RESEARCH ARTICLE

BACTERIAL CONTAMINATION OF SOME VEGETABLES SOLD IN MAJOR MARKETS IN ADO-EKITI, NIGERIA.

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Manuscript Info

**Abstract**
Consumption of vegetables contaminated with pathogens is a common source of infections. This study investigated bacterial contamination of vegetables sold in major markets in Ado-Ekiti, Nigeria. Sixty samples comprising of 20 samples each of *Brassica oleracea*, *Cochorus olitorius*, and *Amaranthus hybridus* were examined. Bacteriological procedures were followed in the isolation and identification of bacteria from culture media. Antimicrobial susceptibility of the isolates was done using the disk diffusion method. Sixty six bacteria were isolated from 60 vegetable samples. Of these isolates, *Salmonella* spp recorded 43.3%, followed by *Citrobacter freundii* 18.3%, *Klebsiella* spp 15.0%, *Enterobacter* spp 11.7%, *Proteus* spp and *Alcaligenes* spp 5.0% each, *Escherichia coli* and *Providencia* spp 3.3% each and *Vibrio* spp 1.7%. The prevalence of the isolates on 20 samples of *Brassica oleracea* decreased in the order of *Salmonella* species 55.0%, *Citrobacter* species 20.0%, *Alcaligenes* species 10%, *Pseudomonas aeruginosa*, *Enterococcus* species, *Escherichia coli*, *Proteus mirabilis* and *Providencia* species 5% each. The most frequent bacteria isolated on 20 samples of *Cochorus olitorius* was *Salmonella* species 50.0%, followed by *Citrobacter* species 15.0%. *Vibrio* species and *Alcaligenes* species recorded 5.0% each. *Enterobacter* species recorded the highest frequency 30.0% on the 20 samples of *Amaranthus hybridus*, followed by *Salmonella* species and *Klebsiella* species 25.0% each, *Citrobacter* species 20.0%, *Pseudomonas aeruginosa* and *Escherichia coli* 5.0% each. All isolates were sensitive to ofloxacin and ciprofloxacin but resistant to augmentin, ampicillin and cefuroxime. It is essential to control the sources of vegetable contamination to minimize risk of infections especially in vegetables consumed raw.

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Introduction:-
Vegetables are leafy outgrowth or plant parts used as food (Yusuf et al., 2004). They contain carbohydrates, proteins, vitamins, minerals as well as trace elements (Farooq et al., 2008; Gharavi et al., 2002 and Itanna, 2002) necessary for nourishment and protection of the body. Vegetables are grown throughout the year relying on rain water in raining season and use of irrigation during dry season. Vegetable consumption has been associated with the decline of hazards of specific diseases (Hung et al., 2004). However, they are often contaminated with enteric microorganisms the incidence of which reflects the sanitary quality of the processing steps of food production as well as the microbial flora of the raw product at the time of processing (Nguyen et al., 1994). Vegetable pollution in agricultural land has been regarded as the major source of infections in humans.

Pre-harvest activities such as cultivation, manuring and irrigation; and post-harvest handling of vegetables such as storage, transportation, food processing for consumption render the produce to microbial contaminations (Erkan and Vural, 2008). As an end result, use of waste water polluted with human and animal feces to wash vegetables or irrigate vegetable garden without proper disinfection has been considered responsible for high rates of vegetable contamination (Erkan and Vural, 2008). Pathogens on the soil may contaminate crops directly when torrential rainfall causes splashes of soil on the leaves of vegetables (Heaton et al., 2008).

Organic fertilizers enrich the soil and cause luxuriant growth of vegetables. However, sewage sludge, animal manures and slurries, and abattoir wastes use to fertilize farmland equally contaminate land with pathogens directly (Natvig et al., 2002; Avery et al., 2005). These processes eventually lead to pathogen entering into the food chain (Machado et al., 2006).

Food-poisoning outbreaks have been traced to the consumption of contaminated vegetables due to unhygienic handling of the farm produce (Brackett et al., 1999). Infections from vegetables are almost inevitable when such produce are eating raw without further processing (Brandl et al., 2006).

