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ISOLATION, PURIFICATION AND IDENTIFICATION OF SOME MULTI-DRUG RESISTANT FOOD-BORNE PATHOGENS IN LEBANESE FRESH PRODUCE

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ABSTRACT: *Fresh produce can be a vehicle for the transmission of multi drug resistant pathogens capable of causing human illnesses and some of them can grow on fresh-cut vegetables. The present study was designed to monitor the occurrence of Esherichia coli, Salmonella and Listeria monocytogenes, in Lebanese fresh produce with the possibility of reducing the contamination using acetic acid and sodium chloride at5°C as dressing sauce and 22°C as a washing solution. A total of about 145 samples of conventional and organic fresh produce were collected from different 10 agricultural fields, 14 grocery stores and 7 market places in Lebanon from north to south areas (during June through December 2013). Salmonella and Listeria monocytogenes were not detected in all the collected samples, however 26.8% of produce items were positive for non-O157:H7 E.coli, the highest contamination with fecal E. coli occurred in leafy green produce (purslane, thyme, parsley and lettuce) except peppermint items were fecal E. coli free . The transition from pre-harvest to post-harvest stage showed an increase in fecal contamination load whereas in farms the prevalence of E. coli was 25.7 % and became 52.9% in market place. However organic fresh produce showed the lowest load in coliform counts and prevalence of E. coli. A total of 23% E. coli isolates are considered to be multidrug resistant since they showed resistance to ≥ 3antibiotic classes (β-lactam, tetracycline and folate inhibitor). It was revealed that 2g of sodium chloride results in a survival of MDR E. coli. A and may limit antibacterial effect of vinegar with low acidity (2% acetic acid) resulting in the reduction of 1.5 log_{10} CFU/ml after 20 min at room temperature , in contrast washing treatment solution with 4% acetic acid reduces 2.42 log_{10} CFU/ml within 10 min. Dressing sauce showed strong inhibition of inoculated Salmonella enteritidis after 3 hours of storage at 5±°C. On the other hand a total reduction of 0.2 and 3 log_{10} CFU/g were noticed with MDR E. coli A at zero time and after 3hrs respectively, where the total inhibition of MDR E. coli A was achieved after 6hrs of storage at 5±1°C.*

KEYWORDS: Lebanese fresh produce, food-borne pathogens, isolation, identification

INTRODUCTION

Fresh produce are important source of vitamins and fibers, decreasing the risk of memory loss, heart diseases and cancers (Cedric et al., 2010), their production and consumption has grown rapidly during the last decade due to increased people demand for healthy and ready-to-eat convenient fruits and vegetables (Alegre et al., 2010 and Olaimat & Holley, 2012). Recently it was noticed an increase in the number of produce-linked food-borne outbreaks (Sewell & Farber, 2001), since contaminated fresh produce can transmit potential human food-borne MDR pathogens (Cedric et al., 2010) namely: *Listeria monocytogenes*, *Salmonella*, *Shigella* and *Esherichia coli* O157:H7 (FDA, 2011). The contamination may occurs, during agricultural

production, harvesting, storage or processing (Pui et al., 2011). Between 1998 and 2006, five types of commodity produce comprised 76% of produce-related outbreaks namely: lettuce/leafy greens (30%), tomatoes (17%), cantaloupe (13%), herbs (basil, parsley, 11%), and green onions (5%) (Lee&Baek,2008).

Washing is one of the most important method for reducing fruits and vegetables contamination, by removing soils, insects, chemical products and some microorganisms from the surface of fresh produce (Ruiz-Cruz et al.,2007). On the other hand washing with contaminated water may represent a source of pathogenic microorganisms (Lapidot et al., 2006). To ensure the safety of fresh produce, the use of sanitizing agents during produce washing is needed, given that ready to eat fresh-cut produce are not subjected to further cooking (microbe-killing steps) (Ruiz-Cruz et al.,2007).

The efficacy of chlorine and chlorine-based derivatives in disinfecting water has been well known for over 30 years (Gómez-López et al., 2008), and the use of chlorinated water for washing fresh-cut produce is widespread throughout the industry (Gil et al., 2009). Lactic acid is one of the sanitizers used alone or in combination with other chemicals, has been shown to be effective in the eradication of bacterial pathogens (Akbas & Olmez, 2007). The bacteriostatic action of 0.1% concentration of acetic acid in the vinegar on food-borne pathogenic bacteria including EHEC O157:H7 was evaluated and it was revealed that the effect was synergically enhanced by sodium chloride (Entani et al., 1998). Lettuce, parsley and peppermint are the main ingredient of fresh cut salad in Lebanon.

