Original Research Article

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

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Abstract

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 contribute 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 feces, 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 under cover 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) (Bowerson, 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

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

**Key:** Location A: FUTA environs, Location B: Ilara-mokin Farm, Location C: Oja-Oba Market
Table 2 Microbial Load of Fresh Vegetable Samples

<table>
<thead>
<tr>
<th>Locations</th>
<th>Sample</th>
<th>Bacteria Count (CFU/ml)</th>
<th>Fungal Count (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUTA GATE</td>
<td>WOROWO</td>
<td>2.57 x 10^7</td>
<td>3.0 x 10^2</td>
</tr>
<tr>
<td></td>
<td>AFRICAN SPINACH</td>
<td>1.02 x 10^5</td>
<td>2.52 x 10^2</td>
</tr>
<tr>
<td>FARM AT ILARA</td>
<td>WOROWO</td>
<td>7.6 x 10^5</td>
<td>5.0 x 10^2</td>
</tr>
<tr>
<td></td>
<td>AFRICAN SPINACH</td>
<td>4.3 x 10^5</td>
<td>7.4 x 10^2</td>
</tr>
<tr>
<td>OJA OBA MARKET</td>
<td>WOROWO</td>
<td>5.08 x 10^5</td>
<td>4.30 x 10^2</td>
</tr>
<tr>
<td></td>
<td>AFRICAN SPINACH</td>
<td>2.04 x 10^5</td>
<td>4.05 x 10^2</td>
</tr>
</tbody>
</table>

Figure 1 Antibiotics sensitivity pattern of bacterial isolates

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 illnesses 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.

References


CAC (Codex Alimentarius Commission)., 2006. Report of the 38th Session of the Codex Committee on Food Hygiene.


