Journal of Advances in Biology & Biotechnology
12(4): 1-6, 2017; Article no.JABB.32352
ISSN: 2394-1081

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of Ready-to-eat Polyethylene Packed Pawpaw (*Carica papaya*) for the Presence of Antibiotic Resistant *Escherichia species*

O. C. Eruteya[1*] and A. Nwayanwu[1]

[1]Department of Microbiology, Faculty of Science, University of Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author OCE designed the study. Author AN handled the laboratory analysis. Author OCE wrote the draft of the manuscript and managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2017/32352
Editor(s):
(1) Maria Serrano, Department of Applied Biology, EPSO, University Miguel Hernandez, Orihuela, Alicante, Spain.
Reviewers:
(1) Pasquale Russo, University of Foggia, Italy.
(2) Lucero Estrada, Cecilia, National University of San Luis, San Luis, Argentina.
(3) Stephen A. Enabulele, Benson Idahosa University, Benin City, Nigeria.
(4) Iyekhoetin Matthew Omoruyi, Benson Idahosa University, Nigeria.
(5) Akindele, Peter Oluwayinka, Federal University of Technology, Akure, Nigeria.
(6) Endang Yuli Purwani, Indonesian Center of Agricultural Postharvest Research and Development, Indonesia.
Complete Peer review History: http://www.sciencedomain.org/review-history/18832

Short Communication

Received 22nd February 2017
Accepted 24th April 2017
Published 28th April 2017

ABSTRACT

Aims: The study examined ready-to-eat cut polyethylene packed pawpaw (*Carica papaya*) sold in the University of Port Harcourt community for the presence of antibiotic resistant *Escherichia species*.
Study Design: The samples were randomly purchased from vendors, who openly display them in trays and composite analysed in duplicate.
Place and Duration of Study: Department of Microbiology, University of Port Harcourt between January and June 2015.
Methodology: A total of 50 ready-to-eat polyethylene packed pawpaw were examined for the presence of presumptive *E. coli* 0157:H7 and non-0157:H7 *E. coli*, using MacConkey-Sorbitol agar and eosin methylene blue agar. Isolated were confirmed on the basis of cultural morphology, physiology and biochemical characteristics. Screening for antibiotic susceptibility was done using

**Corresponding author: E-mail: onoriode.eruteya@uniport.edu.ng;*

the disk diffusion method involving Mueller Hinton agar.
Results: Of the 50 samples examined, 34 (68%) were positive for *Escherichia species*, with counts ranging from 1.6 to 3.5 $\times 10^4$ cfu/g. Presumptive 0157:H7 *E. coli* accounted for 5.88% while non-0157:H7 *E. coli* accounted for 94.12%. The resulting isolates showed varying resistance to augumentin (5.88%), amoxicillin (97.06%), cloxacillin (73.53%), cotrimoxazole (95.58%), erythromycin (5.88%), gentamicin (14.71%), nitrofurantoin (85.29%) and tetracycline (58.82%).
Conclusion: The study clearly reveals that consumption of ready-to-eat cut pawpaw from vendors can be a potential risk for food borne outbreaks because of their contamination level by *E. coli* and the variable resistance patterns in response to different antibiotics used in the study.

Keywords: Antibiotics; Carica papaya; Escherichia coli 0157:H7; pawpaw.

1. INTRODUCTION

Fresh fruits and vegetables are a constant part of the daily diets of Nigerians, and are known for their high nutritional and health values, with fruits and vegetables since they are excellent source of essential nutrients, minerals, vitamins and fiber for humans and are thus vital for health and wellbeing [1,2,3,4,5]. Well balanced diets, rich in fruits and vegetables are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies as well as reducing the risk of several diseases such as atherosclerosis and cancer [1].

Ready-to-eat fruits are sliced fruits in their fresh state that can be bought directly from street vendors or hawkers or at local markets and eaten immediately as they have already been prepared by the vendors [6]. In Nigeria, the consumption of ready-to-eat sliced fruits has increased in the past few years. This is because the sliced fruits are more convenient, easily accessible and most especially cheaper than whole fruits and vegetables as many consumers cannot afford the whole fruits [7,8]. The fruits are peeled, cut into pieces, wrapped with transparent polythene bags and sold to the people. These fruits are sold mainly by unlicensed street vendors or hawkers with poor education levels, untrained in food hygiene and work under crude unsanitary conditions [8].

