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SEROLOGICAL GROUP DIFFERENTIATION OF PSEUDOMONAS AERUGINOSA FROM VARIOUS SOURCES

By

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In recent years Pseudomonas aeruginosa has attracted increasing attention in medical bacteriology. The frequency of severe infections with this organism has risen both in man and in domestic animals (3, 13, 14, 15). This has led to intensified study of the occurrence of the organism in nature and in diverse food-stuffs, etc. (13, 16, 19, 20, 21, 22, 23).

To throw light on various epidemiological and epizootological relationships many workers have tried to differentiate isolated strains of Ps. aeruginosa serologically using specific agglutination or precipitation sera (1, 2, 4, 5, 6, 7, 8, 10, 11, 12, 18, 24) as well as by bacteriophages (15).

The present investigation was undertaken partly as an attempt to differentiate serologically strains of Ps. aeruginosa isolated from various sources and partly to study any relationships that may exist.

MATERIAL AND METHODS

The material consisted of 274 strains of Ps. aeruginosa isolated from pathological processes in human subjects and animals, and from other sources, such as milking utensils, cattle fodder, food-stuffs, and water. All the strains were isolated within a limited area north of Lake Mälar in Sweden over the four-year period 1960—1963.

Out of the 274 strains 215 were of more or less direct animal origin. 148 of these 215 strains were obtained from bovine mastitis in 24 herds and 43 from milking machines and other equipment in the same herds. About one-third of these strains were isolated from a herd (about 75 cows) with a high frequency of pseudomonas mastitis. In some cases more than one strain was isolated from the same quarter on different occasions. The other strains of animal origin were isolated from suppliers milk, autopsy material from cattle and pig, the prepuce of bull, and otitis externa in dog.

The strains of human origin numbered 43. They were isolated from the personnel tending the cattle in herds affected with pseudomonas mastitis and from sporadic cases in various hospitals and private practice within the area (sores, otitis media, urinary-tract infections, faeces, sputum, rhinitis).

Sixteen strains were isolated from cattle feed, consumers milk, ice-cream, and water.

The strains were isolated and subcultured at the Veterinary Bacteriological Laboratory, Västerås. They were then immediately sent to the Veterinary College of Norway, where they were classified serologically by the slide-agglutination test (18). Eight sera were used. Among these, sera I—VII were identical with Sandvik's group sera I—VII; serum VIII was prepared with a strain from bovine mastitis included in the material as antigen.

RESULTS

The results of the serological group differentiation are shown in Table 1. Out of the 274 Ps. aeruginosa strains a total of 241 (88.0 %) were classified; 17 (6.2 %) showed spontaneous agglutination and 16 (5.8 %) were not agglutinated with the sera used.

Group differentiation could be effected in 195 (90.7 %) of 215 strains of animal origin, in 31 (72.1 %) of 43 strains of human origin and in 15 (93.8 %) of 16 strains isolated from other sources.

DISCUSSION

It will be seen from Table 1 that group differentiation by Sandvik's sera I—VII was possible in a large proportion of the material. Accordingly, these sera proved to be suitable for differentiation of Ps. aeruginosa strains in a material that had no

Table 1. Result of serological group differentiation of 274 strains of Pseudomonas aeruginosa.

Tested strains			Classified strains									Non-classified strains	
			Serological groups										
	Origin	No.	I	II	III	IV	V	VI	VII	VIII	No.	Sp[a]	NT[b]
Animal (215 strains)	Bovine mastitis	148	36	34	31	2	6	2	8	12	131	15	2
	Milking utensils	43	6	17	15	2			3		43		
	Suppliers milk	9		3			1		5		9		
	Autopsy material, cattle	10			7				2	1	10		
	Autopsy material, pig	1					1				1		
	Prepuce, bull	1										1	
	Otitis externa, dog	3	1								1		2
Human (43 strains)	Hands of personnel	5			4				1		5		
	Wound secretion	3	1				1				2		1
	Otitis media	22	14		1	2		1		1	19		3
	Urinary-tract infection	10	1		3						4	1	5
	Faeces	1											1
	Sputum	1											1
	Rhinitis	1							1		1		
Other sources (16 strains)	Cattle fodder	2	2								2		
	Consumers milk	1	1								1		
	Ice-cream	1	1								1		
	Water	12	7	1	3						11		1
	Total	274	70	55	64	6	9	3	20	14	241	17	16

[a] = spontaneous agglutination
[b] = non-typable by the sera used

connection with the strains on which Sandvik bases his grouping. By adding one more serum, no. VIII, to these seven sera, more strains could be classified. Further sera were not prepared for this material.

No less than five of the eight sera used were prepared with strains isolated from bovine mastitis as antigen. Excluding those that showed spontaneous agglutination, all but two of the strains isolated from bovine mastitis, milking utensils, and autopsy material from cattle could be classified serologically. *Sandvik* (18) obtained a similar result by sera I—VII; he tested 76 strains

isolated from bovine mastitis and milking machines, among which most of the strains from mastitis belonged to group I. In the material presented here, groups I, II, and III predominated, with uniform distribution among the groups. All the groups were represented in the animal material, however.

