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The impact of roasting and reheating on the microbial load of 'Iwe Ekpang' (Steamed Cassava Batter)

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ABSTRACT

The evaluation of iwe ekpang ['steamed cassava batter'] processed from freshly harvested cassava ['Manihot esculenta'] stored for 24 hours, prepared into fresh, reheated and roasted samples were analysed using Standard Microbiological Techniques[STM] and the antibiotic susceptibility of the isolates using Disc Diffusion Technique[DDT]. The bacteria isolated were Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus. The fungi isolated were mould spp. and Aspergillus sp. The results also revealed that 'Iwe ekpang' C_1 and EK_6 had the highest 7.8 x 10^5 and 1.10 x 10^6 cfu/g, respectively and the highest mycological count was also obtained from 'iwe ekpang' Ek_4 and Ek_6, 4.2 x 10^5 and 4.4 x 10^5 accordingly. The reheated 'iwe ekpang' [Ek_1 and Ek_5] is safer and is within the limit recommended by International Commission on Microbiological Specification for ready to eat foods. Among the antibiotics used, the organism were susceptible to Ofloxacin followed by Perflacin and Gentomycin.The isolates gave gross resistant to Tetracycline, Ampicillin and Nalidixic acid. The P^H range from 5.6 to 7.9. Nevertheless this result reveals most potent drug for treatment of those affected by this organisms in any case of food spoilage or poisoning caused by the tested bacteria.

INTRODUCTION

'Iwe ekpang' is a steamed cassava [*Manihot esculenta*] batter that is often wrapped with plantain leaves. It is usually processed and eaten the same day or the following day. However, 'iwe ekpang' does not stay for two days or three without slimy substance, white patches, and peculiar sour odour. According to [1], spoilage is evidenced by release of offensive odour which is attributed to the action of microorganisms and other chemical changes. Cassava when uprooted as in harvesting sustained bruises and may come with fungal-link. At times 'iwe ekpang' processed with cassava stored for 24 hours or two days often encouraged exposure to deterioration before grating, wrapping and steaming. More so, left over iwe-ekpang is usually stored for a day, two or three days depending on the quantity prepared and the total number of people in the household. The leftover can be reheated or roasted [using open fire] daily where there is no refrigerator or deep freezer. As observed in the literature report, cassava roots are highly perishable and a lot of post-harvest losses occur to this commodity during storage due to high physiological and microbial activities that enter bruises received during harvesting as well as the inherent high moisture content of fresh roots, which promote microbial deterioration and unfavourable biochemical changes in the commodity [2]. Moreso, 'iwe ekpang' with high moisture especially when not properly wrapped and covered is often very perishable and prone to microbial and fungal growth. The objective of this work was to evaluate microbial activities during processing of 'iwe ekpang' and effect of roasting in the open fire as well as reheating methods on the microbial count of leftover 'iwe ekpang' and the susceptibility pattern of the isolates to chemotherapeutic agents.

MATERIALS AND METHODS

1)**Collection of Materials**
Cassava (*Manihot esculenta*) roots and plantain (*Musa paradisiacal*) leaves were obtained from Akpandem Market in Uyo, Nigeria.

2)**Treatment of Samples**
Freshly harvested cassava roots were obtained as mentioned above and subsequently divided into two portions. One portions was kept for 24 hours, while the other half was processed immediately into 'iwe ekpang'. Each portion was processed as illustrated in Fig. 1. Analyses were carried out in the Department of Microbiology, University of Uyo, Nigeria.

METHOD
3)**Determination of pH Values**
The pH of the cassava batter, 'iwe ekpang' and its leftover samples were determined using CD640 digital pH meter [WPA Linton, Cambridge England] after blending 25g of samples.

