Trop J Nat Prod Res, September 2020; 4(9):586-589 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Assessment of Microbial Contamination of Bacteria Isolates from Fermented Cassava (Gari) Sold at Samaru Market, Zaria, Kaduna State and Antibiotic Susceptibility Pattern of Isolated Bacteria

Ayokunnumi F. Obajuluwa[1]*, Isah B. Abubakar[2], Josiah A. Onaolapo[2]

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna, Nigeria
[2]Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

ARTICLE INFO

Article history:
Received 29 July 2020
Revised 05 September 2020
Accepted 19 September 2020
Published online 03 October 2020

ABSTRACT

Fermented cassava is a staple food popularly consumed in Nigeria. It is normally sold in open markets in basins, open sacks and wheel barrows. Contamination of this food product is a potential source of public health hazard more especially when the product is consumed raw or merely soaked in cold water. The aims of this study were to assess the microbial contamination of gari sold in Samaru market, Zaria, Kaduna State, Nigeria and determine the antibiotics susceptibility pattern of the bacteria isolated. Ten samples were collected from different sources and serially diluted. The diluted samples were plated on nutrient agar and MacConkey agar to determine the total viable bacteria counts and coliform counts, respectively. Antibiotics susceptibility testing was carried out using Kirby Bauer agar diffusion method according to Clinical Laboratory Standard International.
The average total bacterial count was 5.20 x 10^4 CFU/g while the average coliform count was 2.62 x 10^4 CFU/g. The following bacterial contaminants were isolated from the samples: *Staphylococcus aureus* 15 (44.1%), *Bacillus* spp. 7 (20.6%), *Escherichia coli* 7 (20.6%), *Pseudomonas* spp. 3 (8.8%) and *Salmonella* spp. 2 (5.9%). The isolates were highly susceptible to gentamicin and ciprofloxacin, but highly resistant to beta-lactam antibiotics used in the study. The presence of antibiotics resistant bacterial strains in gari sold in the market is a potential source of transmission of antibiotic-resistant strains in the community which can lead to treatment failure of infections caused by such organisms.

Keywords: Fermented cassava, Bacteria contamination, Antibiotics, Susceptibility testing.

Introduction

Fermented cassava also known as gari is a creamy-white, granular flour with a slightly fermented flavor and slightly sour taste made from fermented gelatinized fresh cassava (*Manihot esculenta* Crantz) tubers. Gari is a major household food in Nigeria, most part of West Africa and some countries in Central Africa.[1,2] It can be eaten directly either dry or soaked in cold water with sugar, roasted groundnut, peanut, dry fish or coconut as compliment or as a paste made with hot water called 'eba' which is eaten with variety of African soups or stew. Gari is the most commonly consumed in Nigeria among the various fermented cassava products. It accounts for 70% of the entire cassava production in Nigeria.[3] It supplies about 70% of daily calorie of about 50 million people in Nigeria and about 500 million in the world.[4,5] The readily availability for consumption and low price of gari has made it a popular diet. Most gari consumers usually purchase the product from open market.
The production of gari is highly tasking: the cassava tubers are harvested, peeled, removing the covering and the white pulp is grated and packed into closely woven sacks and tied. The sacks are orderly piled up on each other and a wooden board placed below and above the sacks. The wooden boards are tied together with the sacks full of the grated cassava in between. Tension is created by tightening the rope and thus allowing water to drain out of the grated cassava being processed. Traditionally this is left to ferment for three to seven days depending on the type of gari being made. This step is very important as the fermentation process helps to reduce and detoxify the high cyanide content of cassava.[6,7]
After fermentation, the pulp is fried at high temperature to dry the fermented pulp to about 10% moisture content resulting in dextrinization of the starch, the high temperature also eliminates cyanide gas from the gari product.[8,9] After frying, the product is allowed to cool , sieved and packed for marketing. Gari is usually sold in open markets, packaged and displayed in open basins, bowls and sacks. This may predispose the product to microbial contamination from both the atmospheric air and the hands of buyers and sellers.
Microorganisms had been reportedly isolated from gari sold in open markets and these include *Salmonella spp.*, *Klebsiella* spp.,*Pseudomonas* spp., *Bacillus* spp., *Clostridium* spp., *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp.,*Rhizopus* spp and *Cladosporium* spp.[1,10] . The presence of these organisms could constitute public health hazards. There are only few reported studies on the isolation of bacterial drug resistant strains from gari. This study is therefore aimed at assessing the bacterial contamination of gari sold in Samaru market in Zaria and to carry out antibiotics susceptibility testing of the bacterial isolates.

