

Sources of *Pseudomonas aeruginosa* Infection in Burns: Study of Wound and Rectal Cultures with Phage Typing

VERA L. SUTTER, VALERIE HURST

*From the School of Dentistry, University of California, San Francisco Medical
Center, San Francisco, California*

LONGER survival time in severely burned patients has been brought about by control of the initial shock phase with fluid and electrolyte therapy. However, many patients who survive the early shock period now die from infection and other complications.¹ *Pseudomonas aeruginosa* is a major cause of infection and death^{5, 9} or a contributing factor in the death of patients with severe burns.¹⁰

The impression that these infections are the result of contamination by the patient's own intestinal flora is common even though the incidence of intestinal carriage among normal subjects and patients not associated with pseudomonas infections has been reported to be only 3 per cent⁸ to 11 per cent.¹¹ The high infection rate in burns points to hospital contamination as a more probable source of the bacteria.

Study of the epidemiology of pseudomonas infection in burns has been advanced within the past few years by the development of systems for serologic and phage typing for identification of these bacteria.^{3, 4, 6, 8} Lowbury and Fox⁸ demonstrated that pseudomonas infections are probably the result of hospital contamination when they found specific serologic types prevalent in

burns of patients in their hospital. Their studies, however, did not include results of rectal cultures from the burn patients, so that direct contraindication of the role of indigenous flora was lacking.

The present study was undertaken to investigate the role of the rectum as a source of pseudomonas infection in burns. Phage typing was employed for identification of strains cultured from both rectal and burn wound swabs.

Materials and Methods

The 38 patients in this study were burned in a disastrous fire which occurred in a church recreation hall on May 23, 1964. Nine patients were housed in a single ward at San Francisco General Hospital (SFGH), five in Moffitt Hospital, seven in Kaiser Hospital and the remaining 17 in seven other hospitals in the San Francisco Bay Area.

Rectal and multiple wound swabs were taken from the 14 patients at SFGH and Moffitt Hospital approximately five times weekly beginning on the third postburn day and continuing for several days to weeks after patients were colonized with pseudomonas. Rectal swabs were rinsed immediately in liquid "Q" medium, a selective medium for pseudomonas devised by Dr. C. H. Drake^{1a}: asparagine, 0.2 per cent; ethyl alcohol, 2.5 per cent by weight; K_2HPO_4 (anhyd.), 0.1 per cent; $MgSO_4 \cdot$

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TABLE 1. Recovery of *Pseudomonas* from Burn and Rectal Cultures

	Probable Source		
	Hospital	Endogenous	Equivocal
Pseudomonas recovered from			
Burns first	7	0	0
Rectum first	1	1	1
Both, simultaneously	1	1	0
Total	9	2	1

7H₂O, 0.05 per cent; K₂SO₄, 1 per cent, dissolved in distilled water. After 24 to 48 hours' incubation, subcultures were made on SS agar for isolation. Wound swabs were inoculated immediately, first onto blood agar, then rinsed in "Q" medium for enrichment of small numbers of pseudomonas. If pseudomonas were not recovered from blood agar, the "Q" medium was examined by subculture on SS agar.

Cultures from patients in other hospitals were obtained from the respective clinical laboratories of each hospital whenever possible. All *Ps. aeruginosa* isolated were phage typed by the method of Sutter, Hurst and Fennell.¹³

Criteria for assessing whether colonization was the result of hospital acquisition or self infection were chosen as follows: when pseudomonas was recovered from the burns of a patient prior to recovery from rectal swabs, colonization was considered to be a consequence of hospitalization; if rectal swabs were positive first within the first 6 days of hospitalization and the phage type found in the rectum was the same as that later found in the burns, the patient was thought to be self-infected; pseudomonas recovered from either burns or rectum after the sixth day were thought to be hospital-acquired.

