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SEROLOGICAL GROUPING OF *ESCHERICHIA COLI* IN BACTERIURIA OF PREGNANCY

LEELA GANGULI

Bacteriology Department, University of Edinburgh

A DIAGNOSIS of urinary infection is made when freshly voided urine contains 100,000 or more bacteria per ml (Kass, 1955). "Significant bacteriuria", thus defined, is often an asymptomatic condition, and is found in 5-8 per cent. of normal pregnant women. About 40 per cent. of these women, if untreated, will develop a symptomatic episode or acute pyelonephritis later in pregnancy (Kass, 1960; Kincaid-Smith and Bullen, 1965). The causative organism in the majority of women with asymptomatic bacteriuria of pregnancy is *Escherichia coli*. In the present study the O-antigen of *E. coli* strains isolated from 111 such women was identified. Follow-up studies after antimicrobial therapy were done to determine the relation of infection with a particular O-group to the response to therapy, the development of clinical symptoms of urinary infection and the incidence of renal involvement.

MATERIALS AND METHODS

Patients. 9741 consecutive women attending the ante-natal out-patient clinic for the first time had urine specimens examined. Significant bacteriuria was detected in 595 women (6.1 per cent.). Bacteriuric women who were regarded by clinicians as not having active urinary infections were termed "asymptomatic". Only 32 women were found to have symptoms at this time, 563 being asymptomatic. The first 127 of these asymptomatic women who attended early enough in pregnancy to allow treatment to be completed before the 28th wk of gestation were the subjects of this study. All of them had significant bacteriuria demonstrated in two urine samples, taken 1 wk apart, before therapy was given.

Examination of urine. Midstream specimens of voided urine were collected into a sterile container without prior perineal toilet. Urine was examined within the next 2 hr by a quantitative viable count method (Miles, Misra and Irwin, 1938). Follow-up examinations of urine were done in all women one week after the cessation of therapy and at monthly intervals thereafter till delivery.

Sensitivity tests. *Sulphadimidine*.—The disk diffusion method proved unsatisfactory in the assessment of the sensitivity of urinary pathogens to this drug. A standardised tube dilution method was developed with Oxoid sensitivity test broth CM 245 as liquid medium and a measured inoculum of the test strain containing about 2000 viable bacteria in a volume

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of 0.02 ml. Organisms having a minimum inhibitory concentration (MIC) of 52 μ g per ml, or less, of sulphadimidine were considered sensitive (Ganguli, 1966). *Cycloserine*:—Tube dilution sensitivity tests were carried out by the method described by Murdoch, Sleight and Frazer (1959). Organisms having a MIC of 125 μ g per ml, or less, of cycloserine were considered sensitive.

Treatment. This consisted of a 2-wk course of cycloserine, 250 mg twice daily, or sulphadimidine, 500 mg four times a day, given by mouth to alternate patients, without reference to the in-vitro sensitivity of the causal organism. The result of therapy was assessed by culture of the urine during follow-up. The outcome in patients who had no positive cultures after therapy until delivery was termed "successful". If the specimen taken 1 wk after therapy was positive the outcome was termed an "immediate failure". If one or more positive cultures were obtained only at later visits the outcome was termed a "late failure".

Patients who failed to respond to therapy by either of the above criteria had an intravenous pyelogram done at some time between 3 mth and 2 yr after delivery.

E. coli O-antisera. Type cultures representing each of the 145 O-antigens of *E. coli* were obtained from the National Collection of Type Cultures, Colindale, London. Ten O-antigen groups were selected for preparation of antisera, based on reports of their frequency in urinary infections (Rantz, 1962; Vosti *et al.*, 1964). These were O1, O2, O4, O6, O7, O8, O18, O25, O75 and O77. Rabbits were immunised by the method of Ewing (1956) with broth cultures incubated for 5 hr at 37°C and killed by the addition of formaldehyde to a final concentration of 0.2 per cent. Bacterial agglutination tests were carried out on broth cultures of test strains incubated at 37°C for 18 hr and then heated at 100°C for 1 hr in order to inactivate the L and B types of K antigens. The turbidity of these antigen suspensions was adjusted to that of Wellcome opacity tube no. 1 before they were used in agglutination tests. The tests were carried out by the method of Edwards and Ewing (1962). Antigen suspensions that auto-agglutinated when incubated with an equal volume of 0.85 per cent. NaCl were termed "rough".

