Research Paper

Antibiotic Resistance of Enterobacteriaceae Isolated from Fresh Fruits and Vegetables and Characterization of their AmpC β-Lactamases

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ABSTRACT

Enterobacteria may gain antibiotic resistance and be potent pathogens wherever they are present, including in fresh fruits and vegetables. This study tested the antibiotic resistance of enterobacteria isolated from 13 types of local and imported fresh fruits and vegetables (n = 105), using the standard Kirby-Bauer disk diffusion method. Phenotypic and genotypic characterizations of AmpC β-lactamases were determined in cefoxitin-resistant isolates. Ten percent of the enterobacteria tested (n = 88) were pansusceptible, 74% were resistant to at least one antibiotic, and 16% were multidrug resistant. Enterobacteria isolates showed the highest antibiotic resistance against ampicillin (66%), cephalothin (57%), amoxicillin–clavulanic acid (33%), cefoxitin (31%), tetracycline (9%), nalidixic acid (7%), trimethoprim (6%), and kanamycin (5%). Three isolates showed intermediate resistance to the clinically important antibiotic imipenem. Escherichia coli isolated from lettuce exhibited multidrug resistance against five antibiotics. Fifteen isolates were confirmed to have AmpC β-lactamase, using the inhibitor-based test and the antagonism test; the latter test confirmed that the enzyme was an inducible type. Four types of AmpC β-lactamase genes (CIT, EBC, FOX, and MOX) were detected in eight isolates: four Enterobacter cloacae isolates and one isolate each of Citrobacter freundii, Enterobacter asburiae, Enterobacter hormaechei, and Enterobacter ludwigii. It was concluded that fresh fruits and vegetables might play a role as a source or vehicle for transferring antibiotic-resistant bacteria that might spread to other countries through exportation. The clinically significant AmpC β-lactamase was rarely documented in the literature on bacteria isolated from fruits and vegetables, and to our knowledge, this is the first report on the detection of an inducible type in such commodities.

HIGHLIGHTS

• Enterobacteria showed resistance to various clinically significant antibiotics.
• Fourteen isolates of enterobacteria (16%, n = 88) were multidrug resistant.
• An inducible type of AmpC β-lactamase was detected.
• Four types of ampC β-lactamase genes (CIT, EBC, FOX, and MOX) were identified.

Key words: AmpC β-lactamase; Antibiotic resistance; Enterobacteriaceae; Food safety; Fruits; Vegetables

The global problem of antibiotic resistance (8) is costly and can lead to treatment failure (13). Antibiotic resistance describes the inherent ability of a microorganism to grow at a high concentration of an antibiotic, irrespective of the duration of the treatment. There might be an association between agricultural use of antibiotics and human infections with antibiotic-resistant bacteria (26). Resistance to multiple antibiotics has been described in some strains of foodborne pathogens such as Salmonella Typhimurium, Escherichia coli O157:H7, Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, and Clostridium perfringens (29). Studying antibiotic resistance in fresh produce–associated bacteria (pathogenic or commensal) is of particular concern because people often consume fruits and vegetables raw. Commensal bacteria might act as a reservoir for transferring resistance genes to pathogenic bacteria (4, 10, 17). If ingested food contains a significant number of resistant bacteria, this could act as a source of resistance genes for the gut microbiota (22).

Bacterial β-lactamases, which can hydrolyze β-lactam antibiotics, are the main resistance mechanism in enterobacteria (15). β-Lactamases can be split into four classes: class A (e.g., AER, BLA1, CTX-M, KPC, SHV, TEM), class B (metallo-β-lactamases, e.g., BlaB, CepA, IMP, NDM, VIM), class C (ACT, AmpC, CMY, LAT, PDC, etc.), and class D (OXA β-lactamases) (18). AmpC β-lactamases are important enzymes for resistance in
gram-negative bacteria. Their genes can be chromosomal or plasmid-encoded (11), and their expression can be constitutive or inducible. Cefoxitin and imipenem induce AmpC \( \beta \)-lactamases strongly, whereas aztreonam, ceftazidime, cefuroxime, and cefotaxime are weak inducers (28). Induction of AmpC \( \beta \)-lactamase is a complex process (14) that results in the production of copious amounts of these enzymes in the presence of \( \beta \)-lactams (inducers) and, thus, the destruction of the inducer (15).

