Molecular epidemiology of Pseudomonas aeruginosa in an intensive care unit

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SUMMARY

Genotyping was used to analyse Pseudomonas aeruginosa isolates from sink drains and 15 intubated patients as part of a 3-month prospective study of strain transmission in a medical-surgical intensive care unit. Ninety percent of all washbasin drains were persistently contaminated with several P. aeruginosa genotypes. In 60% (9/15) of the patients, P. aeruginosa colonization or infection was hospital-acquired: P. aeruginosa strains isolated from these patients were present in hospital sinks or in other patients before their admission. Since all patients were immobile, personnel were the probable route of transmission of P. aeruginosa in the hospital. The mechanism of strain transmission from sinks to hands during hand washing was investigated in a children’s hospital. When P. aeruginosa was present at densities of $> 10^5$/c.f.u. per ml in sink drains, hand washing resulted in hand contamination with P. aeruginosa via aerosol generation in the majority of experiments or P. aeruginosa was detected using an air sampler above the washing basin. High P. aeruginosa cfu were present at 4.30 h in the eight sinks ($5.4 \times 10^6 - 7.0 \times 10^{10}$ c.f.u./ml), whereas at 13.00 h P. aeruginosa c.f.u. were significantly lower ($3.1 \times 10^2 - 8.0 \times 10^5$ c.f.u./ml). These data reveal that the danger of bacterial contamination of hands during hand washing is highest in the morning. The identified transmission routes demand more effective hygienic measures in hospital settings particularly concerning personnel hands and sink drains.

INTRODUCTION

Pseudomonas aeruginosa is one of the most common nosocomial pathogens and several risk factors associated with P. aeruginosa colonization and infection in hospitalized patient populations have been identified [1–6]. Critically ill patients in intensive care units (ICU), particularly intubated patients receiving mechanical ventilation, are high-risk populations for P. aeruginosa pneumonia with poor prognosis [7]. A large body of information is available on the distribution of P. aeruginosa in the environment of a hospital [8, 9], including ICU’s [10–14]. Predominantly moist areas such as sink drains are persistent sources for P. aeruginosa. Nevertheless, the routes of infection and transmission in hospitals remain controversial. Whilst some authors regard hands of personnel as vehicles from contaminated sinks to the totally immobilized intubated patient [10, 13, 14], others deny this transmission route [2, 12, 15] and suggest pre-existing colon-
ization leading to endogenous infection as the major source of \( P. \text{ aeruginosa} \) and other Gram-negative bacilli in hospitals [16].

Most of these conflicting results may be explained by different design and execution of studies in this field. An improved method, based on a highly strain variable DNA sequence has facilitated investigations on molecular epidemiology of \( P. \text{ aeruginosa} \) [17–22]. This method was used here to compare \( P. \text{ aeruginosa} \) isolates from intubated patients in an ICU with isolates collected from sink drains of the ward. Based on this analysis, we present indirect evidence that the personnel still plays the leading role in \( P. \text{ aeruginosa} \) cross infection, transmitting strains from patient to patient, from patient to sink, and from sink to patient. We further show that the danger of bacterial contamination of hands during hand washing via aerosol generation is highest in the morning.

ACCOMMODATION, PATIENTS, AND METHODS

Accommodation

The old medical-surgical ICU of the Chirurgische Klinik of the University of Tübingen, Federal Republic of Germany, consisted of a 23 bed unit with 10 rooms. Six of the rooms were used for patients, with a maximum of four beds in each room. About 2000 patients were admitted yearly to this ICU. Each room had at least one person performing constant surveillance. Each shift consisted of 10–12 persons and the whole personnel comprised up to 60 persons covering the three daily shifts. The personnel wore freshly laundered uniforms and disposable gloves when handling a patient. Additionally, they are asked to wash hands after each patient contact. Hand disinfection was usually done before and after each shift. The clinic was closed in 1990.

Patients

Sixty-one patients (aged 1–79 years) with several underlying diseases and admitted to the ICU during December 1988 to February 1989 were investigated. These patients had been intubated endotracheally (nasotracheally, orotracheally or by tracheotomy) for 1–35 days and received moistened air. Forty-nine patients remained in one room only, 12 patients were moved to other rooms during their stay at the unit which lasted 1–90 days. The patients were totally immobilized and fed parenterally or enterally with special tube feeding diets (Fresenius AG. Oberursel, Boehringer GmbH, Mannheim, FRG) and mineral water. All patients received treatment with antacids or histamine type 2 receptor blockers (ranitidine, Cascan, Wiesbaden; pirenzepine, Schering, Berlin, FRG) and antibiotics. For treatment of \( P. \text{ aeruginosa} \) colonization or infection the following antibiotics were used: amikacin (Grünental, Stolberg, FRG), azlocillin and ciprofloxacin (Bayer. Leverkusen, FRG), cefsulodine (Takeda, Stolberg, FRG), cefotizoxim (Boehringer. Mannheim, FRG) and ofloxacin (Hoechst, Frankfurt, FRG).

