



World News of Natural Sciences

An International Scientific Journal

WNOFNS 32 (2020) 10-20

EISSN 2543-5426

Microbial safety and molecular characterization of microorganisms associated with sliced water melon fruits in selected markets in Akure, Nigeria

O. O. Oladele* and O. C. Aladesanmi

Department of Biology, Federal University of Technology, P. M. B. 704, Akure, Nigeria

*E-mail address: prophetoladele2014@gmail.com

ABSTRACT

Water melon is one of the most commonly consumed sliced fruits in Nigeria, that is being hawked around particularly in markets. This study was therefore aimed at evaluating microbial safety and characterising microorganisms associated with the sliced water melon fruits in Oja-Oba, Isinkan and Shasha markets in Akure, Nigeria. A total of 54 fruits were randomly sampled from six different fruit vendors (A-F) across the 3 markets. The samples were serially diluted and pour plate technique was used for microbial counts and isolation. Each isolate was characterised using PCR (polymerase chain reaction) analysis. Results showed that bacteria counts ($\times 10^4$ cfu/mL) of the sliced fruits ranged from 0.67 ± 0.67 in Isinkan to 202.33 ± 8.11 in Shasha, while total coliform counts ($\times 10^4$ cfu/mL) equally ranged from 1.67 ± 1.67 in Isinkan to 270.33 ± 33.39 in Shasha. Remarkably, no fungus was observed in both, the control and sliced fruits across the 3 markets. Besides, PCR analysis revealed the presence of *Providencia rettgeri*, *Escherichia coli*, *Bacillus cereus*, *Proteus mirabilis* and *Kersytersia gyiorum* as identified bacteria isolates in the sliced fruits. The high microbial load and the presence of identified isolates consequently suggest the health hazards associated with consumption of such sliced fruits.

Keywords: Microbial contamination, molecular characterization, water melon, vendors, Nigerian markets

1. INTRODUCTION

Watermelon (*Citrullus lanatus*) is a fruit belonging to cucurbitaceae family [1] and possesses both, nutritional and medicinal values [2]. In fact, it is enriched with carotenoid,

vitamin C, citrulline, carbohydrates, water, sugar and dietary fiber [3]. In Nigeria, the fruit is typically produced in the northern part. Nonetheless, they are mostly sold as sliced fruits in Akure metropolis either by mobile vendors who peddle them around or by stationary vendors. Sliced fruits are fruits that have been cut opened, sliced into pieces and still in the fresh state and are either displayed for sale or for serving in retail outlets [4, 5]. They are usually packaged in polyethylene bags and sold by street vendors or at local markets without necessarily being cut or rinse before consumption [6].

However, microbial contamination of sliced fruits can pose threats to human health by causing various diseases, such as diarrhea, abdominal cramps, vomiting as well as death [7]. In Nigeria, several studies have been purportedly carried out with regards to microbial qualities of fresh-cut fruits in various regions including Abeokuta [8], Ota, Ogun State [9], Imo State [10], Ebonyi [11], Ilorin, Kwara State [6], Calabar [12] and Bida, Niger State [13], but none in Akure metropolis.

Thus, it is against this background that this study seeks to investigate microbial safety and molecular characterization of microorganisms associated with sliced watermelon fruits in selected markets in Akure metropolis.

2. MATERIAL AND METHODS

2. 1. Study area and sample collection

Selected markets in this study were the three major markets in Akure namely Isinkan, Shasha and Oja-Oba (**Fig. 1**). Three sliced watermelon fruits were purchased separately from six different fruit vendors (A-F) in each market, making a total of 54 sliced fruit samples that were randomly purchased from the 3 markets. However, wholesome fruit served as control. All samples were collected in sterile universal nylon bags and transported immediately to research laboratory, Department of Biology, Federal University of Technology Akure, Ondo State, Nigeria, for the microbial analysis.

2. 2. Microbial analysis

Sliced watermelon fruit samples and control fruit were aseptically transferred into sterile beakers containing 200 mL of sterile water and homogenized. About 10 g each of the sliced fruit samples were weighed and homogenised in 90 mL of sterile distilled water using an electric blender. Ten-fold serial dilutions of the homogenates were made to $\times 10^4$ dilution factor and 1 mL of the dilution factor was used for microbial count and isolation. 1 mL of the dilution factor was aseptically pipetted into individual sterilized Petri dishes.

