J. Hyg., Camb. (1967), **65**, 229
Printed in Great Britain

Pseudomonas aeruginosa respiratory tract infections in patients receiving mechanical ventilation*

By IAN PHILLIPS
*St Thomas's Hospital Medical School, London, S.E.*1†

(*Received* 1 *February* 1967)

It has been suggested that *Pseudomonas aeruginosa* respiratory tract infection in patients receiving mechanical ventilation arises from contaminated ventilators (Phillips & Spencer, 1965). Efforts to overcome the problem—sterilizing the machines (Bishop, Potts & Molloy, 1962; Sykes, 1964; Alder, Brown & Gillespie, 1966) and the use of filters (Bishop, Roper & Williams, 1963; Bishop, 1966)—have been based on this assumption. The alternative explanation, that the machines might become secondarily contaminated by patients infected by some other, unknown route, has not been excluded however.

This paper describes a study undertaken to determine the direction of spread of *Ps. aeruginosa*—machine to patient or patient to machine. It involved frequent, simultaneous sampling of the flora of the patients' respiratory tracts and of the ventilators in use on them, with the pyocine typing, and in some cases phage typing, of strains of *Ps. aeruginosa* isolated.

INVESTIGATIONS AND METHODS

The patients

All patients requiring tracheostomy and mechanical ventilation (except those recovering from cardiac surgery) during a period of just over one year starting in January 1965, in St Thomas's Hospital, were studied—18 patients in all. The diagnosis, ward and duration of mechanical ventilation for each patient are listed in Table 1. From the time of tracheostomy, swabs were taken at least daily from tracheostomy wounds, and samples of endotracheal aspirate were obtained at similar intervals. In addition, a stool sample or rectal swab was obtained from as many patients as possible before tracheostomy or soon after.

The mechanical ventilators (*Smith-Clarke, Cape Engineering Company*)

Swabs were taken from water-bath humidifiers and the tubing connecting patient to ventilator, between the water-bath humidifier and patient, before use and during use, at daily intervals at least, until contamination with *Ps. aeruginosa* was detected, when the machine was replaced.

After use the ventilators were disinfected by raising the temperature of the water in the humidifiers to boiling, and boiling tubing and metal connectors removable from the machine. The fixed parts of the machine were merely cleaned.

* This paper formed part of a Thesis for the degree of M.D., for the University of Cambridge.

† Present address: Department of Medical Microbiology, Makerere University College, Kampala, Uganda.

Occasionally ventilators were disinfected by the formalin method of Sykes (1964), but facilities were not available for the routine application of this method.

Water-bath humidifiers were left empty until just before use, when they were filled with sterile distilled water and heated to a temperature of about 44° C. In the course of the investigation distilled water was replaced by a solution of chlorhexidine digluconate (pure Hibitane, I.C.I.), 1/5000 in sterile distilled water.

The environment

From time to time, intensive sampling of all parts of the moist environment, and of bed-linen and floor dust, was carried out.

Table 1

Category	Case no.	Diagnosis	Ward	Duration of ventilation (days)	Infection time (days after tracheostomy)	Pseudomonas type from: Patient	Pseudomonas type from: Ventilator	Days between contamination and infection*
Already infected	2	Cor pulm.	MM1	35	—	S^{II}	—†	—
	3	Cor pulm.	MM1	42	—	S^{I}	—†	—
Becoming infected	1	Cor pulm.	MM1	42	26	B	—‡	+26
	4	Post-op.	MS2	1½	4	S^{I}	—†	?
	5	Cor pulm.	MM1	28	2	S^{I}	S^{I}	+2
	6	Polyneur.	FM2	11	3	S^{I}	S^{I}	−3
	7	Post-op.	FM3	1	1	S^{I}	S^{I}	+1
	8	Post-op.	ICU	10	2	S^{I}	S^{I}	−2
	10	Post-op.	ICU	11	5	S^{I}	S^{I}	+1
	11	Diabetic coma	ICU	6	3	Z	Z	+3
	12	Tetanus	ICU	28	10	S^{I}	Not contam.	—
	13	Barbit. poisoning	ICU	2	6	S^{III}	Not contam.	—
	14	Cor pulm.	ICU	14	5	D	D	+1
	15	Flail chest	ICU	14	5	S^{I}	S^{I}	+3
Not infected	9	Cor pulm.	ICU	28	—	Not infect.	Not contam.	—
	16	Cor pulm.	ICU	11	—	Not infect.	Not contam.	—
	17	Mercury poisoning	ICU	3	—	Not infect.	Not contam.	—
	18	Flail chest	ICU	21	—	Not infect.	Not contam.	—

Cor pulm. = cor pulmonale. Post-op. = post-operative. Polyneur. = polyneuritis. Barbit. = barbiturate.

