Evaluation of Bacteria Associated with Ready to Eat Rice in the Niger Delta South-South Nigeria and Their Susceptibility

Christopher M.A1, Nyoyoko V.F2*, Owowo Etanguno E.1 and Uyoh A.E.1

1Department of Microbiology, Akwa Ibom State University, Mkpat-Enin, Nigeria.
2Department of Biological Sciences, Topfaith University, Mkpatak, Nigeria.


ABSTRACT

This study was carried out to investigate the organisms associated with ready-to-eat rice in Ezeke and Madonna Cafeteria, Catholic Prayer Ministry (CPM) Elele cafeteria rice in Niger Delta and their Antibacterial Susceptibility. The study was done using standard microbiological methods. A total of Ten samples were collected aseptically from different cafeteria to avoid contamination and taken to the laboratory for bacteriological analysis which include microbial count using serial dilution and colonies were counted using illuminated colony counter, Gram staining and characterization. Total Aerobic plate count in Madonna and Ezeke cafeteria were 9.1 ± 0.42 and 9.04 ±0.39, respectively. The most predominant bacteria pathogens isolated in this study include Staphylococcus aureus, Bacillus cereus and Escherichia coli. Antibacterial susceptibility pattern of Gram-positive bacteria. Staphylococcus aureus showed 100% resistance to a moxicillin, roceptrin, erythromycin, septrin, zinnacef. Highest sensitivity was observed in gentamycin. Bacillus species was significantly (p< 0.05) sensitive to septrin (69.2%) and significantly (p<0.05) resistant to pefloxacin (11.84%), streptomycin (12,92.3%), erythromycin (13.1 00%) and zinnacef (13, 100%). For antibacterial susceptibility pattern of Gram, negative bacteria. All isolated bacteria were significantly resistant to gentamycin, perfloxacin, tarivid and streptomycin. Shigella species was resistant to all isolated bacteria. Escherichia coli were significantly sensitive to chloramphenicol and augmentin (7.87.5%). Proteus species was significantly sensitive to Chloramphenicol (6,100%) and ciprofloxacin (5,83.3%) This confirmed that pathogenic bacteria can exist in cooked foods even though they may physically appear to be quite wholesome. Proper steps and good hygiene practice should be employed and good measures should be taken to ensure that the occurrence of these organisms in foods is kept within limit to reduce the risk of bacteria.

Keywords
Bacteria, Pathogen, Disease, Food, Rice.

Introduction
Food is one of the vehicles for the transmission of diseases according to IFT, about 250 million people suffer from food-borne diseases and about 5,000 victims die every year. Rice (Oryza spp.) being the second leading cereal after wheat and staple food of half of the world’s population, grown in at least 114 countries with global production of 645 million tonnes. Rice is one of the most important crops in Nigeria and its production represents a significant part of the government strategy to overcome food shortage and improve self-sufficiency for both local consumption and export. Nigeria is the highest importer of rice in Africa and the second highest in the world. Rice is now a daily item on Nigerian menu unlike in the past when Nigerians consumed rice only during ceremonies or festival such as Christmas, Easter, idel-filti and idel-kabi. Today rice is hawked on the street of cities and villages. Nutritionally, a wholesome cereal grain is ideal for diverse nutritional needs. It contains predominately carbohydrate, low in fat and the protein content is comparable to that of wheat, corn, sorghum. Digestibility is high compared to other cereals and provides an excellent source of vitamin E, B (Thiamine, Niacin) and potassium. Rice accounts for about 60-70% of total intake and about 90 – 95% Nigerians consume rice with the former processing better processing and cooking qualities.
Microbiological quality of food indicates the amount of microbial contaminant, it has a high level of contamination indicates low quality of food shortage and it’s handling more likely to transmit diseases. Bacterial count in prepared food and water is a key factor in accessing the quality and safety of food. It also reveals the level of hygiene adopted by food handlers in the course of preparation of such foods. Food and water in particular have been described as vehicle for the transmission of microbial disease among which are those caused by coliforms. These are many reasons why people eat away from home. These include among others absence from home while traveling, studying, while at work or need for a charge in terms of food type or location and as such. Many people resort to buying street vended food, which may be poorly processed. This situation however has resulted to the transfer of food sanitary measures and proper food handling from individuals/families to the food sales point that rarely enforce such practices.

