

Effect of water reservoirs types on the prevalence and antibiotic resistance profiles of *Pseudomonas aeruginosa* isolated from bathroom water in hospitals

Sri Agung Fitri Kusuma,
Tina Rostinawati,
Rini Hendriani¹,
Muhammad Faisal Budiman²,
Ida Parwati³

Department of Biology Pharmacy,
Faculty of Pharmacy, Padjadjaran
University, Sumedang, West
Java, Indonesia, ¹Department of
Pharmacology and Clinical Pharmacy,
Faculty of Pharmacy, Padjadjaran
University, Sumedang, West Java,
Indonesia, ²PT Kimia Farma Tbk,
Bandung, Indonesia, ³Clinical Pathology
Department, Faculty of Medical,
Padjadjaran University, Bandung,
Indonesia

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ABSTRACT

This study was aimed to isolate and characterize *Pseudomonas aeruginosa* antibiotic resistance profiles that isolated from bathroom water of five hospitals in Bandung, Indonesia, with different types of water reservoirs. Total of 25 water samples from bathrooms of five hospitals were collected and analyzed for the existence of *P. aeruginosa* colonies on the surface of MacConkey agar media using a streak plate method and identified using phenotypic identification and a series of biochemical tests. All *P. aeruginosa* isolates were tested against ceftazidime, piperacillin/tazobactam, ciprofloxacin, meropenem, and gentamicin containing in paper disc, using the agar diffusion method. Of all samples, the total number of *P. aeruginosa* isolates was less than that of non-*P. aeruginosa*. In hospitals that use permanent bathtubs, a greater total bacterial count was obtained than those using pails. From 110 isolates, 14.54% were multidrug resistance antibiotics. The majority of the resistant isolates were from hospital B with permanent bathtubs. Of 25 isolates from that hospital, *P. aeruginosa* isolates were resistant to ceftazidime (20%), piperacillin/tazobactam (4%), ciprofloxacin (20%), and gentamicin (20%). The multiple antibiotic resistance index value of *P. aeruginosa* isolates was 0.4–0.6. Thus, it can be concluded that the bathroom water in the hospital with permanent bathtubs were potential reservoirs of antibiotic-resistant *P. aeruginosa*.

Key words: Antibiotic, bathroom, hospital, *Pseudomonas aeruginosa*, resistance, water

INTRODUCTION

The hospital is a place for patients to treat the disease, both inpatient and outpatient. However, this goal does not always have a positive impact on patients, especially in patients who are known to have weak immune systems

Address for correspondence:

Dr. Sri Agung Fitri Kusuma,
Komplek Permata Biru, Cinunuk, Cileunyi, West Java Indonesia.
E-mail: s.a.f.kusuma@unpad.ac.id

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so that they are easily infected with other microorganisms. As a result, it worsens the patient's condition and increases the cost of treatment. Infections that occur in hospitalized patients are called nosocomial infections. This infection can attack the urinary tract (34%), patients after surgery (17%), lower respiratory tract (13%), blood vessels (14%), etc., (21%).^[1] Among the reported causal bacteria of the nosocomial infection, *Pseudomonas aeruginosa* was found as the most common of nosocomial infections causative agent.^[2,3]

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P. aeruginosa infection causes disease in various tissues, including the circulatory system (bacteremia and septicemia), respiratory tract, central nervous system, heart (endocarditis), ears (including external otitis), eyes, bones, urinary tract, gastrointestinal tract, and leather.^[14-6] It was reported that the septicemia caused by *P. aeruginosa* reached an 80% mortality rate.^[7] Several antibiotics such as: antibiotics penicillin group (aminopenicillin, carboxypenicillin, piperacillin), cephalosporin (ceftazidime, cefoperazone, cefepime) groups, imipenem, aztreonam, chloramphenicol, aminoglycosamin, gentamycinamycin, gentinycinamycin, cannocinamycin, and the fluoroquinolone group (ciprofloxacin, ofloxacin, levofloxacin) were administered as the treatments for *P. aeruginosa* infections.^[8] However lately, some studies reported the multidrug antibiotic resistance of *P. aeruginosa* clinical isolates.^[9] The increasing rates of resistant *P. aeruginosa* in hospitalized patients have a significant impact to the increasing of mortality rate and hospital length of stay. The discovery of these several resistance cases, encouraging the spread of *P. aeruginosa* with focusing on their habitat sources, not only in human individual factors, but also the hospital environment deserves serious attention. Although the consensus of the role of contaminated water in *P. aeruginosa* infection transmission is still debated, several studies have demonstrated that water is a major contributor to the spread of *P. aeruginosa* in hospital environments.^[10-12] Water systems can be a source of *P. aeruginosa* infection.^[13-16] Hand washing with contaminated tap water were detected as the contamination source in hospital.^[17] The spread of *P. aeruginosa* might occur from patient to the water system, or vice versa. Thus, the resistance characteristic of *P. aeruginosa* isolates that found from water system in hospital may come from the patients or vice versa. However, the environmental reservoirs of antibiotic resistance factors in the hospital are still unclear. The improvement of hygiene and water management in the hospital environment, especially water in the bathroom, is an important thing that must be considered. It is recognized that *P. aeruginosa* is a bacterium that can increase its virulence factor when forming biofilm layers.^[18] Thus, the ease of cleaning water reservoirs in hospital bathrooms, is thought to affect the level of virulence of *P. aeruginosa*.

