Original Article

International Journal of Dental and Health Sciences
Volume 04,Issue 04

ISOLATION OF BACTERIA ASSOCIATED WITH SPOILED VEGETABLES SOLD IN SOME PARTS OF KANO CITY, NIGERIA

Faisal Muhammad[1,2], Danjuma Shehu Uba[1], Md. Imdadul Haque[1], Usman Aliyu Dutsinma[2], Muhammad Yusha'u[2], ABM Alauddin Chowdhury[1]

[1]Department of Public Health, Faculty of Allied Health Science, Daffodil International University,Dhaka, Bangladesh

[2]Department of Microbiology, Faculty of Science, Bayero University Kano, Kano-Nigeria

ABSTRACT:

The study was carried out to isolate the bacteria associated with spoiled vegetables sold in some parts of Kano city, Nigeria. Samples were collected from two different retail markets. Collected samples were processed by using Standard Plate Count and Coliform count. Eight samples of different vegetables were collected from two different retail markets, were tested for the quantitative and qualitative presence of coliforms and some pathogenic bacteria using standard plate count (SPC). *Escherichia coli* and *Pseudomonas* spp. were dominantly found in both retail market vegetable samples; these organisms were identified by using Morphological and Biochemical characters. Four bacteria belonging to different genera were identified. *Escherichia coli* (36.4%), *Pseudomonas aeruginosa* (27.3%), *Salmonella* (9.1%), and *Staphylococcus aureus* (27.3%) from market A, on the other hand from market B five bacteria were identified *Escherichia coli* (28.6%), *Pseudomonas aeruginosa* (28.6%), *Salmonella* (14.3%), *Shigella* (7.1%) and *staphylococcus aureus* (21.4%). *Escherichia coli* was the most frequently isolated followed by *Pseudomonas aeruginosa*. The findings reveals that there was no significant difference (P>0.05) between number of isolates found in the markets (Market A&B).

Keywords: Isolation, Bacteria, Vegetables, Spoiled, Microbial, Food.

INTRODUCTION:

Spoilage refers to any change in the condition of food in which the food becomes undesirable or unacceptable for human consumption[1]. Microbial spoilage is caused by microorganisms like fungi (moulds, yeasts) and bacteria. They spoil food by growing in it and producing substances that change the colour, texture and odour of the food. Microbial spoilage by moulds and yeasts includes souring of milk, growth of mould on bread and rotting of fruit and vegetables. These organisms are rarely harmful to humans, but bacterial contamination is often more dangerous because the food does not always look bad, even if it is severely infected [2]. Food borne bacterial pathogens, commonly detected in fresh vegetables, were coliform bacteria, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. [2]

Coliforms are facultative anaerobic, Gram-negative, non-spore forming rods that ferment lactose with gas formation within 48 hrs, when grown in lactose broth at 35°C. Common inhabitants of animal and human guts, they are commonly-used bacterial indicator of

*Corresponding Author Address: Dr. Faisal Muhammad . E-mail: fokkanya@yahoo.com

sanitary quality of foods and water and considered as an indicator of microbial pollution [3]. Bacterial spoilage first causes softening of tissues as pectins are degraded and the whole fruit may eventually degenerate into a slimy mass. Starch and sugars are metabolized next and unpleasant odours and flavours develop along with lactic acid and ethanol [4]. Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue [5].

Worldwide vegetables have been associated with outbreaks of food borne disease. Organisms involved include bacteria, viruses and parasites. Contamination of vegetables may take place at all stages during pre and post-harvest procedures. Other possible sources of microorganisms include soil, faeces (human and animal origin), water (irrigation, cleaning), ice, animals (including insects and birds), handling of the product, harvesting and processing equipment and transport [6]. Microorganisms capable of causing human illness and others whose food borne disease potential is uncertain, includes *Aeromonas hydrophila*, *Citrobacter freundii, Enterobacter cloacae* and *Klebsiella spp.* and they have been isolated in lettuce and salad vegetables. Plate count of aerobic mesophilic microorganisms found in food is one of the microbiological indicators for food quality [7]. The study was aimed to isolate different types of bacteria responsible for spoilage of vegetables in Kano city, Nigeria.

MATERIALS AND METHODS:

Sample collection

Total of 8 samples of spoiled Tomato (2), Cabbage (2), Onion (2), and Pepper (2), were collected in sterile polythene bags from two different markets in Kano city, One sample of each vegetable was collected from each of the two retail markets, the samples were collected from the two retail markets in Kano city, namely Kasuwar mata Fagge located at Fagge "D" in Fagge local government Area and Yan Kaba Market in Nassarawa Local Government Area Kano city, Nigeria.

