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# Effect of increasing concentration of antimicrobial agent on microbial load and antibiotic sensitivity pattern of bacterial isolates from vegetables

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# ABSTRACT

Research has shown fresh vegetables to promote good health as well asharbour a wide range of microbial contaminants. The study assesses the effect of increasing concentration of antimicrobial agent (vinegar solution) on the microbial load as well as the Antibiotic Sensitivity Pattern of bacterial isolates on vegetables sold in the Cape Coast Municipality, Ghana. Ten different vegetables were sampled of which 10g of a batch each was washed with 50ml concentration each of vinegar solution of 10%, 20% and 30%. Serial dilution and aerobic colony counting was performed by pour plating on PCA (Plate Count Agar) for each sample and concentration. Isolates were identified by standard biochemical methods whilst the disc diffusion technique was applied in Antibiotic Susceptibility Testing of each bacterial isolate. The mean microbial load ranged from highest of  $2.47 \times 10^8 CFU/ml$  using 10% vinegar to the least  $1.45 \times 10^7 CFU/ml$ washing with 30% vinegar solution. Total microbial counts significantly decreased (P<0.001) with increasing vinegar concentration and in comparison with control (distilled water washing  $3.26 \times 10^{10}$ ). Eight different bacteria species were isolated of which B. cereus (36.25%), was the highest whilst Micrococcus spp., P.aeruginosaandKlebsiellaspp. were the least (2.5%). Proteus spp. and L. monocytogenes were highly resistant (75%) whilst the least resistant organism was Micrococcus spp. (25%). Gentamicin and Amikacin where the most effective (100%) antibioticswhilst Ampicillin was the least effective (12.5%). Increased vinegar concentration has the tendency to reduce microbial loads on vegetables and thus its application is recommended.

Keywords: Antibiotics, Bacterial, Contamination, Resistant, Vegetables, Vinegar.

#### **INTRODUCTION**

Due to increase demand for vegetables and fruits world-wide, developing countries are making substantial gains in their economies trading in these products whilst health awareness and minimal processing has also resulted in increased consumption of these produce in these same countries [1,2].Results from the Global Burden of Disease Project showed that up to 2.7 million deaths worldwide, and 1.8% of the total global disease burden may be attributed to inadequate levels of fruit and vegetable consumption [3]. Vegetables contain all the essential nutrients that can result in the growth of microbes. Although their outer barrier usually prevents contamination [4] their surfaces are usually contaminated based on microbial population of the environment from which they were cultivated[5] as well as microbial physiological and enzymatic activities [6,7]. All over the world, public health agencies are concerned with food safety assurance due to globalization of food markets, growing demand for minimally processed ready-to-eat (RTE) foods and increasing numbers of meals served outside home [8]. Fresh vegetables are subjected to mild treatments and are often stored under conditions that may favor the growth of diverse spoilage and pathogenic microorganisms, such as Listeria monocytogenes[9]. The incidence of foodborne outbreaks caused by contaminated fresh vegetableshas increased in recent years [10]. CDC estimates that each year roughly 1 in 6 Americans (or 48 million people) gets sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases. The 2011 estimates provide the most accurate picture yet of which foodborne bacteria, viruses, microbes ("pathogens") are causing the most illnesses in the United States, as well as estimating the number of foodborne illnesses without a known cause[11]. A novel strain of Escherichia coli O104:H4 bacteria caused a serious outbreak of foodborne illnessfocused in northern Germany in May through June 2011[12]. In the EU/EEA, 885 Haemolytic Uremic Syndrome cases, including 31 deaths, and 3 170 non-Haemolytic Uremic Syndrome cases, including 17 deaths were reported as at the peak of infection [13].

In Ghana, just as in several African countries, overhead irrigation of vegetables with polluted water is very common putting consumers of these irrigated crops at risk, especially those eaten uncooked. Recent outbreaks such as happen in Germany call for an increase awareness of the potential harmful effect of contaminated vegetables but most importantly a means of making them safe for consumption. Thus this study is aimed at determining the effect of increasing concentration ofvinegar (an antibacterial) on microbial loads as well as determining the Antibiotic Susceptibility of isolated bacteria from these vegetables.

#### MATERIALS AND METHODS

**Study Area and Design**: The study was conducted in three major local markets namely, Abura, Kotokraba and the University of Cape Coast Market, all located in the Cape Coast metropolis. The random sampling method was used to purchase vegetables from sellers within the markets from September, 2010 to April, 2011.

**Sampling:** The study sampled ten vegetables i.e. cabbage (*Brassica oleraceaL.*), carrot (*DaucuscarotaL.*), cucumber (*CucumissativusL.*), French beans (*Phaseolus vulgaris*), green pepper (*Capsicum annuumL.*), onions (*Allium cepa*), spring onions (*Allium fistulosumL.*) red pepper (*Capsicum annuum*), lettuce (*Lactuca sativa L.*) and tomato (*Lycopersiconesculentum*).

