International Scholars Journals

African Journal of Parasitology Research ISSN: 2343-6549 Vol. 4 (1), pp. 191-196, January, 2017. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Isolation of enterococci from dried beef crackers (*kilishi*) and its antibiogram

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Accepted 11 January, 2013

The level of safety associated with the consumption of ready-to-eat snack *kilishi* (dried beef cracker) was studied by surveying the total aerobic bioload as well as the density and antibiogram of the enterococcal content. A total of 60 random samples taken from 5 sales outlets in Abuja metropolis were tested for their total aerobic plate count by the spread plate technique on Plate Count Agar (PCA) while the total enterococcal plate count were determined using the pour plate technique on Enterococcus Selective Agar (ECSA). From the results, the TAPC ranged from 1.2 x 10^3- 3.9 x 10^4cfu/g while the enterococcal plate count ranged from 4.6 x 10^1 to 6.7 x 10^1cfu/g respectively. Various sales points within the metropolis recorded different enterococci plate counts. Statistically, no significant difference (p>0.05) occurred in the mean enterococci counts of two of the sales points. Of the eight species of *Enterococcus* isolated in this study (*E. faecium, E. solitaries, E. faecalis, E. asini, E. hirae, E. cecorum, E. casseliflavus*and *E. raffinosus*), *E. faecalis* accounted for 25%.Also, the mean pH (6.2-6.4) and proximate content (moisture content, 12.8-13.7%; crude protein, 60.6-60.9%; fat, 8.3-9.0%; ash, 7.4-7.6%) were not significantly different (p>0.05) in all the samples. The antibiotic pattern of isolates showed a significant resistance pattern (p<0.05) to 33% of commonly used antibiotics (cloxacillin, 93%; streptomycin,70%, penicillin, 98%; colistin, 98% and nalidixic acid, 95%) while 20% of these antibiotics: ofloxacin (98%), nitrofurantoin (95%) and tetracycline (85%) were active against the isolates. The resistance of the enterococci isolates to more than one antibiotic as observed in this study should be of great concern to both food scientists and health practitioners.

Keywords: Kilishi, *Enterococcus*, beef, antibiotics.

INTRODUCTION

Kilishi (Nigerian beef cracker) is a tropical intermediate moisture meat product prepared from beef slices, infused in slurry of groundnut paste and spices and sun-dried. *Kilishi* is comparable to other beef crackers produced and eaten across the globe: odka-Somalia; qwanta-Ethiopia; kaddia-Middle East; jerky-North America and charque-South America (Bennami *et al.,* 2000; Rahman *et al.,* 2005). The relative shelf stability of *kilishi* at room temperature makes it a popular rich nourishing snack (Ogunsola and Omojola, 2008).

Although the production of *kilishi* leaves the product dry, crispy and unfavorable for microbial proliferation, the end product would have a considerable shelf life if it is well packaged and kept sufficiently dry (Jones *et al*., 2001). The safety of meats dried by traditional methods has become a matter of concern as people with little knowledge of food hygiene and safe food handling practices are involved in its preparation. Schjonsby (2002) reported outbreaks of botulism associated with the consumption of dried meat product. *Kilishi c*ould therefore

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contain bacteria including enterococci which are reported to be reservoirs of resistant genes to many antibiotics (Lukasova and Sustackova, 2003).
Monitoring the prevalence of resistance in enterococci in different populations, animals, patients, healthy humans, and even food and meat products makes it feasible to compare the prevalence of resistance and to detect transfer of resistant bacteria or resistance genes from animals to humans and vice versa (van danBogaard, 2000). Enterococci have been reported to be resistant to most antibiotics used in clinical practice. They are known to acquire antibiotic resistance with relative ease and to be able to spread these resistance genes to other species (Kuhn *et al.*, 2000). Enterococci may gain access into raw material and food products from primary habitats such as intestines of animals and humans and from sources associated with unsanitary conditions of the production and handling of foods. The resistant enterococci can be potentially transferred from food animals to human via food chain (Mateu and Martin, 2001). Currently, there is paucity of information on the microbiological quality of commercially available indigenous dried beef snack (*kilishi)* in Abuja. This study will therefore, provide information on the general microbiological quality of *kilishi* sold within Abuja metropolis relating it to the physiochemical properties and establish the antibiotic resistance pattern of the enterococci isolated from it.

MATERIALS AND METHODS

Sample collection

Kilishi was collected randomly from five (5) different sales points within the Abuja metropolis, twelve (12) samples randomly from each sampling site in polyethylene bags and transported to the Microbiology Laboratory, University of Abuja, Abuja. The samples were stored in a desicator until when needed for analysis.