Sample processing

Stock solution preparation

Stock solution was then prepared by weighing about 25g of the sample and dissolved in 225ml of distilled water [8].

Standard plate count (SPC)

Serial dilution was prepared, using the prepared stock solution. Then 9ml of distilled water was measured and placed in each of the 9 test tubes i.e. serial dilutions of 10^{-1}, 10^{-2}, and 10^{-3}, were prepared in triplicates, in test tubes (All the tubes were autoclaved and allowed to cool before dispensing). One ml of the stock solution was measured and dispensed into the 10^{-1} tube and was shaken, 1ml was transferred from 10^{-1} to 10^{-2} and was also shaken, then 1ml from 10^{-2} was transferred to the third test tube with concentration of 10^{-3}, in triplicates [9].

One (1ml) from the first serially diluted test tube was dispensed into petri dish for all the triplicate dilutions, concentration. Then the prepared nutrient agar (NA) and selective differential media was poured into the petri dishes containing 1ml of the serially diluted solutions, and all the plates were allowed to solidify. The plates were incubated at 37oC for 24 hours [10] . The colonies on Nutrient agar were observed, counted and recorded.

The remaining colonies on the selective and differential media were also observed and isolation of the bacteria followed and confirmed by the use of Biochemical tests and gram staining. EMB, MCA, MSA and SSA were used to isolate the organisms. Many biochemical tests were carried out for the confirmation of the isolated bacteria; i.e. Indole [11], Methyl red test [12], Voges-proskauer test [13], Citrate test [14] and Catalase test [11]. In the process of confirmation Gram staining was also carried out using the gram staining techniques [11].

Enumeration of coliform

Lactose broth was prepared by dissolving appropriate gram of the agar into sterile distilled water. The solution was heated under the spirit lamp to dissolved the media and then autoclaved at 121^{o}C for 15 minutes. The media was allowed to cool before dispensing into the test tubes, in order not to kills the microorganisms.

Enumeration of coliform was carried out in accordance with the method done by McCarthy et al., [13]. Lactose broth was dispensed into 9 test tubes containing Durham's tube, each Durham's tube was inverted in each of the 9 test tubes. From serially diluted 9 test tubes 1ml from each was transferred to correspondent test tubes containing the Lactose broth and Durham's tube [14]. The 9 test tubes containing the Durham's tube and lactose broth were incubated at 37^{o}C for 24 hours.

Ethical Approval

The study was Ethically Approved by the Ethical Committee of the Department of Microbiology Bayero University Kano, Nigeria as a part of the Requirement for the Partial fulfilment of a Bachelor degree (B.Sc. Microbiology).

RESULTS:

Isolated Bacteria from the selected markets (A&B)

Table 1 shows the isolated bacteria from the selected markets. It has been reported that from market A samples four different type of bacteria were isolated, the majority (36.4%) of the bacteria isolated was *Escherichia coli,* followed by 27.3% (*Pseudomonas aeruginosa*), 27.3% (*Staphylococcus aureus*) and salmonella (9%). However from market B samples five different bacteria were isolated, little below three-tenth of the bacteria isolated was *Escherichia coli* (28.6%) and *Pseudomonas aeruginosa* (28.6%),

followed by *Staphylococcus aureus* (21.4%). The *Shigella spp.* was found to be the least (7.1%) isolated bacteria in the market B samples. It has been found that that there was no significant difference between number of isolates from market A and B (P>0.05).

Mean Viable Count, Most Probable Number and Correlation Analysis of the samples

Table 2 shows the mean viable count, most probable number count and correlation analysis of the samples. In the samples from market A it has been reported that sample A (tomato) had the highest mean viable count $44.41x10^3$, followed by sample c (Onion) with mean viable count of $39.82x10^3$. On the other hand from market B, sample D (pepper) had the highest mean viable count ($42.09x10^3$), followed by sample c (onion) $28.29x10^3$. According to most probable number count (MPN), sample A (tomato) had the highest MPN count >2400, followed by sample B (cabbage) MPN 1100. On the other hand sample A, C and D (tomato, onion and pepper) had the highest number of MPN 210 and the least was sample B (MPN 150). It has been observed that there was moderate negative correlation between mean viable count of market A and B samples (r=-0.4248). However there was very week negative correlation between most probable number counts of the market A and B samples (r=-0.0743).