Moisture Content
Moisture content was determined as thus.
Weight of the beaker only……a
W eight of the beaker +sample wet……..….b
Weight of the beaker + sample dried…….……..…..c

Calculation $\frac{b - c}{b - a} \times \frac{100}{1}$

MICROBIOLOGICAL METHOD OF ANALYSIS OF CASSAVA BATTER 'IWE EKPANG' AND ITS LEFTOVER
One gram of each blended 'iwe ekpang' was weighed out aseptically from the sterile mortar and introduced into 9mls of sterile distilled water in a test tube. The dilution was serially made up to 10^4. With the aid of wax pencil the bottom of the Petri dish plates were properly labelled with the sample code, dilution factor, media used and date. With the use of sterile pipette, 1ml of the aliquots prepared from each of the 'iwe ekpang' was aseptically transferred from dilution [10^3] into a Petri plates in triplicates [3]. Sterile prepared plate count agar [PCA] and Potato Dextrose Agar [PDA] were used for the pour plate method. Twenty five [25]mls PCA were poured into each the plate labeled for total heterotrophic bacterial count, while 25mls of PDA were poured into each of the plate labeled for total heterotrophic fungal count. These media were aseptically poured at 44^{o}C to avoid killing the microorganisms present in the food samples. The plates of PCA were incubated at 37^{o}C for twenty four [24] hours while plates of PDA were incubated at room temperature for 5-7 days. The immerged colonies were counted and sub-cultured to obtain pure colonies. Gram's staining, microscopic examinations, biochemical tests [urase, catalase, coagulase, indole, motility, citrate, methyl red, vogues proskauer, spore, oxidase, nitrate, gelatin test and carbohydrates fermentation test were carried out for the identification of the isolates.

ANTIBIOTIC SUSCEPTIBILITY TEST
The antibiotic susceptibility test was determined by the standardized disc diffusion method. Commercially prepared single and multidisc with the following antibiotics were used. Ampicillin[Am] [25µg], Tetracycline[Tet] [25µg], Gentamycin[Gen] [10µg], Perflaxin[Pef] [5µg], Cotrimoxazole[Cot] [25µg], Nalidixic acid [Nal][30µg] and Ofloxacin (5µg).

Mueller-Hinton Agar plates were dried in the incubator at 45^{o}C until the surface were free from visible moisture before use for the susceptibility study. One drop of 10 – 18 hour old culture of the test organisms were inoculated by spread plate method. Sterile forceps was used to pick up the disc and placed gently and firmly on the surface of the seeded plate. The discs were evenly distributed to allow an edge not less than 15 mm from the wall of the Petri dish. The inoculated plates were incubated at 37^{o}C for 24 hours.

CONTROL
The control culture (standard organism of a known sensitivity of staphylococcus was plated simultaneously with the test organism on separate plate. The zones of inhibition produced in the two cultures by the same discs were compared. Kirby Bauer classification method of zone inhibition was adopted.

