Epidemiology of *Pseudomonas aeruginosa* in a General Hospital: a Four-Year Study

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A retrospective study was done to determine the epidemiology of infection and/or colonization due to *Pseudomonas aeruginosa* in a Brazilian general hospital. In 1966, 1968, and 1969, there were only two instances where probable cross-contamination was shown; the remaining isolates were unrelated. In late 1971 the hospital experienced a marked increase in *P. aeruginosa* isolation. Contaminated dextrose solutions used in the infant feeding were the apparent cause of the problem which occurred in the premature and special care nurseries. A contaminated oxygen humidifying bottle was the source of a different outbreak in surgery. There was also evidence in four instances that cross-infection and/or contamination had occurred. Pyocin and serological typing revealed that many strains were involved and led to a clear understanding of the complex epidemiological relationships among all the strains.

Infections due to *Pseudomonas aeruginosa* continue to be a problem in many hospitals (2, 11, 19, 21, 23). Since *P. aeruginosa* is frequently found in the hospital environment and in the normal stool flora, it is difficult to trace the source of infection and/or colonization unless a complete study is undertaken (1–3, 24, 25, 27–30, 32). Descriptions of outbreaks and infection and/or colonization problems usually come from large medical centers which have the necessary financial resources for epidemiological studies. We were fortunate to obtain all of the isolates of *P. aeruginosa* from the 359-bed general hospital in Brazil, so a retrospective study could be done to determine the epidemiological relationships among all the strains isolated. We took advantage of the serological typing and pyocin production (9, 13, 31, 33) as a sensitive fingerprinting method for differentiating strains. The report describes the epidemiology of *P. aeruginosa* at this hospital during a 4-year period.

**MATERIALS AND METHODS**

Hospital. This study was made at Ribeirão Preto Hospital in São Paulo, Brazil, a 359-bed general hospital of the University of São Paulo. It has four floors and is divided into services as follows: first floor, neurology; second floor, medicine; third floor, gynecology, neurology, pediatrics, and nurseries; fourth floor, surgery. Most of the beds are located in small wards of 10 beds each. The hospital population is generally of a lower socio-economic background. The sexes are usually in close proximity and may be grouped according to the medical service. There were no known patients with tuberculosis or leprosy in the hospital during this time.

**Media.** Medium 81 contained 28.5 g of Trypticase soy broth without glucose (Bioquest, Cockeysville, Md.), 10 g of KNO₃, and 1,000 ml of distilled water (16). All other media were from commercial sources and were prepared according to the manufacturers instructions.

**Isolation and identification of *P. aeruginosa***. All isolates were identified by the clinical microbiology laboratory at the Brazilian hospital using standard methods. All patient and employee isolates were obtained from individuals manifesting overt symptoms of infection. No attempts were made to evaluate possible colonization of asymptomatic patients or personnel. Isolates with typical colonial morphology, blue-green diffusible pigment, and typical odor required no further identification. Isolates without these characteristics were confirmed on the basis of the production of indol-phenox oxidase, the oxidative utilization of glucose, oxidation of gluconate to 2-ketogluconate, growth on Pseudoeel agar (Bioquest), pyocyanin production on *Pseudomonas* agar P (Difco), and fluorescein production on *Pseudomonas* agar F (Difco).

**Pyocin production patterns.** Pyocin lysates were prepared in medium 81 from each of the isolates by the simplified method recently described by Jones et al. (16), and pyocin production patterns (17) were determined against 27 pyocin indicators of Farmer and Herman (9). Pyocin production patterns were converted into codes as shown in Table 1 (8). The
pattern of each isolate, including multiple isolates from some patients, was determined at least three times, and all isolates with closely related patterns were compared at the same point in time.

