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Pseudomonas aeruginosa infection in hospital: a comparison between 'infective' and 'environmental' strains

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SUMMARY

One hundred and fifty-six infections or episodes of infection associated with *Pseudomonas aeruginosa* in six hospitals over 14 months were investigated. Pyocine typing and serotyping suggested that 145 distinct episodes had occurred, caused by 78 different strains. During this period 15 distinct strains were isolated from the environment at one of the hospitals; 12 of these were apparently unassociated with infection in the same ward during the period, and 4 were of types not encountered in infective processes at any hospital. There appeared to be a rather higher proportion of unclassifiable pyocine inhibition patterns among the environmental strains; in general these strains also produced smaller amounts of haemolysin. If failure to produce haemolysin *in vitro* is correlated with lack of virulence *in vivo*, this may partially explain the sporadic nature of hospital infection with *Ps. aeruginosa*, despite the prevalence of strains of this species in the environment.

INTRODUCTION

Hospital infections caused by *Pseudomonas aeruginosa* appear to be infrequent, except in rather specialized situations. A recent survey (Harris & Gray, 1974) showed that infections due to this species occurred only in approximately 0·5% of patients admitted to the Sheffield Royal Hospital between 1967 and 1972, major infections being virtually confined to debilitated and seriously ill patients nursed in the intensive care unit. However, even in this unit, some patients who seemed eminently liable to become colonized by *Ps. aeruginosa* remained uninfected despite a prolonged stay and the demonstrable presence of the organism in their immediate environment. A somewhat similar situation was described by Shooter *et al.* (1966), who found that there were certain strains which, although encountered in faeces and the environment, did not spread to other patients in the ward.

There are various possible reasons for this anomaly. Firstly, careful aseptic technique on the part of the nursing staff may have prevented transfer of *Ps. aeruginosa* from environment to patients. Secondly, conditions in the tissues of these patients may not have been favourable to the multiplication of *Ps. aeruginosa* or to production of the extracellular toxins on which the pathogenicity of the species is thought to depend (Liu, 1964). A third possibility is that strains of

Ps. aeruginosa may vary in virulence, so that some strains present in the environment may be incapable of initiating infection. In the present study we attempted to determine the extent to which strains present in the environment were responsible for infection, and whether the production of infection was associated with readily identifiable characteristics of the strains.

MATERIALS AND METHODS

One hundred and fifty-six infections associated with *Ps. aeruginosa* were studied. The infections occurred in six Sheffield hospitals over a period of 14 months (January 1973 to February 1974). At hospital A (the Royal Hospital) all 62 infections occurring during the period were investigated. At hospital B (another large general hospital) we studied 61 infections, representing most of those occurring during the period and including all suspected episodes of cross-infection. Hospitals C, D and E are smaller and specialize in obstetrics and gynaecology, paediatrics and plastic surgery respectively. Hospital F deals only with out-patients. All strains of *Ps. aeruginosa* from hospitals C, D and E were derived from suspected instances of cross-infection; the strains from hospital F were a random selection. The numbers of strains studied at these more specialized hospitals were 12, 3, 9 and 9 respectively.

At the Royal Hospital, samples were taken from sink outlets, baths, mop buckets and other moist situations (excluding ventilators and suction equipment) in 7 wards at approximately monthly intervals; 4 of these wards were medical, 2 were surgical and the seventh was the intensive care unit. All strains of *Ps. aeruginosa* were identified according to the criteria of Phillips (1969); they were subsequently pyocine-typed (Gillies & Govan, 1966; Govan & Gillies, 1969) and serotyped on the basis of their O antigens, using Institut Pasteur antisera (Al-Dujaili & Harris, 1974). Colonial morphology was classified as 'typical', 'coliform-like', 'mucoid', 'rugose' or 'dwarf' (Phillips, 1969).

Fifteen strains, of which some had been isolated from patients and others from the environment, were tested for production of various extracellular toxins. The methods used for separating fractions Ia and Ib (pyocyanin and other pigments), fraction II (haemolysin) and fraction III (protease, lecithinase and lipase) were those of Liu, Abe & Bates (1961). For each strain tested, a 'standard preparation' of each fraction was prepared by adjusting the final volume so that the product of one culture plate was contained in 3 ml. Preliminary experiment showed that the greatest biological activity was possessed by fractions II and III. Doubling dilutions of these fractions were titrated against appropriate substrates (freshly drawn human erythrocytes, egg yolk suspension, casein (Hammarsten) and Tween 80, all at a concentration of 5 %. Tests for fraction II were incubated for 1 hr. at 28° C., and those for fraction III for 24 hr. at 37° C. A control fraction of known potency was included in each batch of tests.

