SHORT ARTICLES

MORPHOLOGICAL AND BIOCHEMICAL STUDIES OF 27 STRAINS BELONGING TO THE GENUS AEROMONAS ISOLATED FROM CLINICAL SOURCES

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Until recently, members of the genus Aeromonas have seldom been recognised in material from human sources. Miles and Halnan (1937) were the first to report the isolation of an aeromonad from the faeces of a patient, and in 1954 Hill, Caselitz and Moody isolated a strain post mortem from a female patient with septicaemia and acute metastatic myositis. There have since been numerous reports of isolations from human sources, including blood (see von Graevenitz and Mensch, 1968; Nygaard, Bissett and Wood, 1970).

This report gives the results of a study of the morphological and biochemical characters of 27 strains of aeromonads isolated from human clinical sources.

MATERIALS AND METHODS

Sources of the strains. The 27 strains had been isolated during the routine examination of specimens from hospital in-patients: 24 from the faeces of patients with enterocolitis (including a 6-mth-old baby), two from blood (one from a 38-yr-old patient with chronic myeloid leukaemia and the other from a 9-yr-old boy) and one from bile of a 40-yr-old patient with gallstones.

RESULTS

All 27 strains were motile Gram-negative bacilli with polar monotrichous flagella; occasionally a lateral flagellum was also observed. All gave positive oxidase, indophenol oxidase, catalase, gelatinase, lipase (Sierra, 1957), lecithinase and β-galactosidase reactions. All showed caseinolytic activity, liquefied coagulated serum, reduced nitrate to nitrite, hydrolised starch and degraded dextrose by fermentation. Acid and gas or acid only was produced from dextrin, dextrose, fructose, galactose, glycerol, glycogen, maltose, mannitol, mannose, ribose, sucrose, starch and trehalose.

None of the 27 strains was sensitive to the vibriostatic agent O/129 (2,4 diamino 6,7 di-isopropyl pteridine) (Shewan, Hodgkiss and Liston, 1954) with acetone (Houston, 1967) and dioxan as diluent (Schubert, 1962). All were urease and phenylalanine deaminase negative and after 30 days had failed to attack adonitol, dulcitol, inositol, inulin, melezitose, melibiose, rhamnose, sorbitol, sorbose and xylose. No lysine or ornithine decarboxylase activity could be detected by Möller's (1955) or by Carlquist's (1956) method. Malonate was not utilised and, as judged by the result of tests by the method of Bühman (1961), acetamide was unaffected. The strains did not grow in the presence of 0-5 per cent. cetrimide.

Two of our strains failed to produce haemolysis on horse blood agar plates, and one did not produce indole from tryptophan. The methyl-red test was negative in 19 strains. Six strains produced neither acetoin nor 2,3 mesobutanediol from glucose (read after 1, 2, 3, 4 and 5 days) when tested by the method of Bullock (1961). Seven strains did not grow on Simmons' citrate agar. Six strains did not grow in the presence of KCN. Only one strain was without detectable arginine dihydrolase activity. Six strains did not oxidise gluconate;

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six did not hydrolyse aesculin. There was no acidification of arabinose in eight strains, of cellobiose in three, of aesculin in four, of lactose in two, of raffinose in ten and of salicin in eight. Two strains fermented sorbitol.

In the six gluconate-negative strains, the production of acetoin and 2,3 mesobutanediol could not be detected. Two such strains were aerogenic in glucose (DM28/67 and AS5/68); one of them (AS5/68) did not produce gas in glycerol. Four strains (PJ3/69, JJ37/69, CO57/69 and VM45/70) produced no gas from carbohydrates. Schubert (1964a and b, 1967a and b, 1968, 1969) would identify the first strain (DM28/67) as Aeromonas hydrophila, biotype 2, the second (AS5/68) as Aeromonas punctata, the other four (PJ3/69, JJ37/69, CO57/69 and VM45/70) as Aeromonas punctata subspecies caviae and the remaining 21 strains as Aeromonas hydrophila, biotype 1 (table).

### Table

Intraspecies differentiation of aeromonads by biochemical tests

<table>
<thead>
<tr>
<th>Strains</th>
<th>Gluconate oxidation</th>
<th>Acetoin from glucose</th>
<th>2,3 Mesobutanediol from glucose</th>
<th>Gas from glycerol</th>
<th>Gas from glucose</th>
<th>Identity of organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 strains</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A. hydrophila, biotype 1</td>
</tr>
<tr>
<td>No. DM28/67</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>A. hydrophila, biotype 2</td>
</tr>
<tr>
<td>No. AS5/68</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>A. punctata</td>
</tr>
<tr>
<td>No. PJ3/69</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A. punctata subsp. caviae</td>
</tr>
<tr>
<td>No. JJ37/69</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td></td>
</tr>
<tr>
<td>No. CO57/69</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>No. VM45/70</td>
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</tr>
</tbody>
</table>

### Discussion

The patients from whose faeces aeromonads were isolated all suffered from diarrhoea. The organisms were isolated more than once from four of the patients. Examination of the faecal specimens for the presence of salmonellae, shigellae and, in the case of children, for enteropathogenic serotypes of Escherichia coli was negative. The two patients with positive blood cultures were probably immunologically deficient.

The characteristics of the strains were examined by 66 biochemical and morphological tests. The most useful biochemical tests were: fermentative attack on dextrose, a positive oxidase test, resistance to the vibriostatic agent O/129 and strong extracellular enzyme activity. The most important tests for intrageneric differentiation were oxidation of gluconate (with which the production of acetoin and 2,3 mesobutanediol from glucose was associated) and the production of gas from glucose and glycerol.

### Summary

The morphological and biochemical characters of 27 aeromonas strains of human origin are reported (24 from faeces, two from blood and one from bile). Twenty-one strains were identified as Aeromonas hydrophila, biotype 1, four as Aeromonas punctata subspecies caviae, one as Aeromonas hydrophila, biotype 2 and one as Aeromonas punctata.
I wish to thank Mrs D. Zavodnik for her technical assistance. This study is a part of work done for the Boris Kidrić Fellowship. The gift of a sample of compound O/129 by Messrs Allen and Hanbury, Ltd is gratefully acknowledged.

REFERENCES


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