Prevalence of Antibiotic-Resistant Bacteria in Dried Cassava Powder (Garri) Circulating in Ogun State, Nigeria

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ABSTRACT: Antimicrobial resistance is a serious and complex worldwide problem requiring the creation of a global surveillance system in order to circumvent such negative trend. This study was aimed at determining the prevalence of antibiotic-resistant bacteria in dried cassava powder (garri) circulating in Ogun State, Nigeria. The susceptibility of the isolated bacterial strains were evaluated using standard agar disc diffusion assay. The results obtained revealed a considerably high zone of inhibition ranging from 17mm – 37mm in diameter to the selected quinolones and cephalosporines. The antibiogram studies also showed that *Bacillus subtilis* was the most susceptible to all the antibiotics showing 100% susceptibility to perfloxacin, ciprofloxacin, levofloxacin and cefotaxime, 50% to cefuroxime and 71.4% to ceftriaxone. *E. coli* was however found to show 100% resistance to all the tested antibiotics. In an investigation to determine the most effective antibiotic against the bacterial isolates from dried cassava powder (garri), Levofloxacin was the most effective ($P<0.05$) followed by ciprofloxacin and cefuroxime. The Gram negative bacteria also showed alarming trend of resistance (100%) to all the tested cephalosporines while ceftriaxone was the mostly resisted by the Gram positive bacteria. The prevalence rate of antibiotic resistant bacteria in this study was found to be 49.21%. It can therefore be said that the presence of antibiotic resistant bacteria in garri suggest the need for proper evaluation and monitoring of this important staple food to safeguard against public health.**[**B.T.Thomas, H.I.Effedua, A.Davies and A.Oluwadun. **Prevalence of Antibiotic-Resistant Bacteria in Dried Cassava Powder (Garri) Circulating in Ogun State, Nigeria.** *Academia Arena,* 2012;4(1):9-13] (ISSN 1553-992X). http://www.sciencepub.net.

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1. Introduction

Antimicrobial resistance refers to failure of a given antimicrobial treatment and it is defined as the ability of microorganisms to grow either temporarily or permanently in the presence of an antimicrobials (IFT, 2005). These antimicrobials includes antibiotics, food antimicrobial agents, sanitizers, and other substances that act against microorganisms. Antimicrobial resistance no doubt remain a phenomenon of increasing importance as demonstrated by the emergence of different international antimicrobial resistance surveillance programs (Betty *et al*., 2005). The problem associated with resistance cannot be overemphasized, this is because of its increasing burden to human health. Antibiotics resistant food borne pathogens may develop increased virulence as revealed by the preponderance of evidences which strongly support the suggestion that antibiotic resistance results in a larger number of human infection than would otherwise be the case by increasing the risk of infection in people who have had prior antibiotic exposure (IFT, 2005). It is true that bacterial resistance is gaining popularity world wide due to the problem is posing to the medical and veterinary world. However, outbreaks of infectious diseases all the over the globe picture multi drug resistant organisms as a threat to human health (Singh *et al*., 2007; Okeke *et al*., 2007; Okonkwo *et al.*, 2009).

Garri is a roasted granule of cassava that is widely consumed by both rural and urban areas (FAO, 2010). It is by far the most popular form in which cassava is consumed in Nigeria and indeed in West Africa (Ikediobi *et al*., 1980). It is consumed by both young and old more especially from the bulk of Nigerian population (Ugwu and Odo, 2008). However, the practices that are associated with production, processing and post process handling of garri such as spreading on the floor, display in open bowl in the markets and sales points and use of various packaging materials to haul finished products from rural to urban areas may exacerbate contamination (Ogiehor and Ikenebomeh, 2006). Some of these microbial contaminant are capable of surviving in dried cassava powder (garri) (Thomas *et al.*, 2011). The importance of these bacteria in acquiring and disseminating resistance that could be transmitted to pathogenic or zoonotic bacteria has been inferred (Betty *et al*., 2005). These resistant and multi drug resistant organisms are also capable of spreading from animal to man through food and even become more easy to acquire through garri since this food is consumed raw with or without additives in South Western, Nigeria. Therefore, the present study considered determining the prevalence of

antibiotic-resistant bacteria in dried cassava powder (garri) circulating in Ogun State, Nigeria.

2. Materials and Methods

2.1 Study Areas:

The study area "Ogun State" is a state in South-Western Nigeria. It borders Lagos State to the South, Oyo and Osun State to the North, Ondo State to the East and the Republic of Benin to the West. The State is made up of twenty Local government areas and it have a total area of 16,762km^2 with over four million people.

