J. Hyg., Camb. (1973), **71**, 113
Printed in Great Britain

An epidemiological study of *Pseudomonas aeruginosa* in cattle and other animals by pyocine typing

BY ROSE MUSHIN AND G. ZIV
Kimron Veterinary Institute, Bet Dagan, Israel

(*Received* 20 *June* 1972)

SUMMARY

The high incidence (80·1 %) of *Pseudomonas aeruginosa* pyocine type 1 in bovine mastitis in Israel suggested some degree of selectivity under local conditions. Contrary to other reports, cattle and calves showed a high rate of faecal carriage of this organism. The water supply on farms was often contaminated. The presence of certain pyocine types in the udder, gut or water occasionally led to their transmission from one reservoir to another; however, many types did not seem to spread.

P. aeruginosa was found in association with infections in various animals and was present in many locations such as a mouse breeding house and a chick hatchery.

INTRODUCTION

Pseudomonas aeruginosa is considered to be a natural inhabitant of the human intestine and common enough in water and soil (Editorial, 1969). In recent years the significance of this organism as a pathogen of man (Editorial, 1966) became well established.

A perusal of literature indicated the need for additional information on the association of *P. aeruginosa* with animals. The organism is not considered to be a normal inhabitant of the intestinal tract of most animals, however, various infections were occasionally reported (Hoadley & McCoy, 1968). Attention was given to its role in bovine mastitis, but it is not regarded as a common cause of this disease, only of concern in some herds (Schalm, Carroll & Jain, 1971).

The frequent occurrence of *P. aeruginosa* mastitis in Israel prompted a previous investigation of this problem (Ziv, Mushin & Tagg, 1971). The present study covers a broader field and is concerned with the following topics: (1) the epidemiology of *P. aeruginosa* mastitis in cattle and the faecal carrier rate in cattle and calves, (2) the association of this organism with various animals, and (3) its occurrence in water on farms.

The application of pyocine typing of isolates from various sources allowed the labelling of strains and the assessment of their relationship.

MATERIALS AND METHODS

Isolation of Pseudomonas aeruginosa

P. aeruginosa isolations from udders, rectum and faecal matter from cattle and from water supplies were carried out in our laboratory. Additional cultures from a variety of animals, often from pathological conditions, came on nutrient agar or Kligler iron agar.

Milk samples were obtained from two sources, from bovine udders from farms and from a slaughterhouse. The samples were taken with aseptic precautions, incubated at 37° C. for 24 hr. and plated out on sheep blood agar.

In the examination of material of intestinal origin, preliminary tests indicated the advisability of using large samples. Thus heavily charged rectal swabs or large faecal samples were placed in 5 ml. of 0·03 % cetrimide liquid medium (Brown & Lowbury, 1965), and incubated at 37° C. for 24 hr. A large loopful of each suspension was subsequently transferred to another tube of cetrimide medium for 48 hr. incubation. Water samples were placed in cetrimide medium in the approximate proportion of 1:1. At a later stage of this study, the second series of cetrimide cultures were incubated in a water bath at 42° C. Plating was done from cetrimide tubes on cetrimide agar or SS (Difco) agar.

Identification of Pseudomonas aeruginosa

Preliminary identification of *P. aeruginosa* from blood agar plates was on colony form, type of haemolysis and odour. These colonies and colonies from cetrimide and SS agar were further examined on the basis of biochemical reactions in Kligler iron agar, positive oxidase test and production of chloroform-soluble pyocyanin, or occasionally pyorubin, in the liquid medium A of King, Ward & Raney (1954), which was incubated at 32° C. up to 7 days. These characteristics were sufficient for the identification of *P. aeruginosa*, while non-pigmented strains were further tested for growth in broth at 42° C. on two successive transfers with a straight needle, and on the production of gaseous nitrogen in Durham tubes in nitrate broth. Pyocine production was another useful diagnostic feature.

Pyocine typing

The procedure for pyocine typing followed with some modifications the scheme of Gillies & Govan (1966) and Govan & Gillies (1969), which lists 37 pyocine types. Some isolates, not classified by these workers, were allotted designations UC_1 to UC_9 (Tagg & Mushin, 1971). The major modification in the technique was the introduction of an apparatus ('broomette') for the simultaneous streaking of indicator strains (Tagg & Mushin, 1971). Besides the original eight indicator strains I_1 to I_8 of Gillies & Govan (1966), five additional indicators A to E of Govan & Gillies (1969) for the subdivision of pyocine types 1 and 10 into subtypes *a* to *h* were used. The introduction of two of our own indicator strains, labelled I_A and I_B (Tagg & Mushin, 1971) allowed for an additional division of pyocine types into subtypes labelled + or −, on the basis of the activity of a potential producer on the above two indicators. Accordingly, strains were recorded as 1^{+-}a, 10^{--}h, 3^{++}, and similarly.

