1	**6.7**
M. luteus	1	**6.7**
Total	1	**6.7**
	15	**100%**

in ice for 1 hour. The tubes were centrifuged at 3000×g for 30 minutes. The pellets were washed with 70% ethanol, decanted and dried then 100µl of buffer C (10mM tris, 2mM NaEDTA, acetic acid to adjust pH to 8.0) was added.

Electrophoresis

Loading dye mixed with 10µl of 10bp molecular marker was loaded in the first well. Then 10µl samples and 2µl loading dye were loaded in other wells of agarose. The process was run at 90v for 60minutes in a mupid-ex submarine electrophoresis system and viewed under ultraviolet transillumination.

Plasmid Curing

A volume of nutrient broth was inoculated with aliquot collected from overnight culture grown in nutrient agar containing antibiotics (Chloramphenicol) for 24 hours at 37^0C. The nutrient broth culture was incubated for 3-4 hours to allow minimal growth of the organisms. Sodium deodecyl sulphate (SDS) curing agent sufficient to bring the concentration to 1% (w/v) was added and was incubated at 37^0C for 24- 48hours. Freshly prepared nutrient broth with an aliquot of cured culture was then incubated for 24hours at 37^0C. The antimicrobial sensitivity test for isolates was repeated and the results recorded.

RESULTS AND DISCUSSION

The colony counts of the food samples are shown in table 1.

All the samples had high counts as shown in Table 2 and could be attributed to the hygienic conditions of the handlers, the environment and raw materials used for the cooking. The organisms isolated from the jollof rice were *Klebsiella pneumoniae, E. coli, Bacillus subtilis* and *Micrococcus luteus,* while those isolated from egusi soup samples were *K. pneumonia, Acinetobacter calcoaceticus, Escherichia coli, Lactobacillus acidophilus* and *Bacillus cereus.*

Table 3. Diameter of zones inhibition (mm) of the isolates before curing.

Isolates	GEN	CXM	OFL	AUG	NIT	CPR	CAZ	CRX	SXT
1	19	0	19	0	14	24	0	0	0
2	15	0	20	0	16	22	0	0	16
3	13	0	23	0	17	20	9	0	0
4	14	0	20	0	15	23	0	0	0
5	19	0	21	0	18	24	0	0	13
6	15	0	16	0	14	18	0	0	14
7	14	0	21	0	17	18	0	0	15
8	14	0	20	0	13	17	0	0	0
9	19	0	20	0	14	17	0	0	0
10	15	0	22	0	17	25	0	0	0
11	14	0	19	0	14	22	0	0	0
12	20	0	19	0	17	18	0	0	14
13	12	0	16	0	14	17	0	0	0
14	20	0	16	0	15	19	0	0	13
15	13	0	22	0	15	24	0	0	0

Keys:

1. *K. pneumoniae*- **isolates** 1,3,4,5,7,8
2. *E. coli*- **isolates** 2,9,11,15
3. *A. calcoaceticus*- **isolate** 6
4. *B. subtilis*- **isolate** 10
5. *L. acidophilus*- **isolate** 12
6. *B. cereus*- **isolate** 13
7. *M. luteus*- **isolate**14

GEN- Gentamycine, CXM- Cefixine,
OFL- Ofloxacin, AUG- augumentin,
NIT- Nitrofurantoin, CRX- Cefuroxime,

Table 4. Sensitivity and resistance profile of Bacterial isolates before curing.

ISOLATES	GEN	CXM	OFL	AUG	NIT	CPR	CAZ	CRX	SXT
1	S	R	S	R	S	S	R	R	R
2	S	R	S	R	S	S	R	R	S
3	S	R	S	R	S	S	R	R	R
4	S	R	S	R	S	S	R	R	R
5	S	R	S	R	S	S	R	R	S
6	S	R	S	R	S	S	R	R	S
7	S	R	S	R	S	S	R	R	S
8	S	R	S	R	S	S	R	R	R
9	S	R	S	R	S	S	R	R	R
10	S	R	S	R	S	S	R	R	R
11	S	R	S	R	S	S	R	R	R
12	S	R	S	R	S	S	R	R	S
13	S	R	S	R	S	S	R	R	R
14	S	R	S	R	S	S	R	R	S
15	S	R	S	R	S	S	R	R	R

S- sensitive R- resistant

This finding is similar to reports by Wogu et al. (2011) in which *K. pneumoniae* and *E. coli* were predominant in jollof rice samples from fast food centres in Nigeria but differs from that of Mensah et al. (2002) in which *B. cereus* and *E. coli* were the prevalent bacteria. The presence of these organisms may be attributed to the hygienic conditions of the personnel in contact with the food, the environment and the raw material from which the foods are produced.

