https://dx.doi.org/10.4314/dujopas.v8i1b.1
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 8 No. 1b March 2022*

Isolation of Some Pathogenic Microbes Associated with Spoilt Carrots (*Daucus carota* L.) obtained from Local Markets in Abraka, Delta State, Nigeria

Morka, E.
Department of Microbiology,
Faculty of Science,
Delta State University,
Abraka, Nigeria
Email: emorka@delsu.edu.ng

Abstract
Over the past decades vegetable consumption specifically carrot has been on the rise however, its wastage due to microbial spoilage has been estimated at around 20% annually. In this study, spoilage microbes associated with carrots were identified by employing standard microbiological procedures. Various tests were used to characterize carrots with soft rot symptoms. This study was aimed at assessing microorganisms associated with spoilage of carrots. Seven (7) bacterial species and five (5) fungal species were detected via morphology and biochemical screening. The results showed that Escherichia coli (20%) was recorded the highest prevalence among bacterial isolates while the least prevalence was Shigella sp. (8%). On the other hand, Aspergillus niger was recorded the highest (40%) while the least prevalence of the fungi was Mucor sp. (9%). Results from this study affirmed that both spoilage and pathogenic microorganisms are present in carrots, therefore care must be taken in handling, washing and processing carrots before consumption so as to prevent spoilage that might lead to infections and food-borne outbreaks due to fungi and bacteria.

Keywords: Bacterial isolates, Carrots, Food spoilage, Food-born outbreaks, Fungi

INTRODUCTION
Consumption of vegetables and fruits has been on a rising demand over the past decade as they have now become an essential part of our daily diet. For instance, vegetable salad usually made from carrot,cucumber, lettuce, tomatoes and cabbage (Chukwuma, 2016) is a usual food supplement in Nigeria. Vegetables can either be hawked or purchased directly from the market or mull.

Carrot (*Daucus carota* L.) is a common vegetable variety cultivated globally. It is nutritious and consists of all kinds of vitamins and minerals as documented by Hammad *et al.*(2013). They also consist of carotenoids content and antioxidants, which protect humans from strokes, cardiovascular diseases and cancer (Bishop and Okwori, 2017).Presently, in Nigeria, the public health significance of carrots has been a rising trend.

Carrot cultivation and other varieties of vegetables have limiting factors. Globally, annual vegetables losses have been estimated to around 30 to 40% (Barth *et al.*, 2009). Onuorah *et al.* (2016) also estimated that 20% of harvested carrots for consumption are condemned through

**Author for Correspondence*

spoilage by microorganisms, though additional factors like climatic changes, physical damage, enzymatic reactions and insect invasions are also included. Owais *et al.* (2018) noted that enzymes are responsible for degradation in some vegetables while chemical reactions such as rancidity and oxidation destroy others. However, the most destructive cause of spoilage are microorganisms such as bacteria, moulds and yeast (Jay, 1992) because vegetables are susceptible to microbial spoilage. This susceptibility may arise from differences in the physical compositions like moisture and pH which are linked with high prevalence of spoilage microorganisms. Contamination by microbes occurs during growth phase at fields, greenhouses and orchards; during harvesting to post-harvesting, even at distributive and preparatory stages (Mritunjay and Kumar, 2015; Bishop and Okwori, 2017).

