Reassignment of the Intermediate Strains of *Proteus rettgeri* biovar 5 to *Providencia stuartii* on the Basis of the Somatic (O) Antigens

JOHN L. PENNER AND JOAN N. HENNESSY

Department of Medical Microbiology, Faculty of Medicine, University of Toronto, and Department of Microbiology, Toronto General Hospital, Toronto, Ontario, Canada

The 181 bacteria classified as *Proteus rettgeri* biovar 5 considered to be a group intermediate to *P. rettgeri* and *Providencia stuartii* were found, in all cases, to possess O-specificities characteristic of *Providencia* O-serovars. Isolates of the intermediate group showed biochemical reactions characteristic of *Prov. stuartii* except for positive reactions in tests for urea hydrolysis. The reassignment of the intermediate strains (*P. rettgeri* biovar 5) to *Prov. stuartii* is advocated.

An intermediate group of bacteria possessing characteristics of both *Providencia stuartii* and *Proteus rettgeri* have been described and assigned to a newly proposed biovar for *P. rettgeri* (3). Recent epidemiological investigations have revealed that some of these intermediates were isolated along with *Prov. stuartii* from patients cross-infected in the same institution (7). The intermediate strains differed from the *Prov. stuartii* in positive reactions for urea hydrolysis but reacted identically in 17 other biochemical tests and possessed O-specificities characteristic of *Providencia*. The interpretation was therefore made that the intermediate strains were urease-positive variants of a *Prov. stuartii* endemic strain, and it became evident that it would be more appropriate to allocate these particular intermediates to *Prov. stuartii* rather than to *P. rettgeri*. Furthermore, 14 isolates that were, except for urease-positive reactions, biochemically similar to *Proteus inconspans* biovar B (*Prov. stuartii*) were considered by Ursing to be urease-positive variants of this species rather than *P. rettgeri* (8).

In the present study, the O antigenic structures of the intermediates in our collection now classified as *P. rettgeri* biovar 5 were reexamined for relationships to the *P. rettgeri* included in the other biovars and to the *Prov. stuartii* to determine to which species they were most closely related and with which they should be classified.

MATERIALS AND METHODS

Bacterial isolates. *P. rettgeri* and *Prov. stuartii* isolates were obtained, with a few exceptions, from the urine of patients in hospitals in Canada and the United States.

Biochemical and serological tests. Procedures for determining biochemical reactions have been de-

scribed in detail (1, 3). Christensen medium (Difco) was used in tests for urease activity. Tests were read at 24 h, and positive tests were recorded on observation of a pink color on the agar surface. Serotyping of *P. rettgeri* was performed on the basis of the O antigens previously described (5) and by methods recently reported (4). *Providencia* were serotyped on the basis of O antigens by the scheme reconstituted in our laboratory (6) from the one proposed earlier by Ewing et al. (2).

RESULTS

Typing intermediates in *P. rettgeri* O antisera. Cell suspensions of each of the 181 isolates in our collection assigned to the intermediate group of *P. rettgeri* biovar 5 (Table 1) were tested in *P. rettgeri* O antisera. The results are shown in Table 2. Only seven isolates, all from one hospital, did not agglutinate in any of the 84 antisera. Among the 174 isolates that were typable, a total of nine O-serovars were recognized.

Relations to *Providencia* of the O-serovars among *P. rettgeri* biovar 5 isolates. Antigen preparations of the strains from the serotyping scheme for *P. rettgeri* that were homologous for antisera that agglutinated biovar 5 isolates were each tested in the 63 *Providencia* O antisera. Except for *P. rettgeri* O82, each antigen preparation was agglutinated in either one or two *Providencia* antisera (Table 3). Cross-absorption and cross-titration experiments were performed between the *P. rettgeri* and the corresponding *Providencia* antisera. Between some pairs an "a,b-a,c" relation was identified and, between others, a test of identity was demonstrated. Tests of identity were shown to occur between *Providencia* and those O-serovars of *P. rettgeri* (O83 and O84) that belonged to *P. rettgeri* biovar 5. Except for one other test of iden-

