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Escherichia coli and Staphylococcus aureus contaminations of carrots sold within Zaria, Nigeria and their antibiotic susceptibility profiles

Abstract

Carrots (*Daucus carota*) are root vegetable containing carotene, vitamins and mineral salts. Carrots maybe purple, red, white or yellow in color depending on their varieties and can be eaten fresh, mixed in salad or cooked. Carrots are prone to faecal contamination and often subjected to poor/rough handling in Nigeria. Upon purchase, many people consume them without proper washing. Hence, instead of serving nutritive purposes, they may turn to be a source of food poisoning/infections. *Escherichia coli* have caused a number of diarrheal diseases and *Staphylococcus aureus* is an important agent of food poisoning. Forty (40) fresh carrot samples were randomly purchased from different vendors in Zaria metropolis. Then, 10g of epidermal (layer) scrapings of each sample was aseptically collected, homogenized in 90ml of buffered peptone water and serially diluted. From 10^{-4} dilution, 0.1ml was spread-plated on EMB and MSA plates, followed by 24hrs incubation at 37°C. Characteristic colonies of *E. Coli* and *S. Aureus* were Gram-stained and biochemically identified. A susceptibility testing of the isolates to some selected antibiotics was performed using disc diffusion method. Twenty (20) out of the 40 carrot samples were positive for *E. Coli* (50.0%), whereas *S. Aureus* were isolated from 26 samples (65.0%) with a total mean staphylococcal count of 4.3×10^5 CFU/g. All the *Escherichia coli* isolates were susceptible to Streptomycin (30µg) and Tarivid (10µg), but Septrin (30µg) and Amoxicillin (30µg) had the least activities against them. *Staphylococcus aureus* isolates were most susceptible to Ciprofloxacin (10µg), followed by Gentamycin (10µg) and Streptomycin (30µg), but resistant to Ampiclox (30µg) and Amoxicillin (30µg).

Keywords: *Escherichia coli, Staphylococcus aureus*, susceptibility, antibiotic, carrot, contamination, zaria, nigeria

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Abbreviations: CMKT, community market; CLSI, clinical and laboratory standards institute; CFU/g, colony forming unit per gram: EMB, eosin methylene blue; G, gram; MSA, mannitol salt agar; µg, microgram; MLS, milliliters; MHA, muller-hinton agar; PZ, pz area; SBMKT, sabon-market

Introduction

Carrots are one of the most commonly used vegetables with nutritive value of vitamins (A, B, C, D, K) and mineral (calcium, potassium, phosphorus, sodium, iron). They contain carotenoids and antioxidants, which protect humans from cancers, strokes and cardiovascular diseases.[1] They contain about 88% water, 7% sugar, 0.2% fat, 1% each of protein, fiber and ash[3] and no starch.[4] All vegetables are soft and prone to nematodal, insect, bacterial, fungal or chemical attack. Unsuitable temperature, contaminated materials and poor sanitation enhance their deterioration.[5] Microbial contaminations of vegetables could result from soil, water, air, insects, animals or human contact. Bacterial attacks can occur during early growth to maturity on the field, distribution and sale or storage at home.[1,2] Faecal contamination of vegetables with *Escherichia coli* (*E. Coli*) and *Salmonella* spp had been reported[6] mainly due to contaminated irrigation water.[7] *E. Coli* causes diarrheal diseases via production of enterotoxin or cytotoxin, or plasmid-mediated virulence factors.[8] *Staphylococcus aureus* (*S. Aureus*) causes food poisoning due to contamination of vegetable during handling or from utensils.[5] Post-harvest contamination of vegetable from faeces, human handling, harvesting tools, transport vessels, wild and domestic animals, insects, dust, wash water, ice, transport vehicles, and processing tools must be checked.[9] Since vegetables are increasingly been linked to food borne outbreaks,[7] this research aimed to identify contaminations of carrots by *E. Coli* and *S. Aureus*, and challenge them with antibiotics, as they are capable of causing diarrhoea/food poisoning after ingestion.

