IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 12, Issue 9 Ver. III (September. 2018), PP 69-73
www.iosrjournals.org

Diversity And Antibiogram Of Microbialcontaminants From Ready-To-Eat Foods Hawked In Nsukka Town: Implications For Public Health

U. Okekeaji[1*], V.C. Okore,[1] E.C. Ibezim[1],P.E. Martins[2], K.C. Odoh[2]
[1]*Department of Pharmaceutics, University of Nigeria, Nsukka 410001*
[2]*Department of Microbiology, University of Nigeria, Nsukka 410001*
Corresponding Author: U. Okekeaji

Abstract:*Hawked foods are easy means of getting access to some cooked food as well a formidable source for the spread of pathogenic microorganisms. Different ready-to-eat foods were studied for the incidence of pathogenic along with resistant microbes. Twenty-eight species of microorganisms were isolated and identified with standard cultural and biochemical methods while molecular procedures were used to detect the commonness of impervious genes in some of the microbes. These isolates were Enterobacteraerogenes (1), Staphylococcusaureus (1), Shigelladysenteriae (1), Bacillus spp. (15), Candida spp. (7), and Aspergillus spp. (3). The bacterial contaminants were responsive to amoxicillin, chloramphenicol, erythromycin, gentamicin, levofloxacin, and streptomycin. S. auerus, E. aerogenes and S. dysenteriae showed additional susceptibility to ciprofloxacin, norfloxacin, and rifampicin. Some Bacillus spp. were resistant to ampicillin/cloxacillin, ciprofloxacin, norfloxacin and rifampicin. The fungal contaminants were completely (100%) sensitive to itraconazole. A growing resistance of the isolates for ketoconazole, fluconazole/griseofulvin and nystatin were recorded. The multiple antibiotic resistance (MAR) index was low for bacterial contaminants than the fungal isolates which had higher MAR index. Aspergillus species divulged the prevalence of resistant genes, MDR3. The study revealed that cooked foods hawked in Nsukka town are contaminated with multidrug resistant bacteria and fungi.*

Keywords: *Hawked food, antibiotics, MAR index, Ready-to-eat foods, Resistance genes*

Date of Submission: 04-10-2018 Date of acceptance: 16-10-2018

I. Introduction

Food plays a vital role in the growth ordecline of a nation. This is because of its effect on the health of the population. Hawked food plays an important role in meeting food need of people in many cities and towns. Many personsfeed on hawked food daily. The foods are affordable and easily accessible (Tambekar et al., 2008). Ready-to-eat food can bedefined as food consumed ordinarily in the state in which it is sold by food vendors, hawkers or at local market and not including nut in the shell and whole, raw fruits, vegetables that are intended for peeling or washing before consumption (Eni et al., 2010).They often come with lots of risk. This is because of its consumption at the point of sale without further preparation or cooking (Castro-Rosas etal., 2007). Ready-to-eat food can be cooked, raw, chilled or hot. (Tsang, 2002). Different terms like "convenient", "ready", "instant" and "fast" foods have been used to describe such food.

The present economic condition has resulted in situation where food marketing has turned out to be very substantial in most countries since it contributes to sources of income for individuals involved in selling such foods (Agwa et al., 2012). Microorganisms' ubiquity and their role as causative agents of food-borne illnesses have been recognized as far as in the 19th century.

Food is very vital for the sustenance of the human health. However, the preparation of foods is often associated with inadvertent contamination by microorganisms (Van Ermengen, 1998). The growing rate of resistant organisms has been observed to be the outcome of inapt use of antibiotics. Evidence has shown that the use of antibiotics in livestock has resulted to impervious food-borne pathogens that may be transmitted to humans. Against this backdrop, this study was intended with a view to ascertain the diversity of bacterial and fungal contaminants in foods hawked around Nsukka town.

