Conference of the International Journal of Arts & Sciences,
CD-ROM. ISSN: 1943-6114 :: 10(02):115–120 (2017)

ANTIBIOTIC MULTIRESISTANCE OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM HOSPITAL EFFLUENT WATER

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The present study was carried out to assess the isolation and determination level of antibiotic resistance patterns of *Pseudomonas aeruginosa* from the hospital effluent water. A total of 72 *P. aeruginosa* strains was isolated from the hospital sewages. The resistance of *P. aeruginosa* isolates to 10 different antibiotics was investigated by agar diffusion method. Results revealed a high incidence of resistance to ampicillin (100.0%), nitrofurantoin (100.0%), nalidixic acid (100.0%) and tetracycline (100.0%), and a low incidence of resistance to meropenem (1.4%) and amikacin (5.6%). In the other hand, all of the isolates found sensitive to imipenem. In this study the multiple antibiotic resistance (MAR) index range found very high (from 0.6 to 0.8). Results show that the hospital effluent water has an important amount of antibiotic multi-resistant *P. aeruginosa* strains and these bacteria may result in a potential risk for environment.

Keywords: Pseudomonas aeruginosa, Antibiotic resistance, Hospital sewage, Environment.

Introduction

The usage of antibiotics for treatment infectious disease in the hospital, may lead to spreading antibiotic resistant species in the environment. In the other hand, pollutants such as antibiotics, pharmaceutical wastes and chemicals discharced high amount in the sewages every year. Hospital wastewater can be hazardous to public health and environment since it can contain many kinds of pollutants and also pathogenic microorganisms [1]. Uncontrolled or misusage of antibiotic by human and poultry may results an increase in antibiotic resistance genes in environmental samples such as hospital waste water [2]. Antibiotic resistance in the environment can be transferred from pathogenic bacteria to friendly bacteria, which impair water ecology through change population dynamics and physiology [3]. 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. Pseudomonads are an ubiquitous group of environmental Gram-negative bacterial organisms. *P. aeruginosa* which is opportunistic pathogens that can cause to various diseases such as chronic respiratory infection. *P. aeruginosa* is the third most common pathogen responsible for hospital-acquired (nosocomial) infections [4]. The specific aims of this study were (a) to identify the *Pseudomonas aeruginosa* isolates recovered in hospital effluent water, (b) to determine the MAR indexes of isolated strains.

Material and Methods

Samples from hospital sewage were collected into sterile bacteriological sample bottles and brought to the laboratory in an ice chest immediately [5]. A total of 8 samples were examined for the presence of *P aeruginosa*. 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 *Pseudomonas aeruginosa,* culture were streaked on to *Pseudomonas* Isolation Agar (Difco) using serial dilution method and then incubated at 35°C for 24h. The samples were then plated onto *Pseudomonas* Isolation Agar and then incubated at 35°C for 24-48 h. Presumptive *Pseudomonas aeruginosa* isolates were obtained from *Pseudomonas* Isolation Agar by colony morphology (green to blue-green colonies with pigment that diffuses into the medium). 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. [6]. 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 [7] using Mueller–Hinton agar (Difco). Ten different antibiotics (representing eight classes) 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 ontoMueller–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 index 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) [8].

Results and Discussion

A total of 72 *Pseudomonas aeruginosa* were isolated from hospital effluent water. Members of the genus *Pseudomonas* are important pathogens and agents of human infections, particularly hospital environment. *P. aeruginosa* is the common species found in the hospital sewages that can cause disease plants and animals, including humans. *P. aeruginosa* is considered opportunistic insofar as serious infection often occurs during existing diseases or conditions – most notably cystic fibrosis and traumatic burns. It is also found generally in the immunocompromised patients but can infect the immunocompetent as in hot tub folliculitis. *P. aeruginosa* is responsible for pneumoniae, (16% of nosocomial pneumonia), urinary tract infections (12% of nosocomial urinary tract infections). This bacterium has several virulence factors included a large number of cell associated factors. Treatment of *P. aeruginosa* 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 *P. aeruginosa* isolates a high percentage of bacteria were resistance to ampicillin (100.0%), nitrofurantoin (100.0%), nalidixic acid (100.0%) and tetracycline (100.0%), and a low incidence of resistance to meropenem (1.4%) and amikacin (5.6%). In the other hand, all isolates found sensitive to imipenem. None of the *P. aeruginosa* isolates were resistant to imipenem. The trend of resistance among all isolates for ten antibiotics was found to be the following: AM=FM=NA=TE>CFM>C>ZOX>AN>MEM>IPM (Table 1).

