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O Serotyping of *Providencia stuartii* Isolates Collected from Twelve Hospitals

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A collection of 829 isolates of *Providencia stuartii,* mostly from urological specimens of patients in 12 hospitals, were O serotyped. Hospitals varied in serotype distribution, but most isolates (97%) fell into one or another of 14 O types of *P. stuartii.* One type (O63) was found in 10 hospitals, and six types (O4, O17, O25, O52, O55, O56) were found in 5 or more hospitals. These seven types were more common than others and included 753 (91%) of the isolates. Only four isolates agglutinated in *Providencia alcalifaciens* antisera and, for increased efficiency in serotyping, it is recommended that separate schemes be employed for *P. stuartii* and *P. alcalifaciens.* Strains endemic in different hospitals may differ in serotype and give rise to nosocomial infections that are clinically recognizable when infections occur in obvious clusters. Nosocomial infections occurring in low frequency among patients not located close to each other in the hospital may be detected with the aid of serotyping.

Bacteria of the species *Providencia stuartii* are now well recognized as pathogens of humans, particularly in the urinary tract, and their involvement in nosocomial disease has been reported (2, 3, 6, 7, 14, 16-18). Epidemiological studies of these infections have been difficult because the description of the species was not clear and because means of typing were not readily available. One of the difficulties in classification was the widely held view that strains of this species were unable to hydrolyze urea, and thus urea-positive isolates were usually assigned to another species, namely *Proteus rettgeri.* On the basis of other biochemical reactions, the urea-positive isolates readily differentiated from typical *P. rettgeri* and were placed in a separate biogroup (biogroup 5) of that species (10). However, recent studies have led to proposals to transfer them to *P. stuartii* (1, 5, 9, 13, 15).

The original antigenic scheme described by Ewing et al. (4) for *Providencia,* with some modification (12), was used for O serotyping isolates collected from different hospitals. The present communication is concerned with the distribution of the different O serotypes in hospitals and the implications to the clinician and epidemiologist.

MATERIALS AND METHODS

Bacteria. All isolates of *P. stuartii* found were collected over a period of 6 years from the microbiology laboratories of two Canadian hospitals (A and B), both located in Toronto. The laboratory at hospital B also provides microbiology services to a specialized 50-bed hospital (C) for paraplegic patients. Isolates were also obtained from five other Canadian hospitals (D, E, F, G, and V), in most cases in connection with episodes of urinary tract infections. Isolates associated with episodes of infection were also obtained from two hospitals, one (L) in the U.S.A. (8) and the other (U) in the United Kingdom (E. Dowsett, personal communication). Isolates from hospitals H (Canada) and M (U.S.A.) were submitted for serotyping to support epidemiological studies that have been described previously (13, 16, 18).

Biochemical reactions. Isolates were tested for biochemical reactions as previously described (12). In accordance with recent proposals (1, 5, 9, 15), urea-positive isolates formerly classified as *P. rettgeri* biogroup 5 (10) were included with the *P. stuartii.*

Serotyping. Isolates were typed on the basis of their O antigens according to the scheme (12) reconstituted from the earlier scheme of Ewing et al. (4). Another O specificity was defined in a strain untypable in the 62 different O antisera and was added to the scheme as O63. Bacterial suspensions were prepared by transferring to saline the bacterial lawn grown on a Trypticase soy agar plate after overnight incubation at 37°C. This suspension was autoclaved (1 h at 120°C), cooled, centrifuged, and washed three times in saline, then suspended in a volume of saline equivalent to 0.5 ml per bacterial harvest from one plate. In agglutination tests, a drop of rabbit O antiserum and a drop of bacterial suspension were put into areas encircled (diameter, 0.5 inch [ca. 12.7 mm]) on black glass plates with ceramic ink. The antiserum and the

bacterial suspension were mixed, and the glass plates were placed on a Yankee rotator. After 8 min of rotation at slow speed, the plates were examined for agglutination.

At first, a cell suspension of each isolate was tested in each of the O antisera (diluted 1:5). Later, the use of pooled antisera, as previously described for *P. rettgeri* (11), was adopted. Each pool was prepared by mixing 10 undiluted antisera to provide a dilution of 1:10 of antibody against each O specificity. The observation of agglutination of an isolate in one pool was followed by separate tests in the individual O antisera (diluted 1:5) belonging to that pool.

Epidemiology. The present study was conducted from a bacteriological rather than from an epidemiological standpoint. Our laboratory served primarily as a center for serotyping, and those clinical and/or epidemiological studies that were conducted in hospitals and have been previously reported are indicated above.

