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Pyocine typing as an epidemiological marker in *Pseudomonas aeruginosa* mastitis in cattle

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SUMMARY

Pyocine typing was used for the characterization of 134 *Pseudomonas aeruginosa* strains isolated from bovine mastitis. The scheme of Gillies & Govan (1966) was adopted with some modifications, and the procedure gave 89·6% typability. Pyocine type 1 strains were most commonly encountered and were followed in frequency by types 10 and 3. The introduction of two additional indicator strains allowed for division of these types into subtypes.

In spite of some limitations, discussed in the paper, the pyocine typing scheme proved to be useful in 'marking' *P. aeruginosa* strains and in following their association with bovine mastitis in various herds.

INTRODUCTION

Pseudomonas aeruginosa is widely distributed in nature and consequently the udder of the dairy cow may at times be exposed to this organism. Reports on sporadic cases of bovine mastitis due to this agent appeared in the early literature (Pickens, Welsh & Poelma, 1926; Cherrington & Gildow, 1931; Cone, 1939). However, more recently, attention has been drawn to the increasing incidence of *P. aeruginosa* mastitis and in some instances the disease was reported to be a major herd problem (Plastridge, 1958; Nurmi & Koiranen, 1967), characterized by recurrent infections (van Kruningen, 1963; Schalm, Lasmanis & Carroll, 1967). It was suggested (Mastitis Sub-Committee, 1965) that herd outbreaks might be attributed to the introduction for use in cowsheds of quaternary ammonium compounds to which *P. aeruginosa* proved to be resistant, to contaminated intramammary chemotherapeutic preparations and contaminated milking equipment (Tucker, 1950) and to contaminated water (Redaelli & Perini, 1960; Curtis, 1969).

During investigations of the control of bovine mastitis in Israel (Ziv, 1971) it was found that in several herds, in spite of efficient husbandry and sanitary milking practices, *P. aeruginosa* infected as many as 80% of the cows' udders. This contributed to severe economic losses. In the course of an investigation aiming at tracing the source of infection, the organism was recovered from the skin of the teats and udders, from the milking equipment, from the floor, walls

and bedding in the milking sheds, from the drinking water in utensils but not from the main water supply.

It was of interest to follow the epidemiology of infections and to 'finger-print' the strains of *P. aeruginosa*. The pyocine typing scheme of Gillies & Govan (1966) was adopted with some modifications. The procedure is based on the ability of most *P. aeruginosa* strains to produce pyocines, which are specific antibiotic-like substances with a lethal activity, mainly restricted to other members of this species. Pyocine types are recognized by the patterns of growth inhibition of selected indicator strains.

The present communication records the pyocine typing of *P. aeruginosa* strains, mostly from cases of bovine mastitis, and a few from the cows' environment.

MATERIALS AND METHODS

Origin of Pseudomonas aeruginosa *strains*

Procedures used for the milk sampling and for the isolation and identification of *P. aeruginosa* have been described (Ziv, 1971). The organism was recovered from the secretions of 134 quarters (glands) of 76 cows in 26 dairy herds. In most herds *P. aeruginosa* was cultured from one to three cows only, but in each of four herds about 80 % were infected. Infection of a single quarter of the udder was the most common feature, but 22 cows were infected in two quarters, and nine cows in three or four quarters. In addition, 14 *P. aeruginosa* strains recovered from the previously infected quarters after an interval of 5 months were available for the study of the constancy of infections with particular pyocine types.

Pyocine typing

The procedure for pyocine typing was similar to that described by Gillies & Govan (1966), but we have introduced an apparatus for simultaneous streaking of indicator strains instead of individual application. Furthermore, to the original set of eight indicator strains, I_1 to I_8, received by the courtesy of Dr Gillies and Dr Govan, we have added two of our own indicator strains labelled I_A and I_B. The apparatus used consisted of two parts, one contained ten wells for the indicator strains, which were broth cultures grown for 4 hr. at 37° C., and the other part (named 'broomette') had ten prongs with rounded tapered ends. The prongs were inserted into the wells and then streaked across the plate at a right angle to the line of the original inoculum of the producer strain. The patterns of inhibition ef the indicator strains were recorded after 16 hr. incubation at 32° C.

Drug sensitivity tests

The procedure has been described previously (Ziv & Risenberg-Tirer, 1970). The minimal bactericidal concentrations (MBC) were determined using the following drugs: carbenicillin, polymyxin B, colistin, gentamycin, tetracycline, dihydrostreptomycin, kanamycin, neomycin and nalidixic acid.

RESULTS

Of 134 *P. aeruginosa* strains isolated from bovine udders and typed with the eight indicator strains of Gillies & Govan (1966), 120 (89·6 %) proved to be typable. As shown in Table 1, eight pyocine types were differentiated and type 1, represented by 88 strains, was most frequently encountered. Amongst the other strains, 15 were type 10, 6 were type 3, and the remaining types were 6, 2, 11, 7 and 31. The introduction of two additional indicator strains I_A and I_B provided a useful division of the most frequently encountered types 1, 10 and 3 into subtypes 1^{++}, 1^{+-}, 10^{++}, 10^{--}, 3^{++}, and 3^{+-}.