Salmonella is among the most important foodborne pathogens all over the world contaminating fresh vegetables. Salmonellosis can cause gastroenteritis, bacteremia, typhoid fever and focal infections (Darwin and Miller, 1999). In Nigeria, morbidity associated with illness due to Salmonella is on the increase and has resulted to death in some cases (Adahara et al., 2012; Ibekwe et al., 2008). The prevalence of typhoid fever was found to be relatively high (42 %) in Ado-Ekiti (Oluyege et al., 2015).

Prevalence of enteric fever and bacteria enteritis has continued to increase rapidly in Ekiti State (Ajibade, 2012; David et al., 2015). The carriage rate of the Salmonella typhi among undergraduate students of Ekiti State University, Ado-Ekiti was 33.86% (David et al., 2015; Ajibade, 2012). The researchers reported clinical evidences of high rate of Salmonella infection among the undergraduate students (58.90%). This study evaluated bacterial contamination of Brassica oleracea (Cabbage), Amaranthus hybridus (African spinach/green) and Cochrorus olitorius (Jute leaves) commonly consumed in Ekiti State.

In Nigeria, Brassica oleracea is called “Efo Oyinbo” or “Eso Kabeeji” in Yoruba, “Kabeji” in Igbo and Hausa. Amaranthus hybridus is called “Efo-tete” in Yoruba, “Inine” in Igbo and “Allayahu” in Hausa. Cochrorus olitorius (Jute leaves/saluyot leaves and Jews mallow) is called “Ewedu” in Yoruba, “Kerenkere” in Igbo and “Ayoyo” in Hausa.

Materials and methods:-
Study area
Ado Ekiti is a city in the Southwestern part of Nigeria. It is the State Capital and Headquarters of Ekiti State. Ado-Ekiti is located between latitude 7° 34’ and 7° 44’ north of the equator and longitude 5° 11’ and 5° 18’ east of the Greenwich Meridian on total land area of 36.7 Km² (Olusegun, 2013). Its population is 424,340 (WPR, 2018).

Sample size
A total of Sixty (60) vegetable samples were used in the study. This comprised of ten (10) collections of each of the three vegetables in each of the two selected markets. The vegetables under study were: Brassica oleracea (Cabbage), Amaranthus hybridus (African spinach) and Cochrorus olitorius (Jute leaf) identified at the Science Department of Afe Babalola University, Ado-Ekiti. The markets visited were “Oja Oba” market and “Bisi” market.
Sample collection
Jute leaves were collected at 8.45 a.m., African spinach were collected at 9.22 a.m. while Cabbage were collected at 10.00 a.m. Each of the samples was collected into a sterile polythene bag and transported immediately to the laboratory for analysis.

Sample processing
Each sample was swab with a sterile swab sticks and inoculated aseptically into nutrient broth and incubated for 24 hours at 37°C. Another swab collection was used to inoculate selenite F broth and incubated for 48 hours at 37°C. After 24 hours, the inoculated nutrient broths were subcultured on MacConkey agar while inoculated Selenite F broths were subcultured on deoxycholate agar (DCA). The plates were incubated at 37°C for 24 hours (Cheesebrough, 2006). All isolates were characterized using standard microbiology and biochemical tests as described by Barrow and Feltam (2004); Garrity et al (2005) and Cheesebrough (2006).

Antimicrobial assay
Antibiotics sensitivity test was done using the disk diffusion method (Cheesbrough, 2006). Overnight broth cultures of isolates were adjusted to match that of 0.5 McFarland standards by diluting with distilled water or incubating further. Sterile swab stick was used to spread the inoculums evenly on Muller Hinton agar. Antibiotic disc (Rapid laboratories) of gentamicin, ampicillin, augmentin/clavulanate, ofloxacin, ceftaxidime, cefuroxime, and ciprofloxacin were placed aseptically on the agar and incubated for 24 hours at 37°C (Cheesbrough, 2006). Zone of inhibition was measured in duplicates and sensitivity was determined by comparing the mean inhibition diameter with that of standard antibiotics sensitivity table (Clinical and Laboratory Standards Institute (CLSI), 2003).