This was followed by pouring the already prepared nutrient agar, MacConkey and potato dextrose agar media into the Petri dishes for bacterial, total coliform and fungal counts, respectively. The Petri-dishes were swirled gently for homogeneity of the contents, then allowed to solidify and later incubated upside down at 37 °C for 24 hours for bacteria and total coliform counts.

After 24 hours, the observed colonies were counted with a colony counter, each count expressed as colony forming unit per ml (cfu/mL). For fungal counts, Petri-dishes were incubated upside down at 25 °C for 72 hours, and after 72 hours the observed colonies were counted with a colony counter and each count expressed as a spore forming unit per mL (sfu/mL).

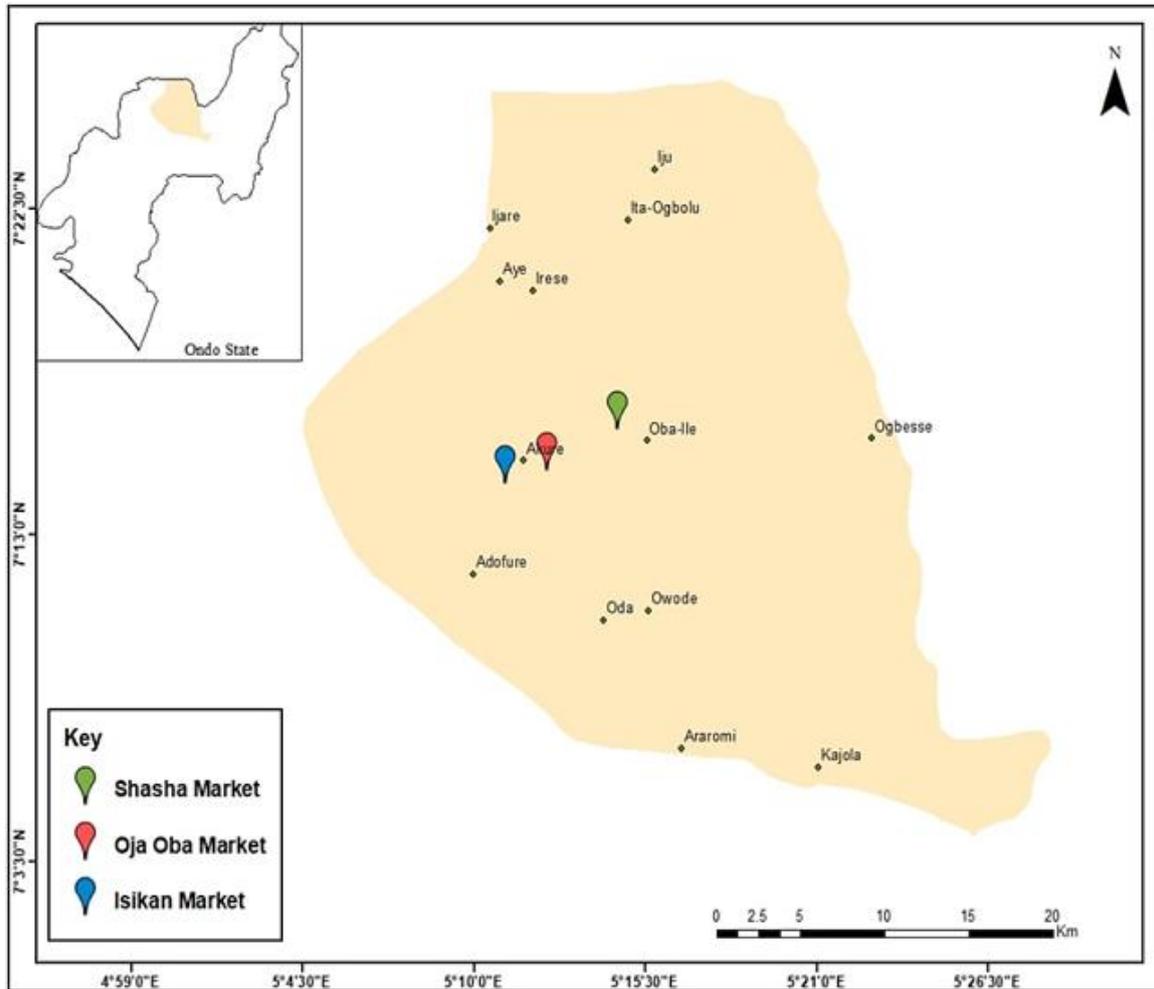


Figure 1. Location of the selected markets in Akure

2. 3. Molecular identification of bacteria isolates

The observed colony of each bacterial isolate was then sub-cultured by streaking on a freshly prepared nutrient agar plates, several times until pure colonies were obtained. Before DNA extraction, the individual bacterial isolates were grown overnight in a liquid nutrient broth at 37 °C and DNA extraction was carried out using DNeasy plant mini kit (Qiagen), according to the manufacturers instruction.

