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Microbial Quality of Some Vegetables Sold in ED DueimTwon, Sudan

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Abstract: This study was probably the first research carried out to investigate the microbiological quality of some vegetables sold in ED DueimTwon, Sudan. Four species of vegetables were used, Arugula (*Eruca sativa*), Mloukhia (*Corchorus olitorius*), Tomato (*Lycopersicon esculentum*) and Green pepper (*Capsicum annuum*). The samples were collected and examined according to standardized methods for total viable bacteria, coliforms and fecal coliform count. The average of total viable count ranged from 1.2×105-5.6×105 CFU mL^{-1} for Arugula; 2.1×105-2.8×107 CFU mL^{-1} for Mloukhia; 3.4×105-4.8×105 for Tomato and 2.3×105-8.0×106 CFU mL^{-1} for Green pepper. However, the maximum level of total and fecal coliform were (93, 21); (28, 11); (75, 15) and (150, 20) MPN 100 mL^{-1}, respectively. Twelve bacteria belonging to five genera were isolated. *Staphylococcus* (33%) was the most predominant isolated followed by *Enterobacteriaceae* (25%), *Bacillus* (17%) and *Streptococcus* (17%). *Micrococcus* (8%) was the least dominant isolated. The results of microbial counts of these vegetable samples in this study indicate that, the agricultural practices, harvesting, hygiene, transporting and selling points are poor and therefore, the higher microbial load could be risked for public health.

Key words: ED Dueim, microbiological quality, vegetables, mloukhia

INTRODUCTION

Vegetables are one of an important part of a balanced diet. Recently, the consumption of fruit and vegetable have been increased significantly, because are dietary sources of nutrients, micronutrients, vitamins and fiber for humans and are thus vital for health and well-being (Eni *et al*., 2010). In addition, therefore, the nutrition policies have strongly promoted the consumption of a diet containing more than 400 g/day of fresh vegetables and fruits as a nutritional goal for health promotion (FAO/WHO, 2004). However, the health of consumers can be adversely affected by consumption of microbiologically unsafe vegetables. Microbiological contamination of vegetables can occur directly or indirectly through contact with soil, dust and water and during cultivation, harvesting, packaging, storage, transporting and marketing (Eni *et al*., 2010; De Giusti *et al*., 2010; FAO/WHO, 2008). In ED Dueim there is limited information available regarding the microbiological quality of vegetables. There is no baseline microbiological data for these vegetables sold in this Town. The objectives of this study were to obtain baseline information on the microbial quality of the most abundant vegetables sold in ED Dueim market to establish baseline data for more research in the future.

MATERIALS AND METHODS

Sample collection: A total of Twenty-five samples of fresh vegetables comprising four types {(Arugula (*Eruca sativa*), Mloukhia (*Corchorus olitorius*), Tomato (*Lycopersicon esculentum*) and Green pepper (*Capsicum annuum*)} were collected from the local main market. The samples were collected in sterile plastic bags and transported to the laboratory for analysis.

Microbial load determination

Total viable, total coliform and fecal coliform counts: All the samples were rinsed thoroughly with distilled water, 10-fold serial dilutions of each rinse water were made and one mL of 10^{-2}, 10^{-4}, 10^{-5}; 10^{-6} dilutions were pipette into sterile Petri-dishes and molten plate count agar (45°C) was added and mixed through and through to allow the distribution. The plates were allowed to solidify, inverted and incubated at 37°C for 24 h for colony formation (Harrigan, 1998). The colonies were counted using a colony counter (Stuart Scientific, UK) after 24 h incubation at 37°C. The total coliform and faecal coliform counts were determined by (MPN) the Most Probable Number (APHA, 1995).

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Isolation of microorganisms: Plat count agar, Nutrient Agar and Peptone Water (Oxoid) were prepared according to Manufacturer's instruction and sterilized by autoclaving at 121°C for 15 min. The predominant bacterial colonies were isolated from plate count agar by pour plate method according to Harrigan (1998); these isolates were purified by streaking twice on nutrient agar and stored in a refrigerator, then further the identification done through Biochemical tests (Cowan *et al*., 1993).

