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Comparative In Vitro Susceptibilities of Eight *Enterobacter* Species, with Special Reference to *Enterobacter sakazakii*

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An agar dilution method was used to measure the MICs of 29 antimicrobial agents against *Enterobacter sakazakii*, *E. cloacae*, *E. aerogenes*, *E. agglomerans*, *E. amnigenus*, *E. gergoviae*, *E. intermedium*, and *E. taylorae* (formerly Enteric Group 19). *E. sakazakii* was the most susceptible species. Results showing resistance to ampicillin are likely to exclude *E. sakazakii*.

Enterobacter sakazakii has been described as a cause of neonatal meningitis (4, 8). An association with bacteremia has also been mentioned. A number of strains were isolated from clinical specimens, including stool, sputum, and superficial wounds, in which they probably represented colonization rather than infection. In addition it was cultured from the environment (4).

We decided to determine the activities of 29 antimicrobial agents against *E. sakazakii*, because the MICs of only a limited number of *E. sakazakii* isolates have been published (4, 8). The data obtained were compared with the susceptibilities of seven other *Enterobacter* species.

The drugs, species tested, and number of isolates are shown in Table 1. The combination of trimethoprim-sulfamethoxazole was tested at a ratio of 1:19.

E. sakazakii strains were obtained from J. J. Farmer, III, Centers for Disease Control, Atlanta, Ga., and E. Aldová, Institute of Hygiene and Epidemiology, Prague, Czechoslovakia, or were part of our collection.

The sources of 157 of 195 *E. sakazakii* isolates were known. Sources that occurred more than twice and numbers of strains were as follows: cerebrospinal fluid, 17; blood, 5; lower respiratory tract, 35; digestive tract, 31; dairy products and kitchen utensils, 21; superficial wounds, 12; upper respiratory tract, 9; urine, 9. *E. amnigenus*, *E. gergoviae*, *E. intermedium*, and *E. taylorae* strains were obtained from C. M. O'Hara, Centers for Disease Control. The other isolates were cultured from patients hospitalized in the St. Radboud University Hospital in Nijmegen.

E. cloacae, *E. aerogenes*, and *E. agglomerans* were identified with the API 20E system (API System S.A., La Balme les Grottes, France). *E. sakazakii* was identified with the API 20E system or the biochemical tests described by Edwards and Ewing (3); in addition to both systems, the production of yellow pigmented colonies on Trypticase (BBL Microbiology Systems, Cockeysville, Md.) soy agar at 25°C after 48 h, of extracellular DNase on toluidine blue agar at 36°C after 7 days, and of α-glucosidase at 36°C after 4 h (7) were tested. The other *Enterobacter* species were identified at the Centers for Disease Control (5). MICs were determined by an agar dilution method. The stock solutions of the drugs were prepared on the day of use. Each inoculum was applied to Mueller-Hinton agar (BBL) with an automatic multipoint inoculator and was prepared by diluting an overnight agar culture in Mueller-Hinton broth in such a way that a spot of broth contained 10^5 CFU. The MIC was the lowest concentration of antibiotic at which there was no growth, one discrete colony, or a fine, barely visible haze after incubation at 35°C for 16 to 20 h. The following control organisms were included on the plates: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853.

Table 1 shows the MICs required to inhibit 50 and 90% of the strains. *E. sakazakii* was quite susceptible to the agents tested, except cephalothin and sulfamethoxazole.

The MICs for 90% of strains tested of 25 agents were at least twofold lower for *E. sakazakii* than *E. cloacae*. This accentuates the differences in the two species. The results of the MICs for *E. gergoviae* are in good agreement with data obtained previously by a disk method (2), except that none of our isolates was resistant to nalidixic acid. This might be due to the relatively low number of strains that were tested.

The data for *E. taylorae* are also in accordance with those presented previously (6), except that susceptibility to polymyxins, as determined by a disk method (94%), was not confirmed in this study (MIC for 50% of strains tested, 128 μg/ml). The resistance that was found might be due to the high inoculum (10^5 CFU per spot) used, because determinations of MICs of these drugs are much affected by the size of the inoculum (9). However, *E. coli* ATCC 25922, which was used as a control, was susceptible (MIC, 1 μg/ml). The resistant *E. taylorae* strains found in this study were susceptible when tested by the same agar diffusion method as described previously (6). It has been noted that a zone of inhibition is only a rough indication of susceptibility to polymyxins; susceptibility must be confirmed by a dilution test, because polymyxins diffuse very poorly (1).

