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THE EPIDEMIOLOGY OF NON-ENTERIC ESCHERICHIA COLI INFECTIONS: PREVALENCE OF SEROLOGICAL GROUPS *

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Escherichia coli is an ubiquitous microorganism which is found in the gastrointestinal tract of every individual, where it usually forms a part of the normal gut flora. Extensive epidemiological, clinical, and bacteriological observations have documented the pathogenic significance of certain serological strains of *E. coli* in infantile diarrhea. However, although they are frequently isolated in infected sites closely related to the gastrointestinal tract, such as the appendix, gall bladder, and peritoneal cavity, little is known about the serological specificity of coliform bacteria in non-enteric infections, particularly those involving the urinary tract. Ewing (1) has emphasized that complete serological typing of *E. coli* should provide accurate information concerning the incidence of specific strains associated with disease and permit evaluation of nosocomial spread.

The concept that some strains of gram-negative organisms may be associated with infection more often than others is not new. Kauffman (2) advanced the hypothesis that certain coliform serotypes are more prevalent in appendicitis, and he also noted that strains of certain serological groups were more frequently isolated from the urine than from feces. Others have reported that strains of certain serological groups are more commonly isolated from infected sites than from fecal specimens (3). Vahlne (4) and Sjöstedt (5) performed serological typing on strains of *E. coli* from a variety of normal and abnormal sources and found that certain types were more commonly associated with human infections but that these types were not limited to the urinary tract. More recently, Rantz (6) suggested that *E. coli* with certain group specific antigens are more invasive for the urinary tract than others. A preliminary report from this laboratory indicated that the majority of non-enteric *E. coli* infections are caused by strains of a few specific serological groups, but did not support the idea that specific strains have a marked predilection for renal tissue (7). This report is an extension of these original observations and offers additional evidence that certain serological groups of *E. coli* are responsible for the majority of non-enteric infections because of their increased prevalence in the environment.

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METHODS

Organisms

The organisms included in this study were obtained from the following sources: *1)* Five hundred and twenty-two strains of *E. coli* were isolated from patients with non-enteric infections hospitalized at King County Hospital (KCH) and University of Washington Hospital (UW) in Seattle, Johns Hopkins Hospital (JH) in Baltimore, and Salt Lake General Hospital (SL) in Salt Lake City. The isolates obtained from KCH were from consecutive patients in whom *E. coli* was of known pathogenic significance (292 strains from 276 patients). The majority of strains was of urinary origin. *2)* Eighty-seven strains were cultured from urine samples of consecutive catheterized pregnant women with low bacterial counts (< 1,000 bacilli per ml). These were considered to represent urethral contamination. *3)* Thirty-five strains were isolated from consecutive untreated patients hospitalized with overt urinary tract infections characterized by significant bacteriuria (100,000 bacilli per ml), from whom simultaneous stool specimens were obtained by rectal swab.

Bacteriology

A modified method of Edwards and Ewing (8) was used for group differentiation of Enterobacteriaceae by biochemical tests. All strains were tested with indol, methyl red, Voges-Proskauer, and Simmons' citrate reagents. The initial 250 strains and all subsequent strains with atypical "IMVIC" patterns were further tested with nine carbohydrates (glucose, lactose, sucrose, mannitol, salicin, inositol, adonitol, sorbitol, and dulcitol) for acid and gas production and with three amino acids (lysine, arginine, and ornithine) for acid production;

they were also tested for hydrogen sulfide and urea production and for motility. As reported previously, there was no correlation between serological groups of *E. coli* and ability to ferment specific carbohydrates (7).

Serology

Grouping sera were made available by the Communicable Disease Center (CDC) Diagnostic Reagents Laboratory, United States Public Health Service. These are unabsorbed sera prepared by the immunization of rabbits with known O groups of *E. coli.* Complete serological typing of *E. coli* cultures is dependent upon determination of the O group followed by characterization of K (envelope or capsular) and H (flagellar antigens). This study is limited to determination of the O (somatic) antigen. O antisera for 137 standard strains of *E. coli* were used in this study. A modification of the scheme for determination of the O antigen described by Edwards and Ewing (8) was used. It may be summarized as follows.

