IN VITRO SUSCEPTIBILITY PROFILE OF BACTERIA ISOLATED FROM FERMENTED AFRICAN OIL BEAN SEEDS (*Pentaclethra macrophylla,* BENTH*)* SOLD IN UYO METROPOLIS

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ABSTRACT

In vitro susceptibility profile of bacteria isolated from traditionally fermented African oil bean seeds (*Pentaclethra macrophylla,* Benth*)* was performed using the Kirby-Bauer disc diffusion method. The pour plate method was used to isolate the bacterial contaminants and characterized. The isolates were *Lactobacillus* sp, *Staphylococcus aureus, Proteus* sp, *Klebsiella* spp, *Bacillus* sp *and Escherichia coli.* Their occurrences varied with the samples analyzed. *Staphylococcus aureus* (with 80% occurrence rate) was the most encountered bacterial contaminants. The results of the antibiotic susceptibility assay of the bacterial isolates have shown that *Lactobacillus* sp was highly sensitive to Streptomycin and Ciprofloxacin with 28.2± 1.2mm and 31±0.4mm zones of inhibition (ZI) respectively but exhibited remarkable resistance to Ceftazidime. *Staphylococcus aureus* AA was moderately sensitive to Ceftazidimeand Gentamycin but expressed resistance to Streptomycin and Ciprofloxacin, *Proteus* sp AA and *Proteus* sp UM were sensitive to Ceftazidime with ZI of 23.1±0.2mm and 24±0.6mm respectively but resistant to Streptomycin. *Klebsiella* sp IT had ZI of 16.1±0.24mm when treated withGentamycin. *Bacillus* spIT was resistant to Gentamycin, Ceftazidime and Ciprofloxacin while *Escherichia coli* BRwas highly (26.3±0.5mm) sensitive to Ceftazidime but resistant to Streptomycin.The multiple numbers of antibiotic resistant bacteria pathogens isolated from fermented African oil bean in Uyo metropolis implies that theproduct could be a potential source of infection if not checked.Strict hygienic measures should be taken during the processing of the oil bean to avoid contaminationof the product.

INTRODUCTION

African oil bean seed (*Pentaclethra macrophylla* Benth), is a woody plant predominant in the rain forest areas of West and Central Africa belonging to the family Leguminosaea, sub-family Mimosoidae (Keay, 1989). African oil bean seed (*Pentaclethra macrophylla, Benth)* is a popular seed which is generally consumed across the States in Nigeria (Abacha, 2012). According to Enuijugha and Akanbi (2005), the unprocessed African oil bean seeds are bitter and possess anti-nutritional factors amongst which are pancine, cyanide, oxalates, phytic acid, saponin, phytate and tannins. Thus to obtain the palatable products, processing and fermentation of the beans are required. The processing involves thermal treatment, boiling, removal from pod, cutting into slices, further boiling, wrapping in banana/ plantain leaves and then fermentation. The application of heat treatment induces a resultant rise in the softening of the seed and availability of nutrients. Processing and fermentation of the oil been seeds drastically reduces the levels of the anti- nutritional compounds present in the seeds while increasing the level of iron, calcium, potassium, thiamine and riboflavin compounds (Enuijugha and Ayodele-Oni, 2003).

In Akwa Ibom State, African oil beans are traditionally and generally produced by local fermentation in homes. Some, for family consumption while others embark on its sale as a small family business. The method of production differs from one producer to another but in any case, production and preparation of African oil bean seeds is usually by mixed fermentation carried out spontaneously by a number of microorganisms. This means Ugba (processed oil

bean seeds) production, like many African fermented foods depends entirely on fermentation by microorganism from diverse source. The fermentation depends on random inoculation of boiled slices of the oil bean seed by microorganism within the immediate environment. Some of these organisms found themselves as contaminants into the food products during processing period. Fermented Ugba has a high rate of susceptibility to microbial spoilage. The product deterioration or spoilage is evidenced by organoleptic changes in colour, texture, odour and taste (Enujiugha and Olajundoye, 2001).