The purpose of the current study was to determine the role that fresh fruits and vegetables may play in harboring and, possibly, in disseminating antibiotic-resistant enterobacteria, through an examination of local and imported samples at the stage of marketing when they are ready for consumption. The study focused on the characterization of AmpC \( \beta \)-lactamase in cefoxitin-resistant isolates because this is an important resistance mechanism in Enterobacteriaceae. Studying microbial quality of fresh fruits and vegetables by evaluating the antibiotic resistance status of their associated bacteria can give us a better understanding of the role of fresh produce in global antibiotic resistance trends and, thus, can contribute to tackling this problem.

**MATERIALS AND METHODS**

**Sample collection and analysis.** Fresh fruits and vegetables (13 types) were obtained from local markets in Oman, from April to September 2014. For each type, local and imported samples were examined, three replicates from each type. In total, 105 samples were analyzed (39 local and 66 imported). Imported samples were selected depending on their availability in the market and their origins were as follows: banana (Musa spp., Philippines), cabbage (Brassica oleracea, The Netherlands), capsicum (Capsicum annuum, Jordan and United Arab Emirates), carrot (Daucus carota, Australia and United States), cucumber (Cucumis sativus, United Arab Emirates), dates (Phoenix dactylifera, Saudi Arabia), lettuce (Lactuca sativa, Iran and Jordan), mango (Mangifera indica, India and Pakistan), papaya (Carica papaya, Thailand and Philippines), pomegranate (Punica granatum, India and Saudi Arabia), radish (Raphanus sativus, China), tomato (Solanum lycopersicum, Jordan, The Netherlands, and Syria), and watermelon (Citrullus, Egypt and Iran) (Supplemental Table S2).

The samples were collected aseptically and refrigerated until analysis (within 12 h). Enterobacteria were recovered on violet red bile glucose agar, whereas *E. coli* was isolated on tryptone bile X-glucuronide medium, as was previously reported (19). At least three bacterial colonies showing the typical morphology on violet red bile glucose agar or tryptone bile X-glucuronide medium were selected to represent each positive sample (enterobacteria were selected so that possible mechanisms of resistance can be inferred from the antibiogram results. All antibiotic discs were from Oxoid (Basingstoke, UK). *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC BAA-1705 (multidrug resistant) were used as quality control strains. Resistance to aztreonam, ceftazidime, or cefotaxime was considered to indicate a positive screening test for the presence of extended spectrum \( \beta \)-lactamase (ESBL), whereas resistance to imipenem (IPM, 10 \( \mu \)g), ertapenem (ETP, 10 \( \mu \)g), or cefoxitin (FOX, 30 \( \mu \)g) was considered to indicate a positive screening test for the presence of metallo-\( \beta \)-lactamase, carbapenemase, or AmpC \( \beta \)-lactamase, respectively.

**Phenotypic confirmatory tests.** Presence of ESBL was checked by comparing the diameter of the growth inhibition zones produced with ceftazidime (CAZ, 30 \( \mu \)g) or cefotaxime with that produced by their clavulanate combination as previously described (31). Modified Hodge test was used to confirm carbapenemase production, and *K. pneumoniae* ATCC BAA-1705 was used as positive control strain (5). Screening for AmpC \( \beta \)-lactamases was considered positive if the inhibition zone was \( \leq \)17 mm. However, there is no standard method to confirm the presence of AmpC \( \beta \)-lactamases (6, 11, 28). AmpC \( \beta \)-lactamase was confirmed using an inhibitor-based test and an antagonism test to overcome problems associated with phenotypic detection of this type of resistance mechanism (11).

The inhibitor-based test was done using cefotetan and cefotetan combined with phenylboronic acid (an inhibitor of AmpC \( \beta \)-lactamase), as was previously explained (6). Bacteria that produced a growth inhibition zone using cefotetan combined with phenylboronic acid that was 5 mm or more larger than the zone diameter around cefotetan alone were considered as a positive confirmatory test for AmpC \( \beta \)-lactamases. An antagonism test was used to detect inducible AmpC \( \beta \)-lactamase using cefoxitin (FOX, 30 \( \mu \)g) and cefotaxime (CTX, 30 \( \mu \)g) discs (15). Blunting of the inhibition zone around the cefotetaxime disc adjacent to cefotetan was considered an indication of the presence of an inducible AmpC \( \beta \)-lactamase because cefotaxim induces AmpC \( \beta \)-lactamases strongly, whereas cefotaxime is a weak inducer (28).