Materials and methods

Study area

The study was conducted in Zaria. Four sampling locations where carrots were mostly sold were randomly selected, which included Samaru Market, PZ Area, A.B.U. Community Market and Sabon-Gari Market.

Collection and processing of carrot samples

In all, 40 fresh carrot samples were collected by randomly purchasing 10 samples from different vendors in each of the four sampling locations in Zaria. The samples were placed in clean separate polythene bags and transferred immediately in cool containers to the laboratory. All the carrots samples were analyzed within 1-2hours after the sampling. Sterilized blades were used to aseptically scrape 10g of the epidermal layer (i.e. phyllosphere) from each carrot sample. Then 10g of each carrot scrapings was added to 90ml of buffered peptone water and homogenized. Serial dilutions were made to obtain 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilutions.

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Cultural and biochemical isolation of *E. Coli* and *S. Aureus*

From the 10^{-4} dilution, 0.1ml each was spread-plated unto Eosin-Methylene Blue (EMB) and Mannitol Salt Agar (MSA) plates and incubated at 37°C for 24hrs. Characteristic colonies were purified and Gram-stained. Staphylococcal count (of colony forming units, i.e. CFU/g) was determined from the MSA plates. The pure cultures were stored on Nutrient Agar at 4°C for further biochemical tests. Suspected *E. Coli* were biochemically confirmed using indole, Methy red, Voges Proskauer, and citrate utilization tests. But mannitol fermentation, catalase and coagulase tests were used to confirm *S. Aureus* as suggested by Cheesbrough.[10]

Antibiotic Susceptibility testing of isolates

The isolates were challenged with some panels of selected antibiotics to determine their susceptibility patterns using agar disc diffusion method on Muller-Hinton Agar (MHA). The inocula were prepared by turbidity standard of 0.5 McFarland.[10] Susceptibility patterns were determined using the Clinical laboratory standards.[11]

Results

Out of 40 carrot samples, 26 were contaminated with *S. Aureus*, with a prevalence of 65.0%. The occurrence of *S. Aureus* was higher, where 20 of the carrot samples were contaminated giving a prevalence of 50.0%. There was a co-contamination of 32.5%, where each carrot sample was contaminated with the two pathogens at the same time (Table 1). Based on sampling locations, the highest carrot contamination with *S. Aureus* occurred on samples obtained from PZ area (80.0%) followed by those from Sabon-Gari Market. Whereas, *E. Coli* had 70% occurrence on carrots obtained from Sabon-Gari Market and Samaru Market each (Table 2). The highest staphylococcal count of carrot samples occurred on samples obtained from Sabon-Gari and Samaru Markets, but the overall mean occurrence in Zaria, Nigeria was 4.3×10^5 CFU/g. Even though carrots samples from Samaru Market had the least prevalence of *S. Aureus* contamination (Table 1), they rather had a higher staphylococcal count in Table 2. Generally, the areas with highest prevalence of these pathogens did not coincide with those of highest staphylococcal counts on the carrots (Table 2) (Table 3).

Table 1 Prevalence of *E. Coli* and *S. Aureus* and their co-contaminations on carrots sold in Zaria, Nigeria

Pathogen	Number (%) positive	Number (%) negative
E. coli	20(50.0)	20(50.0)
S. aureus	26(65.0)	14(35.0)
Co-contaminations	13(32.5)	27(67.5)

Table 2 Occurrences of *E. Coli* and *S. Aureus* based on sampling locations in Zaria, Nigeria

Sampling location	Number of samples examined	Number (%) containing *E. coli*	Number (%) cont$aining *S. aureus*
SMKT	10	7(70)	5(50)
PZ	10	2(20)	8(80)
CMKT	10	4(40)	6(60)
SBMKT	10	7(70)	7(70)
Total	40	20(50.0)	26(65.0)

Table 3 *Staphylococcal* count of epidermal scrapings of carrot samples sold in Zaria, Nigeria

