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Citrobacter koseri. II. Serological and biochemical examination of *Citrobacter koseri* strains from clinical specimens

By B. ROWE, R. J. GROSS and H. A. ALLEN
Salmonella and Shigella Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT

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SUMMARY

165 strains of *Citrobacter koseri* isolated from clinical specimens were studied and their biochemical reactions determined. They were examined serologically by means of a scheme consisting of 14 O antigens. The sources of the clinical specimens were tabulated and the epidemiological information was summarized. The clinical significance of these findings is discussed.

INTRODUCTION

Bacterial strains variously described as *Citrobacter koseri* (Frederiksen, 1970), *Citrobacter diversus* (Ewing & Davis, 1972) and *Levinea malonatica* (Young, Kenton, Hobbs & Moody, 1971) should be regarded as members of a single species (Gross & Rowe, 1974). In this publication the name *C. koseri* has been used for convenience only, pending an agreement on the nomenclature of these organisms.

In most genera within the family *Enterobacteriaceae*, serotyping has been invaluable for the precise identification of strains required in epidemiological investigations. An antigenic scheme for *C. koseri* has been described (Gross & Rowe, 1974) which initially consisted of seven O antigens and was subsequently expanded to 14 O antigens (Gross & Rowe, 1975).

The present study involves the examination of 165 strains from clinical sources. Their biochemical reactions and somatic O antigens have been determined and the clinical and epidemiological significance of these findings is discussed.

MATERIALS AND METHODS

Strains

The sources of 165 strains isolated from clinical specimens are shown in Table 1.

Biochemical tests

The biochemical reactions of the strains were determined by the methods of Cowan & Steel (1965).

Serological tests

The strains were identified serologically using the methods described previously (Gross & Rowe, 1974, 1975).

Table 1. *Results of serological examination of* 165 *strains of* C. koseri

Source of strains	Number of strains with *C. koseri* O antigen															
	O1	O2	O3	O4	O5	O6	O7	O8	O9	O10	O11	O12	O13	O14	O rough	Total
Faeces	49	18	8	—	11	1	3	—	—	2	—	—	1	1	2	96
C.S.F.	2	8	1	—	—	—	7	—	—	—	—	—	—	—	—	18
Blood	4	2	—	—	—	—	—	1	—	—	—	—	—	—	—	7
Urine	1	—	—	1	1	—	—	—	1	—	—	—	—	—	2	6
Sputum	2	—	—	—	—	—	1	—	—	—	—	—	—	—	—	3
Others	12	7	—	2	6	1	2	—	1	1	1	1	—	—	1	35
Total	70	35	9	3	18	2	13	1	2	3	1	1	1	1	5	165

Table 2. *Biochemical reactions of* 165 *strains of* C. koseri

Test	Number of strains positive	% Strains positive	Sign
Motility (Craigie tube)	162	98·2	+
Methyl-red test: 37° C. (2 days)	165	100	+
20° C. (5 days)	165	100	+
Voges–Proskauer reaction 37° C. (2 days)	0	0	−
20° C. (5 days)	0	0	−
Simmons citrate	160 (3)	97·0 (1·0)	+
Malonate	162 (3)	98·2 (1·0)	+
Growth in potassium cyanide	0	0	−
Indole (Kovac's)	165	100	+
H_2S (T.S.I.)	0	0	−
Gluconate	0	0	−
Christensen's urea	93 (52)	56·4 (31·5)	d
Phenylalanine deaminase	0	0	−
Gelatin (stab)	0	0	−
Arginine decarboxylase	44 (121)	26·7 (73·3)	d (+)
Lysine decarboxylase	0	0	−
Ornithine decarboxylase	163 (2)	98·8 (1·2)	+
βGalactosidase (O.N.P.G.)	165	100	+
Glucose (acid)	165	100	+
Glucose (gas)	165	100	+
Lactose	1 (154)	0·6 (93·3)	−(+)
Mannitol	165	100	+
Sucrose	124	75·1	d
Salicin	1 (164)	0·6 (99.4)	−(+)
Dulcitol	97	58·8	d
Inositol	0	0	−
Adonitol	165	100	+
Raffinose	0	0	−
Sorbitol	160 (4)	97·0 (2·4)	+
Arabinose	165	100	+
Rhamnose	165	100	+
Xylose	164 (1)	99·4 (0·6)	+
Trehalose	165	100	+
Inulin	0	0	−
Glycerol	164	99·4	+
Cellobiose	165	100	+
Sorbose	0 (154)	0 (93·3)	−(+)
Maltose	165	100	+

Figures and signs in parentheses indicate reactions delayed beyond 24 hr. incubation.
+ 90 % or more positive.
(+) 90 % or more positive after more than 24 hr. incubation.
d, between 10 % and 90 % positive.
− less than 10 % positive.

RESULTS

Biochemistry

The strains gave the general biochemical reactions of the family *Enterobacteriaceae*. Their additional reactions (Table 2) were those of *C. koseri* as described by Frederiksen (1970) and Gross & Rowe (1974).

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Serology

Five strains were autoagglutinable in saline and were therefore classified as O rough. The remaining strains possessed O antigens included in the antigenic scheme (Table 1).

Epidemiology

The most common serogroups from all sources were O1 and O2, although many of the faecal strains in each of these groups were epidemiologically related. Nevertheless *C. koseri* O1 was isolated from a variety of sources and may prove to be the most common type in the United Kingdom.

