Original Research Article

MICROBIAL ASSESSMENT OF SELECTED, LOCALLY-FERMENTED AND READY-TO-EAT CASSAVA PRODUCTS SOLD IN LOKOJA, NIGERIA.

ABSTRACT

This study was conducted to assess locally-fermented, ready-to-eat cassava products in Lokoja for microbial contamination. Sixty samples comprising; twenty white garri, twenty yellow garri and twenty fufu were subjected to microbial analysis. Samples were serially diluted to 10-4 and appropriate dilutions inoculated by spread plate method unto Nutrient agar, MacConkey agar and Potato Dextrose agar plates for Total aerobic plate count (TAPC), Coliform count (CC) and Fungal count respectively. The TAPC for white garri ranged from 1.0 x 101 to 7.0 x 103, the coliform count ranged from no growth (NG) to 7.1 x 103, while the mean fungal count ranged from 1.0 × 102 to 3.0 × 103. The TAPC for yellow garri ranged from 1.1 x 102 to 9.0 x 103, the coliform count ranged from NG to 6.0 × 103 and the fungal count ranged from 1.0 x 102 to 3.0 x 103. The TAPC of fufu ranged from 1.2 x 101 to 5.0 x 103, the coliform count ranged from NG to 3.0 x 103 and the fungal count ranged from 1.0 × 102 to 7.0 × 102. The bacteria isolated include Bacillus spp., Enterobacter spp., Pseudomonas spp., Staphylococcus aureus, salmonella spp., Escherichia coli and Klebsiella spp. The fungi isolated from the study samples include Aspergillus niger, Cladosporium spp., Fusarium spp., Rhizopus spp., Alternaria spp., Montospora spp., and Penicillium spp. The pH of the samples ranged from 4.02 to 4.96 in white garri, 4.02 to 4.99 in yellow garri, and 5.02 to 6.44 in fufu. Findings show that these widely consumed fermented (ready-to-eat) cassava products presents (may represent) a serious risk and route for transmission of food borne pathogens to consumers and generally huge economic disadvantage to food handlers. Improved manufacturing, packaging and storage practices in garri production and for public health purposes are strongly encouraged.

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Microbial analysis of… via spread plate method.

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1 Introduction

Food is the basic necessity for human survival and attainment of food security is the priority of any country. However it is important that food security should not be seen only in the perspective of availability either

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quantitatively or qualitatively. Therefore, food hygiene and safety should also be given important consideration in order to protect the health of the people as they could serve as vehicle for the transmission of food borne diseases [1] [2]. Food may be available but the source from which it is produced and / or processed may be unhygienic and even the chemicals that may be used to preserve it may cause serious health hazard [1].

Coliforms, particularly *Escherichia coli* are used as indicators of post process contamination and also the presence of *E. coli* in foods serves as an indicator of faecal contamination [3]. Coliforms are group of closely related Gram negative, non-spore forming, rod-shaped aerobes and facultative anaerobes that ferment lactose to produce acid and gas within 48 h at 35^{o}C. They are mostly harmless and lives in soil, water and in the gut of animals with few enteric pathogens including *Salmonellae*, *Shigellae* and enteropathogenic *E. coli* [4].

Filamentous moulds and yeasts are common spoilage organisms of food products and some species of *Penicillia* and *Aspergilli* have been reported as spoilage organisms of a variety of foods on which they may produce a quite number of mycotoxins [5].

Cassava ranks fourth in the list of major crops in developing countries after rice, wheat and maize and it is used for the production of a variety of West African foods [6]. In its natural state, it is toxic to man as it may contain high levels of linamarin, a cyanogenic glucoside. Hence, processing through fermentation, enhance its detoxification, improving the quality and hygienic safety of the food.

Comment [G16]: Please rephrase the sentence. If you read it again, it is sounded like food hygiene and safety serve as a vehicle for the transmission of foodborne disease.