II. Materials And Methods

Study area

This study was carried out in Nsukka, Southeastern Nigeria with a coordinates of 6°51′24″N 7°23′45″E, and elevation of 1,810 ft (550 m). The town is located in tropical rain forest, with a

relatively humid condition occurring highest between March and November. The average annual rainfall is about 2,000 milliliters with heavy rainfall during the rainy season and has a mean daily temperature of 26.7^{o}C.

Sample collection

Seven different samples were purchased from food hawkers at Ogige market, Nsukka town. The food samples were: Agidijollof (spiced mealie meal), Igbangwuoka (maize meal), Echicha (pigeon pea with cocoyam), Okpa (bambara nut meal), Ayarayaji (pigeon pea with yam), Rice and stew, and Ayarayaoka/Ichipe (pigeon pea with maize).

Sample preparation

One gramme (1g) of each of the food samples was homogenized in 9 ml of sterile normal saline. A loopful of each of the samples was inoculated on a surface-dried sterile nutrient agar and Sabouraud's dextrose agar plates for bacteria and fungi respectively. These plates were then incubated at 35˚C for 18-24hand 72h for bacteria and fungi respectively.

Identificationandcharacterization of the isolates

Bacterial isolates were identified using standard microbiological and biochemical tests while the fungal isolates were based on their colonial morphology.

Identification of resistant genes

PCR amplification of the resistant genes, MDR3 and CYP51 of Aspergillus spp. using gene specific primers (MDR3L50: GATGCATCCTGCAAAGTACG, MDR3R50: AGGCTCCTTGGTGCTTGAC, CYP51L50: TTGCGTGCAGAGAAAAGTATG, CYP51R50: GACCTCTTCCGCATTGACAT).

Susceptibilitytesting of the isolates

This was done against ten antibiotics using disc diffusion assay on Mueller-Hinton agar for bacteria and on Sabouraud dextrose agar, by agar well diffusion assay using five antifungal drugs for fungi.They included nystatin (50,000 IU), itraconazole (10 mg), fluconazole (5 mg), griseofulvin (50 mg), and ketoconazole (20 mg).

Multiple antibiotic resistance index (MARI)

The multiple antibiotic resistance index refers to the quantity of antibiotics that a microbial isolate is resistant to. It was calculated as$\frac{a}{b}$; where a, is the number of antibiotics resisted by isolates and b, the total number of antibiotics tested.

III. Results

Of the seven food samples examined, two had no bacterial contaminant, three had both bacterial and fungal contaminants and two had only fungal contaminants. The microbial contaminants were characterized as Bacillus spp.,Staphylococcusaureus, Enterobacteraerogenes, Shigelladysenteriae, Aspergillus and Candida species.

The bacterial isolates were all responsive to gentamycin, chloramphenicol, erythromycin, streptomycin, amoxicillin, and levofloxacin was while the percentage resistance to other antibiotics increases as follows: norfloxacin (5.57%), ciprofloxacin (22.22%), rifampicin (27.78%), and ampicillin (33.33%). The multiple antibiotic resistant indices of the microbial isolates calculated were 0 in five isolates, 0.1 in ten isolates and 0.2 in three isolates for bacteria(Tables 1 and 2).

The fungal contaminants were all sensitive to itraconazole. Ten percent of them were resilient to ketoconazole; 40% to fluconazole and Griseofulvin; 80% to nystatin. The isolates were 100% sensitive to itraconazole. The MARI was 0.6 in three isolates; 0.2 in four isolates and 0.4 in 4 isolates (Tables 3 and 4).

Table 1. Percentage susceptibility of all the bacterial isolates

Antibiotics	Susceptibility (%)	Resistance (%)
Ciprofloxacin	77.78	22.22
Norfloxacin	94.44	5.57
Gentamycin	100	0
Amoxicillin	100	0
Streptomycin	100	0
Rifampicin	72.22	27.78
Erythromycin	100	0
Chloramphenicol	100	0
Ampicillin/cloxacillin	66.67	33.33
Levofloxacin	100	0