TABLE 1. Biochemical reactions of *Proteus rettgeri* and *Providencia stuartii*^a

Substrate	<i>P. rettgeri</i> biovars					<i>Prov. stuartii</i>
	1	2	3	4	5	
Urea	+	+	+	+	+	-
Trehalose	-	-	-	+ or -	+	+
Mannitol	+	+	+	+	+ or -	+ or -
Salicin	+	+	-	-	-	-
Rhamnose	-	+	+	-	-	-
Adonitol	+	+	+	+	-	+ or -
Arabitol	+	+	+	+	-	-
Erythritol	+ or -	+ or -	+ or -	+ or -	-	-

^a All members show positive reactions for phenylalanine deaminase and acid production in inositol.

TABLE 2. Results of serotyping 181 *P. rettgeri* biovar 5 isolates in *P. rettgeri* O antisera

<i>P. rettgeri</i> O-serovar	No. of isolates	No. of sources
6	1	1
10	8	2
12	4	3
26	75	3
42	62	3
64, 80	6	1
82 ^a		
83	9	2
84	9	3
Untypable	7	1

^a O-serovar 82 is included because it is a biovar 5 strain, although O antiserum 82 did not agglutinate other *P. rettgeri* isolates.

TABLE 3. O-antigenic relations between cross-reacting *P. rettgeri* and *Providencia* O-serovars

Serotyping strains		Relation
<i>P. rettgeri</i>	<i>Providencia</i>	
6	9	a,b - a,c
10	63	a,b - a,c
12	44	a,b - a,c
26	17	Unilateral ^a
42	4	a,b - a,c ^a
42	52	Identity ^a
64	25	a,b - a,c
80	25	a,b - a,c
83	24	Identity
84	56	Identity

^a Demonstrated in an earlier study (7).

tity between *P. rettgeri* O42 and *Providencia* O52, and the unilateral relation between *P. rettgeri* O26 and *Providencia* O17 previously reported (7), all other cross-reactants were related through an "a,b-a,c" relation.

Specificities of the O antigens of isolates that agglutinated in both *P. rettgeri* and *Providencia* O antisera. Isolates that were ag-

glutinated by *P. rettgeri* and *Providencia* antisera against serotyping strains shown to be related through an "a,b-a,c" relation were further tested in cross-absorbed antisera. The effect of the cross-absorption was to remove antibody against the cross-reacting "a" component, leaving antibody directed against the O-specificity.

One isolate of *P. rettgeri* biovar 5 and others of biovars 1, 2, and 3 agglutinated in unabsorbed *P. rettgeri* O-antiserum 6 and in *Providencia* O-antiserum 9 (Table 4). When retested in cross-absorbed antisera, it was observed that the biovar 5 isolate agglutinated in *Providencia* O-antiserum 9 absorbed with *P. rettgeri* O6, and the biovars 1, 2, and 3 isolates agglutinated in *P. rettgeri* O-antiserum 6 absorbed with *Providencia* O9. From these results, it was concluded that isolates of biovars 1, 2, and 3 possessed O-specificities indistinguishable from the *P. rettgeri* O6 strain and the biovar 5 isolate possessed an O-specificity like that of the *Providencia* O9 strain. A similar conclusion was reached for isolates agglutinable in unabsorbed *P. rettgeri* O-antiserum 12 and *Providencia* O-antiserum 44. Those of biovars 1, 2, and 3 agglutinated in *P. rettgeri* O-antiserum 12 absorbed with *Providencia* strain O44 and the biovar 5 isolates agglutinated in *Providencia* O-antiserum 44 absorbed with *P. rettgeri* strain O12.

