Citrobacter diversus Isolated from Clinical Material

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Forty-seven strains of *Citrobacter diversus* were isolated during a 12-month period from clinical material obtained from patients in a general hospital in Israel. The majority of cultures (38) were recovered from urine and wound discharges. There was one case of septicemia. The biochemical reactions of all cultures were typical for this species, except one that was anaerogenic, and they could be divided into five biotypes (a through e). Thirty-nine strains were identified serologically and found to belong to one of seven O groups described by Gross and Rowe (1974). All cultures were sensitive to chloramphenicol, kanamycin, gentamicin, polymyxin B, and nitrofurantoin, and most were sensitive to tetracycline and nalidixic acid. All strains were resistant to carbenicillin and ampicillin and produced beta-lactamase.

Of the large group of "atypical" *Citrobacter* strains that are encountered in the clinical laboratory, strains designated by different authors as *Citrobacter koseri* (4), *Levinea malonatica* (10, 11, 14), or *Citrobacter diversus* (3) seem to have emerged as a well-defined species. Although bacteria with the characteristic features of this new species had attracted our attention several years before their final classification, it was only since June 1973 that a systematic search for such cultures was carried out in our laboratories. The results of this survey are presented in this report.

MATERIALS AND METHODS

Initial isolations were made on MacConkey agar at 37°C. Cultures that were indole positive, Simmons citrate positive, oxidase negative, and phenylalanine negative were further tested for all reactions listed in Table 2. Patients considered to have urinary infections due to *C. diversus* had colony counts in excess of 10^5 organisms/ml. Biochemical tests were performed according to methods outlined by Edwards and Ewing (2). Beta-lactamase production was tested by iodometric assay (9) and by the capillary tube method described by Rosen et al. (12). Serological examinations were carried out with O sera (O1 to O7) kindly provided by B. Rowe, Central Public Health Laboratory, London, and with absorbed sera O1 to O4. Antimicrobial sensitivity tests were performed by the method of Kirby et al. (1). Minimal inhibitory concentrations (MIC) were measured by the tube dilution method in brain heart infusion with an inoculum of 10^4 organisms. Results were read after 18 h at 37°C.

Source of strains. Forty-seven strains of *C. diversus* were isolated during a 12-month period from clinical specimens obtained from 38 patients (31 adults and 7 children). Sources of isolations are pre-

sented in Table 1. Most of the strains were isolated as pure cultures from urine of patients with infections of the urinary tract. From one patient with chronic infection, two cultures were obtained at different occasions, and from another the culture was first isolated from urine and then from an infected Foley catheter. From a woman with carcinoma of the bladder and sepsis, two strains were isolated from urine and one was isolated from blood. Multiple isolates from the same patient always belonged to the same sero- and biotype and had identical sensitivity patterns.

Eighteen strains were isolated from pus or wound discharges of 15 patients, some of them with postoperative wound infections. In six patients we tried to establish the presence of *C. diversus* in the intestinal flora. In four patients strains identical to the isolates from wounds according to biotype and serotype were recovered from stool cultures. The remaining strains were isolated from discharge from ears of two patients with otitis externa and one woman with vaginal discharge. *C. diversus* from wound and other discharges grew usually in mixed cultures together with gram-negative bacteria or cocci.

RESULTS

Biological and biochemical characteristics. All 47 strains were gram-negative rods and all except one were motile. They grew on simple media and on MacConkey agar, were oxidase negative, reduced nitrate to nitrite, did not produce hemolysin, and had a strong, fetid odor. All except one culture produced gas from glucose, adonitol, and glycerol. Their biochemical characteristics (Table 2) agree with the findings of other authors (7, 10, 11, 13).

Richard (10) proposed six biotypes of *C. diversus* (a to f) based on fermentation of dulcitol,

TABLE 1. Sources of isolation of 47 strains of *Citrobacter diversus*

Source	No. of strains
Blood	1
Urine	20
Urinary catheter	1
Pus and wound discharge	18
Discharge from ear	2
Vaginal discharge	1
Stools	4

