Survey of *Pseudomonas aeruginosa* Contamination in Human Beings and Laboratory Animals

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Abstract: Several serotypes of *Pseudomonas* (*P.*) *aeruginosa* were isolated from the oral cavities of researchers, but no positive cases were found among the animals they had contacted or in the environment. These results indicated that researchers are not a source of *P. aeruginosa* infection for animals. However, *P. aeruginosa* was detected on the hands of researchers and animal caretakers after they finished their work. The same serotype of *P. aeruginosa* was found in the animals and the environment. These findings demonstrated that the researchers and the animal caretakers were contaminated with *P. aeruginosa* by the animals, and then became infective vehicles.

Key words: *Pseudomonas aeruginosa*, serology, laboratory animal, human being

Introduction

P. aeruginosa is often isolated as indigenous gut flora from healthy mammals including man. In laboratory animals, *P. aeruginosa* is frequently detected in the intestinal tract of specific pathogen-free (SPF) mice or rats, and it is sometimes detected in the inner and middle ear and other sites [10, 14, 16]. Drinking water is considered important in the spreading of contamination within animal colonies [7, 14], but laboratory animals contaminated with *P. aeruginosa* usually have no clinical symptoms. Occasionally there have been reports of naturally occurring pseudomoniasis, otitis media, pyelonephritis and sepsis, etc., in mice [18]. But, inapparent or latent infections have generally been unmasked

by experimental procedures that lower host resistance, such as irradiation, steroid or cyclophosphamide administration, burn stress or other experimental stress [7, 21]. Animals showing clinical signs of pseudomoniasis cannot be used for research, and this is the main reason for eliminating contamination by *P. aeruginosa* in laboratory animals.

In human medicine, *P. aeruginosa* is a major cause of morbidity and mortality in immunocompromised, burned, or otherwise stressed patients [3, 15, 20]. Because of its importance in nosocomial infections, several epidemiological studies have been performed on outbreaks of human *P. aeruginosa* infections [1, 4, 5, 17, 19]. Several epidemiological studies have therefore been undertaken on routes of *P. aeruginosa* infection

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in hospitals. Although pseudomoniasis is a serious problem in both laboratory animal science and human medicine, little has been done to clarify the mode of transmission between laboratory animals and human beings.

Experimental animal facilities are usually located near hospitals, and researchers in a hospital performing animal experiments have frequent contact with both laboratory animals and patients. We therefore surveyed *P. aeruginosa* contamination in researchers working in experimental animal facilities and hospitals. We also examined *P. aeruginosa* in animal caretakers, laboratory animals, and the internal environment of our experimental animal facility. We attempted to determine the pattern of *P. aeruginosa* infection between laboratory animals and human beings by serotyping isolated strains.

Materials and Methods

Animals: Specimens were collected from mice in breeding rooms No.1–4 and from rats in breeding rooms No.5–9. About 350 mice or 270 rats were bred in one breeding room on the average. These animals were housed in groups of 1–5 for mice or 1–3 for rats in autoclaved TPX cages (CLEA Japan Inc., or Tokiwa Kagaku Kikai Co. Ltd., Tokyo, Japan) with stainless steel lids under barrier-sustained conditions with automatically controlled temperature ($22 \pm 2^\circ\text{C}$), humidity (50–70%), and lighting (12-hour light-dark cycle). All the animals were maintained on a laminar flow bench (Tokiwa or ICM Co. Ltd., Ibaraki, Japan), and given commercial food pellets (CE-2;CLEA Japan) and drinking water without sterilization. An automatic watering system was used in the animal facility. Only nude mice kept in the breeding room No. 1 were given autoclaved tap water in a water bottle, and commercial food pellets sterilized by 30 kGy gamma ray irradiation (CRF-1;Oriental Yeast Co. Ltd., Tokyo, Japan). Cages containing sterilized bedding were changed once per week in each breeding room. The water bottles in breeding room No. 1 were changed twice per week. After completion of the breeding work, the floors of each breeding room were cleaned with a vacuum cleaner and the racks of the laminar flow benches and floors were disinfected with a 50 ppm sodium hypochlorite solution. In each breeding room, there were five lami-

nar flow benches, one clean bench used for experiments, one sink supplying tap water, and one air inlet and two exhaust ports. There was one toilet in the corridor near the breeding rooms.

