MULTIPLE ANTIBIOTIC RESISTANCE OF *AEROMONAS HYDROPHILA* ISOLATED FROM HOSPITAL SEWAGE

Fatih Matyar

*Cukurova University, Turkey*

In this study, frequency of antibiotic resistance was investigated in *A. hydrophila* from hospital effluent water in Turkey. A total of 70 *A. hydrophila* strains were isolated. The resistance of *A. hydrophila* isolates to 10 different antibiotics was investigated by agar diffusion method. Results showed that a high incidence of resistance to ampicillin (100.0%), tetracycline (100.0%) and nalidixic acid (87.1%), and a low incidence of resistance to amikacin (15.7%). In the other hand, all isolates found sensitive to nitrofurantoin. Multiple antibiotic resistance (MAR) index values ranging from 0.2 to 0.9. This study suggest that multiple antibiotic resistant *A. hydrophila* strains easilly recovered from hospital effluent waters in Turkey and these sewages may play as an important reservoirs responsible for infectious diseases pathogen *A. hydrophila*. At the same time, these bacteria may result in a potential ecological effect.

**Keywords:** *Aeromonas hydrophila*, MAR index, Hospital sewage.

**Introduction**

In the last years, the usage of antimicrobials for treatment infectious disease in the hospital, may lead to spreading antibiotic resistant pathogen bacteria in the environment. At the same time different pollutants such as pharmaceutical wastes, antibiotic residues and chemicals discharced high amount in the hospital sewages every year. Hospital effluent water can be hazardous to environment and public health since it can contain different pollutants and a lot of pathogenic microorganisms [1]. Hospital-acquired infections such as septisemia contribute significantly to increased mortality in hospitals, particularly intensive care units [2]. Misusage of antibiotic by human and poultry may results an increase in antibiotic resistance genes in environmental samples such as hospital waste water [3]. Antibiotic resistance genes can be found in sediments that are thousands of years old millenia before any modern medicine [4]. Different species of pathogen bacteria and their resistance genes can be found in the area of hospital wastewater discharge. Spreading of these bacteria and their resistance genes in the environment is a growing public health concern. Gene transfer mechanisms, between the bacteria in aquatic environments is very common. The most known mechanism for antibiotic-resistance gen transfer is via mobilization of self-transmissible plasmids in conjunction with transposons, IS-elements and integrons [5]. The members of genus *Aeromonas* are Gram-negative, non spore forming, rod shaped, facultative anaerob bacteria found wide spread in natural habitats such as fresh and brackish water, soil, sewage and wastewater [6]. *Aeromonads* are capable secreting different type of extracellular enzymes including lipase and aerolysin. Both immunocompetent and immunocompromised patients are susceptible *Aeromonas*-acquired infections. Infection caused by *Aeromonas* in humans has includes septicemia, soft tissue infections, acute
gastroenteritis and occasionally pleuropulmonary infections. The specific aims of this study were (a) to identify the *Aeromonas hydrophila* isolates recovered in hospital waste water, (b) to determine the MAR indexes of isolated strains.

**Material and Methods**

Water samples from hospital sewage were collected into sterile bacteriological sample bottles and brought to the laboratory in an ice chest immediately [7]. A total of 9 samples were examined for the presence of *A. hydrophila*. 25 mL of each samples were inoculated in 225 ml alkaline pepton water pH 8.6) and then incubated at 35°C for 24h. For isolation of *A. hydrophila*, culture were streaked on to *Aeromonas* medium base (Ryan) (Difco) using serial dilution method and then incubated at 35°C for 24-72h. For each 500 mL *Aeromonas* medium base, 1 vial of ampicillin selective supplement was used. Final pH was adjusted 8.0 at 25°C. Presumptive *A. hydrophila* isolates were obtained from *Aeromonas* medium base by colony morphology. All the isolates were characterised by phenotypical characteristics, namely Gram staining, oxidase and catalase reactions, motility, OF glucose and gelatin liquefaction tests according to Lemos et al. [8]. Identity was confirmed by using the Becton and Dickinson Crystal E/NF ID system (BBL, Cockeysville, MD, USA). These strains were identified by using the E/NF identification software (BBL, Cockeysville, MD, USA). Antibiotic resistance was determined by an agar diffusion test [9] using Mueller–Hinton agar (Difco). Ten different antibiotics were used. The antibiotics tested and their sensidisk concentrations were amikacin (AN; 30 μg), ampicillin (AM; 10 μg), imipenem (IPM; 10 μg), meropenem (MEM; 10 μg), ceftizoxime (ZOX; 30 μg), cefixime (CFM; 5 μg), chloramphenicol (C; 30 μg), nitrofurantoin (F/M; 300 μg), nalidixic acid (NA; 30 μg) and tetracycline (TE; 30 μg). Overnight nutrient broth cultures of the test strains were used and the turbidity of the inoculum as adjusted in phosphate-buffered saline (pH 7.4) to a 0.5 McFarland opacity standard (Becton and Dickinson). These cultures were then streaked onto Mueller–Hinton agar plates (Difco) using a sterile cotton swab. The antibiotic discs were dispensed using a disc dispenser (Becton and Dickinson) sufficiently separated from each other so as to avoid overlapping of inhibition zones. After 30 min, the plates were inverted and incubated at 37°C for 16–18 h. The isolates were considered sensitive according to the manufacturer’s instructions. For all isolates, we calculated the MAR indexes values (a/b, where a represents the number of antibiotics the isolate was resistant to, and b represents the total number of antibiotics the isolate was tested against) [10].