**Serological typing.** The somatic (O) antigens of important isolates were determined with antisera to the 13 O antigens of Habs (14), kindly supplied by William T. Martin, Center for Disease Control, Atlanta, Ga. A modification of the procedure furnished by Martin was used. Each isolate to be typed was inoculated into 50 ml of medium 81, incubated 24 h at 37 °C, heated at 121 °C for 30 min, and centrifuged for 30 min at 5,000 x g. The cells were washed once in saline, centrifuged, and suspended in 5 ml of saline. Each antiserum was diluted 1:10 in sterile saline, pH 7.0. Each diluted antiserum (0.2 ml) and each cell suspension (0.2 ml) were combined in one section on a REPLI dish (Dyos Plastics, Surrey, England) and incubated at 37 °C for 18 h. The mixtures were observed macroscopically for agglutination and also with a dissecting microscope. When a culture reacted with two antisera it was reported with both reactions (example: Habs 8, 9).

**Definition of hospital strains.** Throughout this paper we use the term "isolate" to refer to a single colony picked from a streak plate containing *P. aeruginosa*. An isolate is referred to as a "strain" when a pyocin production pattern and/or a somatic antigen had been determined. Isolates with identical pyocin production patterns were considered to be the same strain. (All key isolates were serotyped for confirmation.) A "hospital strain" was defined as a strain found in two or more patients or in a patient and in an environmental source (other than his own equipment).

**Epidemiological conclusions.** This was a retrospective study and, therefore, all conclusions must be viewed with this in mind. During the period 1966 to 1969, *Staphylococcus aureus* was the organism of greatest concern in the hospital; little attention was given to the *P. aeruginosa* as a potential nosocomial pathogen by the clinical staff. Our epidemiological conclusions, for this period, were based entirely on the typing results of the culture isolates from 1968 through 1972.

Epidemiological conclusions for the outbreak of 1972 were based on typing data obtained from patient and environmental isolates, and on the extensive epidemiological data that was obtained when the outbreak was investigated. In this paper we define a "colonization" as any culture isolate (other than a stool culture) positive for *P. aeruginosa* obtained from individuals manifesting overt symptoms.

### RESULTS

**Retrospective study 1966 to 1969.** Table 2 shows that during this period *P. aeruginosa* was not a frequent isolate at this hospital. The increase in isolates in 1969 over 1968 in all probability was not significant and may reflect merely an increase in culturing by the hospital staff. All of the isolates were compared by pyocin typing which revealed that very few of the strains came from cross-colonization or from a common source. In 1966 to 1969, each strain was different from every other one, but in 1968 two incidences of probable colonization were shown to have occurred in the nursery, since two common strains were isolated from three infants at one time and two infants at another time. The low colonization rate was probably real for this type of hospital, but it must be pointed out that isolates from cases which were not cultured could not be compared. Perhaps some incidences of cross-infections and/or colonization may have been missed because one culture of the pair had not been obtained. However, data clearly indicate that a common hospital strain was not present. A review of the data suggested that the hospital had infrequent cross-colonizations due to *P. aeruginosa*, and thus the study

### Table 1. Simplified method for converting pyocin production patterns* into digits

<table>
<thead>
<tr>
<th>Pyocin reactions (3)</th>
<th>Representation</th>
</tr>
</thead>
<tbody>
<tr>
<td>++ +</td>
<td>1</td>
</tr>
<tr>
<td>++ +</td>
<td>2</td>
</tr>
<tr>
<td>+ + +</td>
<td>3</td>
</tr>
<tr>
<td>+ + +</td>
<td>4</td>
</tr>
<tr>
<td>++ +</td>
<td>5</td>
</tr>
<tr>
<td>+ + +</td>
<td>6</td>
</tr>
<tr>
<td>+ + +</td>
<td>7</td>
</tr>
<tr>
<td>+ -</td>
<td>8</td>
</tr>
</tbody>
</table>

* The first two digits are defined as the strain's Pyocin type, the complete pattern is defined as the strain's Pyocin Production Pattern. For example, a strain with Pyocin Production Pattern -- + (3), + - + (4), -- + (+8), + - + (1), + - - (3), + - - (2), ++ - (7), -- - (8) would be: Pyocin type 31. Pyocin Production Pattern 61 4813 278.