2.2 Bacterial Isolates and Antibiotic Susceptibility Testing

The isolates were recovered from dried cassava powder (garri) in Ogun State, Nigeria and were identified based on standard microbiological techniques (Cheesborough, 2005). An inoculums size of 10^5 CFU/ml of each of the organism was prepared according to Bauer *et al.*(1996). A loopful of each of the inoculum corresponding to 10^5 CFU/ml was flooded evenly on Mueller Hinton Agar (Difco, USA). The plates were then incubated aerobically at 37°C for 24hours. The interpretation of the zones of inhibition were doneas described by the standard interpretative chart (NCCLS,2002).

Statistical Analysis

Frequency distribution was used for the determination of prevalence rate of the organisms while analysis of variance (ANOVA) was used for the relative measurement of the susceptibility pattern using SPSS Version 20. The level of significance was set at $P<0.05$

3. Results

Out of the sixty three bacterial isolates recovered from the dried cassava powder (garri) examined in this study, *Bacillus subtilis* 28(44.4%) was the most prevalent followed by *Enterococcus faecalis* 17(27%) and *Staphylococcus aureus* 11(17.5%). The least isolated organisms was *Escherichia coli* 2(3.2%). Table 2 and 3 depicts the sensitivity and resistant patterns of these organisms with Levofloxacin showing a markedly significant activities against the bacterial flora of dried cassava powder (garri) than other tested antibiotics. This was followed by Ciprofloxacin and Cefuroxime respectively ($P<0.05$) (Table 4). While Gram negative showed hundred percent resistance to all the tested Cephalosporins. Ceftriaxone was the most resisted antibiotics by the Gram positive organisms. The prevalence rate of antibiotic resistant bacteria in our study was however found to be 49.21%.

Table 1. Antibiotic Sensitivity Pattern of the tested Bacterial Pathogens

	No of strains sensitive to the Antibiotics							
Isolates	N	%	PEF	CIP	LEV	CEF	CFX	CFT
S. aureus	11	17.5	11(100)	5(45.6)	11(100)	5(45.5)	7(63.6)	9(81.8)
E. faecalis	17	27.0	17(100)	12(70.5)	17(100)	10(58.8)	6(35.3)	10(58.8)
P. vulgaris	5.0	7.9	3(60)	2(40)	5(100)	0(0)	0(0)	0(0)
E. coli	2.0	3.2	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
B. subtilis	28	44.4	28(100)	28(100)	28(100)	14(50)	28(100)	20(71.4)
Total	63	(100)	59(93.7)	47(65.1)	61(96.8)	29(46)	41(65.1)	39(61.9)

Table 2. Antibiotic Resistant Pattern of tested Bacterial Isolates

	No of strains resistant to the Antibiotics							
Isolates	N	%	PEF	CIP	LEV	CEF	CFX	CFT
S. aureus	11	17.5	0(0)	6(54.4)	0(0)	6(54.5)	4(36.4)	2(18.2)
E. faecalis	17	27.0	0(0)	5(29.5)	0(0)	7(41.2)	11(64.7)	7(41.2)
P. vulgaris	5.0	7.9	2(40)	3(60)	0(0)	5(100)	5(100)	5(100)
E. coli	2.0	3.2	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)
B. subtilis	28	44.4	0(0)	0(0)	0(0)	14(50)	0(0)	8(28.6)
Total	63	(100)	4(6.3)	22(34.9)	2(3.2)	34(54)	22(34.9)	24(38.1)

KEYS: PEF; Perfloxacin CIP; Ciprofloxacin LEV; Levofloxacin CEF; Cefuroxime CFX; Ceftazidime CFT; Ceftriaxone

Table 3.Determination of the most effective antibiotics against Bacterial flora of DCPs (garri)

Antibiotics	**N**	**% of Sensitive Organisms Mean ± SEM**
Perfloxacin	2	93.7 ± 0.00
Ciprofloxacin	2	65.1 ± 0.10
Levofloxacin	2	96.8 ± 0.10
Ceftriaxone	2	46.0 ± 0.00
Cefuroxime	2	65.1 ± 0.10
Ceftazidime	2	61.9 ± 0.90

F value = 2796.05, P<0.05

Table 4.Determination of the most effective antibiotics against Gram positive bacteria

Antibiotics	**N**	**% of Sensitive Organisms (Mean ± SEM)**
Perfloxacin	2	100 ± 0.00
Ciprofloxacin	2	80.3 ± 0.01
Levofloxacin	2	100 ± 0.00
Ceftriaxone	2	51.8 ± 0.03
Cefuroxime	2	73.2 ± 0.30
Ceftazidime	2	69.2 ± 0.40

F value = 67856.2, P<0.05

Table 5. Determination of the most effective antibiotic against Gram negative bacteria.