Table 1. *Distribution of pyocine types in* 134 Pseudomonas aeruginosa *strains isolated from bovine mastitis*

Pyocine		Strains		
Type	Subtype	Number		%
1	++	73	88	65·7
	+−	15		
10	++	2	15	11·2
	−−	13		
3	++	3	6	4·5
	+−	3		
6		4		3·0
2		3		2·2
11		2		1·5
7		1		0·7
31		1		0·7
Untypable		14		10·5

Table 2. *Distribution of pyocine types in* Pseudomonas aeruginosa *strains isolated from cows' udders in four herds, each with about* 80 % *incidence of infection*

Herd code	No. of infected quarters	Percentage of quarters infected with strains of pyocine type					
		1++	1+−	10−−	3+−	11	Untypable
M	9	0	33	67	0	0	0
P	20	55	0	15	0	10	20
A-6	18	100	0	0	0	0	0
Z	15	60	20	0	20	0	0

A different pyocine type pattern was noted in *P. aeruginosa* strains from each of the four herds with the incidence of infections reaching about 80 % (Table 2). All strains isolated from cows in herd A-6 were pyocine type 1^{++}, and this was also the type of strains recovered from the skin of the udder and teats of these animals and from various fomites such as milking equipment and utensils used for this particular herd. Pyocine type 1 strains were predominant in herds P and Z but were not encountered in herd M, whereas in herds Z and M strains of subtype 1^{+-} were recorded. In herds M, P and Z the other strains were pyocine type 10^{--}, or

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3^{+-}, or the rare type 11; 20 % of strains in herd P were found to be untypable. The above data, although limited in number, indicate that different pyocine type patterns may be found in *P. aeruginosa* strains from various dairy herds, and may serve as useful markers for epidemiological surveys.

Table 3. *The pyocine types of* Pseudomonas aeruginosa *strains isolated from 22 cows each infected in 2 quarters of the udder*

		Pyocine type found in the	
Herd code	Cow no.	First quarter	Second quarter
A	1	1++	1++
P	1	1++	1++
P	8	1++	Untypable
Q	2	1++	1++
G	1	1++	1++
L	2	1++	1++
L	4	1+−	10−−
M	2	1+−	10−−
M	7	1+−	10−−
A-1	1	1+−	10−−
A-2	1	1+−	1+−
Z	2	1++	3+−
Z	3	1++	1++
Z	5	1++	3+−
Z	7	1++	1++
A-6	2, 3, 4, 5 6, 8, 10	1++	1++

Table 4. *The pyocine types of* Pseudomonas aeruginosa *strains isolated from nine cows each infected in 3 or 4 quarters of the udder*

		Pyocine type found in various quarters			
Herd code	Cow no.	First	Second	Third	Fourth
P	5	UT	UT	10−−	
P	9	1++	11	11	1++
P	10	1++	1++		10−−
Q	1	10−−	1++	1++	
T	2		2	2	1++
O	1	1+−		1+−	1+−
Y	1	UT	UT	UT	UT
Y	2	1++	1++	1++	UT
Z	8	3+−	1+−	1+−	

UT = untypable strains.

A comparison was made between the pyocine types of *P. aeruginosa* strains isolated from 22 cows, each infected in two quarters. Table 3 shows that strains of the same pyocine type infected both quarters of 15 (68 %) cows. Further, a comparison was made between pyocine types of strains isolated from nine cows, each with multiple infection of three or four quarters. It was found (Table 4) that in every case, with the possible but unlikely exception of cow Y1, at least two quarters

were infected with *P. aeruginosa* of the same pyocine type. It should be noted that infection of two quarters of the udder was recorded with the rare types 2 and 11. It seems, therefore, that a fair degree of uniformity was shown by the simultaneous presence of organisms of the same pyocine type in different quarters of the udder.

The availability of 14 *P. aeruginosa* isolates from udders of cows which were found to be infected with this species 5 months previously, provided the opportunity of assessing whether the same or different pyocine types appear on repeated examination. Table 5 shows that strains of the same pyocine type were encountered only in five cases.

Results of the drug sensitivity tests did not reveal any significant relationship between the patterns obtained and the various *P. aeruginosa* pyocine types.

Table 5. *Constancy of pyocine types in strains of* Pseudomonas aeruginosa *obtained after 5 months from the same quarters in the same cow*

Herd code	Cow no.	Pyocine type found: On first examination	Pyocine type found: After 5 months
M	2	10 − −	1 + −
O	1	1 + +	1 + +
P	1	1 + +	1 + +
P	1	1 + +	UT
P	2	1 + +	7
P	3	10 − −	1 + +
P	8	UT	1 + +
P	9	1 + +	1 + +
P	9	1 + +	1 + +
P	10	10 − −	UT
P	11	1 + +	1 + +
L	1	1 + +	UT
L	2	1 + +	UT
L	4	10 − −	1 + −

UT = untypable strains.

