Sensitivity of *Citrobacter freundii* and *Citrobacter koseri* to cephalosporins and penicillins

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SYNOPSIS An examination of 99 field and reference strains of *Citrobacter freundii* showed 79% of them to be resistant to cephaloridine and sensitive to carbenicillin, while 96% of 45 field and reference strains of *Citrobacter koseri* examined were sensitive to cephaloridine and resistant to carbenicillin. Susceptibility tests with these two antibiotics are therefore useful in separating the two species of *Citrobacter*.

Werkman and Gillen (1932) proposed the genus *Citrobacter* and included seven named species of which one was *C. freundii*, a species that today is well established while the other six failed to gain acceptance. Frederiksen (1970) proposed the name *Citrobacter koseri* for 30 strains that he studied which were closely related to *C. freundii* but differed sufficiently to warrant recognition as a new species. Booth and McDonald (1971) examined 40 similar strains and agreed with Frederiksen that these strains should constitute a new species of the genus *Citrobacter*.

Ewing and Davis (1972) considered *C. koseri* to be the same taxon as *C. diversus* (Werkman and Gillen, 1932) and in their view *C. koseri* is thus a junior synonym for *C. diversus*. The original description of *C. diversus* was, however, based on only two strains which were described as being non-motile, producing acid and gas from inositol, raffinose, and starch, not attacking dulcitol, and having an indeterminate methyl red reaction, characters that are seldom, if ever, possessed by strains of *C. koseri*.

Young, Kenton, Hobbs, and Moody (1971) proposed a new genus, *Levinea*, for bacteria of the *Citrobacter* group that fail to produce H₂S on triple sugar iron agar (TSI) and divided them into two species, *L. malonatica* (which corresponds to *C. koseri*) and *L. amalonatica*. Previously other strains of the *Citrobacter* group that failed to produce H₂S on TSI agar had been named *Padlewskia* by Macierewicz (1966); but strains of *Padlewskia* and *L. amalonatica* may be atypical strains of *Citrobacter freundii* that fail to produce H₂S on TSI agar and

produce indole, a biotype described by Ewing and Davis (1971). In the present paper we shall refer to reference strains of *C. diversus* and *L. malonatica* that we have examined as *Citrobacter koseri*.

The use of cephalosporins in the differentiation of members of the tribe *Klebsiellae* is well documented. Several authors (Fleming, Goldner, and Glass, 1963; Benner, Micklewait, Brodie, and Kirby, 1965; Koch and Rose, 1966; Ramirez, 1968; Poupard, Dewees, and Morton, 1970; Zabransky, Hall, Day, and Needham, 1969; Sanford, 1969) found strains of *Klebsiella* generally sensitive and strains of *Enterobacter* and *Serratia* resistant to one or other of the cephalosporins (cephalosporin C, cephaloridine, cephalothin). Toala, Lee, Wilcox, and Finland (1970) found their strains of *Enterobacter aerogenes* and *Enterobacter cloacae* resistant to cephalothin and cephaloridine, and Wilfert, Barrett, Ewing, Finland, and Kass (1970) found their strains of *Serratia* highly resistant to cephalosporins.

The use of cephalosporin C in the separation of *Citrobacter freundii* from strains of *Salmonella* has been described by Fleming, Charlebois, and Dunmore (1970) who found all 53 of their strains of *C. freundii* resistant to cephalosporin C while 350 strains of *Salmonella* were sensitive. Similarly Slocombe and Sutherland (1970) found that their 13 strains of *C. freundii* had minimal inhibitory concentrations of cephaloridine in excess of 12.5 µg/ml.