However, fermented seeds are acceptable by many African not just because it is palatable but it serves as a delicacy amongst consuming regions. It is also eaten and used as flavoring agent for soup. It is the commonest and, a very cheap source of protein for people (Mbata and Orji, 2008). However, some strains of microorganisms present in fermented African oil bean seeds could be pathogenic and cause some diseases to the consumers. An even more serious threat may be the emergence of multi-drug resistant pathogens that are resistant to essentially common routine antimicrobial agents (Livermore, 2007). There are a good number of research works on isolation of microorganisms associated with fermented African oil bean seeds (*Pentaclethra macrophylla* Benth*)* in Nigeria,(Ejiofor,1987;Enujiugha and Badejo, 2002; Gadaga,2004; Nwagu *et al.,* 2010; Eze *et al.*,2014) but information on the *In vitro* susceptibility profile of microorganisms isolated from traditional fermented African oil bean seeds (*Pentaclethra macrophylla,* Benth*)* sold in Uyo metropolisis rare. Therefore, rapid detection of the presence of multi-drug resistance by microorganisms associated with food spoilage is of serious concern and public health importance. The present study was conducted to evaluate the *in vitro* susceptibility profile of bacteria isolated from fermented African oil bean seeds (*Pentaclethra macrophylla,* Benth*)* sold in Uyo metropolis.

MATERIALS AND METHODS

Isolation of Bacteria Associated with Fermented Oil Bean Seeds

One gram of each of the sample of fermented African oil bean seed was added into 9ml of peptone water and then shaken vigorously to dislodge adhered bacteria. Zero point one (0.1) ml of the serially diluted sample (10^{-5}) was transferred onto plates of MacConkey Agar (MAC) Nutrient Agar (NA) and Manitoll salt Agar (MSA). Thereafter, the colonies were sub-cultured on to plate of Nutrient Agar and incubated at 37^0C for 18-24hrs. Pure cultures of the bacterial isolates were streaked onto Nutrient Agar slant, incubated at 37^0C for 24hrs and stored in the refrigerator at 4^0C for characterization, identification and other further analysis (Akpomie and Akpan 2013).

Identification and characterization of isolates

The bacterial isolates were identified based on their cultural, morphological and biochemical characteristics described Cheesbrough, (2003) and Holt, *et al., (*1994). Biochemical tests are the test for the identification of bacteria species based on the differences in the biochemical activities of different bacteria.

Antibiotics Susceptibility Test

The susceptibility of bacteria isolates to different antibiotics was performed by means of Kirby-Bauer disc diffusion method according to the guidelines provided by CLSI, (2006). The bacterial isolates were sub-cultured on a freshly prepared Nutrient agar (Oxoid, USA) medium for 18-24 hours at 37ºC to obtain a young culture. Each test organism was picked using a sterile wire loop and inoculated in 1ml of peptone water (Sigma chemical Co.Ltd, England). Zero point one (0.1) ml of each bacterial suspension adjusted to 0.5 McFarland turbidity standard was inoculated using sterile pipette onto each of the plates containing solidified Mueller Hinton Agar (Difco Laboratories, Detroit, Mich), that is, the organism was seeded on Mueller Hilton agar plates. The single disc containing several antibiotics were aseptically placed onto the surface of the Mueller Hinton Agar plates using a sterile forceps and gently pressed to

ensure fixed contact for bacteria. Antibiotics used were; Streptomycin (10 µl), Gentamycin (10 µl), Ceftazidim, (30µl) and Ciprofloxacin (5µl), (Hardy diagnostic, USA).

Statistical Analysis

The values of zones of inhibitions (ZI) recorded were calculated from the means of three measurements of zones of inhibitions from the triplicate cultures and the standard deviation of the mean (Mean± SD).

RESULTS

The bacteria isolated from the fermented oil bean seeds were *Lactobacillus* sp, *Staphylococcus aureus*, *Proteus* sp, *Klebsiella* sp, *Bacillus* spand *Escherichia coli.* Their frequency of occurrencerevealed that *Staphylococcus aureus* (with 80% occurrence rate) was the most encountered isolates from the samples analyzed, closely followed by *Lactobacillus* spp, *Klebsiella* spp and *Baclilus* sp with 3 (60%) occurrence rate each (Table 1)..

