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Source of *Pseudomonas aeruginosa* infection in premature infants

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In January 1965 two babies died in the premature-baby ward of the Queen Victoria Memorial Hospital, Melbourne, from respiratory infection due to *Pseudomonas aeruginosa*. An investigation was promptly commenced in an attempt to confine this potentially serious outbreak.

Most reports of outbreaks caused by Gram-negative bacilli in premature-baby wards have indicated that oxygen equipment, mechanical mucus aspirators (Rogers, 1960; Becker, 1962; Kresky, 1964; Bassett, Thompson & Page, 1965) or the humidifying water of incubators (Sever, 1959; Foley, Gravelle, Englehard & Chin, 1961) are the most likely reservoirs of infection. Other sources of these organisms are water taps, faucet aerators and sink traps. For example, Wilson, Nelson, Phillips & Boak (1961) found *Ps. aeruginosa* in four out of five aerators sampled and Kresky (1964) reported that the same organism persisted in these sites in spite of frequent cleaning. Cabrera & Davis (1961) dramatically terminated an outbreak of *Flavobacterium* meningitis by repair of a leaking sink trap which harboured the organism.

In the study reported here the nursery equipment, wash basins and air conditioners were extensively sampled for *Ps. aeruginosa* and other Gram-negative bacilli. From this emerged a clear picture of heavy contamination of suction equipment and its connecting tubes with a variety of strains of *Ps. aeruginosa*, some of which were similar to those concerned in the fatal infections. Not only was it apparent that infants could be infected by the introduction of contaminated catheters into their respiratory tracts, but it was also demonstrated that the exhaust outlets from the aspirator jars were capable of discharging large numbers of organisms into the ward as an aerosol spray. This report re-emphasizes the importance of contaminated suction equipment as a source of *Ps. aeruginosa* infections and describes how aerial dispersion of this organism was controlled by modifying the design of the suction unit.

EPIDEMIOLOGY

The ground plan of the premature-baby ward (Fig. 1) shows seven bays, separated by 8 ft. partitions, an isolation room and service areas. The smallest babies are nursed in bays 1 and 2, containing respectively four and six incubators ('Insul-cots', The Commonwealth Industrial Gases Limited) similar to the type

shown in Plate 1*a*. As the infants thrive they are moved progressively through the bays in numerical sequence.

Five babies became infected with *Ps. aeruginosa* in January 1965. Three cases presented initially with eye infections and the other two with infections of the nose and throat. Case 1 had been transferred from bay 2 to the isolation room in

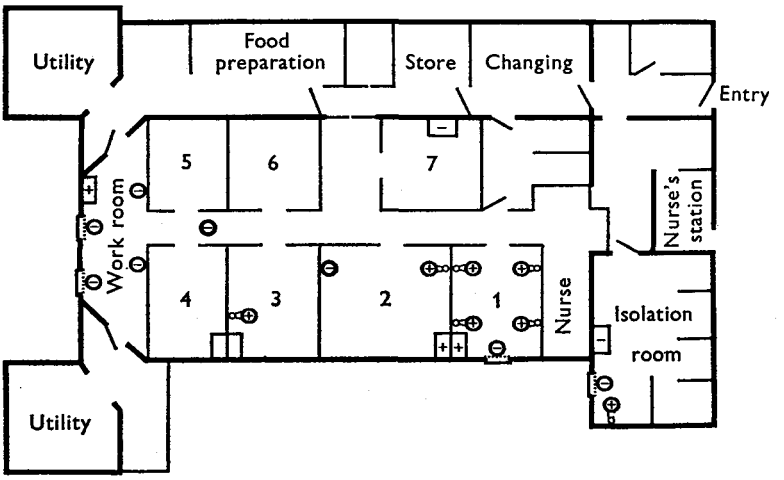

Fig. 1. Plan of premature-baby ward with subdivision into bays, isolation room and other service areas.