Table 2. MAR index of bacterial isolates

Isolates	Number of resisted antibiotics	Number of susceptible antibiotics	MAR index
1	0	10	0
2	1	9	0.1
3	1	9	0.1
4	0	10	0
5	0	10	0
6	1	9	0.1
7	2	8	0.2
8	0	10	0
9	1	9	0.1
10	1	9	0.1
11	1	9	0.1
12	2	8	0.2
13	1	9	0.1
14	2	8	0.2
15	1	9	0.1
16	1	9	0.1
17	1	9	0.1
18	0	10	0

Table 3. Percentage susceptibility profile of all the fungal isolates

Antifungal agent	Susceptibility (%)	Resistance (%)
Fluconazole	60	40
Itraconazole	100	0
Nystatin	20	80
Ketoconazole	90	10
Griseofulvin	60	40

Table 4.MAR index for fungal isolates against antifungal agents

Isolates	Number of resisted antifungal agents	Number ofsusceptible antifungal agents	MAR index
1	3	2	0.6
2	1	4	0.2
3	2	3	0.4
4	2	3	0.4
5	2	3	0.4
6	3	2	0.6
7	3	2	0.6
8	1	4	0.2
9	1	4	0.2
10	1	4	0.2

Identification of resistant genes

PCR amplification of the resistant genes, MDR3 and CYP51 of Aspergillus spp. andDNA preparation yielded about 100bp of the MDR3(Figure 1).

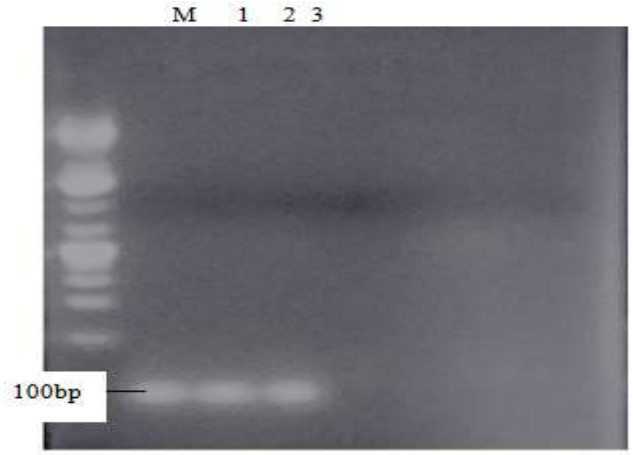

Figure 1. Agarose gel electrophoresis of PCR amplified MDR3 gene of the Aspergillus species.
M = DNA marker, 1-3 =Aspergillus isolates

IV. Discussion

Contaminated ready-to-eat foods are potentially hazardous to public health because they support growth of pathogenic bacteria. Isolation of Bacillus spp.,Enterobacter aerogenes, Staphylococcus aureus, Shigelladysenteriae, Aspergillus spp. and Candida spp. from the ready-to-eat foods,is a sign of poor hygienicpractices among the hawkers. These organisms are known foodborne and opportunistic pathogens that have been implicated in disease outbreaks from food (Tambeker et al., 2008; Oranusi and Braide, 2012). The presence of microbial contaminants (bacteria and fungi) have been reported in ready-to-eat foods in the previous works of Ajao and Atere (2009) in which the bacteriological assessment and hygienic standards of food canteens in Kwara State Polytechnic, Ilorin, Nigeria was carried out, Fowoyo and Igbokwe (2014) whereby the impact of air pollution on the microbial quality of ready-to-eat hawked foods sold in around a cement factory in Lokoja, Nigeria was determined, Madueke et al. (2014) where the microbial analysis of street foods was undertaken and Oranusi and Braide (2012) who took a study of the microbial safety of ready-to-eat foods vended in high ways: Onitsha-Owerri, South east Nigeria. In most cases, foods and ingredients are exposed to contamination from unwashed hands and materials used for wrapping, such as leaves and polythene bags (Agwa et al., 2012). The presence of these organisms in hawked foodsdepictpoor sanitary practices during the processing and packaging of these food. These presence of Bacillus species isin line with previous report on related research (Oladipo and Adejumobi, 2010; Wogu et al., 2011; Oladipo and Fajemilo 2012).Similarly, Kaneko et al. (1999), Das et al. (2010), and Sina et al. (2011) have separately isolated Staphylococcus aureus and Bacillus spp. in ready-to-eat foods. Mensah et al. (2002) reported the presence of Bacillus cereus, Staphylococcus aureus and organisms in the family enterobacteriaceae in street food in Accra, Ghana. The presence of Bacillus spp. in ready-to-eat food could be as a result of food not properly cooked or its spores being resistant to heat. This contributes to their existence, and distribution within the entire food chain from raw agricultural products to finished retailed products (Eglezos et al., 2010). Staphylococcalcontaminants can arise from human contact during handling, processing or vending (Nester et al., 2001; Oladipo and Fajemilo, 2012). Staphylococcus aureus, a microflora present in different parts of human body, is a good pointer of contamination due to poor hygiene practices (Nester et al., 2001). Members of the enterobacteriaceaefamilylike Salmonella and Shigella,are useful gauges of low hygiene standards and post-processing contamination of heat processed food (Gitahi et al., 2012; Oladipo and Fajemilo, 2012). Aspergillus spp. disperse in form of spores which are abundant in the environment and can be introduced through dust and soil (Madueke et al., 2014). They are known with mycotoxin production in food which is a serious problem in public health((Makun et al., 2009)