The *P. rettgeri* O10 and *Providencia* O63 pair of antisera agglutinated isolates of *Prov. stuartii* in addition to isolates of *P. rettgeri* biovars 2, 3, and 5 (Table 5). The isolates of biovars 2 and 3, as in the cases above, identified with the *P. rettgeri* serovar, and those of biovar 5 and *Prov. stuartii* identified with the *Providencia* serovar.

Isolates of *P. rettgeri* biovar 5 and *Prov. stuartii* have been previously reported to agglutinate in unabsorbed *P. rettgeri* O-antiserum 26, in unabsorbed *Providencia* O-antiserum, 17 and in cross-absorbed O-antiserum 17 (7). The only isolate of a different biovar was the O26 strain (biovar 1), and it agglutinated only in homologous antiserum.

The interrelationships among *P. rettgeri* O42, *Providencia* O52, and *Providencia* O4 were more complex. The first two were shown to be antigenically identical but both cross-reacted with the O4 strain on the basis of an "a" component common to all three (6, 7). *P. rettgeri* biovar 5 isolates from two hospitals agglutinated in *Providencia* O-antiserum 4 absorbed with *P. rettgeri* O42 (or *Providencia* O52), but biovar 5 isolates from a third hospital and one biovar 3 isolate agglutinated, like the *P. rett-*

TABLE 4. Reactions in *P. rettgeri* O antiserum 6 and in *Providencia* O antiserum 9^a

Antigen	Unabsorbed antisera		Absorbed antisera ^b	
	<i>P. rettgeri</i> O6	<i>Providencia</i> O9	<i>P. rettgeri</i> O6 (<i>Providencia</i> O9)	<i>Providencia</i> O9 (<i>P. rettgeri</i> O6)
Serotyping strains				
<i>P. rettgeri</i> O6	5,120 ^c	640	1,280	0
<i>Providencia</i> O9	10,240	640	0	2,560
Isolates				
<i>P. rettgeri</i> biovar:				
1	+ ^d	+	+	—
2	+	+	+	—
3	+	+	+	—
5	+	+	—	+

^a Titrations were performed by passive hemagglutination. Initial dilution of antisera was 1:40. Negative results at this dilution are indicated by a zero; titers are expressed as reciprocals of the highest dilutions showing agglutination.

^b Antisera were absorbed with the strains in parentheses.

^c Numbers indicate titration results.

^d Plus or minus indicates slide agglutination results.

TABLE 5. Reactions in *P. rettgeri* O antiserum 10 and in *Providencia* O antiserum 63^a

Antigen	Unabsorbed antisera		Absorbed antisera ^b	
	<i>P. rettgeri</i> O10	<i>Providencia</i> O63	<i>P. rettgeri</i> O10 (<i>Providencia</i> O63)	<i>Providencia</i> O63 (<i>P. rettgeri</i> O10)
Serotyping strains				
<i>P. rettgeri</i> O10	10,240 ^c	5,120	2,560	0
<i>Providencia</i> O63	2,560	10,240	0	2,560
Isolates				
<i>P. rettgeri</i> biovar:				
2	+ ^d	+	+	—
3	+	+	+	—
5	+	+	—	+
<i>Prov. stuartii</i>	+	+	—	+

^a Titrations were performed by passive hemagglutination. Initial dilution of antisera was 1:40. Negative results at this dilution are indicated by a zero; titers are expressed as reciprocals of the highest dilutions showing agglutination.

^b Antisera were absorbed with the strains in parentheses.

^c Numbers indicate titration results.

^d Plus or minus indicates slide agglutination results.

geri O42 strain, in both *P. rettgeri* O42 and *Providencia* O52 antisera after absorption with *Providencia* O4 strain.

Isolates that agglutinated in both *P. rettgeri* O antisera 64 and 80 were noted previously (4). No cross-reacting component was evident between these serovars and it was interesting, therefore, to find in the present study that both strains agglutinated in *Providencia* O-antiserum 25. Evidence was obtained that the O25 strain bears two cross-reacting components—one in common with the *P. rettgeri* O64 and the other in common with O80 (Table 6). The biovar 5 isolates reacted in a fashion identical to *Prov. stuartii* isolates typed as *Providencia* O25, and

the one biovar 2 isolated reacted like the *P. rettgeri* O80 strain.

