Original Article

Drug resistance of coliforms isolated from mint collected from market and home gardens

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Abstract
Mint samples collected different shops and home gardens were assessed for prevalence and enumeration of coliform bacteria. Samples collected from shops harvested copious count in contrast to home grown vegetable. Isolates were identified as *E.coli, Shigella, Enterobacter, Klebsiella,*and *Salmonella*. A blend of responses was observed toward vanomycin, rifampicin, and nalidixic acid with 100% resistance against ampicillin. However, these isolates exhibited gamma (γ) hemolysis, which may be allied with their nonpathogenic attributes.
Key words: Coliforms, Pathogens, *E.coli, Shigella, Enterobacter, Klebsiella, Salmonella*

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INTRODUCTION

Heavy rainfall, environment, fertilizers and irrigation water are sources of contamination of fresh produce (Heaton and Jones, 2008). Fecal coliforms have been identified as a potential public health problem including *E. coli* and *Klebsiella* (Feng *et al.*, 2007). *E. coli* has been declared as pronounced and potential indicator of fecal contamination of fresh produce (Delaquis *et al.*, 2007).Though it maintains the physiology of the healthy host (Conway, 1995) but being opportunistic pathogen, it may cause infections in immunocompromised hosts (Feng *et al.*, 2007). Mint (*Mentha crispa*) is a genus of the family Labiatae, and is used for flavoring, spicing and tea infusions for having digestive, calming, and tonic, antiseptic and anti-asthmatic properties (Jin Park *et al.*, 2002). Environment, irrigation water, and free moisture may contaminate the fresh produce (Wang and Doyle, 1998) at any growth stage (Katusin-Rasem *et al.*, 2001). Manure, sewage sludge used as fertilizer, irrigation water, runoff water from livestock operations, and wild and domestic animals are potential sources of preharvest coliform contamination (Beuchat, 2006). The present study was aimed to investigate the intensity and diversity of coliform bacteria with reference to their pathogenicity and resistance /sensitivity towards various antibiotics. The fecal contamination of the vegetable, which is used without cooking in this country may be relevant for assessing associated health risks.

MATERIALS AND METHODS

Samples were collected from hundred different localities. Half among them were from different sale points. Each sample was processed by dipping five grams of mint in autoclaved bottles containing sterilized water for an hour to shift possibly maximum number of microbial flora from mint into water. Serial dilutions and spread plate technique was used to calculate colony forming units (CFUs) /ml of original solution. First dilution was prepared by taking 0.1 ml of original sample into 9.9 ml autoclaved water. The second dilution was prepared from first dilution likewise (Benson, 2001). Measured amounts of the dilutions were spread on the surface of EMB agar plates. Following subsequent incubation, growth of different types of coliforms was obtained. The plates having 30-300 colonies were selected for enumeration of CFUs. Size, shape elevation, margins, surface texture, consistency,

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pigmentation and optical nature were noted for the category representative and well separated colonies. Following pure culturing of each isolate, their glycerol stocks were prepared and preserved.

Various physiobiochemical tests like Gram's staining, endospore staining, motility, catalase and oxidase tests were performed for characterization of these isolates. Some other tests were also performed for their identification like Indole, Citrate utilization, Methyl red, Voges Proskauer-I and Voges Proskauer-II. The cultural response on EMB agar was also noted to identify different types of coliforms. All the isolates were examined for their degree of pathogenicity by growing over the blood agar medium. Antimicrobial sensitivity disks of ampicillin (25µg), nalidixic acid (30µg), rifampin (5µg) and vanomycin(30µg) were applied for each isolate and the growth inhibition was also verified by using Kirby-Bauer method (Benson, 2001; Pelczar *et al.*, 1986).

Statistical analysis

Analysis of variance (ANOVA) was applied to recognize significant results at $p<0.05$.

RESULTS

Colonial morphologies and some biochemical attributes of the bacterial isolates are shown in table 1. Shop samples gave a mean value of 218×10^2 CFUs/ml of original solution, whereas a mean value of 85×10^2 CFUs/ml of original solution emerged for samples collected from home gardens (Fig 1). All the isolates were found Gram's -ve and non-spore formers, non-reactive for oxidase test with complete catalase reaction. *E.coli* and *Shigella* were found MR and indole +ve and –ve for citrate and VP. *Salmonella* gave –ve reaction for indole and VP, *Klebsiella* gave –ve for MR. Whereas *Enterobacter* was –ve only for indole. *E.coli* remained dominant in all the samples with highest count (71%) in shop samples. Among other isolates, *Salmonella, Enterobacter, Klebsiella, and Shigella* appeared upto 52, 46, 27, and 22%, respectively, for shop samples and they could form only 19, 13, 18, and 10% shares for home samples (Fig. 2).