Antibiotics	**N**	**% of Sensitive Organisms Mean ± SEM**
Perfloxacin	2	42.8 ± 0.00
Ciprofloxacin	2	28.6 ± 0.01
Levofloxacin	2	71.4 ± 0.10
Ceftriaxone	2	00.0 ± 0.00
Cefuroxime	2	0.00 ± 0.00
Ceftazidime	2	0.00 ± 0.00

F value = 260947.2, P<0.05

Table 6. Prevalence of Antibiotic Resistant Bacterial in Dried Cassava powder (garri)

	Sensitivity test result		
Type of organisms based on Gram reaction	Sensitive Organisms	Resistant organisms	Total
Gram negative organisms	3	4	7
Gram positive organisms	29	27	56
Total	32	31	63

Prevalence of resistant bacteria

$$= \frac{\text{total number of resistant organisms}}{\text{Total number of organisms isolated}} \times \frac{100}{1}$$

$$= \frac{31}{63} \times \frac{100}{1} = 49.21\%$$

4. Discussion

With increased consumption of garri in West Africa including Nigeria, more information is needed regarding the safety of this food. Although garri is the most popular form in which cassava is consumed in Nigeria and indeed in West Africa (Ikediobi *et al*., 1980). It may not be free of contamination (Ogiehor and Ikenebomeh, 2006). Frequently low level of microbial contaminants are found in this dried product because of the lack of sufficient moisture necessary for microbial growth and survival (Brown and Jiang, 2008). In our study, garri samples were variously contaminated with different levels of quinolones and cephalosporine resistant bacteria. This observation may not be unconnected to the widespread use of these antibiotics in both human and animal health (Chikwendu *et al*., 2008). Ceftriaxone which was the most resisted antibiotic by the Gram positive organisms in our study has been formerly reported as the most resisted antibiotics by Salmonella in a study conducted by the National Antimicrobial Resistance Monitoring system (CDC, 2004). Hundred percent (100%) resistance of the Gram negative organisms to all the tested cephalosporines noticed in our study may be due to the extensive use in human, animal and agricultural products (Fey *et al*., 2004). *Escherichia coli* isolated in this study was resistant to all the tested groups of quinolones and cephalosporines. These multi drug resistant *Escherichia coli* might have originated from poultry or from susceptible poultry source precursors (Johnson *et al*., 2007). The multi drug resistant pattern of *Escherichia coli* observed in this study is comparable to previous studies (Doleijska *et al*., 2007). The presence of quinolones resistant organisms in our study is similar to the findings of Karlowsky *et al* (2004). It is estimated that nearly 90% of all antibiotic agents is used in foods and animals and are given at subtherapeutic concentrations prophylactically or to promote growth (Abdellah *et al*., 2009). The reduced susceptibility of *Proteus vulgaris* observed may be due to the acquisition of quinolones resistant gene, by the organisms (Van dan-Bogaard and Stobberingh, 2002). The presence of multi drug resistance *Proteus* species are known to cause significant clinical infections and occupy multiple environmental habitats (Mordi and Momoh, 2009). *Bacillus subtilis* showed 100% sensitivity to quinolones and the least resistance to cephalosporines. The ability of this organisms to resist ceftriaxone and cefuroxime may be due to their spore forming potential (Okonkwo *et al*., 2010). The multi drug resistant *Enterococcus faecalis* observed in this study corroborate the finding of Klare (2003) that reported food enterococci as reservoirs and/or vehicles of antibiotic resistance (AR) and possibly virulence factors. During the antibiotic era, an increasing number of food enterococci have developed resistance to various therapeutic agents including vancomycin (Rorbredo *et al*., 2000) Gentamicin (Donabedian, 2003) and Streptogramines (Simjie *et al*., 2002). *Staphylococcus aureus* resistance to the tested antibiotic is similar to that of Motlava *et al* (2004). The high level of resistance in *S. aureus* may be connected to the presence of extended spectrum Beta lactamase enzyme in them (Efuntoye and Amuzat, 2007). The bacterial found in this food may have been the result of contamination before, during or after processing however various levels of antibiotic – resistant bacteria were detected in garri. With greater demand for this food and an increase in the occurrence of multi drug resistant bacterial, there is need to determine the molecular mechanism of resistance of these organisms in order to find the possible way of antagonizing their growth and survival in this mostly consumed food in West Africa.

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