Resistance to metal ions and antibiotics in *Escherichia coli* isolated from foodstuffs

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Summary. Of 39 strains of *Escherichia coli* isolated from foodstuffs, all were resistant to at least one of a panel of four metallic ions tested. The most common resistance (94.9%) was against cadmium, followed by arsenate (76.9%), silver (71.8%) and mercury (61.5%). Multiple resistance to three (35.9%) or four (38.5%) metals was seen more often than resistance to two (18%) or one (7.7%) metal only. The opposite trend was seen in antibiotic resistance; resistance to one (30%) or two (49%) antibiotics was more common than to three or more antibiotics (13%). Resistance to kanamycin correlated with resistance to silver and cadmium ions and resistance to ampicillin or cephalothin was, with one exception, associated with resistance to cadmium ions.

Introduction

Gram-negative bacteria are considered generally to be more resistant than gram-positive bacteria to antimicrobial agents (Hugo and Russell, 1982; Russell, 1982). The transfer of multiple drug resistance through R factors has long been recognised in the enterobacteria, especially *Escherichia coli* (Anderson, 1965; Linton et al., 1981). The plasmids of *E. coli* not only confer resistance to antibiotics and metal ions (Efstathiou and McKay, 1977; Foster et al., 1979; Chopra, 1982; Foster, 1983; Starodub and Trevors, 1989) but, in a few reports, have even been shown to confer sensitivity to mercuric chloride and antibiotics (Foster et al., 1979; Platt et al., 1984). Specific serotypes of *E. coli* have been shown to carry transmissible plasmids for both enterotoxin production (Ent+) and resistance (R) to antibiotics (Walton, 1977; Echeverria and Murphy, 1980). The occurrence of antibiotic resistant *E. coli* in man, sewage and fresh water has been reported (Grabow et al., 1975; Meckes, 1982; Towner, 1982; Antai, 1987).

This study is of the resistance, in *E. coli* strains isolated from foodstuffs, to four heavy metals and 12 antibiotics. Foods containing *E. coli* have often been implicated in specific and non-specific diarrhoea in children (Linton and Hinton, 1988), travellers' diarrhoea (Lee and Kean, 1978) and severe cholera-like illness (Sack et al., 1971). We have attempted to correlate the metal ion and antibiotic resistance in *E. coli* isolates from food.

Materials and methods

*E. coli* strains

Food samples (ice cream, curd, sweets, raw milk and rasmalai) were collected in sterile containers from approved shops in Chandigarh, India. The samples were transported to the laboratory and plated on to MacConkey's agar, within 1-2 h of sampling, for the isolation of *E. coli*. The isolates were identified by standard biochemical tests (Ørskov, 1984).

*Media and chemicals*

Culture media were obtained from Hi-Media Laboratories, Bombay, India. Reagent grade metal salts (silver nitrate, cadmium sulphate, mercuric chloride and disodium arsenate) were from Sarabhai Chemicals, Bombay, India. The antibiotic disks were from Span Diagnostics Limited, Bombay, India.

*Resistance to metal ions*

The method of Novick and Roth (1968) was followed with minor modifications for the determination of MICs of metal ions for 39 *E. coli* isolates; 20-μl inocula of an overnight (16–18 h) culture of the test strain were added to tubes of sterile Tryptone Yeast Extract (TYE) broth containing serial dilutions of the metal salts. The tubes were mixed, incubated at 37°C for 24 h, and then examined for visual turbidity. The lowest concentration of the metal salt at which growth was inhibited (indicated by lack of turbidity) was taken as the MIC. A loopful from each tube with no turbidity was subcultured on to nutrient-agar plates to determine bacteriostatic and bactericidal concentrations.

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Antibiotic susceptibility

The antibiotic resistance of 39 isolates was determined by Stokes' (1975) method with the following disks: ampicillin 10 µg; chloramphenicol 30 µg; tetracycline 30 µg; co-trimoxazole 1.25 µg; kanamycin 30 µg; gentamicin 10 µg; gatifloxacin 60 µg; cephalothin 30 µg; nalidixic acid 30 µg; furazolidone 100 µg; neomycin 30 µg and streptomycin 50 µg.