Statistical analysis
Data generated in this study were subjected to standard statistical test using statistical package for social science (SPSS) version 20. The frequencies of bacteria were recorded in percentages, mean, and standard deviations.

Results:-
A total of 66 different organisms were isolated from the 60 vegetables (Table 1). Of the 60 vegetables examined, the most common bacteria isolated was Salmonella species 26 (43.3%), followed by Citrobacter species 11 (18.3%), Klebsiella species 9 (15.0%), Enterobacter species 7 (11.7%), Alkaligenes species and Proteus species 3 (5.0%) each, Pseudomonas aeruginosa and Escherichia coli recorded 2 (3.3%) each. The least frequent bacterium was Vibro species 1 (1.7%).

Eight (8) different bacteria were identified on Cabbage and on African spinach and 4 bacteria on Jute leaves. The frequencies of the bacteria isolates on 20 samples of Cabbage decreased in the order of Salmonella species 11 (55.0%), Citrobacter species 4 (20.0%), Alkaligenes species 2 (10%), Pseudomonas aeruginosa, Enterococcus species, Escherichia coli, Proteus mirabilis and Providentia species 1 (5%) each. Enterobacter species recorded the highest frequency 6 (30.0%) on the 20 samples of African spinach, followed by Salmonella species and Klebsiella species 5 (25.0%) each, Citrobacter species 4 (20.0%), Pseudomonas aeruginosa and Escherichia coli 1 (5.0%) each. Isolates recovered on 20 samples of Jute leaves decreased in order Salmonella species 10 (50.0%), by Citrobacter species 3 (15.0%) and Vibro species and Alkaligenes species 1 (5.0%) each.

Of the 60 vegetables examined, Salmonella typhimurium recorded the highest frequency 20 (33.3%), occurring virtually in all the vegetables (Table 2). Salmonella typhi and Salmonella paratyphi A each recorded prevalence of 1 (1.7%) while other Salmonella recorded 4 (6.7%).

Out of the 20 samples of cabbage, 11 Salmonella species were isolated (Table 2) with Salmonella typhimurium recording a prevalence of 9 (45.0%) while other salmonella recorded 2 (10%). Five (5) Salmonella species were isolated from the 20 samples of African spinach (Table 2). Salmonella typhimurium recorded the highest frequency of 3 (15.0%) while S. paratyphi A and other salmonella recorded 1 (5.0%) each. Of the 20 samples of Jute leaf, S. typhimurium had the highest frequency of 8 (40.0%), Salmonella typhi recorded frequency of 1 (5%) while 1 (5.0%) was recorded against other salmonella.

Enterobacter species recorded sensitivity to gentamicin, ofloxacin, and ciprofloxacin and resistance to ampicillin, augmentin/clavulanate, ceftaxidime and cefuroxime (Table 3). Citrobacter species recorded sensitivity to only ofloxacin and ceftaxidime. Providentia species equally recorded sensitivity to ofloxacin, ceftaxidime and
ciprofloxacin. *Klebsiella species* and *Pseudomonas aeruginosa* were sensitive to only ofloxacin and ciprofloxacin. *Escherichia coli* recorded sensitivity against gentamicin, ofloxacin, ceftaxidime and ciprofloxacin (Table 3). *Vibrio species* recorded sensitivity against gentamicin, ofloxacin, ceftaxidime and ciprofloxacin (Table 4). *Proteus species*, *Alkaligenes species* and other salmonellae recorded gross resistance against the tested antibiotics; showing sensitivity against only ofloxacin and ciprofloxacin (Table 4). While *Salmonella typhimurium* recorded sensitivity to only ciprofloxacin, *Salmonella typhi* and *Salmonella paratyphi* A recorded sensitivity to ofloxacin, ceftaxidime and ciprofloxacin and resistance to all other tested antibiotics.

