ANTIGENIC ANALYSIS AND BIOCHEMICAL CHARACTERS OF
STRAINS OF *ESCHERICHIA COLI*, COMMONLY ASSOCIATED
WITH HUMAN DIARRHOEA, ISOLATED FROM SPORADIC
CASES OF GASTRO-ENTERITIS IN LOCAL POPULATION
OF DOMESTIC ANIMALS¹

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One hundred and fifty-three strains of *Escherichia coli* were isolated from sporadic cases of gastroenteritis from cows, calves, buffaloes, buffalocalves, goats, and kids. Thirty-six biochemical tests were conducted. On the basis of fermentation reaction of six selected sugars (rhamnose, sucrose, dulcitol, raffinose, salicin, and starch), 153 strains were classified into 28 biotypes. Biotypes I, V, VI, XI, XVIII, and XX appeared more common than the others. These six biotypes accounted for over 60% of the total (153) strains isolated from diarrhoea, dysentery, or white scours. Biotype XX predominated in buffaloes and buffalocalves, and Type VI in cows and calves, and in goats and kids. Again Type VI predominated in diarrhoea and dysentery and Type XI in white scour. Only 137 strains could be identified serologically. Sixty-five strains belonged to 7 human enteropathogenic 'O' groups (O26:B6, O55:B5, O86:B7, O112:B11, O119:B14, O125:B15, and O126:B16). Group O26 predominated in goats and kids, O125 in buffaloes and buffalocalves, and O119 in cows and calves. Another 72 strains belonged to 31 'O' groups, which are not known to be associated with infantile diarrhoea and gastroenteritis of human. Serological heterogenicity was observed within a single biotype and, similarly, strains within a single serotype differed in their biochemical characters.

Bacteriological study of animal gastroenteritis provides opportunity for direct enumeration and identification in animal intestine of forms and types of *Escherichia coli* flora that are known to be commonly associated with human diarrhoea (1–6). Identification and knowledge of 'O' and H antigens of the strains (5–7) enable us to speculate or trace human or extra-human source (5, 8) of new outbreaks of animal gastroenteritis (9–13), and furthers our understanding and knowledge of the spread of diseases common to man and domestic animals. Differences in biochemical characters in strains may

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reveal existence of novel biotypes (1, 14–18) and their possible association with certain serotypes (11, 19) or with animal species (7), their age (20), and type of the disease (11, 21–25), or even their duration (26). It has been suggested that differences in biochemical character of strains in a serotype may arise because of the different origin of serologically identical strains (17).

In the present paper, results of some of the above studies with 153 strains of *Escherichia coli* isolated from faecal samples of sporadic cases of gastroenteritis of domestic animals in Lucknow are presented.

MATERIALS AND METHODS

Isolation and culture. Faecal samples of hospitalised untreated domestic animals of the Lucknow Municipal area were collected from Veterinary Hospitals, Badshahbagh and Havelock Road, Lucknow. The external anal area of the animal was cleansed with cotton moistened with lysol (0.01% v/v). Sterile cotton swab attached to an 8-in-long wire was moistened with sterile 1.0% (w/v) peptone water and introduced gently into the rectum and twisted inside so as to touch the mucosa. The swab was pulled out and transferred to a sterile test tube. It was brought immediately to the laboratory and suspended in 2 ml of sterile nutrient broth (peptone, 20 g; NaCl, 5 g; dextrose, 2 g; disodium hydrogen phosphate, 2.5 g; distilled water to 1 liter and stirred thoroughly. A loopful of the broth was streaked on MacConkey agar plate and incubated aerobically at 37° for 18 hr for the appearance of lactose-fermenting pink colonies. A colony when found was resuspended in nutrient broth and streaked on MacConkey agar once again. Culture from a well isolated colony was made on nutrient agar (20 g Difco agar/liter nutrient broth) slant and subcultured every 15 days. Isolates positive to indole (I) and Methyl Red (M) and negative to Voges-Proskauer (Vi) and citrate (C) were considered as *E. coli*.