\( P. \text{ aeruginosa} \) isolation and quantitation

Samples from sinks of all washbasins and toilets of the ICU were taken twice monthly. Samples from eight sinks of the mixed infectious ward of the children’s hospital, University of Tübingen were taken on 3 days each at 4.30 h and 13.00 h.
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Water samples were drawn using 1 or 10 ml sterile glass pipettes into 10 ml sterile glass tubes. The samples were diluted in 4 ml sterile 0.9\% sodium chloride solution and inoculated on cetrimide agar. For quantitative cultures 10-fold dilutions were made in sterile 0.9\% sodium chloride solution.

Specimens from throat, trachea, wounds, urine and rectum of intubated patients were obtained as soon as possible after admission to the ICU and twice weekly if the patient's stay was prolonged. Samples from patients were collected using sterile flexible intubation tubes or cotton swabs moistened with transport medium (Greiner, Nürtingen, FRG) or 0.9\% sodium chloride solution. \textit{P. aeruginosa} was identified using routine methods including growth on cetrimide agar, biotyping and genotyping.

\textbf{\textit{P. aeruginosa} genotyping}

Genotyping of \textit{P. aeruginosa} with the exotoxin A (ExoA) DNA probe was carried out as described previously [20]. Briefly, purified \textit{P. aeruginosa} DNA was digested with restriction endonucleases, electrophoresed through a 0.6\% agarose gel and transferred to a nylon membrane using the Southern method [23]. The \textit{Escherichia coli} plasmid pCMtox [21] labelled with biotin-11-dUTP (Gibco, Bethesda, USA) by nick translation, was used for hybridization on prehybridized membranes. Isolates were compared visually for differences in probe-reactive fragments. A typical gel is seen in Figure 1.

\textbf{Antibiotic susceptibilities of \textit{P. aeruginosa} isolates}

Genotyped \textit{P. aeruginosa} strains from patients, and environmental strains also present in patients were selected for antibiotic susceptibility testing by an agar diffusion method [24]. The following antibiotics were used: amikacin, tobramycin, gentamicin, mezlocillin, azlocillin, cefoperacon, cefsulodin, ofloxacin, cefoxitin, meroxitin, ceftacidime, rocefine, fosfomicin, pipercillin, and norfloxacin. The strains were incubated on test agar and antibiotic disks were added using a dispenser. After incubation for 24 h at 37 °C, the plates were analysed by measuring the area of inhibition around the antibiotic disks in mm and classified according to the standard classification system of the Hygiene-Institut, University of Tübingen, FRG.

\textbf{\textit{P. aeruginosa} aerosol generation from washing basin sinks}

Eight contaminated washing basin sinks of the mixed infectious ward of the children's hospital in which \textit{P. aeruginosa} was quantitated were used to determine whether sink contamination leads to hand contamination via bacterial aerosols during hand washing. The tap of the wash basin was opened for 30 sec. Thereafter an impactor (Reuter centrifugal sampler, Biotest, FRG) equipped with Standard I agar (Merek, Darmstadt, FRG) was held for 4-8 min at a 15 cm distance from the opening of the sink drain. The agar was incubated for 24 h at 37 °C, and bacterial colonies typed and quantified. Hands which had previously been disinfected with alcohol, were washed for 1 min in the washing basins and dried with a towel. Hand samples were obtained by immersing one hand for 1 min in a sterile plastic bag (National Lab, Hamburg, FRG) containing 100 ml of sterile physiological saline. The saline was filtered through a membrane (Nalgene,
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Fig. 1. DNA hybridization pattern of *P. aeruginosa* strains from two sink drains (lanes 1, 3) and from two patients of an intensive care unit (lanes 2, 4). Purified *P. aeruginosa* DNA was digested with *BglII* (A), *SalI* (B) and *XhoI* (C), electrophoresed and transferred to a nylon membrane using the Southern method. For further detail see Materials and Methods.

Rochester, USA, no. 130-4045), the membrane placed on a cetrimide agar plate and incubated for 24 h at 37 °C. These experiments were carried out on 3 days at each 4.30 h and 13.00 h.