Results

In tests with cadmium sulphate and mercuric chloride, a clear difference was observed between very sensitive (MIC < 20 mg/L) and very resistant (MIC > 80 mg/L) strains, although some strains were moderately resistant (MIC 40–70 mg/L) (fig. 1). However, the demarcation was less apparent with silver nitrate and disodium arsenate. A tailing off (plateau effect) was observed among strains very resistant to disodium arsenate (MIC > 40 000 mg/L) and two peaks were observed with strains moderately resistant to silver nitrate (MIC 20–70 mg/L) (fig. 1). Of the 39 E. coli isolates tested, 94.9%, 76.9%, 71.8% and 61.5% were resistant to cadmium, arsenate, silver and mercury respectively, and 17.9%, 35.9% and 38.5% were resistant to two, three and four metals respectively (table I).

In antibiotic susceptibility tests with 39 isolates 48.7% were resistant to ampicillin, 41% to cephalothin, 15.4% to kanamycin, 7.7% to tetracycline and 2.6% to co-trimoxazole (fig. 2); 13% of the isolates were resistant to three or more antibiotics. Of the 23 isolates resistant to antibiotics, particularly to kanamycin, cephalothin or ampicillin, 59% were also resistant to cadmium sulphate. Strains resistant to tetracycline were also resistant to mercury and arsenate. The strains resistant to kanamycin were resistant to silver as well as to cadmium (table II).

Fig. 1. MICs of (A) silver nitrate (B) mercuric chloride (D) cadmium sulphate and (C) disodium arsenate for 39 food isolates of E. coli. Arrows indicate concentrations above which isolates were considered to be resistant.
Table I. Metal ion and antibiotic resistance patterns of *E. coli* isolates from foods

<table>
<thead>
<tr>
<th>Number of metal ions or antibiotics</th>
<th>Metal ion resistance</th>
<th>Antibiotic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%) of resistant isolates</td>
<td>Resistance patterns (number of isolates)</td>
</tr>
<tr>
<td>1</td>
<td>3 (7-7) Cd (3)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7 (17-9) Cd As (4), Ag Cd (1), Ag As (1), Hg As (1)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14 (35-9) Ag Cd As (6), Ag Hg Cd (5), Hg Cd As (3)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15 (38-5) Ag Hg Cd As (15)</td>
<td></td>
</tr>
</tbody>
</table>

Ag, silver nitrate; Hg, mercuric chloride; Cd, cadmium sulphate; As, disodium arsenate; A, ampicillin; K, kanamycin; Cp, cephalothin; T, tetracycline; Co, co-trimoxazole.

Discussion

The frequency of resistance to metal ions and antibiotics in *E. coli* isolates from various milk-based food samples probably reflects the degree of environmental contamination with these substances and may be related to exposure of bacteria to them (Aiking *et al.*, 1984; Russell and Gould, 1988). Human infections with antibiotic-resistant micro-organisms make treatment difficult by limiting the choice of antibiotics (Al-Jebouri and Al-Meshhadani, 1985). Therefore, any source of antibiotic-resistant or metal-ion-resistant micro-organisms must be viewed with concern, and the presence in these food samples of *E. coli* with multiple resistance to both antibiotics and metals may present a potential health hazard to the consumer. A similar degree of resistance in *Klebsiella pneumoniae* isolated from foodstuffs has recently been reported (Kaur *et al.*, 1988). The finding of co-resistance to metal ions and antibiotics contrasts with an earlier report of Hinton *et al.* (1986) in which higher proportions of antibiotic-resistant enteric organisms had been isolated from pigs.

Correlation between resistance to metal ions and antibiotics has been reported in bacterial species from different origins (Summers *et al.*, 1978; Cenci *et al.*, 1982; Porter *et al.*, 1982). Some correlation between antibiotic resistance and cadmium resistance was also seen in our study (table II). However, this study provides no evidence for the co-transferability of antibiotic and metal ion resistance because only 59% of the strains were resistant to antibiotics whereas all were resistant to metal ions.

Table II. Correlation between antibiotic and metal ion resistance of *E. coli* isolates from foods

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Number of resistant isolates (23)*</th>
<th>Number resistant to metal ion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ag (28)‡</td>
</tr>
<tr>
<td>A</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>T</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Co</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>K</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Cp</td>
<td>16</td>
<td>12</td>
</tr>
</tbody>
</table>

* Sixteen isolates were susceptible to the antibiotics tested.
‡ Numbers in parentheses are total number of isolates resistant to respective metal ion.

Fig. 2. Incidence of antibiotic resistance in *E. coli* food isolates—A, ampicillin; Cp, cephalothin; K, kanamycin; T, tetracycline; Co, co-trimoxazole.
REFERENCES