Biochemical reactions. IMViC reactions, nitrate reduction, gelatin liquefaction, deamination of phenylalanine to phenyl pyruvic acid, organic acid utilization, and carbohydrate fermentation tests were done according to EDWARDS and EWING (27), catalase production according to CRUICKSHANK (28), hydrogen sulphide production according to CLARKE (29), urease activity according to CHRISTENSEN (30), decarboxylase test according to FALKOW (31), and growth in KCN medium according to EDWARDS and FIFE (32).

Motility was determined by a hanging drop preparation and by stab culture (28). After addition of 5 ml of 1% (w/v) 2,3,5-triphenyltetrazolium chloride, to 1 liter of 0.7% nutrient agar the tubed media were stab inoculated and incubated overnight. Motile organisms showed a red diffused zone of growth spreading from the line of inoculation. Non-motile strains showed only red colour along the line of inoculation.

Serological typing. Serological typing of the strains of *E. coli* was done

at the National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli. The following adsorbed O and OB antisera were used to determine the O and K(B) antigens.

OB antisera. O18 : B21, O26 : B6, O44 : K74(L), O55 : B5, O86 : B7, O111 : B4, O112 : B11, O119 : B14, O124 : B17, O125 : B15, O126 : B16, O127 : B8 and O128 : B12.

O antisera. O18, O26, O44, O55, O86, O102, O111, O112, O119, O124, O125, O126, O127 and O128.

For the typing of the strains, slide and tube agglutination tests advocated by EDWARDS and EWING (27) were adopted. Strains that could not be typed at the National Centre (Kasauli) against these sera were sent to the International *Escherichia* Centre, Statens Serum Institute, Copenhagen. All the strains studied in this work have been deposited at Kasauli.

RESULTS

The source of 153 strains of *E. coli* used in this work is given in Table 1. All the strains were found to be similar in MR, VP, citrate, nitrate, ammonia, catalase, H₂S, urease, gelatin, phenylalanine, and KCN reactions.

Table 1. Source of *E. coli* strains.

Source	No. of strain	Diarrhoea	Dysentery	White scour
From 15 calves and cows	31	13	8	10
From 17 buffalo-calves and buffaloes	39	21	8	10
From 38 kids and goats	83	69	14	—
Total:	153	103	30	20

Out of 20 carbohydrates, eight, namely, arabinose, dextrose, laevulose, galactose, trehalose and mannitol, were utilized by all the strains, and mucin and glycogen were utilized by none. Other biochemical fermentation reactions of the strains are given in Tables 2 and 3.

On the basis of results obtained with six selected substrates, the 153 strains could be classified into 28 biotypes (Table 4). Biotypes I, V, VI, XI, XVIII, and XX appear to be more common than the others. They accounted for nearly 60% of the total strains (Table 5). Biotype XX (negative for dulcitol, salicin, and starch) seems to be predominant in buffaloes and buffalo-calves; type VI (negative for starch) in cows and calves, and in goats and kids. Type VI was common in diarrhoea and dysentery cases, and type XI (negative for salicin and starch) in white scour (Table 6).

Out of the 153 strains of *Escherichia coli* only 137 could be typed serologically. It can be seen from Table 7 that 65 strains (47.4%), out of 137, belonged

Table 2. Proportion of strains of *E. coli* (out of 153) producing acid and gas from 12 carbohydrates.

Carbohydrates	Number positive		Positive (%)	
	A G	A	A G	A
Sorbitol	150	3	98.0	1.9
Lactose	147	5	96.1	3.2
Maltose	144	7	94.1	4.5
Xylose	142	9	92.8	5.5
Rhamnose	117	19	76.4	12.4
Sucrose	76	39	49.7	25.4
Dulcitol	108	6	70.5	3.8
Raffinose	84	25	54.9	16.3
Salicin	76	14	49.7	9.2
Starch	2	32	1.3	20.9
Dextrin	11	3	7.2	1.9
Adonitol	6	0	3.9	0.0

A=acid; AG=acid and gas.