RESULTS

A total of 74 *P. aeruginosa* isolates from patients and environmental sources of the ICU was differentiated by genotyping in 33 different *P. aeruginosa* strains.

*P. aeruginosa* isolates from the ICU environment

Samples from all sinks of washing basins and toilets in the ICU were collected twice monthly over 3 months. *P. aeruginosa* was repeatedly cultured from 10 of 11 washbasin sinks (90%), but was never found in the 2 toilet sinks. Genotyping revealed 27 different types in these sinks. Some genotypes were repeatedly isolated from one sink: in 9 of the 10 sinks different, individual genotypes were present for 3–9 weeks. Four genotypes were found in more than one sink. Thus, for example, P8 was isolated on 9 December 1988, 1 January 1989, and 17 February 1989 from sink B, on 31 January 1989 from sink C, and on 17 February 1989 from sink D. These results show that the ICU sinks were highly contaminated with persistent *P. aeruginosa* strains. Five of the 27 different genotypes (P1, P2, P8, P9, P12) isolated from contaminated sinks were also found in patients in the ICU (Table 1). Figure 1 shows the DNA hybridization pattern of two identical sink and patient genotypes.
Table 1. Isolation of P. aeruginosa genotypes from sinks and intubated patients of an intensive therapy unit

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Date</th>
<th>Room</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>21 Dec 88</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>30 Dec 88</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>30 Dec 88</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>1 Jan 89</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>10 Jan 89</td>
<td>C</td>
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<tr>
<td></td>
<td>10 Jan 89</td>
<td>E</td>
</tr>
<tr>
<td>P2</td>
<td>31 Jan 89</td>
<td>A</td>
</tr>
<tr>
<td>P8</td>
<td>17 Feb 89</td>
<td>A</td>
</tr>
<tr>
<td>P9</td>
<td>9 Dec 88</td>
<td>B</td>
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<tr>
<td></td>
<td>31 Jan 89</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>31 Jan 89</td>
<td>D</td>
</tr>
<tr>
<td>P12</td>
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<td>D</td>
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</table>

<table>
<thead>
<tr>
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<td>24 Jan 89</td>
<td>9</td>
</tr>
<tr>
<td>P2</td>
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</tr>
<tr>
<td>P8</td>
<td>3 Feb 89</td>
<td>11</td>
</tr>
<tr>
<td>P9</td>
<td>3 Feb 89</td>
<td>12</td>
</tr>
<tr>
<td>P12</td>
<td>8 Dec 89</td>
<td>15</td>
</tr>
</tbody>
</table>

Fig. 2. Isolation of P. aeruginosa genotype P1 from intubated patients and a sink drain in an intensive care unit (ICU) during December 1988 and February 1989. Horizontal bars represent patient's length of stay in the ICU. Arrows with points on top represent P1 genotype isolation from patients 3-7, and 9 or from an ICU sink. Arrows without points represent nontyped P. aeruginosa isolation from patients 3 and 7.

**P. aeruginosa isolates from ICU patients**

Sixteen of the 61 patients (26%) admitted to the ICU between December 1988 and February 1989 were colonized with P. aeruginosa at various body sites. From these, five patients developed bacterial infections and four patients died within 3–8 weeks thereafter. Strains from 15 of the 16 colonized patients were available for genotyping. Six patients (40%) harboured individual strains which were not isolated from other patients or the environment of the ICU. Nine patients (60%) were colonized by P. aeruginosa strains which were present in hospital sinks or in other patients before their admission (see below).
Spread of a single *P. aeruginosa* genotype to five other patients

Strain PI was initially isolated from wounds of patient 3 at admission to the ICU. On 30 December 1988, the patient was moved from room C to room B. During the course of the study period PI was isolated from five other patients in other rooms of the unit (patients 4–7, 9; Table 1, Figs 2, 3). Since the patients were immobile, the personnel was held responsible for strain transmission in the unit. The question arises whether PI was transmitted directly from patient 3 via the personnel to the other patients or via an environmental reservoir in the unit. Both ways seem unlikely, since, firstly, the antibiotic susceptibility testing of PI isolates from patient 3 revealed increasing resistance patterns from its initial isolation, whereas isolates from the other patients, colonized with PI, yielded less resistant strains (data not shown). Secondly, PI was not found in sinks of the ICU before 31 January 1989, at which time all other patients were already colonized. Therefore the exact transmission route remains unclear.

Spread of *P. aeruginosa* genotypes from colonized patients to the environment

Besides *P. aeruginosa* PI (see above), also P2 which was isolated first from a patient before it was detected in an ICU sink (Table 1). Since the patients were immobile both strains were most certainly transmitted to the sinks via the personnel hands.

