DISTRIBUTION OF ANTIBIOTIC RESISTANT BACTERIA FLORA IN READY-TO-EAT FOOD SAMPLES SOLD IN CALABAR METROPOLIS

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ABSTRACT

The distribution of antibiotic resistant bacteria flora in ready-to-eat food samples sold in Calabar metropolis was investigated. Thirty (30) samples of different ready-to-eat food samples (cooked indomie, sausage rolls, egg-roll, bean cake, fried meat and fish) were purchased at the point of sales in various eateries located in University of Calabar campus, Watt Market and Marian market and were placed into sterile specimen bottles with screw caps, labeled and transported immediately to the laboratory in an ice-packed container for further analysis. The study was conducted within a period of six months. All the procedures were carried out using standard microbiological techniques. The results of the study revealed the presence of Pseudomonas spp, Escherichia spp, Bacillus spp, Staphylococcus aureus, Enterobacter spp, Klebsiella spp, Proteus spp and Shigella spp. Escherichia coli had the highest percentage of occurrence (17.83%) compared to other bacterial isolate in the analyzed ready-to-eat food samples that had; Klebsiella spp (15.50%), Salmonella spp (14.72%), Enterobacter spp (6.98%), Proteus spp (10.85%), Shigella spp (9.30%), Bacillus spp (4.65%), Pseudomonas spp (6.89%) and Staphylococcus aureus (6.20%). Bacteria isolates from the analyzed ready-to-eat food samples showed a higher percentage resistance to augmentin (100%) and amoxicillin (100%) compared to other antibiotics tested against Cotrimoxazole (55.56%) nitrofurantoin (66.67%), gentamycin (66.67%) ofloxacin (44.44%), tetracycline (55.56%), ciprofloxacin (77.78%) and nalidixic acid (22.22%). Among the bacteria isolates from the ready-to-eat food samples, Staphylococcus aureus had the highest percentage resistant to the antibiotics tested against (Ceftriazone, amoxicillin, cotrimoxazole, nitrofurantoin, augmentin and tetracycline) while Klebsiella spp and Shigella spp showed sensitivity to all the antibiotics tested against. However, the study has revealed the distribution and occurrence of multiple antibiotic resistance among bacteria isolates in ready-to-eat food samples sold in Calabar Metropolis. Thus, there is need for good sanitary practices, and intensive surveillance of isolates throughout food pre- and post production stages so as to reduce or eliminate cases of food borne infections, as well as to also detect emerging antimicrobial resistance bacterial phenotypes.

KEYWORDS: Antibiotics, Resistant, Antimicrobial, Bacterial, Food-borne, Infections.

INTRODUCTION

Anti-microbial resistant bacteria have been recovered from both healthy humans (Irons et al, 2012) and a wide variety of food which include vegetables (Mora et al, 2005), confectionary (Okeke et al, 2005), meat and meat products and poultry (Schoeder et al, 2004). Food and water contaminated by faecal material from healthy human may also be an important source of antibiotic resistant organisms that later cause human infections (Schoeder et al, 2004).

Contamination of ready to eat food may occur during and after processing, and this is of primary concern because such organisms may be pathogenic thereby leading to outbreak of food-borne illness (Russel and Path, 2001). Moreover, non-pathogenic organism that can contaminate man’s food chain from time to time may serve as reservoir of genes for antimicrobial resistance in organisms (Goldstein et al, 2001).

A myriad of substances such as drugs radionuclide and solvents are used in hospital, for medical treatment, diagnostics, disinfection and research (Diab et al 2008), and after application many non-metabolized drugs mainly antibiotics are excreted by the patients and from there it enters waste water and then other environment. Antibiotics are whole range of chemical substances that kill or inhibit the growth of bacteria (Brook et al, 2008), most are naturally produced by living organisms while others are produced synthetically (Brook et al. 2008). They are selectively toxic (affecting pathogenic microorganism more adversely than the host), and this may be as a result of the function of specific receptors required for drugs attachment or it may depend on the inhibition of biochemical events essential to the pathogen.
but not to the host (King et al, 2000). The most selective agents of antibiotics are those affecting structures (e.g. cell wall) or functions (e.g. folic acid synthesis) present only in prokaryotic cells (Walsh and Howe, 2002), while the less selective antibiotics are those affecting protein or nucleic acid synthesis which are essential functions for both prokaryotic (bacteria) and eukaryotic cells (the host) (Walsh and Howe, 2002).

