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Investigation of Cephalosporin and Heavy Metal Resistance of *Aeromonas hydrophila* and *Pseudomonas aeruginosa* Strains Isolated from Hospital Sewage in Türkiye

Türkiye’de Hastane Kanalizasyonundan İzole Edilen *Aeromonas hydrophila* ve *Pseudomonas aeruginosa* Suşlarının Sefalosporin ve Ağır Metal Dirençliliğinin Araştırılması

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Abstract: In this study, 89 strains of *Aeromonas hydrophila* and 88 strains of *Pseudomonas aeruginosa* were isolated from the sewage of a university hospital in Turkey. The resistance of these bacterial isolates to 11 different cephalosporin classes’ antibiotics belonging to four generations and to 4 heavy metals was investigated. Cadmium, lead, manganese, and zinc are the heavy metals employed. There was a high incidence of resistance to cefazolin (98.9%), cefaclor (98.9%), and cefprozil (97.8%) among the *A. hydrophila* isolates. Lower resistance to cefoxitin (30.3%), cefepime (30.3%), and ceftazidime (31.4%) were found. Cefazolin, cefuroxime, cefaclor, and cefoxitin resistance was found to be (100%) among the *P. aeruginosa* isolates. Moreover, resistance rates to cefprozil (98.9%), cefixime (96.6%), and ceftizoxime (85.2%) were detected. No isolates of *P. aeruginosa* were showed resistance to ceftazidime, cefepime and ceftipime. Multiple antibiotic resistance (MAR) indexes ranged from 0.27 to 1.0 among *A. hydrophila* isolates and from 0.46 to 0.72 among *P. aeruginosa* isolates. All of the *A. hydrophila* and *P. aeruginosa* isolates showed resistance to cadmium. *A. hydrophila* and *P. aeruginosa* isolates showed low resistance to lead of 6.7% and 2.2% respectively. Results indicate that both species are easily recovered in hospital sewage and these species gained resistance to different generations of cephalosporins and heavy metals.

Keywords

- *Aeromonas hydrophila*
- *Pseudomonas aeruginosa*
- Antibiotic resistance
- Heavy metal resistance

Özet: Bu çalışmada Türkiye’de bir üniversite hastane kanalizasyonundan 89 adet *Aeromonas hydrophila* ve 88 adet *Pseudomonas aeruginosa* suşu izole edilmiştir. Bu bakteriyel izolatların dört kuşağa ait 11 farklı sınıf sefalosporin antibiyotiklerine ve 4 farklı ağır metale karşı dirençliliği araştırılmıştır. Kullanılan ağır metaller kadmiyum, kurşun, manganez ve çinkodur. *A. hydrophila* izolatları arasında sefazoline (%98,9), sefaklor (%98,9) ve sefprozile (%97,8) karşı yüksek insidansla dirençlilik bulunmuştur. Sefoksitin (%30,3), sefepim (%30,3) ve seftazidime (%31,4), karşı dirençlilik daha düşük bulunmuştur. *P. aeruginosa* izolatları arasında sefazoline, sefuroksime, sefaklor ve sefoksitine dirençlilik (%100) olarak bulunmuştur. Ayrıca, sefprozile (%98,7), sefiksime (%96,6) ve seftizoksime (%85,2), oranında karşı dirençlilik tespit edilmiştir. Hiçbir *P. aeruginosa* izolati seftazidim, sefepim ve seftipime karşı dirençlilik göstermemiştir. *A. hydrophila* izolatları arasında çoklu antibiyotik dirençliliği (ÇAD) indeksi 0,27 den 1,0’e kadar ve *P. aeruginosa* ÇAD indeksi 0,46’dan 0,72 aralığında değişiklik göstermiştir. Tüm *A. hydrophila* ve *P. aeruginosa* izolatları kadmiyuma karşı dirençlilik göstermiştir. *A. hydrophila* ve *P. aeruginosa* izolatları kurşuna karşı sırasıyla %6,7 ve %2,2 oranında düşük dirençlilik göstermişlerdir. Sonuçlar her iki türün de hastane kanalizasyonundan kolaylıkla izole edilebildiğini ve bu türlerin farklı kuşak sefalosporinlere ve ağır metallere karşı dirençlilik kazandığını göstermektedir.