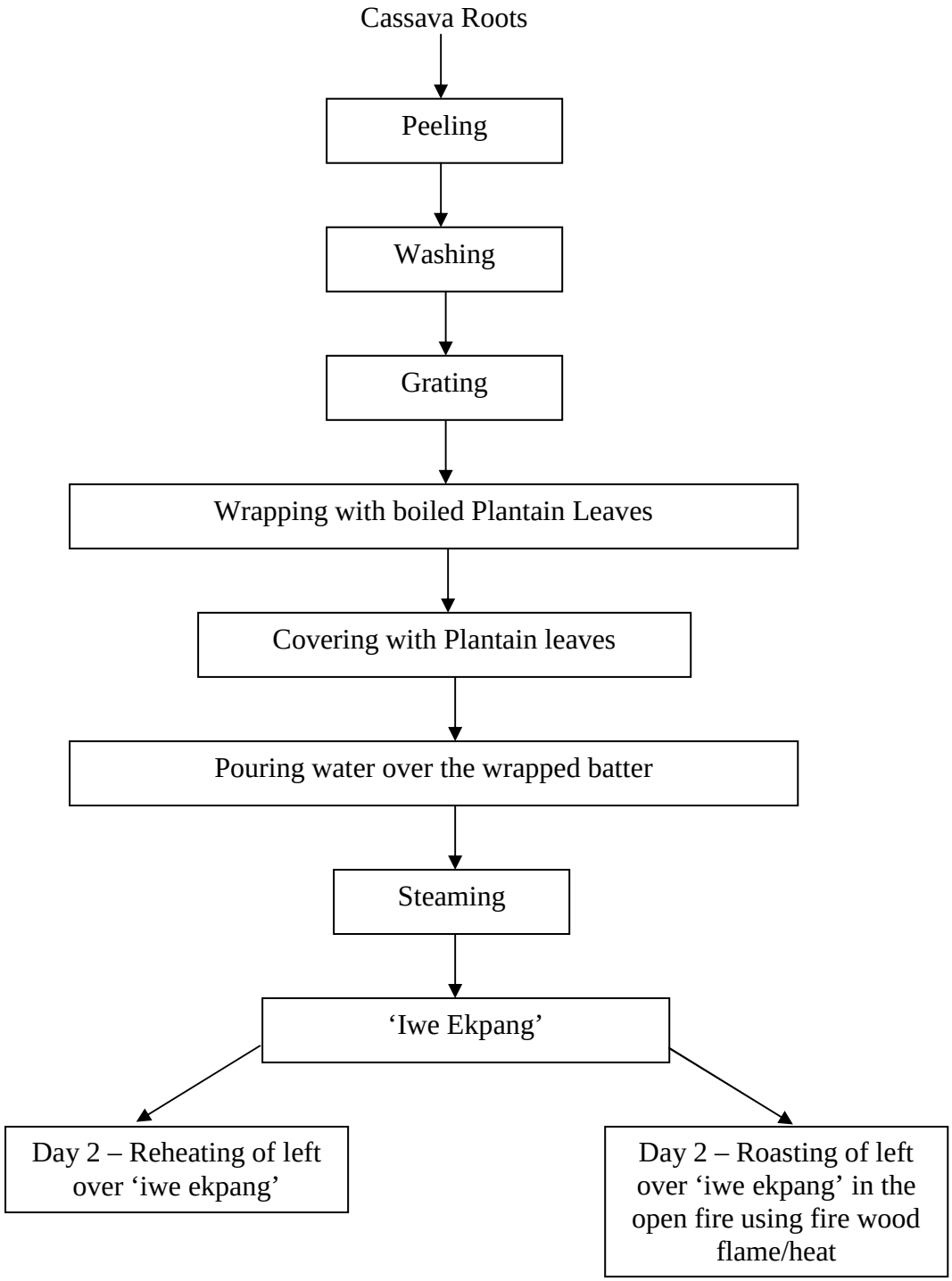

Fig. 1: Flow Chart for Processing of Cassava Roots into 'Iwe Ekpang' and the Treatment of Leftover

RESULTS AND DISCUSSION

Table 1 shows that total heterotrophic bacterial count in the freshly processed product, 'iwe ekpang' [Ek_1] $2.3x10^4$. This was reduced with the application of steaming. However, reheated leftover 'iwe ekpang' [Ek_2] had lowest bacterial count $3.4x10^4$ than roasted 'iwe ekpang'[EK_3] $4.8X10^4$. The bacterial count was in line with the recommendation limits for bacterial contamination for ready to eat foods by the specifications for foods [4], which must be less than 10^5 (cfu/g) of food for total bacterial plate count and mould count. Freshly grated cassava processed from cassava stored for 24 hours (C_2) had the highest total plate count than 'iwe ekpang' processed from it (EK_4). The leftover 'iwe ekpang' when reheated (EK_5) had lower bacteria count than the roasted 'iwe ekpang'