Spreading of *P. aeruginosa* genotypes from hospital reservoirs to patients

As indicated in Table 1, three *P. aeruginosa* strains (P8, P9, P12) were isolated from sinks of various rooms before they were found in three patients, suggesting transmission via the personnel from the sinks to the patients. Also strain P6, which colonized patient 8 one week after admission to the ICU, may have been hospital-acquired, although the source of P6 remained unknown (patient 8 was *P. aeruginosa*-negative in the first week at the ICU in all stool, throat and tracheal cultures).

Transmission of *P. aeruginosa* from sinks to hands

In order to investigate the mechanism of transmission of strains from the sink drains to hands in more detail, *P. aeruginosa* was quantitated in eight contaminated sinks of a mixed infectious ward of the children’s hospital of the University of Tübingen at 4.30 h and 13.00 h (Table 2). Eight different genotypes were obtained from the eight sinks. High *P. aeruginosa* colony counts were obtained at 4.30 h (5.4 × 10^5–7.0 × 10^10 c.f.u./ml), whereas at 13.00 h *P. aeruginosa* c.f.u. were significantly lower (3.1 × 10^2–8.0 × 10^5 c.f.u./ml). Air sampling yielded *P. aeruginosa*-positive cultures when numbers in the contaminated sink drains exceeded 10^6 c.f.u./ml, i.e. in the morning. A similar result was obtained with hand contamination of *P. aeruginosa* (Table 2).

Endogenous *P. aeruginosa* colonization

Gastrointestinal tract colonization may be a source for retrograde oropharyngeal colonization by Gram-negative bacilli especially in antacid treated patients [16]. Fourteen of the 15 patients colonized with *P. aeruginosa* received antacids or...
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Fig. 3. Distribution of P. aeruginosa genotype P1 in an intensive care unit (ICU) during December 1988 and February 1989. A–F. patient rooms; G. storage room; H. kitchen; I. passage; K. toilet.

Table 2. Quantitative P. aeruginosa colony forming units (c.f.u.) in sinks and qualitative detection of genotypically identical organisms with an impactor (RCS) and on hands at different times in a children’s hospital

<table>
<thead>
<tr>
<th>Sink no.</th>
<th>4.30 h c.f.u.</th>
<th>RCS</th>
<th>Hands</th>
<th>1.00 h c.f.u.</th>
<th>RCS</th>
<th>Hands</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.4 x 10⁷</td>
<td>—</td>
<td>+</td>
<td>3.1 x 10²</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>2.1 x 10⁶</td>
<td>ND</td>
<td>+</td>
<td>7.0 x 10²</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>2.7 x 10¹⁰</td>
<td>+</td>
<td>ND</td>
<td>6.6 x 10²</td>
<td>—</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>2.4 x 10⁶</td>
<td>+</td>
<td>ND</td>
<td>1.5 x 10³</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>5.4 x 10⁵</td>
<td>—</td>
<td>+</td>
<td>2.0 x 10⁴</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>5.2 x 10¹⁰</td>
<td>+</td>
<td>+</td>
<td>4.0 x 10³</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>7.0 x 10¹⁰</td>
<td>+</td>
<td>+</td>
<td>8.0 x 10⁹</td>
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<td>ND</td>
<td>+</td>
<td>4.3 x 10²</td>
<td>ND</td>
<td>—</td>
</tr>
</tbody>
</table>

histamine type 2-receptor blockers and one patient was gastrectomized at admission. Actually, 9 of 15 patients (60%) harboured P. aeruginosa strains in their stools. However, only from two patients was the stool strain isolated before an identical genotype appeared in the respiratory tract. In two other patients, strains identical in the gastrointestinal tract and respiratory tract were isolated at the same time. The other patients with stool strains remained negative in the respiratory tract or were colonized with different P. aeruginosa genotypes (data not shown).

DISCUSSION

In the present longitudinal study genotyping of sequential P. aeruginosa isolates from patients and the environment was used to get more insight into routes of infection and strain transmission in an ICU. Two major hypotheses are currently discussed concerning the cause of P. aeruginosa in high-risk patients populations such as intubated patients: first, pre-colonization of patients with P. aeruginosa at the time of admission, and, second, hospital-acquired colonization.