MATERIALS AND METHODS

Materials

This study used antibiotics as follows: gentamicin 10 µg CT0774B (Oxoid), 10 µg meropenem CT0774B (Oxoid), 110 µg piperacillin/tazobactam 100/10 CT0725B (Oxoid), ceftazidime 30 ceftazidime CT0412B (Oxoid), and 5 µg ciprofloxacin CT0425B (Oxoid).

Water samples

Water samples used in this study were collected from bathroom of five different hospitals in Bandung regency,

West Java, Indonesia. The samples from each hospital consisted of different class level (one bathroom of first class, two bathrooms of second class, and two bathrooms of the third class). In addition, each of the hospitals used different water reservoirs in their bathroom, i.e., permanent bathtubs and pails. A volume of 15 ml water sample was taken aseptically using a sterile pipette and stored in a sterile test tube.

Enumeration, isolation, and identification of *Pseudomonas aeruginosa*

The samples were analyzed using a dilution method to achieve bacterial suspension concentration of 10^{-1} – 10^{-3} , then 1 ml of each concentration was inoculated in the petri dish containing 19 ml agar MacConkey and aerobically incubated at 37°C for 24 h. The colony number of *P. aeruginosa* were counted using a colony counter tool and then subcultured to fresh MacConkey agar plates, incubated at 37°C and further identified after 18–24 h. The colonies were verified using phenotypic identification and biochemical tests, i.e., oxidase test, triple sugar iron test, Indole test, motile test, carbohydrate fermentation (a serial fermentation test of glucose, lactose, sucrose, maltose, and mannitol), citrate test, Methyl Red, and Voges–Proskauer test. The identification results were then compared to the *P. aeruginosa* ATCC 27853 as bacterial standard.

Resistance test

All antibiotics resistance characteristics of *P. aeruginosa* isolates were tested against antibiotics containing in paper disc. The bacteria colonies were transferred from a slant agar to a sterile 0.95% NaCl solution and the turbidity was set to achieve turbidity standard, equal to 0.5 McFarland. The suspension in volume of 20 µl then swabbed onto the surface of Mueller Hinton Agar (MHA) using a cotton bud sterile and impregnated with available antibiotics disc. The dishes, then incubated for 37°C for 24 h. Quality control was conducted using *P. aeruginosa* ATCC 27853 and the inhibition zones were compared with the standard inhibitory diameter of each antibiotics, presented in the Clinical and Laboratory Standards Institute library.^[19]

RESULTS AND DISCUSSION

Total colony count

The colony number of *P. aeruginosa* in different isolation sources, i.e., permanent bathtubs and pails was compared. As well as on distribution systems of drinking water, the entire surface of the water reservoirs that in contact with water, can be a place for the colonization of microorganisms and potential to develop biofilm layers.^[20-22] Moreover, the flow-through of cold tap water over a period of 43 days was potent to developed biofilm.^[23] In the present study, out of 25 water samples examined, all samples showed contain of fluorescent colony. The result showed that the total colonies

on hospital with permanent bathtubs were found higher than that of from pails [Table 1].

Bacterial identification

P. aeruginosa having rounded and a greenish yellow colony and grape like-odor on the agar MacConkey. In addition, on the MacConkey agar, *P. aeruginosa* was observed as bacteria strain that produced fluorescence when viewed under ultraviolet light.^[24] These fluorescing colonies were sampling to be further tested and the result revealed that the suspected fluorescent colonies that isolated were confirmed as *P. aeruginosa*, as it performed the same identification result as *P. aeruginosa* ATCC 27853.

Resistance test

Antibiotic resistance in aquatic environment is an important case which must be considered because antibiotic resistance genes can be transferred easily from aquaculture to human.^[25] *P. aeruginosa* is commonly found in aquatic habitats, but their habitat with different ecosystems and sanitation levels, can produce *P. aeruginosa* with different characteristic profiles. Therefore, the profile of *P. aeruginosa* in water samples in each hospital must be evaluated to determine the profile characteristics of bacteria, especially their resistance to the commonly used antibiotics. In this present study, the resistance profiles of the 110 *P. aeruginosa* isolates from bathroom water of total five hospitals were phenotypically characterized against ceftazidime, piperacillin/tazobactam, ciprofloxacin, meropenem, and gentamicin. These antibiotics compile with the standard determination of resistance tests for *P. aeruginosa*. The resistance pattern of the *P. aeruginosa* isolates revealed that

5/110 (4.54%) were resistant to ceftazidime, 5/110 (4.54%) were resistant to ciprofloxacin, 1/110 (0.91%) were resistant to piperacillin/tazobactam, and 5/110 (4.54%) were resistant to gentamycin, as shown in Table 2. The environmental isolates were tended to be more sensitive than clinical isolates, this may due to the clinical isolates had been exposed to antibiotics.^[26] The details of resistance data of all isolates from each hospital are shown in Table 3. Based on data on Table 3, total of 25 *P. aeruginosa* isolates from a hospital with permanent bathtubs demonstrated (Hospital B) their resistance against ceftazidime (20%), piperacillin/tazobactam (4%), ciprofloxacin (20%), and gentamicin (20%). In addition, their intermediate resistance characteristics were also shown on piperacillin/tazobactam (16%) and meropenem (4%).