Cereals and cereal product contain microorganism from insects, soil and other sources. Bacillus and micrococcus were common. The determination of the total mesophilic aerobes, coliforms (Escherichia coli), Bacillus cereus and Samonella have been used in order to ascertain hygienic- sanitary quality [1]. Rice, the staple diet in most Asian countries, may become contaminated during growth, harvesting and other agricultural operations such as processing and handling [2]. In general, members of the Pseudomonadaceae, coliforms, and endospore forming bacteria are the most common members of the rice microflora. Worldwide there is increasing demand for high-quality and safe food, free of chemical and physical contaminants and pathogens. Rice is grown in Kharif or wet season in Pakistan. Rice is arguably the most important foodstuff associated with Bacillus cereus food poisoning. Bacillus cereus comprises 10% of the soil microflora in rice paddies [3]. Bacillus cereus grows and produces emetic toxins in a relatively short time on cooked rice and other starchy foods stored at room temperature.

In Nigeria, there is little or no knowledge of foodborne disease and their transmission among food handlers working in ready-to-eat food centers, ad no rules are provided from the establishment of food handlers. Most proprietors of food centers are not duly licensed, and their staff properly selected. Hence, examining the microbiological quality and quantity of cooked rice from food handlers/centers to reduce the risk of food poisoning is a worthwhile adventure.

An estimated 2.5 billion people patronize food-sales point worldwide, food-borne illness is a major international health problem with consequent economic reduction. According to Doyele and Evans, food born diseases are disease resulting from ingestion of bacteria, toxin and cells produced by microorganism present in food. Once the bacterial have produce toxins, the food can be extensively and properly cooked, killing the bacteria without destroying the toxin. Bacteria such as salmonella species, Escherichia coli and staphylococcus aureus, Bacillus cereus can cause food poisoning and other food-borne diseases such as tuberculosis, typhoid fever and cholera. Some symptoms of food borne illness among others include stomach pains, diarrhea, vomiting, nausea and headache. The global incidence of food borne disease is difficult to estimate but it has been reported that the year 2000 alone, 2.1 million people die from diarrheal disease. A great proportion of these cases can be attributed to contamination of food and drinking water. Rice is a potential reservoir of pathogens that transmit diseases. Several studies have attributed diarrhea and associated death to be consumption of contaminated rice. An epidemiological link between rice foods and diarrhea has been reported [4]. Foodborne bacterial pathogens commonly detected in rice are Bacillus cereus, Clostridium perfringens, Staphylococcus aureus and Salmonella Spp. [5-10]. Certain cooked rice sold by street vendors has been implicated in food poisoning outbreaks [11]. Several types of microorganisms have been known to affect the quality of food, thereby constituting health hazards when rice contaminated with these organisms are consumed. The level of contamination of rice is high in this environment and is a cause of concern because of high consumption of rice especially among children. Microbiological examination of rice especially is very important since this helps to evaluate the safety of food and provide measures to prevent foodborne disease outbreaks. According to Itoandon et al. [12], the presence of mesophilic microorganisms in cooked rice is an indication that pathogenic microbes are likely to be present in such foods. A number of cooked rice sold locally in Nigeria have been shown to be highly contaminated with Bacillus species [12-15], Staphylococcus [16-18] and other bacteria species [19-21].

Aim of the study
This study was carried out to investigate bacteria associated with ready to eat (RTE) rice and their susceptibility.