Sliced fruits commonly consumed in Nigeria include pawpaw, pineapple, watermelon, salad vegetables, cucumbers, carrots and pears [9,10]. *Carica papaya*, commonly known as papaya or pawpaw, is grown throughout the tropics and subtropics for its melon-like fruit, which is usually eaten fresh. The acropetally produced fruits are clustered near the top of small (2-8 m), single-stemmed, herbaceous trees. The edible portion of papaya is composed mostly of water (86.8%), carbohydrates (10.82 g/100 g), fat (0.26 g/100 g), protein (0.47 g/100 g), vitamins and trace metals [11]. Their increased consumption, coupled with the associated risk of disease to which consumers may be exposed, is a matter of great concern, as food borne diseases are increasingly becoming a global public health problem [9,12], resulting in a tangible amount of morbidity and mortality annually worldwide [13].

Pathogens implicated in contamination of fruits and vegetables include; *Escherichia coli*, *Escherichia coli* 0157H7, *Salmonella* spp., *Listeria monocytogenes*, *Aeromonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *Vibrio* spp. and *Pseudomonas* spp. [4,8,14]. Most of the reported outbreaks have been associated with bacterial contamination, particularly members of the enterobacteriaceae [15].

Escherichia coli is a commensal bacterium in humans and animals and has a wide range of hosts. It is commonly present in the environment and is considered an indicator of fecal contamination in food and water. *Escherichia coli* O157:H7 is an emerging disease pathogen whose occurrences have been reported in several parts of the world including Nigeria [16].

Food contamination with antibiotic resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria potentially compromising the treatment of severe bacterial infections [9]. *Escherichia coli* can acquire, maintain, and transmit resistance genes from other organisms in the environment [17].

Therefore the present study was undertaken to compare the prevalence of presumptive 0157:H7 *E. coli* and non-0157:H7 *E. coli* in vended ready-to-eat polyethylene packaged sliced pawpaw and the degree of antibiotic resistance among the *E. coli* strains.

2. MATERIALS AND METHODS

2.1 Sample Source

A total of 50 ready-to-eat polyethylene packaged sliced pawpaw fruits were purchased from vendors around the University community and transported to Microbiology laboratory, University of Port Harcourt in ice container for analysis.

2.2 Isolation Procedure

Twenty five grams of composite samples were aseptically added to 225 ml sterile normal saline in stomacher bags and homogenated for 1 min. This was followed by a ten-fold serial dilution; after which 0.1 ml of appropriate dilutions were seeded on Eosin methylene blue (LAB M, UK) and MacConkey-Sorbitol agar (Fluka analytical, Indian). The plates were incubated at ambient temperature (29± 2℃) for 24 h. Characteristics colourless and green metallic sheen on MacConkey-Sorbitol and Eosin methylene blue agar respectively were sorted in Nutrient agar slated for confirmation.

2.3 Confirmation of Isolates

Characteristic colonies were confirmed on the basis of physiological (Grams reaction) and biochemical (IMViC) reaction.

2.4 Antibiotic Susceptibility Testing

Antibiotic sensitivity patterns of all the *E. coli* and presumptive *E. coli* 0157H7 confirmed biochemically was performed by standard disk diffusion method on Mueller-Hinton agar (Titan, Biotech Ltd, Indian) following the procedures recommended by NCCLS [18]. Eight commonly used antibiotics (µg/disc) viz. augumentin (AUG) 30, amoxycilin (AMX) 25, erythromycin (ERY) 5, tetracycline (TET) 10, cloxacilin (CXC) 5, gentamicin (GEN) 10, cotrimoxazole (COT) 25, and nitrofurantoin (NIT) 300 (Abtek^R, UK) were tested. From an overnight culture in brain heart infusion broth, a 10^8 cell/ml (0.5 McFarland turbidity standards) bacterial culture was prepared in sterile saline, from which 0.1 ml was inoculated onto Mueller Hinton agar, after which antibiotic discs were carefully and aseptically placed on the surface of the agar. The plates were incubated at 37℃ for 24 h. Zone of inhibition was measured in millimeter.