### Table 2. Composite epidemiology of *P. aeruginosa*

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of infections</th>
<th>Epidemiological findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1966</td>
<td>15</td>
<td>All isolates were different.</td>
</tr>
<tr>
<td>1968</td>
<td>15</td>
<td>7 to 30 August: three different infants (one death) acquired the same strain (probably by cross-infection). 25 November: two infants acquired a common strain, different from above (probably by cross-contamination).</td>
</tr>
<tr>
<td>1969</td>
<td>25</td>
<td>All isolates were different.</td>
</tr>
<tr>
<td>August 1971 through April 1972</td>
<td>117</td>
<td>Complex epidemiology.</td>
</tr>
</tbody>
</table>
was terminated. However, in the last month of 1971 there was again a dramatic increase in the number of \emph{P. aeruginosa} isolates (Table 2).

\textbf{Colonization problem, August 1971 to 1972}. In August 1971, the hospital staff was attempting to more closely monitor an increased number of infections and/or colonizations originally thought to be of \emph{S. aureus} origin. More cultures were taken from patients, surgeries, nurseries, and other environmental areas. A significant number of \emph{P. aeruginosa} isolates were thus detected (Tables 2 and 3). \emph{P. aeruginosa} was isolated from 38 different samples of 5% dextrose solution used in infant feeding and in the humidifying water bottles. Control efforts were directed toward these sources which resulted in a dramatic decrease in the isolation of \emph{P. aeruginosa}.

\textbf{Definition of hospital strains}. All the strains from patients and the environment were then compared by the pyocin and/or serological typing. Table 4 shows that 11 hospital strains were defined on the basis of this typing.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Source & Hospital location & Positive for \emph{P. aeruginosa} & \hline
5% Dextrose solution & Premature nursery & 28 & \hline
& Normal nursery & 5 & \hline
Water in oxygen humidifying bottle & Premature nursery & 2 & \hline
& Isolation nursery & 1 & \hline
& Recovery center & 1 & \hline
& Internal medicine ward & 1 & \hline
\end{tabular}
\caption{Environmental survey for \emph{P. aeruginosa}}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Hospital strain & Habs O group & Pyocin type & Complete pyocin production pattern & \hline
1 & 2 & 86 & 86 3134 174 & \hline
2 & 4 & 88 & 86 3257 277 & \hline
3 & 4 & 32 & 32 1211 161 & \hline
4 & 11 & 84 & 84 3131 133 & \hline
5 & 11 & 54 & 54 3111 133 & \hline
6 & 2 & 86 & 86 8567 334 & \hline
7 & 11 & 82 & 82 3131 143 & \hline
8 & 11 & 84 & 84 3111 123 & \hline
9 & 11 & 64 & 64 3131 132 & \hline
10 & 2 & 86 & 86 7287 334 & \hline
11 & 2 & 86 & 86 8587 334 & \hline
\end{tabular}
\caption{Definition of hospital strains}
\end{table}

All other isolates were found in a single patient and, therefore, were probably of little epidemiological significance. Some of the differences in pyocin production involved only two or three reactions out of 27; however, these differences were reproducible and, therefore, were considered significant.

\textbf{Complex epidemiology of the colonization}. It was tempting to conclude that a single strain of \emph{P. aeruginosa} had invaded certain wet environments of the hospital and caused the outbreak. However, detailed study of the cultures revealed that the situation was much more complex. Table 5 shows that six different strains were isolated from the nurseries. All but two strains could be traced to the contaminated dextrose solutions which were considered to be a significant source of organisms in the nurseries. Patients from surgery and the recovery center had acquired a strain different from those in the nurseries (Table 4). The source of this strain was probably the oxygen humidify-