Determination of cross-reactions of the antisera with heterologous O-antigens. Each antiserum was initially diluted to give a final titre of 800 against the homologous O-antigen. One volume of each serum was then tested against one volume of each of the 144 heterologous O-antigens of *E. coli* at a final screening dilution of the serum of 1 in 100. All positive cross-reactions observed at this titre were subsequently titrated. In four out of the ten antisera strong cross-reactions were found and mono-specific sera were prepared from these sera by absorption. In two further sera minor cross-reactions were detected, but these did not interfere with the typing of test strains. The strong cross-reactions were between antigen O50 and serum O1, between antigen O74 and serum O2, between antigens O15 and O40 and serum O8, and between antigen O17 and serum O77. Minor reactions detected were between antigen O100 and serum O6, and between antigens O5 and O71 and serum O7.

RESULTS

Out of 127 women examined, 111 had initial infections due to *Escherichia coli*, the remaining 16 had other infecting organisms and hence were excluded from the analysis. Altogether 331 strains of *E. coli* were obtained at various times during the follow up of the 111 bacteriuric women. After serological tests, 96 (29 per cent.), were found to be auto-agglutinable, or "rough". Of the 235 smooth strains, 141 (60 per cent.) were allocated to an O-group by tests with the ten antisera. Fifty of the remaining 94 smooth but ungrouped strains were subsequently tested by me with all 145 O-antisera at the Salmonella Reference Laboratory, Colindale, with the kind help of Dr Joan Taylor. Of these, 23 were grouped, 8 were found to be "rough", and the rest were ungrouped.

The grouped strains of *E. coli* were related to the patients and it was found that of the 111 women, 54 had the O-group of their initial infecting strain determined. The initial strains from the other 57 were either ungrouped or "rough"; hence further analysis regarding results of therapy and other data was done on the 54 women with bacteriuria due to a known O-group at the onset of the

TABLE I

O-group of infecting strain of Escherichia coli and the response to initial treatment with cycloserine or sulphadimidine in 111 pregnant women with asymptomatic bacteriuria

O-group, or category, of infecting strain	No. (and percentage) of women infected with strain of stated O-group	No. of women in whom outcome of treatment was a	
		success	failure*
O1	3	1	2†
O2	9 (17)	7	2
O4	4 (7)	3	1
O6	11 (20)	3	8
O7	3	1	2
O8	2	1	1
O18	1	0	1
O25	2	1	1
O75	11 (20)	5	6
O77	2	1	1
O83	2	0	2
O5†, O10, O17, O21	1, 1, 1, 1	0	4
All the above groups	54	23	31
Untypable "smooth"	28	21	7
"Rough"	29	17	12
Total	111	61	50

* Either an "immediate" or a "late" failure as defined under *Materials and methods*.

† Infecting strain was resistant to drug given.

study. Groups O6 and O75 occurred most frequently among the initial isolates (table I). Groups O2, O4, O6 and O75 together accounted for infection in 35 (65 per cent.) of the women. When isolates obtained during the follow-up were analysed, these four groups were again frequently encountered.

Result of treatment in infections with strains of known O-group

Treatment with cycloserine or sulphadimidine was successful in eliminating bacteriuria in 23 out of the 54 women whose initial infecting strain was O-grouped. In the other 31 women (57 per cent.), the result was an "immediate" or "late" failure. It was noted that out of 22 women infected with either of groups O6 and O75, 14 (65 per cent.) failed to respond to treatment although the infecting strain did not show drug resistance *in vitro*. This higher incidence of failure of treatment in the O6 and O75 infections as compared with those of other groups (17 out of 32, i.e., 53 per cent.) was not statistically significant.

On the other hand, therapy was successful in 10 out of 13 patients infected with organisms of either group O2 or group O4.

In only 3 of the 31 cases of failure of treatment, the infecting organism was resistant to the drug given as determined by standard tube dilution methods for sensitivity tests (table I).

In 28 out of the 31 patients who failed to respond to treatment, it was possible to group the strains isolated before and after treatment. In three cases the post-treatment isolates were rough. In 12 out of 13 cases of immediate failure a

TABLE II

Relation of O-groups of pre- and post-treatment isolates of E. coli in 28 women with bacteriuria who failed to respond to treatment for 2 wk with cycloserine or sulphadimidine

Result of treatment	Number of women failing to respond to treatment		
	Total	in whom O-groups of pre- and post-treatment isolates were	
		the same (relapse)	different (re-infection)
Immediate failure (i.e., within 7 days)	13	12	1
Late failure (i.e., after 7 days)	15	8	7

relapse of infection due to an organism of the same O-group was found (table II). Of 15 patients in whom the result of treatment was late failure roughly half had a relapse of infection with a strain of the same O-group, and half a re-infection with an *E. coli* strain of a different O-group.