The specific objectives of this research are to:
I. To isolate Bacteria in ready to eat (RTE) rice.
II. To determine the prevalence of bacteria in ready to eat (RTE) rice.
III. To identify and characterize isolate associated with ready to eat (RTE) rice.
IV. To determine Antibacterial Susceptibility

Materials and Methods
Background of Study Site
Madonna University Elele campus is situated in Kelga Local Government Area of Rivers State. It is located in the South-South geopopulated zone of Nigeria. Consumers of cooked rice in Ezeke and Madonna cafeteria, Catholic Prayer Ministry Elele include staff, students, doctors, nurses and Majority of the pilgrims patients.

Collection of Samples
Twenty (20) plates, of ready-to-eat rice were swabbed from two (2) different cafeterias (Ezeke cafeteria and Madonna cafeteria) in Catholic Prayer Ministry Elele. The samples were swabbed using sterile swab stick and taken immediately to the laboratory for microbiological analysis.
**Preparation and Dilution of the Food Samples**

In the preparation and dilution of the food, standard methods were adopted. The plates containing ready-to-eat rice were swabbed from two (2) different cafeterias. The swab stick was labeled with the sample name. Sterile 1% peptone water was poured into swab stick containing the sample and was thoroughly mixed to have an even distribution between the sample and the water. About 9mls (milliliters) of sterile 1% peptone water was pipetted into each of the test tubes and the test tubes were labeled accordingly. 1ml from the stock was pipetted and added into test tube labeled 10⁻¹. Further serial dilution was carried out to 10⁻⁶ dilution factor.

**Cultivation of Microorganisms**

Total Aerobic Plate Counts (TAPC), Coliform Counts (CC), Escherichia coli Counts (EC) and Fungi Counts (FC) were enumerated in nutrient agar, macConkey agar, eosin methylene blue agar and potato dextrose agar respectively. About 0.1ml of the chosen dilutions factor (10⁻⁴) was pour plated aseptically into sterile Petri dishes and incubated for 24 hours [22]. Each of the plates was gently swirled so that there was an even distribution between the sample and the media. The agars were allowed to solidify and the plates inverted and incubated at 37°C for 24 hours. After incubation, the colonies were counted [23].

**Characterization, Isolation and Preservation of Colonies**

Discrete colonies were picked from the plates based on shape, opacity and colour, then sub-cultured on fresh sterile plates. Pure isolates from the corresponding agar slants were characterized and identified using morphological and biochemical characteristics. Molecular characterization was done using the 16SrRNA sequencing.

**Gram Staining of Isolated colonies**

This enables us to differentiate between two groups: Gram negative and Gram-positive bacteria cultures that are morphologically indistinguishable [23].

**Preparation of smear**

The smear was flooded with crystal violet stain and was allowed to stand for 1 minute, after which it was washed with water. The smear was again flooded with Grams’ iodine solution, allowed to stand for 1 minute, and was washed with water. Then the smear was decolorized with ethyl alcohol for 20 seconds after which it was washed with water. The smear was counterstained with safranin for 1 minute and washed with water then allowed to air dry. The gram-positive organisms retain the purple colour of the crystal violet while gram negative appeared red, taking the colour of the safranin [23].

**Principles and Procedures for Biochemical Test**

**Catalase test**

This test is used to detect the presence of the enzyme, catalase, catalase enzyme oxidizes hydrogen peroxide (\(H_2O_2\)) to water and oxygen 2-3 drops of hydrogen peroxide solution was dispersed into slides labeled with the isolates number, using a sterile wooden spatula the isolates were picked and immersed into their respective slides. The rapid appearance of gas bubbles shows catalase positive, while the absence of gas bubbles shows catalase negative [23].

**Oxidase tests**

A piece of filter papers was placed on a sterile Petri-dish and 3 drops of freshly prepared oxidase reagent added. A colony of test organism was removed using a sterile wire loop smeared on a filter paper. The development of a blue-purple colour within a few seconds constitutes a positive result. Thus, test was used in screening colonies suspected to be one of the enterobacteriaceae [22].