Table 3. Biochemical characters of 153 strains of *E. coli*.

Test	No. of strains positive	Positive (%)
Arginine dihydrolase	34*	22.2
	32**	20.9
Lysine decarboxylase	100*	65.3
	19**	12.4
Ornithine decarboxylase	82*	53.5
	32**	20.9
Sodium mucate	92*	60.1
	35**	22.8
Motility	137	89.4

* positive in one day; ** positive in 2-3 days.

to seven human pathogenic 'O' groups to which belong serotypes known to be associated commonly with enteritis of humans. The most commonly occurring human 'O' groups are O26, O119, and O125. The proportion of human pathogenic 'O' groups, isolated from cows and calves was the highest, followed by buffaloes and buffalo calves, and least in goats and kids (Table 8). Group O26 predominated in goats and kids, O125 in buffaloes and buffalo calves, and O119 in cows and calves.

Table 4. Differentiation of 153 strains of *E. coli* into 28 biotypes based on their biochemical reactions towards rhamnose, dulcitol, sucrose, salicin, raffinose and starch.

Biotype	Strain	No.	Biochemical reactions negative for
I	2453, 2454, 2455, 2456, 2457, 2467, 2490, 2509*, 2523, 2524, 2545, 2581**, 2582	13	None
II	2458, 2595, 2596	3	Dulcitol
III	2466	1	Sucrose
IV	2510, 2513, 2514, 2599, 2600	5	Salicin
V	2440, 2441, 2442, 2443, 2447, 2448, 2449, 2450, 2452	9	Raffinose
VI	2432, 2464, 2465, 2468, 2484, 2485, 2487, 2515, 2516, 2522, 2533, 2534, 2537, 2538, 2541, 2542, 2546, 2547, 2551, 2552, 2567, 2568, 2579, 2580, 2585, 2586, 2587, 2588, 2597, 2598	30	Starch
VII	2549, 2550	2	Rhamnose and starch
VIII	2430, 2431, 2493, 2558, 2578	5	Dulcitol and starch
IX	2494	1	Sucrose and starch
X	2478, 2479, 2589	3	Salicin and raffinose
XI	2469, 2470, 2471, 2508, 2520, 2563, 2564, 2565, 2566, 2569, 2570, 2571**, 2572, 2573, 2574, 2577**, 2590**	17	Salicin and starch
XII	2491, 2498, 2502*, 2503, 2504, 2505, 2506, 2507	8	Raffinose and starch
XIII	2486, 2583, 2591, 2592	4	Rhamnose, dulcitol, and starch
XIV	2519, 2531, 2532, 2584	4	Rhamnose, salicin and starch
XV	2446	1	Rhamnose, raffinose and starch
XVI	2482	1	Dulcitol, sucrose and salicin
XVII	2527	1	Dulcitol, sucrose and starch
XVIII	2499, 2500, 2501, 2521**, 2535, 2536, 2544**, 2575, 2576**	9	Sucrose, raffinose and starch
XIX	2497, 2512, 2528, 2539, 2540, 2543**	6	Sucrose, salicin and starch
XX	2480, 2481, 2492, 2495, 2517, 2518, 2553, 2554, 2555, 2556*, 2557, 2559, 2560**, 2561**, 2562	15	Dulcitol, salicin and starch
XXI	2496	1	Salicin, raffinose and starch
XXII	2444, 2548	2	Rhamnose, sucrose, salicin and starch
XXIII	2511, 2593, 2594	3	Dulcitol, sucrose, raffinose and starch
XXIV	2483	1	Dulcitol, sucrose, salicin and starch
XXV	2476, 2477	2	Sucrose, salicin, raffinose and starch
XXVI	2445	1	Rhamnose, sucrose, salicin, raffinose and starch
XXVII	2451, 2525, 2526*	3	Rhamnose, dulcitol, sucrose, raffinose, and starch
XVIII	2529, 2530**	2	Dulcitol, sucrose, salicin, raffinose, and starch
		Total: 153	

* negative in 1-147 'O' sera, ** rough strains.