*Corresponding author. E mail: afobajuluwa@gmail.com
Tel: +234 8036207703

Citation: Obajuluwa AF, Abubakar IB, Onaolapo JA. Assessment of Microbial Contamination of Bacteria Isolates from Fermented Cassava (Gari) Sold at Samaru Market, Zaria, Kaduna State and Antibiotic Susceptibility Pattern of Isolated Bacteria. Trop J Nat Prod Res. 2020; 4(9):586-589. doi.org/10.26538/tjnpr/v4i9.15

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Collection of samples

Ten samples of gari were collected from Samaru market in Giwa Local Government Area, Zaria, Kaduna State, Nigeria. The samples were transported in sterile polythene bag to the laboratory.

Sample analysis

Ten gram (10 g) of each sample of gari were homogenized in 90 mL sterile distilled water (10^{-1} dilution), further serial dilution of sample homogenate to 10^{-4} was carried out also in sterile distilled water. About 0.1 mL aliquot of each dilution was spread plated on plates of Nutrient agar and MacConkey agar for total bacteria viable count (TVC) and coliform count, respectively. The plates were incubated at 37^{o}C for 24 hours. At the expiration of incubation time, colony count was carried out using digital colony counter (Gallenkamp, England), total microbial population was expressed as colony forming units per gram (CFU/g) of sample.

Bacteria identification

Bacterial isolates were identified based on colony morphology, Gram staining and biochemical tests. The following biochemical tests were carried out: catalase, coagulase, indole, citrate utilization, sugar fermentation, MRVP tests.[11]

Antibiotics susceptibility testing

Kirby Bauer agar disc diffusion method was used for the antibiotics susceptibility testing. The test and the interpretation of the inhibition zone diameter (millimeter) were done.[12] The following commonly prescribed antibiotics were tested: Gentamicin 10 µg, tetracycline 30 µg, ciprofloxacin 5 µg, chloramphenicol 30 µg, amoxicillin 20 µg and ampicillin 10 µg. Bacteria isolates with non-susceptibility to at least one agent in three or more antimicrobial categories was classified as multidrug resistant (MDR).

Statistical analysis

The percentage antibiotics susceptibility was calculated using Microsoft Excel, 2007 while SPSS descriptive statistics was used to calculate arithmetic mean.

Results and Discussion

Food contamination especially gari can pose a great public health hazard considering the huge number of people consuming it. The results of the bacterial total viable and coliform counts of gari samples are presented in Tables 1 and 2. The mean total viable count was 5.20 x 10^4 CFU/g while the mean coliform count was 2.62 x 10^4 CFU/g. The following bacterial species were isolated from the gari samples collected: *Staphylococcus aureus* (15), *Bacillus* spp. (7), which are Gram positives and *Escherichia coli* (7), *Salmonella* spp. (2) and *Pseudomonas* spp. (3) which are Gram negatives. Their prevalence is as presented in Figure 1 and *S. aureus* was observed to have the highest percentage prevalence.

The TVC and coliform count observed in this study were high. The microbiological quality of the gari samples were higher than the limits given by African Organization of Standardization which is TVC (10^3 CFU/g); Staphylococcal (10^2 CFU/g); *Escherichia coli, Salmonella, Coliforms and Bacillus cereus* (0 CFU/g) and fungi (10^3 CFU/g) count.[13] The TVC obtained in this study is similar to that reported by Olopade *et al* [14] while some reporters reported higher values.[15,16] A higher coliform count was reported by Akindele and Abimbola [17] The high level of contamination observed in this study may be either from the manufacturing processes especially during handling after the drying, packaging and distribution stages. There could also be introduction of contaminants during handling of the products either when being displayed for selling or during selling especially if the hands were not properly washed before handling. The fact that the gari is usually sold in an open market displayed inside bowls, basins or wheel barrow without covering is also a source of contamination through the air. Generally, the level of sanitation of open markets in Nigeria is very poor. If this food product was displayed in a dirty environment where fly can perch on the food, this can lead to deposit of fecal contaminants resulting in high coliform count. The containers for packaging and the measure used in selling are also potential sources of contamination.

S. aureus was the most prevalent organism the reason being that it is part of the body normal flora. About 30% of people carry *S. aureus* in their noses even though it can also be pathogenic.[18] The presence of other pathogenic organisms isolated from these samples: *Bacillus* spp, *Escherichia coli*, *Salmonella* spp *and Pseudomonas* spp are of serious concern because of their potential health hazards. *S. aureus* infections include bacteremia, endocarditis, pneumonia, osteomyelitis.[19] *E. coli* is a bacteria that is commonly found in the intestine of humans and animals, likewise *Salmonella* spp. Some *E. coli* and *Bacillus* spp can cause food poisoning even though *E. coli* are harmless. Some types of *E. coli*, particularly *E. coli* O157:H7, can cause intestinal infection which is normally caused by ingestion of contaminated food and water.[19]

The results of the antibiotics susceptibility testing showed that gentamicin and ciprofloxacin were the most active followed by chloramphenicol. Both the Gram positive and Gram negative isolates were highly resistant to amoxicillin and ampicillin (100%). The results are presented in Tables 3 and 4. The multidrug resistant isolates observed with the Gram positive and Gram negative isolates were 2 (9.1%) and 7 (58.3%), respectively. Considering the Gram positive isolates, *S. aureus* isolates were highly susceptible to gentamicin, ciprofloxacin and chloramphenicol compared with *Bacillus* spp. which showed lower susceptibility. Both Gram positive and Gram negative isolates were highly resistant to beta lactam antibiotics. However, Gram negative isolates were more resistant to the antibiotics used compared with the Gram positive isolates.