The organisms isolated from the egusi samples were similar with those isolated by Obeta and Abriba (2004). The highest zone of inhibition before curing was recorded

Table 5. Sensitivity and resistance profile of Bacterial isolates after Curing.

ISOLATES	GEN	CXM	OFL	AUG	NIT	CPR	CAZ	CRX	SXT
1	S	R	S	R	S	S	R	R	R
2	S	R	S	R	S	S	R	R	S
3	S	R	S	R	S	S	S*	R	R
4	S	R	S	S*	S	S	R	R	R
5	S	R	S	R	S	S	R	R	S
6	S	R	S	R	S	S	R	R	S
7	S	R	S	R	S	S	R	R	S
8	S	R	S	R	S	S	R	R	R
9	S	R	S	R	S	S	R	R	R
10	S	R	S	R	S	S	R	R	R
11	S	R	S	R	S	S	R	R	R
12	S	R	S	R	S	S	S*	R	S
13	S	R	S	R	S	S	R	R	R
14	S	R	S	R	S	S	R	R	S
15	S	R	S	R	S	S	R	R	R

S- Sensitive
R- Resistance
***means change of sensitivity pattern**

Table 6. Percentage resistance before and after curing.

Antibiotics	No. (%) resistance before curing	No. (%) resistance after curing
Gentamycin	0 (0)	0(0)
Cefixime	15(100)	15(100)
Ofloxacin	0(0)	0(0)
Augumentin	15(100)	14(93.3)
Nitrofurantoin	0(0)	0(0)
Ciprofloxacin	0(0)	0(0)
Ceftriazone	15(100)	13(86.7)
Cefuroxime	15(100)	15(100)
Septrin	9(60)	9(60)

Table 7. Antibiotic resistance profile of bacterial isolates before and after curing.

Isolates	Resistance profile before curing	Resistance profile after curing
1	CXM, AUG, CAZ, CRX, SXT	CXM, AUG, CAZ, CRX, SXT
2	CXM, AUG, CAZ, CRX	CXM, AUG, CAZ, CRX
3	CXM, AUG, CAZ, CRX, SXT	CXM, CAZ, CRX, SXT
4	CXM, AUG, CAZ, CRX, SXT	CXM, CAZ, CRX, SXT
5	CXM, AUG, CAZ, CRX, SXT	CXM, AUG, CAZ, CRX
6	CXM, AUG, CAZ, CRX	CXM, AUG, CAZ, CRX
7	CXM, AUG, CAZ, CRX	CXM, AUG, CAZ, CRX
8	CXM, AUG, CAZ, CRX, SXT	CXM, AUG, CAZ, CRX, SXT
9	CXM, AUG, CAZ, CRX, SXT	CXM, AUG, CAZ, CRX, SXT
10	CXM, AUG, CAZ, CRX, SXT	CXM, AUG, CAZ, CRX, SXT
11	CXM, AUG, CAZ, CRX, SXT	CXM, AUG, CAZ, CRX, SXT
12	CXM, AUG, CAZ, CRX, SXT	CXM, AUG, CAZ, CRX, SXT
13	CXM, AUG, CAZ, CRX	CXM, AUG, CAZ, CRX,
14	CXM, AUG, CAZ, CRX	CXM, AUG, CAZ, CRX, SXT