The aim of the present study is to investigate the contamination of Lebanese fresh produce with potential food-borne pathogens namely: *Esherichia coli, Salmonella and Listeria monocytogenes*, with the possibility of reducing contamination using acetic acid and sodium chloride.

MATERIALS AND METHODS

Sample collection

A total of about 145 samples of conventional and organic fresh produce were collected from different 10 agricultural fields, 14 grocery stores and 7 market places in Lebanon from north to south areas (during June through December 2013). The target commodities included produce items that are mostly eaten raw (Table 1). During collection, samples of conventional produce that were ready for harvest were picked randomly from different locations on the field and immediately put into sterile zip-lock bags without washing and was transported to the microbiology laboratory using ice box. Sample size was from 300 to 500 g for small vegetables while samples of head lettuce consisted of the entire head, all samples were kept stored at 4°C for microbial analyses within 24 h of samples collection (Lynette et al., 2005).

Microbiological analyses

Three subsamples of 25 g each, originating from the composite sample intended for pathogen detection , were weighed and prepared for *E.coli Salmonella* and *L. monocytogenes* assays by the U.S. Food and Drug Administration, *Bacteriological Analytical Manual methods* (Feng &Weagant, 2002).

***Salmonella* detection:** Samples were homogenized in 225 ml lactose broth, followed by incubation at 37°C for 24 h. One milliliter of the lactose pre-enrichment broth was then transferred to tetrathionate broth and incubated at 37°C. After 18 to 24 h, samples were streaked on xylose lysine desoxycholate (XLD) agar. Two or more typical colonies then were transferred to triple sugar iron (TSI) agar slants for further identification and serotyping (Lynette et al., 2005).

***L. monocytogenes* detection:** Twenty five gram (25 g) produce samples were incubated in *Listeria* enrichment broth at 30°C for 24 to 48 h. *Listeria* spp. were isolated using Oxford agar supplemented with esculin and ferric ammonium citrate. Typical colonies were analyzed for beta-hemolysis on 5% sheep blood agar, and colonies displaying beta-hemolysis were streaked on blood agar for further identification, (Lynette et al., 2005).

***E.coli* and coliforms detection:** Twenty five gram (25 g) sample were shaken for 2 min in 225 ml of lauryl sulfate tryptose (LST) broth as an enrichment broth, the coliform count was determined by the three tube most-probable-number (MPN) system using three ten-fold serial dilution in LST broth that were incubated for 48 h at 37°C. LST tubes showing growth and gas production were streaked on eosin methylene blue (EMB) agar plates for *E.coli* colonies isolation. Suspected *E.coli* colonies were confirmed by indol, methyl red, Voges Proskauer and citrate fermentation tests (Avik et al., 2003). Predominant coliforms in fresh produce were determined by identifying the isolated colonies from the highest dilution of the samples on EMB Plates using API stripes (Biomerieux, France). *E.coli* O157:H7 Detection was performed by plating *E.coli* enrichment broth on sorbitol-MacConkey agar supplemented with potassium tellurite and cefixime (Lynette et al., 2005). The confirmation of *E. coli* O157:H7 occurrence in fresh produce was determined by the commercial kit VIDAS® ECO O157 (bioMérieux, France). Five colonies of each pure growth *E.coli* were picked up and inoculated in 1 ml brain heart infusion broth containing 25% glycerol and then stored at -70 for further investigation (Shereen & Asem, 2013).

Identification of isolated bacterial strains

Isolated bacteria from fresh produce were identified using the commercial kit API® ID strip range / Clinical Diagnostics (bioMérieux, France).

Detection of multi-drug resistant food-borne pathogens

- Antimicrobial susceptibility testing

Antimicrobial susceptibility testing and results interpretation were performed according to the recommendation of the Clinical Laboratory and Standards Institute (CLSI). The antibiotic (µg/disc) used in the present study were : gentamicin (CN - 10 µg), levofloxacin (LEV - 5 µg), norfloxacin (NOR - 10 µg), piperacillin /tazobactam (TZP - 100/10 µg), amoxicillin /clavulanic acid (AMC - 20/10 µg) , tetracycline (TE - 30 µg), cefotaxime (CTX - 30 µg), ceftriaxone (CRO - 30 µg), cefepime (FEP - 30 µg), sulphamethoxazole /trimethoprim (SXT-25 µg), cefpodoxime (CPD-10 µg), azetreonam (ATM-30 µg) and imipenem (IPM - 10 µg). Loaded Mueller Hinton agar plates were left for 30 min at room temperature for compound diffusion and then incubated for 24 h at 37◦C. Zones of inhibition were recorded in millimeters and the experiment was repeated twice.