Total Aerobic plate count (TAPC)

Pre-enrichment and homogenization

Enumeration of bacteria in *kilishi* samples was done using Plate Count Agar (PCA) as described by Larry and James (2001). Twenty five gram (25g) of kilishi was macerated and placed in 225ml (1:10) of sterilized buffered peptone water (BPW), this was allowed to soak for 25min and homogenized with a stomacher for 1min.

Serial dilution

A 10-fold dilution of the pre-enrichment broth (1:10) was made up to $10^{-3}$by adding 1ml of the broth to consecutive 9ml of sterilized peptone water.

Inoculation and incubation

Using a standard Pasteur pipette two (2) drops (0.04ml) of 10^{-1}, 10^{-2}, 10^{-3} dilutions were inoculated by the spread plating technique unto each of the triplicate solidified PCA (Britanialab) plates using a sterile bent glass rod. An un-inoculated PCA plate served as the control. Following inoculation, the plates were incubated aerobically at 35^{o}C for 24-48h and colonies that developed (25-250; APHA, 2001) in the triplicate plates of 10^{-2} dilution were counted, calculated and recorded as colony forming unit per gram (cfu/g).

Isolation of enterococci

Enterococci counts of samples were determined on Enterococcus Selective Agar (Fluka *Biochemika*) using pour plating technique following appropriate dilution as described by Larry and James (2001). Triplicate plates were incubated for 24-48h at 37^{o}C; colonies of typical characteristics (pink to dark red colonies) were counted (cfu/g) and recorded. Following incubation, discrete colonies (typical pink to dark red) were randomly picked and purified. Cultures of the isolates were considered to be pure after three (3) successive subcultures on ESCA plates; pure cultures of enterococci isolates (60) were subcultured on ESCA agar slants in Bijou bottles; these were covered with sterile mineral oil and kept in the refrigerator for further studies.

Characterization and identification of enterococcal isolates

The purified isolates were identified following Gram staining on the basis of standard cultural, morphological, physiological and biochemical characteristics (APHA, 2001; Harrigan and McCance, 1976; Snealth *et al*., 1986). The biochemical and physiological characteristics included catalase test growth at different temperatures (45, 50 and 60^{o}C), growth at different salt concentrations (6.5, 10% NaCl), acid and gas production from sugars (glucose, fructose, galactose, lactose, maltose, mannitol, mannose, sorbitol, sucrose, xylose, arabinose, cellobiose and glycerol).

Chemical and proximate analysis

Ten grams (10g) of the samples was homogenated with 90ml of deionized water using a Polytron blender (Brinkman Instruments, New York). The pH of the samples was determined in triplicates using an Orion bench top pH meter (Thermo Electron Corporation, Beverly, USA) after standardization with pH 4 and 7 buffers (BDH, England). The moisture content, crude protein, crude fat, carbohydrate and total ash of *kilishi*

were determined in triplicates as described by AOAC (1990).

Antibiotic sensitivity testing

The sensitivity of sixty (60) enterococcal isolates to various antibiotics was determined using the disk diffusion method described by Belicova*et al*. (2007). Hardened PCA plates were inoculated with 0.1ml of 18h broth culture of the test organisms and spread with bent glass rods to ensure confluent growth. Commercial multidisc (BBL) containing augumentin, amoxycillin, cloxacillin, erythromycin, tetracycline, ampicillin, gentamycin, penicillin, cotrimoxazole, nitrofurantoin, chloramphenicol, ofloxacin, nalidixic acid, streptomycin and colistin were applied to separate plates. These plates were incubated at 37^{o}C for 18-24h and resulting zones of incubation measured. Interpretative categories (susceptible->17mm, intermediate-16-17mm and resistant-<16mm of zone of inhibition) were calculated for each zone of inhibition measurement in accordance with the NCCLS (1993) guidelines tables.

Statistical analysis

Analysis of variance (ANOVA) was carried out for the pH, proximate and microbial counts. The mean scores were computed and significant difference among the mean was determined (Duncan, p=0.05) using 2006 Statistical Packages for social Sciences (SPSS) for windows version 15.0 (SPSS, 2006) while the antibiotic sensitive pattern of the isolates was computed (percentages).