RESULTS AND DISCUSSION

Serotyping *P. stuartii* isolates from 12 hospitals. This communication is concerned essentially with the O serotypes of *P. stuartii* isolates from infected patients and the epidemiological aspects related to the distribution of the serotypes among the isolates. The results of O serotyping 829 isolates collected from 12 hospitals are shown in Table 1. Separate clinical and/or epidemiological studies have been previously reported for hospitals M (13, 16), H (18), and L (8). The first study on *P. stuartii* infections that involved serotyping facilities of our laboratory was conducted in hospital M; this was at the time of our early investigations of the serotyping systems for *P. rettgeri* and *Providencia*. In this hospital, the O17 endemic strain was clearly reflected in a 96-isolate cluster consisting of both urease-positive and urease-negative isolates, and in some cases isolates of both kinds occurred in the same patients. This finding presented the opportunity to direct attention to the importance of classifying such urease-positive isolates as *P. stuartii* rather than as *P. rettgeri* (13). The results in Table 1 show that urease-positive isolates may be found to belong to a number of O serotypes. The confusion that has existed in the classification of these isolates was indicated by the labeling of the cultures we received for typing. Among those labeled *P. rettgeri,* many were urease-positive *P. stuartii.* Indeed, we have not received urease-positive *P. stuartii* labeled as *P. stuartii* until very recently. In some clusters, urease-positive isolates outnumbered urease-negative isolates, and it is evident that failure to classify them correctly will continue to lead to confusion in epidemiological studies.

The epidemiological study in hospital H was a cooperative one involving the infection control staff at that hospital and the staff associated with our laboratory. Evidence for patient-to-patient transmission of a strain of serotype O55 was obtained. This endemic strain constituted a 71-isolate cluster and was distinguished by serotyping from the six-isolate cluster of serotype O49 involved in a separate episode of infections. Isolates from hospitals L and V were received after studies of infections had been described. Serotyping showed that they clustered predominantly in one or two serotypes, thus supporting the clinical observations of cross-infection and providing increased confidence in the serotyping scheme. O serotyping was provided to other

TABLE 1. *Distribution of O serotypes of P. stuartii among isolates collected from 12 hospitals*

O serotype	No. of isolates[a]	No. of isolates from hospital[b]											
		A	B	C	D	E	F	G	H	V	M	L	U
63	234 (15)	78 (1)	31 (1)	49 (6)	17 (7)	7	8	33	1		1		9
4	165 (6)	22 (3)	36	38 (3)		1	3	58	1		6		
17	109 (74)	5 (1)	6				1		1		96 (73)		
55	108 (7)	9	11	2			8		71	7 (7)			
25	65 (9)	16	22 (2)	4 (3)		8	5	3			2		5 (4)
52	41 (7)	4		1 (1)	2			1	1		7 (6)	25	
56	31 (8)	2 (1)	14	3					5		7 (7)		
24	14 (5)	7 (5)				6							1
15	14	3	1	2		8							
43	12	5	3	1		3							
49	6								6				
Others[c]	30 (3)	5		2		1		6	5	3 (3)	1	6	1
No. of serotypes in hospital		14	8	9	2	7	5	5	11	2	7	7	3

[a] Number of urea-positive isolates is indicated in parentheses.

[b] Isolates were collected routinely over a period of 6 years from hospitals A, B, and C. From other hospitals isolates were received in connection with episodes of infection. Clinical and/or epidemiological studies have been previously reported for hospitals H (18), M (16), and L (8). All hospitals are located in Canada except hospitals M and L (U.S.A.) and U (U.K.).

[c] Included are isolates that were smooth and untypable, rough, and of serotypes other than those listed.

hospitals for purposes of evaluating the serotyping scheme in practice, collecting isolates, and assisting in resolving infection problems due to *P. stuartii*.

Most of the 829 isolates could be separated into 14 *P. stuartii* O types. The scheme for O typing *Providencia* includes O types of both species, 17 *P. stuartii* and 46 *P. alcalifaciens*. The majority of the isolates (96%) belonged to one or another of the 11 *P. stuartii* types listed in Table 1. Of the remaining 30 isolates, 8 agglutinated in one or another of the antisera against *P. stuartii* types O26, O44, or O57; 4 agglutinated in antisera against *P. alcalifaciens* types O11, O19, or O34; 8 were smooth but did not agglutinate in any of the 63 antisera; and 10 were considered rough because they autoagglutinated. Of considerable importance in the practice of serotyping was the finding that 807 (99.5%) of the 811 typable isolates agglutinated in antisera against O types of *P. stuartii*. It was, therefore, evident that the species was characterized by its own set of O specificities and that there was only infrequent sharing of these specificities with *P. alcalifaciens*. O serotyping could, therefore, be more efficiently accomplished by separating the *Providencia* antisera according to species and typing each species separately.