**Indole test**

The test was performed to detect the ability of an organism to metabolize tryptophan to indole. The medium was autoclaved at 121°C for 15 min. Peptone water-broth tubes were incubated at 37°C for 24 hours after being inoculated with stock cultures of the isolates. After incubation, 0.5ml of KOVAC’s reagent was added. The result was read immediately, a red ring formation of the surface of the tubes indicated a positive test, while yellow coloration of the surface layer, indicated a negative test [23].

**Utilization of citrate**

This test is based on the ability of the test organism to use citrate as its only source of carbon for metabolism with resulting alkalinity and utilization of ammonia as its site source of nitrogen. 2-3 mls of Simmons citrate agar medium in tubes were inoculated with the test organism, using a sterile wire loop. The inoculated media were incubated at 37°C for 24 hours. Citrate utilization was shown by a change in colour from light green to blue while retention of the original green colour showed citrate negative reaction [23].

**Urease test**

Urease is an enzyme that breaks the carbon-nitrogen bone of amides to form carbon dioxide, ammonia and water. This test is important in differentiating enterobacteria. The test organism was inoculated into a bijou bottle containing 3ml sterile urea broth and phenol red as indicator. It was incubated for 24 hours. A colour change to pink red indicates a positive result [23].

**Coagulase test**

The best used to distinguish between Staphylococcus aureus and Staphylococcus epidermidis. A positive result indicates presence of Staphylococcus aureus. The coagulase causes plasma to clot by converting fibrinogen to fibrin. A drop of sterile distilled water was dropped on a sterile glass slide with a wooden spatula. A suspension of the test organism was emulsified in the drop of distilled water. A drop of reconstituted coagulate plasma was placed adjacent to the drop of bacterial suspension. The two were mixed thoroughly using the wooden spatula. An immediate formation of granular precipitate of white clumps within 15-20 seconds indicates a positive reaction [23].

**Sugar fermentation test**

This is to determine the ability of the organism to attack a specific carbohydrate incorporate in to a basal medium with production of
acid and gas. The glucose and lactose sugars were mixed in phenol red in two conical flasks respectively. The mixtures were then dispersed into bijou bottles, inoculated with the test organism, and incubated at 37°C for 24 hours. A colour change to yellow indicates an acid production while production of gas bubbles indicated gas production [23].

Antibacterial Susceptibility Testing
The antibacterial susceptibility testing used the Kirby-Bauer NCCLS modified disc diffusion technique and all procedures were done using aseptic technique. 0.5 Mc farland standard was prepared. The turbidity of the test inocula can be compared against this barium sulphate standard. When matched with the standard, the inocula should give confluent growth [23]. A small volume of the turbid solution was then transferred to a sterile bijou bottle; peptone water was prepared, dispensed into bijou bottles and autoclaved at 121°C for 15 minutes. Suspensions of the isolates were made in the peptone water [23].

The turbidity of these test suspensions was matched with the turbidity of the Mc farland standard. Mueller Hinton agar was prepared as instructed by the manufacture, then poured into sterile Petri dishes, and allowed to set. Mueller Hinton agar plates were labeled for each organism. A sterile swab was dipped into test suspension and excess fluid was removed by pressing and rotating the swab against the side of the bottle above the level of the suspension and then streaked evenly over the surface of the medium in three directions, rotating the plate approximately 60°C to ensure even distribution. With the Petri dish lid in place, the surface of the agar was allowed to dry for 3-5 minutes. Using a sterile forceps Maxi care antimicrobial disc was aseptically picked and place centrally on the surface of the inoculated plates. The plates were incubated at 37°C for 24 hours and then examined for the appearance of a clear zone of inhibition around the disc. The zone of inhibition was measured in millimeters using a ruler [23].

Statistical Analysis
Result was reported as mean ± standard deviation. All data were subjected to statistical analysis by one-way analysis of variance (ANOVA). The result was considered significant at P < 0.05. Least significant difference test (LSD) was also performed between each treatment and the control. Correlation (association) and regression (changes) analysis done using statistical product and service solution (SPSS) windows version 20.0.