December, when an eye swab yielded heavy growth of 'coliforms'. Cases 2, 3 and 4 were infected in bay 1, where they were nursed until 22 January when case 2 died at 16 days from a suppurative pneumonia and case 3 at 9 days from a lung abscess. After these deaths, the gravity of the situation was realized and case 4 was immediately transferred to the isolation room. One week later case 5 (a carrier detected during routine swabbing of all babies in the ward) was also removed to the isolation room.

BACTERIOLOGICAL INVESTIGATIONS

Various sites in the ward and isolation room were sampled, sometimes on several occasions, with large swabs moistened in nutrient broth. These were plated directly on nutrient agar, horse-blood agar and MacConkey's agar and were also inoculated into nutrient broth. All cultures were incubated aerobically for 24–48 hr. at 37° C. Swabs taken from inside surfaces of air conditioners, window ledges and crevices were, in addition, cultured in cooked-meat medium in a search for anaerobic organisms.

Water (20–50 ml.) from humidifying reservoirs in the incubators was passed through 'Millipore' membranes which were cultured on nutrient agar. Samples of aqueous 'Zephiran' solutions containing aspirated mucus were also filtered through membranes which were then washed with distilled water and cultured in nutrient broth containing 10% 'Lubrol W'.

Air entering the nursery was sampled by exposing agar plates for 10 min. against the grilles of the air conditioners and also by culturing glycerol-moistened gauze strips which had been held in the same position for 24 hr. Nursery air was sampled in the central corridor between bays 4 and 5, using a Casella slit air sampler. The gas discharge from suction units was directed on to horse-blood agar plates for 5 min.

Ps. aeruginosa was identified by morphology, colony characteristics and pigment production on nutrient agar and King's medium (King, Ward & Raney, 1954). An attempt was made, in the absence of pyocines and specific typing sera, to determine whether the strains were identical or dissimilar by testing their sensitivity to a number of bacteriophages. Their antibiotic sensitivity was also investigated.

RESULTS

These are summarized in Table 1, which shows the distribution of *Ps. aeruginosa* in the premature-baby ward. It is evident that this organism was found consistently in the suction tubes, and to a lesser extent in the wash-basin outlets. The suction tubes were also contaminated with other Gram-negative bacilli, including *Klebsiella*, and occasionally with yeasts.

In view of the heavy contamination of the suction tubing, other parts of the 'Twin-O-Vac' suction units (The Commonwealth Industrial Gases Limited) were examined. These were mounted on the dividing partitions and walls and were connected to a piped oxygen supply which delivered oxygen to the intranasal catheter via a water bubbler and, by means of a venturi system, effected suction of mucus from the mouth into a glass jar containing aqueous 'Zephiran' 1 in 1600. Since the inlet tube to the jar did not extend below the disinfectant solution, aspirated material could be expelled directly to the ward. Table 2 records the results of all samplings.

Ps. aeruginosa was isolated regularly from the rubber suction tubing and metal inlets to the jar. Two of the six outlet tubes through which the exhaust air was discharged also harboured the organism at the time of sampling. Impingement plate cultures of exhaust air and oxygen from one of four units tested yielded a heavy growth of *Ps. aeruginosa* and other organisms as shown in Plate 1*b*.

We were not in a position to type the strains by the pyocine or serological methods described by Wahba (1965), but they were tested with bacteriophages available in this department (Holloway, Egan & Monk, 1960). The results, shown in Table 3, indicate that the strains could be separated into six different phage patterns, labelled for convenience A–F, and that the two fatal cases were due to dissimilar organisms. Some strains from the suction tubes corresponded to the strains causing infections, but those from the sink outlets were of unrelated patterns.

All the strains were sensitive to polymyxin, and moderately sensitive to tetracyline and chloramphenicol. Three were distinguished by resistance to streptomycin; these were in phage-group C (Table 3) and were unrelated to the strains causing the infections.