Purchasing ready-to-eat food from the market exposes one to a considerable public health risks (Agwa et al., 2012). Staphylococcus aureusproduces toxin during growth at permissive temperature 6°C and 46°C, and leads to staphylococcal food-borne disease through the consumption of foods infested with it(le Loir et al., 2003). It is associated with hypersalivation, vomiting, nausea and abdominal cramp with or without diarrhoea (Fowoyo and Igbokwe, 2014; Kadariya et al., 2014). Shigella dysenteriae has been linked to severe bacillary dysentery in most developing countries. This could be fatal in children if not diagnosed and treated on time (Adams and Moss, 2008; Abe et al., 2012). Bacillus spp. are associated with production of toxin and food poisoning(Opinion of the Scientific Panel on Bio Hazards on B. cereus and other Bacillus species in Foodstuff, 2005).

Antibiotic resistance has increased worldwide leading to setbacks in the treatment of human infectious diseases (Afroz et al., 2013). Resistance to antibiotics could be as a result of enzymatic inactivation or modification of antimicrobial agent, impermeability of the cell walls or cell membrane, expulsion of the drugs through the efflux pump or alteration in target receptors (Sayah et al., 2005). MAR index values greater than 0.2 indicate high risk of contamination where antibiotics are often used (Osundiya et al., 2013). MAR index of the isolates was low.The presence of resistant microorganisms in food can pose a public health hazard when transferred to humans, especially those with impaired immunity. More so, the eradication of these organisms may be difficult becauseof growing resistance to the antibiotics used in human medicine.

V. Conclusion

This study demonstrated that ready-to-eat food hawked in Nsukka are contaminated with pathogenic and multidrug resistant microorganisms.Although most of these foods are delicacies common in the study area, theirconsumptionmay be harmful and their consumersmay be placed at high health risk as well asthe inefficiency of commonly used clinical antibiotics for the treatment of infections. Therefore, it is essential to create awareness on foodbornediseases to improve the hygienic practices amongst food retailers. In addition, foods vended and the quality of the foods sold to the public should be monitored to avoid outbreak of foodborne diseases.

References

[1]. Abe, A. S., Inuwa, B., Abbas, H., Sule, A. M., Mohammed, H. A.,& Gero, M., 2012, Identification and characterization of bacteria air pathogens for homes in Zaria metropolis,InternationalJournalofScienceandTechnology 2, 7,pp. 443 – 446.

[2]. Adams, M. R.,& Moss, M. O., 2008,Food Microbiology (3rd ed.), The Royal Society of Chemistry, Cambridge, United Kingdom, pp. 182 – 269.