**Identification of *ampC* \( \beta \)-lactamase genes.** Multiplex PCR (24) was used to detect *ampC* \( \beta \)-lactamase genes in 19 isolates that gave positive results using either of the confirmatory tests. Some isolates that were positive in the screening test but not in the confirmatory test were included to check the reliability of the test. The targeted genes and the sequences of primers were as previously described (24). The PCR reaction mix contained PCR beads (puReTaq Ready-To-Go PCR beads, GE Healthcare, Little Chalfont, Buckinghamshire, UK), template DNA at a concentration of 10 ng/\( \mu \)L, sterile Milli-Q water (Millipore, Mississauga, Canada) as required to bring the total volume to 25 \( \mu \)L, and primers (supplied by Macrogen, Seoul, South Korea) for *ampC* genes as follows: 0.6 \( \mu \)M MOXM, MOXM, CITMF, CITMR, DHAMF, and DHAMR; 0.5 \( \mu \)M ACCMF, ACCMR, EBCMF, and EBCMR; and 0.4 \( \mu \)M FOXMF and FOXMR. The thermal profile (Veriti 96-well thermal cycler, Applied Biosystems, Singapore) for PCR reaction was as previously reported (24).

The DNA of *E. coli* ATCC 25922 was used as a negative control. Five-microliter aliquots of PCR products were analyzed by gel electrophoresis with 2% agarose and 0.5 \( \mu \)g/mL ethidium bromide. Gels were visualized by UV using GelDoc (GeneFlash,
Syngene, Frederick, MD). The amplicon size of PCR products was checked and compared with those previously reported (24) to determine the type of ampC β-lactamase genes. The PCR products were sequenced (Macrogen) using the same primers as used for amplification. DNA sequences were aligned and analyzed through ChromasPro program (version 1.41, Technelysium Pty. Ltd., Tewantin, Queensland, Australia) and compared online with those found in the National Center for Biotechnology Information using the BLAST program. The DNA sequences were submitted to the European Nucleotide Archive to be assigned accession numbers (LT548586 and LT548587).

**Statistical methods.** JMP version 14.3 (SAS Institute Inc., Cary, NC) was used to perform statistical tests to identify significant differences (P < 0.05). Wilcoxon–Kruskal-Wallis test (a nonparametric test used for distributions that are not normally distributed) was used to test whether the percentage of resistant enterobacteria differed significantly according to the type of antibiotic and the origin of the sample (local or imported). Steel-Dwass All Pairs test was used to identify differences among levels if they were detected by the Wilcoxon–Kruskal-Wallis test for the percentage of resistant Enterobacteriaceae.

**RESULTS**

**Resistance of Enterobacteriaceae to antibiotics.** Bacterial names, as identified by PCR, can be found in Table S2. Figure 1 shows the percentages of resistant enterobacteria. The diameters of the growth inhibition zones are displayed in Table S3. Enterobacteria had the highest antibiotic-resistance levels against ampicillin (66%), cephalothin (57%), amoxicillin–clavulanic acid (33%), and cefoxitin (31%). Nine percent of enterobacteria were resistant to tetracycline. Smaller percentages (7, 6, and 5%) of enterobacteria were resistant to nalidixic acid, trimethoprim, and kanamycin, respectively. The lowest resistance was to imipenem (3%), chloramphenicol (1%), and ertapenem (1%). All tested Enterobacteriaceae isolates were susceptible to aztreonam, cefotaxime, and ceftazidime. Nine isolates (10%) of all enterobacteria (n = 88) were pansusceptible, whereas 65 isolates (74%) were resistant to at least one antibiotic. Fourteen isolates (16%) showed multidrug resistance, i.e., resistance against three or more antibiotics belonging to different classes (Table S3 and Fig. 2).

