

**Antigenic scheme for *Citrobacter koseri*
(syn. *C. diversus*, *Levinea malonatica*); three new antigens
recognized in strains from Israel**

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(Received 16 September 1980)

SUMMARY

An antigenic scheme for *Citrobacter koseri* was described previously and consisted of 14 'O' antigens. Three additional antigens are now added to the scheme and type strains for these antigens are designated. Their origins and their biochemical and serological reactions are described.

INTRODUCTION

Organisms described as *Citrobacter koseri* (Frederiksen, 1970), *C. diversus* (Ewing & Davis, 1972) and *Levinea malonatica* (Young *et al.* 1971) belong to the same species although their final taxonomic position has not been decided. As a result of studies in Britain (Gross & Rowe, 1975) an antigenic scheme which consists of 14 O antigens has been proposed for these organisms. The scheme has proved valuable in epidemiological studies in Israel where 424 of 484 strains tested (87.6%) could be serotyped using the existing scheme (Altman *et al.* 1976; Sechter *et al.* 1976). The examination of untypable strains has led to the recognition of three additional O antigens. Type strains for these three antigens are now designated and their biochemical and serological reactions are described.

MATERIALS AND METHODS

Strains

Three strains of *C. koseri* were selected as new test strains. Strain number E5143 was isolated from vaginal discharge, as predominant culture; strains E5141 and E9843 were isolated, as almost pure culture, from the urine of adult persons with urinary tract infection.

Biochemical tests

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

Preparation of antisera

O antigen suspensions were prepared for use as vaccines by harvesting the growth from two nutrient agar slopes in 0.9% saline and heating at 100 °C for 2.5 h. After centrifugation the heated organisms were resuspended in 15 ml saline and commercial formalin was added to a final concentration of 0.3%. Rabbits were immunized by intravenous injections of 0.5 ml, 1.0 ml, 2.0 ml, 2.0 ml and 2.0 ml at 5-day intervals. Blood samples of 40 ml were taken at 5 and 10 days after the final injection and the rabbits were exsanguinated 5 days later. The products of all three bleedings were pooled.

Bacterial agglutinations

O antigen suspensions for agglutination tests were prepared by heating overnight broth-cultures at 100 °C for 30 min and then adding commercial formalin to a final concentration of 0.3%. Agglutination tests were prepared in plastic agglutination trays and were incubated at 50 °C for 16 h. Antisera for the three new test strains were tested against O antigen suspensions of the three strains and of the 14 test strains described previously. O antigen suspensions of the three strains were tested against antisera for the 14 test strains described previously.

Absorption studies

Where cross-reactions were found among the test strains, reciprocal absorptions were performed to determine the antigenic relations between the strains.

RESULTS

Biochemistry

The three new test strains were Gram-negative rods. They were oxidase negative, catalase positive, fermentative in the Hugh and Leifson test, and reduced nitrate. Their remaining reactions are shown in Table 1.

Agglutination reactions

Antiserum for strain number E5143 agglutinated O antigen suspension of *C. koseri* 012 to a titre of 200 compared with a homologous titre of 1600. Antisera for the three new strains had no other significant cross-reactions with O antigen suspensions for *C. koseri* 01 to 14.

Absorption studies

Although antiserum for strain E5143 agglutinated *C. koseri* 012 specific antisera could easily be prepared by absorption (Table 2).

Table 1. Biochemical reactions of three new type strains of *C. koseri*

| | O15 E5143 | O16 E5141 | O17 E9843 |
|-----------------------------|-----------------|-----------------|-----------------|
| Motility (Craigie tube) | + | + | + |
| Methyl-red test | | | |
| 37 °C (2 days) | + | + | + |
| 20 °C (5 days) | + | + | + |
| Voges-Proskauer reaction | | | |
| 37 °C (2 days) | - | - | - |
| 20 °C (5 days) | - | - | - |
| Simmons citrate | + | + | + |
| Malonate | + | + | + |
| Growth in potassium cyanide | - ³ | - ³ | - ³ |
| Indole (Kovac's) | + | + | + |
| H ₂ S (T.S.I.) | - | - | - |
| Gluconate | - ³ | - ³ | - ³ |
| Christensen's urea | - ⁷ | - ⁷ | - ⁷ |
| Phenylalanine deaminase | - | - | - |
| Gelatin (stab) | - ²⁸ | - ²⁸ | - ²⁸ |
| Arginine decarboxylase | + ³ | + ⁴ | + ³ |
| Lysine decarboxylase | - ⁴ | - ⁴ | - ⁴ |
| Ornithine decarboxylase | + | + | + |
| β galactosidase (ONPG) | + | + | + |
| Glucose (acid) | + | + | + |
| Glucose (gas) | + | + | + |
| Lactose | + ⁴ | + ³ | - ¹⁴ |
| Mannitol | + | + | + |
| Sucrose | - ¹⁴ | - ¹⁴ | - ¹⁴ |
| Salicin | + ³ | + ³ | + ⁴ |
| Dulcitol | + | - ¹⁴ | - ¹⁴ |
| Inositol | - ¹⁴ | + ⁷ | - ¹⁴ |
| Adonitol | + | + | + |
| Raffinose | - ¹⁴ | - ¹⁴ | - ¹⁴ |
| Sorbitol | + | + | + |
| Arabinose | + | + | + |
| Rhamnose | + | + | + |
| Xylose | + | + | + |
| Trehalose | + | + | + |
| Inulin | - ¹⁴ | - ¹⁴ | - ¹⁴ |
| Glycerol | + | + | + |
| Cellobiose | + | + | + |
| Sorbose | - ¹⁴ | - ¹⁴ | + ¹⁴ |
| Maltose | + | + | + |

Superscript numerals indicate days of incubation if more than 1 day.