Table 1. *Distribution of* Pseudomonas aeruginosa *in premature-baby ward*

Source of cultures	Location	No. examined	No. positive for *Ps. aeruginosa*	No. positive for other Gram-negative bacilli
Suction tubes	Bay 1	4	4	4 (+ yeasts)
	Bay 2	2	2	2 (+ yeasts)
	Bay 3	1	1	1 (*Klebsiella*)
	Isolation room	1	1	1 (+ yeasts)
Oxygen tubes	Bay 1	4	0	0
	Isolation room	1	0	0
Oxygen bubblers	Isolation room	2	0	1
Incubator walls and ports	Bay 1	1	0	0
	Isolation room	1	0	0
Incubator water	Bay 1	3	0	0
	Isolation room	2	0	1
'Zephiran' solution (suction jars)	Bay 1	1	0	1
	Isolation room	1	0	1
Wash-basin outlets	Bay 1	1	1	1
	Bay 2	1	1	1
	Bay 7	1	0	1
	Isolation room	1	0	1
	Workroom	1	1	1
Taps and spray	As above	5	0	0
Air conditioners	Bay 1	1	0	0
	Isolation room	1	0	0
	Workroom	2	0	0*
Ward air	Corridor	1	0	0†

* *Cl. perfringens* and other *Clostridium* species isolated.
† 6 colonies/c.ft. (organisms unidentified).

Table 2. *Contamination of suction units with* Pseudomonas aeruginosa

Location of Unit	Rubber tubing		Jar inlet	Jar outlet	Exhaust oxygen
	Proximal*	Distal†			
Bay 1 Unit 1	+	+	+	+	−
Unit 2	+	+	+	−	+
Unit 3	+	+	+	−	−
Bay 2 Unit 1	+	+	+	−	N.D.
Unit 2	+	+	+	−	N.D.
Isolation room	+	+	+	+	−

* The end joined to the oral catheter.
† The end connected to the inlet tube of suction jar.
N.D. = test not done.

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Table 3. *Phage grouping of* Pseudomonas aeruginosa *strains isolated from premature-baby ward*

Pattern	Phages				Number of isolates	Source of strains
	E 79	F 116	120X	2X		
A	−	+	−	+	10	Case 5 5 suction units (bays 1 and 2) Oxygen exhaust (bay 1)
B	+	+	−	+	7	Cases 3 and 4 Suction unit (isolation room)
C	+	−	−	+	4	2 wash-basin outlets (bay 1 and workroom) 2 suction units (bays 1 and 2)
D	+	+	+	+	3	3 suction units (bays 1 and 2)
E	+	−	+	+	1	Case 2
F	+	−	−	−	1	Wash-basin outlet (bay 3)

MODIFICATION OF THE SUCTION EQUIPMENT

It was obvious that the suction tubes and aspirator jars were the most likely sources of cross-infection in this nursery, the transmission being effected either by gravity flow into the oral catheter, which was connected to the pressure tubing by a simple glass bulb, or by aerial dispersion in the exhaust from the suction jar. The former mechanism was easily prevented by connecting the catheter to a glass bulb fitted with an internally sealed tube to act as a 'no-return' trap for the mucus secretions and replacing the rubber tubing by clear disposable plastic which could be inspected for cleanliness and dryness. The cost of a 4 ft. length was approximately one shilling.

To prevent aerial dispersion from the suction jar a modification of the unit was required, namely the introduction of a liquid trap and fibrous filter in the unit. In order to test the efficiency of various filters and liquid traps an apparatus, illustrated diagrammatically in Fig. 2, was set up.

In this system an atomized culture of *Ps. aeruginosa* was introduced into an aerosol mixing chamber, from which the aerosol was drawn for 7 min. through the suction bottle and then into an exposure chamber. The difference in bacterial counts on nutrient agar plates from the aerosol and exposure chambers gave a measure of the efficiency of filters and liquid traps, either singly or in combination.