Between 50-100 mg (wet weight) of each bacterial isolate was suspended in 200 µL of isotonic buffer which was added to a ZR Bashing™ lysis Tube, then 750 µL lysis solution was added to the tube. A bead fitted with 2 mL tube holder assembly was secured and processed at maximum speed for ≥ 5 minutes.

The ZR BashingBead™ Lysis Tube was centrifuged in a micro centrifuge at $> 10,000 \times g$ for 1 minute. About 400 µL of the supernatant was transferred to a Zymo-Spin™ IV Spin Filter (orange top) in a collection tube and centrifuged at $7,000 \times g$ for 1 minute. Base of the Zymo-Spin™ Spin filter was snapped off prior to use.

About 1,200 μL of bacterial DNA binding buffer was added to the filtrate in the collection tube and 800 μL of the mixture was then transferred to a Zymo-SpinTM IIC column in a collection tube and centrifuged at $10,000 \times g$ for 1 minute. Flow from the collection tube was discarded and was repeated. About 200 μL DNA pre-wash buffer was added to Zymo-SpinTM IIC column in a new collection tube and centrifuged at $10,000 \times g$ for 1 minute and 500 μL bacterial DNA wash buffer was then added to the Zymo-SpinTM IIC Column and centrifuged at $10,000 \times g$ for 1 minute. Zymo-SpinTM IIC column was transferred to a clean 1.5 mL micro centrifuge tube and 100 μL (35 μL minimum) DNA elution buffer was added directly to the column matrix. It was then centrifuged at $10,000 \times g$ for 30 seconds to elute the DNA.

After DNA extraction, PCR analysis was carried out with 27F: AGAGTTTGATCMTGGCTCAG and 1525R: AAGGAGGTGWTCCARCCGCA as primer sequences. PCR cocktail mix consisted of 2.5 μL of PCR buffer, 1 μL of 25 mM MgCl_2 , 1 μL each of forward primer and reverse primer, 1 μL of DMSO, 2 μL of 2.5 mM dNTPs, 0.1 μL of 5 μL Taq DNA polymerase, and 3 μL of 10 ng/ μL DNA. Total reaction volume was made up to 25 μL using 13.4 μL nuclease free water. Initial denaturation at 94 °C for 5 min, followed by 36 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and elongation at 72 °C for 45 s, followed by a final elongation step at 72 °C for 7 min and hold temperature at 10 °C.

Amplified fragments were visualized on safe view-stained 1.5% agarose electrophoresis gels. Size of the amplicon was 1,500 bp and DNA ladder used was 1 kb+ from Bio line. PCR amplification was carried out in Gene Amp PCR system 9700 while Sanger sequencing was performed using 3130XL genetic analyser from applied Bio systems. Genome sequences obtained for each isolate were thereafter submitted to the National Centre for Biotechnology Information (NCBI) gene bank database using Basic Local Alignment Search Tool (BLAST) search program where the isolate was identified.

2. 4. Statistical analysis

Results are expressed as mean \pm standard error ($M \pm S$) for three sliced fruits per vendor ($n = 3$). Data were analysed using SPSS software for windows (IBM SPSS Statistics 21) at 95% significance. Analysis of variance (ANOVA) was used to test for significance in the mean concentrations between vendors in each market.

3. RESULTS

3. 1. Total bacterial counts of sliced watermelon fruits

Total bacterial counts ($\times 10^4$ cfu/mL) of sliced watermelon fruits purchased from vendor A-F in each selected market are presented in **Table 1**. Results show that only sliced fruits purchased from vendors D, E, and F in Oja-Oba recorded bacteria counts when compared with other vendors and their respective bacteria count ($\times 10^4$ cfu/mL) were 1.33 ± 0.67 , 1.67 ± 1.67 , and 15.30 ± 2.91 though counts obtained from vendors D and E were significantly different ($P < 0.05$) from F.