Results
Table 1 shows the mean count of microorganisms isolated from cooked rice sold in Ezeke and Madonna Cafeteria. Analysis of variance showed that, total aerobic plate counts of rice from Ezeke (9.04 ± 0.39) were not significantly different from that of rice sample from Madonna Cafeteria. Similarly, CC for rice sold in Ezeke (8.92 ± 0.37 Log10 CFU/g) was not significantly different (p > 0.05) from that of samples from Madonna Cafeteria (8.70 ± 0.25 Log10 CFU/g), EC of rice from Ezeke (8.58 ± 0.18 Log10 CFU/g) was not significantly different (p > 0.05) from that of samples from Madonna Cafeteria (8.69 ± 0.27 Log10 CFU/g). Fungi counts of the rice were 8.63 ± 0.15 Log10 CFU/g for samples from Ezeke and 8.60 ± 0.13 Log10 CFU/g for samples from Madonna Cafeteria.

From Table 2 bi-variate chi square showed that Staphylococcus species (15, 75%) and Bacillus species (13, 65%) were significantly most prevalent (p < 0.05) in the rice while Shigella species (03, 15%) were significantly the least prevalent organism. Pathogenic organisms isolated include Salmonella species (08, 80%). There was no significant difference (p > 0.05) in the frequency of occurrence of the fungi isolated. Fungi isolated include; Aspergillus species (06, 30%), Mucor species (03, 15%), Penicillium species (05, 25%) and Rhizopus species (04, 20%).

Table 3 shows the antibacterial susceptibility pattern of Gram-positive bacteria. There was significantly higher (p < 0.05) rate of resistance than sensitive bacteria to tested antibiotics. Staphylococcus aureus was showed 100% resistance to amoxicillin, roceptin, erythromycin, septrin and zinnacef. Highest sensitivity was observed to gentamycin. Bacillus species was significantly (p < 0.05) sensitive to septrin (69.2%) and significantly resistant (p < 0.05) to pefloxacin (11, 84.6%), streptomycin (12, 92.3%), erythromycin (13, 100%) and zinnacef (13, 100%).
Table 2: Percentage occurrence of microorganisms isolated from cooked rice sold in Ezeke and Madonna Cafeteria, CPM Elele.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Ezeke Cafeteria</th>
<th>Madonna Cafeteria</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 10</td>
<td>N = 10</td>
<td>N= 20</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus</em> species</td>
<td>08 (80)</td>
<td>5 (50)</td>
<td>13 (65)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>06 (60)</td>
<td>2 (20)</td>
<td>08 (40)</td>
</tr>
<tr>
<td><em>Proteus</em> species</td>
<td>04 (40)</td>
<td>2 (20)</td>
<td>06 (30)</td>
</tr>
<tr>
<td><em>Staphylococcus</em> species</td>
<td>09 (90)</td>
<td>6 (60)</td>
<td>15 (75)</td>
</tr>
<tr>
<td><em>Salmonella</em> species</td>
<td>05 (50)</td>
<td>3 (30)</td>
<td>08 (40)</td>
</tr>
<tr>
<td><em>Shigella</em> species</td>
<td>01 (10)</td>
<td>2 (20)</td>
<td>03 (15)</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus</em> species</td>
<td>03 (30)</td>
<td>3 (30)</td>
<td>06 (30)</td>
</tr>
<tr>
<td><em>Mucor</em> species</td>
<td>01 (10)</td>
<td>2 (20)</td>
<td>03 (15)</td>
</tr>
<tr>
<td><em>Penicillium</em> species</td>
<td>03 (30)</td>
<td>2 (20)</td>
<td>05 (25)</td>
</tr>
<tr>
<td><em>Rhizopus</em> species</td>
<td>02 (20)</td>
<td>2 (20)</td>
<td>04 (20)</td>
</tr>
</tbody>
</table>

Table 3: Percentage antimicrobial susceptibility pattern of gram positive bacteria isolated from cooked rice