In general, all the strains isolated from the same herd could be referred to one serological group. Nineteen strains, for example, isolated in one herd belonged to group I. It could therefore be assumed that the organisms in such a herd were of common origin. More than one group of the organism occurred in 3 of the 24 herds. In a herd of about 75 cows with a high frequency of pseudomonas mastitis both group II and group III were represented and, in addition, a few strains showed spontaneous agglutination. The organisms had probably been introduced into this herd during the pasture season. The pastures were irrigated with water from a faecally contaminated stream, from which groups II and III of the organism were isolated. Ps. aeruginosa was also demonstrated in a cattle man who tended this herd. Organisms of group III were isolated from his hands and nose.

Ps. aeruginosa was in some cases isolated from the same quarter on more than one occasion. Strains from one and the same quarter belonged to the same serological group.

Among the human strains that did not show spontaneous agglutination more than two-thirds could be differentiated into groups, although none of the sera used had been prepared against strains of human origin (18). The latter may have been one reason why a relatively high percentage of the human strains could not be classified, but the explanation can also be that, being isolated mostly from sporadic cases in hospitals and private practice, these strains represent a more comprehensive biological material. Sixteen of the human strains belonged to group I, but here, too, all the groups except one were represented. The result of the investigation presented here thus confirms the earlier observation that group I predominates (17).

Out of 16 strains of "other origin" 15 could be serologically classified. In spite of the small number, there is reason to note that these strains belonged to groups I, II, and III, with the greatest number in group I.

The result of the group differentiation suggests that a relatively small number of representative group-specific sera can be employed for serological classification of pathogenic strains of

Ps. aeruginosa. As occasion may require, the system can be extended by the addition of a number of antisera that might be found necessary for epidemiological studies.

In many pseudomonas infections the source and routes of infection are obscure. Serological variants of the organism occur in human subjects and domestic animals, as well as in divers foodstuffs and water. The result of the investigation presented here suggests many relationships of both theoretical and practical interest. Continued studies in this field are needed to throw further light on various epidemiological and epizootiological relationships.

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SUMMARY

The authors have isolated 274 strains of Pseudomonas aeruginosa from various sources. The strains were differentiated into groups by eight specific agglutination sera. Group differentiation could be effected by these sera in 241 (88.0 %) of the 274 strains, in 195 (90.7 %) of 215 strains of animal origin, in 31 (72.1 %) of 43 strains of human origin, and in 15 (93.8 %) of strains isolated from cattle fodder, foodstuffs, and water.

The results of this and earlier studies indicate that a relatively small number of group-specific antisera can form the basis of a serological classification of pathogenic strains of Pseudomonas aeruginosa of various geographical and epidemiological origin.

The results also suggest many relationships of theoretical and practical interest.

ZUSAMMENFASSUNG

Serologische Gruppendifferenzierung der Pseudomonas aeruginosa verschiedenen Ursprungs.

Der Verfasser hat 274 Stämme von Pseudomonas aeruginosa verschiedenen Ursprungs isoliert. Die Stämme wurden mit Hilfe von 8 spezifischen Agglutinationsseren gruppendifferenziert. Von den 274 Stämmen liessen 241 (88,0 %) sich mit den erwähnten Seren gruppenbestimmen. Von 215 Stämmen tierischen Ursprungs konnten 195 (90,7 %) gruppenbestimmt werden, während von den 43 Stämmen humanen Ursprungs 31 (72,1 %) sich gruppenbestimmen liessen. Von 16 Stämmen die Futtermitteln, Lebensmitteln und Wasser entstammten konnten 15 (93,8 %) gruppenbestimmt werden.

Das Ergebnis von dieser sowie von früheren Untersuchungen deutet darauf hin, dass eine verhältnismässig kleine Anzahl gruppenspezifischer Antiseren die Grundlage für die serologische Klassifizierung der pathogenen Stämme von Pseudomonas aeruginosa verschiedenen geographischen und epidemiologischen Ursprungs bilden kann.

Das Ergebnis besitzt weiter bedeutende sowohl theoretische wie praktische Aspekte.

SAMMANFATTNING

Serologisk gruppdifferensiering av Pseudomonas aeruginosa från olika källor.

Författarna har isolerat 274 stammar av Pseudomonas aeruginosa från olika källor. Stammarna har gruppdifferentierats med åtta specifika agglutinationssera. Av de 274 stammarna kunde 241 (88,0 %) gruppbestämmas med använda sera, av 215 stammar med animalt ursprung kunde 195 (90,7 %) gruppbestämmas, av 43 stammar med humant ursprung kunde 31 (72,1 %) gruppbestämmas och av 16 stammar härrörande från kreatursfoder, livsmedel och vatten kunde 15 (93,8 %) gruppbestämmas.

Resultatet av denna och tidigare undersökningar tyder på att ett relativt litet antal gruppspecifika antisera kan bilda grundlag för serologisk klassificering av patogena stammar av Pseudomonas aeruginosa med olika geografiskt och epidemiologiskt ursprung.

Resultatet antyder vidare många samband av såväl teoretiskt som praktiskt intresse.

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