**FIGURE 1.** Percentage of resistant Enterobacteriaceae isolated from local (nbacteria = 38) and imported (nbacteria = 50) fresh fruits and vegetables for different antibiotics (the amount of the antibiotic in the disc is provided in micrograms in parentheses). AMP, ampicillin (10); AMC, amoxicillin–clavulanic acid (30); ATM, aztreonam (30); CFX, cefotaxime (30); FOX, cefoxitin (30); CAZ, ceftazidime (30); KF, cephalothin (30); C, chloramphenicol (30); ETP, ertapenem (10); IPM, imipenem (10); K, kanamycin (30); NA, nalidixic acid (30); TE, tetracycline (30); W, trimethoprim (5).

**FIGURE 2.** Levels of antibiotic resistance in different species of enterobacteria (n = 88) isolated from fresh fruits and vegetables.
The differences in the percentage of resistant enterobacteria isolated from local as compared with imported samples were not statistically significant (Wilcoxon–Kruskal-Wallis, \( P = 0.8011, \alpha = 0.05 \)), but the type of antibiotics significantly affected the percentage of resistant Enterobacteiriaeae \( (P = 0.0120, \alpha = 0.05) \). Nevertheless, a more detailed test of Steel-Dwass All Pairs failed to detect these differences.

Table 1 shows the resistance of the most abundant enterobacteria. Enterobacter cloacae showed the highest resistance against cefotaxime (80%), followed by Pantoea dispersa (57%) and then Pantoea agglomerans (20%). E. coli isolates were resistant to more types of antibiotics than were other enterobacteria tested in this study. One isolate of E. coli (no. 11, Table S3) showed resistance against five antibiotics: ampicillin, chloramphenicol, kanamycin, nalidixic acid, and tetracycline.

**Phenotypic confirmation of antibiotic resistance of Enterobacteriaceae.** The confirmatory test for ESBL was negative for all isolates. One isolate (no. 52: E. cloacae, isolated from local cabbage) showed intermediate resistance to ertapenem (Table S3). Modified Hodge test for this isolate was negative (indicating the absence of carbapenemase). The positive control strain of K. pneumoniae ATCC BAA-1705 showed a positive result for the Modified Hodge test, as expected. Screening for AmpC \( \beta \)-lactamases was positive for 27 isolates: 14 (37%) from local samples and 13 (26%) from imported samples. Phenotypic confirmation of AmpC \( \beta \)-lactamase was done for all of the 27 isolates and for 3 other isolates (no. 60, 73 and 110) that were susceptible to cefoxitin (to check the reliability of the tests), using the inhibitor-based test and the antagonism test. As expected, the three isolates susceptible to cefoxitin produced negative results with the two confirmatory tests. The inhibitor-based test was positive for 16 isolates, and the antagonism test (Fig. S1) was positive for 19 isolates. Fifteen isolates gave positive results with the two tests (Table 2).

**Types of \( \text{ampC} \) \( \beta \)-lactamase resistance genes.** Table 2 and Figure S2 show the different types of \( \text{ampC} \) \( \beta \)-lactamase genes that were detected in eight isolates: CIT, isolate 117; EBC, isolates 56, 84, 105, and 120; FOX, isolates 57 and 117; and MOX, isolates 52 and 116. Accession numbers were given to the CIT type gene of isolate 117 (Citrobacter freundii, isolated from a tomato obtained from The Netherlands) and to the EBC type gene of isolate 56 (Enterobacter asburiae, isolated from a carrot sample from Oman).

**DISCUSSION**

The food chain may be the source for antibiotic-resistant bacteria that eventually reach humans (17, 29). Therefore, it is essential to study antibiotic resistance in bacteria isolated from food, the environment, and clinical settings (16). Many members of the family Enterobacteriaceae are among the most potent and prevalent pathogens (12), and they are also common in fresh fruits and vegetables (1) that are most often eaten raw (21). Many bacteria isolated in this study, such as E. coli, E. cloacae, and K. pneumoniae, are considered opportunistic pathogens; if they cause infection, their resistance to antibiotics can complicate treatment outcomes. Once ingested, antibiotic resistance genes, if present, may be transferred to the human gut microbiota (7, 27). The highest antibiotic resistance levels of enterobacteria in this study were achieved with \( \beta \)-lactam antibiotics: ampicillin, cephalothin, amoxicillin–clavulanic acid, and cefoxitin. All tested bacteria were susceptible to aztreonam, cefotaxime, and ceftazidime, which indicated the absence of ESBL (15). Enterobacteria isolated from vegetables by Österblad et al. (22) were also susceptible to cefotaxime and aztreonam. However, Ruizy et al. (27) demonstrated the presence of ESBL in all Raheilla spp. that were isolated from fruits and vegetables.