Anahtar kelimeler

- *Aeromonas hydrophila*
- *Pseudomonas aeruginosa*
- Antibiyotik dirençliliği,
- Ağır metal dirençliliği



1. INTRODUCTION

Among the beta-lactam class antibiotics, cephalosporins are the most widely used for the treatment of infectious diseases caused by both Gram-negative and Gram-positive bacteria (Wang et al., 2017). Since they were discovered by Giuseppe Brotzu in 1945, cephalosporins have become the most popular antibiotics worldwide. Cephalosporins, which are derived from cephalosporium mold, are a group of broad-spectrum, semi-synthetic beta-lactam antibiotics. They are divided into three groups: cephalosporins N, C, and P. Semi-synthetic, broad-spectrum cephalosporins have been produced by adding different side chains to the cephalosporin C base. Cephalosporins are classified according to the chronological order in which they were produced. Currently, there are five different generations of cephalosporin that are used in the treatment of infections. Many of the different antibiotic residues are discharged into hospital sewage. Because of the lack of appropriate treatment of hospital sewage water in developing countries, a large number of bacteria, particularly pathogenic species, could gain resistance from these types of antibiotic residues. Moreover, many antibiotic-resistant pathogenic bacteria could be found near the hospital and domestic sewage discharging systems (Choi et al., 2003).

In the last fifty years, the widespread use of antibiotics in human and animal infectious diseases has increased the number of resistant bacteria included as pathogenic species. Many studies have shown the transmission of drug-resistant bacteria from the environment to humans through the food chain or through contact with animals (Witte, 1998). Gene transfer mechanisms in aquatic environments are widely common between bacteria. The most known mechanism for drug-resistant gene transfer is by mobilizing self-transmissible plasmids in conjunction with transposons, IS-elements, and integrons (Tennstedt et al., 2003).

Hospitals discharge a considerable amount of water every day. Mishra et al. (2016) reported that the wastewater generated by developing countries varies from 200 to 400 L/day/bed, while in developed countries, it varies from 400 to 1200 L/day/bed. It is well known that hospital effluent water can contain many hazardous residues such as chemicals (heavy metals), radioactive and pharmaceutical substances, and various toxic remains. Such wastewater, if not treated with appropriate methods, can affect a change in environmental balance (Gautam et al., 2007). Heavy metals have a toxic effect on microorganisms and block enzymatic actions, impair nucleic acids, and disrupt cell membrane functions (Gadd, 1992). As a result of discharging untreated hospital effluent water into aquatic environments, water pollution due to toxic metals and antibiotic residues has posed important issues in aquatic environments because pathogenic microorganisms can gain resistance after exposure to these kinds of agents (Jana & Bhattacharya, 1988). In most countries, including India (Balakrishna et al., 2017) and Spain (Gutierrez-Sanchez et al., 2022), very few studies were carried out for the treatment of hospital wastewater. However, a large amount of untreated wastewater is discharged from hospitals (McCarthy et al., 2021).

Aeromonas hydrophila and *P. aeruginosa*, which are opportunistic Gram-negative pathogens, have recently attracted much interest. There are some studies regarding antibacterial and heavy metal resistance of *A. hydrophila* and *P. aeruginosa* isolated from different sources, such as from aquatic environments (Piotrowska & Popowska, 2014), domestic water (Hassani et al., 1992), aquaculture (Matyar, 2007), fish (Filik et al., 2021), soil (Malik & Jaiswal, 2000), and nosocomial infections (Micek et al., 2015; Song et al., 2018). The present study is the first to determine the prevalence and resistance of *A. hydrophila* and *P. aeruginosa* from hospital sewage in Turkey to cephalosporins and heavy metals. The aims of the present work were investigate the cephalosporin and heavy metal resistivity of the *A. hydrophila* and *P. aeruginosa* isolated from hospital sewage to determine the frequency of bacterial resistivity and possible relationship between antibiotic and metal resistance.