- Double-disk synergy test (DDST)

Disks containing cephalosporins (cefotaxime or ceftriaxone, ceftazidime, cefepime) are applied next to a disk with clavulanic acid (amoxicillin-clavulanic acid or ticarcillin-clavulanic acid). Positive result is indicated when the inhibition zones around any of the cephalosporin disks are augmented in the direction of the disk containing clavulanic acid. The distance between the disks

is critical and 20mm centre-to-centre has been found to be optimal for cephalosporin 30µg disks (Drieux et al., 2008).

ANTIBACTERIAL EFFECT OF VINEGAR AND SALT

- Bacterial strains

The bacterial strains used throughout the present work namely: MDR *E. coli* A that was isolated from Lebanese fresh produce and identified using the commercial kit API® (bioMérieux, France) and *Salmonella enteritidis* that was kindly provided by the American University of Beirut, Microbiology laboratory, were maintained on nutrient agar slants at 4°C and with monthly transfer to fresh media, for long preservation 25% glycerol was added (Shereen & Asem, 2013).

- Bacterial suspension and inoculation

MDR *E. coli* A was cultured twice in tryptic soy broth overnight at 37°C before use in experiments, and a portion (500 µl) of the overnight culture was inoculated into 50 ml of tryptic soy broth supplemented with 1% (w/v) glucose for 24 h at 37°C for acid adaptation. The bacterial culture was combined in plastic centrifuge tube and cells was harvested by centrifugation at 2,600 × g for 20 min. After the supernatant was discarded, the pellet was washed twice with 0.2% sterile peptone water. The final pellet was resuspended in 0.2% sterile peptone water to a concentration calculated to yield 10^6 to 10^7CFU per milliliter of sample. Prepared culture of MDR *E. coli* A was added to the treatment solutions and then completely mixed with the sample for 30 s for uniform distribution (Min-Suk et al., 2003).

- Inoculum treatment solutions

Commercial vinegar (containing 5% acetic acid) and salt were purchased at a grocery store. Samples were prepared to achieve various concentrations of acetic acid (0, 2, and 4 % [v/v]) by adding 0, 40, and 80 ml of vinegar, without and with 2 g of salt (fixed amount) to a sterilized 250-ml glass bottle with a screw cap and bringing the volume up to 100 ml with sterilized distilled water. The samples were made 12 h prior to inoculation and were held at room temperature and examined at 0,10, 20, 30 min, these experiments were repeated three times (Min-Suk et al., 2003).

- Bacterial enumeration and enrichment

Aliquots (1 ml) of samples were serially diluted (10^1 to 10^5) with 9 ml of 0.2% sterile peptone water. Following 10-fold serial dilution, 0.1 ml of diluents was spread-plated onto eosin methylene blue agar as selective media for the enumeration of *E. coli*. The plates were incubated at 37°C for 48 h, and then cells were enumerated (Min-Suk et al., 2003).

ANTIBACTERIAL EFFECT OF DRESSING

- Inoculum cocktail preparation

Following duplicate sub-culturing of pathogen isolates MDR *E. coli* A and *Salmonella enteritidis*, 2.0 ml of culture medium from each isolate was dispensed into a sterile conical flask. Tubes were immediately centrifuged for 15 min at 1623×g. After gently pouring off the resulting supernatant, each pellet was suspended in 20 ml of 0.1% (w/v) peptone water and washed twice more by centrifugation in identical fashion. Following the third centrifugation, the pellet was suspended in 20 ml of 0.1% peptone water. In order to prepare the cocktail of pathogens, equivalent volumes of all isolates were mixed together in a virgin sterile conical vial and vortexed vigorously prior to further work (Calix-Lara et al., 2012).

- Dressing sauce preparation and testing

Commercial lettuce was purchased from a local supermarket and immediately transported to microbiology laboratory and stored at 4°C; all products was used within 2 days of purchase. To prevent interference from background microbiota inner leaves of lettuce were washed with tap water, and than immersed in a chlorine solution (230 ppm of sodium hypochlorite) at 30°C for 15 min. Finally, lettuce leaves were washed with sterile distilled water to leach chlorine , aseptically weighed, separated into portions of 25g and inoculated with cocktailed *salmonella enteritidis* and MDR *E. coli* A (Eduardo et al., 2007). Inoculated pathogens were allowed to adhere to lettuce surfaces for 60min at 25°C in a biological cabinet prior to dressing sauce application. Sample of dressing sauce were prepared by mixing 1.5 ml of 4% acetic acid with 2 g of salt or water as control . All the ingredients were thoroughly hand mixed with autoclaved forks (Eduardo et al., 2007). After 30min of occasional blending, treated samples were held at 5°C. Following 0, 3, 6, 24 and 48h samples were aseptically loaded into sterile conical flasks mixed with 225 ml 0.1% peptone water and shacked at 150rpm for 10min. Aliquots (1 ml) of samples were serially diluted in 0.1% peptone water, plated onto eosin methylene blue (EMB) agar and incubated at 37°C for 48 h for the enumeration of *E. coli*, while samples for *Salmonella* enumeration were plated on xylose lysine desoxycholate (XLD) agar and then incubated at 37°C for 24 h. controls, treated with sterile water not containing acetic acid and salt were prepared and processed in identical fashion (Min-Suk at el., 2003).