Also in Isinkan, bacterial count was only observed in sliced fruits from vendors A (2.67 ± 0.88), D (0.67 ± 0.67), and F (1.00 ± 0.58) and the 3 were significantly different ($P < 0.05$) from vendor C (14.07 ± 3.06). Similarly in Shasha, bacterial counts were only observed in sliced fruits from vendors C (61.67 ± 12.20), D (202.33 ± 8.11), and E (163.00 ± 22.52) were significantly different ($P < 0.05$) from each other.

3. 2. Total coliform counts (TCC) of sliced watermelon fruits

The total coliform counts ($\times 10^4$ cfu/mL) of the sliced watermelon fruits purchased from different vendors across the selected markets are presented in **Table 2**. Interestingly, only sliced fruits purchased in Oja-Oba from vendor F recorded TCC (39.00 ± 3.46) when compared with other vendors and the control. In the same vein, coliform was not detected in sliced fruits purchased from vendors B, E, and F in Isinkan as TCC of sliced fruits from vendors A (7.67 ± 0.881) and D (1.67 ± 1.67) were both significantly different ($P < 0.05$) from vendor C (70.33 ± 7.31). Meanwhile in Shasha, sliced fruits purchased from vendors A, B, and F recorded no TCC, while TCC of sliced fruits purchased from vendors C (205.67 ± 5.49), D (270.33 ± 33.39), and E (230.67 ± 39.30) were not significantly different ($P > 0.05$) from each other.

Table 1. Total bacterial counts of sliced watermelon fruits from selected markets

Vendors	Total bacteria count ($\times 10^4$ cfu/mL)		
	Oja-oba	Isinkan	Shasha
A	0.00 ± 0.00^a	2.67 ± 0.88^a	0.00 ± 0.00^a
B	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
C	0.00 ± 0.00^a	14.07 ± 3.06^b	61.67 ± 12.20^b
D	1.33 ± 0.67^a	0.67 ± 0.67^a	202.33 ± 8.11^d
E	1.67 ± 1.67^a	0.00 ± 0.00^a	163.00 ± 22.52^c
F	15.30 ± 2.91^b	1.00 ± 0.58^a	0.00 ± 0.00^a
Control	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a

Means having the same alphabet within the column are not significantly different from each other using ANOVA and Duncan's test at $\alpha=0.05$.

Table 2. Total coliform counts in sliced watermelon fruits from selected markets

VENDORS	Total Coliform Counts ($\times 10^4$ cfu/mL)		
	Oja-oba	Isinkan	Shasha
A	0.00 ± 0.00^a	7.67 ± 0.881^a	0.00 ± 0.00^a
B	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
C	0.00 ± 0.00^a	70.33 ± 7.31^b	205.67 ± 5.49^b
D	0.00 ± 0.00^a	1.67 ± 1.67^a	270.33 ± 33.39^b

E	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	230.67 ± 39.30 ^b
F	39.00 ± 3.46 ^b	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
Control	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a

Means having the same alphabet within the column are not significantly different from each other using ANOVA and Duncan’s test at $\alpha=0.05$.

3. 3. Total fungal count of sliced watermelon fruits

Total fungal count ($\times 10^4$ sfu/mL) of the sliced watermelon fruits, purchased from different vendors in the selected market, are presented in **Table 3**.

Table 3 Total fungi counts in sliced watermelon fruits from selected markets

VENDORS	Total Fungi counts ($\times 10^4$ sfu/mL)		
	Oja-oba	Isinkan	Shasha
A	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
B	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
C	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
D	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
E	0.00 ± 0.00 ^a	0.00 ± 1.00 ^a	0.00 ± 0.00 ^a
F	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
Control	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a

Means having the same alphabet within the column are not significantly different from each other using ANOVA and Duncan’s test at $\alpha=0.05$.

3. 4. Molecular identification of bacteria isolates

The 16S rRNA gene of each bacterial isolate was amplified using suitable primer and the amplified 16S rRNA gene sequences were then compared with sequence in National Centre for Biotechnology Information (NCBI) gene bank database using Basic Local Alignment Search Tool (BLAST) program. The Amplified fragments visualized on safe view-stained 1.5% agarose electrophoresis gels are presented in **Figure 2**.