**Results and Discussion**

A total of 70 *Aeromonas hydrophila* were isolated from hospital sewage. Members of the genus *Aeromonas* are important opportunistic pathogens and agents of human and animal infections, particularly hospital and aquatic environment. *Aeromonas* spp. is can easily found in chlorinated and non-chlorinated drinking water sources and is able to survive in a different food products stored between -2 and +10 °C. Furthermore, *A. hydrophila* secretes different type of enzymes including aerolysin. Antibiotic resistance of food and clinical isolates of *Aeromonas* spp. have been extensively studied but little is known about the hospital effluent waters. Treatment of *A. hydrophila* infections can be difficult due to its natural resistance to antibiotics. When more advanced antibiotic drug regimens are needed adverse effects may result. Among the *A. hydrophila* isolates a high percentage of bacteria were resistance to ampicillin (100.0%), tetracycline (100.0%) and nalidixic acid (87.1%) and a low incidence of resistance to amikacin (15.7%), meropenem (34.3%), cefepime (34.3%) and imipenem (35.7%). In the other hand, all of the isolates found sensitive to nitrofurantoin. The trend of resistance among all isolates for ten antibiotics was found to be the following: AM=TE>NA>CPO>IN>IPM=MEM>FEP>AN>FM (Table 1).

The high degree of resistance to ampicillin and tetracycline in the present study was similar to the findings of Chandrarathna et al. [11]. As shown Table 1, *A. hydrophila* isolates showed a high resistance to antibiotics. If the bacterial isolates were resistant to two or more antibacterial agents, they were
evaluated as multiple antibiotic resistant (MAR). Among the *A. hydrophila* isolates MAR index ranging from 0.2 to 0.9. 15.7% of *A. hydrophila* isolates showed resistance to seven antibiotics (data not shown). High degree of MAR index is results significant problems for aquatic environments. Bacteria are capable to transfer some genetic elements such as plasmids and transposons. They have gained resistance to different antibacterial agents via gene transfer methods. Many hospitals, discharge antibiotics wastes in large quantities to the sewage. Antibiotic multi-resistant bacteria in aquatic environment have great importance all over the World.

**Table 1.** Percentage of resistant isolates of *Aeromonas hydrophila* from hospital effluent in Turkey to 10 antibiotics belonging 8 classes.

<table>
<thead>
<tr>
<th>Classes of antibiotics</th>
<th>Antibiotics</th>
<th>Percentage of resistant isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>Amikacin (AN, 30 µg)</td>
<td>15.7</td>
</tr>
<tr>
<td>Penicillins</td>
<td>Ampicillin (AM, 10µg)</td>
<td>100.0</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>Imipenem (IPM, 10µg)</td>
<td>35.7</td>
</tr>
<tr>
<td></td>
<td>Meropenem (MEM, 10µg)</td>
<td>34.3</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Cefepime (FEP, 30µg)</td>
<td>34.3</td>
</tr>
<tr>
<td></td>
<td>Cefpirome CPO, 30µg)</td>
<td>51.0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Chloramphenicol (C, 30µg)</td>
<td>38.6</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>Nitrofurantoin (F/M, 300µg)</td>
<td>-</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Nalidixic acid (NA, 30µg)</td>
<td>87.1</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Tetracycline (TE, 30µg)</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Figure 1.** MAR index of *Aeromonas hydrophila* from hospital effluent water.
Conclusion

*A. hydrophila* is an important agent that causes infectious diseases in human and different animals. This bacterium is easily spreading in the hospital sewages and have become a significant cause of hospital acquired infections. This study investigated that the antibiotic and resistance patterns of *A. hydrophila* from the hospital sewage. The results showed that *A. hydrophila* can easily recovered from hospital sewage and multiple antibiotic resistant *A. hydrophila* is poses a serious risk to public health.

Acknowledgement

This work was supported by Research Fund of the Cukurova University. Project Number: FBA-2018-10863.

References