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These were collected under normal purchasing conditions, from randomly selected sellers. A minimum of two composite sample of each vegetable were collected aseptically in a sterilized container and sent to the laboratory immediately and analyzed. The elapsed time between sample collection and analysis did not exceed 10hours. Sample collection was undertaken in intervals of three weeks for three replicates.

#### Laboratory Methods and Procedures

All laboratory work was undertaken in the Laboratories of the Department of Laboratory Technology of the University of Cape Coast, Cape Coast, Ghana.

**Sample Preparation:** 10g of each vegetable was aseptically weighed and thoroughly washed with 50ml of sterile distilled water. Three other 10g weight of each vegetable were weighed and washed with 10%, 20% and 30% vinegar solutions separately. 10ml of the washed solutions were then inoculated into peptone water and incubated for a period of 16-18hrs at  $37^{\circ}$ C.

**Quantification of Bacteria:** Serial dilutions from the resulting growth from the peptone water medium were pour-plated on Plate Count Agar (PCA) and incubated for 24hrs at 37°C under aerobic condition. The number of estimated Colony Forming Units (CFU) for each sample was then counted using the Quebec colony counter (Reichert, USA).

**Isolation of Organisms:** All pure isolated colonies were sub-cultured onto blood agar plates (for growth of heterotrophic bacteria) and MacConkey agar plates (for coliforms) for 24hrs at 37°C for colony isolation and morphological identification.

**Identification of Organisms:** Pure isolated colonies were Gram differentiated and then biochemically identified using Indole, Catalase, Citrate, Oxidase, Coagulase, and Urease tests.

Antibiotic Susceptibility Test (AST): Antibiotic susceptibility were determined by agar diffusion technique on Mueller-Hinton agar (Kirby-Bauer NCCLS modified disc diffusion technique) using 8 antibiotics discs (Biotec Lab. UK) corresponding to drugs commonly used in the treatment of human and animal infections caused by bacterial; Gram negative antibiotics includes: Ampicillin (AMP) (10µg), Cefuroxime (CRX) (30µg), Cotrimoxazole (COT) (25µg), Cefotaxime (CTX) (30µg), Tetracycline (TET) (30µg), Amikacin (AMK) (30µg), Gentamicin (GEN) (10µg), and Chloramphenicol (CHL) (30µg) whilst Gram positive antibiotics includes: Ampicillin (AMP) (10µg), Cefixime (CXM) (30µg), Cloxacillin (CXC) (5µg), Cotrimoxazole (COT) (25µg), and Erythromycin (ERY) (15µg).

**Statistical Analysis:** Data obtained in the study were descriptively and statistically analyzed using Statview from SAS Version 5.0. The means were separated using double-tailed Paired Means Comparison. ( $P \le 0.05$ ) = Significant and ( $P \ge 0.05$ )= Not significant.

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RESULTS



Mean microbial load after washing with test concentrations of vinegar is shown in Fig. I. Mean microbial load of distilled water washing ranged from  $2.70 \times 10^{10}$  CFU/ml–  $3.90 \times 10^{10}$  CFU/ml (data not shown). Fig. II shows bacteria isolated and their frequencies. Eighty-seven (87%) of the bacterial isolates were pathogenic whilst 13% were non-pathogenic. Percentage of different bacterial isolated from each sampled vegetableis depicted in Fig. III. Fig. IV shows percentage of vegetables that served as a source for each of the bacterial isolates. Fig. V shows bacterial

isolates and their frequency of resistance to tested antibiotics. Fig. VI depicts antibiotics and their activity on isolated bacteria.



Fig. II. Frequency of bacterial Isolates



Fig. III. Percentage Of Different Bacterial Isolated From Each Sampled Vegetable



Fig. IV. Percentage of Vegetables That Served as a Source for Each of the Bacterial Isolates



Fig. V. Bacterial Isolates and their Frequency of Resistance to Tested Antibiotics



Fig. VI. Antibiotics and their Activity on Isolated Bacteria

#### DISCUSSION

The research studied the effect of increasing concentrations of vinegar as an antimicrobial agent in reducing bacterial contamination of ready to eat vegetables using distilled water as a negative control as well as antibiotic susceptibility pattern of isolated bacteria.