Booth and McDonald (1971) reported that their strains of *C. koseri* were sensitive to cephaloridine; Smith, Dayton, and Chirps (1973) found their strains of *Citrobacter diversus* sensitive to cephalothin and resistant to carbenicillin, and Jones, Ragsdale,

Kutscher, and Sanford (1973) found 93% of their strains of *C. diversus* were inhibited by 5 µg/ml or less of cephalothin whilst 95% of *C. freundii* strains tested were resistant to this concentration. Washington, Yu, and Martin (1970), however, found that most of their H₂S-negative *Citrobacter* strains were resistant to cephalothin (96%) and sensitive to carbenicillin (80%). The biochemical results they obtained for their 25 strains, however, suggest that although the strains are H₂S-negative they correspond more closely to *C. freundii* than *C. koseri*, as most strains (80%) failed to produce indole, all grew in KCN medium, 60% failed to utilize malonate, and none fermented adonitol. A high proportion (76%) of their strains decarboxylated ornithine which is unexpected for strains of *C. freundii*. If it is accepted that these strains described by Washington, Yu, and Martin (1970) are H₂S-negative variants of *C. freundii* then their antibiotic sensitivity results also accord with the findings of

other authors. Slifkin and Engwall (1969) found 93% of their H₂S-negative strains, which they classified as *Citrobacter intermedium* (Werkman and Gillen, 1932), sensitive to cephalothin. Possibly these strains belonged to the H₂S-negative biotype of *C. freundii* since they were able to grow in KCN medium and 83% were sensitive to ampicillin but as these strains were sensitive to cephalothin they were unlike the typical H₂S-negative strains of *C. freundii*.

Materials and Methods

Of the 144 strains examined in this study, 54 field strains of *Citrobacter freundii* and 36 of *Citrobacter koseri* were isolated in St Thomas' Hospital, London, over a period of three years. A further 22 field strains of *C. freundii* were received for identification by the Computer Trials Laboratory at Colindale. Thirty-two NCTC strains of *Citrobacter* were examined and included strains listed in the NCTC

Group	No. of Strains	Antibiotic Sensitivity			Biochemical Tests					Distribution of Strains				
		Cephalo-ridine	Carbenicillin	Ampicillin	Malonate	Arginine	Indole	Ornithine	H ₂ S (TSI)	No. Reference Strains	Designation	NCTC Number	No. Field Strains from St Thomas' Hospital	No. Field Strains from Other Sources
A	63	R	S	S	—	+	—	—	+ ¹	6	<i>C. ballerupensis</i> 7822 ¹	12	4	22
										1	<i>C. freundii</i> 6272			
										5	<i>C. ballerupensis</i> 7820	13	0	14
											<i>C. ballerupensis</i> 6021	4	9	18
											<i>C. freundii</i> 6269 ¹¹			
										0		1	4	5
										0		1	0	1
										0		1	0	1
										2	<i>C. freundii</i> 6266 ¹¹¹	0	0	2
										2	<i>C. freundii</i> 8581 ^{1v}	7	0	9
B	15	R	S	R	—	+	—	—	+	0		2	0	2
										1	<i>C. ballerupensis</i> 6848	1	0	2
										1	<i>C. freundii</i> 6271	0	0	1
										1	<i>C. freundii</i> 8165	0	0	1
										0		4	0	4
C	12	R	R	R	—	+	+	—	+	0		1	0	1
										2	<i>L. amalonatica</i> 10805 ^v	0	4	6
D	1	S	R	R	—	+	+	+	—	0		1	0	1
										1	<i>C. freundii</i> 6071	0	0	1
E	2	S	R	S	+	+	—	—	—	1	<i>C. freundii</i> 9094 ²	0	0	1
										0		1	0	1
F	5	S	S	S	—	+	—	—	+	0		1	0	1
										0		1	0	1
										0		2	0	2
										0		0	1	1
G	1	S	S	R	—	+	—	—	+	0		1	0	1
										0		1	0	1

Table I Biochemical and antibiotic resistance patterns of *Citrobacter freundii*^a

¹Most typical antibiotic sensitivity and biochemical pattern

²Possess *Escherichia coli* antigens

³All strains grow in KCN medium and do not ferment adonitol

⁴Also 7823, 7824, 7829, 8782

¹¹Also 9067¹, 9072¹, 9122¹

¹¹¹Also 6267

^{1v}Also 9750

^vAlso 10806

catalogue under *Citrobacter ballerupensis*, *C. freundii*, *C. diversus*, *C. koseri*, *Levinia malonatica*, and *L. amalonatica* (tables I and II).

