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An investigation into the properties of klebsiella strains isolated from ankylosing spondylitis patients

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SUMMARY

Thirty-nine strains of klebsiella isolated from ankylosing spondylitis patients were examined by the methods of Cowan & Steel (1974), those described by Edmondson *et al.* (1980) and by capsular typing. No significant difference was detected by any of these methods between these strains and those examined by other workers from non-ankylosing spondylitis patients and other environments.

INTRODUCTION

Evidence from a number of sources has recently been accumulating, suggesting that klebsiellas have a role in the pathogenesis of ankylosing spondylitis (AS). Some of the evidence has been cultural. *Klebsiella pneumoniae* has been isolated more frequently from faecal cultures of AS patients during active phases of the disease than during inactive phases (Ebringer *et al.* 1978); *K. pneumoniae* was isolated in 13 of 17 episodes (76%) in AS patients with uveitis compared with an overall klebsiella isolation rate of 30% in AS patients without uveitis (Ebringer, Cawdell & Ebringer, 1979) and Eastmond *et al.* (1980) produced results which indicated that *K. aerogenes* has a role in the development of non-granulomatous anterior uveitis and peripheral arthritis in AS patients although the results did not support a role for the organism in the actual spinal disease. Kuberski *et al.* (1981) showed increased recovery of faecal klebsiellas from Pima Indians with active AS and Reiter's syndrome. On the other hand, Warren & Brewerton (1980) were unable to confirm these observations.

Other evidence has been of a serological nature in as much as cross-reactions between klebsiellas and HLA-B27 lymphocytes have been reported by various workers. The significance of this lies in the undisputed association of AS with HLA-B27 (Caffrey & James, 1973; Brewerton *et al.* 1973; Schlosstein *et al.* 1973), and strongly suggests that molecular mimicry could play a role in the pathogenesis of AS. Welsh *et al.* (1980) demonstrated cross-reactions between rabbit anti-human

B27 lymphocyte sera and some gram-negative bacteria including klebsiella and Avakian *et al.* (1980) showed cross-reactions between mono-specific HLA-B27 typing sera and klebsiella antigens. Another group of workers has demonstrated that antisera raised against certain strains of *K. pneumoniae* are cytotoxic for B27 positive lymphocytes from AS patients, but not for B27 positive controls (Seager *et al.* 1979; Geczy & Yap, 1979; Geczy, Alexander & Bashir, 1980).

The purpose of this paper is to describe and identify the klebsiellas isolated by one of us (D.C.) from AS patients, in order to see if there is any detectable distinguishing feature about them, as opposed to those found in non AS patients and other environments (Cooke *et al.* 1979*a*, *b*, 1980; Edmondson *et al.* 1980).

MATERIALS AND METHODS

Organisms

These were isolated by culturing standard aliquots of faecal samples onto medium containing 1 % inositol, 1 % lab lemco, 1 % peptone, 1·2 % agar, with bromothymol blue indicator, and incubated at 37 °C for 18 h. Inositol fermenting colonies were initially identified using API 20E. Thirty-nine strains were selected at random for further examination.

Capsular serotyping

CIE tests were used primarily, when strains were found to be untypable by this method, the Quellung test was performed. All these tests were performed by Dr P. B. Mortimer of the Public Health Laboratory, Coventry and Warwickshire Hospital, Coventry.

Further identification tests

Most of these were performed according to the methods of Cowan & Steel (1974). Where more than one method is described the following were chosen: carbohydrate test method 1; catalase 1; oxidase 1; arginine, ornithine and lysine decarboxylase 2; urease 1; Voges Proskauer 2; gelatin hydrolysis 1 and 5; malonate utilization 1; indole production 2; gluconate oxidation 1; H_2S production 1. Two further tests, namely growth at 10 °C and pectin liquefaction were performed according to the methods of Edmondson *et al.* (1980) with some modification. For growth at 10 °C, one loopful of a broth culture was used as the inoculum, a stirred water-bath was used and the results were read visually. In the pectin liquefaction tests 0·2 % agar was used.

RESULTS

All 34 tests for the identification of enterobacteria (Cowan & Steel, table 7·9e) were carried out and the results confirmed that all the organisms isolated were klebsiellas.

In the Cowan and Steel scheme five tests are used for identifying klebsiella species, i.e. indole production, dulcitol fermentation, methyl red test, Voges

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Table 1. *Groups of klebsiellas separated by four tests*

	Reaction in tests				
	FC	Growth at 10 °C	Indole production	Pectin liquefaction	No. of strains
1	+	−	−	−	2
2	−	+	−	−	9
3	−	+	+	+	11
4	−	+	+	−	6
5	−	−	−	−	11
6	−	−	+	−	1

FC = faecal coliforms

Proskauer test and gelatin liquefaction. It is upon this classification that we have based the naming of our strains.

Thirteen strains could be identified as *K. oxytoca* (senso stricto): 1 as *K. oxytoca* except for a failure to ferment dulcitol, 11 as *K. aerogenes* (*K. pneumoniae* senso lato), a further 13 as *K. aerogenes* except for a failure to ferment dulcitol, and 1 as *K. pneumoniae* (senso stricto), according to these 5 tests.

The organisms were also examined with the four tests used by Edmondson *et al.* (1980), i.e. the faecal coliform test, the production of acid and gas in bile-salt broth at 44·5 °C, growth in nutrient broth at 10 °C, indole production and pectin liquefaction. The results of these tests are shown in Table 1, and with one exception (the strain which was positive in the indole tests and negative in the other 3) they all fell into one of the five groups described by Edmondson *et al.* (1980). The atypical strain was the atypical *K. oxytoca* strain previously mentioned.

Capsular types

CIE tests were performed upon all strains and Quellung tests were done on those organisms found to be not typable by the CIE method. The results are as follows, the CIE test being the one used unless otherwise stated by including Q in the designation. Numbers of strains are in parentheses:

$\frac{21}{2}$(1)Q, 7(1)Q, 8(1)Q, $\frac{5}{9}$(1), 12(1), 18(2), 23(1)Q, 31(1)Q, 35(1), 35(2)Q, 41(2), 43(1)Q, 43(2), 44(1), 47(2), 49(1), 51(1), 53(1), 60(2), 61(1), $\frac{20}{61}$(1), 62(1)Q, 71(1), 74(2).

DISCUSSION

The results of the tests described in this paper indicate that whilst the isolates are all definitely identifiable as klebsiellas, there is no obvious way in which they differ from those found in hospital and other environments (Cooke *et al.* 1979*a*, *b*, 1980; Edmondson *et al.* 1980).

All these strains fell into the five groups described by Edmondson *et al.* (1980) except one which was positive in the indole test and negative in the other 3. The strain was the atypical *K. oxytoca* strain and it so happens is the strain (Middlesex hospital patient 100) used by Welsh *et al.* (1980) in the demonstration of cross-

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reactions between rabbit anti-human HLA-B27 lymphocytes and klebsiella, and Avakian *et al.* (1980) in their demonstration of cross-reactions between non-specific HLA-typing sera and klebsiella. However, we do not feel this is of particular significance.

Neither can capsular type be considered to be of any relevance since no less than 23 different types were found among the 39 strains, 8 of which were found to be untypable. The Middlesex hospital 100 strain was found to be type 7 by the Quellung method.

The conclusion drawn from this study is that if there is anything peculiar to the strains of klebsiellas associated with AS patients, it is not to be found in the tests routinely applied to the identification and classification of these organisms.

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