Table 5. Frequency of occurrence of the common *E. coli* biotypes among 153 strains in diseased animals.

Animals	Total No. of strains	Biotypes							
		I	V	VI	XI	XVIII	XX	Total	%
Buffaloes and buffalo-calves	39	2	5	1	2	0	8	18	46.1
Cows and calves	31	2	4	10	5	2	2	25	80.6
Goats and kids	83	9	0	19	10	7	5	50	60.2
Total	153	13	9	30	17	9	15	93	60.75

Table 6. Association of the common biotypes with forms of disease in animals.

Diseases	No. of strains	Biotypes						Total	%
		I	V	VI	XI	XVIII	XX		
Diarrhoea	103	13	3	21	11	4	10	62	60.1
Dysentery	30	0	6	7	0	3	3	19	63.3
White scour	20	0	0	2	6	2	2	12	60.0
Total	153	13	9	30	17	9	15	93	60.75

Table 7. Occurrence in the diseased animals of 'O' groups commonly associated with human enteritis.

Strain	No. of strains	Antigens		
		O	K	H
2432, 2453, 2454, 2476, 2477, 2505, 2506, 2507, 2528, 2538, 2539, 2540, 2597	13	26:	B6:	H-
2527*, 2547**	2	26:	B6:	NM
2487, 2515, 2516	3	55:	B5:	H-
2447, 2448, 2485	3	86:	B7:	H-
2492, 2495, 2513, 2514	4	112:	B11:	H-
2430, 2440, 2441, 2442, 2443, 2457, 2458, 2486, 2494, 2497, 2531, 2533, 2535, 2536, 2555, 2568, 2572, 2580, 2589, 2595, 2596	21	119:	B14:	H-
2449, 2452, 2455, 2456, 2464, 2465, 2467, 2478, 2479, 2510, 2532, 2534, 2548, 2565, 2599, 2600	16	125:	B15:	H-
2545, 2546, 2584	3	126:	B16:	H-
Total	65			

* 2527 (calf); ** 2547 (goat); H-=unknown; NM=non-motile.

Table 8. Distribution of 38 'O' groups (137 strains) of *E. coli* isolated from diseased animals.

Animals	No. of strains	'O' groups	Proportion of human 'O' groups
Goats and kids	73	OX1, OX3, OX6, 3, 6, 9, 10, 21, 26 , 37, 49, 55, 58, 75, 88, 94, 102, 103, 110, 119 , 125 , 126 , 138, 146 (=24)	4 : 24
Buffaloes and buffalo-calves	36	9, 49, 61, 76, 83, 86 , 88, 101, 102, 103, 112 , 119 , 123, 125 , 141, 147 (=16)	4 : 16
Cows and calves	28	26, 45, 49, 55 , 86 , 91, 108, 117, 119 , 125 , 134, 140 (=13)	5 : 13

NB: The O antigen types, OX1, OX3, OX6, are provisional; those printed bold indicate human 'O' groups.

Table 9. Biotypes of frequently occurring human enteropathogenic *E. coli* 'O' groups isolated from diseased animals.

Frequently occurring 'O' groups	Serotypes	No. of strains	Biotypes
O26	O26 : B6 : H-	13	I, VI, XII, XIX, XXV.
	O26 : B6 : NM	2	VI, XVII.
O119	O119 : B14 : H-	21	I, II, V, VI, VII, VIII, IX, X, XI, XIII, XIV, XVIII, XIX.
O125	O125 : B15 : H-	16	I, IV, V, VI, VII, X, XIV, XXII.