Though vegetables are important sources of nourishment to human beings (Kaur *et al*., 2017), specifically vitamins, and could serve as an important ingredient in enhancing health and proper diets. However, they are notable sources of chemical and microbial contaminants (Uzeh *et al*., 2009). Velusamy *et al.* (2010) stated that vegetables have been linked with illnesses arising from food borne because notable pathogens like *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enteric* (Kim *et al*., 2013) grow on them. Unfortunately, carrots and other vegetables are consumed for their enormous nutritional benefits without thoughts of possible contamination with disease causing microorganisms (Oranusi *et al*., 2012). Pathogens like *Bacillus cereus*, *Listeria monocytes* and *Clostridium botulinum* are dominant flora of the soil, whereas species of *Salmonella*, *Campylobacter*, *Shigella*, and *Escherichia coli* resides in the colon of animals, including man. These organisms are notable contaminants of vegetables and raw fruits through faecal, untreated irrigation and surface water, and sewage channels (Kaur *et al*., 2017). According to Mukherjee *et al.* (2006), the level of food borne outbreaks caused by spoilt fruits and vegetables has been on a rising side in recent years, thus, a quest to isolate and identify these pathogens that causes spoilage should be recommended as a control measure (Akinyele and Akinkunmi, 2012). Recent studies on carrots includes growth and yield responses of carrot (*daucus carota* L.*)* to different levels of oil palm refuse bunch ash in an ultisols environment by Law-Ogbomo (2018); as well as Modeling the carrot slices (daucus carota L.) drying characteristics using microwave oven done by Nwajinka and Okonjo (2019). Most of these studies did not focus on the handling practice and consequences of human action on exposure to pathogens. Hence, by reason of the increase in number of patients attending Abraka General hospital as observed during my preliminary survey, it became necessary to look at exposure of humans to pathogenic microbes associated with vended vegetables with carrots as a likely source of transmission of diseases to consumers arising from poor processing and handling practices. Thus, the study on isolation of some pathogenic microbes associated with spoilt carrots (*daucus carota* L.) obtained from local markets in Abraka, Delta state, Nigeria

MATERIALS AND METHOD

Study Area: The research was done in Abraka town at Ethiope East Local Government Area of Delta State. Abraka is located 5^0 47′ 0″ North and $6^0$6′0″ East of Delta State, Nigeria.

Collection of samples

Ten (10) carrot samples with soft rot symptoms were purchased from five (5) vended sites including Abraka main market, small market, Ekrejeta, Umege and Oria Market. They were kept in sterile polythene bags before transporting to microbiology laboratory at site 2 of Delta State University where analysis was done. The carrots were washed with clean running water

which was followed by cutting off at the margin of rotted tissue segments (1g) with a sterilized knife and grinded with mortar and pestle.

Bacteriological screening
Carrot samples weighing 1g was inoculated into McCartney bottles containing 9ml of sterilized water. This was followed by agitation of the bottles for proper mixture of the contents. Ten-fold serial dilutions was performed and dilution factor of 10^{-3} of diluted samples was used with 1ml plated out unto solidified nutrient agar medium and incubation followed immediately at 37 ^{o}C for 24 hours.

Isolation of coliforms
Coliforms were isolated by membrane filtration technique through a membrane filtration funnel with a 50ml capacity. The membrane filtration funnel was positioned at a fixed portion attached to a vacuum pump allowing passage of water into porous and sterilized membrane filter (0.45 µm). With an aid of sterile forceps, the filters were positioned on MacConkey agar plates after influx of 100ml of carrot samples. The media was prepared and was followed by autoclaving at 121 ^{O}C for 15 mins at 15Ib prior inoculation with the filters.

Purification of bacterial isolates
The streak plate technique was done as reported by Cheesbrough (2004). With the aid of a sterile forceps, isolates were picked and streaked directly to molten nutrient agar labelled plates thereafter incubation was done at 37 ^{o}C for 24 hours to identify a pure colony. Discrete colonies from these subcultures were enumerated. The bacterial isolates obtained were characterised using morphological, biochemical and sugar fermentation tests.

Fungal characterization
Ten-fold serial dilutions with dilution factor of 10^{-3} plated out with 1ml of samples inoculated into prepared and solidified potato dextrose agar (PDA) plates. The PDA consists of 30 mg/l of chloramphenicol which hinders bacteria growth. Incubation was done for two (2) days at room temperature. All fungal isolates were characterized based on macroscopic and microscopic examination