3. RESULTS AND DISCUSSION

3.1 Occurrence of *E. coli* in Examined Samples

Of the 50 samples of ready-to-eat vended pawpaw examined, 34 (68%) were positive for *Escherichia coli,* with counts ranging from 1.6 to 3.5 $\times 10^4$ cfu/g. This findings is comparable to previous reports of 48%, 70% and 75% occurrence of *E. coli* in pawpaw examined in Calcutta, Umuahia and Minna respectively [19,20,21]. These high incidences of *E. coli* gives credence to report by Adesetan et al. [9], that microbiological studies carried out on street vended foods from many developing countries, revealed a high bacteria count. Daniyan and Ajibo [21] have linked most contamination of sliced fruits, especially pawpaw which are usually washed after slicing to faecally polluted water used for washing utensils (e.g. Knives, trays, and pans), wrapping material and the exposure of these products to low temperature. The results of the cultural, physiological and biochemical reactions of the *E. coli* isolated is presented in Table 1. Presumptive *Escherichia coli* 0157:H7 accounted for 5.88% (4 of 68) while non-0157:H7 *E. coli* accounted for 94.12% (64 of 68).

Table 1. Cultural, physiological and biochemical characteristics of *Escherichia species*

Isolate	Cultural morphology on Eosin methylene blue agar	Cultural morphology on MacConkey-Sorbitol agar	Grams reaction	Indole	Methyl-red	Voges-Proskauer	Citrate
Presumptive *E. coli* 0157:H7	Green metallic sheen	Colourless	- (rod)	+	+	-	-
Non-0157:H7 *E. coli*	Green metallic sheen	Pink	- (rod)	+	+	-	-

Table 2. Distribution of resistant non-0157:H7 *E. coli* and presumptive *E. coli* 0157:H7 to common antibiotics

Isolates	No.	AUG	AMX	CXC	COT	ERY	GEN	NIT	TET
Presumptive *E. coli* 0157:H7	4	1	2	2	1	1	1	2	2
Non-0157:H7 *E. coli*	64	3	64	48	64	3	9	56	38
% resistance		5.88	97.06	73.53	95.59	5.88	14.71	85.29	58.82

AUG=augmentin; AMX=amoxicillin; CXC=cloxacillin; COT=cotrimoxazole; ERY=erythromycin; GEN=gentamicin, NIT=nitrofurantoin and TET= tetracycline

A number of authors have reported the presence of *E. coli* along with other bacteria in vended ready-to-eat pawpaw in different parts of Nigeria [5,8,22,23]. However, Adekanle et al. [24] did not detect *E. coli* in 20 pawpaw samples examined in Sagamu, South-West, Nigeria. The *E. coli* counts reported in this study is comparable to 3.2 to 3.7×10^3 cfu/g and 1.8 to 3.4×10^5 cfu/g reported by Daniels et al. [22] and Allamin et al. [5] respectively.

3.2 Antibiotic Susceptibility

Food contamination with antibiotic resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria potentially compromising the treatment of severe bacterial infections [9].

The resulting isolates showed varying resistance to augumentin (5.88%), amoxicillin (97.06%), cloxacillin (73.53%), cotrimoxazole (95.58%), erythromycin (5.88%), gentamicin (14.71%), nitrofurantoin (85.29%) and tetracycline (58.82%) (Table 2). These findings corroborated reports by Sabaté et al. [25] that treatment for *E. coli* infection has been increasingly complicated by the emergence of resistance to commonly antimicrobial agents.

The sensitivity of the isolated *E. coli* to augumentin is in agreement with results reported by Marwa et al. [26] and Osterbald et al. [27] that most *E. coli* isolates from food were sensitive to Amoxicillin/clavulanic acid; whereas, Oje et al. [28] and Lateef et al. [29] reported a 100% resistance of *E. coli* isolated from ready-to-eat foods from outlet in Ekiti State University and its Environs and orange juice respectively to augumentin. The *E. coli* in this study however, had a high resistance to cotrimoxazole contrary to report of high sensitivity of food isolates by Marwa et al. [26] and Srinu et al. [30] and a 40% resistance of *E. coli* from orange juice reported by Lateef et al. [29]. The *E. coli* isolated from orange juice had a comparable resistance to amoxicillin (100%), cloxacillin (60%) and gentamicin (20%) [29]. On their part, Rasheed et al. [31] have reported lower percentage resistance of *E. coli* from non fruit sources to tetracycline (12.6%), cotrimoxazole (11.3%) and gentamicin (4.6%).