Human beings: Among persons in direct contact with mice and rats researchers were involved only in their animal experiments, but animal caretakers were in charge of feeding, watering, and cage-changing. Researchers and animal caretakers have to wear clean laboratory clothing, caps and gloves, and also must always disinfect their hands with 50% isopropyl alcohol before entering the breeding rooms.

Isolation and typing of organisms: Specimens were collected from animals, human beings, and the environment related to the animals. Samplings were performed repeatedly in June, September and December, 1990 and in March, 1991. Specimens related to the animals were as follows: fresh feces, swabs from the automatic watering nozzle and water from the water bottles. Specimens from the environment relating to the animals were as follows: swabs from the tap water, water collected from the bottom of the sink before the start of breeding, and the swabs from the inside doorknob, the air inlet, the interior of the exhaust ports, prefilters and racks in the laminar flow bench, the floors in breeding rooms, and the floors in the corridor and the toilet after breeding was finished.

Specimens from human beings included swabs from the hands and the oral cavity of the researchers and the animal caretakers before they entered and after they left the breeding room. Altogether 79 researchers and four animal caretakers were examined repeatedly.

All specimens were first inoculated into NAC broth (Eiken Chemical Co. Ltd., Tokyo, Japan) and incubated at 37°C for 48 hours, and then passaged on NAC agar (Eiken) at 37°C for 48 hours [13]. Suspected colonies were identified by Pile Tube No. 2 (Eiken). Serological classification of *P. aeruginosa* was performed with *P. aeruginosa* antisera (Denka Seiken Co. Ltd., Tokyo, Japan) [9].

Monitoring microbes: To determine the microbiological status in breeding rooms No. 1–9, SPF CD-1 mice and Wistar rats (Charles River Japan Inc., Kanagawa, Japan) were kept in each breeding room for 3 months as sentinels. We tested these animals monthly for infections with Sendai virus, mouse hepatitis virus, *Mycoplasma pulmonis*, Tyzzer's organism, *Bordetella*

bronchiseptica, *Corynebacterium kutscheri*, *Pasteurella pneumotropica*, *Streptococcus pneumoniae*, *Escherichia coli* O115 a,c:K(B), *Salmonella* spp., *Staphylococcus aureus*, *P. aeruginosa*, *Syphacia obvelata* and *S. muris* [11].

Results

Table 1 shows the results of *P. aeruginosa* detection from mice and rats reared in breeding rooms No.1–9. *P. aeruginosa* was detected in fecal or drinking water specimens from four of the nine breeding rooms. *P. aeruginosa* in 43 out of 51 fecal specimens (84%) and 33 out of 50 drinking water specimens (66%) was detected in breeding room No. 1. The isolation rates were higher than those for the other breeding rooms. In breeding rooms No. 3, 8 and 9, only a few positive cases were found in animal feces. From June, 1990 to March, 1991, as a result of monitoring microbes, all pathogens except *P. aeruginosa* were negative in all

cases.

P. aeruginosa detection results for human beings are shown in Table 2. In among researchers, 19 and 17 positive oral cavity specimens were found before entering and after leaving the breeding rooms, respectively. One and two positive specimens from the hands were found before entering and after leaving the breeding rooms, respectively. From the animal caretakers, no *P. aeruginosa* was detected in the oral cavity at any time. Seven positive specimens from the hands were found just after they finished work and left the breeding rooms.

In the environment in breeding room No. 1, *P. aeruginosa* was detected in the water in the sinks in all four specimens, and in the racks of the laminar flow bench in one out of eight specimens (Table 3). The disinfectant solution for the hands (35 specimens), the disinfectant solution for the shoes (four specimens), the tap water nozzles (eight specimens), the water in the sinks (eight specimens), the floor in the toilet (42 specimens), the floor in the corridor (23 specimens), and the

Table 1. Detection of *Pseudomonas aeruginosa* on mice and rats

Specimens	Breeding room No.									Total
	Mice				Rats					
	1	2	3	4	5	6	7	8	9	
Feces (%)	43/51 ^{a)} (84)	0/149 (0)	3/98 (3)	0/6 (0)	0/82 (0)	0/80 (0)	0/88 (0)	1/86 (1)	1/63 (2)	48/694 (7)
Drinking water (%)	33/50 (66)	0/149 (0)	0/90 (0)	0/6 (0)	0/83 (0)	0/84 (0)	0/87 (0)	0/81 (0)	0/62 (0)	33/692 (5)

^{a)}: Number positive / No. examined.