Each strain was typed at least twice, using composite growth picked up by a loop sweep from a 24 hr. agar plate. Colonies showing a distinct dissociation were on occasions individually typed. In the tabulation of the distribution of pyocine types, the isolation of two or more types from a single specimen was recorded, while identical types were only once listed.

Table 1. *Distribution of pyocine types in* Pseudomonas aeruginosa *strains isolated from bovine mastitis on farms and from bovine udders in a slaughterhouse*

Pyocine	116 strains from farms			50 strains from slaughterhouse		
Type and subtype	No.	%		No.	%	
$1^{++}a$	7	6·0		1	2·0	
$1^{++}b$, $1^{+-}b$	17	14·7		13	26·0	
$1^{++}c$, $1^{+-}c$, $1^{--}c$	9	7·8		10	20·0	
$1^{+-}d$	2	1·7	80·1	0	0	72·0
$1^{+-}f$, $1^{--}f$	10	8·6		0	0	
$1^{++}h$, $1^{+-}h$, $1^{--}h$	45	38·7		9	18·0	
$1^{+-}uc$, $1^{--}uc$	3	2·6		3	6·0	
$10^{++}b$	0	0		2	4·0	
$10^{+-}c$	1	0·9	3·5	1	2·0	10·0
$10^{++}h$	3	2·6		2	4·0	
3^{++}, 3^{+-}	7	6·0	9·4	3	6·0	16·0
Other types	4*	3·4		5†	10·0	
Unclassifiable	6‡	5·2		0	0	
Untypable	2	1·7		1	2·0	

uc = unclassifiable subtypes.
* = types: 2^{--}, 5^{++}, 13^{--}, 35^{--}, one of each type.
† = types: 2^{--}, two; 4^{+-}, 6^{++}, 30^{--}, one of each type.
‡ = three were UC_2.

RESULTS

Pyocine types from bovine mastitis on farms

A total of 116 *P. aeruginosa* strains were isolated from cows' udders, some of which were re-examined on a few occasions, within a time interval of at least 2 months. As shown in Table 1, pyocine type 1 represented by 80·1 % isolations was predominant, and the most frequently encountered subtype was 1h, pattern $1^{++}h$ being the most common. The remaining 23 strains belonged to six pyocine types, or to the unclassifiable or untypable group.

Pyocine types from bovine udders from a slaughterhouse

Of a total of 50 *P. aeruginosa* strains (Table 1) isolated from udders in a slaughterhouse, 72·0 % were pyocine type 1, with a scatter of subtypes. Type 10 was responsible for 10 % isolations, while the remaining isolates were represented by five pyocine types and one untypable strain.

Pseudomonas aeruginosa *in faeces of cattle, calves and in water*

The survey of animal faecal carriers included cattle and calves from nine farms and a slaughterhouse. Contrary to reports found in the literature and to be discussed later, the faecal carrier rate was high (Table 2), reaching 60·0 % in calves and 32·2 % in cattle. It fluctuated in being lower in calves on farms and much higher in calves in a slaughterhouse, as compared with cattle in these locations.

A perusal of data from individual farms also indicated fluctuation in the faecal carrier rate. *P. aeruginosa* was not recovered from faecal specimens from groups

https://doi.org/10.1017/S0022172400046271 Published online by Cambridge University Press

Table 2. *Incidence of* Pseudomonas aeruginosa *in bovine faecal carriers and in water samples*

Location	Source	Total no. examined	Positive No.	Positive %
Nine farms	Cattle	185	45	24·3
	Calves	33	6	18·2
Six farms	Water	16	9	56·3
Slaughterhouse	Cattle	59	19	32·2
	Calves	35	21	60·0
	Water	2	1	

Table 3. *Incidence of* Pseudomonas aeruginosa *faecal carriers in groups of calves of various ages*

Calves	Age in months < $1\frac{1}{2}$	3–5*	4*	> 4*	6–9 (a)	6–9 (b)	12 (c)	12 (d)
No. examined	13	11	5	19	5	5	6	4
No. positive	2	3	3	15	0	0	0	4
% positive	15·4	27·3	60·0	78·9	0	0	0	100

* From slaughterhouse, other calves from farms. Figures in parentheses refer to groups of calves from different farms.

of twenty and twelve cows on two farms with cases of subclinical mastitis. On the other hand, on a farm where only recently a few cases of *Pseudomonas* mastitis were diagnosed, the faecal carrier rate in cows ranged from 10 % to 50 % and for calves up to 6 weeks of age it was 15 %.

