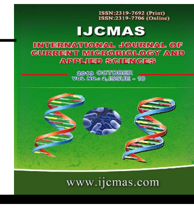

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Original Research Article

Microbiological analysis and antimicrobial sensitivity pattern of microorganisms isolated from vegetables sold in Akure, Nigeria.

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A B S T R A C T

Fresh vegetables is a good source of various components of food that enhances a healthy living, but evidence shows that they promote the growth of microbes which also deteriorate the food and subsequently causes adverse effect on the health of consumers. Microbial investigation was conducted on two different vegetable samples; *Senecio biafrae* (worowo) and *Amaranthus cruentus* (African spinach) collected from three different locations in Akure, Nigeria. The bacterial count ranged from 2.57 x 10^5 to 7.6 x 10^5 cfu/ml for *Senecio biafrae* and 1.0 x 10^5 to 4.3 x 10^5 cfu/ml for *Amaranthus cruentus* samples while the fungal count ranged from 3.0 x 10^2 to 5.0 x 10^2 sfu/ml for Senecio biafrae and 2.52 x 10^2 to 7.4 x 10^2 sfu/ml for *Amaranthus cruentus* samples. Eight bacteria belonging to different genera, namely: *Staphylococcus aureus, Bacillus sp, Escherichia coli, Pseudomonas aeruginosa, Enterobacter aerogenes, Serratia marcescens, Salmonella sp* and *Citrobacter sp* were identified while six fungi, namely: *Aspergillus niger, Thysanophoralongspora sp, Penicilum notatum, Neurospora crassa, Aspergillus fumigatus* and *Trichoderma sp* were also identified and isolated. Ciprofloxacin and augmentin exerted a pronounced inhibitory effect on all the bacterial isolates while nystatin and mycoten also inhibited all the fungal isolates with considerable zones of inhibition. All fungal isolates were resistant to griseofulvin with ketoconazole showing the lowest inhibitory zone (2.0 mm) on *Aspergillus niger*. Therefore, consumer's consciousness on the type and load of pathogens associated with vegetables and antibiotics of choice as a therapeutic measure against the infection caused by the implicated microorganisms needs to be re-awakened.

Keywords

Vegetables, zone of inhibition; pathogens; antibiotics and therapy.

Introduction

Vegetables are the fresh and edible portions of herbaceous plants, which can be eaten raw or cooked (Dhellot *et al.*, 2006). Green leafy vegetables are valuable sources of nutrients for growth in man and animal especially in rural areas where they Contributes substantially to protein, minerals, vitamins, fibers and other nutrients which are usually in short supply in daily diets (Mohammed and Sharif, 2011).

Problems linked with pathogens in fresh produce, including the associated public health and trade implications, have been reported in a number of countries worldwide(CAC,2006). The inner tissues of healthy plants and animals are free of microorganisms, however, the surfaces of raw vegetables and meats are contaminated with a variety of microorganisms and this depends on the microbial population of the environment from which the food was taken, the condition of the raw product, the method of handling, the time and conditions of storage (Pelczar *et al.,* 2006). Contamination may also occur during post-harvest handling, including at points of preparation by street vendors, in food-service establishments, home and also with viruses or parasites can result from contact with faeces, sewage and irrigation water (Cliver, 1997; Speer, 1997).

Senecio biafrae (local name "worowo") belong to the group of vegetables that grow in large quantity as undercover in tree crop plantation, this leafy vegetable is also considered for its high medicinal value as the juice extracted from the leaves are wholly applied to fresh wounds or cuts as styptic in the rural community for man and animal use (Viana *et al.*, 2003; Okpara *et al*., 2006). *Amaranthus cruentus* L. belongs to the Amaranthaceae family and is an erect herb with oblong green leaf; it is widely distributed throughout Africa with the young leaves, growth points and whole seedlings harvested and cooked for use as vegetable (Iheanacho and Udebuani, 2009).

Most of the reported outbreaks of gastrointestinal disease linked to the fresh produce have been associated with microbial contamination, particularly with members of the *Enterobacteriaceae* family (Hamilton *et al.*, 2006; Tyler and Triplett, 2008). This research will serve as a guide to the microflora and the drug of choice that can be used in treating infections that can arise from the consumption of such vegetables.