1. Preparation of antigen. A trypticase soy broth culture which had been incubated overnight at 37° C was used in preparation of the *E. coli* antigen. The broth culture was heated at 100° C for one hour in a water bath to destroy the thermolabile K antigen which may inhibit agglutination. If the bacterial suspension appeared granular or flocculent after heating, it was defined as rough. All rough cultures were discarded and an additional broth culture was prepared from the same strain of *E. coli* after it had been serially transferred on blood agar for two successive days. If this culture remained rough after heating, no further attempt was made to type the particular strain and it was reported as ungroupable.

2. Agglutination with pooled specific O antisera. The prepared antigens (smooth colonies) of *E. coli* were first tested with pools containing combinations of the 138 available O antisera according to the procedure described by Ewing, Tatum, Davis and Reavis (9, 10). After testing the first 150 strains in our laboratory, a separate pool containing O antisera 1, 4, 6, 50, and 75 was made and subsequently all strains were first tested with this pool. If no agglutination occurred, the strain was then tested with eight pools containing the other 133 O antisera. Individual sera were diluted 1 : 50, and after antigen was added, each antiserum in the pool was present in final concentration of 1 : 500. Test tubes (100 × 12 mm) containing prepared antigen and pooled antisera were incubated at 50° C overnight. If there was still no agglutination with any of the pools, the broth culture was autoclaved at 121° C for 2 hours in an attempt to destroy the A variety of K antigen. The autoclaved culture was then tested with the pooled antisera, and if agglutination did not occur, the strain was reported as *E. coli,* O group undetermined (ungroupable smooth strain). If there was agglutination in a pool, the antigen was tested with individual components contained in the pool in the same manner described above.

3. Titrations with specific O antisera. A titer was run with each O antiserum showing agglutination at a 1 : 500 dilution in the pool. Antisera were diluted serially from 1 : 40 to 1 : 20,480 and were tested against diluted antigen. The antigen-antiserum mixture was incubated at 50° C overnight. The highest dilution of antiserum showing significant agglutination was recorded. All titers 1 : 1,280 were considered significant. Owing to cross-reactions between various O groups, some strains showed significant titers with more than one antiserum. Cross-absorbed sera were not available to determine the specific O groups of these strains.

RESULTS

Prevalence of O groups in non-enteric infections. The somatic antigen (O Group) of 522 strains of *E. coli* isolated from patients with urinary tract infections, bacteremia, pulmonary infections, and other non-enteric purulent foci was identified (Table I). Strains of *E. coli* of 54 different O groups were encountered, but four distinct serological groups—1, 4, 6, and 75—accounted for 44.8 per cent of all isolates. Other serological groups of particular frequency were 07, 025, 050, and the inter-O group cross-reaction of 050 with 01. A total of 401 of the 522 strains (76.8 per cent) could be characterized with available typing sera. Of the ungroupable strains, 8.8 per cent could not be characterized serologically because the strains were rough, and 14.4 per cent of isolates

TABLE I

Prevalence of serological groups of Escherichia coli isolated from non-enteric infections of man

O group	No. isolated	Per cent
1	27	5.2
4	48	9.2
6	104	20.0
75	54	10.4
7	15	2.9
25	13	2.5
50	12	2.3
50 or 1	11	2.1
Misc. cross-reactions *	33	6.3
42 Misc. O groups †	84	15.9
O group undetermined ‡	75	14.4
Rough‡	46	8.8
Total groupable	401	76.8
Ungroupable‡	121	23.2
Total	522	100.0

* Agglutination in 2 or more sera at dilution of 1 : 1,280 or greater.

