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Citrobacter koseri (syn. *C. diversus*): biotype, serogroup and drug resistance patterns of 517 strains

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SUMMARY

The names *Citrobacter koseri* and *C. diversus* are synonyms for a species of enterobacterium with a particular ability to cause neonatal meningitis. 517 strains belonging to this species were examined using biotyping and serotyping techniques. 40 % of the strains belonged to serogroups O2 and O1 and 72 % belonged to biotypes d and a. Strains isolated from cerebrospinal fluid belonged to several different serogroups and biotypes but serogroups O2 and O3 and biotypes d and e were the most common. All the strains were resistant to ampicillin, 42 % were resistant to neomycin/kanamycin and 38 % were resistant to cephaloridine. 37 % of the strains were resistant to three or more drugs.

INTRODUCTION

Frederiksen (1970) examined a collection of 30 strains belonging to the genus *Citrobacter*, but differing in several biochemical tests from *C. freundii*. He considered that these strains should be regarded as a new species and proposed the name *C. koseri* for them. Booth & McDonald (1971) examined 40 strains which were biochemically similar to those described by Frederiksen and also proposed that they should be regarded as a new species. Young *et al.* (1971) studied 108 strains and proposed the recognition of a new genus, *Levinea*, having two species, *L. malonatica* and *L. amalonatica*. The biochemical reactions of *L. malonatica* were similar to those of *C. koseri*. Ewing & Davis (1972) described a strain which was biochemically similar to *C. koseri* but they considered that the name *C. diversum* (Werkman & Gillen, 1932) was an earlier synonym but required a change to *C. diversus* for grammatical reasons. Representative strains from all these authors were examined biochemically and serologically by Gross & Rowe (1974) and they were all shown to belong to a single species. This was confirmed by Crosa *et al.* (1974) and Sakazaki *et al.* (1976). There is still no agreement on the proper name for the species and the names *C. koseri* and *C. diversus* are both included in the Approved List of Bacterial Names (Skerman *et al.* 1980).

Serogrouping and biotyping schemes have been described for *C. koseri* (Gross & Rowe, 1975; Gross *et al.* 1981; Richard, Brisou & Lioult, 1972). We now report the results of the application of these typing methods in a study of 517 strains of *C. koseri*.

Table 1. *Source of* 517 *isolates of* Citrobacter koseri

Faeces or rectal swab	251
C.S.F. or brain	46*
Respiratory tract, nose and throat	44
Urine	25
Wound	19
Blood	17
National Collection of Type Cultures	9
Vaginal swab	8
Skin, hands	5
Food	4
Humans, other sources or not known†	86
Animal	3
Total	517

* Isolated from 41 patients with meningitis.

† Includes nine isolates from various specified human clinical specimens and 77 isolates of human origin where the nature of the specimen was not specified.

Table 2. *Outbreaks of* C. koseri *meningitis in infants*

Location	No. cases	Serogroup	Biotype	Reference
U.K., Slough	3	O2	d	Gross, Rowe & Easton, 1973
U.K. Birmingham	4	O2	e	Gwynn & George, 1973
U.K., Manchester	3	O1	d	Ribeiro, Davis & Jones, 1976
U.S.A., Chicago	2	?	?	Vogel, Ferguson & Gotoff, 1978
U.S.A., Connecticut	2	?	?	Center for Disease Control, 1979
U.S.A., Florida	5	O2	d	Graham *et al.* 1981

MATERIALS AND METHODS

Bacterial strains

The 517 strains studied were isolated during the period 1971–81. Most strains were isolated from human clinical specimens as shown in Table 1; 196 were isolated in the United States, 188 in the United Kingdom, 80 in Venezuela and 28 in Israel. Strains from four published outbreaks of neonatal meningitis were included (Table 2).

Biochemical tests

The strains were identified as *C. koseri* (*C. diversus*) as described by Frederiksen (1970) and Ewing & Davis (1972). Standard biochemical test methods were used (Cowan, 1974).

Biotyping

The strains were assigned to biotypes based on the fermentation of dulcitol, rhamnose, sucrose and sorbose (Richard, Brisou & Lioult, 1972).

Table 3. *Serogroups and biotypes of* 517 C. koseri *strains*

	Biotype						
Serogroup	a	b	c	d	e	NT*	Total
O1	39	—	7	98	4	—	148
O2	11	1	—	118	30	—	160
O3	*3*	—	—	*11*	*23*	*1*	*38*
O4	4	—	—	*1*	7	—	*12*
O5	*25*	—	—	*1*	*3*	*1*	*30*
06	8	—	1	2	—	—	11
07	3	—	20	1	—	—	24
O8	3	—	—	—	—	—	3
O9	1	—	1	—	1	—	3
O10	7	—	—	—	1	—	8
O11	—	—	9	—	—	—	9
O12	4	—	4	3	4	—	15
O13	3	1	—	—	—	—	4
O14	4	—	1	—	1	—	6
O15	—	—	3	—	—	—	3
O16	1	—	1	5	—	—	7
O17	1	—	—	—	—	—	1
O?	8	2	5	1	5	—	21
O rough	3	—	2	5	4	—	14
Total	128	4	54	246	83	2	517

* Two strains were lost before biotyping could be done.

Serotyping

The strains were tested for agglutination in antisera for *C. koseri* O groups 1–17 using previously described methods (Gross & Rowe, 1975; Gross *et al.* 1981).

Drug resistance testing

All strains isolated from cerebrospinal fluid or post mortem brain specimens and all other strains isolated between 1974 and 1981, a total of 374 strains in all, were tested for resistance to 12 antimicrobial drugs. The methods used were those of Anderson & Threlfall (1974). Resistance to ampicillin, cephaloridine, cephalexin, chloramphenicol, gentamicin, neomycin/kanamycin, streptomycin and tetracyclines was tested by a strip diffusion method. Resistance to sulphonamides, trimethoprim, furazolidone and nalidixic acid was tested by an agar dilution method.

