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O Antigen Distribution and Sensitivity to the Bactericidal Effect of Normal Human Serum of *Proteus* Strains from Clinical Specimens

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Abstract. O antigen distribution and sensitivity to the bactericidal effect of normal human serum were determined in 485 *Proteus* strains isolated from urine, blood, wounds, and feces. Of all strains, 62% could be grouped according to 25 O antigens. The most frequent one, O 3, appeared in 13% of all the strains. In blood cultures this antigen was found significantly more often. No other O group dominated. Of all strains, 11% were spontaneously agglutinating but such strains were significantly more common in urinary specimens from children with neurogenic bladder disorders.

Strains sensitive to the bactericidal effect of normal human serum were significantly more prevalent in urines than in fecal specimens. Most of the sensitive strains were spontaneously agglutinating and were isolated from the urines of children with neurogenic bladder disorders. This may be an example of 'antigenic drift' of the bacteria as a response to host defense mechanisms in the urinary tract.

Introduction

Studies on urinary tract infections (UTI) in children have shown that the sensitivity of the urinary *E. coli* strains to the bactericidal activity of normal human serum (SBS) varies for different forms of UTI [12]. Furthermore, comparison of these strains has shown differences in O antigen distribution and frequency of spontaneously agglutinating strains in symptomatic and asymptomatic bacteriuria [8].

The present study was undertaken to analyze the O antigen distribution and the SBS in *Proteus*, another gram-negative rod causing UTI.

Material and Methods

Bacterial isolates of *P. mirabilis* and *P. vulgaris* were collected from clinical specimens submitted to the Bacteriological Laboratory, Göteborg. Duplicates were excluded.

The serologic system of Kauffmann and Perch [4] was used for typing. In this system *P. mirabilis* and *vulgaris* are divided into 49 0 serotypes.

Clinical Sources

From children with significant bacteriuria (≥ 100,000 bact./ml) 139 strains were collected. Of these strains 49 were analyzed separately because these children had neurogenic bladder disorders (NBD), mostly due to myelomeningocele. From adults with significant bacteriuria 113 strains were obtained. From positive blood cultures, mostly from adult patients, 100 strains were studied and from wounds where *Proteus* was the only microorganism isolated, 33 strains were obtained. Fecal strains were collected from 38 healthy persons and from 62 patients with diarrhea where no *Salmonella* or *Shigella* were found.

O serotyping was performed using the technique described by Lincoln et al. for *E. coli* [7]. An overnight nutrient broth [1] culture of each strain to be tested was autoclaved for 1/2 h or boiled for 2 1/2 h. One drop of this suspension was mixed in Perspex agglutination trays with one drop of *Proteus* O antiserum properly diluted (final dilution 1:800–1:1600) in each well. Agglutinations were read after overnight incubation at 50°C. Monovalent antisera were prepared in rabbits as earlier described [6] from standard strains and were available against the following O antigens: 1,3,5–8, 10–17, 19, 20, 23–30, 32, 33, 35, 36, 38, 40, 41, 44, 49. Some strains were not typable within the O 1–49 system and in order to decrease the number of nontypable isolates, some of these strains were selected for preparation of antisera. These antisera were designated O A-C and gave no agglutination reaction with the standard strains O 1–49 or with the heterologous O A-C strains in control experiments. Strains still nontypable were designated O Na and strains showing autoagglutination in saline (spontaneous agglutination) were labeled Sa.

Sensitivity to the bactericidal effect of normal human serum (SBS) of the strains was determined according to Olling et al. [12] with a rating [3] of 'resistant' (meaning that less than 50% of the bacteria were killed), 'intermediately sensitive' (50–99% were killed), or 'very sensitive' (more than 99% were killed) during 30 min incubation in each case.

For statistical evaluation Fisher's permutation test was used [11].

Results

O Antigen Distribution

With the available antisera 299 (62%) of the total *Proteus* strains could be classified according to 25 O antigens. There were 132 strains (27%) that were not typable (O Na) and 54 (11%) that were spontaneously agglutinating (Sa). The results are summarized in Table 1. Fecal *Proteus* strains isolated from healthy persons and patients with diarrhea could not be differentiated with regard to O antigens or the SBS distributions and therefore these strains are presented as one group.

Table 1. 0 antigen distribution (in percent) among *Proteus* strains from different clinical sources

0 antigen	Urines			Blood	Wounds	Feces	Total material	
	children		adults					No.
	with NBD[a]	without NBD					percent	of strains
3	12	11	15	23	9	6	13	65
10	0	13	4	7	6	6	7	32
13,30[b]	0	2	1	4	3	6	3	14
23	6	3	2	6	0	5	4	19
24	0	1	1	7	6	3	3	14
27	6	3	11	1	6	2	5	24
28	0	9	6	3	6	2	4	22
29	2	1	3	5	0	1	3	12
30	6	3	8	5	0	4	5	24
A	4	3	2	1	3	2	2	11
Misc.0[c]	15	14	9	9	10	25	13	62
0 Na	20	33	27	18	45	28	27	132
Sa	29	4	11	11	6	10	11	54
No. of strains	49	90	113	100	33	100	100	485

[a] neurogenic bladder disorders
[b] strains giving strong agglutination with 0 13 and 0 30 antisera
[c] 0 antigens 6,7,9,12,13,16,19,25,26,32,35,38,40,B,C.