All reference strains were tested in the Computer Trials Laboratory in 50 tests described previously (Bascomb, Lapage, Willcox, and Curtis, 1971). A smaller number of tests was performed on all the field strains, using the same methods, at St Thomas' Hospital. Sensitivities of the organisms to ampicillin, carbenicillin, cephalosporin C, and cephaloridine were assessed at St Thomas' Hospital by the determination of minimal inhibitory concentrations (MICs). Suitable concentrations of the antibiotics were incorporated in Diagnostic Sensitivity Test agar (Oxoid CM 261). Inocula consisted of approximately 10^4 organisms and the results were read after overnight incubation at 37°C . Strains were considered sensitive to ampicillin, carbenicillin, and cephaloridine if the MICs were $10\text{ }\mu\text{g/ml}$ or less and to cephalosporin C if the MICs were $100\text{ }\mu\text{g/ml}$ or less.

Results

CITROBACTER FREUNDII

Of 99 strains of *Citrobacter freundii*, 78 (79%) were resistant to cephaloridine, sensitive to carbenicillin, and either moderately sensitive or resistant to ampicillin (groups A and B, table I). This group of strains comprised 19 of 23 reference strains, 42 out of 54 field strains from St Thomas' Hospital, and 17 of the 22 field strains from elsewhere. Characteristic MICs for this typical pattern were greater than $100\text{ }\mu\text{g/ml}$ for cephaloridine, $1\text{--}25\text{ }\mu\text{g/ml}$ for carbenicillin, and $5\text{ to }50\text{ }\mu\text{g/ml}$ for ampicillin.

Twenty-one of the strains of *Citrobacter freundii*

showed different sensitivity patterns; 12 were resistant to all three antibiotics and of these four strains were biochemically typical and eight biochemically atypical (group C, table I).

A further three strains were biochemically atypical and showed the sensitivity pattern expected for *C. koseri* (groups D and E, table I), and five other strains were sensitive to all three antibiotics (group F, table I); one of these five strains was biochemically typical but the other four were biochemically atypical. The remaining strain was biochemically typical but sensitive to both cephaloridine and carbenicillin although resistant to ampicillin (group G, table I). Of these nine cephaloridine-sensitive strains, two had cephalosporin C MICs of $25\text{ }\mu\text{g/ml}$, two of $50\text{ }\mu\text{g/ml}$, and the remainder were resistant to cephalosporin C, with MICs of $100\text{ }\mu\text{g/ml}$ or more. The distribution of these strains is given in table I.

CITROBACTER KOSERI

Forty-three of 45 strains (96%) of *Citrobacter koseri* were sensitive to cephaloridine and resistant to carbenicillin and ampicillin. These comprised all the strains from St Thomas' Hospital and seven of nine reference strains. Characteristic MICs were $1\text{--}25\text{ }\mu\text{g/ml}$ for cephaloridine, greater than $100\text{ }\mu\text{g/ml}$ for carbenicillin, and $25\text{ to }100\text{ }\mu\text{g/ml}$ for ampicillin. Two of the reference strains had cephaloridine MICs of $25\text{ }\mu\text{g/ml}$ and were classed as resistant, but were sensitive to cephalosporin C and were typical in all other respects (table II).

Discussion

The findings substantiate the basic division of the genus *Citrobacter* into two species, *C. freundii* and *C.*

No. of Strains	Antibiotic Sensitivity			Biochemical Tests				H_2S (TSI)	Distribution of Strains					
	Cephaloridine	Carbenicillin	Ampicillin	Malonate	Arginine	Indole	Ornithine		No. Reference Strains	Designation	NCTC Number	No. Field Strains from St Thomas' Hospital	No. Field Strains from Other Sources	Total Strains
43	S	R	R	+	+	+	+	— ¹	6	<i>C. ballerupensis</i>	7229	34	0	40
										<i>C. koseri</i>	10770 ¹			
										<i>C. diversus</i>	10849			
										<i>L. malonatica</i>	10810 ¹¹			
				+	+	+	—	—	1	<i>C. koseri</i>	10769	0	0	1
				—	+	+	+	—	0			2	0	2
2	R ²	R	R	+	+	+	+	—	2	<i>C. koseri</i>	10768 ¹¹¹	0	0	2

