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Escherichia coli flagellar serotyping is as reliable as it has always been!

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In a recent paper in this Journal by Marshall *et al.* (1985), which described the bacterial endonuclease DNA analysis (BRENDA) of several *Escherichia coli* 0126 strains, the following statement was found in the summary: 'The isolates from the outbreak produced indistinguishable DNA electrophoretic patterns in spite of their assignment to seven different H serotypes . . . These results support the epidemiological evidence that a single-strain outbreak had occurred, and they cast doubt on the value of H typing for this particular investigation.' The same 0126 strains that were enterotoxigenic (ST) were treated in two earlier papers (Bettelheim & Reeve, 1982; Bettelheim, 1984) and also on these two earlier occasions the authors raised doubt about the stability of *E. coli* H typing results.

Two of the present authors (F.Ø. and I.Ø.) were, quite naturally, also puzzled by these unusual results and suggested to K.A.B. that the strains should be re-examined, but this time in Copenhagen.

When re-examined, they came out as 0126:H12 ST-positive, a well-known ETEC serotype (Rowe, Gross & Takeda, 1983), all belonging to the same biotype, except for one immotile strain.

However, the first attempt to H-type the strains in Copenhagen was a failure, as they were all spontaneously agglutinable in the battery of pooled H antisera. This phenomenon of spontaneous agglutinability is not unknown at the International Escherichia Centre. When spontaneous agglutinability hinders H determination, we carry out the H examination by the more cumbersome way of antiserum immobilization controlled by light microscopy (Ørskov, 1954). This examination showed that all motile outbreak strains belonged to H12 (with a known cross-reaction to H1) (Ørskov & Ørskov, 1984).

To further support these new H-typing results we also carried out in Copenhagen immobilization tests in agar plates with H-antisera added to the agar (agar concentration 0·3 %) – a method well established in salmonella serology.

As expected, the motile strains were also immobilized during growth in H-serum agar with H12 antiserum added, but were not immobilized by the different H antisera earlier suggested.

A definite explanation of the earlier false typing results cannot be given. It is

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not rare for motile cultures used for H-typing to show a tendency to spontaneous agglutination that may be more pronounced in one laboratory than in another; this tendency, in association with the occurrence in H antisera of antibodies against several surface components other than flagellae, may have been responsible for the false positive H-antigen reactions found. Why the motile cultures, when examined in New Zealand by the standard agglutination method, did not react in H12 (and in H1) antiserum is difficult to explain.

In conclusion: H typing in *E. coli* is still as reliable as it has always been; occasionally the agglutination technique should be supplemented with other methods.

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