4. CONCLUSION

The findings of this study highlight the potential health risk to the community due the presence of *E. coli* and presumptive *E. coli* 0157:H7 contamination showing variable susceptibility and resistance patterns in response to commonly used antibiotics employed in this study. The study has brought to the fore the need for a more intense and well planned community awareness programs advocating use of uncontaminated water, hygiene-based handling and marketing procedures to prevent any major outbreak associated with contaminated fruits otherwise the already poor public health system in these community may be further burdened.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kalia A, Gupta RP. Fruit microbiology. In: Hui YH, Cano J, Gusek MP, Sidhu W, Sinha JW, editors. Handbook of fruit and fruit processing 1st ed. USA: Blackwell Publishing; 2006.
2. Anitha M, Swathy SR, Venkateswari P. Prevalence of disease causing microorganisms in decaying fruits with analysis of fungal and bacterial *species*. Int. J. Res. Health Sci. 2014;2(2):547-54.
3. Brooks AA. Evaluation of microbial contamination of street-vended fruit salad

in Calabar, Nigeria. Int. J. Curr. Microbiol. Appl. Sci. 2014;3(7):1040-6.

4. Senjuti JD, Feroz F, Tahera J, Das KK, Noor R. Assessment of microbiological contamination and the *in vitro* demonstration of the antibacterial traits of the commonly available local fruit blend within Dhaka metropolis. J. Pharmacog. Phytochem. 2014;3(1):73-7.
5. Allamin IA, Borkoma MB, Ibn Abbas M, Waziri MA. Bacteriological quality of sliced fruits sold at road site in Maiduguri, Borno State. Int. J. Environ. 2015;4(2):236-42.
6. Oranusi S, Olorunfemi OJ. Microbiological safety evaluation of street vended ready-to-eat fruits sold in Ota, Ogun State, Nigeria. Int. J. Res. Biol. Sci. 2011;1(2):27-32.
7. De Roever C. Microbiological safety evaluations and recommendation on fresh produce. Food Contr. 1998;9(6):321-47.
8. Afolabi O, Oloyede A, Abibu W, Adeyanju A. Microbial safety of polyethylene packaged sliced fruits sold in Abeokuta, South-West Nigeria. J. Natural Sci. Res. 2015;5(13):16-21.
9. Adesetan TO, Egberongbe HO, Ilusanya OAF, Bello OO. Antimicrobial sensitivity of bacterial isolates from street vended fruits in Ijebu area of Ogun State, Nigeria. Int. Res. J. Microbiol. 2013;4(9):220-5.
10. Mbata CA, Nwagu C, Adegoke OA, Nyenke CU, Wali AN. Bacteriological status of water melon (*Citrullus Lanatus*) sold in Mile III Market Port Harcourt. Int. J. Eng. Innovation Res. 2016;5(1):46-9.
11. Ogbogu CL, Ojiagu DK, Anyamene NC. Isolation and characterization of microorganisms involved in the postharvest loss of *Carica papaya* (Papaya) and *Mangifera indica* (Mango) in Awka, South-Eastern Nigeria. Int. Agric. Biosci. 2014;3(5):225-9.
12. Hannan A, Rehman R, Saleem S, Khan MU, Qamar MU, Azhar H. Microbiological analysis of ready-to-eat salads available at different outlets in Lahore, Pakistan. Int. Food Res. J. 2014;21(5):1797-1800.
13. Hanson LA, Zahn EA, Wild SR, Döpfer D, Scott J, Stein S. Estimating global mortality from potentially foodborne diseases: An analysis using vital registration data. Population Health Metrics. 2012;10(5):1-7.
14. Nwachukwu E, Chukwu CM. Effect of chemical treatments on the microbial load of fruits and vegetables. Int. J. Appl. Microbiol. Biotechnol. Res. 2013;1:16-19.