Results

Recovery of Pseudomonas from Burn and Rectal Cultures. *Pseudomonas aeruginosa* was recovered from burn and rectal swabs of all but two of the 14 patients at

TABLE 2. Strains of *Pseudomonas* Found Among Burn Patients

Strain Designation	Pattern of Phage Lysis
A	P2/6/7/8:C7/9/13
B	P2/7/8:C9/13
C	P2/7:C13
D	P2:C1/6
E	P2/8:C9
F	P2:C9/16
G	P2/6/8:C1/6/7/9/16
I	P2/8:C1/6/7/9
K	P2/7:C1/6/13
L	P8:C7/9
M	C1
P	P2/8:C13/22
NT	No reactions

SFGH and Moffitt Hospital (Table 1). Since the burns of seven of these patients yielded pseudomonas before positive cultures were obtained from rectal swabs, colonization of such burns appeared to be the result of hospital contamination. Among three patients who had positive rectal cultures first, the burns of one appeared to be hospital contaminated; another seemed to become infected endogenously; and one was equivocal. The first patient had negative cultures until the eighth day; the rectal culture became positive on the ninth day; then the burn and rectal cultures were positive on the tenth day. The wounds in second patient appeared to be endogenously colonized because the first rectal culture taken on the fourth postburn day was positive for pseudomonas while burn cultures did not become positive until the sixth day. Burn and rectal cultures on the third patient were negative through the fifth day; on the sixth day the rectal culture was positive; then both burn and rectal cultures were positive on the seventh day.

Of the two patients from whom pseudomonas were recovered simultaneously from both burn and rectal cultures, one was thought to represent hospital contamination since recovery of pseudomonas was not achieved until the tenth day; the other

TABLE 3. Acquisition of Strains of *Pseudomonas* by Patients at SFGH

Type	Number of Patients in Whom <i>Pseudomonas</i> Appeared First in:			Total No. Patients from Whom Type was Isolated
	Burn	Rectum	Burn and Rectum	
A	7	2	0	9
B	2	1	0	3
C	3	0	1	4
D	2	1	2	5
E	2	1	0	3
F	1	1	0	2
G	0	1	0	1
I	1	0	0	1
M	1	1	0	2
NT	2	1	2	5

was considered indicative of endogenous colonization.

Phage Typing of Strains. Numerous patterns of lysis were encountered among the strains isolated (Table 2), and some patients harbored as many as three or four different types at one time. To facilitate discussion, phage patterns encountered have been arbitrarily designated as types A, B, C, etc. Table 3 lists the multiple types found among the nine patients at SFGH and gives further evidence that hospital contamination was more important than fecal contamination in colonization of burns. The results indicated that the majority of strains encountered were acquired first in the burn wounds. For example, Type A was recovered from all nine patients at SFGH and in seven it was recovered first from the burns. In three of four patients with type C, burn cultures were positive first with simultaneous burn and rectal recovery of this type in the fourth patient.

Examples of culture and phage typing results on five of the nine patients at SFGH are shown in Table 4. The first of these patients, Ln, serves to illustrate the probable acquisition of two strains by hospital contamination. Both types A and C appeared first in the burns. Type A was never isolated from rectal swabs, while type C was

recovered from rectal swabs only after the burns were colonized.

Patient Ed, whose burn infection was probably of endogenous origin, may have been the ward's source of the type A strain because the first rectal culture obtained on the fourth postburn day yielded type A pseudomonas. Burn cultures yielded type A pseudomonas from the sixth to the eighth day and blood cultures were positive for this strain on the seventh and eighth days. Cultures taken on the ninth postburn day yielded another type which was probably hospital acquired.

Patient Lf illustrates rectal acquisition of type A pseudomonas on the sixth postburn day with wound cultures positive for this type on the seventh day. It is uncertain whether this was an endogenous strain or acquisition of the hospital strain. Since this strain was the predominant type on the ward and was not apparent in this patient until the sixth day of hospitalization, rectal and subsequent burn colonization may have been the result of hospital contamination. A nontypable strain appeared in the burns on the eighth day and in the rectum on the ninth. The sequence of colonization indicates that this strain was hospital acquired. Type D appeared in rectal and burn cultures on the thirteenth day and was also considered to be hospital acquired.

TABLE 4. *Phage Types of Pseudomonas Found in 5 Patients at SFGH*

Patient	Site	Date of Culture													
		May					June								
		26	27	28	29	31	1	2	3	4	6	8	9	10	11
Ln.	Rectum	O	O		O		O	O	O	O	O	O	O	O	C
	Burns	O				O	O	A	A	A	O	A		A	C
Ed.	Rectum		A		A			D							
	Burns	O	O		A	A	A	D	Expired						
Lf.	Rectum		O		A		O	NT	NT	NT	D	D	D	D	D
	Burns	O		O		A	NT	NT	NT	NT	NT	NT	NT	NT	NT
Va.	Rectum		F		F	O	NT	NT	NT	NT					
	Burns	O	F	A	A	NT	A	A	A	F			Expired		
Ar.	Rectum		O		O	O	G	G	G	G	O	G	D	G	NT
	Burns	O				O	O	G	G	O	O	G	O	O	O

O = negative culture; A, C, D, F, G = types of pseudomonas recovered; NT = nontypable strain(s) recovered.