TABLE 2. Biochemical reactions of 47 strains of *Citrobacter diversus*

Test	No. of strains reacting:		
	Positive	Late positive ^a	Negative
Methyl red (37 C)	47	0	0
Voges-Proskauer (37 C)	0	0	47
Growth in KCN	0	2	45
Utilization of:			
Citrate	47	0	0
Malonate	47	0	0
Production of:			
Indole	47	0	0
H ₂ S on triple sugar iron agar	0	0	47
Beta-galactosidase	47	0	0
Urease	14	23	10
Phenylalanine deaminase	0	0	47
Arginine dehydrolase	0	47	0
Lysine decarboxylase	0	0	47
Ornithine decarboxylase	47	0	0
Gelatinase	0	0	47
Reduction of NO ₃ to NO ₂	47	0	0
Fermentation of:			
Glucose, acid	47	0	0
Glucose, gas	46	0	1
Adonitol	47	0	0
Arabinose	47	0	0
Dulcitol	16	0	31
Glycerol	47	0	0
Inositol	0	39	8
Lactose	0	47	0
Maltose	47	0	0
Mannitol	47	0	0
Raffinose	0	0	47
Rhamnose	47	0	0
Salicin	0	47	0
Sorbitol	47	0	0
Sorbose	1	3	43
Sucrose	23	9	15
Trehalose	47	0	0
Xylose	47	0	0

^a Positive reaction after 3 or more days.

sucrose, sorbose, and rhamnose. All our cultures could be divided accordingly and were found to belong to biotypes a to e (Table 3). The most frequent ones were a, e, and d.

Serological characteristics. Gross and Rowe (5) have identified seven O groups of *C. diver-*

sus. Of the 47 cultures examined by us, 39 reacted with the O1 to O7 sera. Twenty-six of these possessed the common antigens of groups O1 to O4 and were further identified with absorbed sera. Six cultures reacted with the O2 nonabsorbed serum but not with respective absorbed serum; two cultures could not be grouped with the O1 to O7 sera. The number of cultures in each of the O groups and the biotypes found are presented in Table 4.

Sensitivity to antimicrobial agents. All strains were resistant to ampicillin, amoxycillin, and carbenicillin. All produced beta-lactamase when tested by iodometric assay and by the capillary tube method. Eighteen strains examined further by the tube dilution method could be divided into two groups. One group of 14 strains was inhibited by 60 to 120 µg of ampicillin and 4 to 8 µg of cephalothin per ml. The remaining four strains showed pronounced resistance to ampicillin (MIC, 10,000 to 20,000 µg/ml) and were resistant to cephalothin (MIC, 60 to 120 µg/ml). All 47 strains were sensitive to chloramphenicol, kanamycin, gentamicin, polymyxin B, and nitrofurantoin. Seven strains were resistant to tetracycline (MIC, 120 to 240 µg/ml), and six were resistant to nalidixic acid (MIC, 120 to 240 µg/ml). Of these, five showed resistance to both agents. All these strains were isolated from the urine of patients with chronic urinary tract infections. On further examination it was found that four strains belonged to two sero- and biotypes and that nosocomial infection in two patients was a possibility.

DISCUSSION

An increased awareness of *C. diversus* has resulted in a number of recent investigations concerning the taxonomy and clinical importance of this organism. This bacterium is not frequently encountered in the clinical laboratory. Of 2,998 isolates from urine cultures during the 12 months of this survey, only 20 were *C. diversus*. Isolations from pus and wounds were similarly rare in comparison to other bacteria. As reported by other investigators (7, 10), urinary tract infections appear to be one of the main sources of *C. diversus*. In the current series, nearly half of the strains were isolated from urine. One patient had septicemia in addition to urinary tract infection. Isolations from blood as well as from cerebrospinal fluid have been previously reported (6). In isolations from other sites, the clinical significance of *C. diversus* was often difficult to ascertain due to the complexity of underlying disease or the presence of other bacteria. In four patients the same

TABLE 3. *Biotypes of Citrobacter diversus* among 47 isolations: frequency and source

Fermentation of:				Biotype	No. of cultures	Source (no.)
Dulcitol	Sucrose	Sorbose	Rhamnose			
—	—	— (+)	+	a	17	Urine (7), stool (4), wound (3), pus (2), blood (1)
—	—	+	+	b	1	Wound (1)
+	—	— (+)	+	c	6	Wound (3), urine (1), pus (1), catheter (1)
+	+	— (+)	+	d	10	Wound (5), urine (4), otitis (1)
—	+	— (+)	+	e	13	Urine (8), wound (2), pus (1), otitis (1), vaginal discharge (1)

TABLE 4. Frequency of O groups among 47 isolations of *Citrobacter diversus*

O group	No. of cultures	Biotypes
1	12	a, b, d, e
2	12	a, d, e
3	1	a
4	1	a
5	9	a, c, d, e
6	3	a, e
7	1	c
(2)	6	a
Not identified	2	c

sero- and biotype that was present in wounds could be isolated from stools. This bacterium belongs most probably to the intestinal flora, but its frequency in the intestine has not been investigated.