0 3 was the most common 0 antigen, appearing in 13% of the total material. Such strains were isolated from all sources, but significantly more often from the blood cultures ($P < 0.01$, 23%). The second most common 0 antigen, 0 10, was found in 7%. Spontaneously agglutinating strains were isolated from all clinical sources, but most frequently from the urines of children with neurogenic bladder disorders ($P < 0.01$).

Correlation of SBS and 0 Antigens

Most strains (69%) were serum resistant and only 14% very sensitive, as can be seen in Table 2. Few of the 0 typable and nontypable strains were sensitive and no major differences in SBS distribution were found between different 0 groups. Significantly more ($P < 0.01$) of the spontaneously agglutinating strains were very serum sensitive, with 85% of the strains in this group.

Correlation of SBS and Source of Strains

The urinary strains from children with NBD were significantly more sensitive ($P < 0.01$) than the fecal strains as seen in Table 3. Also when the spontaneously agglutinating strains were disregarded a similar tendency ($0.05 > P > 0.01$) was found.

Strains isolated from blood cultures were very sensitive in 12% and, discounting spontaneously agglutinating strains, significantly more sensitive than strains of fecal origin ($P < 0.01$). No significant difference was found between strains from feces and wounds.

Table 2. Frequency in percent of *Proteus* strains with various 0 antigens, according to SBS rating

O antigen	SBS rating			No. of strains
	Resistant	Intermediate	Sensitive	
3	85	10	5	65
10	75	22	3	32
13,30[a]	86	14	0	14
23	84	16	0	19
24	64	36	0	14
27	84	8	8	24
28	68	27	5	22
29	58	33	9	12
30	75	8	17	24
A	45	45	10	11
Misc.0[b]	84	11	5	62
0 Na	72	23	5	132
Sa	11	4	85	54
Total	69	17	14	485

[a] strains giving strong agglutination with 0 13 and 0 30 antisera

[b] 0 antigens 6, 7, 9, 12, 13, 16, 19, 25, 26, 32, 35, 38, 40, B, C.

Table 3. Frequency in percent *Proteus* strains according to SBS rating in relation to their clinical source

Clinical source	SBS rating			No. of strains
	Resistant	Intermediate	Sensitive	
Urines				
Children				
with NBD[a]	55	10	35	49
without NBD	76	14	10	90
Adults	69	12	19	113
Blood	61	27	12	100
Wounds	70	24	6	33
Feces	76	15	9	100
Total	69	17	14	485

[a] neurogenic bladder disorders

Discussion

In the search for potential virulence factors for *Proteus* bacteria, studies have been made on urease activity [10] and pili [15]. In the present study the 0 antigen and the serum bactericidal sensitivity have been analyzed for the same purpose.

The most prevalent *Proteus* 0 antigen in this work, 0 3, has also commonly been found in investigations of other authors on strains from urines, wounds, and feces [5,9,14]. We found 0 3 to be significantly more frequent among *Proteus* strains isolated from blood cultures in accordance with a report by Sedlák [14] of three cases of bacteremia with 0 3, 0 13, and 0 26 (3b) *Proteus* strains. The high frequency of 0 3 strains in the blood may reflect an increased virulence of bacteria with this antigen.

Most strains in this study were resistant to the bactericidal activity of normal serum but we found that serum sensitive *Proteus* strains also exist, as did Roantree and Rantz [13] in their analysis of six strains. As a group, however, *Proteus* bacteria seem to be more resistant than *E. coli* [8,12].

Serum sensitive and/or spontaneously agglutinating urinary *E.coli* are much more common in asymptomatic bacteriuria in otherwise healthy girls than in the urine of girls with symptomatic UTI [8]. These spontaneously agglutinating strains are considered to be cell wall degraded and probably appear as the result of the activity of the host defence, mainly secretory IgA, in the urinary tract [2]. Such an 'antigenic drift' might be a way for the bacteria to avoid the defense mechanisms of the host. Serum-sensitive *Proteus* strains were usually also spontaneously agglutinating and were found mostly in urines from children with neurogenic bladder disorders who have a high tendency for recurrent and prolonged bacteriuria. Classification of the UTI with regard to symptoms and level is difficult in the latter group of patients due to their neurologic disorders and thus comparison to children with uncomplicated asymptomatic bacteriuria must be done with caution. However, the two groups of patients have in common bacteriuria with no or minor symptoms and a high frequency of serum-sensitive and spontaneously agglutinating bacteria, i.e., *E.coli* and *Proteus* respectively.

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