Data Analysis

The average coliform counts were calculated, and statistically significant differences between varieties of fruits and vegetables and between organic and conventional farms were determined using one sample test. The same statistical tool was used to compare the prevalence of E. coli among different produce varieties and origin. While paired samples test was used to compare the antibacterial effect of different acetic acid and sodium chloride combinations. The criteria for statistical significance was based on a (p<0.05).

RESULTS AND DISCUSSION

A total of 145 conventional and organic produce samples were collected during a period of 7 months originating from different 10 agricultural fields, 14 grocery stores and 7 market places in Lebanon (Table 1) More than 50% of the produce items collected consisted of lettuce (17.2 %),

tomato (13.1%), parsley (12.4 %) and cucumber (9.6 %) and the remainder included spinach, thyme, peppermint, purslane, arugula and radish.

In a trial to test the microbiological quality of produce**,** Coliform bacteria were detected in 71% of all the samples, and the overall average counts in the conventional produce from agricultural area, grocery and market place were 1.3 $\log_{10}$ MPN/g (±0.5 SD), 2.2 $\log_{10}$ MPN/g (±0.7 SD) and 2.0 $\log_{10}$ MPN/g (±0.4 SD) respectively (Table 2). Parsley, thyme and purslane collected from grocery and market place had slightly higher means counts of coliform, showing more than 1. $\log_{10}$ MPN/g greater counts than same types of fresh produce collected from agricultural area and these differences were statistically significant. In addition when the coliform counts were compared between ten different fresh produce types having the same origin, the differences were obvious and statistically significant (p<0.05).On the other hand when we determined the predominant bacteria in 145 samples, *E.coli* was the most common identified coliform bacteria (38%), besides *Enterobacter cloacae*, *Enterobacter sakazaki* and others were also found in some fresh produce. The coliform level on most of fresh produce reported in the present study were from 1 to 3 $\log_{10}$ CFU/g, these results consistent with those of other studies that examined microbial levels on fresh produce items (Lynette et al., 2005), but lower than those reported by Ruiz-Cruz et al.(2007) whereas levels on samples of tested fresh produce ranged from 10^4 to 10^6 $\log_{10}$ CFU/g.

As for Pathogen detection in fresh produce, all samples were analyzed for *Salmonella*, *L. monocytogenes*, *E.coli* o157:H7 and *E.coli* non- o157:H. *Salmonella*, *L. monocytogenes* were not detected in any of the 145 tested items. Only one isolate was suspected to be *E.coli* o157:H7 since it showed colorless colony on sorbitol-MacConkey however it showed negative result after confirmation by the commercial kit VIDAS® ECO O157 (bioMérieux, France). Data in Table 3 revealed that purslane was the most fecal *E.coli* contaminated produce, 5samples out of 10 was contaminated (50%), whereas peppermint was completely sterile, free produce fecal *E.coli* contamination (0%). On the other hand thyme, parsley and lettuce showed slightly high contamination with fecal *E.coli* (46.1, 38.6 and 36% respectively). However tomato and radish showed moderate contamination (21and 23%) followed by spinach, cucumber and argula that showed a lower fecal *E.coli* contamination (14.2%). Data in Table 4 showed that conventional fresh produce collected from market places showed high level of fecal *E.coli* contamination 18 samples out of 34 (52.9%), on the other hand conventional fresh produce collected from agricultural fields and grocery stores showed moderate fecal contamination (25.7 and 22% respectively), Whereas organic fresh produce showed a lower fecal *E.coli* contamination (3.8%), and these differences were statically significant. For decades, *E. coli* has been used as the reference indicator for fecal contamination, and a number of surveys have reported its isolation from fresh fruits and vegetables (Jay, 2000). In a recent study that tested conventionally grown fresh produce at retail, only one sample tested positive for *E. coli* out of 50 samples that included alfalfa sprouts, broccoli, caulif ower, lettuce, celery, and mung bean sprouts (Thunberg et al., 2002). The percentage of *E. coli*–positive samples found in a survey of conventionally grown fresh vegetables in Japan (including cabbage, lettuce, onions, spinach, and celery) was 2% (Kaneko et al., 1999). However, in the present study *E. coli* was found in 26.8% of conventional fresh vegetables in Lebanon, this result is consistent with the prevalence of 25% this bacterium in ready-to-use lettuce (Soriano et al., 2000). *E.coli* O157:H7 infection in approximately 19 outbreaks in the United States (Olsen et al., 2000). A number of surveys have attempted to detect *E. coli* O157:H7 in fresh fruits