2. MATERIALS AND METHODS

2.1. Sampling

Hospital sewerage samples were collected from 0-20 cm using sterile 250 ml bottles (APHA, 1992). All of the samples were transported to the laboratory in an ice chest and processed within 3 hours of collection. A total of 24 samples were examined for the presence of *Aeromonas hydrophila* and *Pseudomonas aeruginosa* from Cukurova University Balcali Hospital.

2.2. Isolation of *Aeromonas hydrophila* and *Pseudomonas aeruginosa*

For the isolation of *A. hydrophila* and *P. aeruginosa*, 25 ml of each water sample was inoculated into 225 ml of alkaline peptone water (pH 8.6) with 1% NaCl (w/v) and incubated at 30 °C for 24 h. Then, the samples were plated onto selective media for each species (*Aeromonas* medium base (Ryan) (Oxoid) and *Pseudomonas* isolation agar (Difco)) and then incubated at 30 °C for 24-72 h. For each 500 mL *Aeromonas* medium base, 1 vial of ampicillin selective supplement was used. The final pH was adjusted to 8.0 at 25 °C. Presumptive *A. hydrophila* isolates were obtained from the *Aeromonas* medium based on colony morphology (good growth; opaque colonies with dark centers). *A. hydrophila* was identified based on the findings of Gram-negative rods, fermentation of D-glucose, a positive oxidase test, motility and the absence of growth in 6.5% sodium chloride (Ko et al., 2000).

The isolates were considered to be presumptive *P. aeruginosa* if they were Gram-negative rods that produced green to blue-green fluorescent colonies on *Pseudomonas* isolation agar, were oxidase- and catalase-positive and were glucose oxidative and positive for citrate.

Presumptive *A. hydrophila* and *P. aeruginosa* colonies were subcultured on tryptone soy agar (Oxoid) at 30 °C for 24 hours to obtain at least two consecutive pure cultures. The identity was confirmed using the Becton and Dickinson Crystal E/NF ID system (BBL, Md, USA). These strains were identified using E/NF identification software (BBL, Md, USA). A number of isolates (20%) were re-examined to determine the reproducibility of the test.

2.3. Antibiotic resistance tests

In this study, antibiotic resistance was determined using the disc diffusion method (NCCLS, 1997) and Mueller-Hinton agar (Difco). A total of eleven different antibiotics were selected from the cephalosporin class and represented 4 different generations used in this study, as shown below. Cefazolin (CZ, 30 µg), cefuroxime (CXM, 30 µg), cefoxitin, FOX 30 µg), cefaclor (CEC 30 µg), cefprozil (CPR, 30 µg), ceftizoxime (ZOX, 30 µg), cefixime (CFM, 5 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), cefepime (FEP, 30 µg), and ceftiofime (CPO, 30 µg) were used. The isolates were determined to be sensitive according to the information supplied by the manufacturer (BBL, Md, USA).

The reference strains *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as control organisms for verification of the antibacterial effect of the discs, as recommended by NCCLS (1997). All discs were purchased from Becton Dickinson (BBL, Md, USA).

For all isolates, the MAR (multiple antibiotic resistance) index values were calculated (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). A MAR index value >0.2 is observed when the isolates are exposed to high-risk sources of human or animal contamination, where antibiotics use is common; in contrast, a MAR index value ≤ 0.2 is observed when antibiotics are seldom or never used (Krumperman, 1983).

2.4. Determination of the minimal inhibitory concentration (MIC) of heavy metals

In this work, the MIC of four different heavy metals (Cd^{+2} , Pb^{+2} , Mn^{+2} and Zn^{+2}) was determined for each strain using Mueller-Hinton agar (Difco) containing each heavy metal in concentrations ranging from 12.5 µg/mL to >3200 µg/mL. The following four heavy metals were used in the compounds $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, $\text{MnCl}_2 \cdot 2\text{H}_2\text{O}$, and ZnCl_2 (Merck). The isolates were considered resistant if the MIC values exceeded that of the control organism. An *Escherichia coli* K-12 strain was used as the control organism as described by Ansari and Malik (2007).