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Garri and fufu happen to be one of the finished products of fermented cassava and if not properly and carefully handled during processing and / or storage, it could serve as vehicle for transmission of food borne pathogens. Moreover, there could be economic losses and widespread of food borne illnesses as a result of contamination by these microorganisms. Therefore, this study is aimed at evaluating locally fermented ready-to-eat cassava products (garri and fufu) for any microbial contamination and microbiological safety with attempt at awareness creation on food safety to consumers and relevant additions to the body of knowledge on this all important staple foods in order to inform right agricultural and health policies. Furthermore, the obtained result will aid policy makers in making necessary quality hazard, storage techniques as well as processing line crucial for the production of fermented staple food from cassava in Nigeria and West Africa at large.

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2 Materials and Methods

2.1 Sample collection

A total of 60 samples of locally-fermented, ready-to-eat cassava products, comprising 20 white garri, 20 yellow garri and 20 fufu were collected

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aseptically from the five major markets in Lokoja Kogi state which includes; Ganaja, Adankolo, Lokongoma, Old and New International Markets. In each of the markets, four samples (i.e. from 4 vendors) of each of the ready-to-eat locally fermented cassava products were collected in sterile nylon bags and transported to Salem University Advanced Microbiology Laboratory for analyses.

2.2 Microbiological Analysis.

Microbiological analysis was carried out using conventional microbiological procedures. The analysis involved total aerobic plate count, fungal count and coliform count. This was determined by the spread plate method using standard microbiological techniques.

2.2.1 Isolation and Identification of Microbial Isolates

Ten gram (10g) of each sample were homogenized in 90 ml sterile distilled water (10^{-1} dilution). Serial dilution of sample homogenate to 10^{-4} was carried out also in sterile distilled water for colony count. Approximate 0.1ml aliquot of appropriate dilutions were spread plated on plates of Nutrient agar and Potato Dextrose agar supplemented with 0.2µg of chloramphenicol (all from Bio-laboratory, Hungary). All Nutrient agar and MacConkey agar plates were incubated at 37^0C for 24-48 h while all potato dextrose agar plates where incubated at 25^0C for 72-120 h. All plates were prepared in duplicate. Culture plates were examined, while enumeration and identification of colonies was carried out at the end of the incubation period. The total microbial population was expressed as colony forming unit per gram of the sample (cfu/g).

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Comment [G31]: This method is for the enumeration. Not for the isolation and identification part. Please change the subtitle.

2.2.2 Detection of Hygiene indicator organisms and specific food borne pathogens

Samples were plated on MacConkey agar, Manitol Salt agar and Salmonella-Shigella agar (Oxoid, England) after pre-enrichment in Selenite F broth and incubated at 35^0C for 24-48 h, for isolation of Escherichia coli, Staphylococci and Salmonellae respectively.

2.2.3 Coliform Test

One gram samples were also inoculated into Lactose broth in screw capped test tubes with inverted Durham tubes and incubated at 37°C for 24-48 h. Tubes showing gas production and/or color change of dye were reported as presumptive coliform test positive. These positive tubes were streaked out on duplicate plates of Eosin Methylene Blue (EMB) agar for confirmatory test and incubated at 37°C and 44°C respectively for 24 h. Growth of characteristic colonies on EMB medium represent confirmatory positive test which were Gram stained and inoculated into lactose broth for complete coliform test. Gas production and/or color change of dye plus Gram negative non spore bearing rod represent presence of coliform.

2.2.4 Isolation and Identification of bacteria and fungi

Pure cultures of suspected colonies were obtained by repeated subculture on nutrient agar plates and potato dextrose agar plates for bacteria and

Comment [G32]: Directly? Samples of the food products?

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In addition, the usage of 1 g of sample is questionable. The method here is incomplete/wrongly written as MPN is often used for coliform test.

fungi isolates respectively and stored on slants at 4°C until characterized. The isolation and identification of the bacteria were carried out using standard microbiological techniques including; Gram stain, catalase test, coagulase test, indole test, citrate test, oxidase test [7]. All fungi isolates were identified following previously described methods [8].