and vegetables, in a study that included 3,200 vegetables, no O157:H7-positive sample was detected, and in another survey of 890 fruits and vegetables, this pathogen could not be found either (Johannessen et al., 2000). Consistent with these previous studies, this investigation did not found any evidence of O157:H7 contamination in Lebanese. Moreover *Salmonella*, L. *monocytogenes*, were not detected in any of the 145 Lebanese tested items, these results are similar to those represented in the U.S, Food and Drug Administration's survey (Lynette et al., 2005). In contrast, Sait Aykut et al. showed that the prevalence of contamination of *Salmonella* and *L. monocytogenes* are (14%) and (8.5%) respectively on leafy green vegetables grown around Ankara which have more contamination percentage than the tested raw vegetables in Catalonia, Spain (0.74% *Salmonella*, 1.48% *L. monocytogenes*)(Badosa et al., 2008), and the tested leafy salad samples in Sao Paulo, Brazil (3% *Salmonella*, 0.6% L. *monocytogenes*) (Froder et al., 2007). Among fresh produce items, purslane appears to be more susceptible to bacterial contamination followed by thyme, parsley and lettuce showing 50, 46.1, 38.6 and 36% respectively of samples contaminated with fecal *E.coli* these results are similar to those showed by Avik et al. where leafy green and lettuce showed high level of fecal contamination, in addition recent evidence suggests that food-borne pathogens can be internalized into leafy green and lettuce leaves (Solomon et al., 2002), except for peppermint items that show no fecal *E.coli* contamination and this is maybe due to the strong antibacterial effect of this leafy green to wide spectrum of bacteria (Friedman et al., 2002). Farms are all about wide open space and can not seal them off into sterile biospheres resulting in contamination of fresh produce, in addition to adjacent land use practices and water safety all come into play that affect the quality and safety of fresh fruits and vegetables, in the present study 25.7% of fresh produce originating from agricultural lands were fecal contaminated. However the level of contamination increase during the post –harvest phase such as handling, transportation and distribution resulting in an increase in the level of contamination to 52.9% in the present study, Lynette et al reported a significant increase in the fecal coliform load for both root crops and leafy vegetables from farm to market.

In an attempt to detect the antimicrobial resistance of fecal *E.coli* isolates, *E.coli* Isolates were considered to be multidrug resistant since they showed resistance to ≥ 3antibiotic classes (β-lactam, tetracycline and folate inhibitor). The antibiotics to which resistance was detected are: amoxicillin /clavulanic acid (AMC), piperacillin /tazobactam (TZP), tetracycline (TE) and sulphamethoxazole /trimethoprim (SXT). The remainder fecal *E.coli* isolates showed resistance only to amoxicillin / clavulanic acid (AMC) (fig.1). ESBL *E.coli* strains were not detected, since no synergistic effect appears between amoxicillin /clavulanic acid (AMC), cefotaxime (CTX) and ceftriaxone (CRO) using Double-disk synergy test (DDST) .The present study demonstrates that *E.coli* isolates from fresh produce were resistance to 30% of the antibiotics used in the treatment of urinary tract infection in Lebanon. A previous study carried out during 2000-2001, reported similar antimicrobial resistant patterns in uropathogenic *E.coli* isolates from patients (Shehabi et al., 2004). In addition to a recent study that found bacterial isolates from fresh vegetables exhibited higher resistance rates than our study to ampicillin, cephalothin, trimethoprime-sulfamethoxazole, aminoglycosides, tetracycline, fluoroquinolones, amoxycillin-clavulanic acid, and chloramphenicol (Hassan et al., 2011).

The antibacterial effect of vinegar and salt against MDR *E. coli* A were detected. The survival of multi-drug resistant *E. coli* A in various solutions containing acetic acid and sodium chloride at 22±1 °C is presented in Table 5.