Only a few studies have reported the presence of AmpC \( \beta \)-lactamase in bacteria isolated from fresh fruits and vegetables. van Hoek et al. (30) detected ESBL and AmpC \( \beta \)-lactamase in bacteria isolated from vegetables. E. coli isolated from lettuce was found to produce ESBL and/or AmpC \( \beta \)-lactamase, in which \( \text{ampC} \) genes included ACC, MOX, CIT, DHA, and FOX types (20). Our results showed that screening for AmpC \( \beta \)-lactamases was positive for 27 isolates that were resistant to cefoxitin. The inhibitor-based test confirmed the presence of AmpC \( \beta \)-lactamase in 16 isolates, and the antagonism test confirmed its presence in 19 isolates. Fifteen isolates gave positive results with the two tests. Utilizing different tests to confirm the presence of AmpC \( \beta \)-lactamase can potentiate the confirmation results. The positive result of the inhibitor-based test indicates the presence of AmpC \( \beta \)-lactamase (6), whereas the antagonism between cefoxitin and cefotaxime indicates the presence of

### Table 1. Percent resistance of the most abundant enterobacteria against various antibiotics

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>n</th>
<th>AMP</th>
<th>AMC</th>
<th>FOX</th>
<th>KF</th>
<th>C</th>
<th>ETP</th>
<th>IPM</th>
<th>K</th>
<th>NA</th>
<th>TE</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter cloacae</td>
<td>15</td>
<td>87</td>
<td>80</td>
<td>80</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>13</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>11</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>7</td>
<td>24</td>
<td>47</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Pantoea agglomerans</td>
<td>10</td>
<td>50</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pantoea dispersa</td>
<td>7</td>
<td>43</td>
<td>29</td>
<td>57</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Antibiotics (with amount of the antibiotic in the disc provided in micrograms in parentheses): AMP, ampicillin (10); AMC, amoxicillin–clavulanic acid (30); FOX, cefoxitin (30); KF, cephalothin (30); C, chloramphenicol (30); ETP, ertapenem (10); IPM, imipenem (10); K, kanamycin (30); NA, nalidixic acid (30); TE, tetracycline (30); W, trimethoprim (5).
an inducible type of AmpC \(\beta\)-lactamase (15). Variations in the sensitivity and accuracy of these two tests have previously been reported (6, 11, 24).

Resistance to cefoxitin can occur due to the presence of enzymes other than AmpC \(\beta\)-lactamases or due to the presence of mutations in porin channels, which can lead to a reduction in outer membrane permeability. Cefoxitin was found to be a substrate for active efflux pumps in clinical enterobacteria (11). This may explain why some phenotypically cefoxitin-resistant isolates in this study were not proven to be functional (14). Polsfuss et al. (25) detected a CIT-type \(ampC\) gene by multiplex PCR in a clinical Enterobacteriaceae isolate, but screening for AmpC \(\beta\)-lactamase using cefoxitin was negative. Sequence analysis of the CIT-type \(ampC\) gene did not reveal any mutation affecting the function or the structure of the enzyme. However, the authors noted that mutation in the regulatory regions might result in low, or no, expression of the structural gene, resulting in phenotypic susceptibility to cefoxitin.

### Table 2.

**Confirmation of the presence of AmpC \(\beta\)-lactamase in enterobacteria using the inhibitor-based and antagonism tests and the type of \(ampC\) resistance genes**