Antibiotics when in these waste water may be present at levels that can not only alter the ecology of the environment but also give rise to antibiotic resistance (Diab et al, 2008). Acquired resistance to antibiotics may arise by cellular mutation or by acquisition of genetic elements in the form of plasmids or transposons (Diab et al, 2008). The occurrence of strongly selective environments such as water and food promotes, not only the growth of resistant bacteria, but also leads to an increase in the frequency of resistant bacterial genes and genetic elements such as plasmids (Khachatourians et al, 2003). Waste water when not effectively treated may contain pathogenic drug-resistant bacteria which constitute the most dangerous single risk factor for dissemination of pathogenic and drug resistant bacterial species in the environment (Cabrera et al 2004). These resistant bacteria species may be transmitted to humans and farm animals hereby causing infection that cannot be treated by conventional antibiotics (Chitris, 2004). Therefore, this forms the main rationale why this study is focused on investigating the distribution of antibiotic resistant bacteria flora in ready-to-eat-foods in Calabar metropolis.

MATERIALS AND METHODS

The study area
The study was carried out in Calabar, which is the capital of Cross River State, Nigeria. It is bounded at north by Odukpani and Akpabuyo Local Government Area, at east by the Republic of Cameroon, at the west by Akwa-Ibom State, and the south by the Atlantic Ocean. Calabar with an approximate population of about one million two hundred thousand (1,200,000) inhabitants (2006 census), is situated seventy-seven (77) kilometers up the Calabar river and over an area of about 604km².

Calabar has an average high temperature of 29⁰C and an average low temperature of 25⁰C. The precipitation of Calabar is about 51mm on the average during the dry season and 445mm on average during the wet season (weather base, 2011). Calabar is located approximately between longitude 80°19'E and 80°21'E and latitude 40°55'N and 40°58'N. Calabar inhabited people are from three (3) ethnic groups which are the Quas, Efuts and the Efiks.

Sample collections

Ready-to-eat-food samples
30 samples of different ready-to-eat-foods comprising of moi-moi (beans pudding), indomie, meat and fish, sausage rolls, egg-roll, doughnut and bean cake were purchased at the point of sales in various eateries located in University of Calabar Campus, Watt market and Marian market and were placed into sterile specimen bottles with tight screw caps, labeled and transported immediately to the laboratory in an ice-packed container for further analysis.

Media
The media used in this study were Nutrient agar, Muller Hinton agar, Motility Indole Ornithine (MIO) (Hardy diagnostics, USA); MacConkey agar, Simmon Citrate Medium (Acumedia, USA). These media were prepared according to the manufacturers instruction.

Chemical and reagents
Chemical used in this study were of analytical grade. They include absolute alcohol, acetone, methanol (Sigma, USA) neutral red, methyl red indicators, phenol red indicator (Titan Biotech, India). Reagents used were oxidade strips, indole kovacs and were products of Hardy diagnostics, USA.

Sample preparation

Ready-to-eat-food samples
10 grams of the ready-to-eat-food samples were aseptically weighed into 90ml of sterile distilled water in a 100ml conical flask. The sample was vortexed to homogenized and allowed to stand for 10minutes. From this initial dilution, 10-fold serial dilutions were carried out in clean sterile test tubes containing 9ml of sterile distilled water.

Plaiting procedures
0.1ml of desire dilutions 10⁻³ – 10⁻⁵ was spread plated in duplicate into nutrient agar and MacConkey agar plates supplemented with 50µg/ml of nystatin Plates were incubated at 37⁰C for 24hours bacterial counts were then recorded.

Purification of Isolates
Following enumeration of total heterotrophic bacteria, representatives of observed colonies were selected and sub-cultured repeatedly into nutrient agar for purification. Purified isolates were stocked in nutrient agar slants for further studies.

Characterization and identification
Purified bacterial isolates were characterized by gram staining and biochemical tests using the scheme in Bergey’s manual of determinative bacteriology (Holt et al, 1994; Cheesbrough, 2000).