[3]. Afroz, H., Sultana, F., Fakrddin, Md., Kamrunnahar, Khan, Z. U. M., & Datta, S., 2013, Isolation of Escherichia coli and Staphylococcus aureus from full cream powder milk sold under market conditions at Dhaka, Bangladesh and their antibiotic susceptibility,Journal of Advanced Scientific Research, 4, 3,pp. 27 – 31.

[4]. Agwa, O. K., Uzoigwe, C. I.,& Wokama, E. C., 2012, Incidence and antibiotic susceptibility of Bacillus cereus isolated from ready-to-eat foods sold in some markets in Port Harcourt, Rivers State, Nigeria,Asia Journal of Microbiology, Biotechnology, and Environmental Sciences,14, 1, pp. 13 – 18.

[5]. Ajao, A.T., & Atere, T.G., 2009, Bacteriological assessment and hygienic standard of food canteens in Kwara State Polytechnic, Ilorin, Nigeria,AfricanScientistJournal, 3, 10, pp. 173 – 180.

[6]. Castro-Rosas, J., Cerna-Cortés, J. F., Méndez-Reyes, E., Lopez-Hernandez, D.,Gómez-Aldapa, C. A., & Estrada-García, T., 2012, Presence of faecal coliforms, Escherichia coli anddiarrheagenic E. coli pathotypes in ready-to-eat salads, from an area where crops are irrigated with untreated sewage water,International Journal of Food Microbiology, 156, 2, pp. 176 – 180.

[7]. Das, A., Nagananda, G. S.,Bahattachaya, S.& Bhardway, S., 2010, Microbial quality of street vended Indian Chacets sold in Bangalore,JournalofBiologicalScience, 10,pp. 255 – 260.

[8]. Eglezos, S. Huang, B., Dykes, E. A.,& Fegan, N., 2010, The prevalence and concentration of Bacillus cereus in retail food products in Brisbane, Australia,Food Borne Pathogens and Diseases,7, 7, pp.867 – 870.

[9]. Eni, A. O., Oluwawemitan, I. A.,&Oranusi, U. S., 2010, Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria,African Journal of Food Science, 4, 5, pp. 291 – 296.

[10]. Fowoyo, P. T.,& Igbokwu, O. E., 2014, Impact of air pollution on the microbial quality of ready to-eat hawked foods sold in around a cement factory in Lokoja, Nigeria,AmericanJournalofResearchCommunication 2**,** 11, pp. 138 – 157.

[11]. Gitahi, M. G., Wangoh, J., & Njage, P. M. K., 2012, Microbial safety of street food in industrial area, Nairobi,Research Journal of Microbiology, 7, pp. 297 – 308.

[12]. Kadariya, J., Smith, T. C.,& Thapaliya, D., 2014,Staphylococcus aureus and Staphylococcus food borne diseases: An ongoing challenge in Public Health,Biomed Research International pp. 1 – 9.

[13]. Kaneko, K., Hayashidani, H., Ohtoma Y., Kwuge, J., Kato, M., Takahashi, K., Shiraki, Y., & Ogaua, M., 1999, Bacterial contamination of ready-to-eat foods and fresh products in retail shops and food factories,JournaloffoodProduction, 62, pp. 644 – 649.

[14]. le Loir, Y., Boron, F.,& Gautier, M., 2003, Staphylococcus aureus and food poisoning,Genetic and Molecular Research, 2, 1, pp. 63 – 76.

[15]. Madueke, S., N., Awe, S., & Jonah, A., L., 2014, Microbial analysis of streets foods along Lokoja – Abuja Express way, Lokoja,AmercianJournalofResearchCommunication, 2, 1, pp. 196 – 211.

[16]. Makun, H. A., Gobodi, T. A., Akanya, O. H., Salako, A. E., & Ogbadu, G. A., 2009, Health implications of toxigenic fungi found in Nigeria Staples: guinea corn and rice,AfricanJournalofFoodScience, 3, 9, pp. 250 – 256.