Dilution factor	Sample location	No. tested	Range Of staphylococcal count(Cfu/G)	Mean staphylococcal count (Cfu/G)
	SMKT	10	2.0×10^5-7.0×10^5	4.8×10^5
	PZ	10	2.0×10^5-6.0×10^5	3.9×10^5
104	CMKT	10	1.3×10^5-9.0×10^5	3.3×10^5
	SBMKT	10	3.0×10^5-8.0×10^5	4.9×10^5
Total	-	40	-	4.3×10^5

SMKT, Samaru Market; CMKT, A.B.U. Community Market; SBMKT, Sabon-Market; PZ , PZ area

From a panel of 10antibiotics of which 20 isolates of *E. Coli* were challenged with, 100% susceptibility to Streptomycin (30μg) and Tarvid (10μg) was observed. Also, 90% susceptibility to Chloramphenicol (30μg) and Gentamycin (10μg) were recorded. On the other hand, *E. Coli* had the highest resistance to Augmentin (30μg) and Amoxicillin (30μg) (Table 4). Similarly, 26 isolates of *S. Aureus* were challenged with a panel of 10 antibiotics. The highest susceptibility was 92.3% to Ciprofloxacin (30μg), following by 88.5% to Gentamycin (10μg). However, the highest resistance of *S. Aureus* was 84.6% to both Amoxicillin (30μg) and Ampiclox (30μg) as indicated in (Table 5).

Table 4 Antibiotic susceptibility patterns for *Escherichia coli* isolates from carrot samples sold in Zaria, Nigeria

Antibiotics/ Disc potency (µg)	Number(%) susceptible	Number(%) intermediate	Number (%) resistant
Augmentin (30)	7(35.0)	3(15.0)	10(50.0)
Ciprofloxacin (10)	15(75.0)	3(15.0)	2(10.0)
Amoxicillin (30)	8(40.0)	2(10.0)	10(50.0)
Chloramphenicol (30)	18(90.0)	1(5.0)	1(5.0)
Streptomycin (30)	20(100.0)	0(0.0)	0(0.0)
Pefloxacin (30)	13(65.0)	5(25.0)	2(10.0)
Septrin (30)	7(35.0)	6(30.0)	7(35.0)
Gentamycin (10)	18(90.0)	1(5.0)	1(5.0)
Tarivid (10)	20(100.0)	0(0.0)	0(0.0)
Sparfloxacin (10)	16(80)	2(10)	2(10)

n=20 isolates

Table 5 Antibiotic susceptibility patterns of *S. Aureus* isolates from carrot samples sold in Zaria, Nigeria

Antibiotics/ Disc potency (µg)	Number(%) susceptible	Number(%) intermediate	Number (%) resistant
Pefloxacin (10)	21(80.8)	4(15.4)	1(3.8)
Streptomycin (30)	22(84.6)	4(15.4)	0(0.0)
Erythromycin (10)	16(61.5)	3(11.5)	7(26.9)
Zinnacef (20)	14(53.8)	2(7.7)	10(38.5)
Septrin (30)	13(50.0)	2(7.7)	11(42.3)
Gentamycin (10)	23(88.5)	0(0.0)	3(11.5)
Amoxicillin (30)	4(15.4)	0(0.0)	22(84.6)
Rocephin (25)	21(80.8)	2(7.7)	3(11.5)
Ciprofloxacin (10)	24(92.3)	1(3.8)	1(3.8)
Ampiclox (30)	2(7.7)	2(7.7)	22(84.6)

n=26 isolates

Discussion

Though there are a lot of health benefits from vegetable consumption and promoting increased demands on these produce,[12] they are also not without consequences if hygienic practices are not observed. The major threats carrots pose on consumers all over the world (and Nigeria in particular) is the risk of infections/food poisoning. Some of these consumers do little or no further washing/ processing of purchased carrots before consumption; hence, they stand at increased risks of infections.[12] Heaton and Jones have emphasized on potential risks of infections and outbreaks following the consumption of vegetables. *E. Coli* can be transmitted to humans via foods and vegetables contaminated with animal faeces. Such commonly implicated vegetable include carrots, lettuce, radish sprout, alfalfa sprout, potatoes, spinach, green onions, cabbage, pepper, cucumber and so on.[13] Of the two pathogens examined, *S. Aureus* contamination of carrots occurred higher than *E. Coli*. Though *S. Aureus* is a normal flora of the skin and nares, it can also cause a number of infections. Human contact and carriers of *S. Aureus* help