* + = ventilator contaminated before patient infected; − = patient infected before ventilator contaminated.

† Not studied. ‡ lost before typing.

https://doi.org/10.1017/S002217240004571X Published online by Cambridge University Press

Bacteriological methods

Isolation and identification of Ps. aeruginosa

Sputum and endotracheal aspirate were homogenized by treatment with buffered pancreatin (Oxoid). All samples were cultured on blood agar, and in 0·03 % cetrimide in nutrient broth, subcultured after 24 hr. at 37° C. to blood agar.

Ps. aeruginosa was usually identified by its well-known typical colonial form and odour, and the identification was confirmed by the demonstration of pyocyanin production on enhancement medium (Pseudomonas Agar P, Difco). Strains with less typical colonial forms were identified by the following sequence of tests: examination of colonies on blood agar in ultra-violet light; Gram stain; oxidase test (Gaby & Hadley, 1957); oxidation of glucose (Hugh & Leifson, 1953); and pyocyanin production on enhancement medium. The identity of the single non-pigmented strain in this series was confirmed by three further tests—for gluconate oxidation (Haynes, 1951); arginine dihydrolase activity (Thornley, 1960); and growth in nutrient broth at 42° C. in three successive subcultures (Haynes & Rhodes, 1962).

Pyocine typing

The method described by Darrell & Wahba (1964) was used with a slightly modified set of indicator strains (Wahba, 1964) supplied by Dr M. T. Parker of the Central Public Health Laboratory, Colindale.

Phage typing

A number of strains with identical pyocine typing patterns were phage typed by Mrs E. H. Asheshov of the Central Public Health Laboratory, Colindale.

RESULTS

The patients

The eighteen patients studied may be divided into three groups—those who were already infected when mechanical ventilation was started (two patients, both with chronic bronchitis and cor pulmonale), those who became infected during treatment (12 patients), and those who never became infected (four patients). These groups are listed separately in Table 1.

The time taken for infection to develop varied from 1 to 26 days, but in all except two cases was less than 1 week.

Only one patient out of seven examined was found to be a faecal carrier of *Ps. aeruginosa* before treatment started: she subsequently developed respiratory tract infection, but the organism had a different pyocine typing pattern from that from the faeces.

The ventilators

Bacteriological study of the ventilators was possible in 15 of the 16 cases in which the patient was not already infected when tracheostomy was performed.

Before the use of chlorhexidine, 37 % of 125 samples from humidifiers contained

Ps. aeruginosa, but after its use, all of 111 samples were sterile. *Ps. aeruginosa* was found in 14·5% of 355 swabs taken from respirator tubing.

In six of the ventilators *Ps. aeruginosa* was never isolated from any part: these included the machines used on the four patients who did not become infected. In the nine cases where contamination was detected *Ps. aeruginosa* was isolated from the ventilator before the patient in seven, and from the patient before the machine in two (Table 1).

The environment

There was frequent contamination with *Ps. aeruginosa* of moist sites, e.g. drains, soap, nailbrushes, ointments, distilled water. Sheets in use on infected patients and floor dust close to these patients, were also often contaminated (Table 2).