H=flagellar antigen not known; NM=non-motile.

Strains differed serologically within a biotype and *vice versa*. For instance, thirty strains of the biotype VI (Table 4) belonged to at least seven human 'O' groups (Table 7). Twenty-one strains, belonging to 'O' antigen group 119, were distributed into 13 biotypes (I, II, V, VI, VII, VIII, IX, X, XI, XIII, XIV, XVIII, and XIX) (Table 9).

Apart from the seven human 'O' groups, 31 'O' groups to which belong serotypes that are not known to be commonly associated with neonatal diarrhoea and gastroenteritis of humans were identified. They accounted for 72 strains (52.6%) out of 153 studied (Table 10).

DISCUSSION

Detection of gastrointestinal diseases caused by pathogenic *E. coli* common to man and animals (zoonoses) has not received the attention of workers in India it deserves. This is because in most instances attempts at diagnosis

Table 10. 'O' groups* of *E. coli* isolated from the diseased animals, not known to be commonly associated with human enteritis.

No. of strains	Serotypes	No. of strains	Serotypes
2496	OX1 : K- : H16	2466	O88 : K- : H12
2498	O3 : K- : H8	2573, 2574	O88 : K- : H?
2512	OX3 : K- : H?	2585, 2586, 2587, 2588	O91 : K- : H14
2591, 2592	OX3 : K- : NM	2562	O98 : K- : NM
2469, 2470, 2471	OX6 : K- : H21	2525	O101 : K- : NM
2499, 2500, 2501	O6 : K- : H12	2563, 2564, 2593, 2594	O102 : K- : H?
2582	O6 : K- : H9	2446	O103 : K- : H8
2598	O6 : K- : H21	2517, 2518, 2541, 2542	O103 : K- : H21
2451, 2503, 2504	O9 : K(A) : NM	2578	O103 : K- : H?
2529	O9 : K- : H10	2566	O108 : K- : H7
2508	O10 : K- : H?	2557, 2558	(O110) : K- : H34
2549, 2550, 2551	O21 : K- : H12	2431	(O117) : K- : H47
2490, 2491	O37 : K- : H8	2553, 2554, 2559	O123 : K-NM
2575	O45 : K- : H8	2484	(134) : K- : H21
2450, 2569, 2570	O49 : K- : H12	2511, 2522	O138 : K81 : H48
2579, 2583	O49 : K- : H?	2253, 2524	O140 : K- : H16
2519, 2520	O58 : K- : H16	2493	(O141) : K : H48
2444	O61 : K- : H8	2552	O146- : K- : H?
2567	O75 : K- : H10	2445	O147 : K- : H?
2480, 2481, 2482, 2483	O76 : K- : H21		
2468	O83 : K- : H16		

Total number of strains=72; * No. of 'O' groups=31;

? indicates negative in H sera; O antigen types in paranthesis indicate low titres.

and characterization of bacteria have ended with the identification of specific 'O' group antigens known to be associated with human diarrhoeal infection. While this may serve the initial purpose, namely, of suggesting possible occurrence of cases of zoonoses, they need to be confirmed by analysis of flagellar antigens of the bacteria wherever possible. This at any rate was the view until a few years ago (5). Additionally, according to current knowledge, it would seem that identification and knowledge of specific chromosomal or extra-chromosomal biochemical markers (*e.g.*, sugar reactions, drug resistance, phage sensitivity) of the isolated organisms may materially contribute to refinements of the existing technique of tracking source and origin of zoonoses (33, 34).