DISCUSSION

Bacteriophage typing and serotyping have been employed by various workers to differentiate strains of *P. aeruginosa*. However, these procedures require the preparation and maintenance of a collection of phages and diagnostic sera. The introduction of pyocine typing schemes (Darrell & Wahba, 1964; Gillies & Govan, 1966) provided simpler techniques for use in diagnostic laboratories and were adopted by various workers for epidemiological studies.

In the present survey the method of Gillies & Govan, applied to the pyocine typing of *P. aeruginosa* strains from bovine mastitis in Israel, gave 89·6% of typable strains. No record has been found in the literature of this procedure being applied in surveys of bovine pseudomonas mastitis in other countries. Govan & Gillies (1969) gave the figure 7·6% of untypable strains in an investigation of pyocine typing of clinical strains in Scotland, while J. R. Tagg and R. Mushin

(unpublished data) listed 8·6 % in their series from hospitals in Australia. Pyocine type 1 strains were most commonly encountered in the above three surveys, concerned with man and animal. In the present study strains of pyocine type 10 and 3 came next on the list of frequency of appearance, as compared with pyocine types 3 and 5, and 3 and 10 in the Scottish and Australian surveys, respectively.

The addition of two indicator strains I_A and I_B allowed the division of the more commonly occurring pyocine types 1, 10 and 3 into subtypes.

It is of interest to note the results of pyocine typing of material from other sources in Israel. Thus of 15 *P. aeruginosa* strains from laboratory mice, 12 were type 3^{+-} and three were untypable, while 35 strains from human sources were predominantly type 1^{++} (49 %), less frequently types 1^{+-} and 10^{++} and occasionally types 35, 6, 3^{+-} or untypable (G. Ziv, unpublished).

The examination of *P. aeruginosa* strains from two or more quarters of the same udder, and from the same quarters after an interval of 5 months, showed at times the presence of different pyocine types. The present results are comparable with those reported by Gillies & Govan (1966) and Govan & Gillies (1969) who found that in a series of *P. aeruginosa* strains from human cases, some belonged to different pyocine types from those originally isolated from the same site of the patient. Evidence pointed to the possibility of multiple infections rather than to instability of the pyocine types.

In conclusion, in spite of the occurrence of some untypable strains and the limited number of pyocine types, the scheme seems to be of practical value in characterizing *P. aeruginosa* strains associated with bovine mastitis.

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REFERENCES

Cherrington, V. A. & Gildow, E. M. (1931). Bovine mastitis by *Pseudomonas aeruginosa*. *Journal of the American Veterinary Medical Association* **79**, 803.

Cone, J. F. (1939). *Pseudomonas aeruginosa* in bovine mastitis. *Journal of Agricultural Research* **58**, 141.

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

Darrell, J. H. & Wahba, A. H. (1964). Pyocine typing of hospital strains of *Pseudomonas pyocyanea*. *Journal of Clinical Pathology* **17**, 236.

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.

Kruiningen van, H. J. (1963). Successful treatment of a herd outbreak of *Pseudomonas* mastitis. *Cornell Veterinarian* **53**, 240.

Mastitis Sub-Committee of the Technical Development Committee (1965). Controlling bovine mastitis. *Veterinary Record* **77**, 612.

Nurmi, E. V. & Koiranen, L. (1967). Bacteriological review of milk samples from clinical mastitis. *Nordisk Veterinaermedicin* **19**, 36.

PICKENS, E. M. M., WELSH, F. & POELMA, L. J. (1926). Pyocyaneus bacillosis and mastitis due to *Pseudomonas aeruginosa*. *Cornell Veterinarian* **16**, 186.

PLASTRIDGE, W. (1958). Bovine mastitis – a review. *Journal of Dairy Science* **41**, 1141.

REDAELLI, G. & PERINI, G. (1960). Contributo allo studio della mastite bovina da '*Pseudomonas aeruginosa*'. *Archivio Veterinario Italiano* **11**, 273.

SCHALM, O. W., LASMANIS, J. & CARROLL, E. J. (1967). Experimental *Pseudomonas aeruginosa* mastitis in cattle. *American Journal of Veterinary Research* **28**, 697.

TUCKER, E. W. (1950). Pseudomonas infection of the bovine udder apparently contracted from contaminated equipment and materials. *Cornell Veterinarian* **40**, 95.

ZIV, G. (1971). Mastitis control in the family-type dairy herd in Israel: I. Effects of milking hygiene and antibiotic treatment on new infections. *Refuah veterinarith* **28**, 1.

ZIV, G. & RISENBERG-TIRER, R. (1970). The in vitro activity of several antibiotics against Pseudomonas of bovine udder origin. *Zentralblatt für Veterinärmedizin* B **17**, 963.