Table 2. Detection of *Pseudomonas aeruginosa* in human beings

Specimens		Before entering the breeding room	After leaving the breeding room	Total
Researcher	Oral cavity	19/211 (9.0) ^{a)}	17/227 (7.4)	36/438 (8.2)
	Hand	1/214 (0.5)	2/238 (0.8)	3/452 (0.7)
Animal caretaker	Oral cavity	0/34 (0)	0/24 (0)	0/58 (0)
	Hand	0/33 (0)	7/99 (7.1)	7/132 (5.3)
Total	Oral cavity	19/245 (7.8)	17/251 (6.8)	36/496 (7.3)
	Hand	1/247 (0.4)	9/337 (2.7)	10/584 (1.7)

^{a)}: Number positive / No. examined (%).

Table 3. Detection of *Pseudomonas aeruginosa* in the internal environments of breeding rooms

Isolation sites	Breeding room No.									Total
	Mouse				Rat					
	1	2	3	4	5	6	7	8	9	
Door knob	0/4 ^{b)}	0/4	0/4	0/2	0/4	0/4	0/4	0/4	0/4	0/34
Air inlet	0/4	0/4	0/4	0/2	0/4	0/4	0/4	0/4	0/4	0/34
Exhaust port	0/8	0/8	0/8	0/4	0/8	0/8	0/8	0/8	0/8	0/68
Tap water in the nozzle	0/4	0/4	0/4	0/2	0/4	0/4	0/4	0/4	0/4	0/34
Water in the sink ^{a)}	4/4	0/4	0/4	0/2	0/4	0/4	0/4	0/4	0/4	4/34
Laminar flow bench:										
Prefilter	0/8	0/20	0/20	0/6	0/20	0/20	0/20	0/20	0/20	0/154
Rack	1/8	0/20	0/20	0/6	0/20	0/20	0/20	0/20	0/20	1/154
Floor	0/16	0/15	0/15	0/7	0/15	0/15	0/15	0/15	0/15	0/128

^{a)}: Water specimens were collected from the bottom of the sink. ^{b)}: Number positive / No. examined.

Table 4. Isolation and typing of *Pseudomonas aeruginosa*

Specimens or isolation sites	Positivity and serotype						
	B	C	D	E	G	I	Untypable
Animals: Feces	- ^{d)}	-	1	-	44	-	3
Animals: Drinking water	-	-	-	-	33	-	-
Researchers: Oral cavity	19	5	-	1	4	4	6
Researchers: Hands (Before ^{a)})	1	-	-	-	-	-	-
Researchers: Hands (After ^{b)})	-	-	-	-	2	-	-
Animal caretakers: Hands (After)	-	-	-	-	7	-	-
Interior of breeding room:							
Water in the sink ^{c)}	-	-	-	-	4	-	-
Rack of laminar flow bench	-	-	-	-	1	-	-
Outside of breeding room:							
Floor	-	-	-	-	1	-	-
Total	20	5	1	1	96	4	9

^{a)}: Before entering the breeding room. ^{b)}: After leaving the breeding room. ^{c)}: Water specimens were collected from the bottom of the sink. ^{d)}: Not isolated.

containers for dead animals (12 specimens) outside of the breeding rooms were also examined. However, only one specimen from the floor in the corridor was positive (data not shown).

The serotypes of *P. aeruginosa* isolated from the animals, human beings, and the environment are shown in Table 4. Most of the *P. aeruginosa* isolated from the oral cavities of researchers was type B and a few were types C, E, G, I or untypable. However, most of the *P. aeruginosa* from the animal feces, drinking water, the

hands of the human beings after leaving the breeding rooms, and the environment was type G.

The correlation of *P. aeruginosa* detection among the human beings, animals they contacted and the internal environment of the breeding rooms is shown in Table 5. Representative examples are shown by the serotypes of the isolated *P. aeruginosa*. In examples 1–3, the organism was detected in their oral cavities and on their hands before entering the breeding rooms, but the organism was not detected in the animals they

Table 5. Serotypes of *Pseudomonas aeruginosa* isolated from human beings, animals and environment