2.3 Determination of pH of the locally fermented ready-to-eat cassava products

The pH was determined using digital pH meter calibrated with standard buffer solutions.Ten grams of each sample were weighed and homogenized in 20ml of sterile distilled water in a beaker for 1 min. The solution decanted and the pH of the suspension measured.

2.4 Statistical analysis

The mean of the total viable microbial count was subjected to analysis using SPSS version 20. The mean microbial load cfug^{-1} and pH of the samples were presented in tables.

3 Results and Discussion

The mean TAPC, fungal count, and coliform count results are shown in Table 3, and it reveals the samples had TAPC that are within the range of

Comment [G36]: What is the purpose of determining the pH of the food samples?

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10^1 to 10^3. The fungal and coliform counts are about the same order. The result of this study was in line with the earlier reported data [9] [10] however, it was slightly lower than another previously reported [11], with counts that ranged from 10^3 to 10^4. Moreover a study conducted in Ebonyi, Ogun and Oyo states in Nigeria reported microbial burden as high as 10^6 to 10^7 respectively [12] [13], while another related work reported a fungal count as high as 10^4 to 10^6 [14]. The disparity in the microbial count from these studies could be as a result of the processing method, the quality of water used in the production process and the length of exposure during sale. A researcher equally observed back slopping used by some processors to reduce the length of time for fermentation to compromise the quality of the product [13], suggesting it could be the lead cause of high counts observed in their study. Meanwhile, the total aerobic plate count and fungi counts of this study samples were within the acceptable limit. Ready-to-eat foods with plate counts of $\leq 10^3$ are within the acceptable limit while counts of 10^4 to 10^5 are tolerable and counts that are $>10^6$ are totally unacceptable [15]. Coliform was detected in most of the samples at high counts 10^2 to 10^3, and the presence of *E. coli* calls for serious concern. It signifies poor sanitary condition and indicative of faecal contamination during the production process and / or storage of the fermented ready-to-eat food under study. It is also indicative of the potential presence of enteric pathogens and therefore makes the study samples of poor quality for human consumption.

Bacterial isolates from the study samples were *Bacillus* sp., *Enterobacter* sp., *Pseudomonas* sp., *Staphylococcus aureus*, *salmonella* sp.,

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Escherichia coli and *Klebsiella* sp. (Table 1). Most of the isolates were glucose positive, indole negative, catalase positive and a reasonable number of both Gram positives and Gram negatives (Table 4). The isolation of diverse microbial species from this ready-to-eat fermented foods did not completely agree with the earlier findings [9] [10] [13] as each author had dissimilarities in bacterial presence in their study samples. Most of these studies reported the presence of *Staphylococcus aureus*, *Bacillus* sp., *Pseudomonas* sp., *E. coli* and *Klebsiella* sp. The observation of diverse bacteria isolates could be attributed to the fact that these studies was carried out at different regions and from different sample markets of which environmental conditions of the study areas could affect the distribution of organisms. Buyer's attitude towards the exposed food products in the market could also contribute to the microbial load and diversity as they touch the products with bare hands and taste it before they buy. The presence of *Salmonella* in this study calls for concern as this organism is the common cause of human food poisoning, and salmonellosis can affect all species of domestic animals and man. It is important to draw to our attention that the young, aged, stressed, debilitated and pregnant individuals are more susceptible while the immunosuppressed and those suffering from malnutrition are at risk for *salmonella* infection [16]. The presence of *Bacillus* and *Staphylococcus aureus* also calls for concern because some strains of these organisms are known to be toxigenic and often implicated in food borne intoxication [17] [18]. *Bacillus* a common environmental contaminant and a spore former can withstand environmental stress and this may account for its presence

in the samples. Meanwhile, *Staphylococcus aureus* is of human origin and their presence could therefore be from the food handlers, utensils and the environment. Moreover, garri and fufu is a common food widely consumed by all in Nigeria and increasing intake of it, especially dry garri as snacks or with cold water is an added practice that exposes the populace to serious health risk due to the microbial status of the product.