RESULTS
The microbial counts obtained from the carrot samples were presented in Table 1. The bacterial counts were within the range of 1.0 ± 0.18 to 4.5 ± 0.26 x 10^{3}CFU/gfor CAI and CAC respectively. The coliform count ranged from 1.0 ± 0.26 to 4.8 ± 0.37 x 10^{3}CFU/gfor samples CAG and CAD. The fungal counts ranged from 0.8 ± 0.22 to 5.5 ± 0.40 x 10^{3}CFU/g for samples CAH and CAA respectively. Table 2. Showed the biochemical characterization of bacterial isolates. Seven (7) bacterial species were isolated which included: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, *Erwinia* sp., *Enterobacter* sp., *Salmonella* sp. and *Shigella* sp. Table 3 revealed the frequency level of occurrence for bacteria species from carrot samples. *E. coli* (20%) showed higher occurrence followed by *S. auerus*(15%) while *Shigella* sp. (8%) had the lowest percentage occurrence. The following fungi were isolated as shown in Table 4: *Aspergillus niger*, *Rhizopus* sp., *Fusarium* sp., *Cladosporium* sp. and *Mucor* sp. *Aspergillus niger*(40%) was highest in the order of dominance while *Mucor* sp. (9%) had least occurrence as represented in Table 5.

Table 1: Microbial counts (CFU/g)of the carrot samples ($\times 10^3$)

Samples	Total bacteria count (CFU/g)	Total coliform count (CFU/g)	Total fungal count (CFU/g)
CAA	2.6 ± 0.24	2.5 ± 0.11	5.5± 0.40
CAB	4.0 ± 0.15	2.4 ± 0.02	1.0± 0.32
CAC	4.5 ± 0.26	3.4 ± 0.18	3.8± 0.38
CAD	2.9 ± 0.32	4.8 ± 0.37	2.1± 0.55
CAE	3.6 ± 0.22	1.2 ± 0.22	3.1± 0.18
CAF	3.9 ± 0.14	2.7 ± 0.41	2.1± 0.09
CAG	4.0 ± 0.54	1.0 ± 0.26	1.8± 0.14
CAH	4.0 ± 0.35	2.4 ± 0.13	0.8± 0.22
CAI	1.0 ± 0.18	2.3 ± 0.19	2.1± 0.10
CAJ	1.1 ± 0.21	1.5 ± 0.16	1.5± 0.17

Keys: CAA – CAJ = Carrot samples A – J

Table 2. Cultural and Biochemical characterization of bacterial isolates

Shape	Appearance	Cell shape	Grams stain	CT	MT	IN	OX	CAT	Glucose	LAT	Acid	H_2S	Gas	Tentative Genera
Ovoid	Discrete	Cocci	+	_	_	_	_	+	+	+	_	_	-	*Staphilococcus aureus*
Iregular	Mucoid	Rod	_	+	_	_	_	+	+	_	+	+	+	*Salmonella* sp
Ovoid	Discreet	Rod	_	+	+	_	_	+	+	+	_	_	_	*Shigella* sp
Ovoid	Discreet	Rod	_	+	+	_	_	+	+	_	+	+	+	*Erwinia* sp
Ovoid	Discreet	Rod	_	_	+	_	_	+	+	_	_	_	+	*Enterobacter* sp
Ovoid	Discreet	Rod	_	+	+	_	+	+	_	_	_	_	_	*Pseudomonas* sp
Ovoid	Discreet	Rod	_	_	_	+	_	+	+	+	+	_	_	*Escherichia coli*

Keys: CT= Citrate, MT= Motility, IN= Indole, OX= Oxidase, CAT= Catalase, LAT= Lactose

Table 3. Frequency of occurrence of bacteria isolates from carrot samples

Bacteria	%
Escherichia coli	20
Staphylococcus aureus	18
Pseudomonas sp.	14
*Erwinia*sp.	15
Enterobacter sp.	10
Salmonella sp.	15
Shigella sp.	8