[17]. Marambio, E., Cordano, A. M., Insunza, M., Fernandez, M.,&Astroga, J., 2005, Determination of bacterial pathogen in food for export and their raw material,Proceedings of a Final Research Coordination Meeting held in Mexico City, Mexico IAEA-TECDOC-1431.

[18]. Mensah, P., Yeboah-Manu, D., Owusu- Darko, K., & Ablordey, A., 2002, Street food in Accra, Ghana: how safe are they? Bulletin of the World Health Organisation, 80, 7, pp. 546 – 554.

[19]. Nester, E. W., Anderson, D. G., Roberts, C. E., Pearsall, N. N.,& Nester, M. T., 2001,Microbiology: A human perspective(3rd ed.),New York: McGraw Hill.

[20]. Oladipo, I. C., & Adejumobi, O. D., 2010, Incidence of antibiotic resistance in some bacterial pathogens from street vended food in Ogbomoso, Nigeria. Pakistan Journal of Nutrition,9, 11, pp. 1061 – 1068.

[21]. Oladipo, I. C.,& Fajemilo, Y. O., 2012, Physiological studies and antibiotic resistant profile of bacterial pathogen isolated from some Nigerian fast food,American Journal of Food Technology, 7, pp. 746 – 753.

[22]. Opinion of the Science Panel on Biological Hazards on B cereus and other Bacillus species in Food stuff, 2005,The EFSA Journal 175, pp. 1 – 48.

[23]. Oranusi, U. S., & Braide, W., 2012, A study of microbial safety of ready-to-eat foods vended in high ways: Onitsha-Owerri, South east Nigeria,InternationalResearchofMicrobiology, 3, 2, pp. 66 – 71.

[24]. Osundiya, O. O., Oladele, R. O.,& Oduyebo, O. O., 2013, Antibiotic resistance (MAR) indices of Pseudomonas and Klebsiella species isolates in Lagos University Teaching Hospital,African Journal of Clinical and Experimental Microbiology, 14, 3, 1 pp.64 – 168.

[25]. Sayah, R. S., Kaneene, J. B., Johnson, Y.,& Miller, R., 2005, Pattern of antimicrobial resistances observed in E. coli isolated from domestic- and wild-animal fecal samples, human septage and surface water,American Society of Microbiology, 71, pp. 1394 – 1404.

[26]. Sina, H., Baba, M. F., Kayode, A. P., Noumaw, P. A., Sezan, A., Houn houigan, J. D., Kotchoni, S. O., Prevost, G., & Baba, M. L., 2011, Characterization of Staplylococcus aureus isolated from street foods: toxin profile and prevalence of antibiotic resistance,JournalofAppliedBiologicalScience, 46, pp. 3133 – 3143.

[27]. Tambekar, D. H., Gultan, S. R., Jaisingkar, R. S., Wangikar, M. S., Banginwar, V. S.,& Mogarekar, M. R., 2008, Household water management: A systematic study of bacteriological contamination between source and point of use,American-EurasianJournalofAgriculturalandEnvironmentalScience 3, 2, pp. 241 – 246.

[28]. Tsang, D., 2002, Microbiological Guidelines for Ready-to-eat Foods, Road and Environmental Hygiene Department Hong Kong. pp. 115 – 116.

[29]. Wogu, M. D., Omoruyi, M. I., Odeh, H. O. & Guobadia, J. N., 2011, Microbial load in ready-to-eat rice sold in Benin city, Journal of Microbiology and Antimicrobial, 3, pp. 29 – 33.

[30]. Van Ermengen, E., 1998, Ueber eǹnen anaroben Bacillus und seine Bezichungen zum Botullsmus,Z__Hyg. Infektolo,26, pp. 1 – 8.

U. Okekeaji "Diversity And Antibiogram Of Microbialcontaminants From Ready-To-Eat Foods Hawked In Nsukka Town: Implications For Public Health ""IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 12.9 (2018): 69-73