<table>
<thead>
<tr>
<th>Bacteria no.</th>
<th>Source</th>
<th>Local/imported</th>
<th>Bacteria identity (PCR)</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inhibitor based</td>
<td>Antagonism</td>
</tr>
<tr>
<td>52</td>
<td>Cabbage, Oman</td>
<td>Local</td>
<td><em>Enterobacter cloacae</em></td>
<td>Positive</td>
</tr>
<tr>
<td>54</td>
<td>Cabbage, Netherlands</td>
<td>Imported</td>
<td><em>Kluyvera intermedia</em></td>
<td>Positive</td>
</tr>
<tr>
<td>56</td>
<td>Carrot, Oman</td>
<td>Local</td>
<td><em>E. asburiae</em></td>
<td>Positive</td>
</tr>
<tr>
<td>57</td>
<td>Carrot, Oman</td>
<td>Local</td>
<td><em>E. cloacae</em></td>
<td>Positive</td>
</tr>
<tr>
<td>60</td>
<td>Carrot, Australia</td>
<td>Imported</td>
<td><em>Pantoea agglomerans</em></td>
<td>Negative</td>
</tr>
<tr>
<td>65</td>
<td>Capsicum, Oman</td>
<td>Local</td>
<td><em>P. agglomerans</em></td>
<td>Negative</td>
</tr>
<tr>
<td>66</td>
<td>Capsicum, Oman</td>
<td>Local</td>
<td><em>P. agglomerans</em></td>
<td>Negative</td>
</tr>
<tr>
<td>71</td>
<td>Capsicum, UAE</td>
<td>Imported</td>
<td><em>E. ludwigi</em></td>
<td>Positive</td>
</tr>
<tr>
<td>72</td>
<td>Cucumber, Oman</td>
<td>Local</td>
<td><em>P. dispersa</em></td>
<td>Negative</td>
</tr>
<tr>
<td>73</td>
<td>Cucumber, Oman</td>
<td>Local</td>
<td><em>P. dispersa</em></td>
<td>Negative</td>
</tr>
<tr>
<td>74</td>
<td>Cucumber, Oman</td>
<td>Local</td>
<td><em>P. dispersa</em></td>
<td>Negative</td>
</tr>
<tr>
<td>75</td>
<td>Cucumber, UAE</td>
<td>Imported</td>
<td><em>E. ludwigi</em></td>
<td>Positive</td>
</tr>
<tr>
<td>76</td>
<td>Lettuce, Oman</td>
<td>Local</td>
<td><em>E. cloacae</em></td>
<td>Positive</td>
</tr>
<tr>
<td>76</td>
<td>Radish, Oman</td>
<td>Local</td>
<td><em>E. cloacae</em></td>
<td>Positive</td>
</tr>
<tr>
<td>91</td>
<td>Banana, Oman</td>
<td>Local</td>
<td><em>P. dispersa</em></td>
<td>Positive</td>
</tr>
<tr>
<td>105</td>
<td>Papaya, Oman</td>
<td>Local</td>
<td><em>E. cloacae</em></td>
<td>Positive</td>
</tr>
<tr>
<td>107</td>
<td>Papaya, Oman</td>
<td>Local</td>
<td><em>E. cloacae</em></td>
<td>Negative</td>
</tr>
<tr>
<td>108</td>
<td>Papaya, Thailand</td>
<td>Imported</td>
<td><em>P. eucrina</em></td>
<td>Negative</td>
</tr>
<tr>
<td>109</td>
<td>Papaya, Thailand</td>
<td>Imported</td>
<td><em>P. dispersa</em></td>
<td>Negative</td>
</tr>
<tr>
<td>110</td>
<td>Papaya, Thailand</td>
<td>Imported</td>
<td><em>E. cloacae</em></td>
<td>Negative</td>
</tr>
<tr>
<td>111</td>
<td>Papaya, Philippines</td>
<td>Imported</td>
<td><em>E. cloacae</em></td>
<td>Negative</td>
</tr>
<tr>
<td>114</td>
<td>Tomato, Oman</td>
<td>Local</td>
<td><em>Serratia marcescens</em></td>
<td>Negative</td>
</tr>
<tr>
<td>116</td>
<td>Tomato, Oman</td>
<td>Local</td>
<td><em>E. ludwigi</em></td>
<td>Positive</td>
</tr>
<tr>
<td>117</td>
<td>Tomato, Netherlands</td>
<td>Imported</td>
<td><em>Citrobacter freundii</em></td>
<td>Negative</td>
</tr>
</tbody>
</table>

\(^a\) Nine genes were identified in eight isolates. ND, not detected; UAE, United Arab Emirates; —, not applicable.