3. RESULTS AND DISCUSSION

3.1. *Aeromonas hydrophila* and *Pseudomonas aeruginosa* isolates

In this work, a total of 89 *A. hydrophila* and 88 *P. aeruginosa* isolates were obtained from hospital sewage. Rhodes et al. (2000) isolated 72 mesophilic aeromonads from untreated hospital sewage in their work. Rahman et al. (2009) isolated 201 *Aeromonas* spp. in their study, which was performed in a duckweed aquaculture-based hospital sewage water recycling system in Bangladesh. In another study, 97 *Aeromonas* spp. strains were isolated from Southern Turkey's fresh and brackish water by Matyar et al. (2007). In Brasil, 25 *P. aeruginosa* isolates were recovered from raw hospital effluent (Magalhães et al., 2016). Hospital wastewater contains high amounts of pathogenic microorganisms including *P. aeruginosa* and *A. hydrophila*. As a member of the genus *Aeromonas*, *A. hydrophila* is

found in a wide range of habitats, including soil and different types of aquatic sources. This microorganism is even found in hospital water supply systems (Picard & Goulled, 1987). *A. hydrophila* is capable of secreting different types of extracellular enzymes, including lipase and aerolysin (Li et al., 2015). Both immunocompetent and immunocompromised patients are susceptible to *Aeromonas*-acquired infections. Infections caused by *Aeromonas* in humans include septicaemia, soft tissue infections, acute gastroenteritis, hepatobiliary tract infections, and occasionally pleuropulmonary infections (Janda & Abbott, 1998). Kueh et al. (1992) researched the probability of wound infection with sewage-related microorganisms following exposure to contaminated seawater. The authors determined that *A. hydrophila*, *Vibrio cholerae*, *Vibrio alginolyticus* and *Vibrio parahaemolyticus* were among the bacteria that were able to cause different kinds of infections. Furthermore, the authors observed that these bacteria were present in coastal waters and even in unpolluted regions.

The other species isolated in this study, *P. aeruginosa* is a bacterium that is common in humid and aquatic environments such as on plants and in soil, fresh and brackish water. This bacterium can also colonize hospital environments. These environments may include shower rooms and sinks, and thus, it is a common cause of hospital-acquired infections (Kerr & Snelling, 2009). As an opportunistic human pathogen, *P. aeruginosa* is a leading aetiological factor of nosocomial infections, especially in immunocompromised patients (Cross, 1985). This bacterium shows a noteworthy capability to resist different classes of antibiotics either intrinsically or following the acquisition of different resistance genes.

3.2. Resistance patterns against cephalosporins

As shown in Table 1, a high percentage of *A. hydrophila* isolates were found to be resistant to first- and second-generation cephalosporin. A total of 98.9%, 98.9%, and 97.8% of *A. hydrophila* isolates were found to be resistant to cefazolin, cefaclor, and cefprozil, respectively. Conversely, *A. hydrophila* isolates showed relatively low resistance to third- and fourth-generation cephalosporin: 46.1%, 46.1%, 41.1%, and 31.1% were resistant to ceftizoxime, cefixime, cefotaxime and ceftazidime (third-generation cephalosporins), respectively, and 43.8% and 30.3% were resistant to ceftipime and cefepime (fourth- generation cephalosporins), respectively. The trend of resistance among all *A. hydrophila* isolates to the eleven antibiotics was the following:

CZ=CEC>CPR>CXM>ZOX=CFM>CPO>CTX>CAZ>FOX=FEP.

In a study performed in German intensive care units, a dramatic increase in resistance to third-generation cephalosporin was found among *E. coli* isolates between 2001 to 2008 (Meyer et al., 2010).

Table 1. Percentage of resistant isolates of *Aeromonas hydrophila* and *Pseudomonas aeruginosa* from hospital sewage in Türkiye to 4 different generations of cephalosporins. The number of isolates is shown at the top of each column.