Mean microbial load ranged from 2.70×10<sup>10</sup>-3.90×10<sup>10</sup> CFU/ml after washing with distilled water;  $2.10 \times 10^8 - 3.25 \times 10^8$  CFU/ml with 10% vinegar solution;  $1.65 \times 10^7 - 2.60 \times 10^7$  CFU/ml 20% vinegar solution and  $1.10 \times 10^7 - 1.90 \times 10^7 \text{CFU/ml}$  30% vinegar solution. For vinegar washes, highest amount of contaminants were found on the vegetables after washing with 10% vinegar concentration followed by 20% vinegar solution, with the least contaminant found on the vegetables after washing with 30% vinegar solution. There was a significant difference (P<0.001) in microbial load between 10% vinegar wash and 20% and 30% vinegar wash. Although 30% vinegar washing reduced microbial load further than 20% vinegar wash, the difference was not significant (P>0.68). Increasing the concentration of vinegar solution from 10% through to 30% resulted in 93.26%–95.02% reduction in the microbial load of the various vegetables. Cabbage had the highest amount of contaminants after washing with distilled water and the different concentrations of vinegar solution. Highest percentage microbial load reduction due to increase in vinegar concentration was observed in green pepper (95.02%) whilst lowest reduction was observed in tomato (93.26%). Lowest microbial load for all the vegetables were obtained when 30% vinegar solution was used in washing each of the vegetables. Research has shown that the efficacy of the method used for microbial load reduction is usually dependent on the type of treatment, type and physiology of the target microorganisms, characteristics of produce surfaces, exposure time and concentration of cleaner/sanitizer, and temperature [14]. Thus increasing concentration of vinegar expectedly reduced microbial loads. However, the resultant 93% reduction effect of just 10% increase without any observed effect on the vegetables is very significant and very important in the fight to curb vegetable infections and associated disease outbreaks. The observed decrease in microbial loads with increase vinegar concentration can be attributed to a further reduction in pH creating an acidic medium that is toxic to most microbes. There was significant difference (P < 0.001) in either of the vinegar washes compared with distilled water. There was no significant difference in any of the replicates (P>0.85) showing similar levels of contamination of the vegetables in all replicates.

Eight different bacteria species were isolated. B. cereus(36.25%) was the highest and most frequent isolate present on 90% of all vegetables sampled. S. aureus (25.00%) on 80% of vegetables, L.monocytogenes (13.75%) on 50%, E. coli (12.50%) on 40%, Proteus spp. (5.00%) on 40% and Klebsiella spp. (2.50%), P.aeruginosa (2.50%) and Micrococcus (2.50%) on 20% of vegetables each. All these bacteria have been isolated from fruits and vegetables in other studies [15-17].Some of these bacteria isolates may be part of the natural flora of the fruits and vegetables or contaminants from soil, irrigation water, the environment during transportation, washing/rinsing water or handling by processors [18]. Pseudomonas spp. and Bacillus spp. are part of the natural flora and are among the most common vegetable spoilage bacteria though some Bacillus species (B. cereus) are capable of causing food borne infections. The presence of S. auerus, a pathogenic organism of public health concern, in most of the samples and the presence of other pathogenic and opportunistic bacteria like Klebsiella spp., in some of the vegetables, further highlights the need for proper decontamination of vegetables through proper washing before eating. Surfaces of vegetables may be contaminated by S. auerusthrough human handling and other environmental factors. Human skin and nasal cavity is the main reservoir of staphylococcus that can survive for several weeks when contaminating surfaces. Contamination of foodstuffs during distribution and handling may allow bacterial growth and subsequently production of toxins that may represent a potential risk to humans [19]. Results shows that cabbage and lettuce carried higher incidence of *E. coli* and *S.aureus*. The higher microbial loads on lettuce and cabbage may be due to the large surface area of the leaves. Having foliar surfaces with many folds and fissures provide good shelter for microorganisms and the fragility of leaves allow the penetration and reproduction of bacteria in their inner tissues [20]. This has serious health implications considering that *S. auerus* one of the major causes of community-acquired infections whilst presence of *E. coli* indicates faecal contamination of food with its potential foodborne outbreaks as occurred in Germany early 2011.

The result of the antibiotic susceptibility testing showed varied response of isolated bacterial to antibiotics tested. Majority of isolates showed  $\geq$ 50% resistance to the antibiotics tested.*E. coli, S. aureus, B. cereus and Klebsiellaspp.* had 62.5% resistance whilst *Proteus* spp. and *L. monocytogenes* showed 75% resistance which is similar to research undertaken on bacterial isolates in sachet water sold in the streets of Cape Coast [21]. Ampicillin was the least effective antibiotic that was similar to observations[22]. Amikacin and Gentamicin were however 100% effective when tested on bacterial isolates confirmingearlier studies[21]. The presence of antibiotic resistant bacteria on vegetables is of health significance because of the danger in promoting multiple antibiotic resistant organisms in humans. The prevalence of drug resistant organisms poses a great challenge to clinician as the consumption of vegetables containing these antibiotic resistant organisms may serve to prolong the treatment of food borne diseases.

### CONCLUSION

The data obtained proves that vegetables can be highly contaminated with antibiotic resistant bacterial and that  $\geq 20\%$  vinegar concentration effectively reduces about 93% of bacterial contamination. Thus this study provides a first-hand indication of which microorganisms might be present in fresh vegetables and how to eliminate them.

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