Fungal isolates from the samples collected were detailed in Table 2. More so, among all fungal isolates; most authors reported the presence of *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. in their study [14] [9] [10], which agrees with the result of this study. Filamentous fungi are common environmental contaminants usually implicated in ready-to-eat foods because they produce spores and this could explain their presence in the study samples. More so, species of *Aspergillus, Penicillium* and *Fusarium* are known to produce mycotoxins [19] [20] [21] and their presence in the study samples calls for serious concern.

Comment [G41]: How do you explain the spores to be present in the food?

The pH value of white garri and yellow garri ranged from 4.02 to 4.96 and 4.02 to 4.99 respectively while that of fufu ranged from 5.02 to 6.44 (Table 3). It was observed from the study that the pH values of fufu were higher than that of the garri samples. The pH values recorded in this study was within the range of those reported in related studies [9] [10] [13] for the garri samples. Moreover, a study conducted in Ebonyi state, Nigeria reported higher pH values of 5.47 to 6.61 [12] which is in disagreement with this study. This disparity observed could be attributed to the length of fermentation and storage time. A study reported a reduction in pH of

cassava mash from 6.2 to 3.2 over a period of 10 fermentation days under ambient temperature of 28 – 32^{o}C [22]. [14] It was also revealed from a study that the longer the storage time, the higher the pH. Another study attributed the increase in pH to be as a result of production of acidic metabolites by microorganisms during their growth and proliferation [23].

Conclusion

This study has shown that the fermented staple food under study were contaminated with both bacterial and fungal species, with presence of coliforms. Some of the isolated organisms are well-known causes of food borne diseases and food intoxications. With these findings, the important of hygiene during processing and/or storage of these food products cannot be overemphasized. It is also important that the food handlers properly covers the food product during storage and also reduce the length of its exposure in the market place. The use of specific starter culture, effective HACCP application and good manufacturing practice from farm to fork is also suggested to help reduce the level of contamination and possibly ward off food borne pathogens.

Comment [G42]: Conclusion should be a summary of your results followed by recommendation.

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This was not at all mentioned in the discussion.
Effective HACCP application applies in food manufacturing companies but not locally/artisanal produced food.

Comment [G44]: This is not applicable.

References

1. Ojo EO, Adebayo PF. Food security in Nigeria: An overview. European Journal of Sustainable Development, 2012; 1(2): 199-122.

2. Feroz F, Shimizu H, Nishioka T, Mori M, Sakagami Y. Bacterial and fungal counts of dried and semi-dried foods collected from Dhaka, Bangladesh and their reduction methods. Biocontrol Science, 2016; 21(4): 243-261

3. Dogan-Halkman HB, Cakir I, Keven F, Worobo RW, Halkman KA. Relationship among faecal coliforms and Escherichia coli in various foods. European Food Research and Technology, 2003; 216: 331-334.

4. Nkere CK, Ibe NI, Iroegbu CU. Bacteriological Quality of foods and water sold by vendors and in restaurants in Nsukka, Enugu state, Nigeria: A comparative study of three microbiological methods. J Health Popul Nutr, 2011; 6: 560-566.

5. Samson RA, Hoekstra ES, Frisvad JC, Filteenborg O. Introduction to food and air borne fungi. 6th ed. Central bureau voor schimmelcultures. Utrecht, The Netherlands; 2002. P. 210.

6. Edward VA, Egounlety M, Huch M, Vanzyl PJ, Singh S, Nesengani ND, et al. Isolation and screening of microorganisms from garri fermentation process for starter culture development. African Journal of Biotechnology, 2012; 11(65): 12865-12877.

7. Cheesbrough M. District laboratory practice in tropical countries. 2nd ed. Cambridge University Press; 2006.

8. Okafor JI, Eze EA. Medical mycology. In: Ezeonu IM, Okafor JI, Ogbonna JC, editors. Laboratory exercises in microbiology: a practical manual for students of tertiary institutions.1st ed. Ephrata Printing and Publishing Company, Nsukka; 2011. pp 108-116.