Generation of cephalosporins	Antibiotic	Percentage of resistant isolates	
		<i>Aeromonas hydrophila</i> N=89	<i>Pseudomonas aeruginosa</i> N=88
I.	Cefazolin (CZ, 30 µg)	98.9	100.0
II.	Cefuroxime (CXM, 30µg)	48.3	100.0
	Cefoxitin (FOX, 30 µg)	30.3	100.0
	Cefaclor (CEC, 30 µg)	98.9	100.0
	Cefprozil (CPR, 30 µg)	97.8	98.9
III.	Ceftizoxime (ZOX, 30 µg)	46.1	85.2
	Cefixime (CFM, 5 µg)	46.1	96.6
	Cefotaxime (CTX, 30 µg)	41.6	20.5
	Ceftazidime (CAZ, 30 µg)	31.4	-
IV.	Cefepime (FEP, 30 µg)	30.3	-
	Cefpirome (CPO, 30 µg)	43.8	-

N: The numbers of isolates

Matyar et al. (2010) found a high percentage of *Aeromonas* spp. resistant to cefazolin (66.6%). The incidence of resistance to antibiotics among *Aeromonas* spp. isolated from aquatic environments is rapidly increasing (Rhodes et al., 2000).

Among the *P. aeruginosa* isolates all of the strains showed resistance to the first- generation cephalosporin cefazolin (100%). Therewithal, all of the *P. aeruginosa* isolates showed resistance to three of four second-generation cephalosporins tested: cefuroxime (100%), cefoxitin (100%) and cefaclor (100%). Similarly, a high percentage of *P. aeruginosa* isolates were found resistant to two of four third-generation cephalosporins: cefixime (96.6%) and ceftizoxime (85.2%). Conversely, these isolates showed a low resistance to cefotaxime (20.5%) (a third-generation cephalosporin). None of the *P. aeruginosa* isolates showed resistance to ceftazidime (a third-generation cephalosporin), cefepime or cefpirome (fourth-generation cephalosporins). The trend of resistance among *P. aeruginosa* isolates for cephalosporin class antibiotics was found to be the following:

CZ=CXM=FOX=CEC>CPR>CFM>ZOX>CTX>CAZ=FEP=CPO (Table 1).

These results revealed that the resistance to fourth-generation cephalosporins, was relatively infrequent among the *A. hydrophila* and *P. aeruginosa* isolates. Matyar et al. (2010) found in their study, which was performed at Iskenderun Bay, Turkey, that a high percentage of *Pseudomonas* isolates were resistant to cefazolin (84.8%) and cefuroxime (71.7%). The findings of this study were similar with the results of Matyar et al. In contrast, Blandino et al. (2004) found that 33.3% and 16.7% of 108 clinical isolates of *P. aeruginosa* were resistant to ceftazidime and cefepime, respectively. Kato et al. (2015) indicated that the most used antibiotic class against *Pseudomonas* was cephalosporin from 2009 to 2010 and in 2012 in a university hospital in Japan. Recent studies have shown that among *P. aeruginosa* isolates the resistance to antimicrobials can be transferred by transduction (Masuda & Ohya, 1992), conjugation (Watanabe et al., 1991) or transposition (Yobe et al., 1996). Ben-Mahrez et al. (1999), which indicates that clinical isolates of *P. aeruginosa* expressed an inducible chromosomal cephalosporinase in their study.

3.3. Multiple antibiotic resistance index

In this study, *A. hydrophila* and *P. aeruginosa* isolates, which were resistant to three or more antibiotics, were designated as multiple-antibiotic resistant. A high frequency of multi-resistant *A. hydrophila* was found in this study. MAR index values ranged from 0.27 to 1.0 for this species (Figure 1). Matyar et al. (2007) found that MAR index values ranged from 0.2 to 0.8 for *A. hydrophila* isolates from environmental water in their work performed in southern Turkey.