Table 1: Isolated Bacteria from the selected markets (A&B)

Bacteria	Sampling Area		Statistics
	Market A %	Market B %	
Escherichia coli	36.4	28.6	X^2=16.919
Pseudomonas aeruginosa	27.3	28.6	
Salmonella spp.	9.1	14.3	P>0.05
Shigella spp.	--	7.1	
Staphylococcus aureus	27.3	21.4	

Table 2: Mean Viable Count, Most Probable Number and Correlation Analysis of the samples

Samples	Sampling Area				Correlation analysis
	Market A %		Market B %		
	Mean Viable Count (cfu/ml)	MPN	Mean Viable Count(cfu/ml)	MPN	
A	$44.41x10^3$	>2400	$17.57x10^3$	210	MVC (r) = -0.4248
B	$31.19x10^3$	1100	$7.116x10^3$	150	MPN (r) = - 0.0743
C	$39.82x10^3$	290	$28.29x10^3$	210	
D	$24.14x10^3$	150	$42.09x10^3$	210	

* A: **Tomato *B: Cabbage *C: Onion *D: Pepper**

Table 1: Shows the mean average score for all the photos ranked by the laypeople and the orthodontist

DISCUSSION:

This study was aimed to isolate bacteria from spoiled vegetables sold is some parts of Kano city, Nigeria. Samples were collected from two different retail markets namely; Kasuwar mata Fagge, Fagge local government Area and Yan Kaba market in Nassarawa local government Area. Collected samples were analyzed by using Standard Plate Count and Coliform Count by pour plate techniques [10]. From the result low bacterial count was found in the market B samples (Yan Kaba market in Nassarawa local government Area), on the other hand market A (Kasuwar mata Fagge in Fagge local government Area) has the higher bacterial count, and from statistical analysis that was also observed (Table 2) the correlation analysis for the market A & B samples was -0.4248 which shows that one of the market has the higher bacterial load than the other and the correlation between them.

Morphological and Biochemical character were used to identify the bacteria found in all the samples. The most probable number count of the four samples from both market A & B were compared statistically (correlation analysis) and was found to be -0.0743 which shows that one of the market has the higher bacterial load than the other. *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella spp*. were found in the samples from markets A. Nevertheless all the above bacteria were found in the samples from market B in addition with *Shigella* spp. (Table 1). However most of the bacteria isolated in our study has been isolated previously in vegetables and fruits in other studies [15-20]. Less than two-fifth (36.4%) of the samples from market A contained *Escherichia coli and salmonella* 9.1%. On the other hand only 57.1% of the market B samples showed *Escherichia coli* and *Pseudomonas aeruginosa*. Statistical analysis (chi-square test) on the number of isolates from market A & B shows that there was no significant difference between the two markets chi-square found to be 16.919 (Table 2). These result showed that microbiological qualities of the vegetables from market A was higher than that of market B samples[21]. *Escherichia coli* and *Pseudomonas aeruginosa* were found to be the most frequently proliferating pathogens in the vegetable samples (Table 1), and *Shigella* spp. (7.1%) were found only in market B*. Escherichia. coli, Salmonella* spp and *Shigella* spp revealed the possibility of spreading enteric diseases to the consumers. Similar results were also reported by Khan *et al*., [22] and Nipa *et al.,*[23] from the similar vegetables. The high microbial contamination observed in the vegetables from market A in this study may be a reflection of storage conditions and how long these produce were kept before they were obtained for sampling. Bacteria on storage materials may transfer to produce and cross contamination between produce is probable particularly where produce are pre-washed with the same wash water

by the vendor or processor. More importantly, bacteria on the produce may multiply over time depending on the storage conditions [24, 25]. Some of the bacteria isolated in this study may be part of the natural flora of the vegetables or contaminants from soil, irrigation water, and the environment during transportation, washing/rinsing water or handling by processors [26]. *Pseudomonas* spp. are part of the natural flora and are among the most common vegetable spoilage bacteria [27].

CONCLUSION:

The findings of this study revealed that five bacteria isolates viz. *Escherichia coli, Pseudomonas aeruginosa, Salmonella, Shigella* and *Staphylococcus aureus* were isolated from the spoiled vegetable samples. Statistical analysis on the number of isolated bacteria from all the markets shows that there was no significance difference between the two markets. It was found that the *Escherichia coli* and *Pseudomonas aeruginosa* were the dominating species that played a huge role in the spoilage of vegetables in some part of Kano city, Nigeria.

Recommendations: The vegetable producers (farmers) and retailers should improve the way of storing, handling, transporting and preserving their products so as to make their product free from spoilage microorganisms.

- Contamination can arise as a consequence of treating soil with organic fertilizers, such as sewage sludge and manure, and from the irrigation water, as well as from the ability of pathogens to persist and proliferate in vegetables, due to that chlorinated water has to be used.
- Most of the reported outbreaks of gastrointestinal disease linked to the fresh produce have been associated with bacterial contamination, particularly with members of the Enterobacteriaceae family; due to this reason care has to be taken before consumption vegetables by washing it with clean water.