It is of interest to note the high rate of isolation of *P. aeruginosa* from water. Of sixteen samples of water from six farms, 56·3 % were positive, and each farm was represented. On two farms, where bovine faecal carriers were not detected, one out of two, and two out of three water samples were positive for this organism.

Faecal carrier rate of Pseudomonas aeruginosa *in groups of calves of various ages*

Table 3 presents the results of examination of faecal matter from calves under 6 weeks till 1 year of age. In a slaughterhouse the faecal carrier rate was as high as 78·9 % for calves over 4 months old. On farms, calves were faecal carriers when under 6 weeks and when 1 year old. The negative specimens came from three groups of calves, between 6 and 9 months, and under 12 months of age.

Pyocine types from bovine faecal carriers and from water on farms and in a slaughterhouse

In Tables 4 and 5, the figures referring to pyocine types of *P. aeruginosa* strains from bovine faecal carriers represent single and multiple isolates and therefore are higher than the figures which indicate the incidence of the species in Tables 2 and 3.

The survey of pyocine types in faecal carriers on farms (Table 4) showed that the occurrence of type 1 (36·2 %) was much lower than in udders (80·1 %). In all,

Table 4. *Distribution of pyocine types of* Pseudomonas aeruginosa *strains isolated from bovine faecal carriers and from water on farms*

Pyocine	58 strains from cattle			10 strains from calves		9 strains from water	
Type and subtype	No.	%		No.	%	No.	%
1^{++}a	1	1·7		0		1	
1^{++}b, 1^{+-}b	5	8·6		1		0	
1^{+-}c	2	3·4		0		0	
1^{++}f, 1^{--}f	3	5·2	36·2	0	30·0	0	44·4
1^{+-}g	1	1·7		0		1	
1^{++}h, 1^{--}h	2	3·4		0		1	
1^{+-}uc	7*	12·0		2		1	
10^{++}c, 10^{++}d	0	0	1·7	0	0	2	22·2
10^{++}h	1	1·7		0		0	
3^{++}	12	20·7		3		0	
2^{--}	5	8·6	50·0	0	4	0	0
Other types	12†	20·7		1‡		0	
Unclassifiable	2	3·4		0	0	0	0
Untypable	5	8·6		3	30·0	3	33·3

Figures referring to strains from faecal carriers represent single and multiple isolates.
* Five subtypes had pattern + − − + −.
† Types: 5^{+-}, 27^{++}, 29^{--}, 35^{--}, two of each type; 8^{--}, 11^{--}, 31^{--}, 37^{+-}, one of each type.
‡ Type: 35^{--}.
Unclassifiable types were UC_3.

Table 5. *Distribution of pyocine types of* Pseudomonas aeruginosa *strains isolated from bovine faecal carriers and from water* in a slaughterhouse*

Pyocine	22 strains from cows		36 strains from calves	
Type and subtype	No.	%	No.	%
1^{++}b	0		2	
1^{++}c, 1^{+-}c	5		3	
1^{+-}d	1		0	
1^{--}f	0	63·6	2	36·1
1^{++}g	1		0	
1^{++}h, 1^{+-}h	3		5	
1^{+-}uc, 1^{--}uc	4		1	
10^{+-}c	1	13·6	2	11·1
10^{+-}d, 10^{+-}f	2		2	
3^{++}	3	13·6	15	41·7
Other types	0	0	3†	8·3
Untypable	2	9·1	1	2·8

Figures referring to strains represent single and multiple isolates from faecal carriers.
* Two pyocine types: 1^{++}a and 36^{+-}.
† Types: 6^{++}, two; 12^{+-}, one.

there was a varied collection of pyocine types, comprising twelve types, and of unclassifiable and untypable strains. Strains from calves and water showed a limited range of pyocine types, and the occurrence of type 1 was listed as 30·0 % and 44·4 % respectively. From the faecal carriers and water samples a compara-

https://doi.org/10.1017/S0022172400046271 Published online by Cambridge University Press

Table 6. Pseudomonas aeruginosa *in cows, calves and in the water on particular farms*

Farm code	Pyocine types in: Faeces: Cows	Calves	Udder	Water
B	1^{+-}uc*	1^{+-}uc*	—	—
	1^{+-}c	1^{+-}b	—	—
	1^{--}f	3^{+-}	1^{--}f	—
	10^{++}	UT	UC	UT
P	UC, UT	0	1^{++}h	10^{++}a, 10^{++}c
S	1^{++}a	1^{++}a	UC	—
	1^{++}h	—	—	—
	27^{++}, 37^{+-}	—	—	—

* The same subtype, + − − + −. UT = untypable. UC = unclassifiable.