Table 1: Total viable count of gari samples

Sample	Viable Count (CFU/g)
A	4.98 x 10^4
B	3.26 x 10^4
C	3.41 x 10^4
D	1.09 x 10^5
E	1.19 x 10^4
F	5.54 x 10^4
G	4.71 x 10^4
H	4.42 x 10^4
I	4.99 x 10^4
J	8.57 x 10^4

Table 2: Total coliform count of gari samples

Sample	Coliform count (CFU/g)
A	3.48 x 10^4
B	2.76 x 10^4
D	1.80 x 10^3
E	7.21 x 10^3
F	4.44 x 10^4
G	3.72 x 10^4
H	3.76 x 10^4
I	3.11 x 10^4
J	3.90 x 10^4

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Antibiotics susceptibility testing result of Gram negative isolates

Antibiotics	No of isolates (%) Sensitive	Resistant
Ampicillin	0	12 (100.0)
Ciprofloxacin	7 (58.3)	5 (41.7)
Amoxicillin	0	12 (100.0)
Tetracycline	7 (58.3)	5 (41.7)
Gentamicin	8 (66.7)	4 (33.3)
Chloramphenicol	6 (50.0)	6 (50.0)

The findings of this study were similar to previous study,[20] but the *Bacillus* spp. in that report were resistant to gentamicin. Isolation of antibiotics resistant bacteria in this study may pose public health challenge. These resistant strains might have been contacted through cross-contamination with antimicrobial resistant strain during the processing of gari. The fact that gari can be consumed raw without cooking poses greater danger with the risk of the resistant strain being transferred from one person to another.

Table 4: Antibiotics susceptibility testing result of Gram-positive isolates

Antibiotics	*S. aureus* n = 15 No. of isolates (%) Sensitive	Resistant	*Bacillus* spp. n =7 No. of isolates (%) Sensitive	Resistant
Ampicillin	0	15 (100)	0	7 (100)
Ciprofloxacin	15 (100)	0	6 (85.7)	1 (14.3)
Amoxicillin	0	15 (100)	0	7 (100)
Tetracycline	2 (13.3)	13 (86.7)	7 (100)	0
Gentamicin	15 (100)	0	6 (85.7)	1 (14.3)
Chloramphenicol	15 (100)	0	4 (57.1)	3 (42.9)