Antibiotic susceptibility testing
The antibiotic susceptibility test was determined by the disc diffusion method as described by Bauer et al. (1996). Ten different commercially prepared antibiotic discs (Abtek Biological Ltd, Uk) were used and the concentration of each is as follows; amoxicillin (25µg), cotrimoxazde (25µg), nalidixic acid (30µg), nitrofurantoïn (30µg), gentamycin (10µg), ofloxacin (30µg).
augmentin (30µg), tetracycline (30µg), doxycycline (30µg), trimethoprin/sulfamethazole (25µg).

After 18 hours incubation of the isolates in Mueller Hinton agar at 37°C, the size of the zone of inhibition was measured and interpreted by comparing with 0.5 Mcfarland standard antibiotic sensitivity chart to determine their resistance patterns.

RESULTS

Biochemical characterization and identification of isolate

Bacteria isolates from the ready-to-eat food samples were identified as Escherichia coli, pseudomonas spp, Enterobacter spp, Klebsiella spp, Salmonella spp, Bacillus spp, Staphylococcus aureus, Proteus spp and Shigella spp.

Frequency and percentage occurrence of bacteria isolate from the ready-to-eat food samples

Table 1 presents the result of frequency of occurrence of bacteria isolate from ready-to-eat food and water samples respectively. It showed that Escherichia coli had the highest frequency of occurrence (23%) compared to other isolate counterparts from ready-to-eat food samples that had; Klebsiella spp (20), Salmonella spp (19), Enterobacter spp (18), Proteus spp (14), Shigella spp (12), Pseudomonas spp (9), and Bacillus spp (6).

Fig 1 presents the result of percentage occurrence of bacteria isolate from ready-to-eat food samples, it showed that Escherichia coli had the highest percentage occurrence (17.83%), compared to other isolates that had; Klebsiella spp (15.50%), Salmonella spp (14.72%), Enterobacter spp (13.95%), Proteus spp (10.85%), Shigella spp (9.30%), Pseudomonas spp (6.98%), Staphylococcus aureus (6.20%) and Bacillus spp (4.65%).

Antibiotic susceptibility profile of bacteria isolates from analyzed the ready-to-eat food sample

Table 2 presents the result of antibiotic susceptibility profile of bacteria isolate from the analyzed ready-to-eat food sample.

It showed that bacteria isolate from the ready-to-eat food samples. Its showed a higher percentage resistance to augmentin (100%) and amoxycillin (100%) compared to other isolates tested against; cotrimoxazole (55.56%), nitrofurantoin (66.67%), gentamycin (66.67%), ofloxacin (44.44%), tetracycline (55.56%), ciprofloxacin (77.78%) and ceftriazone (77.78%) while the bacteria isolate showed the least percentage resistance to nalidixic acid (22.22%).

Distribution and proportion of antibiotic resistance among bacterial isolates from samples

Table 3 presents the result of the distribution and proportion of antibiotic resistance among bacterial isolates from ready-to-eat food samples. It showed that 67.44% of the isolates showed resistance to ceftriazone, 83.72% showed resistance to amoxicillin, 50.39% showed resistance to cotrimoxazole, 55.81% showed resistance to nitrofurantoin, 68.22% showed resistance to gentamycin, 24.81% showed resistance to Nalidixic acid, 10.85% showed resistance to ofloxacin, 64.34% showed resistance to augmentin, 79.07% showed resistance to tetracycline, and 59.69% showed resistance to ciprofloxacin.

Fig 2 presents the result of antibiotics resistance pattern in Escherichia coli from ready-to-eat food samples. It showed that the isolate had a higher percentage of occurrence (100%) when tested against amoxicillin, while the isolate was all sensitive to ofloxacin compared to other antibiotics tested against.

Fig 3 presents the result of antibiotic resistance pattern in Klebsiella Spp from ready-to-eat food samples. It showed that the isolate had a higher percentage of resistance with Ciprofloxacin (85%) and least resistance to ofloxacin and cotrimoxazole (20% each).

Fig 4 presents the result of antibiotic resistance pattern in Proteus Spp from ready-to-eat food samples. It showed that the isolate showed a higher percentage resistance to ceftriazone (100%), amoxycillin (100%) and augmentin (100%), while the least resistance was observed with nalidixic acid (21.42%) and ofloxacine (21.42%) compared to other antibiotic.