Examples	No. of isolated cases	Human beings				Animals	Environment
		Before entering the breeding room		After leaving the breeding room			
		Oral cavity	Hand	Oral cavity	Hand		
Researcher (1)	1	B ^{a)}	- ^{b)}	-	-	-	-
Researcher (2)	1	-	B	-	-	-	-
Researcher (3)	1	G	-	-	-	-	-
Researcher (4)	1	-	-	I	-	-	-
Researcher (5)	6	B	-	B	-	-	-
Researcher (6)	3	C	-	C	-	-	-
Researcher (7)	1	I	-	E	-	-	-
Researcher (8)	1	G	-	G	-	-	-
Researcher (9)	1	I	-	I	-	G	G
Researcher (10)	10	-	-	-	-	G	G
Researcher (11)	2	-	-	-	G	G	G
Animal caretaker (12)	1	-	-	-	-	G	-
Animal caretaker (13)	8	-	-	-	-	G	G
Animal caretaker (14)	7	-	-	-	G	G	G

a) : Serotype. b) : Not isolated.

had contacted or in the environment. In examples 4–8, *P. aeruginosa* was detected in the oral cavities before entering the breeding rooms and after leaving, and it was also not detected in the animals or in the environment. In examples 9, 10, 12 and 13, *P. aeruginosa* of the same serotype was detected in the animals they had contacted, but not from their oral cavities or hands before entering and after leaving the breeding rooms. In examples 11 and 14, *P. aeruginosa* was detected on their hands after leaving the breeding rooms, and the same strains of type G organisms were detected in the animals and the environment.

Discussion

In 1963 Flynn [7] isolated *P. aeruginosa* from an animal caretaker. After the animal caretaker was replaced, contamination with *P. aeruginosa* declined in the animal colonies, which indicated that human beings are involved in the spread of *P. aeruginosa*. However, in the early 1960s, in reports on *P. aeruginosa* related to laboratory animals, serological classification of the isolated strains was not performed, so that the epidemiological study of *P. aeruginosa* was incomplete.

In 1957 Habs [8] first reported the serotypes of *P.*

aeruginosa, and thereafter many researchers became involved in this work [9, 12]. *P. aeruginosa* serotyping is the test of choice since it is the most convenient, can be performed within a few minutes, and is highly dependable. *P. aeruginosa* serotyping became an important tool in epidemiological studies on *P. aeruginosa*. We have also isolated *P. aeruginosa* from the feces and drinking water of mouse colonies, and, through serotyping, we studied *P. aeruginosa* in laboratory animals epidemiologically [14, 16]. We found in these studies that the same serotype of *P. aeruginosa* was isolated from the same mouse colony. These results were the same as those obtained in the present study, indicating that the spread of *P. aeruginosa* among animals is quite easy. Batema [2] has reported that *P. aeruginosa* not only exists in laboratory animal facilities, but also spreads between facilities.

Many epidemiological studies on *P. aeruginosa* infection have been undertaken in the field of human medicine. Allen [1] has surveyed *P. aeruginosa* carriers among patients in intensive care units in hospitals, and studied the contamination correlation of the organism in the staff and the environment. The results showed that the *P. aeruginosa* isolated from the fingers of most of the staff and utensils was derived from posi-

tive patients. Olson [17] also proved that *P. aeruginosa* isolated from most patients did not originate in the staff or the environment in intensive care units in hospitals, but was originally carried in by the patients.

As shown above, many epidemiological studies on *P. aeruginosa* are isolated and small in scale in the fields of both laboratory animal science and human medicine. No report on how *P. aeruginosa* spreads, especially between human beings and laboratory animals, has previously been published. When we isolated *P. aeruginosa* from the caretaker in a mouse colony, the serotype of the isolates was not same as that of the isolates from the animal feces [14]. But in that case, only two individuals were examined, and the possibility of spreading has not been clarified. In this study, we analyzed the possibility of *P. aeruginosa* spreading between human beings and laboratory animals, and a total of 14 examples were compiled. In examples 1 to 8 for researchers, *P. aeruginosa* was detected in their oral cavities or on their hands before entering and after leaving the breeding rooms, but not detected on the animals they had contacted or the environment. It appears that researchers are not the infective source of *P. aeruginosa*. On the other hand, in examples 9, 10, 12 and 13, *P. aeruginosa* was detected in the animals, but the same serotype of *P. aeruginosa* was not detected on human beings who had contacted these positive animals. This therefore does not prove the spread of *P. aeruginosa* from animals to human beings. In example 11 for researchers and example 14 for animal caretakers, *P. aeruginosa* was detected in a few cases on the hands of researchers and animal caretakers after they contacted positive animals. The evidence suggested that human beings may be contaminated by *P. aeruginosa* from laboratory animals.

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