Table 2. *Environmental studies*

Site	No. of samples	% positive for *Ps. aeruginosa*	Pyocine types
Respirator tubing	355	14·5	As patients
Water-bath humidifiers	125	37	As patients
Water-bath humidifiers plus chlorhexidine, and nebulizers	111	0	—
Sheets—Infected patients	20	50	As patients
Non-infected patients	20	0	—
Floor dust—Infected patients	20	75	As patients
Non-infected patients	20	0	—
Air (Slit-sampler)	Numerous	0	—
Ointments	16	20	Miscellaneous
Baths, sinks, soap, nail-brushes	Numerous	Frequent	Miscellaneous
Boiled catheters for endotracheal suction	25	0	—
Anaesthetic equipment	Numerous	0	—
Distilled water	6	50	Miscellaneous

Typing results

Pyocine typing

The commonest pyocine inhibition pattern was one not described by Darrell & Wahba: it resembled their type D except that indicator strain B 10 was consistently inhibited. It was labelled type S. Other patterns seen were Darrell & Wahba's types B and D, and inhibition of indicator strain 577 alone, labelled by us type Z.

Nine of the patients who became infected were found to have strains of type S: the ventilators used in these cases had type S organisms in six instances, while two were not contaminated and one was inadvertently not studied. Of the three patients who did not have type S strains, one had type B, another type D, and the third type Z. In the last two cases, the ventilators had organisms with identical patterns; in the first the strain was lost before typing.

Pyocine types of organisms from sheets and dust were the same as those of the organisms isolated from the respective patients. In contrast, organisms isolated from other sites were of a variety of pyocine types, distinct from those of the patients' organisms.

Phage typing

Pyocine type S was shown to include organisms with three different phage-typing patterns, which were labelled S^{I}, S^{II}, and S^{III} (Table 3).

Type S^{II} was isolated from one of the patients who was already infected (case 2) and type S^{III} from a patient whose ventilator was not shown to be contaminated (case 13). The remainder were S^{I}.

On the basis of pyocine typing and phage typing, 15 strains out of 22 isolated from these patients and their ventilators (excluding multiple isolations) were indistinguishable.

Table 3. *Phage-typing patterns of pyocine type S strains*

Strain	Phage-typing pattern
S^{I}	7/16/44/68/F 8/109/119x/1214
S^{II}	7/44/68/F 8/109/352/1214
S^{III}	7/21/31/F 8/109/119x/352/Coll 11

DISCUSSION

The three groups into which these patients fall each illustrate something of the probable epidemiology of *Ps. aeruginosa* respiratory tract infection following tracheostomy and mechanical ventilation.

The first group comprised two patients who were already infected before tracheostomy was performed: both had chronic bronchitis and cor pulmonale. It seems likely that such patients as these were responsible for some of the initial contamination of our mechanical ventilators.

The second group was made up of four patients who did not become infected. It is striking that in each case contamination of the ventilator was never demonstrated, a situation found in only two instances in the ventilators of those patients who did acquire infection.

The third and most important group was made up of the 12 patients who did become infected. In 11 of the 12, simultaneous sampling of tracheo-bronchial secretions and of the ventilators was carried out repeatedly until infection was established. In seven cases contamination of the ventilator was demonstrated before respiratory tract infection; in two infection developed in the absence of demonstrable contamination; and in two more infection was detected before contamination.

In the interpretation of these findings, it should be noted that the method of sampling was relatively inefficient, as in the interest of practicability it was possible only to sample four sites in the ventilator, chosen on the basis of a previously demonstrated high likelihood of contamination when other parts were contaminated. Thus detection of contamination depended on widespread colonization of the moist parts of the machine from limited foci not touched by our standard regime of disinfection. In contrast, sampling of the patients' tracheo-bronchial secretions was presumed to be more sensitive. The failure to detect contamination of the ventilator with *Ps. aeruginosa* before colonization of the patient's respiratory

tract in four instances is therefore not surprising, and the success in seven cases the more striking.

The results of pyocine and phage typing lend further support to the hypothesis of machine-to-patient spread. The finding of 15 indistinguishable strains out of 22 isolated—and these of an unusual pattern—is in striking contrast to the general distribution of pyocine typing patterns. Among strains of *Ps. aeruginosa* isolated from the respiratory tracts of 52 other patients in hospital during the same time, only two were of type S, the majority being types A, B or D, a distribution similar to that described by Darrell & Wahba (1964). Self-infection, in view of the predominance of a single pyocine type and the demonstration of faecal carriage in only one patient, seems unlikely. The most convincing explanation is cross-infection, and the only evident common source the ventilators. Patients nursed in distant wards acquired the same organism, which was otherwise only demonstrated in their immediate vicinity and in the ventilators, and never in the general environment of the hospital. Even where patients were nursed in the same ward they were usually separated in time, so that other infected patients are not a likely direct source.