DNA samples loaded in lane 1 and 4 had low intensity bands and the identified isolates are *Providencia rettgeri* and *Escherichia coli*, respectively, while those in lanes 2, 3, and 5 had intense visible bands and their respective identified isolates are *Bacillus cereus*, *Proteus mirabilis* and *Kersytersia gyiorum*.

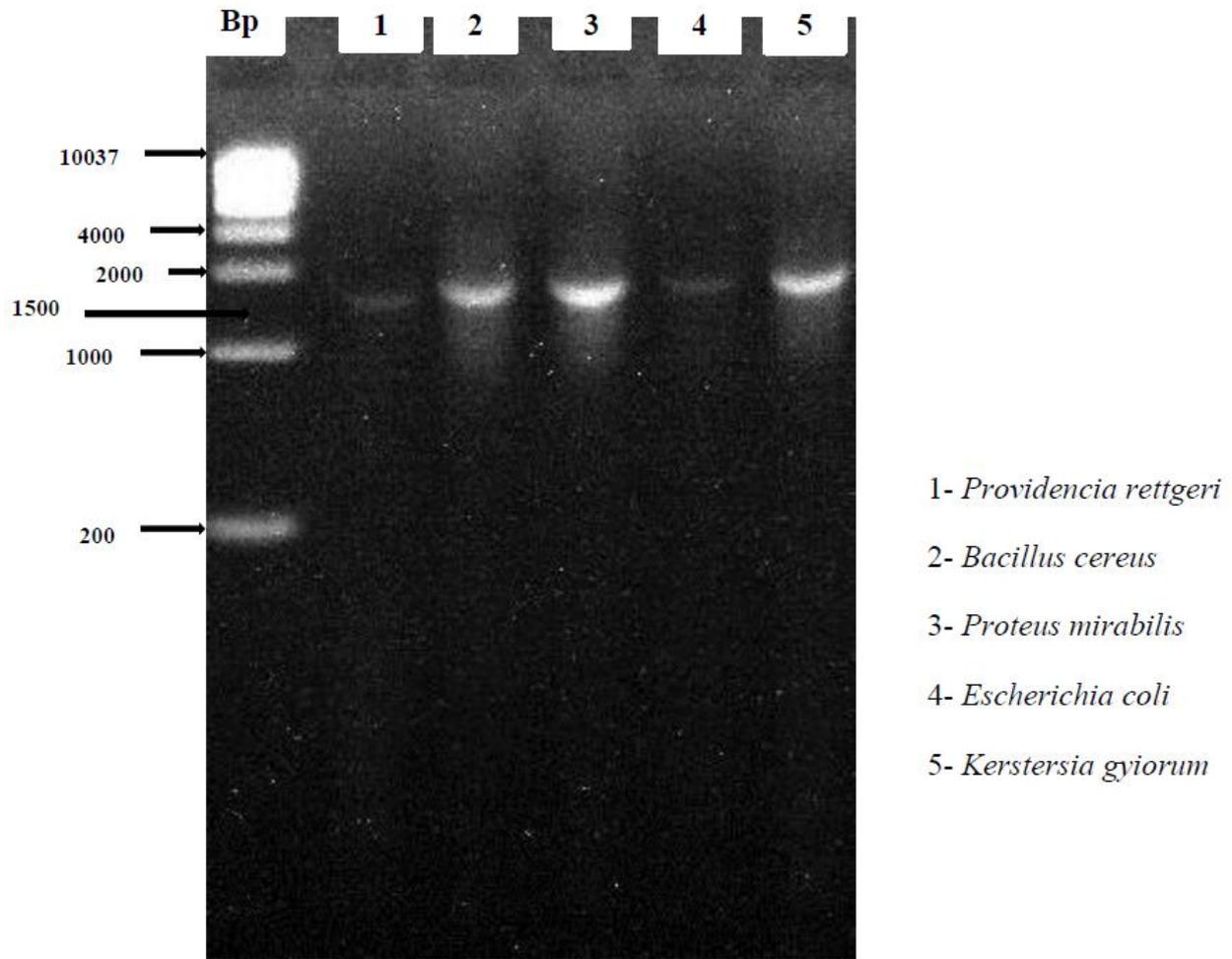


Figure 2. Agarose gel electrophoresis of the bacterial genomic DNA samples

4. DISCUSSION

High bacteria counts observed in sliced fruits from some vendors across the 3 markets are similar to the results obtained in similar studies in Nigeria [5, 6, 8, 14] and could be attributed to unhygienic practices of fruit vendors and unclean vending environment. The high bacteria count could also be a result of contamination of sliced fruits during washing, slicing, and packaging. This was the observation of Eni *et al.* [15] who reported that the bacterial loads present in fruits are a direct reflection of the sanitary quality of cultivation water, harvesting, transportation, storage, and processing of produce. In fact, fruits exposed to various types of cutting result in a six- to seven-fold increase in microbial loads [16]. This is because cutters and slicers used in preparation of fruits can be potent sources of contamination, since they usually provide inaccessible sites, which harbour bacteria.

Another health concern was the high coliform counts observed among the vended fruits, particularly in Shasha market. This agreed with the works of [8] who reported that the sliced water melon samples sold in Abeokuta were observed to contain a high number of coliform organisms. This could, however, be traced to a high level of faecal contamination of water used

in washing sliced fruits and coliform has been earlier considered as a hygiene indicator, especially for faecal contamination [17]. Remarkably, no fungus was observed in both, the control and sliced fruits purchased from all vendors across the 3 markets. Absence of any fungus in this study contradicts the earlier works by [8] who reported *Penicillium* spp., *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* spp., *Fusarium* spp., *Saccharomyces cerevisiae* and *Neurospora* spp. as observable fungi isolates from polyethylene packaged, sliced and ready-to-eat pawpaw, watermelon and pineapple fruits sold by street vendors in Abeokuta.

Isolation of *B. cereus*, *E. coli* and *P. mirabilis* from sliced fruits in this study could be supported by the earlier work of ([18] who reported the presence of these isolates in their findings. Also, these isolates had been previously isolated from fruits in Abakaliki, Ebonyi State [11], Abeokuta, Ogun State [8] and Owerri, Imo State [19]. Occurrence of *B. cereus* in the sliced fruits cannot be connected with the reports of [13] that fresh cut fruits sold in