RESULTS AND DISCUSSION

The microbial load (aerobic plate and enterococci count) of *kilishi* collected from five sale points are shown in Table 1. The result obtained shows that the aerobic count ranged from $1.2x10^{3}$-$3.9x10^{3}$cfu/g while the enterococci count ranged from $4.7x10^{1}$-$6.7x10^{1}$cfu/g. From the data, 30% of the samples had aerobic plate counts <1000 cfu/g while 27% had aerobic plate counts >10,000 cfu/g which is significantly higher (p<0.05 However, the total aerobic count obtained in this study were acceptable when compared with 5.4-8.0 $\log_{10}$ reported by Jones *et al.* (2001) as being the acceptable limits of the microbial quality of some ready-to-eat food. The high counts of enterococci as obtained in this study may be due to the fact that *kilishi* (a supposedly a dried product) have some physiological properties (moisture content, carbohydrate and protein) that may favor microbial proliferation during sales. Bennani *et al.* (1995); Bennani *et al*. (2000)and Rhaman *et al*. (2005) reported enterococcal counts of 2.0 $\log_{10}$cfu/g in their study on dried beef products. The high enterococcal count recorded in this study is a reflection of poor handling practices by *kilishi* producers. Shjonsby (2002) reports that high amount of enterococci could be as a result of poor handling procedures as well as use of wide variety of substrates and methods by people with little or no knowledge of safe food handling practices; such view is in agreement with the findings of this study. Again, there is every possibility that the high count was due to the exposures of the meat during processing and sales. Jean-Claude and Pascale (2007) reported enterococci to be found in the air, water, soil, vegetation etc.

In Table 2 is shown the identified enterococci isolated from kilishi as well as their distribution pattern. In all, eight (8) species of *Enterococcus* were identified following screening of sixty (60) enterococci isolated during the preliminary stages. These isolates included: *Enterococcus faecium, E. solitaries, E. faecalis, E. asini, E. hirae, E. cecorum, E. casseliflavus* and *E. raffinosus*. Their presence suggests that they could be used as indicator organisms to determine the bacterial quality of food products since they survive in adverse conditions where other organisms of sanitary importance are inhibited. The fact that *E. faecium* and *E. faecal is* were isolated confirmed the report of Jett *et al*. (1994) and Rice *et al.* (1995) that enterococci can easily be isolated from animal products such as meat, milk and other sources include soil, sand, sea, water, dust and air.

The pH of the samples ranged from 6.2-6.4 and these were not significantly different (p>0.05). The pH values for *kilishi* were slightly above the maximum accepted limits of 6.0 suggested for fresh meat (Bennani *et al*., 2000 and Jones *et al*., 2001). This suggests that the pH is affected by the sauce and processing. The moisture content (%) of the samples ranged from 12.8-13.7; ash (7.4-7.6%); crude protein (9.8-10.2%); fat (8.0-9.0%) and carbohydrate (9.8-10.2%). Generally, no significant difference (p>0.05) was recorded among the means in the proximate content of the samples as analyzed. Data obtained showed a correlation between the water content and the aerobic plate counts. Water and high amount of carbohydrates (sugar) support the growth of microorganisms (Mbofung, 1993). Cross correlation of the physiochemical variables showed a significant relationship between moisture content, pH, carbohydrates, ash content, protein content and fat content. High levels of ash content expressed as dry weight have been reported by Jones *et al*. (2001) suggesting the presence of sand and dirt and condiment used for the processing of the meat. Fat content plays an important role in flavor development of meat, as meat ages, the fat deteriorates through microbial attack and tissues enzyme activities which cause the development of free acidity and oxidation of unsaturated bonds. This results in the development of bad odour and deterioration of taste. The antibiotic sensitivity pattern of the sixty (60) enterococci isolated in this study were resistant to five

Table 1. Microbial load of kilishi collected from five sale points within Abuja metropolis.

	Enumeration (cfu/g)[1,2]	
Sale point	Aerobic plate count	Enterococci count
A	$3.9x10^{3}±1.1x10^{3}$ **a**	$6.4x10^{1}±1.3x10^{1}$ **a**
B	$3.3x10^{3}±8.4x10^{2}$ **ab**	$6.7x10^{1}±1.1x10^{1}$ **a**
C	$2.4x10^{3}±3.5x10^{2}$ **ab**	$4.6x10^{1}±0.7x10^{1}$ **a**
D	$1.8x10^{3}±3.\ 6x10^{2}$ **bc**	$6.9x10^{1}±1.9x10^{1}$ **a**
E	$1.2x10^{3}±2.3x10^{2}$ **c**	$4.7x10^{1}±1.8x10^{1}$ **a**

[1]Each data is the mean+ standard error of 12 determinations
[2]Different letters within the same column are significantly different at $p<0.05$.