Table 2 shows that only 1 of 195 strains of *E. sakazakii* was inhibited by more than 8 μg of ampicillin per ml. This concentration corresponded to a zone diameter of 14 mm in the disk diffusion test recommended by the U.S. Food and Drug Administration and the National Committee for Clinical Laboratory Standards and is the breakpoint which separates susceptible and intermediately susceptible strains from each other (1). Results showing resistance to ampicillin are likely to exclude *E. sakazakii*. Inhibition by a high concentration of cephalothin only (256 to 512 μg/ml or more, which corresponds to the absence of a zone of inhibition in the disk agar diffusion test) is also likely to exclude *E.*

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TABLE 1. Antimicrobial susceptibility patterns of *Enterobacter* species

Drug	MICs (μg/ml) for:											
	E. sakazakii (195)[a]			*E. cloacae* (29)			*E. aerogenes* (25)			*E. agglomerans* (27)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
Ampicillin	0.25–>128	2	4	8–>128	>128	>128	8–>128	>128	>128	1–>128	32	>128
Cefaloridin	2–128	8	16	4–>128	>128	>128	4–>128	>128	>128	2–>28	4	>128
Cephalothin	2–>128	64	128	2–>128	>128	>128	2–>128	>128	>128	2–>128	16	>128
Cefamandole	≤0.125–>128	2	4	0.5–>128	8	>128	0.5–>128	4	>128	0.25–>128	2	>128
Cefoperazone	≤0.125–16	1	2	≤0.125–>128	1	16	≤0.125–>128	0.25	128	≤0.125–>128	0.5	128
Ceforanide	≤0.125–>128	1	2	0.25–>128	16	>128	0.25–>128	8	>128	0.25–>128	4	>128
Cefotaxime	≤0.03–0.5	0.125	0.125	≤0.03–16	0.25	8	0.06–32	0.125	0.5	≤0.03–64	0.25	32
Cefoxitin	0.5–>128	8	16	2–>128	128	>128	2–>128	>128	>128	2–>128	8	>128
Cefsulodin	2–>128	32	32	64–>128	128	>128	64–>128	128	>128	1–>128	64	>128
Ceftazidime	≤0.03–1	0.125	0.25	0.125–32	0.25	2	0.125–2	0.25	1	0.06–128	0.25	4
Ceftizoxime	≤0.125–1	≤0.125	≤0.125	≤0.125–128	≤0.125	8	≤0.125–128	≤0.125	0.25	≤0.125–>128	≤0.125	32
Ceftriaxone	≤0.03–0.5	0.06	0.125	≤0.03–16	0.25	8	≤0.03–64	0.06	0.5	≤0.03–128	0.25	32
Cefuroxime	0.25–32	4	8	2–>128	8	>128	4–>128	8	>128	1–>128	4	>128
Chloramphenicol	1–>128	8	16	4–>128	16	>128	4–>128	8	>128	2–>128	8	128
Ciprofloxacin	≤0.06–0.25	≤0.06	≤0.06	≤0.06–2	≤0.06	0.125	≤0.06–0.125	≤0.06	≤0.06	≤0.06–8	≤0.06	1
Doxycyclin	1–32	4	4	1–64	4	32	2–64	4	16	0.125–>128	2	128
Gentamicin	0.06–1	0.25	0.5	0.25–>128	0.5	8	0.5–32	0.5	32	0.25–4	0.5	1
Imipenem	≤0.06–2	0.125	0.25	≤0.125–2	0.5	1	≤0.125–4	1	2	0.125–4	0.5	0.5
Moxalactam	≤0.06–1	≤0.06	0.125	≤0.03–8	0.06	1	≤0.03–2	≤0.03	0.25	≤0.03–128	0.125	8
Nalidixic acid	1–16	4	4	2–>128	4	16	2–16	4	8	0.5–>128	4	64
Neomycin	0.125–32	1	2	1–2	1	1	0.5–128	1	2	0.5–8	1	2
Norfloxacin	≤0.06–1	0.125	0.125	≤0.06–4	≤0.06	0.5	≤0.06–0.5	0.125	0.25	≤0.06–32	≤0.06	2
Pipemidic acid	1–8	2	2	1–>128	2	8	2–8	2	2	1–>128	2	32
Piperacillin	0.125–8	2	2	1–>128	4	>128	1–>128	4	>128	0.5–>128	4	32
Polymyxin B	0.25–64	1	1	0.5–128	1	2	0.5–>128	1	2	0.5–4	1	1
Rifampin	2–16	8	8	16–32	32	32	16–64	32	64	8–128	32	64
Sulfamethoxazole	16–>128	>128	>128	64–>128	>128	>128	>128	>128	>128	>128	>128	>128
Trimethoprim	0.06–32	0.25	1	0.5–16	2	8	0.25–32	2	16	≤0.03–128	2	128
Trimethoprim-sulfa	0.25–32	2	4	2–32	8	32	2–64	4	32	0.5–>128	4	>128

[a] Number of strains is given in parentheses.