(EK_6). These plate counts of EK_6 were higher than the specification of less than 10^5 (cfu/g). In Table 2 total fungal count in samples EK_1, EK_2 and EK_3 were lower than C_1. High total fungal count was observed in samples C_2, EK_4 and EK_6 whereas there was no fungal activities in sample EK_5. The bacterial isolated as observed has shown *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. While fungal isolate was *Aspergillus* sp. And these are in agreement with [5]. Four isolates of distinct organisms were identified. Table 3 shows the isolates and percent occurrence. These were high ranging from 53.33 percent for *Aspergillus* sp. and *Bacillus subtilis*. with 66.67%. Microorganisms were isolated from the freshly harvested cassava and cassava roots stored for 24 hours as well as 'iwe ekpang' (steamed cassava batter) prepared from the samples of cassava roots. The microorganisms isolated were present in the two cassava samples as well as 'iwe ekpang' processed from the two cassava samples. However, the microbial growth was favoured by the increase in moisture content from 56 percent in freshly harvested cassava to 68% in 'iwe ekpang' processed from it. It also increased from 54% in cassava roots stored for 24 hours to 62% in 'iwe ekpang' processed from it. Consequently, pH level ranges from 5.6 in freshly harvested cassava to 7.9 in 'iwe ekpang' processed from it and from 5.8 in cassava roots stored for 24 hours to 7.7 in 'iwe ekpang' processed from it. Nevertheless, the increase in percent moisture and the pH level (especially alkalinity) of the samples affected the growth pattern (that supports the persistence of the microorganisms isolated from the cassava samples and in the processed product ('iwe ekpang'). This is in confirmation with reports from [2]. Although the same microorganisms were isolated from the reheated and roasted 'iwe ekpang' the count was greater in roasted than the reheated 'iwe ekpang'. This means that the microbial contamination is in conformity with of [5]. That boiling at standard temperature and pressure for 30 – 60 minutes did not completely destroy the enterotoxin as measured by the ability of the toxins to induce vomiting in monkeys. It is not unusual for some people to vomit after eating freshly processed cooked or reheated or roasted 'iwe ekpang'. Also, in this study the bacterial plate count in roasted 'iwe ekpang' connotes with literature report of Rose (1983) that large number of *Staphylococcus* cells ($5 \times 10^5 - 10^6$ cfu/g) are necessary for disease symptoms to be manifested. 'Iwe ekpang' as wrapped with plantain leaves does not allow adequate heat to penetrate the food for effective destruction of the organisms. Also exposure of this batter, the product ('iwe ekpang') to the environment and the handlers health during processing contribute to the state of the food and contamination. The report of [6],[7]and[8] confirmed that the most important sources of this organism *Staphylococcus* in foods and beverage are the nasal canals and infected hand.

Table 1: Total bacterial count from 'iwe ekpang' analysed

Parameter	Sample code	Total Bacterial count[cfu/g
Cassava batter processed from		
freshly harvested cassava roots	C_1	$7.8x10^4$
'Iwe ekpang 'processed from		
freshly harvested cassava roots [from C_1]	EK_1	$2.3x10^4$
Reheated 'Iwe ekpang'[fromEK_1]	Ek_2	$3.4x10^4$
Roasted 'Iwe ekpang' from [EK_1]	EK_3	$4.8x10^4$
Cassava batter processed from		
cassava stored for 24 hours	C_2	$6.4x10^4$
'Iwe ekpang' processed from		
cassava stored for 24 hours [C_2]	EK_4	$2.2x10^4$
Reheated 'Iwe ekpang' [fromEK_4]	EK_5	$3.0x10^4$
Reheated 'Iwe ekpang' [fromEK_4]	EK_6	$1.10x10^5$

Table2: Total Micological Count from 'Iwe ekpang'

Parameter	Sample code	Total Fungal count[cfu/g]
Cassava batter processed from freshly harvested cassava roots	C_1	$3.3x10^4$
'Iwe ekpang' processed from freshly harvested cassava roots[from C_1]	EK_1	$2.0x\ 10^4$
Reheated 'Iwe ekpang' [fromEK1]	Ek_2	$1.0x\ 10^4$
Roasted 'Iwe ekpa [fromEK_1]	EK_3	$3.0x10^4$
Cassava batter processed from cassava stored for 24 hours	C_2	$4.0x10^4$
'Iwe ekpang' processed from cassava stored for 24 hours [from C_2]	EK_4	$4.2x10^4$
Reheated 'Iwe ekpang' [fromEK_4]	EK_5	Nil
Reheated 'Iwe ekpang'[from EK_4]	EK_6	$4.4x10^4$

Table 3: Occurence of Fungi and Bacteria isolates isolated from all 'Iwe ekpang'

Organism	Number of occurrence	% of Occurrence
Staphylococcus aureus	7	26.92
Bacillus subtilis	8	30.77
Pseudomonas aeruginosa	7	7.69
Mould sp	3	11.58
Aspergillus sp.	6	23.08

Table 4: Organism Isolated from each sample of 'iwe ekpang'

Parameter	Sample code	Organism isolated
Cassava batter processed from freshly harvested cassava roots	C_1	*S. aureus, B subtilis, P aeruginosa Mould* sp and *Aspergillus* sp
'Iwe ekpang' processed from freshly Harvested cassava roots [from C_1]	EK_1	*S. aureus, Bacillus subtilis and Aspergillus sp.*
Reheated 'Iwe ekpang [FromEk_1]	EK_2	*B. subtilis* and *Aspergillus* sp
Roasted 'Iwe ekpang [FromEk_1]	EK_3	*S. aureus, B. subtilis* and *Aspergillus* sp and *mould*
Cassava batter processed from stored For 24 hours	C_2	*S. aureus, B. subtilis, Ps aeruginosa* and *Aspergillus* sp
'Iwe ikpang' processed from stored for 24 hours [form C_2]	EK_4	*Staphylococcus aureus B subtilis* and *mould* sp.
Reheated 'Iwe ekpang' [From Ek_4]	EK_5	*B. subtilis* and *S. aurues*
Roasted 'Iwe ekpang [From Ek_4]	EK_6	*S. aurues, B. subtilis* and *Aspergillus sp.*