Table 1. *Predominant pyocine and sero-types encountered in* 145 *infections or episodes of infection caused by* Ps. aeruginosa

Pyocine type; serotype	Number of Sporadic infections	Episodes of cross-infection	Patients in each episode
5 ; 3	8	0	0
3 ; 6	7	1	4
10 ; 11	5	1	2
1(c) ; 8	7	0	0
1(d) ; 5	4	0	0
1(b) ; 11	3	0	0
1(b) ; 4	3	0	0
1(d) ; 7	3	0	0
1(f) ; 9	3	0	0
8 ; 3	0	1	5
1(c) ; 5	0	1	2
Other combinations (67)	98	0	0

RESULTS

The results of pyocine and serotyping suggested that 145 distinct infections or episodes of infection had occurred, the strains responsible showing 78 different pyocine-serotype combinations. Most infections appeared to be sporadic (Table 1). Strains responsible for infection commonly belonged to pyocine type I and serotype 3 or 6.

There were only 4 episodes of undoubted cross-infection, and the maximum number of patients in an episode was 5. Strains associated with episodes of cross-infection were apparently not necessarily associated with sporadic infection elsewhere. All proved episodes of cross-infection occurred either in intensive care units or in neurosurgical wards.

The strains isolated from the inanimate environment at the Royal Hospital could be divided into 15 distinct pyocine-serotype combinations. Table 2 shows the extent to which strains encountered were responsible for infection in the same ward, and the number of infections caused in other wards (either in the same or another hospital) by apparently identical strains. Twelve of the environmental strains were unassociated with infection in the same ward during the period, even though one was isolated consistently from the same environmental site for 5 months. Furthermore, 5 strains were of a pyocine-serotype combination which was not represented among the 'infective' strains isolated at any of the hospitals. The 3 strains associated with subsequent infection were all isolated during the first month of the investigation, and may originally have been derived from patients admitted to the wards before the survey began. However 10 of the 12 strains apparently unassociated with infection were isolated after sampling had been in progress for 5 months or longer. It was not possible to determine with certainty the origin of these strains, but since persistence of a known infective strain in the environment for more than a month after the patient's discharge was uncommon, it is unlikely that these strains were derived from prior infection.

Table 2. *Clinical infections caused by strains of* Ps. aeruginosa *present in the environment of 7 wards in hospital A*

Pyocine; serotype	Present in (no. of wards)	Number of infections caused in: Same ward	Other wards (hospital A)	Other hospitals
10 ; 11	2	2	3	2
6 ; na	1	0	1	0
10 ; 13	1	0	0	1
uc ; 10	2	0	0	1
uc ; 3	3	0	2	4
1(a) ; 4	1	0	0	0
1(g) ; 6	1	0	0	0
uc ; 4	1	0	0	0
uc ; na	1	0	0	0
uc ; 1	1	1	1	0
uc ; 6	1	3	3	0
10 ; 5	1	0	0	0
1(a) ; 7	1	0	0	2
1(b) ; 4	1	0	0	3
1(d) ; 5	1	0	0	4

uc, Unclassifiable pyocine inhibition pattern; na, non-agglutinable.

The great majority of strains, whether isolated from patients or the environment, possessed a typical colonial morphology; a few tended to produce a 'coliform-like' colony. Mucoid strains were isolated only from the sputum of patients suffering from cystic fibrosis; no other colonial types were encountered either in patients or in the environment.

Six of the 15 environmental strains (including 2 of the 5 unassociated with infection in the same ward) produced pyocine inhibition patterns which were unclassifiable according to the Gillies & Govan scheme; only 15 (19·2%) of the 78 distinct strains isolated from infective lesions showed unclassifiable patterns.

The results obtained when selected strains were investigated for production of extracellular toxins are shown in Table 3. In general there was little correlation between the extent of protease, lecithinase and lipase production and the source from which the strain was derived. However haemolysin titres were generally higher in those strains which had been responsible for infection. Strains 1, 24, 32, 23 and 61, although showing similarities of pyocine and serotype, were derived from unconnected patients in different wards. No specific relation could be demonstrated in this small number of strains between toxin production and either pyocine type or serotype.