Table 4 shows results which are typical of many tests performed with this apparatus. It will be seen that the high count of airborne bacteria was progressively reduced, first by the glass-wool filter, more effectively by the water trap and completely by a combination of both.

As a result of these experiments the design of the 'Twin-O-Vac' unit was altered. The conversion of the standard to modified unit consisted of extending the inlet tube close to the bottom of the jar and attaching to the exhaust outlet a 3 in. metal filter packed lightly with glass wool (Corning Glass Co., no. 28C). Aqueous

chlorhexidine (300–400 ml. of 1 in 10,000) is used as a bacteriostatic liquid trap and is changed at least once a week. The standard and modified units are illustrated in Plate 2*a*, *b*.

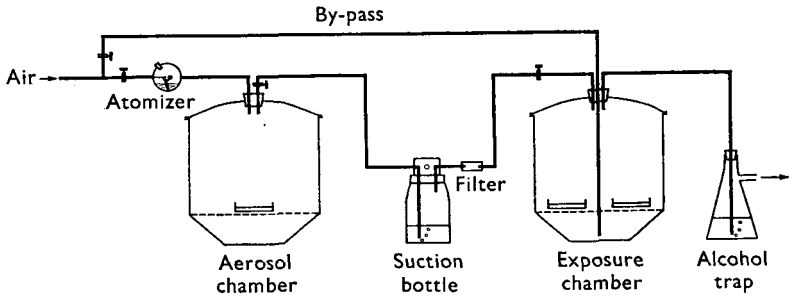

Fig. 2. Diagram of apparatus used for testing fluid traps and fibrous filters attached to suction jar.

Table 4. *The efficiency of removal of bacterial aerosols by modified suction equipment*

Suction equipment	Colony counts* of *Ps. aeruginosa* from Aerosol chamber	Exposure chamber
Standard	Uncountable	Uncountable
Modified with glass-wool filter	Uncountable	300–400
Modified with water trap (400 ml.)	Uncountable	17
Modified with glass-wool filter and water trap	Uncountable	0

* Nutrient agar plates were exposed during passage of aerosol through the apparatus for 7 min.

DISCUSSION

It is well known that *Ps. aeruginosa* survives and multiplies in moist conditions and this study has shown that the lumen of rubber suction tubes provided a consistent source of infection (Table 1). The wash-basin outlets yielded three isolations out of five samplings, but none of these strains corresponded to those associated with the infections (Table 3). The taps, including a spray device, the humidifying water in the incubators and the rubber oxygen tubes were all negative for *Ps. aeruginosa*, although one oxygen bubbler and the water from one cot reservoir produced heavy growth of other organisms. It seemed evident that the suction units and their tubing provided the main source of infection in this ward, a finding similar to that of Bassett *et al.* (1965). These authors abandoned the use of aspirators, parts of which are difficult to sterilize, in favour of disposable or boilable mucus extractors.

Apart from the obvious role of contaminated mucus gravitating into the oral catheter and thus initiating a contact infection, there exists also the problem of its dispersion as an aerosol spray in the ward. This could lead to the establishment of

widespread foci in the ward or nursery environment. For various reasons connected with the nursing of the babies in the hospital concerned, it was decided to continue the use of mechanical suction with the following recommendations:

(1) The standard 'Twin-O-Vac' unit was replaced with one possessing a liquid trap and a glass-wool or similar fibrous filter.

(2) Clear disposable plastic tubing was substituted for rubber tubing and was replaced daily.

(3) A glass bulb trap was used to prevent gravitational flow of mucus from the suction tubing to the oral catheter.

(4) The catheter and glass bulb were autoclaved before use.

(5) The suction jar was dismantled and cleaned weekly and recharged with 300–400 ml. of 1 in 10,000 aqueous chlorhexidine.

Since the introduction of these precautions no further infections due to *Ps. aeruginosa* have occurred, and swabs from the plastic tubing have yielded negative cultures.