In explanation of the importance of these findings, some discussion of the meaning of the word 'infection' in this context is relevant. Often the presence of *Ps. aeruginosa* in the respiratory tract is of little clinical importance. However, eight of these patients died, and in six of the seven cases where an autopsy was performed there was evidence of bronchopneumonia. In only three was bacteriological study possible: in two of these *Ps. aeruginosa* was the only organism isolated from areas of consolidation, while in the third *Ps. aeruginosa* was confined to the trachea and main bronchi and *Staph. aureus* was isolated in pure culture from the areas of bronchopneumonia, although it had not been found in the endotracheal aspirate. These cases leave no doubt that the finding of *Ps. aeruginosa* in the sputum may be an indication of true infection. It follows that patients treated by mechanical ventilation must be protected against acquiring infection from the ventilators, either by the use of ventilator components which are readily sterilizable or disposable, or by the use of filters to isolate the patient from the machine.

SUMMARY

Eighteen patients treated by tracheostomy and mechanical ventilation were studied in an attempt to determine whether contaminated ventilators could act as a source of *Pseudomonas aeruginosa* isolated from the respiratory tract. Twelve became infected. In seven instances it was possible to demonstrate prior contamination of the ventilator. Furthermore, eight of the patients had indistinguishable organisms on the basis of pyocine and phage typing, and six of the ventilators harboured the same organisms. The most likely explanation is cross-infection via contaminated machines.

I thank members of the consultant staff of St Thomas's Hospital for permission to study these patients and for helpful discussion, Sister S. Smith for collecting many of the specimens, Miss Susan Madden for careful technical help, Mrs E. H. Asheshov for phage typing and Dr M. Ridley and Dr M. T. Parker for continued good advice.

REFERENCES

ALDER, V. G., BROWN, A. M. & GILLESPIE, W. A. (1966). Disinfection of heat-sensitive material by low-temperature steam and formaldehyde. *J. clin. Path.* **19**, 83.

BISHOP, C. (1966). *Pseudomonas aeruginosa* cross-infection. *Lancet* i, 267.

BISHOP, C., POTTS, M. W. & MOLLOY, P. J. (1962). A method of sterilization for the Barnet respirator. *Br. J. Anaesth.* **34**, 121.

BISHOP, C., ROPER, W. A. G. & WILLIAMS, S. R. (1963). The use of an absolute filter to sterilize the inspiratory air during intermittent positive pressure respiration. *Br. J. Anaesth.* **35**, 32.

DARRELL, J. H. & WAHBA, A. H. (1964). Pyocine typing of hospital strains of *Pseudomonas pyocyanea*. *J. clin. Path.* **17**, 236.

GABY, W. L. & HADLEY, C. (1957). Practical laboratory test for the identification of *Pseudomonas aeruginosa*. *J. Bact.* **74**, 356.

HAYNES, W. C. (1951). *Pseudomonas aeruginosa*—its characterization and identification. *J. gen. Microbiol.* **5**, 939.

HAYNES, W. C. & RHODES, L. J. (1962). Comparative taxonomy of crystallogenic strains of *Pseudomonas aeruginosa* and *Pseudomonas chlororaphis*. *J. Bact.* **84**, 1080.

HUGH, R. & LEIFSON, E. (1955). The taxonomic significance of fermentative versus oxidative metabolism of various Gram-negative bacteria. *J. Bact.* **66**, 24.

PHILLIPS, I. & SPENCER, G. (1965). *Pseudomonas aeruginosa* cross-infection, due to contaminated respiratory apparatus. *Lancet* ii, 1325.

SYKES, M. K. (1964). Sterilizing mechanical ventilators. *Br. med. J.* i, 561.

THORNLEY, M. J. (1960). The differentiation of *Pseudomonas* from other Gram-negative bacteria on the basis of arginine metabolism. *J. appl. Bact.* **23**, 37.

WAHBA, A. H. (1964). Epidemiology of hospital infection with *Pseudomonas pyocyanea*. Thesis, University of London.