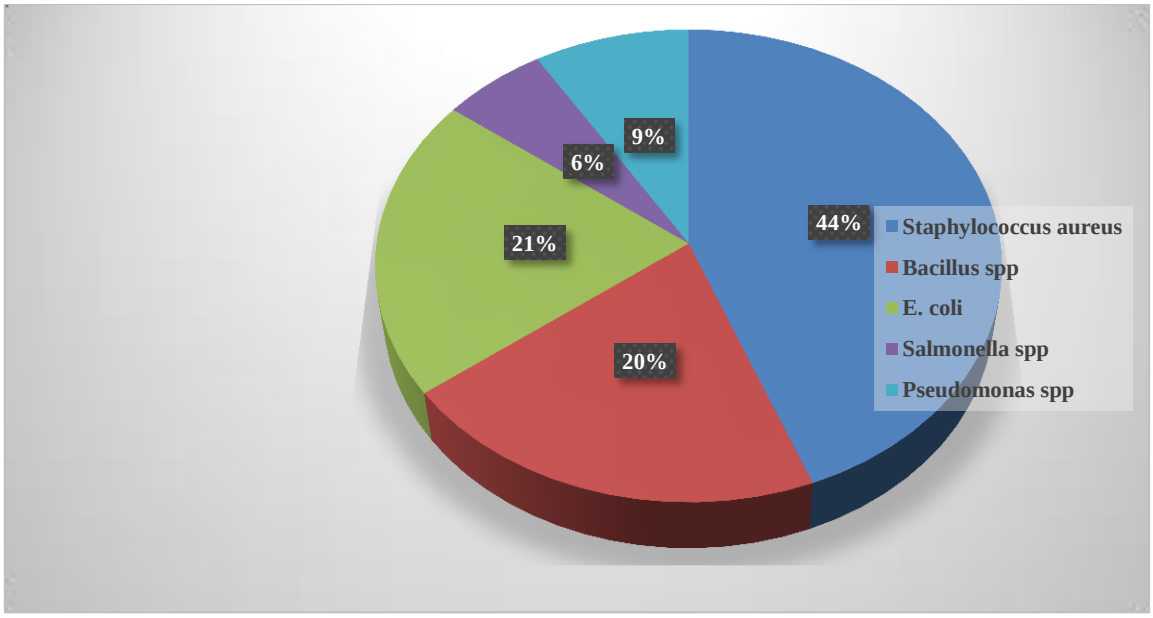

Figure 1: Percentage Prevalence of bacterial isolates from gari samples

Conclusion

The sampled gari sold in Samaru market were contaminated with *S. aureus*, *Bacillus* spp., *E. coli*, *Pseudomonas* spp. and *Salmonella* spp. Among these were bacterial drug resistant strains which are potential public health risk. Gentamicin and ciprofloxacin were the most active antibiotics against the isolates. The market women and the entire public should be educated on the health implication of consuming contaminated food.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Ogiehor IS, Ikenebomeh MJ, Ekundayo AO. The bioload and aflatoxin content of market garri from some selected states in southern Nigeria: public health significance. J Afr Health Sci. 2007; 7(4):223-227.
2. Kostinek M, Specht I, Edward VA, Schillinger U, Hertel C, Holzapfel WH, Franz CM. Diversity and technological properties of predominant lactic acid bacteria from fermented cassava used for the preparation of Gari, a traditional African food. Syst and Appl Microbiol. 2005; 286:527-540.
3. IITA. Cassava in tropical Africa. A reference manual. International Institutes for Tropical Agriculture, Ibadan Nigeria, 1990. 113-117 p.
4. Oluwole OB, Olatunji OO, Odunfa SF. A process technology for conversion of dried cassava chips into garri. Nig Food J. 2004; 22:65-73.
5. Abu JO, Badifu GTO, Akpapunam MA. Effect of Crude palm oil inclusion on some physicochemical properties of garri –A fermented cassava Food product. Nig Food J. 2006; 24(1):73-75.
6. Ray RC and Sivakumas PS. Traditional and novel fermented foods and beverages from tropical root and tuber crops: review. Int J Food Sci Technol. 2009; 44:1073-1087.
7. Huch M, Hanak A, Specht I, Dortu CM, Thonart P, Mbugua S, Holzapfel WH, Hertel C Franz CMAP. Use of *Lactobacillus* strains to start cassava fermentations for Garri production. Int J Food Microbiol. 2008; 128:258-267.
8. Akindahunsi AA, Oboh G, Oshodi AA. Effect of fermenting cassava with Rhizopusoryzae on the chemical composition of its flour and garri. Riv Ital Delle Sost. Grasse. 1999; 76:437-440.
9. Azam-Ali S, Judge E, Fellows P, Battcock M. Small-scale food processing: A directory of equipment and methods. 2nd edition, ITDG Publishing, 2003. 256. 2003 p.
10. Amadi JE and Adebola MO. Effect of moisture content and storage conditions on the storability of garri. Afr J Biotechnol. 2008; 724: 4591- 4594.
11. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press. 2002. 135-142, 158-159 p.
12. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing approved standard M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA. 2013. 44-50, 70-72 p.
13. African Organization of standardization (ARS). Garri-specification. African Standard. 1st edition. Nairobi, Kenya. 2012. CD-ARS 840:2014(E):4
14. Olopade BK, Oranusi S, Ajala R, Olorunisola SJ. Microbiological quality of fermented cassava (Gari) sold in Ota, Ogun State, Nigeria. Int J Curr Microb Appl Sci. 2014; 3(3):888-895.
15. Orji JO, Nnachi AU, Ojiochie CO, Efunshile AM, Ezejiofor OI, Ezeama CO, Nwaneli CU, Mbachu I. Bacteriological quality and public health implication of fermented cassava (Garri) sold in Okwor and Nkalagu markets in Ebonyi State, Nigeria. Int J Sci Technol Res. 2016; 5(11):85-91.
16. Ajayi OA, Adegbenro AA, Akinwumi OO. Quality Assessment of Some Gari famples From Iwo, Osun State, Nigeria, FUTA J Res Sci. Special Edition. 2017; 13(3):403-411.
17. Akindele ST, Abimbola WA. Microbial evaluation of Gari sold in Ijebu community. IOSR J Environ Sci Toxicol Food Technol. 2018; 12(7):35-38.
18. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Healthcare Quality Promotion (DHQP) 2011.
19. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Healthcare Quality Promotion (DHQP) 2014.
20. Adewunmi GA, Quadri RA Oguntoyinbo FA. Antibiotics susceptibility pattern of Bacillus species isolated from solid substrate fermentation of cassava for gari production. Afr J of Microb Res. 2009; 3(11):840-843.