Table 5: P^H Level and Moisture Content each Sample of 'iwe ekpang'

Parameter	Sample code	P^H	Moisture
Cassava batter processed from freshly harvested cassava roots	C_1	5.6	56
'Iwe ekpang' processed from freshly harvested cassava roots [from C_1]	EK_1	7.9	68
Reheated 'Iwe ekpang [FromEk_1]	Ek_2	7.8	65
Roasted 'Iwe ekpang [FromEk_1]	EK_3	7.2	62
Cassava batter processed from stored For 24 hours	C_2	5.8	54
'Iwe ikpang' processed from stored For 24 hours [from C_2]	EK_4	7.7	62
Reheated 'Iwe ekpang' [From Ek_4]	EK_5	7.5	64
Roasted 'Iwe ekpang [From Ek_4]	EK_6	7.6	61

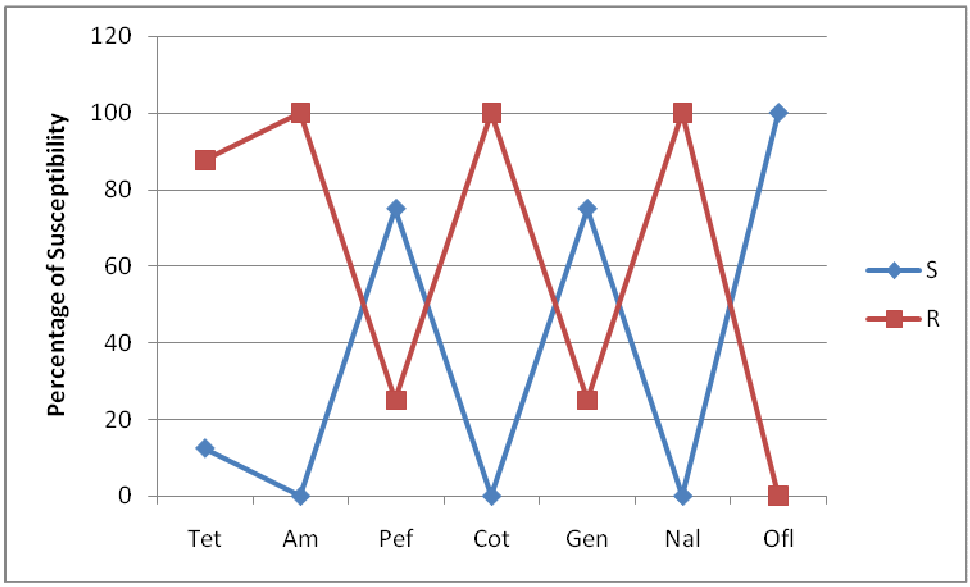

Fig2: **Susceptibility Patterns of *Staphylococcus aureus* Isolated from Steamed Cassava Batter**
S=Sensitivity, R=Resistance