RESULTS

Biochemical tests

All the strains gave the biochemical reactions of *C. koseri*. We have previously described in detail the reactions of 165 strains (Rowe, Gross & Allen, 1975) and we shall not attempt to give details of the 517 strains examined here.

Biotyping and serotyping

The biotypes and serogroups of the strains are shown in Table 3. Serogroups O2 and O1 were by far the most common making up 40% of all strains. Biotypes d

Table 4. *Serogroups and biotypes of* 41 C. koseri *from C.S.F*

	Biotype					
Serotype	a	b	c	d	e	Total
O1	0	0	0	3	0	3
O2	0	0	0	10	6	16
O3	0	0	0	6	7	13
O4	0	0	0	0	2	2
O7	2	0	2	0	0	4
O8	2	0	0	0	0	2
O11	0	0	1	0	0	1
Total	4	0	3	19	15	41

Table 5. *Drug resistance of* 374 *isolates of* C. koseri

	No. resistant	%
Ampicillin (A)	374	100
Chloramphenicol (C)	6	2
Neomycin/Kanamycin (K)	158	42
Streptomycin (S)	67	18
Sulphonamides (Sm)	68	18
Gentamicin (G)	65	17
Tetracyclines (T)	7	2
Furazolidone (Fu)	0	0
Nalidixic acid (Nx)	10	3
Trimethoprim (Tm)	1	0
Cephaloridine (Ce)	141	38
Cephalexin (Cx)	6	2
Total tested	374	100

and a were by far the most common and included 72% of the strains. For comparison the biotypes and serogroups of 41 strains from C.S.F. are shown in Table 4. Among these strains serogroups O2 and O3 and biotypes d and e were the most common. The serogroups and biotypes of strains from four published outbreaks of neonatal meningitis are shown in Table 2.

Drug resistance tests

The incidence of drug resistance among 374 strains of *C. koseri* is shown in Table 5. All strains were resistant to ampicillin, 42% were resistant to neomycin and 38% were resistant to cephaloridine. Three patterns of resistance predominated; 51% of strains were resistant to ampicillin only, 18% were resistant to ampicillin, cephaloridine and neomycin, and 16% were resistant to ampicillin, cephaloridine, gentamicin, neomycin, streptomycin and sulphonamides (Table 6).

DISCUSSION

The epidemiological deductions that can be made from these studies are limited by the availability of information concerning the patients. For example the age

Table 6. *Drug resistance patterns of* 374 *isolates of* C. koseri

A	192	A Nx Su	1
A Ce	6	A Ce Cx Nx	1
A G	1	A Ce Cx Su T	1
A K	25	A Ce K S Su	1
A Nx	7	A C Ce S Su T	1
A T	3	A C K S Su T	1
A C K	1	A Ce G K S Su	59
A Ce K	67	A C Ce Cx G K S	1
A Ce Tm	1	A C Ce G K S Su	1
A Cx Nx	1	A C Ce Cx G K S Su	1
A G S	1	A Ce Cx G K S Su T	1
		Total strains tested	374

and sex of the patients were frequently not obtained. Nevertheless it is clear both from the literature and from the present study that *C. koseri* is an important cause of neonatal meningitis. We were able to find six published descriptions of hospital outbreaks of neonatal *C. koseri* meningitis in Britain and the United States and strains from four of these incidents were included in the study (Table 5). Graham and his colleagues (1981) suggested on the basis of their study of one outbreak that strains of *C. koseri* O2 biotype d might have a particular ability to cause meningitis. The present study suggests that although strains of this serogroup and biotype might be the most common of *C. koseri* causing meningitis, strains of several other serogroups and biotypes may also be important. Several of the published studies showed that *C. koseri* caused intestinal colonization of infants in hospital and was able to spread from patient to patient, possibly by way of nurses hands. These findings suggest that measures to reduce such colonization might help to prevent the occurrence of meningitis among neonates in hospital.

Our finding of *C. koseri* in 25 urine, 19 wound and 17 blood cultures suggests that *this organism may resemble other members of the Enterobacteriaceae in its ability* to act as an opportunistic pathogen. In a recent report describing two cases of *C. koseri* urinary tract infection it was suggested that *C. koseri* might be an important primary cause of urinary tract infection in infants (Barton & Walentik, 1982). Unfortunately we were able to discover the ages of only seven of the patients with *C. koseri* urinary tract infection in the present study and all seven were adults. Further studies are required to establish the role of *C. koseri* in urinary tract infection.

Our drug resistance findings agree substantially with those of other workers in that *C. koseri* strains appeared to be invariably resistant to ampicillin. Holmes *et al.* (1974) and Southern & Bagby (1977) reported that most strains of *C. koseri* were resistant to ampicillin and sensitive to cephaloridine and suggested that drug resistance was valuable in distinguishing between *C. koseri* and *C. freundii*. In the present study, however, 38 % of strains were resistant to cephaloridine. Indeed, a high level of multiple resistance was found with 140 (37 %) strains being resistant to three or more drugs. Multiple resistance was particularly common among strains isolated in the U.S.A.

In conclusion, *C. koseri* has the ability to spread within hospital wards causing

intestinal colonization and creating a reservoir of infection. It causes neonatal meningitis and septicaemia as well as other forms of sepsis and it readily acquires multiple drug resistance. The isolation of *C. koseri* from the stools of infants in hospital should be regarded as a warning and measures should be taken to prevent further colonization.

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