**Table 1**: The frequency of various organisms isolated from the vegetables.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Vegetables</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cabbage (% n = 20)</td>
<td>Jute leaf (% n = 20)</td>
</tr>
<tr>
<td><em>Salmonella spp</em></td>
<td>11(55.0)</td>
<td>10(50.0)</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>4(20.0)</td>
<td>3(15.0)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1(5.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td><em>Klebsiella spp</em></td>
<td>4(20.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td><em>Vibrio spp</em></td>
<td>0(0.0)</td>
<td>1(5.0)</td>
</tr>
<tr>
<td><em>Enterobacter spp</em></td>
<td>1(5.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td><em>Alcaligenes species</em></td>
<td>2(10.0)</td>
<td>1(5.0)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>1(5.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td><em>Proteus spp</em></td>
<td>1(5.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td><em>Providencia spp</em></td>
<td>1(5.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>26 (130.0)</td>
<td>15 (75.0)</td>
</tr>
</tbody>
</table>

**Table 2**: The occurrence of *Salmonella species* on the vegetables under study.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Cabbage (% n = 20)</th>
<th>African spinach (% n = 20)</th>
<th>Jute-leaf (% n = 20)</th>
<th>Total N = 60</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella typhi</em></td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1(5.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>9 (45.0)</td>
<td>3 (15.0)</td>
<td>8 (40.0)</td>
<td>20 (33.3%)</td>
</tr>
<tr>
<td><em>Salmonella paratyphi A</em></td>
<td>0 (0.0)</td>
<td>1 (5.0)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Other Salmonella</td>
<td>2 (10.0)</td>
<td>1(5.0)</td>
<td>1(5.0)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>11 (55.0%)</td>
<td>5 (25.0%)</td>
<td>10 (50.0%)</td>
<td>26 (43.3%)</td>
</tr>
</tbody>
</table>

**Table 3**: Mean zone of inhibition and sensitivity status of *Enterobacter species*, *Citrobacter species*, *Providentia species*, *Klebsiella species*, *Pseudomonas aeruginosa* and *Escherichia coli* against tested antibiotics.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Mean inhibition diameter (±SE) of bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Enterobacter species</em></td>
</tr>
<tr>
<td></td>
<td>Zone of inhibition</td>
</tr>
<tr>
<td>GEN(5µg)</td>
<td>15.3±1.04</td>
</tr>
<tr>
<td>AMP(10µg)</td>
<td>0</td>
</tr>
<tr>
<td>AUG(30µg)</td>
<td>3.0±0.02</td>
</tr>
<tr>
<td>OFL(5µg)</td>
<td>23.0±1.13</td>
</tr>
<tr>
<td>CAZ(30µg)</td>
<td>14.0±0.81</td>
</tr>
<tr>
<td>CRX(30µg)</td>
<td>8.9±0.51</td>
</tr>
<tr>
<td>CPR (5µg)</td>
<td>21.1±1.24</td>
</tr>
<tr>
<td><em>Klebsiella species</em></td>
<td>9.7±0.73</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1.2±0.01</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>2.4±0.03</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>18.4±1.06</td>
</tr>
</tbody>
</table>
A bacterial study on vegetables sold in Ado-Ekiti, Nigeria, revealed the presence of various bacteria including E. coli, S. aureus, and Shigella spp. The study was conducted to assess the risk of foodborne infections associated with the consumption of vegetables irrigated with clean disinfected water. The researchers isolated a total of 66 bacteria isolates belonging to 10 genera, categorized into three species: Vibrio species, Proteus species, and Alcaligenes species, along with Salmonella typhimurium, Salmonella paratyphi A, and other Salmonella species.

The study employed a protocol for cleaning and disinfection of vegetables, where the vegetables were soaked in a disinfectant solution and rinsed in clean water before consumption. This process aimed to reduce contamination and prevent the spread of foodborne diseases.

Discussion:

Sixty-six (66) bacteria isolates belonging to ten bacteria genera were recorded from 60 different collections of vegetables in the present study. Oluyege and Famurewa (2015) isolated similar bacteria from cooked foods sold in eateries in Ado-Ekiti, Nigeria. These bacteria included enteric bacteria such as E. coli, S. aureus, and Shigella spp., which are of concern due to their potential pathogenicity.