Fig 5 presents the result of antibiotic resistance pattern in Pseudomonas Spp from ready-to-eat food samples. It showed that the isolate had a higher percentage resistance to ceftriazone (100%), amoxycillin (100%) and Tetracycline (100%), while the least percentage resistance was observed with ofloxacin (55.67%).

Fig 6 presents the result of antibiotic resistance pattern in Salmonella Spp from ready-to-eat food samples. It showed that the isolate had a higher percentage resistance to tetracycline (100%), and least percentage resistance were all sensitive to Ofloxacin compared to other antibiotics tested against.

Fig 7 presents the result of antibiotic resistance pattern in Enterobacter Spp from ready-to-eat food samples. It showed that the isolate had a higher percentage resistance when tested against Amoxycillin (100%), Gentomycine (100%) and Tetracycline (100%), and were all sensitive to Nalidixic acid, compared to other antibiotics tested against.

Fig 8 presents the result of antibiotic resistance pattern in Shigella Spp from ready-to-eat food samples. It showed that the isolate had a higher percentage resistance when tested against cotrimoxazole (83.33%) and Tetracycline (83.33%) and were all sensitive To ofloxacin as compared to other antibiotics tested against.
Fig 9 presents the result of antibiotic resistance pattern in *Bacillus Spp* from ready-to-eat food samples. It showed that the isolate had a higher percentage resistance to ceftriazone (100%), amoxycillin (100%) and least percentage resistance to ofloxacin (33.33%), as compared to other antibiotic tested against.

### Table 1: Frequency of occurrence of bacteria isolate from ready-to-eat food samples.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Frequency</th>
<th>% Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>23</td>
<td>17.83</td>
</tr>
<tr>
<td><em>Enterobacter spp</em></td>
<td>18</td>
<td>13.95</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>8</td>
<td>6.20</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>20</td>
<td>15.50</td>
</tr>
<tr>
<td><em>Salmonella spp</em></td>
<td>19</td>
<td>14.73</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>9</td>
<td>6.89</td>
</tr>
<tr>
<td><em>Proteus spp</em></td>
<td>14</td>
<td>10.85</td>
</tr>
<tr>
<td>Shigella spp</td>
<td>12</td>
<td>9.30</td>
</tr>
<tr>
<td><em>Bacillus spp</em></td>
<td>6</td>
<td>4.65</td>
</tr>
<tr>
<td>Total</td>
<td>129</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 2: Antibiotic susceptibility profile of bacteria isolate from ready-to-eat food sample.

<table>
<thead>
<tr>
<th>Antibiotics tested</th>
<th>Disc potency (µg/ml)</th>
<th><em>Escherichia coli</em></th>
<th><em>Enterobacter spp</em></th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Klebsiella spp</em></th>
<th><em>Salmonella spp</em></th>
<th><em>Pseudomonas spp</em></th>
<th><em>Proteus spp</em></th>
<th><em>Shigella spp</em></th>
<th><em>Bacillus spp</em></th>
<th><em>Staphylococcus aureus</em></th>
<th>% Resistance of organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>25</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>100</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>25</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>55.56</td>
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<tr>
<td>Ciprofloxacin</td>
<td>30</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>22.22</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>44.44</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>30</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>100</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>55.56</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>30</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>77.78</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>30</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>77.78</td>
</tr>
</tbody>
</table>

% Resistance of single organisms = 30 90 60 40 80 90 100 80 30

### Table 3: Distribution and proportion of antibiotic resistance among bacterial isolate from ready-to-eat food samples.