P. rettgeri serovars O83 and O84, both biovar 5 strains, showed tests of antigenic identity with *Providencia* O-serovars 24 and 56, respectively. Antisera against these did not agglutinate *P. rettgeri* of biovars 1 to 4 in our collection.

P. rettgeri serovar O82. *P. rettgeri* O82 antiserum did not agglutinate *P. rettgeri* isolates of biovars 1 to 4 but did agglutinate two *Prov. stuartii* isolates untypable in the 63 *Providencia* O antisera and, hence, it would appear appropriate to include this O-specificity with the *Providencia*.

TABLE 6. Reactions in *P. rettgeri* O antisera 64 and 80 and in *Providencia* O antiserum 25^a

Antigen	Unabsorbed antisera			Absorbed antisera ^b			
	<i>P. rettgeri</i> O64	<i>P. rettgeri</i> O80	<i>Providencia</i> O25	<i>P. rettgeri</i> O64 (<i>Providencia</i> O25)	<i>P. rettgeri</i> O80 (<i>Providencia</i> O25)	<i>Providencia</i> O25 (<i>P. rettgeri</i> O64)	<i>Providencia</i> O25 (<i>P. rettgeri</i> O80)
Serotyping strains							
<i>P. rettgeri</i> O64	10,240 ^c	0	1,280	5,120	0	0	640
<i>P. rettgeri</i> O80	0	5,120	640	0	5,120	320	0
<i>Providencia</i> O25	2,560	1,280	5,120	0	0	1,280	2,560
Isolates							
<i>P. rettgeri</i>							
biovar:							
2	— ^d	+	+	—	+	+	—
5	+	+	+	—	—	+	+
<i>Prov. stuartii</i>	+	+	+	—	—	+	+

^a Titrations were performed by passive hemagglutination. Initial dilution of antisera was 1:40. Negative results at this dilution are indicated by a zero; titers are expressed as reciprocals of the highest dilutions showing agglutination.

^b Antisera were absorbed with the strains in parentheses.

^c Numbers indicate titration results.

^d Plus or minus indicates slide agglutination results.

O-specificities of intermediates untypable in *P. rettgeri* antisera. The seven *P. rettgeri* biovar 5 isolates that were untypable in *P. rettgeri* O antisera were agglutinated in *Providencia* O55 antiserum. Therefore, like all others of this intermediate group, they possessed O-specificities characteristic of a *Providencia* O-serovar.

DISCUSSION

A group of bacteria considered intermediate between *Prov. stuartii* and *P. rettgeri* were assigned to *P. rettgeri* biovar 5 because they showed urease activity and because their exact position in reference to *Prov. stuartii* was not known (3). The results of the present study have shown that all biovar 5 isolates in our collection have O-specificities characteristic of *Providencia* strains. Agglutination in *P. rettgeri* antisera was attributable to antibody directed against either cross-reacting components or against O-specificities of *P. rettgeri* that were serologically indistinguishable from those of *Providencia* O-serovars.

It was also apparent from biochemical studies that these intermediate bacteria, except for their reaction in urea, were like the *Prov. stuartii* (3, 7, 8). Hence, support for maintaining the *P. rettgeri* biovar 5 subdivision is based on the positive reactions of these bacteria in urea, a characteristics of the genus *Proteus*. This allows a single biochemical test to be a criterion sufficient to separate otherwise identical bacteria into two genera. Consideration should therefore be given to a reassignment to

Prov. stuartii of the bacteria now included in biovar 5 of *P. rettgeri*.

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REPRINT REQUESTS

Address reprint requests to: Dr. John L. Penner, Department of Medical Microbiology, Banting Institute, 100 College St., Toronto, Canada, M5G 1L5.

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