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Location & Duration & No. of patients or source & \emph{P. aeruginosa} isolated & \hline
Isolation nursery & September 1971 to April 1972 & 2 Infants & Hospital strain 1 & \hline
& & 1 Infant & Hospital strain 4 & \hline
& & 2 Infants & Hospital strain 5 & \hline
Premature nursery & September 1971 to April 1972 & 11 Dextrose bottles & Hospital strain 1 & \hline
& & 5 Infants & Hospital strain 2 & \hline
& & 9 Dextrose bottles & Hospital strain 2 & \hline
& & 1 Infant & Hospital strain 4 & \hline
& & 3 Dextrose bottles & Hospital strain 4 & \hline
& & 3 Dextrose bottles & Hospital strain 5 & \hline
& & 2 Infants & Hospital strain 6 & \hline
Normal nursery & March 1972 & 4 Dextrose bottles & Hospital strain 1 & \hline
& & 1 Dextrose bottle & Hospital strain 3 & \hline
& & 8 Patients & Hospital strain 7 & \hline
Surgery and recovery center & October 1971 to March 1972 & 1 Oxygen humidifying bottle & Hospital strain 7 & \hline
3 Different floors & October 1971 to March 1972 & 4 Patients & Hospital strain 11 & \hline
\end{tabular}
\caption{Epidemiology of the \emph{P. aeruginosa} isolates}
\end{table}
ing bottle in the recovery room. Three pairs of patients (two patients in neurology had strain 8, two patients in the renal unit had strain 9, and two patients in pediatrics had strain 10) acquired strains which appeared to be from cross-colonization, but were clearly not related to the outbreak. Strain 11 was isolated from four different patients in different parts of the hospital (Table 4). This could not be satisfactorily explained unless it originated from some common source which escaped sampling. The epidemiology of the _P. aeruginosa_ organisms, therefore, was very complex and could not be determined without a retrospective study of all of the isolates, and very sensitive typing methods.

Multiple isolates from the same patient. One of the best ways to test the stability of an epidemiological marker is to obtain multiple isolates from the same patient and compare them. In 15 patients the pyocin production pattern remained the same when multiple cultures were obtained; however, seven patients yielded strains with different pyocin production patterns. It first appeared that the organism's pyocin type had changed, but serological typing indicated that the more likely explanation was colonization by multiple strains, since each strain was a different class and type and also had a different somatic antigen.

**DISCUSSION**

Infection occurring during hospitalization is a worldwide problem. Many microorganisms can cause nosocomial infections, but _S. aureus_ caused the most concern in this country for many years. Between 1964 and 1967, studies at Boston City Hospital revealed a decrease in the isolation of _S. aureus_ but a striking increase in the isolation of gram-negative bacteria which were antibiotic resistant (12). Now _P. aeruginosa_ is a primary concern in institutions caring for the sick (11, 18–21, 23), because such infections are usually serious and often fatal (2, 24, 27, 29, 30).

Hospital infections due to _S. aureus_ have always been a problem at the hospital of the University of São Paulo, but _P. aeruginosa_ had been considered to be a minor problem. In 1971 to 1972, the staff realized that _P. aeruginosa_ colonization had increased significantly, particularly in the nurseries. In the hospital there had been only 15 patient isolates of _P. aeruginosa_ in 1966, 15 in 1968, and 25 in 1969. However, from August 1971 through 10 April 1972, there were 117 patient isolates. An environmental survey yielded an additional 38 isolates.

In the nurseries there were six different strains of _P. aeruginosa_. The strain found in an oxygen humidifying bottle was also found in two premature infants, both with upper respiratory tract infections. All but two of the 33 isolates from the bottles of 5% dextrose were proven to be hospital strains. The sterile water and dextrose used to prepare these solutions were cultured and yielded no growth; thus, contamination must have occurred during or after preparation of these solutions. Unit sterilization was not practiced, but bottles, nipples, and nipple protectors were all sterilized separately. Each baby's bottle was used for 24 h and kept near his bed at all times.

It might be concluded that the presence of _P. aeruginosa_ in the bottles represented contamination by the colonized patient. The following facts indicate that the dextrose solution contaminated the infants: (i) all but 6 of the 56 isolates from the nurseries were grouped into six hospital strains; (ii) one of these strains (hospital strain 1) had been cultured from colonized babies over a period of 6 months and was found in bottles 23 days after it was last cultured; (iii) the strains were found in five bottles in the normal nursery; (iv) the strains were found in babies in the premature nursery (hospital strain 3); (v) or the strains were found in both the premature and the isolation nursery (hospital strain 1). The fact that certain strains were present in more than one nursery over a period of several months indicates their continuous residence in some reservoir accessible to all three nurseries. All nurseries were attended by the same personnel, and the 5% dextrose formula was made at a common nursery station. There were no attempts to recover _P. aeruginosa_ from personnel or from apparently healthy babies.