<table>
<thead>
<tr>
<th>Isolates identified</th>
<th>No.</th>
<th>Ce (%)</th>
<th>AMX (%)</th>
<th>COT (%)</th>
<th>NIT (%)</th>
<th>GEN (%)</th>
<th>NAL (%)</th>
<th>OFL (%)</th>
<th>AUG (%)</th>
<th>TET (%)</th>
<th>CIP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>23</td>
<td>10(43.48)</td>
<td>23(100)</td>
<td>23(100)</td>
<td>12(52.17)</td>
<td>12(52.17)</td>
<td>12(52.17)</td>
<td>12(52.17)</td>
<td>12(52.17)</td>
<td>12(52.17)</td>
<td>12(52.17)</td>
</tr>
<tr>
<td><em>Klebsiella spp</em></td>
<td>20</td>
<td>13(65)</td>
<td>12(60)</td>
<td>11(55)</td>
<td>4(20)</td>
<td>5(25)</td>
<td>3(15)</td>
<td>0(0)</td>
<td>4(20)</td>
<td>11(55)</td>
<td>17(85)</td>
</tr>
<tr>
<td><em>Proteus spp</em></td>
<td>14</td>
<td>14(100)</td>
<td>14(100)</td>
<td>10(71.43)</td>
<td>13(92.90)</td>
<td>10(71.43)</td>
<td>3(21.40)</td>
<td>3(21.40)</td>
<td>14(100)</td>
<td>13(92.9)</td>
<td>11(78.57)</td>
</tr>
<tr>
<td><em>Pseudomonas spp</em></td>
<td>9</td>
<td>9(100)</td>
<td>9(100)</td>
<td>9(100)</td>
<td>9(100)</td>
<td>9(100)</td>
<td>9(100)</td>
<td>9(100)</td>
<td>9(100)</td>
<td>9(100)</td>
<td>9(100)</td>
</tr>
<tr>
<td><em>Salmonella spp</em></td>
<td>19</td>
<td>12(63.16)</td>
<td>12(63.16)</td>
<td>10(52.63)</td>
<td>10(52.63)</td>
<td>10(52.63)</td>
<td>10(52.63)</td>
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<td>10(52.63)</td>
<td>10(52.63)</td>
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</tr>
<tr>
<td><em>Enterobacter spp</em></td>
<td>18</td>
<td>10(55.56)</td>
<td>18(100)</td>
<td>0(0)</td>
<td>9(50)</td>
<td>18(100)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>8(44.44)</td>
<td>18(100)</td>
<td>7(38.89)</td>
</tr>
<tr>
<td><em>Shigella spp</em></td>
<td>12</td>
<td>5(41.67)</td>
<td>8(66.67)</td>
<td>10(83.33)</td>
<td>7(58.33)</td>
<td>5(41.67)</td>
<td>2(16.67)</td>
<td>0(0)</td>
<td>6(50)</td>
<td>10(83.3)</td>
<td>7(58.33)</td>
</tr>
<tr>
<td><em>Bacillus spp</em></td>
<td>6</td>
<td>6(100)</td>
<td>6(100)</td>
<td>6(100)</td>
<td>6(100)</td>
<td>6(100)</td>
<td>6(100)</td>
<td>6(100)</td>
<td>6(100)</td>
<td>6(100)</td>
<td>6(100)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>8</td>
<td>8(100)</td>
<td>8(100)</td>
<td>8(100)</td>
<td>7(87.5)</td>
<td>6(75)</td>
<td>4(50)</td>
<td>8(100)</td>
<td>8(100)</td>
<td>6(75)</td>
<td>8(100)</td>
</tr>
</tbody>
</table>

**Total number and percentage resistance exhibited to an antibiotics**

**AMX = Amoxycillin (25µg), COT = Cotrimoxazole (25µg), NIT = Nitrofurantoin (30µg), GEN = Gentamicin (10µg), NAL = Nalidixic acid (30µg), OFL = Ofloxacin (30µg), AUG = Augmentin (30µg), TET = Tetracycline (30µg), Ce = Ceftriazone (30µg), CIP = Ciprofloxacin (10µg)**
Fig. 2: Antibiotic resistance pattern in *Escherichia coli* from ready-to-eat food samples.

Fig 3: Antibiotic pattern in *Klebsiella sp* from ready-to-eat food samples.

Fig 4: Antibiotic resistance pattern in *Proteus spp* from ready-to-eat food samples.

Fig 5: Antibiotic resistance pattern in *Pseudomonas spp* from ready-to-eat food samples.

Fig 6: Antibiotic resistance pattern in *Salmonella* from ready-to-eat food samples.