the open space are prone to contamination by spores of *Bacillus*, since their spores are widespread in nature coupled with it's the ability to form endospores. Unfortunately, *B. cereus* has been implicated in a number of food poisoning outbreaks [20]. *E. coli* has been regarded as a primary indicator for microbiological quality of food and water. Therefore, the presence of *E. coli* suggests that the sliced watermelon fruits have been contaminated with faecal material and thereby renders them unsafe for human consumption. Likewise, the presence of *P. mirabilis* in this study is of public health importance because the isolate is known to be the main cause of all *Proteus* infections accounting for about 80–90% of them [21]. In fact, the isolate is mostly known as opportunistic human pathogens and its roles in pathogenesis of human beings have been extensively studied and reviewed [22-24].

Contamination of the sliced watermelon fruits with *P. rettgeri* could be linked with the use of faecal contaminated water to wash and process the fruits. This is because the organism is commonly found in both, water and land environments. Watery droppings from houseflies could also serve as a source of contamination, since fruits are exposed to flies during processing and the organism has earlier been isolated from the gut of Housefly [25]. The isolate is an opportunistic pathogen of medical importance [26] and has been reported for causing urinary tract infections [27] and ocular infection [28]. More so, an outbreak of the food poisoning associated with *P. rettgeri* has been reported in China [29-30].

Besides, isolation of *K. gyiorum* in the sliced fruits could be described as novel because this is the first time the organism would be isolated from the sliced fruits, although it was first described in 2003 by Coenye *et al.* [31] and was then isolated from faecal samples, sputum and leg ulcer. Recently, the organism was also isolated inside the gut of a housefly [27]. Hence, contamination of the sliced fruits with *K. gyiorum* could be linked to either the unhygienic practices of the vendors, washing and processing water, or houseflies.

5. CONCLUSION

This study indicates that majority of the polyethylene packaged sliced water melon fruits sold across the selected markets in Akure were adjudged unfit and unsafe for public consumption because of microbial contamination. In fact, the bacteria and total coliform counts observed in the study exceed the recommended standard (1×10^2 cfu/mL) per g of food sample. This implies that the fruit samples could serve as a vehicle in the transmission of pathogens to the consumers of these contaminated fruits. Hence, fruit vendors should be educated on

personal hygiene and a proper sanitary way of the processing their fruits in order to reduce the microbial contamination.