Persistence of the same O-group in chronic bacteriuria

Grouping was done on serial isolates of infecting strains of *E. coli* obtained during the follow-up of some women who had chronic bacteriuria throughout pregnancy. The duration of follow-up varied from 16 to 30 wk depending on the period of gestation when the patient was first seen in hospital. In table III, it is seen that in 15 (no. 1-14 and 22) of the 31 patients who failed to respond to treatment, the same O-group persisted in significant numbers in the urine for periods up to 30 wk. All 31 women received at least one course of therapy and 15 of them, who had clinical symptoms, received one or more further courses of therapy with drugs selected on the basis of the results of the in-vitro sensitivity tests. Re-treatment did not displace the resident infecting strain of *E. coli* except in one woman in whom it was replaced by infection with a strain of *Proteus*.

In many instances, these further courses of chemotherapy coincided in time with the occurrence of an intermittency of bacteriuria.

TABLE III

*Persistence of strain of original infecting O-group in 31 pregnant women with chronic bacteriuria who failed to respond to treatment with cycloserine or sulphadimidine**

Patient no.	O-group of original infecting strain of <i>E. coli</i>	Bacteria cultured from urine at weeks after therapy					Results of pyelogram
		1	5	9	13	17	
1	O7	—	O7	O7	O7	O7	Abnormal
2	O7	O7	—	O7	O7	O7	Abnormal
3	O21	—	O21	O21	O21	O21	Not done
4	O6	O6	SL	O6	O6	O6	Normal
5	O6	O6	O6	O6	O6	O6	Abnormal
6	O5	O5	O5	O5	O5	—	Not done
7	O77	O77	O77	O77	...	...	Normal
8	O75	—	—	O75	O75	O75	Normal
9	O2	—	O2	O2	O2	...	Abnormal
10	O18	O18	O18	O18	...	...	Not done
11	O17	O17	O17	O17	...	...	Not done
12	O83	O83	O83	—	...	...	Abnormal
13	O1	O1	O1	—	...	...	Not done
14	O1	—	O1	O1	—	—	Normal
15	O75	—	O75	N	O4	...	Normal
16	O8	—	O8	—	...	...	Not done
17	O2	O2	—	—	—	...	Not done
18	O6	O6	R	R	R	R	Normal
19	O6	O6	O2	O2	O2	...	Not done
20	O6	—	O18	O18	—	...	Abnormal
21	O4	—	O7	O7	...	...	Normal
22	O25	—	O25	O25	...	...	Not done
23	O83	—	O1	UT	UT	...	Not done
24	O75	—	UT	UT	UT	...	Not done
25	O6	—	R	UT	UT	...	Abnormal
26	O6	—	N	N	...	...	Normal
27	O75	—	—	—	—	N	Abnormal
28	O75	—	R	UT	R	...	Normal
29	O6	R	N	—	—	...	Not done
30	O75	—	—	R	R	...	Normal
31	O10	—	—	—	—	R	Not done

* Patients no. 3, 4, 9, 10, 12, 18, 20, 24, 25, 26, 27, 29 and 31 were treated with cycloserine and the other patients with sulphadimidine. The follow-up period includes an additional 13 wk between the original isolate and the first isolate after therapy.

O1, O2, etc. = Isolation of *E. coli* of stated O-group; — = urine not infected; N = bacteria other than *E. coli*; R = rough strain of *E. coli* isolated; UT = *E. coli* strain untypable with available antisera; SL = strain lost; ... = specimen of urine not examined.

Incidence of clinical symptoms

Although all 54 women with infections with a strain of known O-group were asymptomatic at the onset of study, 15 developed symptoms of an acute urinary infection on one or more occasions during the follow-up period after therapy.

All 15 were found to be in the group of the 31 women who failed treatment. Symptoms were referable to the upper or lower urinary tract and the clinical diagnosis of acute pyelonephritis was made in only a few cases, most of which required admission to hospital. All women with symptoms were found to have an infected urine at that time. However, no particular O-group was found to be more frequently associated with these symptomatic episodes than other O-groups.

Radiological findings

Intravenous pyelogram studies were carried out on 18 of the 31 women who failed to respond to treatment. About half of those examined showed structural abnormalities of the renal tract. It was noted that 4 of the 8 women with abnormalities had a persistent bacteriuria throughout pregnancy that was due exclusively to bacteria of the same O-group, and yet had no clinical symptoms. The infecting O-group in three of them was O6 and in the fourth was O75. The renal lesion in two of these four cases was bilateral chronic pyelonephritis; in one it was a renal cyst and in one unilateral hydronephrosis.

DISCUSSION

A complete serological identification of *Escherichia coli* requires the determination of its O, H and K antigens. For the purposes of the present study a determination of the O-antigen alone was considered sufficient. Serological tests were done only on a single colony from each urine specimen since Vahlne (1945) and Vosti, Monto and Rantz (1962) had found that over 90 per cent. of specimens of infected urine contained only bacteria of a single O-group per specimen regardless of the number of colonies examined.