The reduction of MDR *E. coli* A at room-temperature was clearly differentiated ($p<0.05$) among the combined treatments . MDR *E. coli* A was strongly reduced and became undetectable after 30min in all treatment solutions except in control and in sodium chloride solution. MDR *E. coli* A was effectively reduced (reduction of 4 $\log_{10}$ CFU/ml) in 2% acetic acid within 10 min. In contrast, after10 min MDR *E. coli* A was not detected when treated with 4% acetic acid. The antibacterial activity of 2% acetic acid was decreased when combined with 2g salt resulting in the reduction of 1.5 $\log_{10}$ CFU/ml after 20 min . On the other hand synergistic effect was showed when 4% acetic acid was combined with 2g salt resulting in additive reduction of about 0.83 $\log_{10}$ CFU/ml when 4% acetic acid was used alone.

To ensure the safety of fresh produce, the use of sanitizing agents during produce washing is needed, given that ready to eat fresh-cut produce are not subjected to further cooking (microbe-killing steps) (Ruiz-Cruz et al.,2007). Overall, acidified products may limit microbial growth or survival, and the extent of this survival depends on the types of microorganisms harbored in the food and the type and amount of acid, in the present study the effect of 4%acetic acid against MDR *E.coli* isolate increased in presence of sodium chloride, and strongly eliminates the bacterium from treatment solution at room temperature. However, in low acidic medium (2% acetic acid), sodium chloride may result in survival of *E.coli* and these results are similar to Zhao

and Doyle(1994) who showed that *E. coli* O157:H7 survived slightly longer in real mayonnaise (pH 3.9) than in the reduced-calorie formulation made with less acid (pH 3.8)in addition to another study that indicates that the addition of small amounts of acetic acid (0.5%) to mustard retards the reduction of *E. coli O157:H7* and *L. monocytogenes* and these antagonistic effects may be changed if mustard is used alone or in combination with ≥1% acetic acid (Shereen & Asem, 2013).

In the present study the antibacterial effect of dressing against MDR *E. coli* A and *Salmonella enteritidis* was evaluatd. It was noticed that the survival of inoculated MDR *E. coli* A and *Salmonella enteritidis* on lettuce leaves dressed with vinegar and salt after 0, 3, 6, 12, 24, 48 hrs is reflected in Figure 2 and 3.The effectiveness of vinegar and salt in reducing inoculated *Salmonella enteritidis* was showed, resulting in the reduction of 0.6 $\log_{10}$ CFU/g after direct contact between innoculated bacterial cells on lettuce leaves and dressing solution. Strong inhibition of inoculated *Salmonella enteritidis* was observed after 3 hours of storage at 5±1 °C (Figure 2). On the other hand reduction of 0.2 $\log_{10}$ CFU/g and 3 $\log_{10}$ CFU/g at time 0hr and 3hrs respectively on MDR *E. coli* A was showed, where the total inhibition of MDR *E. coli* A was achieved after 6hrs of storage at 5±1 °C (Figure 3). These results agree with Min-Suk et al.(2003) who showed that the numbers of *E. coli* O157:H7 and *L. monocytogenes* were reduced much more rapidly at 22°C than at 5°C.

CONCLUSION

The present study showed that the prevalence of fecal *E.coli* was significantly higher in leafy green produce and those from market place origin. In addition acidified products may limit microbial growth or survival, and the extent of this survival depends on the types of microorganisms harbored in the food, the amount of acid and the storage temperature.

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Figures and Tables

Produce varieties	% of samples (No. of samples)				
	Conventional			Organic	Total
	Agricultural Area	Grocery	Market Place		
Lettuce	11.4 (4)	16 (8)	26.4 (9)	15.3 (4)	17.2 (25)
Parsley	22.8 (8)	12 (6)	11.7 (4)	-	12.4 (18)
Spinach	8.5 (3)	8 (4)	-	-	4.8 (7)
Peppermint	11.4 (4)	10 (5)	8.8 (3)	-	8.2 (12)
Thyme	14.2 (5)	12 (6)	5.8 (2)	-	8.9 (13)
Purslane	8.5 (3)	8 (4)	8.8 (3)	-	6.8 (10)
Arugula	5.7 (2)	8 (4)	5.8 (2)	23 (6)	9.6 (14)
Radish	5.7 (2)	8 (4)	11.7 (4)	11.5 (3)	8.9 (13)
Tomato	8.5 (3)	10 (5)	11.7 (4)	26.9 (7)	13.1 (19)
Cucumber	2.8 (1)	8 (4)	8.8 (3)	23 (6)	9.6 (14)
Total	35	50	34	26	145

Table 1.Distribution of conventional and organic samples, according to produce varieties