Acknowledgements

The authors are grateful to molecular laboratory of IITA for providing facilities for the PCR analysis.

References

- [1] A.J. Edwards, B.T. Vinyard, E.R. Wiley, E.D. Brown, J.K. Collins, and P. Perkins-Veazie, Consumption of watermelon juice increases plasma concentrations of lycopene and β -carotene in humans. *Journal of Nutrition* 133 (2003) 1043-1050
- [2] A.M. Gwana, M.M. Bako, B.Y. Bagudu, A.B. Sadiq, and M.M. Abdullahi, Determinations of phytochemical, vitamin, mineral and proximate compositions of varieties of watermelon seeds cultivated in borno state, north-eastern Nigeria. *International Journal of Nutrition and Food Science* 13 (4) (2014) 238-245
- [3] B.D. Bruton, W.W. Fish, W. Roberts, and T.W. Popham, The influence of rootstock selection on fruit quality attributes of watermelon. *Open Food Science Journal* 3 (2009) 15-34
- [4] C. De Rover, Microbial safety evaluations and recommendation on fresh produce. *Food Contamination* 9(6) (1998) 321-347
- [5] K.I. Auta, B. Madinatu, H.C. Yayock, and B. Solomon, Microbial quality assessment of sliced pineapple and watermelon sold in some selected markets in Kaduna metropolis, Kaduna State. *Journal of Biological Science* 3 (7) (2017) 13-29
- [6] M.B. Odebisi-Omokanye, M.A. Oke, A.M. Ahmed El-Imam, A.K. Ajijolakewu, and B.I. Salaudeen, Microbiological Quality and Safety of pre-cut fruit retailed in Ilorin, Kwara State, Nigeria. *Fountain Journal of Natural and Applied Science* 4 (1) (2015) 19-26
- [7] F. Qadri, A.M. Svennerholm, A.S. Faruque, and R.B. Sack, Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment and prevention. *Clinical Microbiology Review*, 18 (2005) 465-483
- [8] A. Oluwatoyin, O. Adejare, A. Wasiu, and A. Adeola, Microbial safety of polyethylene packaged sliced fruits sold in Abeokuta, South-West Nigeria. *Journal of Natural Science Research* 5(13) (2015) 16-21
- [9] S. Oranusi and O.J. Olorunfemi, (2011). Microbiological safety evaluation of street vended ready-to-eat fruits sold in Ota, Ogun state, Nigeria. *International Journal of Research in Biological Science* 1(3) (2011) 27-32
- [10] K.S. Chukwuka, I.O. Okonko, and A.A. Adekunle, Microbial ecology of organisms causing pawpaw (*Carica Papaya* L.) fruit Decay in Oyo State, Nigeria. *American-Eurasian Journal of Toxicological Science* 2(1) (2010) 43-50
- [11] O.J. Okechukwu, C.I. Orinya, E.O. Okonkwo, C.V. Uzoh, U.O. Ekuma, G.A. Ibiam, and E.N. Onuh, The microbial contamination of ready-to-eat vended fruits in Abakpa

