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


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,
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 \(218 \times 10^2\) CFUs/ml of original solution, whereas a mean value of \(85 \times 10^2\) 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 up to 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.
BACTERIA ON MINT

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.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Color/Size</th>
<th>Margin/Configuration</th>
<th>Elevation/Consistency</th>
<th>Opacity/Motility</th>
<th>Gram’s/Endospore staining</th>
<th>Catalase/Oxidase</th>
<th>MR/Citrate</th>
<th>Indole/VP</th>
<th>Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>Metallic sheen/1-2mm</td>
<td>Smooth/Round nucleated</td>
<td>Covex/Butterious</td>
<td>Opaque/+</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>γ</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>Brown/2mm</td>
<td>Smooth/Round nucleated</td>
<td>Covex/Mucoid</td>
<td>Opaque/+</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>γ</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Colorless/2mm</td>
<td>Smooth/Round</td>
<td>Covex/Mucoid</td>
<td>Opaque/+</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>γ</td>
</tr>
<tr>
<td>Shigella</td>
<td>Colorless/2mm</td>
<td>Wavy/Round</td>
<td>Flat/Mucoid</td>
<td>Transparent/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>γ</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>Brown/mm</td>
<td>Wavy/Round</td>
<td>Covex/Mucoid</td>
<td>Transparent/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>γ</td>
</tr>
</tbody>
</table>

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). Enterobacter 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 (Fernández-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.

REFERENCES