15. Kumar A, Bhushan V, Verma S, Srivastav G, Kumar S. Isolation and characterization of microorganisms responsible for different types of food spoilages. Int. J. Res. Pure Appl. Microbiol. 2011;1(2):22-31.
16. Esumeh FI, Isibor JO, Egbagbe IDS. Screening for *Escherichia coli* 0157:H7 in diarrheic patients in Benin City, Nigeria. J. Microbiol. Biotechnol. Res. 2011;1(4):1-4.
17. Zhao S, Blickenstaff K, Bodeis-Jones S, Gaines SA, Tong E, McDermott PF. Comparison of the prevalence and antimicrobial resistances of *Escherichia coli* isolates from different retail meats in the United States, 2002 to 2008. Appl. Environ. Microbiol. 2012;78(6):1701–7.
18. National Committee for Clinical Laboratory Standards (NCCLS). Performance standard for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; tentative standard. NCCLS document M31-T. Villanova, PA: NCCLS; 1997.
19. Mukhopadhyay R, Mitra A, Roy WR, Guha AK. An evaluation of street-vended sliced papaya (*Carica papaya*) for bacteria and indicator microorganisms of public health significance. Food Microbiol. 2002;19:663-7.
20. Nwachukwu E, Osuocha HU. Microbiological assessment of ready-to-eat sliced pawpaw (*Carica papaya*) and watermelon (*Citrullus lanatus*) vended in Umuahia, Nigeria. Int. J. Curr. Microbiol. Appl. Sci. 2014;3(6):910-6.
21. Daniyan SY, Ajibo CQ. Microbiological examination of sliced fruits sold in Minna Metropolis. Int. Res. J. Pharm. 2011;2(7): 124-9.
22. Daniel AA, Danfulani S, Barnabas BB, Peter G, Ajewole AE. Microbiological quality of sliced fresh fruits sold in Bida, Nigeria. Global J. Biol. Agric Health Sci. 2014;3(3):178-180.
23. Nwankwo IU, Osaro- Matthew RC, Uchendu WO. Microbiological examination of fruits sold at Isi-gate Umuahia, Abia State. J. Biol. Sci. 2015;2(11):1-16.
24. Adekanle MA, Effedua HI, Oritogun KS, Adesiji YO, Ogunledun A. A study of microbial analysis of fresh fruits and vegetables, in Sagamu markets South-West, Nigeria. Agrosearch. 2015;15(2):1–12.
25. Sabaté M, Prats G, Moreno E, Ballesté E, Blanch AR, Andreu A. Virulence and antimicrobial resistance profiles among

Escherichia coli strains isolated from human and animal wastewater. Res. Microbiol. 2008;159:288-93.

26. Marwa EAA, Tamer ME, Magdy AM. Antibiotic resistance profile of *E. coli* strains isolated from clinical specimens and food samples in Egypt. Int. J. Microbiol. Res. 2012;3(3):176-82.
27. Osterbald M, Pensala O, Peterzens M, Heleniusc H, Huovinen P. Antimicrobial susceptibility of Enterobacteriaceae isolated from vegetable. J. Antimicrob. Chemother. 1999;43(6):503–9.
28. Oje OJ, David OM, Adeosun OM, Adebayo AA, Famurewa O. Multiple antibiotic-resistant *Escherichia coli* in ready-to-eat foods from food outlets in Ekiti State University and its environ. British Microbiol. Res. J. 2016;13(1):1-11.
29. Lateef A, Oloke JK, Gueguim-Kana EB. Antimicrobial resistance of bacterial strains isolated from orange juice products. Afr. J. Biotechnol. 2004;3(6):334-8.
30. Srinu B, Anumolu VK, Kumar E, Madhava RT. Antimicrobial resistance pattern of bacterial foodborne pathogens. J. Chem. Pharmac. Res. 2012;4(7):3734–6.
31. Rasheed MU, Thajuddin N, Ahamed P, Teklemariam Z, Jamil K. antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. Rev. Inst. Med. Trop. Sao Paulo. 2014;56(4):341-346.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18832