RESULTS

All the vegetables sampled in this study were microbial contaminated. However, the total viable count of vegetables varied with type of samples (Table 1). Microbial count ranged from 1.2×10^5-5.6×10^5 CFU mL^{-1} for Arugula; 2.1×10^5-2.8×10^7 CFU mL^{-1} for Mloukhia; 3.4×10^5-4.8×10^5 for Tomato and 2.3×10^6 -8.0×10^6 CFU mL^{-1} for Green pepper. Table 1 also shows the total coliform and fecal coliform of these samples; the maximum levels for Arugula were (93, 21) MPN 100 mL^{-1}; (28, 11) MPN 100 mL^{-1} for Mloukhia; (75, 15) MPN 100 mL^{-1} for Tomato and (150, 20) MPN 100 mL^{-1} for Green pepper, respectively.

Organisms isolated from vegetable samples: According to the cultural, morphological and biochemical characteristics of the organisms isolated, a five bacterial genera was isolated (*Staphylococcus*, *Enterobacteriaceae*, *Bacillus*, *Streptococcus* and *Micrococcus*) (Table 2). A total of twelve bacteria isolates were identified to their genera as shown in Table 3. Staphylococcus (33%) was the most frequently isolated followed by *Enterobacteriaceae* (25%), *Bacillus* (17%) and *Streptococcus* (17%). *Micrococcus* (8%) was the least frequently isolated. It was appeared at different frequencies in the vegetable samples (Fig. 1). Some of them were appeared more than once and others appeared at one time. *Staphylococcus* genus was present in all leafy vegetables sampled and just in Green pepper in fruity vegetables while *Enterobacteriaceae* was found in all fruity vegetables sampled and only in Mloukhia in leafy

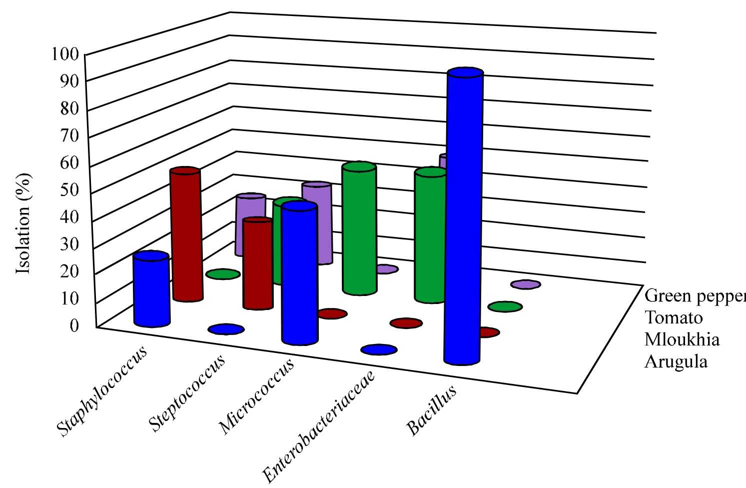

Fig. 1: Frequency isolation (%) of bacterial genera from different vegetables samples in ED Dueim town

Table 1: Total viable counts (TVCs), Total coliform (TC) and faecal coliform (FC) counts of fresh vegetables

Vegetable samples	TVCs (CFU mL^{-1})	TCMPN 100 (mL^{-1}) (Max.)	FC MPN 100 (mL^{-1}) (Max.)
Eruca sativa (Arugula)	1.2×10^5-5.6×10^5	93	21
Corchorus olitorius (Mloukhia)	2.1×10^5-2.8×10^7	28	11
Lycopersicon esculentum (Tomato)	3.4×10^5-4.8×10^5	75	15
Capsicum annuum (Green pepper)	2.3×10^5-8.0×10^6	150	20

Table 2: Biochemical identification tests

Unknown isolates	Gram stain test	Shape	Spore forming ability	Motility test	Aerobic growth	Catalase test	Oxidase test	O/F test	Acid from gluc. test	Genus
1	+	Cocci	-	-	+	+	-	F	+	*Staphylococcus*
2	+	Cocci	-	-	+	-	-	F	+	*Staphylococcus*
3	-	Rode	-	+	+	+	-	F	+	*Enterobacteriaceae*
4	+	Rode	+	+	+	+	-	F	+	*Bacillus*
5	+	Cocci	-	-	+	-	-	F	+	*Streptococcus*
6	+	Cocci	-	-	+	+	-	O	+	*Micrococcus*

Table 3: Occurrence of bacterial genera isolated from vegetables sampled in ED Dueim town

Bacterial genera isolates	Total No. of isolates	% of individual genera to the total No. of isolates	Leafy vegetables		Fruity vegetables	
			Arugula	Mloukhia	Tomato	Green pepper
Staphylococcus	4	33	+	+	-	+
Enterobacteriaceae	3	25	-	+	+	+
Bacillus	2	17	+	-	+	-
Streptococcus	2	17	-	-	+	+
Micrococcus	1	8	+	-	-	-
Total	12	100%	3	3	3	3

vegetables sampled. However, *Bacillus* genus was isolated from Arugula in leafy vegetables and from Tomato in fruity vegetables sampled. *Streptococcus* was present in fruity vegetable sampled but not in any of the leafy vegetables while the *Micrococcus* was isolated from leafy vegetables from Arugula samples.