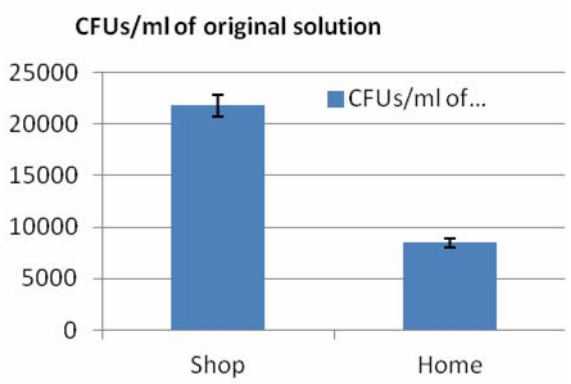

Figure 1 Mean of CFUs/ml of original solution in different samples

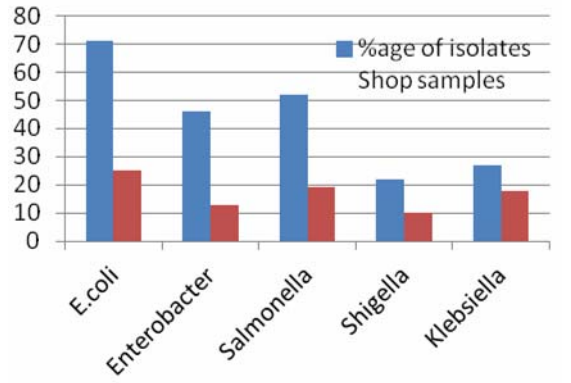

Figure 2 Occurrence of various isolates in samples collected from shops and homes.

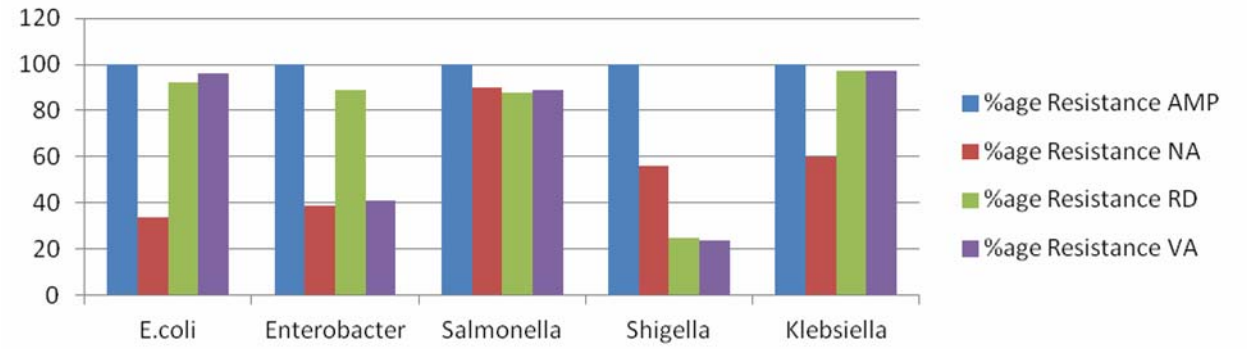

Figure 3 Percentage of the antibiotics resistant isolates against various drugs.

Note: AMP: Ampicillin with disk potency 25µg, NA: Nalidixic acid with dish potency 30 µg, RD: Rifampicin with dish potency 5 µg, VA: Vanomycin with dish potency 30 µg

Table I: Colonial following growth of the isolates on EMB agar and biochemical characteristics of bacteria isolated from the mint samples.

Isolate	Color/ Size	Margin/ Configuration	Elevation/ Consistency	Opacity/ Motility	Gram's/ Endospore staining	Catalase / Oxidase	MR/ Citrate	Indole / VP	Hemolysis
E.coli	Metallic sheen/ 1-2mm	Smooth/ Round nucleated	Covex/ Butterious	Opaque /+	-/-	+/-	+/-	+/-	γ
Enterob-acter	Brown/ 2mm	Smooth/ Round nucleated	Covex/ Mucoid	Opaque /+	-/-	+/-	+/+	-/+	γ
Salmonella	Colorless/ 2mm	Smooth/ Round	Covex/ Mucoid	Opaque /+	-/-	+/-	+/+	-/-	γ
Shigella	Colorless/ 2mm	Wavy/ Round	Flat/ Mucoid	Transpar ent/-	-/-	+/-	+/-	+/-	γ
Klebsiella	Brown/ mm	Wavy/ Round	Covex/ Mucoid	Transpar ent/-	-/-	+/-	-/+	+/+	Γ

All the isolates were found resistant against ampicillin. Among *E.coli* 92, 96 and only 34% isolates were found resistant against RD, VA and NA, respectively. Most of the isolates of *Enterobacter* (89%) were resistant against RD, whilst only 39 and 41% were resistant to NA and VA. The drugs RD and VA were found very effective against *Shigella* contrary to *Klebsiella* (Fig 3).Surprisingly not a single isolate was found hemolytic.