Extended antigenic scheme

It is proposed that the three new test strains be designated as *C. koseri* 015 to 017. The extended scheme is summarized in Table 3.

Table 2. *Antigenic relationship between strains E5143 and C. koseri O12*

| Antiserum | Absorbing suspension | Antigen suspension | |
|----------------------|----------------------|----------------------|-------|
| | | <i>C. koseri</i> O12 | E5143 |
| <i>C. koseri</i> O12 | None | 1600 | — |
| | E5143 | 1600 | — |
| E5143 | None | 200 | 1600 |
| | <i>C. koseri</i> O12 | — | 800 |

Table 3. *Extended antigenic scheme*

| Antigen | Test strain | Absorption required |
|---------|-------------|---------------------|
| O1 | NCTC 10786 | O2 and O4 |
| O2 | E1597/71 | O1 |
| O3 | NCTC 10768 | O1 and O2 |
| O4 | E1558/72 | O1 |
| O5 | NCTC 10769 | O13 |
| O6 | NCTC 10770 | O8 |
| O7 | E1599/71 | O9 |
| O8 | E2641/72 | O2 and O6 |
| O9 | E2567/72 | O11 |
| O10 | E2576/72 | — |
| O11 | E2569/72 | O9 |
| O12 | E2577/72 | O15 |
| O13 | E508/74 | O5 |
| O14 | E1020/74 | — |
| O15 | E5143/76 | O12 |
| O16 | E5141/76 | — |
| O17 | E9843/78 | — |

DISCUSSION

Strains of *C. koseri* or *C. diversus* have been recognized by several authors as a cause of meningitis in the newborn. Gross, Rowe & Easton (1973) described three cases, two of which were fatal, and Gwynn & George (1973) described a further four cases in the same year. A further incident was described in France two years later (Duhamel *et al.* 1975) and Tamborlane & Soto (1975) described a further case in the U.S.A. at about the same time. Ribeiro, Davis & Jones (1976) subsequently described an outbreak of three cases which had taken place in England in 1973. In three of these incidents more than one case of meningitis occurred in the same ward and it was clear that the organism was able to spread from person to person. In one outbreak the use of serotyping showed that two different organisms were involved (Gross, *et al.* 1973). The use of serotyping has therefore led to a better understanding of the epidemiology of these organisms.

Among the three new O-groups, O15 was frequently isolated in Israel and represented 12% of all the *C. koseri* isolated in this country. Most of the cultures of this O-group were isolated from urine, but some also from wounds, vaginal discharge, secretion of ear or nose, sputum, bile and faeces. The other two O-groups were represented by single isolates.

Studies in other geographical areas may reveal strains of *C. koseri* which cannot be typed with the existing scheme. If such strains appear to be numerous or are shown to cause outbreaks of infection it may be useful to include them as additional new O-groups in the current scheme.

REFERENCES

- ALTMAN, G., SECHTER, I., CAHAN, D. & GERICHTER, C. B. (1976). *Citrobacter diversus* isolated from clinical material. *Journal of Clinical Microbiology* **3**, 390.
- COWAN, S. T. & STEEL, K. J. (1965). *Manual for the Identification of Medical Bacteria*. Cambridge University Press.
- DUHAMEL, M., CUVELIER, A., COUSIN, J. & FOURNIER, A. (1975). Septicémie et méningite néonatales à *Levinea malonatica*. *Nouvelle Presse Médecine* **4**, 428.
- EWING, W. H. & DAVIS, B. R. (1972). Biochemical characterization of *Citrobacter diversus* (Burkey) Werkman and Gillen and designation of the neotype strain. *International Journal of Systematic Bacteriology* **22**, 12.
- 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). Publications of the Faculty of Science of the University of J. E. Purkyně, Brno **47**, 89.
- GROSS, R. J. & ROWE, B. (1975). *Citrobacter koseri*. I. An extended antigenic scheme for *Citrobacter koseri* (syn. *C. diversus*, *Levinea malonatica*). *Journal of Hygiene* **75**, 121.
- GROSS, R. J., ROWE, B. & EASTON, J. A. (1973). Neonatal meningitis caused by *Citrobacter koseri*. *Journal of Clinical Pathology* **26**, 138.
- GWYNN, C. M. & GEERGE, R. H. (1973). Neonatal citrobacter meningitis. *Archives of Disease in Childhood* **48**, 455.
- RIBEIRO, C. D., DAVIS, P. & JONES, D. M. (1976). *Citrobacter koseri* meningitis in a special care baby unit. *Journal of Clinical Pathology* **29**, 1094.
- SECHTER, I., CAHAN, D., OHAD, O., GERICHTER, C. B. & ALTMAN, G. (1976). The antigenic structure of *Citrobacter koseri* (syn. *C. diversus*, *Levinea malonatica*) isolated in Israel, 1974–75. *Israel Journal of Medical Sciences* **12**, 704.
- TAMBORLANE, W. V. & SOTO, E. V. (1975). *Citrobacter diversus* meningitis: a case report. *Paediatrics* **55**, 739.
- YOUNG, V. M., KENTON, D. M., HOBBS, B. J. & MOODY, M. R. (1971). *Levinea*, a new genus of the family *Enterobacteriaceae*. *International Journal of Systematic Bacteriology* **21**, 58.