SUMMARY

1. A small but serious outbreak of *Ps. aeruginosa* infection in a premature baby ward has been described.

2. Heavy contamination of the suction apparatus and tubing was demonstrated to be the main reservoir of infection.

3. It was also shown that this apparatus could disseminate the organism as an aerosol.

4. The measures adopted to eliminate the source and prevent dissemination of the infectious agent have been described.

We wish to thank Dr A. Wheildon, superintendent of the Queen Victoria Memorial Hospital, and the nursing sisters in the premature-baby ward for most helpful collaboration, also Mr H. Berkshire, the clinical photographer at the hospital, and officers of The Commonwealth Industrial Gases Limited. Dr B. W. Holloway of this Department assisted in phage-grouping the *Pseudomonas* cultures. One of us (J.C.F.) is supported by a grant from the National Health and Medical Research Council.

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EXPLANATION OF PLATES

PLATE 1

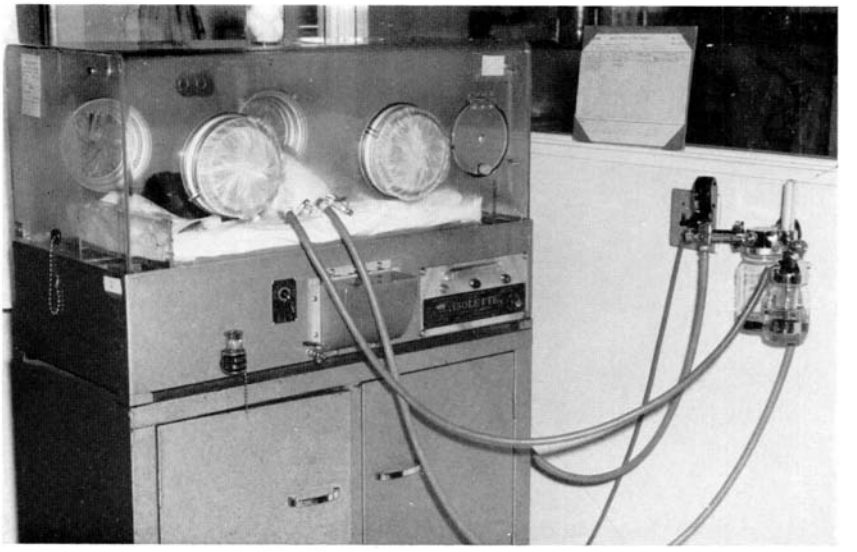
(*a*) Incubator ('Insul-cot' or 'Isolette') showing wall-mounted suction units ('Twin-O-Vac') for delivery of oxygen and removal of mucus by suction. Note the connexion between unit and incubator is by rubber tubing.

(*b*) Horse-blood agar plate exposed to exhaust oxygen from suction jar for 5 min. Twenty of the colonies were *Ps. aeruginosa*.

PLATE 2

(*a*) Standard 'Twin-O-Vac' suction unit showing oxygen humidifier and suction jar with rubber tubing connexion to the incubator.

(*b*) Modified unit showing liquid trap, glass-wool filter and clear plastic tubing.



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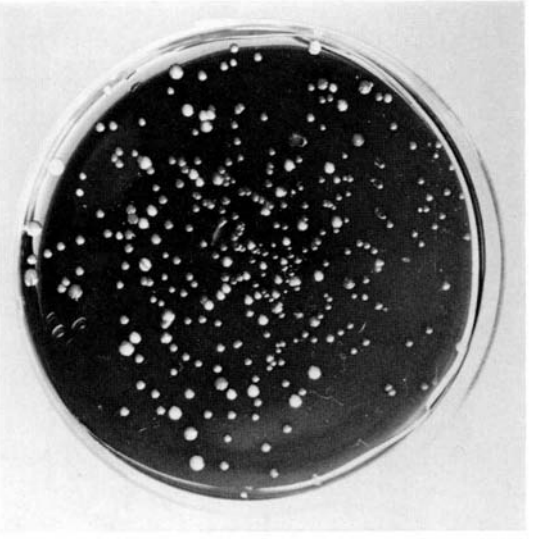