9. Olopade BK, Oranusi S, Ajala R, Olorunsola SS. Microbiological quality of fermented cassava (Gari) sold in Ota Ogun state Nigeria. International Journal of Current Microbiology and Applied Sciences, 2014; 3(3): 888-895.

10. Akindele ST, Abimbola WA. Microbial evaluation of Garri sold in Ijebu community. IOSR Journal of Environmental Science, Toxicology and Food Technology, 2018; 12(7): 35-38.

11. Adebayo CO, Aderiye BI, Akpor OB. Assessment of bacterial and fungal spoilage of some Nigerian fermented and unfermented foods. African Journal of Food Science, 2014; 8(3): 140-147.

12. Orji JO, Nnachi AU, Ojiochie CO, Egwuatu CC, Efunshile AM, Ezejiofor OI, Ezeama CO, Nwanchi CU, Mbachu I. Bacteriological quality and public health implications of fermented cassava (Garri) sold in Okwor and Nkalagu markets in Ebonyi state, Nigeria. International Journal of Scientific and Technology Research, 2016; 5(11): 85-91.

13. Adebayo-Oyetoro AO, Oyewole OB, Obadina AO, Omemu MA. Microbiological safety assessment of fermented cassava flour “Lafun” available in Ogun and Oyo states of Nigeria. International Journal of Food Science, 2013; Article ID 845324, 5 pages.

14. Aguoru CU, Ondo MA, Omoni VT, Ogbonna IO. Characterization of moulds associated with processed garri stored for 40 days at ambient temperature in Markurdi, Nigeria. African Journal of Biotechnology, 2014; 13(5): 673-677.

15. International Commission on Microbiological Specifications for Foods (ICMSF) 1996. Microorganisms in Foods 5: Microbiological Specifications of Pathogens.

16. Rahman HS, Othman HH. Salmonella infection: The common cause of human food poisoning. Progress in Bioscience and Bioengineering, 2017; 1(1): 5-10.

17. Oranusi S, Galadima M, Umoh VJ, Nwanze PI. Food safety evaluation in boarding schools in Zaria, Nigeria, using the HACCP system. Scientific Research and Essay, 2007; 210, 426-433.

18. Behling RG, Eifert J, Erickson MC, Gurtler JB, Kornacki JL, Line E, Radcliff R, Ryser ET, Stawick B, Yan Z, editors. Principles of Microbiological troubleshooting in the industrial food processing environment, food microbiology and food safety. Behling food safety associates Madison, WI, USA, 2010.

19. Greco MJ, Franchi ML, Golba SLR, Pardo AG, Pose GN. Mycotoxins and mycotoxigenic fungi in poultry feed for food-producing animals. The Scientific World Journal, 2014; Volume 2014, Article ID 968215, 9 pages.

20. Bennett JW, Klich M. Mycotoxins. Clinical Microbiology Reviews, 2003; 16(3): 497-516.

21. Pitt JI, Basilico JC, Abarca ML, Lopez C. Mycotoxins and toxigenic fungi. Medical Mycology, 2000; 38(1): 41-46.

22. Oduah NO, Adepoju PA, Longe O, Elemo GN, Oke OV. Effects of fermentation on the quality and composition of cassava mash (Garri). International Journal of Food Nutrition and Safety, 2015; 6(1): 30-41.

23. Ogugbue CJ, Mbakwem-Aniebo C, Akubuenyi F. Assessment of microbial air contaminants of post processed garri on sale in markets. African Journal of Food Science, 2011; 5 (8): 503-512.