- : Not available samples

Table 2. Levels of coliform contamination in produce varieties

Produce varieties	Coliform count Mean log MPN/g ± SD				
	Conventional			organic	*p*-value
	Agricultural Area	Grocery	Market Place		
Lettuce	1.4±0.9	1.4±1.0	2.0±1.12	0.0	.066
Parsley	1.7±1.4	2.3±0.8	2.3±0.7	-	.009*
Spinach	1.8±1.2	2.9±0.3	-	-	.144
Peppermint	0.3±0.5	2.3±0.9	1.9±0.6	-	.126
Thyme	1.2±1.2	2.5±0.7	2.4±0.0	-	.037*
Purslane	1.2±1.0	2.6±0.9	2.6±0.8	-	.041*
Arugula	2.1±0.7	3.1±0.0	1.3±0.5	0.2±0.7	.066
Radish	1.5±0.1	2.3±1.3	1.5±1.1	0.0	.068
Tomato	0.5±0.3	1.4±1.3	2.2±1.0	0.0	.127
Cucumber	1.3±0.1	0.9±0.7	1.9±1.0	0.0	.080
overall	1.3±0.5x	2.2±0.7x	2.0±0.4x		

* Means of the bacterial counts in fresh produce indicate statistically significant differences (p <0.05) between locations. X indicates statistically high significant differences between overall counts of fresh produce from agricultural area, grocery and market place. Statistical analysis was only done on conventional produce types that supplied > 80% of the samples.
- : Not available

Table 3. Incidence of fecal *E.coli* in collected fresh produce according to produce varieties

Varieties of the samples (No.)	No. (%) positive *E.coli* samples
Lettuce (25)	9 (36)
Parsley (18)	7 (38.8)
Spinach (7)	1 (14.2)
Peppermint (12)	- (0)
Thyme (13)	6 (46.1)
Purslane (10)	5 (50)
Arugula (14)	2 (14.2)
Radish (13)	3 (23)
Tomato (19)	4 (21)
Cucumber (14)	2 (14.2)
Total (145)	39 (26.8)
p-value	0.02

Table 4. Incidence of fecal *E.coli* in collected fresh produce according to produce type and origin

Produce origin (No.)			No. (%) positive E.coli samples
Conventional	Agricultural Area	(35)	9 (25.7)[a]
	Grocery	(50)	11 (22)[a]
	Market Place	(34)	18 (52.9)[b]
Organic		(26)	1 (3.8)[c]

Data of produce origin having different letters (a through c) were significantly different ($p < 0.05$)

Table 5. Antibacterial effect of acetic acid combined with sodium chloride on MDR fecal *E.coli*

	Log10 CFU/ml (growth)						
Species	Time (min)	vinegar + 0g salt			vinegar + 2g salt		
		0%	2%	4%	0%	2%	4%
MDR *E.coli*	0	5.44[a]	4.32[b]	3.85[d]	4.15[e]	4.14[e]	3.02[f]
	10	5.46[a]	<1.47[c]	-	4.01[e]	3.98[e]	-
	20	5.39[a]	-	-	3.92[e]	3.90[e]	-
	30	5.45[a]	-	-	3.53[e]	-	-

l squares means lacking a common superscript differed significantly ($p < 0.05$). - : No l al growth