Materials and Methods

Source of samples

Samples were collected from three different locations, a local market in Akure (oja-oba), vegetable vendors at the Federal University of Technology, Akure, Nigeria (FUTA) environs and from farmers at ilara-mokin town in Akure, Ondo State, Nigeria.

Collection and processing of Samples

Six samples, three each of *Senecio biafrae* and *Amaranthus cruentus* were collected from each location and packed into sterile plastic containers, transported to the laboratory and processed immediately to prevent deterioration. From each vegetable sample, 25 g was aseptically weighed and 150 mls of sterile distilled water was added and blended using Nakai Japan Magic blender, (model 462). The blender compartment was flooded with boiled water after each blending and allowed to cool before loading the next vegetables, according to the method of Sarkono *et al*. (2010).

Isolation and Enumeration of microorganisms

This was carried out according to the pour plate method of isolation as described by Uzeh *et al*. (2009).

Antibiotics sensitivity test

The disc diffusion method as described as Khan *et al*. (2002) was used to determine the antibacterial activities of standard or commercially produced antibiotics against the test isolates, while the Kirby-Bauer method as described by Willey *et al.* (2008) was used to determine the sensitivity pattern of the fungal isolates to Griseofulvin (500mg), Ketoconzole (200mg), Mycoten (200mg) and Nystatin (500mg).

Results and Discussion

The high microbial load obtained in urban farm could be directly linked to the recorded a greater waste water used in irrigation that could be from sewage water for watering the field or the use of manure used for fertilization and the unhygienic condition of the area where the vegetables were being grown. The result correspond to the findings of Beuchat (1997) who reported that the presence of many pathogens in the soil was thought to be from historical application or environmental presence of feaces or untreated sewage and pathogens existing in the soil or water can be the source of both pre- and post-harvest contamination respectively. The slight variation in the microbial load from other sources can be traced to the prewashing with 'refreshing water'.

Among the organisms encountered during this study *Staphylococcus aureus* showed the highest occurrence in all the three locations. It is an opportunistic pathogen and enterotoxigenic strains which are known to cause serious food borne disease and has been reported that ingestion of the thermostable enterotoxins, rather than the bacterium itself is responsible for foodborne illness (Mead *et al.,* 1999). Common symptoms of staphylococcal intoxication include nausea, vomiting, retching, abdominal cramping , sweating, chills, prostration , weak pulse, shock, shallow, respiration, and subnormal body temperature. Recovery from this intoxication (which is rarely fatal) usually occurs uneventfully within 24- 48hrs. *S aureus* is commonly found in the nose and throat and on the hair and skin of more than 50% of healthy individual any food like vegetables that are frequently handled may therefore easily become contaminated. Staphylococcal foodborne illness may occur from minor skin infections, such as pimples to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS) (Bowersox, 1999). Other organisms encountered during the study and their respective health implications include *Bacillus subtilis* causing both emetic (Melling *et al.,*1976) and diarrheal (Granum, 1997). The occurrence of *Bacillus subtilis* in vegetables agrees with the findings Mead *et al.,* (1999) who reported that estimated 27,000 cases of foodborne illness are due to *Bacillus species*. The occurrence of *E.coli* in the vegetables may also be linked to animal dung manure is used ion cultivatin the vegetables. The difficulties in the treatment of food and water associated gastrointestinal diseases due to *E. coli* have been reported coupled with their resistivity to antibiotics (Patoli *et al.,* 2010). Other organisms encountered include *S.typhii* has been reported to be responsible for typhoid fever (Nester *et al.,* 2004). *Pseudomonas aeruginosa* is a prominent inhabitant of soil and organism is responsible for diseases of vegetables like angular leaf spot of many vegetables it has become an important cause of infection and It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs), and bacteremia (Aloush *et al.,* 2006). The presence of*, penicillium notatum,*

Table.1 Percentage occurrence of organisms associated with the vegetables in each location