Table II Biochemical and antibiotic resistance patterns of *Citrobacter koseri*³

¹Most typical antibiotic sensitivity and biochemical pattern

²Sensitive to cephalosporin C

³All strains ferment adonitol and do not grow in KCN medium

¹Also 10786

¹¹Also 10811

¹¹¹Also 10771

koseri, though individual strains may be difficult to allot to one or the other species by biochemical tests. Tolerance of KCN and acid production from adonitol show the best correlation (tables I and II). Tests for cephaloridine and carbenicillin resistance are additional aids for their separation, and our findings substantiate those of other workers.

The results also confirm that *Citrobacter diversus* (Ewing and Davis, 1972), the *Citrobacter* strains of Booth and McDonald (1971), the *Levinea malonatica* strains of Young, Kenton, Hobbs, and Moody (1971), and the *Citrobacter koseri* of Frederiksen (1970) can be considered to belong to the same taxon.

Occasional strains of both taxa may be resistant to both cephaloridine and carbenicillin and both reference strains of *Levinea amalonatica* that we examined fell into this category. One reference strain of *C. freundii* and five field strains from sources other than St Thomas' Hospital were biochemically identical to the two reference strains of *L. amalonatica* that were examined. This reference strain of *C. freundii* showed the sensitivity pattern expected for strains of *C. koseri* (group D, table I) and one field strain was sensitive to all three antibiotics (group F, table I); however, both were resistant to cephalosporin C. The remaining four strains were resistant to both cephaloridine and carbenicillin (group C, table I). If this dual resistance were observed in additional isolates conforming to *L. amalonatica* then this would be a further useful character in distinguishing *L. amalonatica* from *C. koseri*, and from *C. freundii* if it is sufficiently different from this organism to warrant specific status as *Citrobacter amalonatica*. Field strains of *Levinea amalonatica*, however, do not appear to be very common in Europe; the five included in this paper were all that we received among 170 strains of *Citrobacter* isolated in the UK and sent to the Computer Trials Laboratory for identification. Richard, Brisou, and Lioult (1972) also found only eight strains among 63 strains of the genus *Levinea* of European or African origin that they examined.

As the field strains from St Thomas' Hospital were isolated over a period of three years during which cross-infection appears to have been minimal, and field strains of *C. freundii* from other sources and reference strains of both taxa in general possess the same patterns of antibiotic resistance it does not appear likely that a single strain has been repeatedly investigated. The wide range of biochemical patterns observed would support this conclusion. However, typical strains may acquire resistance, presumably by resistance transfer, and this may account for the finding of occasional strains of *C. freundii* resistant to carbenicillin. Successful transfer of carbenicillin resistance to a strain of *Escherichia coli* from four

biochemically typical strains of *C. freundii* recently isolated from St Thomas' Hospital (in group C, table I) has been carried out by Slocombe and Sutherland (1974) who were unable, however, to transfer carbenicillin resistance from two of their own strains of *C. freundii* isolated some years ago.

The determination of ampicillin sensitivities did not help to distinguish *C. freundii* from *C. koseri* since *C. freundii* is usually only marginally more sensitive with MICs of the order of 10 to 25 µg/ml, whereas *C. koseri* has ampicillin MICs of 25 to 50 µg/ml. Similarly, the determination of cephalosporin C in addition to cephaloridine MICs contributed little. Five strains of *C. freundii* sensitive to cephaloridine were resistant to cephalosporin C with MICs of 100 µg/ml or more, but a further four cephaloridine-sensitive strains were also sensitive to cephalosporin C with MICs of 25 to 50 µg/ml. The two field strains of *C. koseri* with cephaloridine MICs of 25 µg/ml, and classed therefore as resistant, had cephalosporin C MICs of 50 µg/ml and were thus classed as sensitive. A typical result would therefore have been obtained for seven of these 11 strains by the use of cephalosporin C rather than cephaloridine, but the results would still have been atypical for the other four strains.

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