Results support conclusions reached in other studies that newborn infants, especially premature infants, are more susceptible to colonization by _P. aeruginosa_ (1, 2, 26). Exposure to the organism, resulting in colonization or infection, usually occurs after birth (5, 6) and should be prevented, if possible. Without a complete epidemiological picture one can only speculate on the original source of strain. The first chronological appearance of three of the six hospital strains found in the nurseries were in a patient in the isolation nursery.

It is significant that the strain affecting nine patients (hospital strain 7) was found in an oxygen humidifying bottle in the surgical recovery center. The hospital has a central oxygen distribution system; tent therapy is utilized for infants and small children, and intranasal administration is used for all other patients. Be-
before reaching the patient, the oxygen bubbles into a bottle fixed on the wall and half filled with water. Originally there had been no hospital rule for changing the water and disinfecting the bottle, consequently the water in some of the bottles could have been present for months.

Four hospital strains (strains 6, 8, 9, and 10) were found in two different patients but not in any environmental cultures. All were from infected and/or colonized patients residing in the same ward or nursery and all were probably due to a cross-contamination. Although hospital strain 9 was isolated from four patients but not detected in any other source, the time interval from its first isolation to its last (6 months) implies its continuous survival in the environment, in a carrier, or in colonized patients whose hospital residency and contact overlapped. The surgical patients spent some time in the recovery center, but the pediatric wards and the neurological wards, in which the other two patients resided, were on different floors. Both of these patients had conditions that could have required surgery or intensive care, which could have caused their residency in the recovery center at some time. Hospital records did not clarify this situation, but our hypothesis is that all four patients acquired this strain in the recovery room, and that the strain was eliminated before environmental cultures were taken.

A review of our study and the localization of the hospital strains indicate correlation between the Pseudomonas problem in this hospital and other hospitals. Newborn infants, particularly premature infants and surgical patients, were infected and/or colonized with hospital strains to a much greater degree than medical patients. Many patients, including all those from internal medicine wards, were colonized with heterogeneous strains (no proven hospital source) of P. aeruginosa. These patients probably acquired their organisms in the hospital as a result of their therapy or disease. The strains were undoubtedly endogenous and not hospital strains as such. Medical patients, such as those with cancer, diabetes, and respiratory or coronary disorders, often require long-term and/or repeated hospitalization which increases their chances of colonization with one or more strains of P. aeruginosa (4, 15). The possibility of community-acquired infection or colonization present at the time of admission is also a strong possibility.

All isolates of our proposed hospital strains had the same serotype. This supports the validity of grouping isolates by their pyocin production patterns. Yet isolates possessing the same somatic antigen did not necessarily have the same pyocin production pattern. Groupings on the basis of serological typing alone are too broad since all 77 isolates making up the 11 hospital strains fall into three serotypes. Studies which have evaluated serological types of P. aeruginosa in a given hospital show that three or four different serotypes prevail (21, 31).

An interesting ramification of this study was the examination of multiple isolates from the same patient. In 15 of the 23 patients, all isolates from a given patient were the same strain. Yet seven other patients appeared to be colonized with two or more different strains. All combinations or variables of body site and time interval between cultures existed for those patients whose strains were all the same, as well as for patients yielding different strains. Only two patients yielded more than two different strains and both were medical patients. Two patients each yielded one hospital strain from a particular body site, then consequently yielded a different hospital strain from the same site. The occurrence of multiple strains of P. aeruginosa in the same patient has been reported by others (4, 7, 13, 22, 32).

The method for differentiating strains on the basis of the pyocin production pattern appears stable and reliable (9, 13, 31, 33) and includes those which are phenotypically disassocialants (5). Differentiation by serotyping has not been specific enough, although it is accurate, even with disassocialants (2, 15). Thus an accurate epidemiological marker, such as pyocin typing, enables the microbiologist/epidemiologist to assess the interaction of all isolates within the hospital, as well as the situations and techniques which predispose to infection or colonization.

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LITERATURE CITED