In the present study 60 per cent. of smooth strains of *E. coli* were allocated to one or other of 10 O-groups when tested with 10 out of the 145 available O-typing antisera. Groups O2, O4, O6 and O75 made up the majority of primary and recurrent isolates. The common O-groups found in other reported studies of urinary infection are seen in table IV. A predominance of these O-groups in my study emphasises the fact that the majority of urinary infections are caused by strains of only a few of the known serological groups. Jackson, Kozij and Jao (1965) also found, as I did, that group O75 was frequently associated with urinary infections during pregnancy.

There was no correlation between infection with a particular O-group and the occurrence of clinical symptoms. Moreover, the results of radiological examination revealed that the presence of clinical symptoms could not be regarded as a reliable guide to the presence of serious renal disease since half the women with renal disease and chronic bacteriuria remained asymptomatic throughout pregnancy. McGeachie (1965) and Jackson *et al.* found that strains of groups O4 and O6 caused symptoms more often than the others. Rantz (1962) found an exceptionally high incidence of pyelonephritis associated with O4 and O6 infections. Kunin and Halmagyi (1962), however, failed to find any association between the frequency of symptoms and the presence of infection with particular O-groups.

Almost all women who had a recurrence of bacteriuria within one week after therapy had a relapse of infection with the same O-group. In patients with recurrences occurring after this period only about half showed a relapse of infection with the same O-group. Pryles and Glagovsky (1965) found that 85 per cent. of recurrences following treatment of urinary infection in children were caused by strains of a different O-group from that causing the initial infection. However, these children were not similar to my patients with regard to the severity of infection, the type and duration of therapy and the method of follow-up. These results on relapse and re-infection are based on the identification of one colony per specimen. The findings of Spencer *et al.* (1968) confirm the

TABLE IV
O-groups of *E. coli* most frequently encountered in reported studies of urinary infection

Authors	Number of typing sera used	Number of strains tested	O-groups commonly found
Rantz (1962)	129	156	O2, O4, O6, O75
Turck and Petersdorf (1962)	137	293	O1, O4, O6, O75
Kunin and Halmagyi (1962)	144	57	O1, O2, O4, O6, O7, O30, O75
Vosti <i>et al.</i> (1964)	131	181	O1, O4, O6, O7, O16/62, O25, O50, O75
Pryles and Glagovsky (1965)	151 (including Ox groups)	288	O1, O4, O6, O7, O75
Jackson <i>et al.</i> (1965)	64	500	O1, O4, O6, O75
McGeachie (1965)	12	316	O1, O2, O4, O6
Maiztegui and Kass (1965)	152	163	O4, O6, O62, O75
Present study	10	331	O2, O4, O6, O75

view that the population of infecting organisms in a urine sample is serologically homogeneous. They found bacteria of only one O-type per specimen in acute infections and bacteria of more than one O-type in specimens from only 12 per cent. of cases of recurrent infections following therapy.

In follow-up studies in 14 women with chronic bacteriuria the same strain was repeatedly isolated, in spite of antibiotic treatment, for periods up to 30 wk. In some of the women a high level of serum antibody to the O-antigen of the infecting strain was detected in haemagglutination and bacterial agglutination tests (Ganguli, 1966). Maiztegui and Kass (1965) had similar findings in 36 pregnant women, but did not state the duration of excretion in their cases.

Winterbauer, Turck and Petersdorf (1966) and Spencer *et al.* suggest that bacteria of the O-groups commonly found in urinary infections, particularly groups O4, O6 and O75, are acquired and spread in hospital, but the pregnant women in my study were not in contact with each other and were hospitalised only at the end of the study period.

My results show that certain serological groups, i.e., O2, O4, O6 and O75, of *E. coli* cause the majority of urinary infections in pregnant women. The infections may remain entirely asymptomatic, but the serious nature of the disease is shown by its resistance to short courses of chemotherapy and the degree of renal damage in some of the patients.

SUMMARY

Escherichia coli strains isolated from the urine of 111 pregnant women with significant asymptomatic bacteriuria were studied. Sixty per cent. of the 235 smooth strains isolated were successfully grouped in agglutination tests with antisera to ten O-groups. Groups O2, O4, O6 and O75 accounted for the majority of the primary and recurrent isolates. Fifty-four women with initial infections due to a strain of a known O-group were treated and examined monthly until delivery. Over half of these 54 women failed to respond to therapy and 14 of them continued, in spite of further chemotherapy, to excrete bacteria of the same O-group for periods up to 30 wk.

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