C. diversus has a distinct pattern of susceptibility to antimicrobial agents. All strains of this series were resistant to ampicillin, amoxycillin, and carbenicillin and produced beta-lactamase. All strains were sensitive to chloramphenicol, kanamycin, gentamicin, polymyxin B, and nitrofurantoin, and the majority were sensitive to tetracycline and nalidixic acid. Five strains, all from urine of patients with chronic infections of the urinary tract, were resistant to these last agents. In comparison, *Escherichia coli* strains isolated from similar clinical material during the same time period and under the same antibiotic pressure showed a very different pattern of sensitivity. Of a series of 300 strains, 69% were resistant to ampicillin, 47% to chloramphenicol, 55% to tetracycline, 35% to kanamycin, and 6% to nitrofurantoin. Lund et al. (8) reported significant differences in the sensitivity to ampicillin and cephalothin between *C. diversus* and indole-negative *C. freundii*.

ADDENDUM

After preparation of this paper, another group of 25 cultures of *C. diversus* from clinical material was

examined. Of them, nine cultures belonged to O-group 2, seven to O-group 6, five to O-group 1, three to O-group 5, and one to O-group 7.

LITERATURE CITED

- Bauer, A. W., W. M. Kirby, J. C. Sherris, and M. Turk. 1966. Am. J. Clin. Pathol. 45:493-496.
- Edwards, P. R., and W. H. Ewing. 1972. Identification of *Enterobacteriaceae*. Burgess Publishing Co., Minneapolis.
- Ewing, W. H., and B. R. Davis. 1972. Biochemical characterization of *Citrobacter diversus* (Burkey) Werkman and Gillen and designation of the neotype strain. Int. J. Syst. Bacteriol. 22:12-18.
- Frederiksen, W. 1970. *Citrobacter koseri*, a new species within the genus *Citrobacter*, with a comment on the taxonomic position of *Citrobacter intermedium* (Werkman and Gillen). Publ. Fac. Sci. Univ. J. E. Purkyně Brno 47:89-94.
- Gross, R. J., and B. Rowe. 1974. The serology of *Citrobacter koseri*, *Levinea malonatica* and *Levinea amalonatica*. J. Med. Microbiol. 7:155-161.
- Gross, R. J., B. Rowe, and J. A. Easton. 1973. Neonatal meningitis caused by *Citrobacter koseri*. J. Clin. Pathol. 26:138-139.
- Jones, S. R., A. R. Ragsdale, E. Kutscher, and J. P. Sanford. 1973. Clinical and bacteriologic observations on a recently recognised species of *Enterobacteriaceae*, *Citrobacter diversus*. J. Infect. Dis. 128:563-565.
- Lund, M. S., J. M. Matsen, and D. J. Blazevec. 1974. Biochemical and antibiotic susceptibility studies of H₂S-negative *Citrobacter*. Appl. Microbiol. 28:22-25.
- Perrette, C. J. 1954. Iodometric assay of penicillinase. Nature (London) 174:1012.
- Richard, C. 1972. Le genre *Levinea* (famille des *Enterobacteriaceae*). Bull. Liaison (Inst. Pasteur Paris) 54:85-92.
- Richard, C., B. Brisou, and J. Lioult. 1972. Étude taxonomique de *Levinea*, nouveau genre de la famille des entérobactéries. Ann. Inst. Pasteur Paris 122:1137-1146.
- Rosen, I. G., J. Jacobson, and R. Rudderman. 1972. Rapid capillary tube method for detecting penicillin resistance in *Staphylococcus aureus*. Appl. Microbiol. 23:649-650.
- Smith, R. F., S. L. Dayton, and D. D. Chipps. 1973. Recognition of *Citrobacter diversus* in the clinical laboratory. Appl. Microbiol. 25:157-158.
- Young, V. M., D. M. Kenton, B. J. Hobbs, and M. R. Moody. 1971. *Levinea*, a new genus of the family *Enterobacteriaceae*. Int. J. Syst. Bacteriol. 21:58-63.