Table 1: Distribution of bacteria in the food samples across the sampled markets

Market	Sample	Salm	Kleb	Staph	Bacillus	E.coli	Strept	Pseudo	Entero
Adankolo	White	+	+	+	-	-	-	-	-
	Yellow	-	-	+	+	-	-	-	-
	Fufu	-	+	+	-	-	-	+	-
Lokongoma	White	+	-	-	-	+	-	-	-
	Yellow	+	-	+	+	-	-	-	-
	Fufu	-	-	+	+	-	-	-	-
New	White	-	-	+	-	+	+	-	-
	Yellow	+	-	+	-	+	-	-	-
	Fufu	-	+	+	-	-	+	-	-
Old	White	+	-	+	+	+	-	-	-
	Yellow	-	-	+	-	+	-	-	+
	Fufu	+	-	+	-	-	+	-	-
Ganaja	White	-	-	+	-	+	-	-	-
	Yellow	-	-	+	-	+	-	-	-
	Fufu	-	-	+	-	-	-	+	-

Comment [G45]: Please think of a better way for you to present the headings of the bacteria. You should write their names in full and properly.

Comment [G46]: White what? Yellow what?

Key: Salm = Salmonella sp., Kleb = Klebsiella sp., Staph = Staphylococcus aureus, Pseudo = Pseudomonas sp., Entero = Enterococcus sp., + = present, - = absent

Table 2: Distribution of fungi in the food samples across the sampled markets

Market	Sample	Asper	Peni	Mold	Mucor	Rhi	Fus	Clado	Alter	Monto
Adankolo	White	+	-	-	-	-	-	-	-	-
	yellow	+	+	+	-	-	-	-	-	-
	Fufu	-	+	-	-	-	-	-	-	-
Lokongoma	White	-	+	-	+	-	-	-	-	-
	yellow	-	-	-	-	+	-	-	-	-
	Fufu	-	-	-	-	-	+	-	-	-
New	White	-	+	+	+	-	-	-	-	-
	yellow	-	-	+	+	-	+	-	-	-
	Fufu	-	+	-	-	+	-	-	-	-
Old	White	-	-	-	+	+	-	-	-	-
	yellow	-	-	-	-	-	-	+	+	-
	Fufu	-	-	-	-	-	+	-	-	-
Ganaja	White	+	-	-	-	-	+	-	-	-

Comment [G47]: Please think of a better way for you to present the headings of the fungi. You should write their names in full and properly.

	yellow	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**-**	**+**
	Fufu	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**-**	**-**

Key: Asper = Aspergillus sp., Peni = Penicillium sp., Rhi = Rhizopus sp., Fus = Fusarium sp., Clado = Cladosporium sp., = Alter = Alternaria sp., Monto = Montospora sp., + = present, - = absent.