<table>
<thead>
<tr>
<th>Disc code</th>
<th><em>Staphylococcus</em> species</th>
<th><em>Bacillus</em> species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible%</td>
<td>Resistant%</td>
</tr>
<tr>
<td>PEF</td>
<td>2(13.3)</td>
<td>13(86.7)</td>
</tr>
<tr>
<td>CN</td>
<td>9(60)</td>
<td>6(40)</td>
</tr>
<tr>
<td>AM</td>
<td>-</td>
<td>15(100)</td>
</tr>
<tr>
<td>RO</td>
<td>-</td>
<td>15(100)</td>
</tr>
<tr>
<td>CPX</td>
<td>7(46.7)</td>
<td>8(53.3)</td>
</tr>
<tr>
<td>S</td>
<td>6(40)</td>
<td>9(60)</td>
</tr>
<tr>
<td>SXT</td>
<td>-</td>
<td>15(100)</td>
</tr>
<tr>
<td>E</td>
<td>-</td>
<td>15(100)</td>
</tr>
<tr>
<td>Z</td>
<td>-</td>
<td>15(100)</td>
</tr>
</tbody>
</table>

**KEY**

PEF - Pefloxacin  
CN - Gentamycin  
APX - Ampiclox  
AM - Amoxacillin  
RO - Rocepitin  
CPX - Ciprofloxacin  
S - Streptomyacin  
SXT - Septin  
E - Erythromycin  
Z - Zinnacef
Table 4: Percentage antimicrobial susceptibility pattern of gram negative bacteria isolated from cooked rice.

<table>
<thead>
<tr>
<th>Disc code</th>
<th>E coli</th>
<th>Proteus</th>
<th>Salmonella</th>
<th>Shigella</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sus%</td>
<td>R%</td>
<td>Sus%</td>
<td>Res%</td>
</tr>
<tr>
<td>SXT</td>
<td>2(25)</td>
<td>6(75)</td>
<td>3(50)</td>
<td>3(50)</td>
</tr>
<tr>
<td>2(66.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1(12.5)</td>
<td>7(87.5)</td>
<td>-</td>
<td>6(100)</td>
</tr>
<tr>
<td>3(100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td>4(50)</td>
<td>4(50)</td>
<td>2(33.3)</td>
<td>4(67)</td>
</tr>
<tr>
<td>3(100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPX</td>
<td>2(25)</td>
<td>6(75)</td>
<td>1(16.7)</td>
<td>5(83.3)</td>
</tr>
<tr>
<td>3(100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>3(37.5)</td>
<td>5(62.5)</td>
<td>4(67)</td>
<td>2(33.3)</td>
</tr>
<tr>
<td>3(100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AU</td>
<td>1(12.5)</td>
<td>7(87.5)</td>
<td>-</td>
<td>6(100)</td>
</tr>
<tr>
<td>3(100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>-</td>
<td>8(100)</td>
<td>-</td>
<td>6(100)</td>
</tr>
<tr>
<td>3(100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEF</td>
<td>-</td>
<td>8(100)</td>
<td>-</td>
<td>6(100)</td>
</tr>
<tr>
<td>3(100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OFX</td>
<td>-</td>
<td>8(100)</td>
<td>-</td>
<td>6(100)</td>
</tr>
<tr>
<td>3(100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>-</td>
<td>8(100)</td>
<td>-</td>
<td>6(100)</td>
</tr>
<tr>
<td>3(100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

KEY
SXT - Seprin
C - Chloramphenicol
SP - Sparfloxacin
CPX - Ciprofloxacin
AM - Amoxacillin
AU - Augmentin
CN - Gentamycin
PEF - Pefloxacin
OFX - Tarivid
S - Streptomycin

Table 4 shows the antibacterial susceptibility pattern of Gram-negative bacteria. All isolated bacteria were significantly (p < 0.05) resistant (100 %) to gentamycin, perfoxacin, tarivid and streptomycin. Shigella species was resistant to all isolated bacteria. E. coli was significantly sensitive (p < 0.05) to chloramphenicol (7, 87.5%), and augmentin (7, 87.5%). Proteus species was significantly sensitive (p < 0.05) to Chloramphenicol (6, 100%) and ciprofloxacin (5, 83.3%). Shigella species was significantly resistant (p < 0.05) to all antibiotics tested.