- main market, Abakaliki, Ebonyi State, Nigeria. *Journal of Pharmacy and Biological Science* 11 (6) (2016) 71-80
- [12] G.E. John, U. Edeghor, and C. Origbu, Bacteriological profile of cut fruits sold in Calabar metropolis. *World News Natural Science* 23 (2019) 43-55
- [13] A.A. Daniel, S. Danfulani, B.B. Barnabas, G. Peter, and A.E. Ajewole, Microbiological quality of sliced fresh fruits sold in Bida, Nigeria. *Global Journal of Biology and Agricultural Health Science* 3(3) (2014) 178 -180
- [14] I.A. Allamin, M.B. Borkoma, A.M. Ibn, and M.A. Waziri, Bacteriological quality of sliced fruits sold at road site in Maiduguri, Borno State. *International Journal of Environment* 4(2) (2015) 236-42
- [15] A.O. Eni, I.A. Oluwawemitan, and U.S. Oranusi, Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria. *African Journal of Food Science* 4(5) (2010) 291-296
- [16] T.O. Adesetan, H.O. Egberongbe, O.A.F. Ilusanya, and O.O. Bello, Antimicrobial sensitivity of bacterial isolates from street vended fruits in Ijebu area of Ogun State, Nigeria. *International Research Journal of Microbiology* 4 (9) (2013) 220-225
- [17] M.A. Halablab, I.H. Sheet, and H.M. Holail, Microbial quality of raw vegetables grown in Bekaa valley, Lebanon. *American Journal of Food Technology* 6 (2011) 129-139
- [18] C.A. Mbata, C. Nwagu, O.A. Adegoke, C.U. Nyenke, and A.N. Wali, Bacteriological status of water melon (*Citrullus Lanatus*) sold in Mile III Market Port Harcourt. *International Journal of Engineering Innovation Research* 5(1) (2016) 46-49
- [19] E. Nwachukwu, E.F. Ezeama, and B.N. Ezeanya, Microbiology of polyethylene packaged sliced watermelon (*Citrullus lanatus*) sold by street vendors in Nigeria. *African Journal of Microbiology Research* 2 (2008) 192-195
- [20] K. Dierick, E. Coillie, I. Swiecicka, G. Meyfroidt, H. Devlieger, A. Meulemans, G. Hoedemaekers, L. Fourie, M. Heyndrickx, and J. Mahillon, Fatal family outbreak of *Bacillus cereus*-associated food poisoning. *Journal of Clinical Microbiology* 43(8) (2005) 4277-4279
- [21] C.M. O'Hara, F.W. Brenner, and J.M. Miller, Classification, identification and clinical significance of *Proteus*, *Providencia* and *Morganella*. *Clinical Microbiology Review* 13(4) (2000) 534-46.
- [22] R.M. Morgenstein, B. Szostek, and P.N. Rather, Regulation of gene expression during swarmer cell differentiation in *Proteus mirabilis*. *FEMS Microbiology Review* 34 (2010) 753-763
- [23] C.E. Armbruster and H.L.T. Mobley, Merging mythology and morphology: the multifaceted lifestyle of *Proteus mirabilis*. *Nature and Review Microbiology Journal* 10(11) (2012) 743-754
- [24] A. Różalski, A. Torzewska, M. Moryl, I. Kwil, A. Maszewska, K. Ostrowska, D. Drzewiecka, A. Zabłotni, A. Palusiak, M. Siwińska, and P. Stączek, *Proteus* sp. – an opportunistic bacterial pathogen – classification, swarming growth, clinical significance and virulence factors. *Folia Biologica et Oecologica* 8 (2012) 1-17

- [25] A.K. Gupta, D. Nayduch, P. Verma, B. Shah, H. V. Ghate, M.S. Patole, and Y.S. Shouche, Phylogenetic characterization of bacteria in the gut of houseflies (*Musca domestica* L.). *Microbial Ecology* 79(2011) 581–593
- [26] D.I. Huiling, L. Sisi, L. Qingyang, S. Lei, S. Ayaka, M. Hecheng, and Y. Shinji, *Providencia* in retail meats from Guangzhou, China and Osaka, Japan: prevalence, antimicrobial resistance and characterization of classes 1, 2 and 3 integrons. *Veterinary Medical Science* 80(5) (2018) 829–835
- [27] B.D. Jones and H.L. Mobley. Genetic and biochemical diversity of ureases of *Proteus*, *Providencia* and *Morganella* species isolated from urinary tract infection. *Infectious and Immunity* 55 (9) (1987) 2198–2203
- [28] A.F. Koreishi, B.A. Schechter, and C.L. Karp, Ocular infections caused by *Providencia rettgeri*. *Ophthalmology* 113(8) (2006) 1463–1466
- [29] Y.J. Cui, Y.G. Zhu, R.H. Zhai, D.Y. Chen, Y.Z. Huang, Y. Qiu, and J.Z. Liang, Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. *International Journal of Environment* 30 (2010) 785-791
- [30] G. Zhang, An outbreak of foodborne infection attributed to *Providencia rettgeri* in Shanghai. *Journal of Preview Medicine* 17 (2005) 128-132
- [31] T. Coenye, M. Vancanneyt, M.C. Cnockaert, E. Falsen, J. Swings, and P. Vandamme, *Kerstersia gyiorum* gen. nov., sp. nov., a novel *Alcaligenes faecalis*-like organism isolated from human clinical samples, and reclassification of *Alcaligenes denitrificans* Rügger and Tan 1983 as *Achromobacter denitrificans* comb. Nov. *International Journal of Systems and Evolutionary Microbiology* 53(6) (2003) 1825–1831