\(^b\) Accession number given by the European Nucleotide Archive.
Bacteria that give positive confirmatory results for AmpC β-lactamase should be reported as resistant to all cephalosporins even though the isolates appear to be sensitive to extended-spectrum β-lactams. This is supported by the clinical success of treating bloodstream infections caused by pathogens harboring AmpC β-lactamase when imipenem was used for the treatment and the clinical failure when treating similar infections by agents such as cefotaxime or ceftazidime (6). This is because, clinically, induction of AmpC β-lactamase leads to the production of large amounts of these enzymes even with a weak inducer (9).

Enterobacteria, isolated from vegetables by Österblad et al. (22), were susceptible to imipenem. In this study, three isolates showed intermediate resistance to imipenem: *E. coli* (no. 8), *E. ludwigi* (no. 75), and *E. cloacae* (no. 84), isolated from cabbage, cucumber, and lettuce, respectively. The last two isolates were confirmed to possess an inducible AmpC β-lactamase, which may have resulted in their reduced susceptibility to imipenem (28). Presence of metallo-β-lactamase can also lead to resistance to imipenem (31). Oteo et al. (23) investigated the emergence of imipenem resistance in clinical *E. coli* isolates during therapy. Resistance was found to be associated with the loss of two porin proteins: OmpF and OmpC. Plasmid-mediated AmpC β-lactamase and ESBL were also found. Finding reduced susceptibility to imipenem in bacteria isolated from fresh produce is significant because carbapenems such as imipenem are considered the last available line of antibiotics and are reserved for severe infections (24, 31). However, identification of mechanisms responsible for this reduced susceptibility requires further confirmation and investigation. Resistance to carbapenem can occur through different mechanisms, such as upregulation of efflux pumps, modification of outer membrane permeability, hyperproduction of AmpC β-lactamases or ESBLs, or the production of specific carbapenem-hydrolyzing β-lactamases, i.e., carbapenemases (16).

Multiple antibiotic resistance against three or more antibiotics belonging to different classes was achieved by 14 isolates (16%, no. 4, 5, 7, 8, 11, 15, 52, 56, 75, 80, 84, 91, 114, 117). Many of them (6 isolates) were *E. coli*; the others included *C. freundii*, *E. asburiae*, Enterobacter cancerogenus, *E. cloacae*, *E. ludwigi*, *P. dispersa*, and *Serratia marcescens*. Further characterization of *E. coli* isolates that possessed multiple antibiotic resistance against five antibiotics (ampicillin, chloramphenicol, kanamycin, nalidixic acid, and tetracycline) will be of great interest but was beyond the scope of this article. Even though it cannot be known exactly where contamination of the analyzed samples with antibiotic-resistant bacteria occurred, results indicate that antibiotic resistance in fresh fruits and vegetables grown in Oman is statistically similar to that present in the imported samples brought to the local market from different countries in the world.

Contaminated fresh fruits and vegetables are difficult to sanitize, especially when the bacteria become internalized within the plant tissue (21, 32). However, it is essential to minimize contamination of fresh produce with pathogenic or antibiotic-resistant bacteria. This can be achieved by implementing good agricultural and manufacturing practices. Various agents can be used for sanitation purposes, such as chlorine, electrolyzed water, benzalkonium chloride, organic acids, ozone, antagonistic bacteria, and bacteriocins (21). A detailed review of the decontamination of fruits and vegetables eaten raw can be accessed online (3).

In conclusion, enterobacteria isolated from fresh fruits and vegetables showed resistance to various antibiotics. Thus, fruits and vegetables may act as a source or vehicle for transferring antibiotic-resistant bacteria or their genes to different environmental niches. Some isolates exhibited intermediate resistance to the clinically important antibiotic imipenem. To our knowledge, this is the first report that describes the detection of an inducible type ampC β-lactamase in bacteria isolated from fresh fruits and vegetables, which is considered an important resistance mechanism. More efforts should be applied to determine the role of fruits and vegetables in the globalization of antibiotic-resistant bacteria so as to better tackle and manage the problem of antibiotic resistance in the future. Moreover, studies should be conducted to investigate at which stages or critical points contamination of fresh produce with antibiotic-resistant bacteria is most likely to occur and to determine the sources of contamination so as to establish a better control system that assures safer fresh fruits and vegetables from farm to fork.

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SUPPLEMENTAL MATERIAL

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REFERENCES