Discussion
When cooked rice is kept at ambient temperature for several hours, it may serve as a medium for the growth and proliferation of food borne pathogens, which causes food borne illness. Good sanitation can improve storage of cooked rice. The microbial counts observed in all cooked rice samples from the two different cafeterias can be attributed to handling procedure during sale or because of opening and covering of coolers, which enhances contamination from the atmosphere. The presence of Escherichia coli and Salmonella species points to possibility of faecal contamination of cooked rice by food handlers. Escherichia coli have been associated with outbreak of diarrhea in adult but mostly children less than five years of age. The presence of Salmonella species in food indicates that many students and members of this community are more likely to suffer from gastroenteritis associated with Salmonella species. The presence of Staphylococcus species may be indications of poor sanitary method, because it is the normal flora of the body, which is likely from food handlers.

Antibacterial susceptibility pattern of Gram-positive bacteria. There was significantly higher (p < 0.05) rate of resistance than sensitive bacteria to tested antibiotics. Staphylococcus aureus was showed 100% resistance to amoxacillin, roceptin, erythromycin, septrin and zinacef. Highest sensitivity was observed to gentamycin. Bacilllus species was significantly (p < 0.05) sensitive to seprin (69.2%) and significantly resistant (p < 0.05) to pefloxacin (11, 84.6%), streptomycin (12, 92.3%), erythromycin (13, 100%) and zinacef (13, 100%) [23].
Antibacterial susceptibility pattern of Gram-negative bacteria. All isolated bacteria were significantly (p < 0.05) resistant (100 %) to gentamycin, perfluoxacin, tarivid and streptomycin. Shigella species was resistant to all isolated bacteria. E. coli was significantly sensitive (p < 0.05) to chloramphenicol (7, 87.5%), and augmentin (7, 87.5%). Proteus species was significantly sensitive (p < 0.05) to Chloramphenicol (6, 100%) and ciprofloxacin (5, 83.3%). Shigella species was significantly resistant (p < 0.05) to all antibiotics tested [23]. The consumers of ready-to-eat rice sold in Ezeki and Madonna cafeteria, CPM Elele are at risk of acquiring food borne diseases.

Conclusion
The high occurrence of Staphylococcus species indicated contamination from handlers. Thus, standard sanitary measures should be carried out when preparing any meal especially with cooked rice to avoid food borne illness and food poisoning. Most of the bacterial were resistant to antibiotic. This confirmed that pathogenic bacteria could exist in cooked foods.

The result drawn from this prevalent organism associated with ready-to-eat rice suggests that there is need to check the free attitudes shown by cooks and food handlers during preparation and handling of ready-to-eat rice in Ezeki and Madonna cafeteria, CPM Elele. Proper steps and good hygiene practice should be employed and good measures should be taken.

Recommendations
The isolation of pathogenic microorganisms associated with plate containing ready-to-eat rice sold in Ezeki and Madonna cafeteria, CPM Elele was an indication of potential health hazard. Therefore the following measures should be adhered to in order to reduce the risk of food borne illnesses. The CPM authority should organize workshops to enlighten food handlers on the standard practices that should be adopted for the proper handling and cooking of foods for sale in Elele. They should also monitor the health status of the food handlers including the cleanliness of the cooking utensils and environment. Food handlers should wash their hands after cleaning tables, coughing or sneezing and after touching any item that can contaminate their hands. Ready-to-eat rice should not be exposed to carriers like flies, which could be agent of food borne disease. Thus, if all these rules are adhered to, we must have very good and pathogenic-free ready-to-eat rice in Ezeki and Madonna cafeteria, CPM Elele.

References
15. Okonko JO, DConbraye E, Babatunde SO. Microbiological quality of sea food processors and Water used in two different sea processing plant in Nigeria. EJEAfche. 2009; 8: 621-629.


© 2022 Christopher MA, et al. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License