Acknowledgments:The authors would like to thank Department of Microbiology Bayero University Kano, Nigeria and Department of Public Health Daffodil International University, Dhaka-1207 Bangladesh for their help and guidance throughout this research.

REFERENCES:

1. Akinmusire, O. O. Fungal species associated with the spoilage of some edible fruits in Maiduguri Northern Eastern Nigeria. Advances in Environmental Biology, 2011; 5: 157-161
2. Tournas, V. H. Spoilage of vegetable crops by bacteria and fungi and related health hazards. Critical Review of Microbiology, 2005; 31(1): 33-44.

3. Kornacki, JL, Johnson JL. Enterobacteriaceae, coliforms, and E. coli as quality and safety indicators. Compendium of Methods for the Microbiological Examination of Foods. 4 th ed. F. P. Downes/K. Ito editors. APHA. Washington, DC. 2001.
4. Rawat, S. Food spoilage: Microorganisms and their prevention. Asian Journal of Plant Science and Research 2015; 5(5):47-56.
5. Tournas, VH, Katsoudas E. Mould and yeast flora in fresh berries, grapes and citrus fruits. International Journal of Food Microbiology, 2005; 105(1): 11-17.
6. Johannessen GS et al. Bacteriological analysis of fresh produce in Norway. Int. J. Food Microbiol. 2002; 77:199-204.
7. Aycicek H et al. Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey. Int. J. Hyg. Environ. Health. 2006; 209:197-201.
8. Peter, F. et al. J. Biol. Chem., in the press 1992.
9. Fawole, M.O, Oso, B.A 'Laboratory manual of microbiology' Spectrum Books Limited, Ibadan, Nigeria 2001.
10. Brown, G. E. A. A Report on the prevalence of Bacteria specie in Retail Smoked fish within Bauchi Metropolis 2004.
11. Cheesbrough M. Microscope. In: District Laboratory Practice in Tropical Countries. Part 1, Low price Egyptian edition. The Anglo-Egyptian Bookshop & Cambridge University Press,2004; pp: 110.
12. Mac Faddin, Jean F. Biochemical tests for identification of medical bacteria. Williams and Wilkins Co., 1976.
13. McCarthy, J.A. et al. Evaluation of the reliability of coliform density test. Am. J. Publ. Health 1958; 48:1628-1635.
14. American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater. 20th Ed., Washington, D.C. 1998.
15. Adebolu TT, Ifesan BO. Bacteriological quality of vegetables used in salads. Niger. J. Microbiol. 2001; 15:81-85.
16. Dunn RA et al. Outbreak of Shiegella flexneri linked to salad prepared at a central commissary in Michigan. Public Health Reports 1995; 110: 580-586.
17. Olayemi AB. Microbiological hazards associated with agricultural utilization of urban polluted river water. International J. Enviro. Health Res. 2007; 7:149-154.
18. Omemu AM, Bankole MO. Ready-to-eat (RTE) vegetable salad: effect of washing and storage temperature on the microbial quality and shelf-life. In: Microbes as Agents of Sustainable Development. 29th Annual Conference & General Meeting of the Nigerian Society for Microbiology UNAAB, Abeokuta 6-10th November 2005 p. 28

19. Tambekar DH, Mundhada RH. Bacteriological quality of salad vegetables sold in Amravati City (India). J. Biol. Sci. 2006; 6:28-30.
20. Uzeh RE, et al. The microbial quality of prepacked mixed vegetable salad in some retail outlets in Lagos, Nigeria. Afr. J. Food Sci. 2009; 3: 270-272
21. Prescott, MI, Harle, JD, Klein, DA. Microbiology of Food. Fifth edition, McGraw- Hill Ltd, New York, USA. 2002; 964.
22. Khan MR et al. A bacteriological profile of salad vegetables in Bangladesh with special reference to coliforms. Letter Appl. Microbiol. 2008; 14(3):88-90.
23. Nipa MN, et al. Prevalence of multi-drug resistant bacteria on raw salad vegetables sold in major market of Chittagong City, Bangladesh. Middle-East J. Sci. Res. 2011;10 (1):70-77.
24. Montville TJ, Mathews KR. Food Microbiology: An Introduction, 2nd ed. American Society of Microbiology (ASM) Press. 2008.
25. Abadias M, et al. Microbiological quality of fresh, Minimally-processed fruit and vegetables, and sprouts from retail establishments. Int. J. Food Microbiol. 2008; 123:121-129.
26. Ofor MO, et al. Microbial Contaminants in Fresh Tomato Wash Water and Food Safety Considerations in South-Eastern Nigeria. Life Sci. J. 2009;1:80-82.
27. Vanderzant C, Splittstoesser DF. Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington D. C., 1992;l:44.