Table 7. Pseudomonas aeruginosa *pyocine types from pathological material from various animals*

Animal	No.	Pyocine type
Goose	19*	UT*
Goose	2	1^{++}b, 1^{+-}b
Turkey	1	1^{++}e
Chicken	1	1^{++}b
Lamb	2	10^{++}b, 3^{++}
Cow	1	1^{+-}c
Calf	8	UC(3), 1^{+-}c(2), 1^{++}b, 3^{++}(2)
Mink	1	27^{++}
Fox	1	UT
Otter (*Lutra* sp.)	1	3^{++}
Skunk (*Vormela* sp.)	1	1^{+-}c

* All of one type as shown with an additional indicator. Figures in parentheses are numbers of each pyocine type. UC = unclassifiable. UT = untypable.

tively high number of untypable strains was isolated. Some of them proved to be sensitive to phage typing which was carried out by the courtesy of Mrs R. Ziv. Especially in dealing with atypical non-pigmented strains, a pyocine or a phage pattern were of diagnostic significance.

Data presented in Table 5 indicate that pyocine type 1 was frequently encountered amongst cows in a slaughterhouse (63·8 %), while amongst calves the figure was lower (36·1 %). In the material from calves pyocine type 3 was most common (41·7 %). The examination of two water samples yielded two pyocine types from one of them, namely 1^{++}a and 36^{+-}.

Pyocine types in cows, calves and water on particular farms

Table 6 gives a representative set of data on the occurrence of pyocine types in cows and calves of the same herds, namely in the udder and the intestine, and in water supplies. Occasionally the same pyocine type was recovered from different

https://doi.org/10.1017/S0022172400046271 Published online by Cambridge University Press

Table 8. *Distribution of pyocine types in* 88 Pseudomonas aeruginosa *strains isolated from laboratory mice*

Pyocine	Strains		
Type and subtype	No.	%	
1^{+-}a	1	1·1	
1^{+-}b	2	2·2	
1^{+-}c, 1^{--}c	13	14·7	38·6
1^{++}h	1	1·1	
1^{--}f	3	3·3	
1^{+-}uc, 1^{--}uc	14*	15·9	
5^{+-}	21	23·9	
13^{--}	7	10·0	35·3
Other types	3†	3·3	
Unclassifiable	12‡	13·6	
Untypable	11	12·5	

* Eleven subtypes had pattern: — — — + —.
† Types: 2^{--}, 11^{--}, 29^{+-}, one of each type.
‡ Two were UC$_4$ and six were UC$_7$.

sources, such as the faeces of cows and calves, from udder and faeces, or possibly from water and faeces (untypable strain). However, usually, there was a greater range of types in the faecal material.

Pyocine types from pathological material from various animals

The material was obtained at post-mortem examinations from animals with various pathological conditions, such as bacteraemia, diarrhoea or lung infection. A set of 19 cultures, isolated from a flock of geese dying of bacteraemia (Table 7), was untypable with the indicators of Gillies and Govan (1966). However, an additional indicator was sensitive to all the strains examined, thus demonstrating their similarity.

Specimens from various animals yielded *P. aeruginosa* of different pyocine types, some unclassifiable or untypable. The only strain which was not encountered in the cultures listed in previous Tables was subtype 1^{++}e from a turkey.

Pyocine types in laboratory mice

The high incidence of *P. aeruginosa* in colonies of mice in a breeding unit provided an opportunity of sampling an additional source. The mice were of inbred lines raised under SPF (specific pathogen free) conditions, however *P. aeruginosa* became an established inhabitant. The specimens were faeces and water from drinking bottles in the cages, and occasionally organs from mice obtained at post-mortem examinations.

It can be seen in Table 8 that amongst 88 strains examined, pyocine type 1 was predominant (38·6 %) and type 5^{+-} was next in the frequency of appearance (23·9 %). The occurrence of unclassifiable or untypable strains, 13·6 and 12·5 % respectively, was comparatively high.