Table 1 Bacterial isolates from fermented African oil bean seed and frequency of occurrences

Isolate	Market centers					Frequency of occurrence (%)
	AA	BR	IT	UE	AN	
Lactobacillus spp	+	-	+	-	+	3 (60)
S.aureus	+	-	+	+	+	4 (80)
Proteus spp	+	-	-	+	-	2 (40)
Klebsiella spp	-	+	+	+	-	3(60)
Escherichia coli	-	+	+	-	-	2 (40)
Baclilus spp	-	+	+	-	+	3 (60)

Keys: AA=Akpan Andem market, BR =Barrack road, IT=Itam market, UE=Udoette market, AN=Anua market

The *Lactbacillus* sp AA was highly sensitive to Streptomycin and Ciprofloxacin with zones of inhibition (ZI) of 28.2± 1.2mm and 31±0.4mm respectively but resistant to Ceftazidime, *Staphylococcusaureus* AAwas moderately sensitive to Ceftazidimeand Gentamycin with ZI = 13.±1.2mm and 16.±0.6mm and but expressed resistance to Streptomycin and Ciprofloxacin. *Proteus s*p AA and *Proteus* sp UM were highly sensitive to Ceftazidime with ZI of 23.1±0.2mm and 24±0.6mm respectively but showed resistance to Streptomycin. *Klebsiella* sp IT had ZI of 16.1±0.24mm when treated withGentamycin, *E.coli* BRwas highly (26.3±0.5mm) sensitive to Ceftazidime butexpressed total resistancetoStreptomycinwhile *Baclilus* sp ITwas observed to be sensitive toStreptomycinwith ZI of20.±0.2mm but resistant to Gentamycin,Ceftazidimeand Ciprofloxacin(Table 2)

DISCUSSION

Many of the bacterial contaminants isolated from the bean seeds analyzed have previously been reported by Ogueke *et al.*, (2010) and, Okorie and Olasupo, (2013). The presence of these isolates in fermented African oil bean seeds is of public health implications. *Lactobacillus* speciies are often considered to be commensal or beneficial participants in human microbial ecology, involved actively in the fermentation process as well as the use and important of lactobacilli as additives in both human and animal diets (Anyanwu *et al.*, 2016). However, Harty *et al.*, 1994 reported that lactobacilli also cause some human diseases (e.g. dental caries, rheumatic vascular disease, septicaemia and infective endocarditis (IE)). *Lactobacillus* species have recently been identified as potential emerging pathogens in elderly and immuno-compromised patients, particularly those receiving broad spectrum antibiotic therapy (Kubiszewska *et al*, 2014) Moreover, Sullivan and Nord,(2006) reported bacteraemia caused by Lactobacilli.

Table 2: Antibiotics sensitivity profiles of the bacterial isolates

Bacterial lsolates	Antibiotics used and their zones of inhibitions			
	STR (ZI in mm)	GEN (ZI in mm)	CIP (ZI in mm)	CEF(ZI in mm
Lactobacillus sp AA	22.1±0.2	18.±1.5	12.±0.2	21.±0.82
Lactobacills sp IT	28.2± 1.2	17.±1.12	31±0.4	NZ
Lactobacillus sp	17.3.±1.6	10.±0.15	12.±0.2	11.±0.31
S. aureus AA	9.3 ±1.5	16.±0.6	9.±0.2	13.±1.2
S. aureus IT	8.1±0.12	14.±0.11	12.±0.2	12.±0.2
S. aureus UE	11.±0.31	15.±0.3	10.±0.2	13.±0.48
S.aureus AN	10.±1.41	12.4.±0.16	11.±0.2	15.±0.2
Proteus spp AA	11.±1.6	18.1.±0.22	16.±0.01	23.1±0.2
Proteus spp UM	9.1±0.03	14.1.±1.12	19.±1.7	24±0.6
Kleb. spp BR	12.±0.12	17.2.±0.32	8.±0.12	9.±0.45
Kleb. spp IT	14.±0.06	16..1±0.24	11.±4.21	10.±1.4
Kleb. spp UE	15.5.±0.49	14.±0.17	11.±0.2	9.±0.2
E.coli BR	NZ	13.±1.02	16.±0.2	*26.3*±0.5
E.coli IT	15.±0.2	18.±0.2	14.±0.2	19.±0.09
Baclilus spp BR	18.±2.3	15.±1.24	9.±1.0	10.±1.2
Baclilus spp IT	20.±0.2	12.±0.32	10.±0.2	12.±0.23
Baclilus spp AN	14.±0.2	14.±1.2	15.3.±0.2	13.6.±2.2