DISCUSSION

E.coli was isolated from all the mint samples, except one obtained from a house. CFUs counts was higher for samples collected from shops in contrary to the findings of Erkan and Vural (2008) who reported higher coliform and *E.coli* counts in home grown samples. Johannessen *et al.* (2002) detected *E. coli* from different leafy vegetables, causing several health risks (Delaquis *et al.*, 2007). The presence of *E. coli* shows the contact of samples with fecal material (Wheeler Alm *et al.*, 2003). Leafy vegetables are most commonly linked to *E. coli* infection due to livestock grazing (Stopforth *et al.*, 2004). Environmental hygiene, handling and transportation play very critical role in food safety (Erkan and Vural, 2008) suggesting washing practices with potable water (Blumenthal *et al.*, 2000). Otherwise irrigation water itself may be potential source of other pathogens (Anonymous, 2002).Use of untreated waste (Amoah *et al.*, 2009) and manure enhances survival of coliforms especially *E. coli* (Islam *et al.*, 2005). Fecal contaminated water used for irrigation and, washing of fresh produce can be a source of *E. coli* (Wachtel *et al.*, 2002; Wang and Doyle, 1998; Steele and Odumeru, 2004) and even chlorination can't affect the behavior of *E.coli* (Rodgers *et al.*, 2004).

Singh *et al.* (2007) utilized 212 samples of mint to study food borne pathogens and isolated salmonella from 11 samples and *E. coli* in 47 samples. Katusin-Rasem *et al.*(2001) worked on mint leaves to study microbial contamination and found 7300 C.F.U/m1 to 7800 C.F.U/m1. *Salmonella* was also found in all samples as reported previously in other leafy vegetables (Golberg *et al.*, 2011) and tomatoes associated with fresh produce-related infection, in USA (Sivapalasingam *et al.*, 2004). In the present study, *Salmonella* was found resistant to all the drugs tested as reported previously (Singh *et al.*, 2007; Miranda *et al.*, 2009; Nipa *et al.*, 2011). *Enterobacte*r has been isolated in high amount from shop samples as isolated from raw vegetables by many workers (Johannessen *et al.*, 2002; Rudi *et al.*, 2002). Most of the isolates of *Enterobacter* were found resistant against ampicillin and rifampicin, contrary to nalidixic acid and vanomycin. In other studies coliforms and *Enterobacter* have been found 76.5% resistant against ampicillin isolated from raw vegetables (Jin Park *et al.*, 2002; Hassan *et al.*, 2011).

Klebsiella was found in the entire purchased sample, contrary to the findings of Johannessen *et al.* (2002) who isolated coliforms from different leafy vegetables. *Klebsiella* being ESBLs (Extended spectrum beta- lactamase) is multidrug resistant (Gundogan *et al.*, 2011; Polishko *et al.*, 2011; Todar, 2012) was found resistant to most of the drugs. All the isolates present in the samples were found non-hemolytic, but this property cannot be attributed for their non- fatality (Taylor and Barkham, 2002).

Shigella had been isolated by Bansal *et al.* (2004) in a product of mint. *Shigella* has been found highly resistant to ampicillin as compared to other drugs used in the study. Recently, it has been found that *Shigella* has developed resistance to not only to ampicillin but many other drugs as well (Ashkenazi *et al.*, 2003).

The present study indicates that organic foods may act as source of transmission of for antibiotic resistance (Fern+índez-Fuentes *et al.*, 2012) supporting Schwaiger et al. (2011) for finding highly resistant bacteria from farm as compared to market. Moreover, it recommends the use of washed fresh produce as they harbor pathogen to human which may cause health hazards (Berger *et al.*, 2010). This work also prefers the use of fresh home grown vegetables as post-harvest practices add to the contamination as concluded by Nipa *et al.*(2011) that coliform and fecal coliforms might be present in raw salad vegetables with multidrug resistance.

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