Bacterial contamination of vegetables in the markets could have been from the farmland, transportation from the farm to the markets or handling by the marketers. Consumption of cabbage, Jute leaves, and African spinach sold in the major markets in Ado-Ekiti, Nigeria, may be associated with infections unless the preparation before consumption is done under hygienic conditions. Heat treatment of African spinach is usually minimal because of the need to preserve vitamins which may not withstand too much heat. Such vegetable foods may pose health hazards to the consumers. Of greater concern is cabbage often consumed raw in salad. If cleaning process do not sufficiently decontaminate the vegetable, food poisoning becomes inevitable. It is advocated that vegetables be rinsed in clean water, soaked in a disinfectant solution and afterwards rinsed in clean water before consumption (Rosas et al., 1984). This process will reduce contaminating microorganisms to the barest minimum that may not cause infection. The risk of infection to consumers of vegetables that are irrigated with clean disinfected water may be reduced to the minimum. The high technology tertiary treatments and disinfection systems (Hamilton et al., 2006) to achieve such water may not be readily available to farmers; neither the clean water either. This is a challenge in developing countries. Greater effort is therefore needed to prepare vegetables in a most hygienic manner before consumption for food safety purpose.
The high recorded prevalence of typhoid and bacteria enteritis in Ado-Ekiti (Ajibade, 2012; David et al., 2015) might not be unconnected with presence of Salmonella species on vegetables. The present study appears to support it as the organisms were isolated on all the vegetables investigated.

Organisms isolated in this study were enteric bacteria. This is either a reflection of the sanitary situation in the land or use of contaminated water for irrigation of farmland or vegetable garden (Barber et al., 2007). It is therefore important to control the sources of vegetable contamination.

Decrease in risk for human illness associated with raw vegetables can be further achieved through controlling points of potential contamination such as during harvesting; during processing or distribution; or in retail markets (Beuchat et al., 1997). To reduce the microbiological contamination of vegetables after harvest, proper washing of vegetables with clean water is essential (Eni et al., 2010). Water supplemented with varying concentrations of organic acids, such as citric and, acetic acids (vinegar), has been shown to reduce microbial populations on fruits and vegetables (Eni et al., 2010). After harvest, vegetables should be transported to the market in proper containers (Rosas et al., 1984) in sanitized vehicles allowing adequate air circulation (Brackett et al., 1999).

Most of the isolates recorded gross resistance against the tested antibiotics. Infections caused by the isolates may prove difficult to manage if antibiotic sensitivity testing is ignored. Virtually all the isolates were inhibited by ofloxacin and ciprofloxacin and 100% resistant to ampicillin, augmentin/clavulanate and cefuroxime is recorded. This agreed with the findings of Oluyege and Famurewa (2015) on enteric pathogens isolated from cooked foods sold in eateries in Ado-Ekiti. Salmonella typhi in the present study was resistant to ceftaxidine and cefuroxime. This result is in agreement with the findings of David et al (2015) who recorded resistance of Salmonella typhi isolated from the oropharynx of some students of Ekiti State University against the antibiotics.

Sufficient heat treatment of vegetables may be needed in preparation of vegetables for consumption to prevent enteric bacterial infection. Vegetables eaten raw such as Cabbage may pose threats to the consumers because of contamination with bacteria.

In conclusion, the general populace may need to be informed of the health risks associated with the consumption of contaminated vegetables. The consumers should always observe the basic principle of food and personal hygiene in handling of vegetables. As vegetables are not usually exposed to sufficient heat to avoid loss of contain nutrients, the nutritional benefits may need to be balanced with the risk of enteric bacterial infection especially in vegetables consumed raw and in places where control of contamination of vegetables is a challenge.

Conflict of interest:
Not declared.

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References:
2. CLSI document M100-S23 (M02-A11) (2003): Disc diffusion supplemental tables. Performance standards for antimicrobial susceptibility testing. The complete standard may be obtained from the Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19807