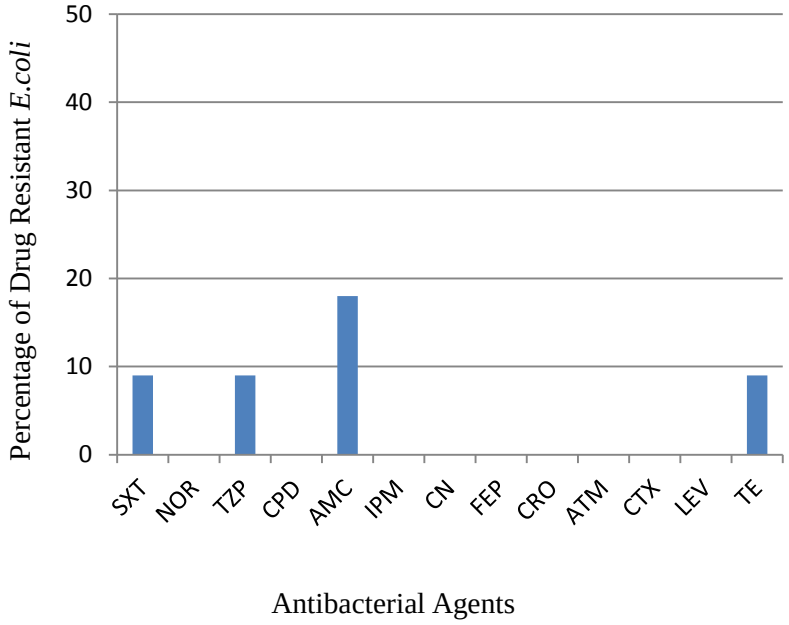

Figure 1: Antimicrobial resistance pattern of fecal *E.coli* isolates

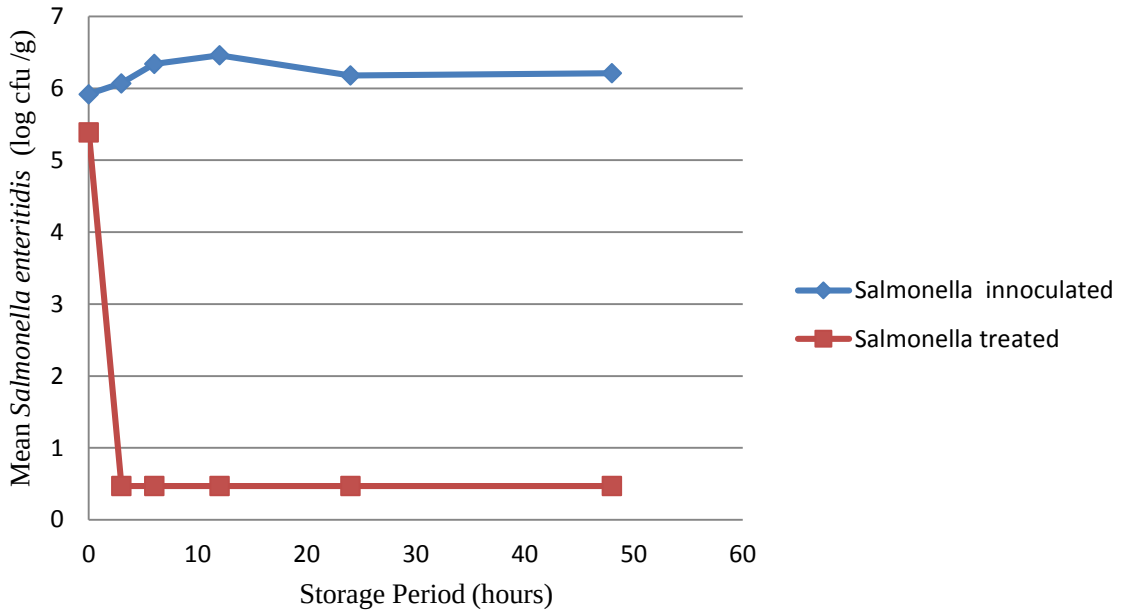

Figure 2: Survival of *Salmonella enteritidis* on lettuce leaves dressed with vinegar, salt and stored at 5°C

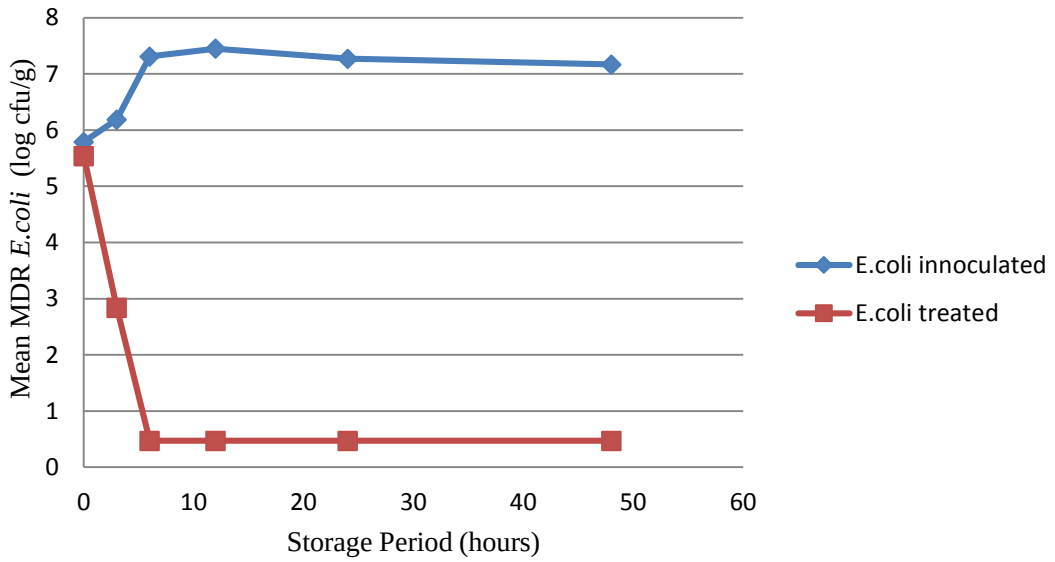

Figure 3: Survival of MDR *E.coli* on lettuce leaves dressed with vinegar, salt and stored at 5°C