Table 3: Mean microbial load in cfu/g and pH

Sample Outlet		White garri TAPC	CC	FC	pH	Yellow Garri TAPC	CC	FC	pH	Fufu TAPC	CC	FC	pH
Adankolo	1	3.2×10^2	2.0×10^3	4.0×10^2	4.20	2.0×10^3	1.1×10^3	3.0×10^2	4.34	1.3×10^2	2.0×10^3	4.0×10^2	6.23
	2	1.0×10^1	3.0×10^3	3.0×10^2	4.66	4.2×10^2	1.0×10^3	8.0×10^2	4.83	2.3×10^2	4.0×10^2	7.0×10^2	6.44
	3	3.0×10^2	2.7×10^3	3.0×10^2	4.64	3.8×10^2	2.0×10^3	3.0×10^2	4.02	3.0×10^2	4.0×10^2	2.0×10^2	6.02
	4	1.8×10^1	3.0×10^3	4.0×10^2	4.91	2.0×10^3	4.0×10^2	4.0×10^2	4.33	3.0×10^2	NG	5.0×10^2	5.93
Lokongoma	1	1.8×10^2	NG	4.0×10^2	4.36	3.2×10^2	4.0×10^2	7.0×10^2	4.54	2.0×10^2	1.4×10^3	1.0×10^2	5.64
	2	2.5×10^2	2.7×10^3	3.0×10^2	4.43	3.1×10^2	NG	2.0×10^2	4.30	4.0×10^2	3.4×10^2	4.0×10^2	5.52
	3	9.6×10^2	2.0×10^3	3.0×10^2	4.22	4.7×10^2	1.4×10^3	5.0×10^2	4.49	1.6×10^1	2.0×10^3	4.0×10^2	5.75
	4	1.5×10^2	2.0×10^3	6.0×10^2	4.11	3.1×10^2	3.4×10^2	1.0×10^3	4.67	1.2×10^1	3.0×10^3	4.0×10^2	5.46
New	1	7.0×10^3	NG	3.0×10^3	4.40	1.3×10^2	6.0×10^3	4.0×10^2	4.52	3.2×10^2	2.7×10^3	3.0×10^2	5.62
	2	1.0×10^1	7.0×10^3	4.0×10^2	4.43	1.6×10^2	1.0×10^3	6.0×10^2	4.23	1.0×10^3	3.0×10^3	3.0×10^2	5.41
	3	3.8×10^2	2.0×10^3	3.0×10^2	4.59	9.0×10^3	8.0×10^2	9.0×10^2	4.12	8.8×10^2	NG	6.0×10^2	5.03
	4	1.2×10^2	3.0×10^3	4.0×10^2	4.17	1.2×10^2	2.0×10^2	4.0×10^2	4.04	3.0×10^3	2.7×10^3	3.0×10^2	5.14
Old	1	1.0×10^3	8.0×10^2	4.0×10^2	4.08	1.1×10^2	2.0×10^2	5.0×10^2	4.50	1.0×10^3	2.0×10^3	4.0×10^2	6.35
	2	1.2×10^2	7.1×10^3	3.0×10^2	4.87	1.5×10^2	3.0×10^2	9.0×10^2	4.12	1.2×10^2	2.0×10^3	3.0×10^2	6.23
	3	1.3×10^2	3.0×10^2	4.0×10^2	4.96	1.6×10^2	NG	5.0×10^2	4.73	1.5×10^2	3.0×10^2	1.0×10^2	6.10
	4	6.0×10^3	NG	4.0×10^2	4.15	5.0×10^3	1.0×10^2	1.0×10^2	4.94	2.0×10^3	NG	6.0×10^2	6.06
Ganaja	1	1.2×10^2	2.0×10^2	3.0×10^2	4.96	1.8×10^2	4.2×10^3	4.0×10^2	4.56	1.1×10^2	2.0×10^2	1.0×10^2	5.64
	2	1.9×10^2	2.0×10^2	1.0×10^2	4.02	1.3×10^2	3.1×10^3	3.0×10^3	4.15	1.5×10^2	2.0×10^3	3.0×10^2	5.43
	3	1.5×10^2	NG	6.0×10^2	4.14	1.2×10^2	1.2×10^2	5.0×10^2	4.77	1.6×10^2	8.0×10^2	1.0×10^2	5.02
	4	2.0×10^2	NG	1.0×10^2	4.25	5.0×10^3	NG	1.2×10^2	4.99	5.0×10^3	2.0×10^2	6.0×10^2	5.13

Key: TAPC = Total Aerobic Plate Count, CC = Coliform Count, FC = Fungal Count, NG = No Growth.

Comment [G48]: Incorrect calculation

357 **Table 4: Biochemical characteristics of bacterial isolates**

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Bacteria found	Gram stain	H_2O_2	Glucose	Citrate	Indole	Endospore	Oxidase	Coagulase	Catalase
***Salmonella* sp.**	-		+	-	-	-	-	-	+
***Klebsiella* sp.**	-		+	+	-	-	-	-	+
S. aureus	+		+	+	-	-	-	+	+
***Bacillus* sp.**	+		+	+	-	+	+		+
E. coli	-		+	-	+	-	-	-	+
***Streptococcus* sp.**	+		+			-		-	-
***Pseudomonas* sp.**	-	-	-	+	-	-	+	-	+
***Enterococcus* sp.**	+	-	+	-	-	-	-	-	-