Table 9. *Distribution of pyocine types in* 46 Pseudomonas aeruginosa *strains isolated in a chick hatchery*

Source of specimen	Pyocine: Type and subtype	No. of strains
Dead embryo	1^{+-}c	2
1-day-old chicks	1^{+-}c	2
	1^{++}b, 1^{+-}b	2
	1^{++}h	2
	3^{++}	3
	5^{+-}, 5^{--}, UC, UT	4
Eggs	1^{+-}c, 5^{--}	2
Solution before dipping eggs	1^{+-}c	4
	1^{++}h	1
Solution after dipping eggs	1^{+-}c	21
Miscellaneous	1^{+-}h, UC, UT	3

UC = unclassifiable. UT = untypable.

For experimental purposes some mice were irradiated and many died of *Pseudomonas* septicaemia. Of twenty-four strains, the majority were either 1^{+-} or untypable.

Pyocine types in chick hatchery

Another set of *P. aeruginosa* cultures came from a chick hatchery. As seen in Table 9, the predominant type was pyocine 1^{+-}c, which was found in 4 samples of a solution before the dipping and in 21 after the dipping of eggs. It was also isolated from two dead embryos and from an egg shell. The pyocine types were similar to those encountered in other locations, but few were unclassifiable or untypable.

DISCUSSION

The present study indicated a close association of *P. aeruginosa* pyocine type 1 with bovine mastitis on farms in Israel. The incidence was high, 80·1 %, while the previously reported figure by Ziv, Mushin & Tagg (1971) was 65·7 %. The introduction of subtypes a to h (Govan & Gillies, 1969) and of a subdivision into + and − subtypes (Tagg & Mushin, 1971) allowed for more exact fingerprinting of strains. In this series of strains, pattern 1^{++}h was most frequently encountered. The above data, with some deviation, were comparable with those recorded for cattle in a slaughterhouse, the incidence of type 1 being 72·0 %.

The faecal carrier rate of pyocine type 1 in adult cattle and in calves on farms was lower, being 36·2 % and 30·0 % respectively, and in a slaughterhouse 63·6 % and 36·1 % respectively. This pyocine type was found in four out of nine *P. aeruginosa* strains from water on farms. In colonies of laboratory mice type 1 was recovered from 38·6 % of samples from these animals and from water bottles in their cages. It was also isolated from clinical material from a variety of animals, but in this series of cultures there was a disproportionate weighting of a number of untypable strains from one source. In a survey of *P. aeruginosa* in a chick hatchery type 1 was predominant, especially type 1^{+-}c which was found in various locations.

https://doi.org/10.1017/S0022172400046271 Published online by Cambridge University Press

It is well documented that pyocine type 1 is the most commonly encountered type in material from human sources. In Israel, specimens from two hospitals accounted for the incidence of 37 % and 45 % (unpublished data). The figure in a survey in Scotland was 34·2 % (Govan & Gillies, 1969), in Australia 31 % (Tagg & Mushin, 1971) and in clinical isolations in U.S.A. 52·1 % (Heckman, Babcock & Rose, 1972).

It seems that although the high incidence of pyocine type 1 in bovine mastitis in Israel is the reflection of its common occurrence in the environment, the exceptionally high figure indicates a certain selectivity. The prolonged presence in the cow's udder may be partly due to the chronic character of *Pseudomonas* mastitis.

Attention was given to statements found in the literature that faecal carriers of *P. aeruginosa* appeared occasionally amongst animals associated with man. In a survey by Hoadley & McCoy (1968) the percentage of positive samples for man was 11·5 %, while transient carriage (9·45 %) in calves under four weeks of age, only on some farms, and occasional carriage in some other animals in proximity to man was observed. A study by Matthews and Fitzsimmons (1964) on the incidence of *P. aeruginosa* in the intestine of calves indicated that it was exceptional for this organism to be present in these animals by the time they attained the age of 8 weeks.

Our results, contrary to other reports, indicated a high faecal carrier rate both in adult cattle and in calves. The highest carrier rates, 78·9 % and 60·0 % were encountered in a slaughterhouse in calves over the age of 4 months and at 4 months, and on farms the organism appeared in young calves under 6 weeks and at 1 year of age. The faecal carrier rate in adult cattle in the above locations was 32·2 % and 24·3 %.