Of the isolates studied, 58% were from human faeces; in most of these cases the patients were free from symptoms but strains of *C. koseri* from cases of enteritis in young adults were also examined. An outbreak of diarrhoeal disease in students in a University hostel occurred during February 1974 (Communicable Diseases Scotland, 1974) and affected a total of 120 patients. Bacterial strains from 80 of these were initially reported as *Shigella boydii* 7 because of their reaction with *Sh. boydii* 7 antiserum. Because only one strain of this serotype had been received at the Salmonella and Shigella Reference Laboratory from sources within the United Kingdom during the two-year period 1972–3, the authors examined representative strains for confirmation. Subsequently eleven strains were examined and all proved to be *Citrobacter*. Eight were found to be *C. koseri* O5, one was *C. koseri* O6, one was *C. koseri* O13 and one was *C. freundii*. A serological cross-reaction between *Sh. boydii* 7 and *C. koseri* O5 has been described (Gross & Rowe, 1974). This is an example of the widespread sharing of antigens among the *Enterobacteriaceae* and emphasizes the need for accurate biochemical characterization of all strains. Further strains were not available for study but the evidence suggests that a large proportion of the patients were excreting *C. koseri* O5 at the time of their diarrhoea.

Strains were also examined from blood culture in cases of septicaemia and from cerebro-spinal fluid (C.S.F.) in cases of meningitis. Three outbreaks of neonatal meningitis were known to occur and provided strong evidence of a pathogenic role for *C. koseri*.

Outbreak 1

Four cases of meningitis, two of which were fatal, occurred in a premature baby unit (Gross, Rowe & Easton, 1973). Further examination of the organisms isolated from C.S.F. showed that three cases were due to *C. koseri* O2 and one to *C. koseri* O7. *C. koseri* was not isolated from any other source in the hospital but an organism which proved to be *C. koseri* O7 was isolated two years later from the antral wash-out of a nurse who had been a member of the theatre staff at the time of the outbreak.

Outbreak 2

Four cases of meningitis occurred in a special care baby unit (Gwynn & George, 1973) and only one of those affected made a full recovery. The organisms isolated from C.S.F. proved on further examination to be *C. koseri* O2. Biochemically and serologically similar strains were isolated from the faeces of a number of unaffected babies in the same unit. A further case of meningitis occurred in another hospital in the same city but in this case the strain isolated from blood culture proved to be *C. koseri* O8.

Outbreak 3

Two babies in a special care unit died of meningitis and septicaemia. The organisms isolated from blood and C.S.F. proved to be *C. koseri* O1 and this serogroup was also isolated from the faeces of many other babies in the same unit. A further patient developed meningitis several months later and *C. koseri* was again isolated.

DISCUSSION

The biochemical reactions of *C. koseri* are similar to those of other members of the Enterobacteriaceae, particularly to those of *Enterobacter*, *Escherichia coli* and *C. freundii*. Strains of *C. koseri* can be distinguished from *Enterobacter* by tests for growth in potassium cyanide medium (KCN), oxidation of gluconate and hydrolysis of gelatin and by their Voges–Proskauer reaction; *C. koseri* gives negative results in all these tests while strains of *Enterobacter* may be positive in all or some of them. Strains of *E. coli* fail to utilize Simmon's citrate or malonate and they differ from *C. koseri* in both these respects. Strains of *C. koseri* can be distinguished most readily from *C. freundii* by their ability to ferment adonitol, an ability invariably lacking in strains of *C. freundii* and, in addition, by their failure to grow in KCN or to produce H_2S from triple sugar iron agar (TSI). Bacteriologists should be alert to the possibility of incorrect classification.

Further information is needed to assess the clinical importance of *C. koseri*, although it seems clear that these organisms occur frequently in clinical specimens. Ewing & Davis (1972) studied 137 strains from human material, but details were not given concerning the site of infection. Frederiksen (1970) described 30 strains, one of which was from C.S.F., ten from blood cultures and five from infected wounds. Young *et al.* (1971) examined 58 strains from human nose, urine, sputum and faecal specimens but none was from blood or C.S.F. Booth & McDonald (1971) studied 40 strains, mostly from urine, sputum and pressure sores, and suggested that the organisms act mainly as secondary invaders. Jones, Ragsdale, Kutscher & Sanford (1973) described 15 strains isolated from patients in a single hospital and considered that these strains were usually commensal or saprophytic, although they were capable of causing disease, especially in the compromised host.

The present study shows that *C. koseri* is an inhabitant of the human intestine. Like *E. coli* it can be isolated from the faeces of healthy persons, and may be

disseminated in a hospital ward by person to person spread. Further study is needed to establish the role of *C. koseri* in diarrhoea; strains have been isolated from sporadic cases but such information is difficult to evaluate. Nevertheless, the finding of *C. koseri* O5 in so many cases of diarrhoea in an outbreak in a students' hostel suggests that this serogroup may cause enteritis. Unfortunately in this outbreak faecal specimens from healthy students were not cultured.

C. koseri can be a cause of septicaemia and meningitis. In this study all the strains from blood and C.S.F. came from infants; some were sporadic cases but several outbreaks occurred in babies in hospital units. These organisms may spread extensively within such units and deserve special attention as a cause of neonatal meningitis. *C. koseri* must be regarded as potentially dangerous whenever it is found in hospital units, particularly those housing young babies.

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