In examining the distribution of the serotypes among the isolates, it was seen that serotype O63 was isolated in all but two of the hospitals and, on the basis of this wide distribution, may be regarded as a type more common than others. Six other types, O4, O17, O25, O52, O55, and O56, were common to five or more hospitals, and 91% of the isolates fell into one or another of these seven serotypes (listed uppermost in Table 1). The significance of this finding was that it indicated that most *P. stuartii* infections of the human urinary tract were caused by a small number of O serotypes and that these common types were included in the serotyping scheme in its present state of development. In this connection, it was interesting to note that the common types O52 and O55 were also found among the *P. stuartii* typed during a study reported by Solberg and Matsen (14).

The hospital with the largest number of O serotypes among the isolates was A with 14, but this heterogeneity was considered to reflect the larger collection (156 isolates), the longer period (6 years) of collection, and the fact that all isolates found were included. Because the O63 isolates in hospital A, as well as in hospitals B and C, were collected over long periods of time, they were not comparable to the clusters of O55 isolates from hospital H or those of O17 from hospital M that were collected over shorter periods and associated with clinically apparent cross-infections. The introduction of O63 into hospitals could conceivably be more frequent because it appeared to be more widely distributed, but its predominance in these hospitals could not be accounted for satisfactorily on this basis. In hospital D, one patient after another acquired this strain over an 11-month period (A. E. Franklin, personal communication). Although the 13 patients, except in a few instances, were located in different rooms or wards or were in the hospital during different periods of time, two observations may be cited to support the view that the separate introduction into the hospital of this serotype by individual patients was unlikely. In the case of some patients, a urine culture positive for O63 was obtained after one or more urines had been shown negative for *Providencia*, and case-to-case transmission could not be ruled out because, in one instance at least, patients in adjacent beds had urines positive for O63. The low rate of isolation, approximately one isolate per month, was not by itself a signal sufficient to alert hospital staff to the possible occurrence of nosocomial infection. Rather, it was on the basis of serotyping data that an infection problem due to this serotype became evident. In hospital A, the 78 isolates of O63 collected over a 6-year period reflected, as in hospital D, a low rate of isolation. In most cases, the isolates were from patients not in close proximity to each other. Cross-infections were not apparent and not reported in this hospital, and the extent of the endemic nature of the O63 strain was not realized because these isolates were grouped as untypable until they were found to agglutinate in antiserum prepared against the newly defined O-type 63 strain. Numerous isolates of O63 were also collected from hospitals B and C, and the long-term paraplegic and geriatric patients, from whom most of these were cultured, could clearly not have served as the individual vehicles for repeated reintroductions of the O63 strains into the hospital. The persistence of O63 in hospitals A, B, and C, as in D, was therefore more likely due to patient-to-patient transmission or to transmissions from an unidentified hospital environmental source.

Therefore, two patterns of nosocomial infections could be distinguished. The first was reflected in hospitals H, M, L, and U, where the infections were typically episodic and thus identified by a higher rate of isolation over a relatively short period of time. The infected patients were in close proximity, being located in one or in a small number of wards in the hospital, and the outbreak was apparent to attending staff. The other pattern, characteristic of infections caused by O63 in hospitals A and D, was unapparent and insidious. Infected patients were, for

the most part, separated either by location or time of stay in the hospital. Because of the low frequency of isolation and because isolates were from cases not obviously connected epidemiologically, the hospital strain remained unrecognized until the infections were linked by serotyping data. Since as few as two isolations per month from different patients could be a reflection of this pattern, the isolates should be completely characterized biochemically, and serotyped if the biochemical reactions are identical or almost identical.

Factors other than those considered in this study need to be examined for an understanding of the epidemiology of *P. stuartii* infections. Serotyping of isolates from 12 hospitals has demonstrated that hospitals vary in their serotype distribution but that the serotypes of isolates cultured from urine specimens most often belong to a relatively small number of common types. If this is the case in general, a laboratory with the 11 *P. stuartii* O antisera listed in Table 1 could distinguish among the majority of its isolates. The finding of 134 urease-positive isolates among the 829 should emphasize the importance of correct classification of urease-positive *P. stuartii* in epidemiological studies. Strains endemic in different hospitals may be of different serotypes and include both urease-negative and urease-positive isolates, and they may give rise to nosocomial bacteriuria that is either clinically recognizable when infections occur in obvious clusters or detectable only with the aid of serotyping when cases are separated and apparently unconnected. The establishment of serotyping facilities in more microbiology laboratories, particularly in reference centers, should be encouraged.

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