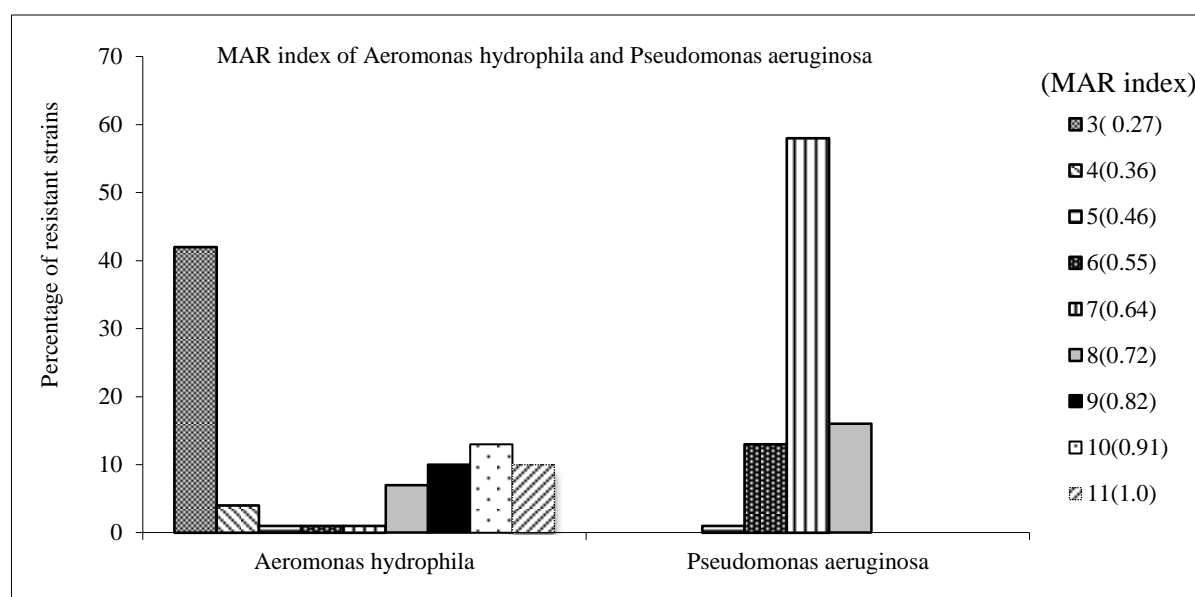


Figure 1. Antibacterial multi-resistance of *Aeromonas hydrophila* and *Pseudomonas aeruginosa* isolated from hospital sewage in Turkey.

The findings of this study were similar to those of Radu et al. (2003), who detected a high percentage of multiple resistant *Aeromonas* spp. from retail fish in Malaysia. In this work, MAR index values ranged from 0.46 to 0.72 for *P. aeruginosa* isolates. Magalhães et al. (2016) found that MAR index values were between 0.21-0.86 for *P. aeruginosa* isolated from a stream receiving effluents from ineffective hospital wastewater treatment plants. Because of multiple inherent resistance mechanisms and the presence of a lipopolysaccharide outer membrane and efflux pumps (Fair & Tor, 2014), Gram-negative bacteria create a problem for the treatment of infectious diseases. Uncontrolled or misuse of different classes of antibiotics for the treatment of infectious diseases promotes resistance to antibiotics among bacteria. In addition, the hospital sewage environment may be a powerful incubator for new combinations of virulence properties of bacteria. In the last decade, there has been an increased effort toward controlling the use of antibiotics and promoting public awareness of the cautious use of antibiotics.

3.4. Heavy metal resistance

In the present study, resistance to four heavy metals (Cd^{+2} , Pb^{+2} , Mn^{+2} and Zn^{+2}) was investigated for all of the *A. hydrophila* and *P. aeruginosa* isolates. The trend in heavy metal resistance was $\text{Cd} > \text{Mn} > \text{Zn} > \text{Pb}$ for *A. hydrophila* and $\text{Cd} = \text{Mn} > \text{Zn} > \text{Pb}$ for *P. aeruginosa* isolates (Table 2). The resistance to four heavy metals for *A. hydrophila* and *P. aeruginosa* isolates was as follows: to cadmium, 100.0% and 100.0%; to manganese, 97.8% and 100.0%; to zinc, 51.7% and 100.0%; and to lead, 6.7% and 2.2%, respectively. In a study performed in bacterial isolates from sea water by Altug & Balkis (2009), it was found that the trend in resistance to seven heavy metals was $\text{Cu} > \text{Mn} > \text{Ni} > \text{Zn} > \text{Pb} > \text{Cd} > \text{Fe}$. Matyar et al. (2012) in their study in Iskenderun Bay found a trend in heavy metal resistance of $\text{Cu} > \text{Cd} > \text{Mn} > \text{Cr} > \text{Pb}$. The findings of present research were similar with the results of Matyar et al.