MICs (μg/ml) for:											
E. amnigenus (11)			*E. gergoviae* (11)			*E. intermedium* (10)			*E. taylorae* (10)		
Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
4–>128	8	8	1–>128	4	16	4–>128	8	128	8–>128	128	>128
4–>128	8	16	1–128	4	64	4–>128	128	>128	16–>128	>128	>128
64–>128	128	>128	2–128	16	128	32–>128	128	>128	128–>128	>128	>128
0.5–>128	2	16	0.25–16	2	8	1–32	2	32	2–64	8	64
≤0.125–32	0.5	2	≤0.03–4	0.125	1	0.125–4	0.5	4	≤0.125–2	0.25	0.5
2–>128	4	64	0.25–4	0.5	4	2–>128	8	128	8–>128	32	>128
0.06–16	0.125	0.5	≤0.03–1	0.125	0.25	0.06–2	0.125	0.5	0.125–0.5	0.125	0.25
4–>128	8	64	4–>128	8	128	8–>128	>128	>128	8–>128	>128	>128
32–>128	64	128	32–128	64	128	32–>128	32	128	64–>128	>128	>128
0.125–64	0.25	0.25	0.06–1	0.125	0.25	0.25–16	0.5	0.5	0.125–1	0.25	0.5
≤0.125–64	≤0.125	0.5	≤0.125–0.5	≤0.125	0.5	0.125–4	0.125	2	≤0.125–0.5	0.25	0.25
0.06–16	0.125	0.5	≤0.03–0.25	0.06	0.125	0.125–0.5	0.25	0.5	0.06–0.5	0.125	0.25
2–>128	8	128	2–32	8	16	4–>128	8	64	8–>128	16	>128
2–16	4	16	4–>128	8	32	8–16	8	16	4–16	8	8
≤0.06	≤0.06	≤0.06	≤0.06–0.125	≤0.06	≤0.06	≤0.06–0.125	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06
1–8	2	4	0.5–32	4	8	1–4	2	4	2–8	4	4
≤0.06–0.5	0.25	0.5	0.125–16	0.25	2	0.25–1	0.5	0.5	0.125–0.5	0.5	0.5
0.125–4	0.5	1	0.25–2	0.5	0.5	0.25–2	0.5	2	≤0.06–4	2	2
0.125–32	0.125	2	≤0.06–1	0.125	0.25	0.125–32	0.125	4	≤0.06–1	0.25	1
2–8	2	4	2–16	4	16	2–8	4	8	4–8	4	8
0.25–2	1	1	0.25–16	1	8	1–8	2	4	0.5–4	1	2
≤0.06–0.25	≤0.06	0.25	≤0.06–0.5	≤0.06	0.125	≤0.06–0.25	0.125	0.25	≤0.06–0.25	≤0.06	0.125
0.5–8	1	4	1–16	2	4	2–4	2	4	2–4	2	4
1–2	1	2	0.5–>128	1	4	2–8	2	4	0.25–2	2	2
0.5–64	1	1	0.5–2	1	1	0.5–128	1	2	1–>128	128	>128
8–32	16	32	8–32	16	16	16–32	16	32	8–32	16	32
>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
0.5–2	1	2	0.25–8	0.5	4	0.125–2	0.25	0.5	0.5–8	1	2
2–8	2	4	0.5–8	2	4	0.5–4	1	2	2–8	4	4

TABLE 2. Differentiation of 195 strains of *E. sakazakii* and 111 strains of *E. cloacae* by differences in MICs of ampicillin and cephalothin

MIC (μg/ml)	No. of strains inhibited by the following drugs:			
	Ampicillin		Cephalothin	
	E. sakazakii	*E. cloacae*	*E. sakazakii*	*E. cloacae*
0.25	2			
0.5	1			
1	14			
2	114	2	1	
4	55	2		1
8	8	5	5	
16		3	13	2
32		3	57	1
64		13	81	4
128	1	16	36	5
>128		67		
256			1	7
512			1	10
1,024				17
2,048				27
>2,048				37

sakazakii. These observations are in agreement with previous data published by Farmer et al. (4).

Susceptibility testing of 50 *E. sakazakii* and 50 *E. cloacae* strains by the National Committee for Clinical Laboratory Standards disk diffusion test (data not shown) confirmed the presumptions based on the agar dilution method. The use of one of these characters (resistance to ampicillin or growth around a cephalothin disk) is recommended to exclude *E. sakazakii* and restrict the number of times that additional steps for the identification of *E. sakazakii* are needed.

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