† O groups 3, 5, 9, 10, 11, 12, 14, 15, 16, 18ac, 20, 21, 22, 23, 26, 28ac, 29, 30, 39, 42, 43, 51, 55, 59, 60, 62, 82, 83, 85, 86, 91, 93, 101, 102, 110, 113, 117, 120, 121, 125ab, x2, and x9

‡ Ungroupable strains include rough strains and strains which did not agglutinate with any of the antisera even after autoclaving.

did not agglutinate with any of the 137 antisera used in the present study even after autoclaving. It is apparent that the majority of strains cultured from patients with non-enteric *E. coli* infections belonged to but a few serological groups. In addition to these common O groups, 42 miscellaneous O groups were identified but accounted together for only 15.9 per cent of the 522 isolates. O group 6 was the single most common isolate and accounted for 26 per cent of all groupable strains; serological groups 06, 075, 04, and 01 accounted for 58.2 per cent of groupable trains. Conspicuous by its absence among the 522 non-enteric strains was O group 2 which was reported as one of the most frequent urinary isolates by Rantz (6) and Ewing and Davis (10). However, this discrepancy is more apparent than real, and in the present studies O group 2 was commonly involved in cross-reactions with other O groups. The inter-O group relationship of 050 with 01 was another cross-reaction which occurred frequently. Cross-absorbed O antisera were not available, and it was not possible to define the O group of such strains by titration alone. Rough strains were repeatedly plated on blood agar in an attempt to produce version to a smooth form; this was usually not successful.

Prevalence of O groups in different hospitals (Table II). Serological distribution of groupable strains of *E. coli* was similar in Seattle, Salt Lake City, and Baltimore. This included strains from patients hospitalized at King County Hospital, University of Washington Hospital, Johns Hopkins Hospital, and the Salt Lake General Hospital. The latter had an extraordinarily high incidence of O group 7, but there were too few groupable strains from this hospital to attribute any significance to this observation. The same few specific serological groups accounted for the majority of non-enteric infections regardless of hospital or geographic site sampled. Again, O group 6 was the most common strain isolated from all hospitals.

Prevalence of O groups by site of infection. Although most strains were isolated from patients with urinary infections, the same serological pattern was found among 92 groupable strains of *E. coli* cultured from bacteremia, pulmonary infections, deep abscesses, and other purulent foci (Table III). It is apparent that there was no significant difference between serological patterns among urinary and extra-urinary coliforms. O antigens 6, 75, 4, and 1 accounted for 57 per cent of groupable urinary strains and 60.9 per cent of extra-urinary isolates. Ungroupable strains were found with approximately the same frequency among urinary and non-urinary *E. coli.* Table III also illustrates the distribution of serological groups among urinary contaminants. These organisms were isolated from urine specimens of 87 pregnant women who were catheterized at term. All of these specimens contained less than 1,000 *E. coli* per ml urine and may be considered representative of the urethral flora. The same serological distribution of the common O groups was present as was encountered in clinical infections, and groups 6, 75, 4, and 1 comprised 72.3 per cent

TABLE II

Prevalence of serological groups of Escherichia coli isolated in different hospitals *

O group	KCH		UW		JH		SL	
	No.	%	No.	%	No.	%	No.	%
6	62	28.4	13	19.7	22	27.9	7	18.4
75	30	13.8	11	16.7	7	8.9	6	15.8
4	26	11.9	9	13.6	10	12.7	3	7.9
1	14	6.4	6	9.1	4	5.1	3	7.9
7	6	2.8	2	3.0	1	1.3	6	15.8
25	8	3.7	3	4.5	1	1.3	1	2.6
50	8	3.7	2	3.0	2	2.5	0	0
50 or 1	5	2.3	2	3.0	4	5.1	0	0
Misc. cross-reactions	17	7.8	3	4.5	8	10.1	5	13.2
Misc. O groups	42	19.2	15	22.8	20	25.3	7	18.4
Total	218	100.0	66	99.9	79	100.2	38	100.0

* Abbreviations: KCH = King County Hospital, Seattle; UW = University of Wasington Hospital, Seattle; JH = Johns Hopkins Hospital, Baltimore; SL = Salt Lake General Hospital, Salt Lake City.