Table 4: Microscopic and Macroscopic characterization of fungal isolates

Cultural morphology	Microscopic characteristics	Fungal species
Presence of numerous black dots	Dichotomous branching. Septate and hyaline detected. Long, smooth conidiophores with hyaline, usually darker at the apex. Numerous black spores.	*Aspergillus niger*
Appeared whitish to cream coloration, turned bluish brown with presence of sporodochia	Short and multi-branched. Septate hyphae. Cylindrical, fusiform, curved shape pedicellate foot cell, blunt and short apical cell. Appeared in pairs or single with globose, hyaline, smooth and rough walled.	*Fusarium* sp
Colonies appeared olive-green to brown or black colonies	Branched chains. Septate with brown hyphae. Conidiophores are erect and dark pigmented. Conidia appeared cylindrical in shape. Fragile spore chains	*Cladosporium* sp
White to grey and fast-growing. Older colonies appeared grey to brown	Branched. Non septate.Smooth, short with green coloration of conidiophores. Appeared simple, branched which forms an apical, globular sporangia supported and elevated by a column-shaped columella	*Mucor* sp
Appeared dense with aerial mycelium. Previously white before turning to grey	Branched. Non septate with stolons. Greyish black, flattened and globose sporangia, appeared powdery with numerous spores	*Rhizopus* sp

Table 5. Frequency by occurrence of fungal species from sampled carrots

Fungi	%
Aspergillus niger	40
Rhizopus sp	20
Fusarium sp.	16
Cladosporium sp	15
*Mucor*sp	9

Discussion

The results obtained from this study revealed a high microbial load in carrot samples obtained from markets in Abraka town which was similarly reported by Uzeh*et al.*(2009) and Oji (2016) who reported high levels of microbial load in carrots. According to Oji (2016) whose findings on bacteriological assessment of salad vegetable in Eke Awka market, Anambra State, Nigeria recordedthat carrots had the highest bacterial count of 3.26 x 10^7 cfu/g among the vegetables investigated from a rangeof 1.83 x 10^7 to 3.26 x 10^7cfu/g. This might be due to post-harvest handling, processing, distribution, contaminated water used in washing and processing by

the retailers. The bacteria isolated from carrot samples were in line with those recorded by Adebayo-Tayo *et al.* (2012), Harding *et al.* (2017) and Ehimemen *et al.* (2019) in which they characterized the microbial communities associated with vegetables. The study hasshown that vegetables and fruits can be attacked by microorganisms.

Seven (7) bacteria species including *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, *Erwinia* sp., *Enterobacter* sp., *Salmonella* sp. *and Shigella* sp. were isolated. The result was similar to those isolated by Oji (2006). *E. coli* (20%) was the highest in occurrence followed by *S. auerus* (15%) while *Shigella* sp. (8%) had the lowest percentage occurrence. The high incidence of *E. coli* might be due to contaminated water used during irrigation as observed by Oranusi and Olorunfemi (2011) and Kumar (2012). It could also be from water used in washing the carrots during post-harvest processing and unhygienic practices by the vendors and buyers. *S. aureus*, which is prevalent in the skin, could possibly be transferred to carrots via direct human contact by hawking or roadside marketing. The carrots are frequently touched during selection process thereby causing the spread of *S. aureus* from unsterilized hands to the carrots as noted by Bishop and Okwori (2017).

These pathogens are transmitted to humans when consumed via contaminated carrots and vegetables. Heaton and Jones (2008) discussed on common risks of infections and outbreaks of food-related diseases following vegetable consumption therefore, postharvest contamination from handling and processing must be monitored due to rise of food borne outbreaks associated with vegetables (Erickson, 2010).

Five (5) fungal species were reportedly isolated from the study which included: *Aspergillus niger*, *Rhizopus* sp., *Mucor* sp., *Cladosporium* sp. and *Fusarium* sp. The fungi species were similarly identified by Adebayo-Tayo *et al.* (2012), Iniekong *et al.* (2015) and Onuorah *et al.* (2016) who isolated similar fungal groups from carrots and other vegetables sold in the market. Many of these fungi isolates linked vegetables and fruits have shown to cause spoilage. These included *Fusarium* sp., *Aspergillus* sp., and *Cladosporium* sp. (Harding *et al.*, 2017).