Table 1. Percentage of resistant isolates of *Pseudomonas aeruginosa* from hospital sewages in Turkey to 10 antibiotics belonging 8 classes

		P. aeruginosa N=72
Classes of antibiotics	Antibiotics	Percentage of resistant isolates
Aminoglycosides	Amikacin (AN, 30 µg)	5.6
Penicillins	Ampicillin (AM, 10µg)	100.0
Carbapenems	Imipenem (IPM, 10µg)	-
	Meropenem (MEM, 10µg)	1.4
Cephalosporins	Ceftizoxime (ZOX, 30µg)	81.9
	Cefixime (CFM, 5µg)	97.2
Chloramphenicol	Chloramphenicol (C, 30µg)	88.9
Nitrofurantoin	Nitrofurantoin (F/M, 300µg)	100.0
Quinolones	Nalidixic acid (NA, 30µg)	100.0
Tetracyclines	Tetracycline (TE, 30µg)	100.0

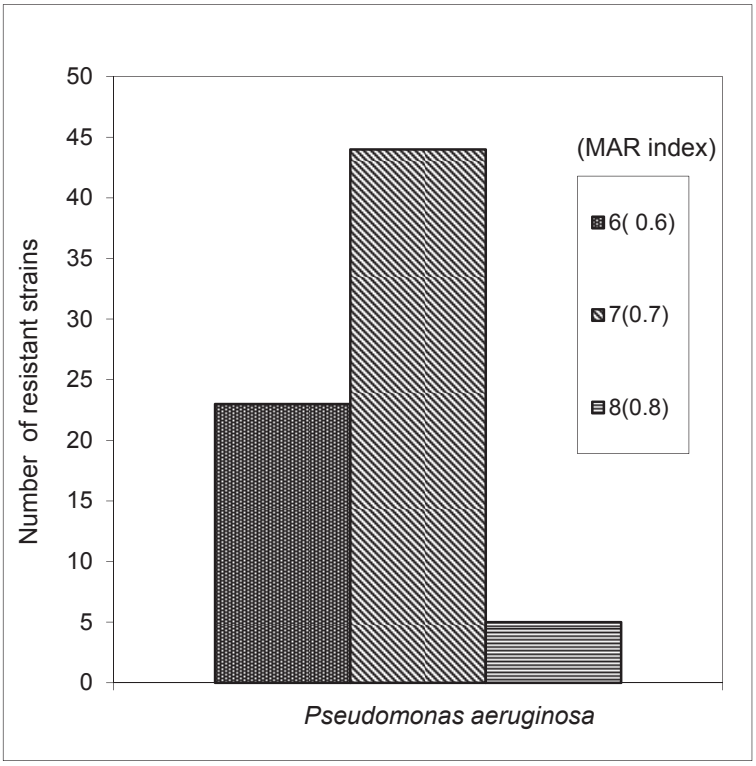

Figure 1. MAR index of *Pseudomonas aeruginosa* from hospital sewages

The high degree of resistance to ampicillin and chloramphenicol in the present study was similar to the findings of Malik and Aleem [9]. As shown Table 1, *P. aeruginosa* 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 *P. aeruginosa* isolates MAR index ranging from 0.6 to 0.8. 61.1% of *P. aeruginosa* isolates showed resistance to seven antibiotics (data not shown). High degree of MAR index is results significant problems for aquatic environments. It is known gen transfer methods since 1928. Bacteria have plasmids and transposons as extrachromosomal genetic elements. They have gained resistance to different antibacterial agents via gene transfer methods.

Conclusion

P. aeruginosa is a major cause of hospital accuired infectious diseases. There is a serious risk of spread and infection with multiple antibiotic resistant *P. aeruginosa*. Several multiple antibiotic resistant bacteria including *P. aeruginosa* are spreading in the hospital sewages and have become a significant cause of hospital accuired infections. This study investigated that the antibiotic and resistance patterns of *P. aeruginosa* from the hospital effluent water. The results showed that the these environments were of poor microbiological quality.

Acknowledgement

This work was supported by Research Fund of the Cukurova University. Project Number: FBA-2017-8910.

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