As previously mentioned, *P. aeruginosa* is common enough in water and soil (Editorial, 1969). Human faeces and sewage were shown to be its normal habitat, while natural waters were found to be devoid of this organism (Ringen & Drake, 1952). A contaminated warm water system used for spray udder washing was considered to be a source of infection of cattle (Curtis, 1969). In a collection of *P. aeruginosa* strains (Csiszar & Lanyi, 1970), 218 were from water and sewage, and some originated from a municipal water supply. In our survey nine out of sixteen water samples from farms carried this organism.

Pyocine typing was found to be useful in examining the epidemiological aspects of our survey. *P. aeruginosa* types isolated from cows' udders, from faecal material and from water on particular farms were compared. Occasionally the same pyocine type appeared in udder and faeces or in water and faeces. Shooter *et al.* (1966) in their survey of faecal carriage of *P. aeruginosa* in hospital patients, observed that although numerous strains appeared in faeces, many did not seem to spread. Similarly, in our study, the high incidence and selectivity for pyocine type 1 in cows' udders and the presence of many other pyocine types in faecal material did not point to a frequent transmission from one reservoir to another. Apparently more data should be collected on the dissemination of *P. aeruginosa* under various environmental conditions.

Thanks are expressed to our colleagues for their co-operation in supplying us with *P. aeruginosa* cultures from a variety of sources.

This work was supported by the Kimron Research Prize for one of us (R.M.).

REFERENCES

BROWN, V. I. & LOWBURY, E. J. L. (1965). Use of an improved cetrimide agar medium. *Journal of Clinical Pathology* **18**, 752.

CSISZAR, K. & LANYI, B. (1970). Pyocine typing of *Pseudomonas aeruginosa*: association between antigenic structure and pyocine type. *Acta Microbiologica Academiae Scientiarum Hungaricae* **17**, 361.

CURTIS, P. E. (1969). *Pseudomonas aeruginosa* contamination of warm water system used for pre-milking udder washing. *Veterinary Record* **84**, 476.

EDITORIAL (1966). *Pseudomonas aeruginosa. Lancet* i, 1139.

EDITORIAL (1969). Resistant *Pseudomonas. Lancet* ii, 473.

GILLIES, R. R. & GOVAN, J. R. W. (1966). Typing of *Pseudomonas pyocyanea* by pyocine production. *Journal of Pathology and Bacteriology* **91**, 339.

GOVAN, J. R. W. & GILLIES, R. R. (1969). Further studies in the pyocine typing of *Pseudomonas pyocyanea. Journal of Medical Microbiology* **2**, 17.

HECKMAN, M. G., BABCOCK, J. B. & ROSE, H. D. (1972). Pyocine typing of *Pseudomonas aeruginosa*: clinical and epidemiological aspects. *American Journal of Clinical Pathology* **57**, 35.

HOADLEY, A. W. & MCCOY, E. (1968). Some observations on the ecology of *Pseudomonas aeruginosa* and its occurrence in the intestinal tract of animals. *Cornell Veterinarian* **58**, 354.

KING, E. O., WARD, M. K. & RANEY, D. E. (1954). Two simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine* **44**, 301.

MATTHEWS, P. R. J. & FITZSIMMONS, W. M. (1964). The incidence and distribution of *Pseudomonas aeruginosa* in the intestinal tract of calves. *Research in Veterinary Science* **5**, 171.

RINGEN, L. M. & DRAKE, C. H. (1952). A study of the incidence of *Pseudomonas aeruginosa* from various natural sources. *Journal of Bacteriology* **64**, 841.

SCHALM, O. W., CARROLL, E. J. & JAIN, N. C. (1971). In *Bovine Mastitis*. Philadelphia: Lea & Febiger.

SHOOTER, R. A., WALKER, K. A., WILLIAMS, V. R., HORGAN, G. M., PARKER, M. T., ASHESHOV, E. H. & BULLIMORE, J. F. (1966). Faecal carriage of *Pseudomonas aeruginosa* in hospital patients. Possible spread from patient to patient. *Lancet* ii, 1331.

TAGG, J. R. & MUSHIN, R. (1971). Epidemiology of *Pseudomonas aeruginosa* infection in hospitals. 1. Pyocine typing of *Ps. aeruginosa. The Medical Journal of Australia* **1**, 847.

ZIV, G., MUSHIN, R. & TAGG, J. R. (1971). Pyocine typing as an epidemiological marker in *Pseudomonas aeruginosa* mastitis in cattle. *Journal of Hygiene* **69**, 171.