Usually, spoilage fungi are also known to be toxigenic or pathogenic and they have been reportedly isolated from vegetables or fruits (Al-Hindi *et al.*, 2011). At the time of storage and refrigeration, certain moulds may harbour mycotoxins which are injurious to human and animal health. Fungi pathogens could also cause allergies. *Aspergillus niger*(40%), which had the highest percentage occurrence in this study are notable producers of different toxic metabolites, like naphthopyrones and malformins (Al-Hindi *et al.*, 2011). Ochratoxins which is also produced by *Aspergillus niger*, is a mycotoxin which causes hazard to man and other animals health.

Moreover, the harvesting stage of carrot from soil can attract spoilage pathogens. According to Oji (2016) vegetables sold in markets can cause food poisoning which is dangerous to consumer's health. The risk factors associated with carrots globally is the risk of food poisoning and infections. This might be due to improper handling and washing before consumption as reported by Bishop and Okwori (2017). Contamination could also be connected to the supply stage, conditions of storage, distribution, marketing stage and transportation (Akinmusire, 2011; Akintobi*et al.*, 2011).

CONCLUSION

This study revealed that carrot has a plethora of bacteria and fungi which cause spoilage and are also pathogenic to human health. There is, therefore, need to ensure that care is taken in handling, washing and processing carrots before consumption so as to prevent food spoilage that might lead to infections and food-borne diseases caused by fungi and bacteria. It is also expedient to control food spoilage microorganism in order to reduce economic loss due to food spoilage.

REFERENCES

Adebayo-Tayo, B.C., Odu, N., Esen, C.U. and Okonko, T.O. (2012). Microorganisms associated with spoilage of stored vegetables in Uyo metropolis, AkwaIbom State, Nigeria. *Nature and Science*, **10**(3): 23-32

Akinmusire, O.O. (2011). Fungal species associated with the spoilage of some edible fruits in Maiduguri Northern Eastern Nigeria. *Advances in Environmental Biology*, **5**(1):157-161. 3.

Akintobi, A.O., Okonko, I.O., Akano, O.R., Agubiade, S.O. and Onianwa, O. (2011). Isolation and identification of fungi associated with the spoilage of some selected fruits in Ibadan, South Western Nigeria. *Academia Arena*, **3**(11): 1-10 4.

Akinyele, B. J. and Akinkunmi, C. O. (2012). Fungi associated with the spoilage of berry and their reaction to magnetic field. *Journal of Yeast and Fungal Research*, **3** (4):49-57.

Al-Hindi, R.R., Al-Najada, A.R. and Mohamed, S.A. (2011). Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. *African Journal of Microbiology Research*, **5**(4): 443-448.

Barth, M., Hankinson, T.R., Zhuang, H. and Breidt F. (2009). Microbiological spoilage of fruits and vegetables. In: Compendium of the microbiological spoilage of foods and beverages. Springer, New York, NY, Pp 135-183.

Bishop, H.G. and Okwori, G.O. (2017). *Escherichia coli* and *Staphylococcus aureus* contaminations of carrots sold within Zaria, Nigeria and their antibiotic susceptibility profiles. *Open Access Journal Science*, **1**(4):110–113.

Chessbrough, M. (2004).*District laboratory practice in Tropical countries*. Cambridge University Press, London, Pp 112-115.

Ehimemen, N. H., Mukhtar, M. F. and Salisu, N. (2019). Prevalence of Bacterial Loads on some Fruits and Vegetables Sold in Kaduna Central Market, North western Nigeria. *Journal of Applied Sciences*, **19**(1), 20-24.

Erickson, M.C. (2010). Microbial risks associated with cabbage, carrots, celery, onions, and Deli salads made with these produce items. *Comprehensive Reviews in Food Science and Food Safety*, **9**(6):602–619.

Hammad, A.A., AbdEl-Khalek, H.A., Youssef, K.A. and Abd El-Kader, M.R. (2013). Microbiological, nutritional and sensorial changes in fresh carrot juice preserved by irradiation. *Food Science and Quality Management*, **11** (2):61-69

Harding, M.W., Butler, N., Dmytriw, W., Rajput, S., Burke, D.A. and Howard, R.J. (2017). Characterization of microorganisms from fresh produce in Alberta, Canada reveals novel food-spoilage fungi. *Research Journal of Microbiology,* **12** (1): 20-32.