TABLE III

Prevalence of O groupable strains of Escherichia coli isolated from patients with significant bacteriuria, those with extra-urinary infections, and those with bacterial counts <1,000/ml

O group *	Significant bacteriuria		Extra-urinary infection		Bacterial counts <1,000/ml in urine	
	No.	%	No.	%	No.	%
6	77	26.3	24	26.1	28	38.9
75	39	13.3	15	16.3	5	7.0
4	32	10.9	14	15.2	14	19.4
1	19	6.5	3	3.3	5	7.0
7	12	4.1	4	4.4		
25	7	2.3	5	5.4		
50	11	3.8	1	1.1	2	2.8
50 or 1	8	2.7	1	1.1		
Misc. cross reactions	26	8.9	6	6.5		
Misc. O groups	62	21.2	19	20.6	18	25.0
Total	293	100.0	92	100.0	72	100.1

* O antisera for testing *E. coli* from patients with bacterial counts <1,000/ml in the urine were limited to O groups 6, 75, 4, 1, and 50, i.e., miscellaneous O groups include remaining O groups and cross-reactions.

of the groupable *E. coli* cultured from urethral contaminants.

Prevalence of O groups in feces. The somatic antigen (O group) of 1,300 smooth colonies of *E. coli* isolated from the feces of 30 consecutive hospitalized patients with negative urine cultures and 35 consecutive hospitalized patients with significant *E. coli* bacteriuria was identified. At least 20 colonies of *E. coli* were picked at random from each of the initial stool cultures. No patient was receiving antimicrobial therapy when the urine or stool cultures were obtained. In all instances urine and stool cultures were obtained simultaneously. The serological distribution of O groups of *E. coli* isolated from the feces of the 30 abacteriuric patients is shown in Table IV. Again,

TABLE IV

Prevalence of serological groups of Escherichia coli in feces

O group	30 Abacteriuric patients		35 Patients with *E. coli* bacteriuria	
	No. colonies	%	No. colonies	%
6	99	16.5	197	28.1
75	100	16.7	51	7.3
4	20	3.3	56	8.0
1	90	15.0	42	6.0
Subtotal	309	51.5	346	49.4
Misc.*	291	48.5	354	50.6
Total	600	100.0	700	100.0

* Includes all other O groups, O group undetermined strains, and cross-reactions.

O groups 6, 75, 4, and 1 accounted for the majority of isolates, and 309 of 600 smooth colonies (51.5 per cent) consisted of these common O groups. Similarly, these four specific O groups accounted for the majority of isolates from stool specimens of the 35 patients with significant *E. coli* bacteriuria and 346 of 700 smooth strains (49.4 per cent) were of O groups 6, 75, 4, or 1.

Relationship of antigens of urinary and fecal Escherichia coli. Thirty-five patients with significant *E. coli* bacteriuria had stool cultures performed on the same day bacteriuria was discovered. Twenty-five of these 35 harbored organisms with the same serological group in both stool and urine. In 21 instances this organism belonged to one of the common O groups and was the predominant isolate in 17 instances in which the stool contained several different O antigens. These results suggest that the coliform flora of urine and stool are serologically similar and that those *E. coli* which infect the urinary tract emanate from the gut.

DISCUSSION

The demonstration and refinement of serological techniques by Kauffmann and his co-workers (2, 11–13) have led to renewed interest in the relationship of specific serologic strains of *E. coli* to pathogenicity. Many early investigators, attempting to implicate strains of *E. coli* in specific disease processes, confined their investigations to

the use of biochemical tests for differentiation of these organisms. It has been shown subsequently that cultural characteristics and biochemical tests correlate poorly with serological groups of *E. coli*. Further interest has been stimulated by extensive epidemiological studies which have demonstrated the frequent occurrence of particular serotypes in diarrheal disease. There are few data, however, relating antigenicity to pathogenicity in nonenteric coliform infections. Studies by Rantz in this country and by Kauffmann, Ujváry, Vahlne and Sjöstedt abroad have suggested that certain serological groups of *E. coli* are more frequently associated with clinical infections.