In another study, pre-colonization with P. aeruginosa was observed in about one-third of ICU patients at admission of a general hospital [3]. The rectum was
the most commonly observed colonization site and preceded oropharyngeal colonization with mostly identical *P. aeruginosa* pyocin types and serotypes in all patients infected at both sites. Also other investigators [15] explain the failure of usual means of disinfection in hospitals to eliminate *P. aeruginosa* infection by the high faecal carriage of the organism in hospitalized patients. The present study confirms the high faecal carriage of *P. aeruginosa*, however, in only two cases did gastrointestinal colonization precede oropharyngeal colonization with identical *P. aeruginosa* genotypes, suggesting that the endogenous transmission of *P. aeruginosa* in patients receiving stress ulcer prophylaxis is possible but does not seem to be the major tracheal colonization route.

Hospital-acquired colonization or infection was proven in 60% of the intubated patients in the present study, since *P. aeruginosa* strains from patients were either found in specimens from other patients or from ICU sink drains at a time before admission of the patients. This rate is in good accordance with previous studies where *P. aeruginosa* acquisition rates between 33% and 78% [3, 13, 15, 25, 26] during hospitalization were found. Whereas in most other studies the source of the acquired strains within the hospital have not been determined, genotyping in the present study allowed us to demonstrate this acquisition in much more detail.

Although the exact bacterial transmission routes remained unclear in the present study, it has to be noted that the failure to isolate an organism, and especially *P. aeruginosa*, does not necessarily mean that it is not there but could be a consequence of the sampling technique. As was shown previously, survival times of *P. aeruginosa* strains in aerosols are short [20]. The same is true for hand carriage of *P. aeruginosa*. Thus, the investigator of *P. aeruginosa* transmission routes has to be in close contact with a hypothetical *P. aeruginosa* source from which an aerosol is generated or in close contact with a person who hypothetically transmits the bacteria for rapid culturing of appropriate samples. Therefore, attempts to show temporal relationships between environmental contamination and appearance of an organism in a patient are inherently flawed.

Sink drains have been previously suspected as major sources of *P. aeruginosa* infection in hospitals mainly based on the vast contamination of these sites with the organism [11, 13, 25] and on several typing methods for *P. aeruginosa* which show indistinguishable strains in patients and sinks [10, 11, 14]. A controversial opinion is presented by other investigators, based on the observation that *P. aeruginosa* strains in sink drains did not cause infection [13], normally reveal high level antibiotic resistance which is not found in patient isolates [12], and lack serotype identity between strains from environmental sources and patients [2, 15]. These data suggest little clinical importance of the *P. aeruginosa* sink contamination.

The present results show again that washbasin sinks are heavily contaminated with *P. aeruginosa* genotypes which sometimes persist over long periods of time. As noted by others, backsplash and aerosols [10, 11, 13, 14, 27, 28] may contaminate hands during hand washing: after activation of the tap for three 10-second intervals, *P. aeruginosa* and other organisms were recovered on agar plates up to 2 m distant from the tap [20]. Similar experiments were carried out by Chadwick and colleagues [11] and Gerba and colleagues [27]. Kohn mimicked transmission of organisms from the drain of the sinks to the hands with the use of...
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methylene-blue [25]. Also in the present study, hand contamination with *P. aeruginosa* sink strains was demonstrated. In addition, the time point when hand washing is most likely to cause contamination was related to numbers exceeding $10^5$ *P. aeruginosa* c.f.u./ml sink water.

In the light of this mechanism, decontamination of sinks by installing heating elements may be successful at reducing bacterial transmission and subsequent hospital-acquired infection [20, 25, 29]. Significantly reduced bacterial counts were found after taking these means [14] and only 7 of 48 samples from heated sinks grew *P. aeruginosa* compared with three-quarters of those from unheated ones [13]. In a recent study from our laboratory, heating of washing basin sinks to 70 °C with a new, safe and inexpensive device inhibited bacterial growth in sinks, generation of *P. aeruginosa* aerosols, and resulted in hand cultures negative for *P. aeruginosa* after washing [20].

Since the intubated patients of the present study are virtually immobile and identical strains were found widely distributed in the unit, other transmission routes than via the personnel are difficult to believe. Using the same genotyping method for *P. aeruginosa*, we showed [22] that 48% of hospitalized paraplegic patients with *P. aeruginosa* urinary tract infections harboured strains identical to those found in the environmental water reservoirs of the specialized wards. Nevertheless, the precise transmission route has not been detected, since hand cultures of the personnel have not been taken. In the light of previous results showing that 42-5% of the personnel of a children’s hospital carried *P. aeruginosa* at least once during a 4-week study period [20], sampling of *P. aeruginosa* from personnel hand cultures as well as from gloves after wearing would have been supportive of the present hypotheses.

In summary, the present study reinforces previous opinions of major transmission routes for *P. aeruginosa* in a hospitalized critically ill patient group using precise bacterial typing methods and emphasises improved cross infection control.

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