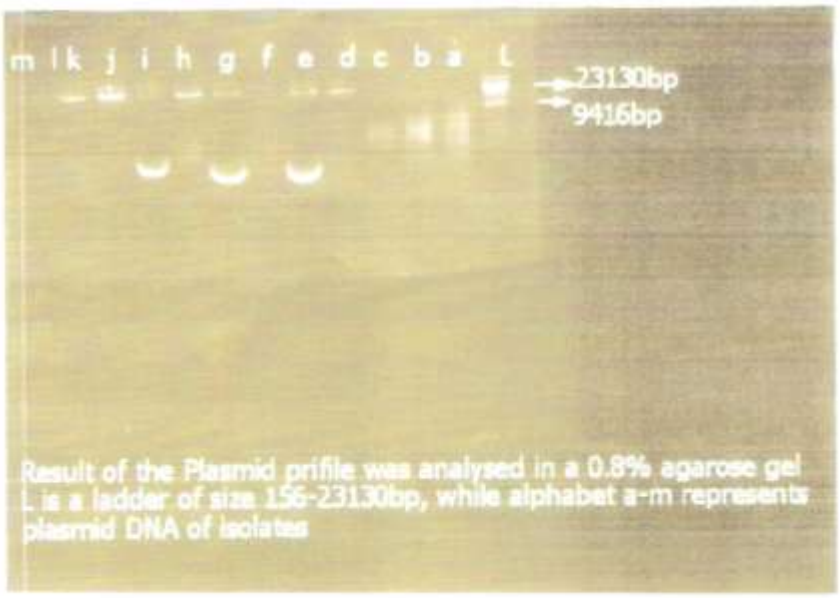

Plate 1: Gel of Plasmid Isolation

Plate 1 shows the result of plasmid analysis of resistant isolates analyzed with 0.8% agarose gel. L represents the DNA ladder. It was observed that after plasmid curing isolates 4, 5, 7, 8, 9, 10 and 11 harbour plasmids with DNA size ≤23130bp.

for Ciprofloxacin (25mm) on *B.subtilis* while there was resistance against Cefixime (0mm), Augumentin (0mm), Ceftriazone (0mm) as shown in table 3. Table 4 showed that all the isolates were sensitive to Gentamycin, Ofloxacin, Nitrofurantoin, Ciprofloxacin and resistance to Cefixine, Augumentin, Ceftriazone and Cefroxime before plasmid curing while after curing all isolates were sensitive to Gentamycin, Ofloxacin, Nitrofurantoin, Ciprofloxacin and resistant to Cefixine and Cefuroxime (table 5).

Table 6 showed that after curing, there was little reduction in the percentage resistance of the isolates to Augumentin and Ceftriazone. The resistance to the other drugs remained the same. The reason for the resistance might be attributed to misuse and overuse of these antibiotics leading to the emergence of resistant strains of bacteria.

Since there was still resistance of most of the organisms to the antibiotics after curing the resistance might be **chromosomally** mediated (Obi et al., 1998). The isolates were resistant to more than one drug. They were resistant to Cefuroxime, Cefixime and Ceftriazone (Cephalosporins), Augumentin (β- lactam inhibitor) and septrin (Sulfonamide) so they are multi-drug resistant (Wasfy et al., 2000).

Plate 1 showed that the isolates harbored plasmids which is indicative of cryptic plasmids. Lyon et al. *(*1984) and Gillespie et al. (1984) isolated small plasmids with no attributable functions but probably responsible for important accessory functions not associated with resistance to any antimicrobial agents tested.

With 0.8% agarose gel electrophoresis, analysis of the bacterial isolates as seen in plate 1, it was found that isolates 4, 5, 7, 8, 9, 10 and 11 harboured plasmids with DNA sizes ≤ 233130bp while isolates 1,2,3,12 and 13 did not harbor plasmids after plasmid curing. Plate 1 revealed that some isolates (5, 7 and 9) harboured more than two plasmids with DNA sizes< 23130 bp which is indicative of cryptic plasmids which are not responsible for drug resistant but in the near future could acquire such resistance.

The study revealed that chromosomal mediated resistance was more prevalent (53.3%) while that of plasmid mediated resistance was less prevalent (46.7%). Resistance to these multi-drugs could be as a result of chromosomal DNA and not plasmid mediated. This is contradictory to the findings of Adeleke et al. (2003) that resistance to high levels of antibiotics is ascribed in most instants to the presence of plasmids.

CONCLUSION

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 foodborne

illness due to these organisms, appropriate antibiotics should be given especially in a combined therapy regimen. It is encouraged that food safety personnel should enforce adherence to rules and regulations on food safety by these restaurants and cafeteria.

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