Table 2. Distribution pattern of Enterococcus spp. isolated from kilishi from five sale points.

		Sale points/distribution pattern of isolates				
Isolates	Number of isolates[1]	A	B	C	D	E
E. faecium	13	3	2	5	1	2
E. solitaries	6	3	1	1	-	1
E. faecalis	15	5	2	3	4	1
E. asini	8	-	2	-	5	1
E. hirae	5	1	1	-	-	3
E. cecorum	4	-	2	-	1	1
E. casseliflavus	5	-	2	2	1	-
E. raffinosus	4	-	-	1	-	3

[1]Sixty (60) enterococci were isolated from five sale points.

Table 3. pH and proximate composition of kilishi obtained from five sale points.

		Proximate content (%)[1,2]				
Sale point	pH	Moisture content	Ash	Crude protein	Crude fat	Carbohydrate
Federal Sec	6.3±0.08 **a**	13.0±0.60 **a**	7.4±0.34 **a**	60.6±0.11 **a**	9.0±0.57 **a**	9.9±0.61 **a**
Jabbi	6.4±0.09 **a**	13.1±0.44 **a**	7.4±0.36 a	60.9±0.18 **a**	8.5±0.51 **a**	9.9±0.43 **a**
Garki	6.3±0.08 **a**	13.4±0.55 **a**	7.6±0.43 a	60.8±0.12 **a**	8.3±0.64 **a**	9.8±0.38 **a**
Utako	6.2±0.10 b	13.7±0.47 **a**	7.6±0.28 **a**	60.7±0.20 **a**	8.0±0.60 **a**	10.0±0.64 **a**
Airport	6.3±0.08 **a**	12.8±0.51 **a**	7.4±0.29 **a**	60.9±0.16 **a**	8.7±0.51 **a**	10.2±0.33 **a**

[1]Each data is the mean + standard error of 12 determinations
[2]Similar letters within the same column are not significantly different at $p>0.05$.

(33%) of the fifteen antibiotics tested. These included Penicilin (98%), Colistin (98%), Nalidixic acid (97%), Cloxacilin (93%), and Streptomycin (70%). This agrees with the work of Lukasova and Sustackova (2003), and Aarestrup *et al*. (2000) who reported resistance of these commonly used antibiotics in medical practice among enterococci. Only three (20%) of the antibiotics tested were very active against the isolates obtained in this study. The isolates showed high susceptibility to ofloxacin, nitrofurantoin and tetracycline. None of the isolates tested was resistant to ofloxacin while 98% of the isolates were susceptible to the antibiotic. There was no resistance to nitrofurantoin as 95% of the isolates demonstrated susceptibility to the antibiotic. Only 10% of the isolates were resistant to tetracycline while 85% of isolates were susceptible to tetracycline. The isolates also showed intermediate susceptibility which is worthy of note to some antibiotics which included chloramphenicol (45%), gentamicin (43%), augumentin (40%), amoxycilin (38%), streptomycin (30%) and erythromycin (28%).

In this study, the resistance to more than one antibiotic was observed in all the enterococcal isolates from *kilishi*. Stovcik *et al*. (2008) reported that the high level of resistance to tetracycline and erythromycin in isolates of animal origin is probably related to the wide use of these classes of antibiotics in livestock production. However, as observed in this study the isolates were obtained from processed meat and they were most likely to have come from post production contamination. The very high susceptibility of isolates to nitrofurantoin agrees with the

Table 4. Antibiotic sensitivity pattern of enterococci isolated from kilishi from five sale points.

	Percentage (%)		
	Resistant	Intermediate	Susceptibility
Augumentin	22	40	38
Amoxycillin	18	38	43
Cloxacillin	93	7	0
Erythromycin	13	28	58
Tetracycline	10	5	85
Ampicilin	48	7	45
Gentamycin	50	43	7
Penicillin	98	2	0
Cotrimoxazole	52	5	43
Nitrofurantoin	0	5	95
Chloramphenicol	30	45	25
Ofloxacin	0	2	98
Nalidixic acid	97	3	0
Streptomycin	70	30	0
Colistin	98	2	0

works of Belicova *et al*. (2007) where isolates from Bryndza cheese showed very high susceptibility to the antibiotic. Previous research have pointed out that it is possible to achieve low contamination of street-vended food supplies which *kilishi* falls into if proper control points such as proper packaging and handling, are successfully established (Mosupye and von-Holy, 1999). It is possible to employ good hygiene practices in the production of *kilishi* so as to minimize and or eliminate the risk posed by these contaminations.

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