In this study, the minimal inhibitory concentrations (MIC) of the *A. hydrophila* and *P. aeruginosa* isolates ranged from 25 µg/ml to 3,200 µg/ml. *P. aeruginosa* isolates showed higher resistance to manganese and zinc than did the *A. hydrophila* isolates. Conversely, *A. hydrophila* isolates showed higher resistance to lead than did the *P. aeruginosa* isolates. Resistance to cadmium was similar between *A. hydrophila* and *P. aeruginosa* isolates. Tolerance to the highest MIC (>3,200 µg/ml) of manganese was only found in *A. hydrophila* isolates. A total of 93.3% of *A. hydrophila* isolates showed resistance to the highest MIC of manganese. It is known that aside from antimicrobial residues, there are a significant number of radioactive elements and heavy metals in hospital effluent water.

Table 2. Heavy metal tolerance in *Aeromonas hydrophila* and *Pseudomonas aeruginosa* from hospital sewage in Turkey.

Heavy metal/ species	Number of total isolates	Metal concentrations (µg/mL) with the number of tolerant isolates									Resistant isolates	
		25	50	100	200	400	800	1600	3200	>3200	n	%
Cadmium												
		a										
<i>Aeromonas hydrophila</i>	89				10	40	11	13	4	11	89	100.0
<i>Pseudomonas aeruginosa</i>	88					69	4	8	2	5	88	100.0
Total	177										177	100.0
Lead												
		a										
<i>Aeromonas hydrophila</i>	89						83		6		6	6.7
<i>Pseudomonas aeruginosa</i>	88						86		2		2	2.2
Total	177										8	4.5
Manganese												
		a										
<i>Aeromonas hydrophila</i>	89							2	4	83	87	97.8
<i>Pseudomonas aeruginosa</i>	88								23	65	88	100.0
Total	177										175	98.9
Zinc												
		a										
<i>Aeromonas hydrophila</i>	89				43	16	12	17		1	46	51.7
<i>Pseudomonas aeruginosa</i>	88					25	46	13	3	1	88	100.0
Total	177										134	75.7

a = Minimal inhibition concentration of standard strain *Escherichia coli* K12.

n= Total number of tolerant isolates.

The high heavy metal resistance detected in the isolates could result from these types of contaminants. Metal-resistant *A. hydrophila* isolates also showed high resistance to three antibiotics: cefazolin, cefaclor, and cefprozil. However, *P. aeruginosa* isolates that were metal resistant showed high resistance to seven antibiotics: cefazolin, cefuroxime, cefoxitin cefaclor, cefprozil, cefixime, and ceftizoxime. The correlation between heavy metal resistance and resistance to antibiotics is very common in the same organism (and also in the same transposon, plasmid, or integron), which is evidence that industrial pollution most likely selects for antibiotic resistance and *vice versa* (Baker et al., 2006).

In the present research, antibiotic and heavy metal resistance patterns of *A. hydrophila* and *P. aeruginosa* isolates from the effluent water of a university hospital were investigated. The results of this study suggest that the hospital effluent water is a reservoir for antibiotic and heavy metal-resistant *A. hydrophila* and *P. aeruginosa* isolates. Contaminated water with antibiotics, disinfectants, pesticides, and heavy metals might encourage selective activities and result in antibiotic and heavy metal resistance.

The presence of opportunistic species responsible for serious infectious diseases in hospital wastewater could cause significant health problems for humans and animals that live close to these water effluents. In the past 25 years, only two new cephalosporin-beta-lactamase inhibitor combinations, ceftolozane/tazobactam in 2014 and ceftazidime/avibactam in 2015, have been approved to treat systemic bacterial infections caused by multi-drug resistant Gram-negative bacteria (Liscio et al., 2015). Through gene exchange or other means, resistance to new antibacterial agents could cause newly found antibiotics to be ineffective against bacteria. This situation will force pharmacologists to discover new and effective antibiotics in the near future.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

AUTHOR CONTRIBUTION

Single Author.

ETHICAL STATEMENTS

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of the present study are available from the corresponding author upon reasonable request.

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