Fig 7: Antibiotic resistance pattern in *Enterobacter spp* from ready-to-eat food samples.
antibiotics prescriptions in hospitals are given. Nevertheless, however revealed that Escherichia coli contamination and is a threat to public health (Moro et al., 2000; Mora et al., 2005). Its presence is a major health concern especially in cases of verotoxin producing E. coli (VTEC) serogroup 0157, a major cause of haemorrhagic colitis (Moro et al., 2000). Faecal contamination of food and water cannot be prevented entirely, particularly in this setting where hygienic standard of food production and water treatment process is low and not monitored (Famurewa et al., 2003). The frequency of isolation of Salmonella and Shigella spp was also reasonably high and supports individual studies and laboratory records that have however revealed that typhoid fever is endemic in Nigeria (Moro et al., 2001). The percentage occurrence of Klebsiella spp, Shigella spp and Enterobacter spp were a bit high, their contamination could be attributed to the poor hygiene practices of the food handlers before and after food processes upon returning from toilet, lack of disinfection of table tops before and after daily use by customers as well as poor water treatment process (Fang et al., 2013) as this postulation is supported by previous report on the isolation of Salmonella spp, Klebsiella spp, Shigella spp, Staphylococcus aureus and Escherichia coli from the hands of food vendors and food canteens in Nigeria (Famurewa et al., 2003).

Antibiotics susceptibility profile of bacteria isolates from the ready-to-eat food samples showed that all the isolates had multiple resistance to the antibiotics they were tested against. This observation was in agreement with that of Gundogan et al., (2006) who reported to have isolated Klebsiella spp and Escherichia coli with multiple antibiotic resistance from meat, chicken and meat ball samples. Also other studies by Lateef et al., (2005); Majolagbe et al., 2011, have reported to isolate E. coli, Staphylococcus aureus, Salmonella spp and Streptococcus spp from water, food and clinical samples with multiple antibiotic resistance.

The relatively high level of resistance of the isolates to antimicrobial agents, is a reflection of misuse or abuse of these agents in the environment (Abbar and Kaddar, 2001). Antibiotics prescriptions in hospitals are given without clear evidence of infection or adequate medical attention. Broad spectrum antibiotics sometimes given in place of narrow spectrum are drugs as a substitute for culture and sensitivity testing, with consequential risk being super infections and the selection of drug resistant mutants (Prescott et al., 2005). In developing countries, drugs are available to the public and thus people may practice self-administration of antibiotics and further increase the prevalence of drug-resistant strains, also the long standing practice of using low doses of antibiotics for a long period of time for growth promotion and arbitrary use of antibiotics in animal husbandry is a strong contributor to the development of antibiotic resistant bacteria in the environment.

The ability of some of the isolates such as Bacillus spp, Pseudomonas spp, Enterobacter spp, Salmonella spp, Proteus spp. E. coli and Staphylococcus aureus to show

DISCUSSION
In this study, bacteria isolate from ready-to-eat food and water samples were identified as Escherichia coli, Pseudomonas Spp, Enterobacter spp, Klebsiella spp, Salmonella spp, Bacillus spp, Staphylococcus aureus, Proteus spp, and Shigella spp. This observation was not surprising as it corroborates with that of Oluyege et al., 2009; Oladipo and Adejemobi, 2010; and Majolagbe et al., 2011, who all identified Staphylococcus aureus, Bacillus Marcescens, Streptococcus faecalis, Pseudomonas putida, Aeromonas hydrophila, Enterobacter aerogenes, Klebsiella spp, and Proteus spp from ready-to-eat food samples in Ado-ekiti and Ogbomoso, Nigeria respectively.

Escherichia coli was the most common bacteria isolate from the collected ready-to-eat and samples with a percentage occurrence of 17.83% as compared to other isolate from the samples. This observation corroborates with that of Oluyege et al., (2009), who reported a higher occurrence of Escherichia coli in ready-to-eat food samples analyzed. Escherichia coli when found in water and food supplies, is an indicative of a recent faccal contamination and is a threat to public health (Moro et al., 2000; Mora et al., 2005). Its presence is a major health concern especially in cases of verotoxin producing E. coli (VTEC) serogroup 0157, a major cause of haemorrhagic colitis (Moro et al., 2000). Faecal contamination of food and water cannot be prevented entirely, particularly in this setting where hygienic standard of food production and water treatment process is low and not monitored (Famurewa et al., 2003). The frequency of isolation of Salmonella and Shigella spp was also reasonably high and supports individual studies and laboratory records that have however revealed that typhoid fever is endemic in Nigeria (Moro et al., 2001). The percentage occurrence of Klebsiella spp, Shigella spp and Enterobacter spp were a bit high, their contamination could be attributed to the poor hygiene practices of the food handlers before and after food processes upon returning from toilet, lack of disinfection of table tops before and after daily use by customers as well as poor water treatment process (Fang et al., 2013) as this postulation is supported by previous report on the isolation of Salmonella spp, Klebsiella spp, Shigella spp, Staphylococcus aureus and Escherichia coli from the hands of food vendors and food canteens in Nigeria (Famurewa et al., 2003).