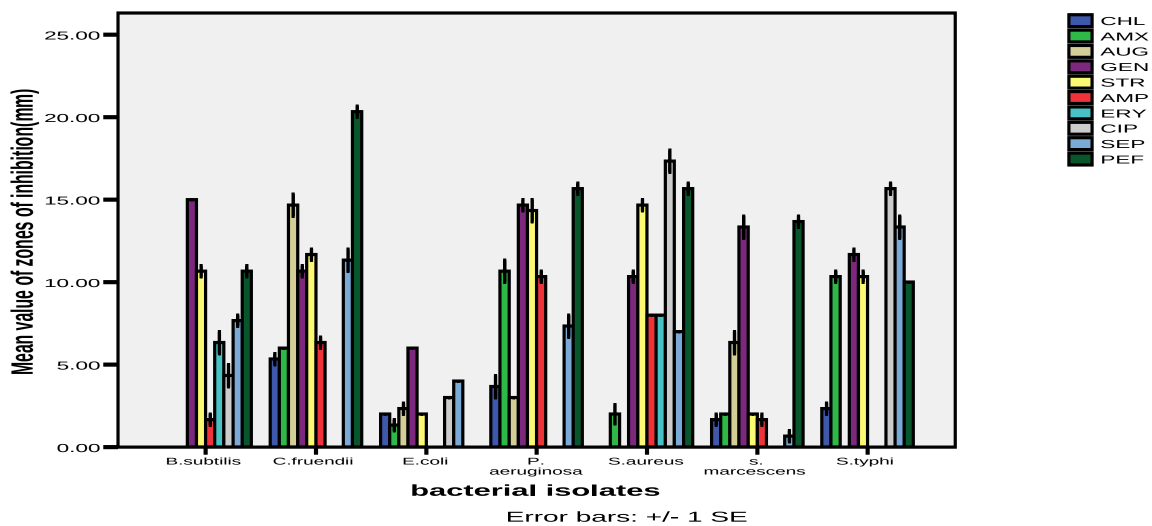
Organisms	***Senecio biafrae***			***Amaranthus cruentus***		
	Location A % occurrence	Location B % occurrence	Location C % occurrence	Location A % occurrence	Location B % occurrence	Location C % occurrence
Bacteria						
Staphylococcus aureus	47.0	33.3	70.0	50.0	33.3	52.6
Salmonella typhi	23.5	15.5	0.0	25.0	12.5	10.5
Citrobacter fruedii	29.5	0.0	20.0	0.0	0.0	0.0
Escherichia coli	0.00	16.7	0.0	15.0	16.7	26.3
Bacillus subtilis	0.00	12.5	10.0	20.0	16.7	10.5
Pseudomonas aeruginosa	0.00	20.8	0.0	0.0	12.5	0.0
Serratia marcescens	0.00	16.7	0.0	0.0	8.3	0.0
Fungi						
Neurospora crassa	33.3	0.00	0.0	0.0	0.0	0.0
Aspergillus niger	22.2	57.1	0.0	0.0	33.3	0.0
Aspergillus fumigatus	44.4	0.0	0.0	66.7	16.7	0.0
Penicillium notatum	0.00	0.0	0.0	33.3	0.0	0.0
Trichoderma viridae	0.00	42.9	100.0	0.0	0.0	100.0
Thysanophora longispora	0.00	0.00	0.0	0.0	50.0	0.0

Key: Location A: FUTA environs, Location B: Ilara-mokin Farm, Location C: Oja-Oba Market