Accordingly, as a first step, biochemical characters of the 137 serologically identified strains of *E. coli*, locally isolated from cases of gastroenteritis of animals, were studied. Among these 65 (42%) strains belonged to seven of

12 'O' antigen groups to which belong serotypes that are commonly associated with human cases of gastroenteritis (27). Only one human pathogenic serotype (O26:B6:NM) consisting of two strains was completely identified in faeces of calf and goat. If the knowledge of H antigens of all the 65 strains were available, perhaps a larger number of 'human' pathogenic serotypes could have been identified. It was of interest to note that of the seven human 'O' antigen groups detected, only three, namely, O26, O119, and O125, predominated, accounting for as many as 52 of the 65 strains.

Apart from these seven human 'O' groups, 31 other 'O' groups, comprising 72 strains (52.5%), were identified (Table 10). This gives an incidence ratio of one human 'pathogenic' to about four 'non-human' pathogenic 'O' antigenic groups (Tables 7 and 10). EWING (5) observed in the course of a similar survey and study of 139 cultures of *Escherichia coli* from animal sources that belonged to 12 common human pathogenic 'O' antigen groups, only 38 (27.3%) were serotypes that are known to occur in association with diarrhoeal diseases in infants suggesting that majority of the cultures from animal sources studied by him were serotypes not known to be commonly associated with diarrhoea in human or about which there is very little information regarding such an association.

It was of interest to note that in our studies human 'O' group 86 was absent (Table 8) in goats and kids, and O126 from buffaloes and buffalo calves. In addition, several 'O' antigen groups that occurred in buffaloes were not present in cows, and *vice versa*, even though about the same number of cultures were examined from each of the two species of animals. There is, therefore, a suggestion in these findings, that certain pathogenic 'O' groups may be closely associated with particular forms of the disease or the species of animals that suffer from it. Greater proportion of human 'O' groups was obtained from cows and calves than from other species.

In quest of biotypes within a serotype, most workers seem to have taken into consideration the ability (or inability) of *E. coli* strains to ferment one or more substrates (notably carbohydrates). There is no general agreement or unanimity in the choice as to the minimum number, or types of substrates, that should be used for tests to identify biotypes of *E. coli* within a serotype. For instance, only sucrose, sorbose, and salicin were employed by MANSSON (16); sucrose, maltose, dulcitol, salicin, rhamnose, and adonitol by WRIGHT and VILLANUEVA (19); saccharose and salicin by SHIRLAW (22); sorbitol by RAPPAPORT and HENIG (15); sorbitol, dulcitol, salicin, and adonitol by KAUFFMANN and ØRSKOV (35); adonitol, dulcitol, inositol, rhamnose, salicin, saccharose, and xylose by KAUFFMANN and PERCH (1); and only sucrose by CHARTER and TAYLOR (17).

In the present study, in order to facilitate detection of maximum number of biotypes by the use of a minimum number of fermentation tests, only those substrates were considered suitable for inclusion in such tests which, on examination, were found to give variable reactions (Table 2), *i.e.*, be de-

graded (acid and gas production) by at least 10% but not more than 90% of the strains. Such substrates were rhamnose, raffinose, salicin, dulcitol, sucrose and starch. By use of the above battery of tests it became possible to detect 28 different biochemical types or biotypes from among the 137 strains, classified into 38 'O' groups. More than one biotype could be detected within a single serotype. Also, within a single biotype (biotype VI) all the seven human enteropathogenic 'O' antigen groups (O26, O55, O86, O112, O119, O125, and O126) were represented. It may be noted that, whereas nearly all (30 out of 32) starch-splitting strains were pathogenic (known to be associated with gastroenteritis of man or animals), all pathogenic strains were not starch splitting.

Biotypes of *E. coli* which predominated are: I (no substrates degraded), V (negative for raffinose), VI (negative for starch), XI (negative for salicin and starch), XVIII (negative for sucrose, raffinose, and starch), and XX (negative for salicin, sucrose, and starch). These biotypes accounted for nearly 60% of the 153 strains. Biotype VI predominated in cows and calves, but XX in buffaloes and their calves, even though almost the same number of strains were examined in two species of animals. Biotype VI also predominated in goats and kids.

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