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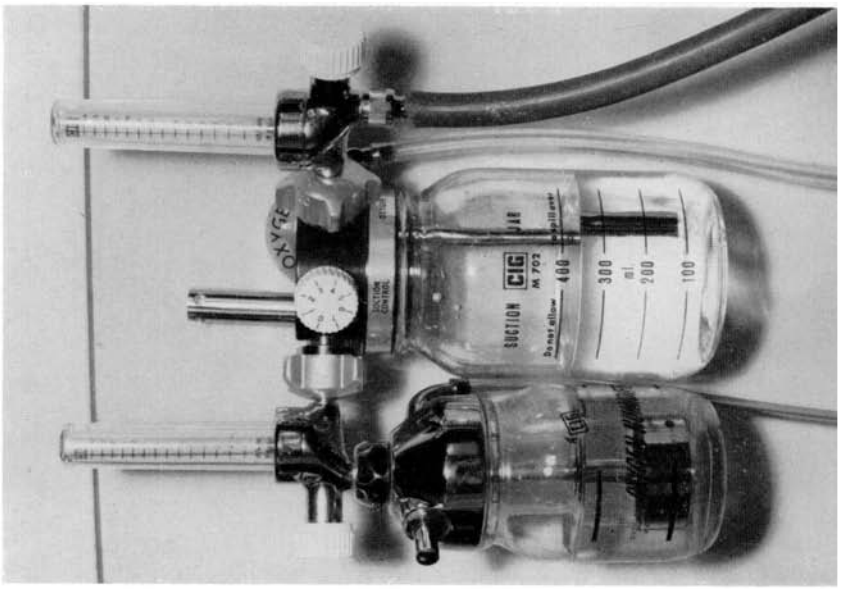

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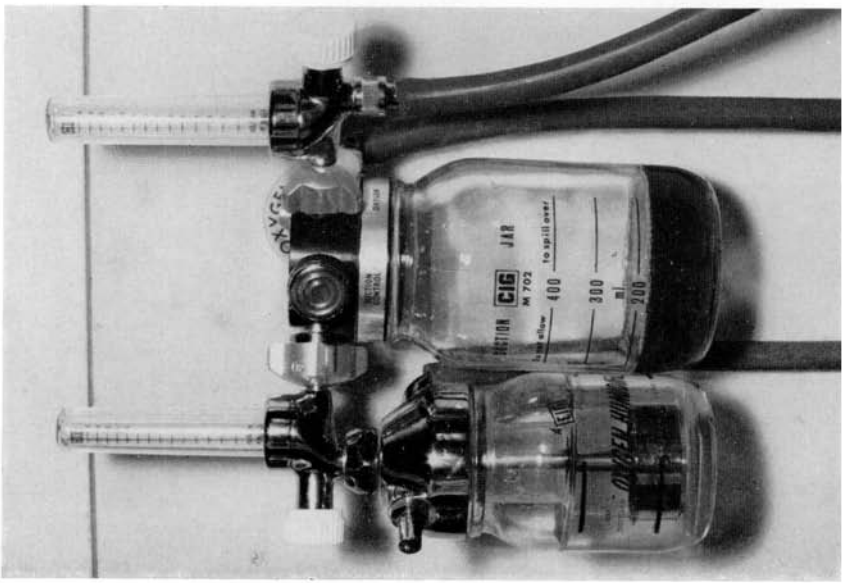

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FREDERICK GRIFFITH, 1879–1941

The main part of this issue is published as a memorial to Frederick Griffith. To commemorate the twenty-fifth anniversary of his death we are reprinting one of his most important papers on 'The Significance of Pneumococcal Types' (*J. Hyg. Camb.* (1928), **27**, 113–59). This is followed by contributions by some of his friends.

(*Facing p.* 129)

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