Table. 2 Microbal Load of Fresh Vegetable Samples

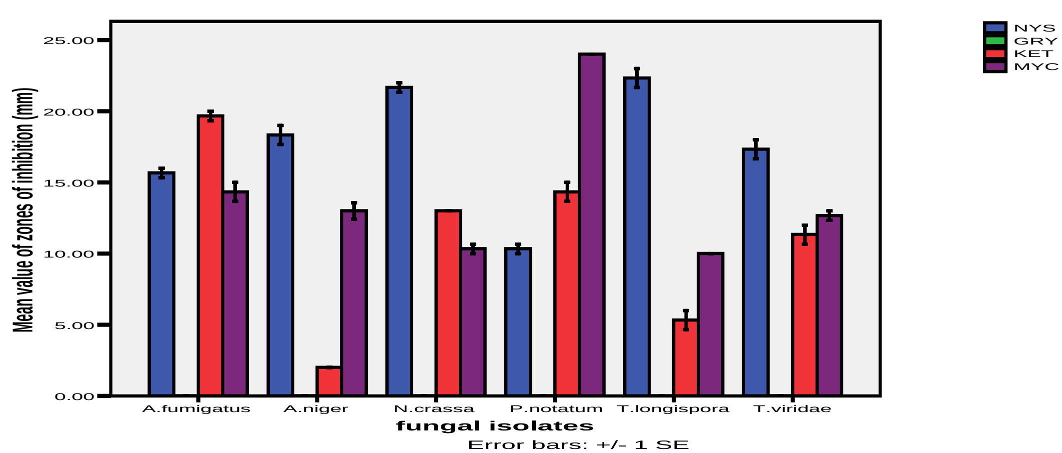
Locations	Sample	Bacteria Count (CFU/ml)	Fungal Count (CFU/ml)
FUTA GATE	WOROWO	2.57×10^5	$3.0x10^2$
	AFRICAN SPINACH	1.02×10^5	$2.52x10^2$
FARM AT ILARA	WOROWO	7.6×10^5	$5.0 x10^2$
	AFRICAN SPINACH	$4.3x 10^5$	7.4×10^2
OJA OBA MARKET	WOROWO	5.08×10^5	$4.30 x10^2$
	AFRICAN SPINACH	$2.04x10^5$	$4.05 x10^2$

Figure. 1 Antibiotics sensitivity pattern of bacterial isolates



CHL – chloramphenicol, AMX – Amoxacillin, AUG – Augmentin, GEN – Gentamycin, STR – Streptomycin, AMP – Ampicillin, ERY – Erythromycin, SEP – Septrin, PEF – Pefloxacin

Figure.2 Antibiotic sensitivity pattern of fungal isolates



NYS – Nystatin, GRY – Griseofulvin, KET – Ketoconazole, MYC – Mycoten

Aspergillus niger and *Aspergillus fumigates, T. longispora, T. viridae* could be due to the fact that these organisms are spore formers and are known as common environmental contaminants; nevertheless, they have been implicated as food borne pathogens (Peraica and Domijan,2001; Aboloma,2008; Oluwafemi, and Simisaye, 2005; Katherine *et al.*,2006). This study reveals these antibiotics can be used in treating the food born ilnesses associated with the isolated test organisms.

Infections caused by resistant pathogens result in significant morbidity and mortality, and contribute to escalating healthcare costs worldwide. Despite the availability of newer antibiotics, emerging antimicrobial resistance has become an increasing problem in many pathogens throughout the world (Keith and John, 2005). For instance, *S. aureus* exhibits remarkable versatility in their behaviour towards antibiotics and its capacity to produce human diseases had not diminished even with the introduction of antibiotics (Obiazi *et al.,* 2007). Although, outbreaks of *S. aureus* resistant to beta-lactam antibiotics have been frequently associated with devastating foodborne infections.

Buhlmann *et al.,* 2008. The variation in the susceptibility of these organisms to antibiotics may be connected to their previous exposure to the antibiotics and thereby varying the degree of resistance in addition to this the gram reaction of the organisms also influences their susceptibility to the antibiotics used. *E.coli* a gram negative organism gave a zone of inhibition of 6mm while *S.aureus* a gram positive organism gave a zone of inhibition of 10mm upon exposure to gentamycin. This finding may be due to the presence of peptidoglycan in the cell wall of gram negative organisms which may prevent the penetration of any dilluent (antibiotics) into the cell. The high performance some of these antibiotics can also be due to their molecular sizes a factor which enhances their solubility in diluents thus promoting their penetration power through cell wall into the cytoplasm of the target microorganism. This finding is in line with Maillard (2002) and Poole (2002) who respectively opined that the high efficacy of antibiotics may be traced to their molecular sizes.

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