Antibiotics susceptibility profile of bacteria isolates from the ready-to-eat food samples showed that all the isolates had multiple resistance to the antibiotics they were tested against. This observation was in agreement with that of Gundogan et al., (2006) who reported to have isolated Klebsiella spp and Escherichia coli with multiple antibiotic resistance from meat, chicken and meat ball samples. Also other studies by Lateef et al., (2005); Majolagbe et al., 2011, have reported to isolate E. coli, Staphylococcus aureus, Salmonella spp and Streptococcus spp from water, food and clinical samples with multiple antibiotic resistance.

The relatively high level of resistance of the isolates to antimicrobial agents, is a reflection of misuse or abuse of these agents in the environment (Abbar and Kaddar, 2001). Antibiotics prescriptions in hospitals are given without clear evidence of infection or adequate medical attention. Broad spectrum antibiotics sometimes given in place of narrow spectrum are drugs as a substitute for culture and sensitivity testing, with consequential risk being super infections and the selection of drug resistant mutants (Prescott et al., 2005). In developing countries, drugs are available to the public and thus people may practice self-administration of antibiotics and further increase the prevalence of drug-resistant strains, also the long standing practice of using low doses of antibiotics for a long period of time for growth promotion and arbitrary use of antibiotics in animal husbandry is a strong contributor to the development of antibiotic resistant bacteria in the environment.

The ability of some of the isolates such as Bacillus spp, Pseudomonas spp, Enterobacter spp, Salmonella spp, Proteus spp. E. coli and Staphylococcus aureus to show
100% resistance to some of the antibiotics such as ceftriaxone, amoxyccilin, augmentin, tetracycline and cotrimoxazole could possibly be as a result of selection pressure created by the use of antimicrobials in food producing animals as well as the indiscriminate use of antibiotics by human (Chui et al 2002; Threlfall et al, 2000). The co-existence of resistance genes with mobile elements such as plasmids, transposons and intergrons facilitates the rapid spread of antibiotic resistance genes among bacteria and this could possibly be the reason for the high percentage resistance (Sunde, 2005).

The total resistance (100%) shown by some of the isolates to ceftriaxone, (a cephalosporin and widely used as broad spectrum antibiotic) which acts by inhibiting cell wall synthesis in growing or dividing cells (Kathleen and Arthur, 2000) is likely due to the presence of β-Lactamase which acts by cleaving β-Lactam ring of cell wall, thus inhibiting antibiotics like ceftriaxone (Warren 2006) while the resistance to other antibiotics by the isolates could as well be due to the fact that antibiotic resistance microorganisms may be associated with reduced penetration of antibiotic into the cell, or from active processes such as changes in the transport of those compounds into or from the bacteria cells (Hermansson et al., 2007).

Most of the bacteria isolates from the analyzed ready-to-eat samples showed significantly higher (p<0.05) percentage resistance to the antibiotics tested. This observation was not surprising, as this could have possibly resulted from inappropriate or uncontrolled use of antibiotic in farming practices, indiscriminate use of antibiotics by humans, indiscriminate dumping of hospital wastes and antibiotic materials in the environment, indiscriminate use of manure as well as human excreta in the environment (Warburton et al, 2002).

CONCLUSION

The study has revealed the distribution and occurrence of multiple antibiotic resistance among bacteria isolates in ready-to-eat food samples sold in Calabar Metropolis. The study thus emphasizes the need for intensive surveillance of isolates throughout the food production continuum as to prevent food borne infections, as well as detect emerging antimicrobial resistance bacterial phenotypes especially in this our developing world.

REFERENCES

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