LETTER-TO-THE-EDITOR

Antibiotic Resistance Conferred by Conjugative Plasmid in *Escherichia coli* Isolated from Community Ponds of Kathmandu Valley

Sir:

Bacteria can develop a variety of cellular mechanisms for acquiring resistance against antibiotics, including genetic mechanisms. Both commensal and pathogenic bacteria become resistant to every new antibiotic within few years of introduction. In recent years, antibiotic-resistant bacteria are being widely spread in nature. This can be due to the irrational use of sub-lethal concentrations of antibiotics in animal feeds and abuse of these substances in medical practice. The presence of antibiotic-resistant species of the *Enterobacteriaceae* family in aquatic animal and human environments has given rise to considerable concern over the recent years. Faecally-contaminated natural water systems have revealed the presence of enteric micro-organisms with genetic factors, which control antibiotic resistance. Of them, enteric bacteria with plasmids are a growing public health concern. Conjugative and transductional transfer (R plasmid) of these factors among microbial strains in the aquatic environment has already been demonstrated (1,2,3).

An important cause of rapid emergence of drug-resistant pathogens in Nepal is the irrational use of antimicrobial agents. On the other, antibiotics are taken at inappropriate dosages and duration. Laboratory facilities are often not available for isolation and sensitivity testing of pathogens. Infection control procedures in hospitals are also often inadequate, resulting in the spread of potential pathogens and resistant strains in the environment. Selection of drugs and information about drug resistance are not communicated to those who prescribe antimicrobials (4). Hence, a preliminary study was conducted to investigate the transmission of antibiotic-resistant bacteria with R plasmids in community ponds of Kathmandu.

In this study, multidrug-resistant strains of *Escherichia coli*, isolated from four different community ponds of Kathmandu valley, were characterized. Nine (40%) of 22 *E. coli* isolates from four different ponds were sensitive to all 5 antimicrobials (ampicillin, chloramphenicol, nalidixic acid, tetracycline, and trimethoprim). Table 1 shows the resistance patterns of the isolates to different drugs.

The Table 1 shows that the most common R pattern encountered was with ampicillin, tetracycline, and trimethoprim which occurred in 6 of the 22 isolates, followed by the tetracycline pattern which occurred in 2 of the 22 isolates. The rest of all other resistant patterns, viz. ampicillin, tetracycline, trimethoprim, chloramphenicol, nalidixic acid; ampicillin, tetracycline; tetracycline, trimethoprim; chloramphenicol; ampicillin, trimethoprim, was obtained in only one isolate respectively. Six (27.2%) of the 22 isolates were resistant to 3 different antibiotics, and 3 (13.6%) isolates were resistant to 2 different antibiotics, whereas the rest 3 (13.6%) isolates were resistant to only one antibiotic tested (Table 2).

Nine isolates, which were either resistant to ampicillin or to trimethoprim but sensitive to nalidixic acid, were tested by conjugation experiment (Table 3).

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**Table 1. Antibiotic resistance patterns encountered in *E. coli* isolates**

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Ampicillin</th>
<th>Tetracycline</th>
<th>Trimethoprim</th>
<th>Chloramphenicol</th>
<th>Nalidixic acid</th>
<th>No. of occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>9</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>6</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>2</td>
</tr>
</tbody>
</table>

R=resistance; S=sensitive
Table 2 shows that 5 (55.5%) of the 9 isolates possessed conjugative types of plasmids, and transferred at least two resistance markers, viz. ampicillin, tetracycline, trimethoprim and ampicillin, trimethoprim, successfully to the recipient (E. coli K12) bacterium. The plasmid profiles of donors and transconjugants clearly showed the transformation of plasmids from donors to recipients. Most isolates possessed the plasmid size ranging from 34 to 98 MDa. In most transconjugants, plasmids having a molecular weight of 98 MDa were found to be transferred (Table 3). These plasmids can be responsible for the transformation of ampicillin, tetracycline and trimethoprim resistance patterns to recipients, since most transconjugants acquiring this plasmid from donors were resistant to these 3 antibiotics. In only one transconjugant, i.e. transconjugant 8, the plasmid having a molecular weight of 70 MDa was found to be transferred. In no cases were the smaller plasmids transferred. Similarly, resistance was not transferred by conjugation in two isolates having resistance patterns ampicillin, tetracycline, trimethoprim; one isolate having resistance pattern ampicillin, tetracycline, and one isolate having resistance pattern tetracycline, trimethoprim, despite possessing the large plasmid (Table 3).

This study has demonstrated that strains of faecal E. coli with R plasmids (transferable antimicrobial resistance) are present in the community ponds of Kathmandu valley. Water from these ponds is used for recreational purpose, and, thus, such bacteria are likely to be transmitted to human. Combined resistance to ampicillin, tetracycline, and trimethoprim was most common, and resistance to these three antimicrobials was transferred in conjugation experiments. Results of studies on plasmids showed that this resistance pattern was transferred by the single plasmid of a molecular weight of 98 MDa (5). Bacterial resistance to antimicrobial agents is a major public health problem in many tropical countries (6). In the countries of South Asia, this problem has been particularly important in enteric pathogens, with both India and Bangladesh reporting outbreaks of Shigella with multiple antimicrobial resistance (7). Numerous factors might have involved for the release of resistant strains in the environment. Limited sanitation facilities (8), indiscriminate use of antibiotics (6,9) and lack of well-managed sewerage systems are important contributing factors. In conclusion, the results of our study suggest that most community ponds of the developing countries may be the reservoir of drug-resistant bacteria as observed in the case of Nepal.

REFERENCES


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