The present epidemiological studies substantiate previous suggestions that strains of a few serological groups of *E. coli* are responsible for the majority of coliform infections occurring outside the gastrointestinal tract. These serological groups were the most prevalent strains regardless of hospital or geographic site sampled. The serological spectrum of *E. coli* was the same in cultures from urine, blood, sputum, and purulent exudates, indicating that there was no specific coliform, characterized by its somatic antigen, with an unusual propensity to selectively incite infection in renal tissue. Furthermore, strains of these prevalent O groups were also commonly isolated in stool specimens from hospitalized patients with and without urinary tract infections. The very fact that *E. coli* of common O groups frequently exist in the normal urethra without associated urinary tract infection also provides evidence against the concept of "epidemiological virulence" proposed by Fekety and Bennett in regard to staphylococcal infections (14). The results of the present studies suggest that the reason certain serological groups of *E. coli* are more common in urinary infections may be a consequence of their relative ubiquity in the environment rather than increased virulence for man. The alternate hypothesis that these strains are actually more virulent and invasive cannot be excluded on the basis of the data presented; virulence and prevalence may well be related and inseparable factors.

The great majority of isolates from infected urines were of the same serological group, whereas stool specimens were frequently polygroupic. Stated in another way, only a single serological group of *E. coli* was usually found in multiple colonies picked from any one urine culture, while of the 65 stool cultures examined in the present study, over 50 per cent contained more than one serological group of *E. coli* per 20 colonies studied. Similar observations have been reported by Vosti, Monto and Rantz (15). In addition, the antigenic composition of the fecal coliform flora may change from day to day (16) and may also be altered by antibiotic therapy. Therefore, in making any comparison between the frequency of serological groups of *E. coli* present in the stool and urine of the same patient, it is important to obtain simultaneous cultures. This may explain Rantz's preliminary observation that *E. coli* of certain O groups occurred more commonly in urinary than in fecal specimens. This is not the case in the present study, and the fecal flora of hospitalized patients was serologically similar regardless of the presence of urinary tract infection.

One possible explanation for the observation that stools may contain *E. coli* of different serological groups while urines are usually monogroupic may relate to the growth characteristics of these organisms in infected environments, exemplified by urinary tract infections, as opposed to noninfected sites. The discovery of *E. coli* of more than one serological group in the urethral flora would help to clarify this point. These studies are in progress.

Finally, mixed *E. coli* urinary tract infections with strains of more than one serological group occur not infrequently in patients with chronic bacteriuria, and changes in the flora of the urine often represent selection of resistant strains after exposure to antimicrobials. However, these changes in serological groups of coliforms in bacteriuric patients may also occur in the absence of antimicrobial therapy. This may represent endogenous re-infection from the gut or may be merely a consequence of the limitations inherent in picking a limited number of colonies for serological grouping. In other words, it is conceivable that many coliform infections are associated with several serologically distinct strains de novo and that an alteration in the coliform flora does not necessarily connote re-infection from either endogenous or exogenous sources. With more precise techniques for identification of specific strains of *E. coli* in the offing, this problem should be resolved in the near future.

SUMMARY

Serological grouping with determination of the somatic antigen was performed on 1,344 strains of *Escherichia coli* isolated from enteric and nonenteric sources.

Fifty-four different O groups of *E. coli* were encountered among 522 strains studied from patients with urinary infections, bacteremia, abscesses, pulmonary infections, and other nonenteric purulent foci. Four distinct serological groups—1, 4, 6, and 75—accounted for 58 per cent of groupable infections. Coliforms cultured in hospitals in Seattle, Salt Lake City, and Baltimore had similar distribution of common O groups.

These studies suggest that certain strains of *E. coli* are responsible for the majority of nonenteric infections because of their greater prevalence in the environment.

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