Keys: STREP =Streptomycin, GEN = Gentamicin, CIP= Ciprofloxacin, CEF= Ceftazidine, ZI =Zone of inhibition, NZ =No zone of inhibition

Staphylococcus aureus which was most frequently isolated (80% occurrence rate) from the samples studied is a member of the normal flora of the respiratory tract, gastrointestinal tracts and human skin. However, the public health implication according to Evenson *et al.,* (1988), and Nema *et al., (*2007), indicates that enterotoxin producing strains of *S. aureus* is a leading cause of food intoxication as it can produce extremely potent gastrointestinal toxin. *Escherichia coli, Klebsiella* sp. and Proteus spp are member of Enterobactericeae found in gastrointestinal tract of humans and animals. Ogueke *et al.,* (2010) in their work also reported the presence of some member of this family in fermented African oil bean seeds. Their presence in the African oil bean seed is an indication of fecal contamination either through direct or indirect means.

The isolation of *Bacillus* spp in this work agrees with the work of Mbata and Orji,(2008) and, Nwagu *et al.,* (2010) and Eze *et al.,* (2014) who reported the presence of *Bacillus* species in African oil bean seed undergoing controlled fermentation using traditional protocol. . They further stressed that during the fermentation process, *Bacillus subtilis* plays significant roles in modifying the substrate biochemically, nutritionally and organoleptically. This indicates that the use of *Bacillus* sp could successfully lead to good and proper fermented African oil bean seeds quality. Although *Bacillus* species is used in controlled fermentation of African oil bean seed , Nwagu *et al.,* (2010) reported that their population increased with time and finally noted that spoilage may occur due to continuous activities of *Bacillus* spp..

Staphylococcus aureus and other gram- positive bacteria expressed multidrug resistance profile. This report agrees with previous work by Clarence *et al.,* (2009). Members of Enterobacteriaceae isolated encountered in study also exhibited resistance to some routine antibiotics. Theiry *et al.,* (2006) and Shaikh *et al*., (2015) reported that resistance to the currently available antibiotics among the Enterobacteriaceaeis a serious challenge while Udoh *et al*., (2018) also reported theproduction of extended spectrum beta-lactamases (ESBLs) by members of the Enterobacteriaceae. According to Akujobi and Ewuru (2010), many drug resistant cases especially multidrug resistance have been attributed to the production of these extended spectrum beta-lactamases (ESBLs). This group of enzymes enables and endowed bacteria to hydrolyze antibiotics. The phenomenon could also be mediated by genetic mutation or plasmid transfer among antibiotic resistant bacterial strains. Thus, fermented African oil

bean seed samples could be a source of resistant bacteria that could cause food- borne disease outbreak in a community at large if not prevented.

CONCLUSION AND RECOMMENDATION

The multiple numbers of antibiotic resistant bacteria pathogens isolated from fermented African oil bean in Uyo metropolis implies that the product could be a potential source of infection if not checked. The presence of microorganisms of medical importance such as S. *aureus, E. coli and Bacillus* spp which are potentially life threatening pathogens indicates the potential health hazard faced by the consumers of this product. The public health implication of these findings is highlighted as these isolates that expressed resistance to these routine antimicrobial drugs cause several infections. Therefore, the processors/handlers/sellers should practice strict hygienic measures right from when the seeds are fermented, processed, spiced, packaged, and storage so as to avoid introducing any contaminant into the product. Also consumers should be enlightened on dangers of eating food products that are contaminated by some potential microorganisms.

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