DISCUSSION

Controversy still exists over the importance of contamination of the hospital environment by *Ps. aeruginosa*. For example, Teres *et al.* (1973) considered sinks to be a major source of infection in a respiratory-surgical intensive care unit; however, Lowbury *et al.* (1970), studying a similar unit, had found that although

Table 3. *Biological activity of fractions II and III of selected 'infective' and 'environmental' strains of* Ps. aeruginosa

Strain no.	Pyocine; serotype	Source	Ass. infections	Reciprocal of titres: Haemolysin	Protease	Lecithinase	Lipase
1	3 ; 6	Patient	8	16	32	4	2
24	3 ; 6	Patient	8	32	32	16	16
23	3 ; 6	Patient	8	32	16	4	2
32	1(c) ; 8	Patient	7	16	16	8	< 2
61	1(c) ; 8	Patient	7	16	8	2	2
150	1(a) ; 5	Patient	2	16	8	16	< 2
158	10 ; 11	Patient	5	32	4	16	< 2
58	1(a) ; 7	Patient	2	8	8	4	4
157	uc ; 6	Patient	6	8	8	8	2
17	10 ; 13	Environment	1	2	16	16	8
26	1(g) ; 6	Environment	0	4	4	2	2
15	uc ; na	Environment	0	2	32	8	2
171	34 ; 3	Environment	0	2	8	8	2

Ps. aeruginosa could be demonstrated in the inanimate environment between outbreaks of infection, in only half of these interim periods did the strains isolated from the environment belong to the same type as a strain that caused infection during the next outbreak. At other times, sinks and other moist environmental sites were colonized by endemic strains which could not be incriminated in episodes of infection. Our findings tend to support the view of Lowbury and his colleagues. Of course it is not possible to say that strains isolated only from the environment during the relatively short period of the investigation have never caused infection or would never do so if given the opportunity. However, in general terms, it appears that certain differences exist between these strains and those which were commonly responsible for infection. For example, although the number of strains isolated from the environment was relatively small, it appeared to contain a larger proportion of strains producing unclassifiable pyocine inhibition patterns than the group of strains isolated from patients. The fact that the inhibition patterns do not appear in the scheme of Gillies and Govan suggests that such strains were also uncommon as causes of infection in Edinburgh at the time the scheme was devised. This does not necessarily imply that the production of specific pyocines is related to pathogenicity. It is more likely that the strains identified by these methods are incapable of elaborating sufficient toxin, and that this deficiency is unrelated to any specific pyocine inhibition pattern. Wretlind, Hedén, Sjöberg and Wadström (1973) investigated a large number of strains isolated from various infections. They found that all strains were capable of producing haemolysin, protease, lecithinase and lipase, irrespective of whether they had originated from life-threatening infections or trivial colonizations; production of the toxic factors was not linked to specific types. However, these authors did not investigate quantitative aspects of toxin production, nor did their series include any environmental strains. In the limited number of strains which

we have investigated, production *in vitro* of haemolysin seems to be the factor which was most closely associated with the ability to produce infections. However it cannot be inferred that the haemolysin is necessarily the most important factor in the pathogenesis of *Ps. aeruginosa* infection, since the extent of its production *in vivo* appears to be influenced by a number of host factors, notably the glucose content of tissues (Liu, 1964). It is therefore necessary to interpret with caution the apparent difference in haemolysin production between 'infective' and 'environmental' strains. The production *in vitro* of the enzymes which collectively form Liu's fraction III was much less closely correlated with the ability to produce infection. However, once again, production of these enzymes in the patient may be greatly increased if tissue conditions are especially favourable; for instance, it has been shown that protease production may be enhanced by accumulation of lactic acid in the tissues (Liu, 1964). In certain experimental models, importance has been attached to the growth-rate of different strains (Klyhn & Gorrill, 1967) and the time interval required for production of significant amounts of the various toxins (Carney & Jones, 1968). It is possible that the relative importance of different factors in the pathogenesis of *Ps. aeruginosa* infection varies according to the circumstances in which infection occurs, and further experimental work appears to be needed in relation to specific problems. Meanwhile the present study suggests that haemolysin production may be a suitable *in vitro* marker of virulence; if this prediction is fulfilled by *in vivo* experiments, it may be possible to identify potentially dangerous strains in the hospital environment.

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