in its transfer unto vegetables, especially those vegetables hawked or exposed along roadsides. Outbreaks caused by *Staphylococcus* have implicated vegetables like carrots, alfalfa sprouts, lettuce, parsley, radish and onion sprouts as vehicles.[9] Carrots ready-for-consumption in this part of the world are majorly exposed along roadsides or hawked while exposed in streets or markets. Buyers touch the carrots severely to select their choices. This behaviour increases the transfer of *S. Aureus* from contaminated hands unto the carrots sold.

More so, the consumption of vegetables like carrots is linked with increased risks of enteropathogenic infections[12] by *E. Coli, S. Aureus* and *Salmonella enterica* with consequent foodborne outbreaks due to them.[15] The occurrences of *E. Coli* especially *E. coli* O157:H7[13,14] and *S. Aureus*[16] have been reported in ready-to-eat raw salad. There is an inadequate awareness of the plant-microbe interactions, epiphytic and immigrant micro-organism on leaves and phyllosphere of vegetables[12] which is prompting the continuous spread of pathogens to humans. Many sources of contamination of vegetables include soil, faeces, water for irrigation, dust, insects, inadequately composted manure,

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wild or domestic animal wastes, and human handling.[9] The use of inadequately treated water for irrigation purpose in carrot cultivation could increase the spread of *E. Coli* and other entero-pathogens.[12] Such water is obtained from shallow streams exposed to refuse-dumping and human/animal wastes. Other irrigation farms use untreated night soil as manure or organic fertilizer,[17] wastes from abattoirs.[18] Sewage and surface run-offs[12] can contaminate fresh vegetables. The presence of infections or outbreaks following consumption of vegetables like carrots can affect the demand of such produce because consumers are rational in their choices. This will also affect the income of local farmers and the economy at large. It is important to adequately wash carrots with clean water and peel off the epidermal layers before consumption. This practice will help to remove pathogenic organisms that have adhered to the carrots. Safe and treated water should be advocated for vegetable irrigation farming. The unhygienic hawking of carrots or roadside selling should be disallowed and regulations should be enforced on hygienic processing selling/distribution of carrots in the country.[19,20]

Conclusion

Carrots are widely and increasing being consumed in all parts of the world because they constitute an important source of nutrients. However, they are prone to so many contamination routes: from the cultivation farms, through the chains of distribution, to the final consumers. In Nigeria, carrots are often sold along roadsides or hawked in markets or streets. Some sellers used the same wash water repeatedly for the carrots sold, which are also exposed to other airborne contaminations. Bacterial contamination was evident by the presence of *E. Coli* and *S. Aureus* isolated from the epidermal (phyllosphere) scrapping of sampled carrots in Zaria, Nigeria. The prevalence of *E. Coli* was 50.0%, but *S. Aureus* occurred higher with a prevalence of 65.0% and a mean staphylococcal count of 4.3×10^5 CFU/g. These pathogens can cause infections/food poisoning when contaminated carrots are consumed. After subjecting the bacterial isolates to some selected antibiotics, *E. Coli* were most susceptible to Streptomycin (30μg) and Tarvid (10μg), Chloramphenicol (30μg) and Gentamycin (10μg). On the other hand, the *E. Coli* had the highest resistance to Augmentin (30μg) and Amoxicillin (30μg). Also, the *S. Aureus* isolates were most susceptible to Ciprofloxacin (30μg), and Gentamycin (10μg), but mostly resistant to Amoxicillin (30μg) and Ampiclox (30μg). Hygienic practices should be observed in the cultivation and distribution of carrots. It is equally necessary to adequately wash carrots with clean water, peel off the epidermal layer and rinse again before consumption. Government can discourage the unhygienic processing/hawking of carrots and other vegetables along roadsides and streets in Nigerian through firm regulations.

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Conflict of interest

The authors declare that there is no any financial or conflict of interest.

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