Heaton, J.C. and Jones, K. (2008). Microbial contamination of fruit and vegetables and the behavior of enter pathogens in the phyllosphere: a review. *Journal of Applied Microbiol*ogy, **104**(3):613–626.

Iniekong, P.U., Eleazar, C.I. and Ohanu, M.E. (2015). Studies on fungi responsible for the spoilage and deterioration of some edible fruits and vegetables. *Advances in Microbiology*, **5** (4):55947

Jay JM. 1992. Bioassay and Related Methods. In: Modern food microbiology. Springer Netherlands, pp 166-184.

Kaur, A., Sood, A., Kaur, S. and Bhowate, P. (2017). Bacterial population associated with fruits and vegetables and its treatment using antimicrobial rinsing. *International Journal of Current Microbiology Applied* Science, **6**(12): 2099-2107.

Kim, S.J., Cho, A.R. and Han J. (2013). Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. *Food Control*, **29** (1): 112-120.

Ksouri, A., Dob, T., Belkebir, A., Krimat, S. and Chelghoum, C. (2015). Chemical composition and antioxidant activity of the essential oil and the methanol extract of Algerian wild carrot *Daucuscarota* L. sspcarota (L.). *Thell. Journal of Mater Environmental Science*, **6**(3): 784-791.

Kumar, V. (2012). Incidence of *Salmonella* sp. and *Listeria monocytogenes* in some salad vegetables which are eaten raw: A study of Dhanbad city, India. *International Journal of Engineer Science Research*, **2** (10): 2277-2685.

Mritunjay, S.K., Kumar, V. (2015). Fresh farm produce as a source of pathogens: A review. *Research Journal of Environmental Toxicology*, **9** (2): 59-70.

Mukherjee, A., Speh, D., Jones, A.T., Buesing, K.M. and Diez-Gonzalez, F.(2006). Longitudinal microbiological survey of fresh produce grown by farmers in the upper Midwest. *Journal of Food Protection*, **69**: 1928–1936.

Nwajinka, C.O. and Okonjo, E.O. (2019). Modeling the carrot slices (*Daucus carota* L.) drying characteristics using microwave oven. *CIGR Journal*, **21**(4): 231–235

Law-Ogbomo, K.E., Osaigbovo, A.U. and Omokaro, K.E. (2018). Growth and yield responses of carrot (Daucus carota L.) to different levels of oil palm refuse bunch ash in an ultisols environment. *Journal of Agriculture and Environment***14** (1): 67-74

Oji, P.C. (2016). Bacteriological analysis of salad vegetable in Eke Awka Market, Anambra State, Nigeria. *International Journal of Scientific and Research Publications*, **6** (6): 2250-3153

Oranusi, S. and Olorunfemi, O.J. (2011). Microbiological safety evaluation of street vended ready-to-eat fruits sold in Ota, Ogun state, Nigeria. *International Journal of Research Biological Science*, **1** (3): 27-32.

Oranusi, S., Braide, W. and Nwankwo, O.E. (2012). Microbial and geohelminth population in different parts of carrot (*Daucus carota* L.). *Current Trends in Microbiology*, **8** (1): 21-27

OwaisY., Iftikhar A., Mohd I. B. and Sushree T. (2018). Assessment of microbial spoilage and techniques to avert the deterioration in fruits and vegetables. *International Journal of Chemical Studies*; **6**(3): 2230-2235

Samson, R.A. and Varga J. (2007). Aspergillus systematics in the genomic era. CBS Fungal Biodiversity Centre, Utrecht, p. 206.

Uzeh, R.E., Alade, F.A. and Bankole, M. (2009). The Bacterial quality of pre-packed mixed vegetable in some retail out lets in Lagos, Nigeria. *African Journal of Food Science*, **3** (9) :270-272.

Velusamy, V., Arshak,, K., Korostynska, O., Oliwa, K. and Adley, C.(2010). An overview of foodborne pathogen detection: In the perspective of biosensors. *Biotechnology Advance*, **28**: 232-254.