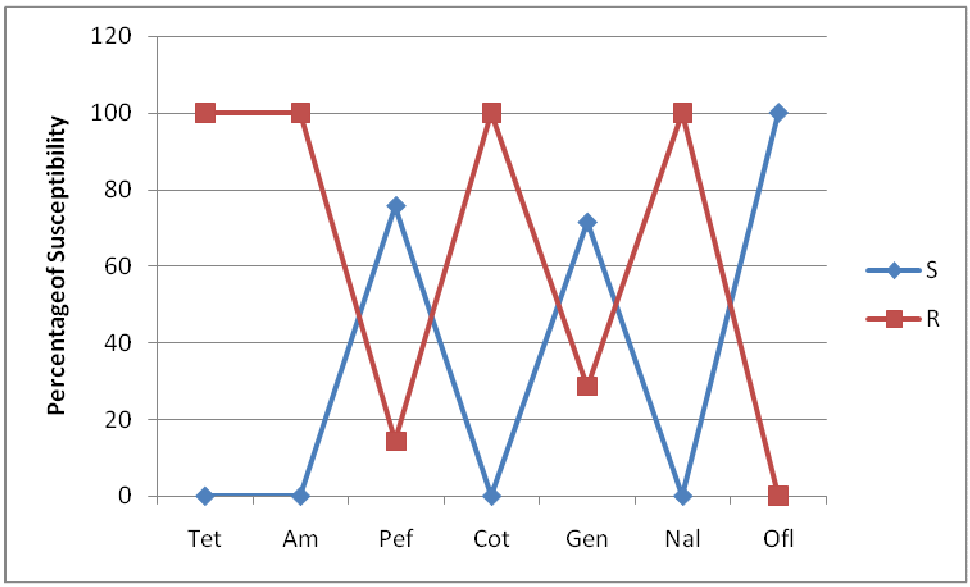

Fig3: Susceptibility Patterns of *Bacillus aureus* Isolated from Steamed Cassava Batter
R=resistance, S= Sensitive.

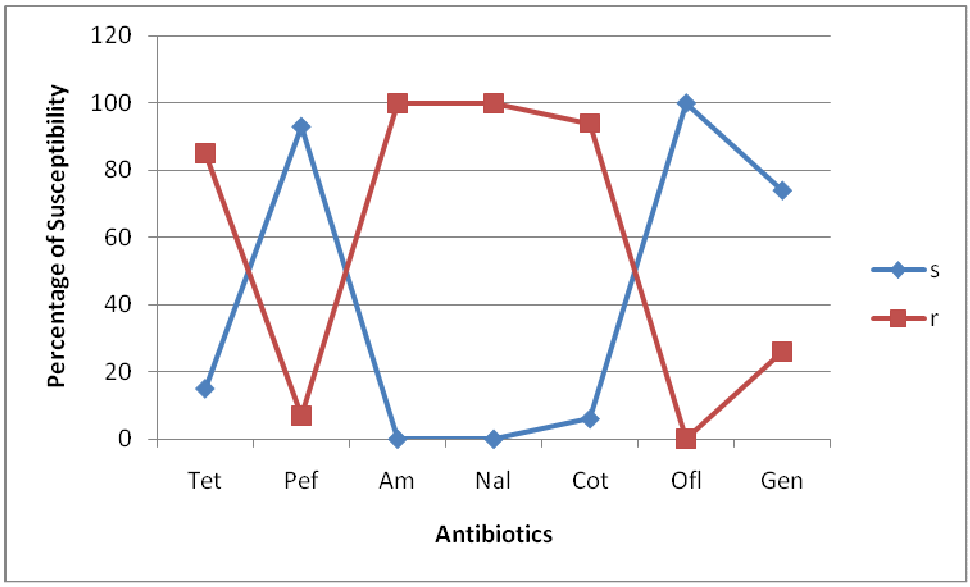

Fig4: Susceptibility Patterns of *Pseudomonas aeruginosa* Isolated from Steamed Cassava Batter
R=resistance, S= Sensitive.

The degree of sensitivity pattern varied with isolates. The most sensitive antibiotics was Ofloxacine followed by Perflacine, and Gentamycin. The resistant patterns of *S. aureus*, *Ps aeruginosa* and *B. subtilis* to penicillin ampicillin cotrimoxazole and nalidixic acid fig 2,3and,4 may be due to indiscriminate use and abuse of drugs, adulteration of drugs and mutation of microorganisms. Multiple antibiotic resistance amongst members of bacterial isolates has been well documented by [10].

CONCLUSION

The disease symptoms associated with the consumption of 'iwe ekpang' (steamed wrapped cassava batter), is not only caused by the high level of cyanide consumption in 'iwe ekpang' processed from freshly harvested cassava and cassava stored for 24 hours or eaten the same day but it is also caused by the consumption of 'iwe ekpang' roasted in the open fire which contain *Staphylococcus* spp., and *Bacillus* sp. and *Aspergillus* sp. and could not be destroyed at the temperature and duration for which 'iwe ekpang' was cooked, roasted and reheated. It is better to reheat the

leftover 'iwe ekpang' to the boiling point than to roast in the open fire before eating and misused of antibiotics should be avoided.

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