All the resistant isolates were sourced from hospital B which using permanent bathtubs. A profile of multiple antibiotic resistance (MAR) of hospital B isolates is shown in Table 4. The MAR index was 0.4–0.6 and the similar study of *P. aeruginosa* isolates from effluent water gave the same range.^[27] The position of a bathtub drain which higher than the minimum the surface of the water, tend to form a biofilm which functioned as pathogens reservoir and a source of water contamination. *P. aeruginosa* cells may persist for several weeks in the form of biofilms. The biofilm forms might improve their potential to produce virulence factors, including the antibiotic resistance mechanism. Although the level of *P. aeruginosa* resistance was not as high as reported in other studies, but it must be considered that the resistance genes of the bacteria can be transmitted to other *P. aeruginosa* cells. This fact can endanger the individual which frequently contacted with the contaminated water in the hospital bathroom. The acquired antibiotic resistance can be transmitted through mobile genetic element.^[28] Each individual infected with resistant *P. aeruginosa*, resulting in the difficulty of infection treatment and must invent the antibacterial agents for alternative therapy.^[29] Therefore, hospital management must have focused in the monitoring and evaluating the bacterial parameters in the aquatic environment to improve the water hygiene. The frequency and precision in the process of cleaning the bathroom are very important to do. This study recommended the use of the pails compared to the permanent bathtub in the bathroom of the hospital. Because the pails are more easily to be cleaned, therefore, the cleaning frequency may often do, compared to

Table 1: The prevalence of *Pseudomonas aeruginosa* viable colony

Hospital	Type of water reservoir	Total viable colony count (CFU/ml)	
		<i>P. aeruginosa</i>	Non <i>P. aeruginosa</i>
A	Pails	340	100
B	Permanent bathtubs	≥12,171,333	≥6,244,394
C	Pails	1402	370,938
D	Permanent bathtubs	564,533	1,616,040
E	Pails	49,327	19,552

P. aeruginosa: *Pseudomonas aeruginosa*

Table 2: Interpretative of antibiotics resistance profile (Clinical Laboratory Standards Institute, 2013)

Antibiotics	Code	Disc content (µg)	Interpretive criteria		
			Resistant (%)	Intermediate (%)	Sensitive (%)
Ceftazidime	CAZ	30	5/110 (4.54)	0/110 (0)	105/110 (95.45)
Piperacillin/tazobactam	TZP	110	1/110 (0.91)	5/110 (4.54)	104/110 (94.54)
Ciprofloxacin	CIP	5	5/110 (4.54)	0/110 (0)	105/110 (95.45)
Meropenem	MEM	10	0/110 (0)	2/110 (1.81)	108/110 (98.18)
Gentamycin	CN	10	5/110 (4.54)	4/110(3.63)	101/110 (91.81)

Table 3: Details of interpretive resistance profile of *Pseudomonas aeruginosa* from five hospitals

Antibiotics	Interpretive criteria	Hospital				
		A	B	C	D	E
CAZ	R	0	5	0	0	0
	I	0	0	0	0	0
TZP	S	18	20	20	25	22
	R	0	1	0	0	0
CIP	I	0	4	1	0	0
	S	18	20	19	25	22
MEM	R	0	5	0	0	0
	I	0	0	0	0	0
CN	S	18	20	20	25	22
	R	0	0	0	0	0
	I	0	1	0	0	1
	S	18	24	20	25	21
	R	0	5	0	0	0
	I	4	0	0	0	0
	S	14	20	20	25	22

R: Resistant, I: Intermediate, S: Sensitive, TZP: Piperacillin /tazobactam, CIP: Ciprofloxacin, CN: Gentamycin, MEM: Meropenem, CAZ: Ceftazidime

Table 4: Multiple antibiotic resistance index of *Pseudomonas aeruginosa* isolated from the water reservoir in bathroom of hospital B

Isolate code	Antibiotics	MAR index
1a	CIP,CN	0.4
1b	CIP,CN	0.4
1e	CIP,CN	0.6
3a	TZP, CIP, CN	0.4
3d	CIP,CN	0.4

TZP: Piperacillin /tazobactam, CIP: Ciprofloxacin, CN: Gentamycin, MAR: Multiple antibiotic resistance

the permanent bathtub. In addition to the cleaning process, the use of antiseptics can decrease the level of contamination.

CONCLUSION

The confirmed existence of multidrug-resistant *P. aeruginosa* found in the bathroom water of hospitals generates a high probability risk both directly on the individual health associated with the water source and indirectly impact on the health of the wide community.

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Conflicts of interest

There are no conflicts of interest.

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