In Patient Va burns initially were apparently colonized with a type of pseudomonas (F) found in rectal cultures, but there was subsequent colonization with the hospital strain (A) and a nontypable strain.

Table 4 also shows results on Patient Ar whose burn colonization was probably the result of hospital contamination even though rectal cultures were positive first prior to colonization of her burns. Because rectal swabs were negative until the eighth day of hospitalization, it is thought rectal colonization with type G occurred as a consequence of hospitalization. The strain acquired in the rectum then colonized the burns and Type A, also a hospital strain, was subsequently added. Rectal cultures

later yielded type D and nontypable strains, both interpreted as being hospital acquired.

Further evidence that most of these patients acquired pseudomonas from their environment is apparent from comparison of the types found at SFGH with those found at other hospitals (Table 5). Phage typing of cultures from 28 of the 38 patients showed type A, found in all nine patients at SFGH, was found in only three of the 19 patients ultimately hospitalized elsewhere. Two of these were at SFGH for 1 to 3 days prior to transfer. The initial hospitalization of the third patient is not certain. Types B, C, E and F were not seen in any hospital but SFGH. Type D, seen in five patients at SFGH and five elsewhere,

TABLE 5. *Types of Pseudomonas Found Among Patients in Several Hospitals*

Type	A	B	C	D	E	F	G	I	K	L	M	P	NT
Hospital:													
SFGH	9	3	4	5	3	2	1	1	1		2		5
Moffitt	1			1			1	2	1	2	1		1
Kaiser	1			4							3	2	
Others	1							3	1	1	1		
Total	12	3	4	10	3	2	2	6	3	3	7	2	6

is a type encountered commonly in the hospitals of the San Francisco Bay Area.

Discussion

Establishing patients' hospital environment and contacts as primary sources of infection in burns is an important step in prevention. As long as infections by pseudomonas are thought to arise from indigenous flora, disease caused by these bacteria will be considered inevitable in patients with extensive burns. When it is recognized that these infections are of exogenous origin and preventive measures taken, disease rates can be decreased.⁷

The number of patients studied in detail in this series was small, but the data suggest that most were infected from sources other than their own intestinal tracts. Rectal swabs as a source of fecal material limits the significance of negative findings since the sample in most instances was scant. Stool specimens were not available from most patients during the early post-burn period. However, results are in general agreement with those of Kefalides and coworkers⁵ who found that 65 per cent of patients harbor *Ps. aeruginosa* on burned skin as early as the third postburn day, while less than 20 per cent had pseudomonas in their stools.

It is possible that these patients may have harbored pseudomonas in sites other than the intestinal tract, but this appears unlikely because others^{7, 8} have shown that pseudomonas is rarely found in the nose, throat or on the skin of normal individuals. Preliminary studies in this laboratory agree with these findings.

Multiple types of organisms appearing concurrently or sequentially in several patients raises the question of reliability of phage typing, but retyping of representative strains confirmed the stability of phage types. The data indicate that patients were contaminated with several different types of pseudomonas, but another explanation is

that the numerous types encountered were the result of contamination by two or more strains with subsequent *in vivo* lysogenization. Smith¹² demonstrated cross lysogenization with strains of staphylococci isolated from a herd of cows with mastitis. Mixtures of staphylococci of different types grown together resulted in type changes consistent with types isolated from the infections. This same phenomenon may occur with pseudomonas in infected burns, and this warrants further investigation.

Summary and Conclusions

Cultures were made of burn and rectal swabs taken from patients soon after hospitalization and for several days to weeks after colonization with *Ps. aeruginosa*. Results, coupled with phage typing of the pseudomonas isolated, suggest that most burn and rectal colonization by pseudomonas results from cross-contamination within the hospital.

Twelve patients with positive cultures were studied in detail; initial colonization of the burns in nine was a consequence of hospitalization; two patients were probably self-infected; and another was equivocal.

Eventual colonization of these wounds with additional phage types occurred and also appeared to be the result of hospital contamination.

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