DISCUSSION

Previously, there was no available information on the microbial quality and the prevalence of a microorganism in fresh vegetables sold in ED Dueim Town, Sudan. This study provided baseline information on the microbial load and occurrence levels of a microorganism in selected vegetables present to consumers from public markets. The results obtained in this study shown that bacterial, coliform and fecal coliform counts observed from vegetables (Table 1) are recorded higher counts. The total viable count was ranged from 1.2×10^5 to 2.8×10^7 CFU mL^{-1}. This high load cfu mL^{-1} of viable counts of vegetables could be attributed to the unhygienic practices right from the farm to the market and exposed to potential microbial contamination at every step, including cultivation, harvesting, transporting, packaging, storage and selling to the final consumers. Generally, there is no pre-treatment given to fresh produce before transporting to market and hence number of contaminants is so large. Similar findings were observed from various studies in different countries (Titarmare *et al*., 2009; Eni *et al*., 2010; Abdullahi and Abdulkareem, 2010). They have been reported total viable count of fruits and vegetables collected from varied retail markets were ranged from 1.3×10^6-1.82×10^7 CFU mL^{-1}, 1.3×10^6-3.0×10^7 and from 3.0×10^5 to 5.7×10^8, respectively. Bacterial isolated from selected vegetables in this study are belonged to five genera identified (Table 2). *Staphylococcus* (33%) was the most frequently isolated followed by *Enterobacteriaceae* (25%), *Bacillus* (17%), *Streptococcus* (17%) and *Micrococcus* (8%). Bacteria of belonging to the same genera were also isolated and identified by other researchers from fruits and vegetables in different countries (Osamwonyi *et al*., 2013; Eni *et al*., 2010; Rajvanshi, 2010; Tambekar and Mundhada, 2006; Uzeh *et al*., 2009; Adebolu and Ifesan, 2001). The microorganisms present in fruits and vegetables are a direct reflection of the sanitary quality of the cultivation water, harvesting, transportation, storage and processing of the produce (Beuchat, 1996; Ray and Bhunia, 2007). Some of the bacteria isolated in this study may be part of the natural flora of the fruits and vegetables or contaminants from soil, irrigation water and the environment during transportation, washing/rinsing water or handling by processors (Ofor *et al*., 2009). *Bacillus* sp. are part of the natural flora and are among the most common vegetable spoilage bacteria (Vanderzant and Spittstoesser, 1992) and the presence of Bacillus in the vegetables may be said to be due to environmental factors? The survival of *Bacillus* depends on several factors such as nature of the organism, resistance to a new physical environment and ability to form spores (Godon, 1977). The dominance of Staphylococcus among the bacterial genera identified from the vegetables was an indication of poor hygienic practices by both the farmers and sellers.

Coliforms and fecal coliform are usually indicators whose presence will normally indicate the probable presence of pathogenic organisms. There is the high count of coliforms and fecal coliform in these vegetables (Table 1) which could be attributed to the use of animal feces as fertilizer for the vegetables in the respective farms and gardens, many possible sources for raw vegetables to become contaminated with coliforms and fecal coliform, including soil, water and the environment. *Escherichia coli* is a fecal organism which forms part of the normal intestinal flora of the digestive system in humans and a wide variety of animals and is commonly found in animal manures (Desmarchelier and Fegan, 2003). Its presence in vegetables indicates the possibility of fecal contamination from manures and inferior post-harvest washing by processors to remove soil and debris. Or it may be due to cross contamination by food handlers through poor hand washing, or contamination of utensils and preparation surfaces. *E. coli* is not normally pathogenic to humans; its presence indicates that fecal contamination is occurring at some point during the production process and other pathogenic micro-organisms associated with fecal contamination may be present. From the results obtained, it was obvious that eating these vegetables can expose the consumer to many risks. Therefore, it is recommended that these vegetables be thoroughly washed before consumption, especially where they are not going to be cooked before consumption.

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