FAECAL FLORA IN SPONDYLOARTHROPATHY

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SUMMARY

Enterobacteria, in particular Klebsiella spp., have been implicated in the aetio-pathogenesis of ankylosing spondylitis. A comprehensive examination of the faecal flora of 82 patients with ankylosing spondylitis, either primary (67), or in association with inflammatory bowel disease (4), reactive arthritis (6) or psoriatic arthritis (5), was performed and compared with that of a control population (36) of healthy individuals. The range of flora identified was similar in both populations and there was no increased isolation rate of Klebsiella or other proposed arthritogenic organism in those with spondyloarthritis. In those patients in whom Klebsiella was identified, its presence was not related to disease activity, the erythrocyte sedimentation rate or C-reactive protein.

Key words: Spondyloarthritis, Ankylosing spondylitis, Klebsiella, Faecal flora, Bacteria, Infection.

ANKYLOSING spondylitis (AS) and reactive arthritis have several features in common both in the clinical expression of disease and in the strong association with HLA-B27. The observation that reactive arthritis often followed an enteric infection led to speculation that AS might also be triggered by a gut infection.

Sero logical cross-reactivity between HLA-B27 and Klebsiella pneumoniae [1–3] prompted several groups to study the faecal flora of individuals with spondyloarthropathy. An increased prevalence of faecal Klebsiella in AS patients with active disease was noted [4–6], but if the patients were not categorized by disease activity, the isolation of Klebsiella in patients was similar to that of controls. Several investigators worldwide have not confirmed this association of Klebsiella with AS [7–12].

The isolation of Klebsiella from the stools of patients with inactive disease was associated with a flare-up of the disease 2–3 months later and it was suggested that K. pneumoniae could be an exacerbating agent in AS [5].

Although Klebsiella are gut commensal organisms, they have been reported as a cause of neonatal enterocolitis [13] and a variety of infections in hospital patients [14]. The gastrointestinal tract is usually assumed to be the source of these bacteria and the source of Klebsiella in the gut is likely to be food.

The aims of the study were (1) to examine the range of faecal flora of patients with spondyloarthritis in a semi-quantitative manner and to compare it with that of healthy controls, and (2) to determine whether there was any association between a particular organism and clinical disease activity, the erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP).

MATERIALS AND METHODS

Patient details

The patient group (n = 82) included those with primary AS and those with AS in association with inflammatory bowel disease (IBD), reactive arthritis (ReA) and psoriatic arthritis (PSA) (Table I).

Controls

The controls were 36 healthy individuals with no present or past history of rheumatic disease.

Only patients and controls who had not taken antibiotics for the previous 3 months were included as antibiotic therapy is well known to suppress the normal faecal flora and allow the emergence of resistant or abnormal organisms.

Assessment of clinical disease activity

Patients were assessed for the duration of early morning stiffness (EMS), their requirement for a non-steroidal anti-inflammatory drug (NSAID), the presence of worsening back pain over the previous 2–3 weeks and the presence of iritis or synovitis. Using a classification of disease activity similar to that used in previous studies [4, 7], patients were classified into one of three categories: (a) inactive disease—little or no back pain or EMS (< 30 min), and requiring no or infrequent use of a NSAID; (b) probably active—significant EMS (30–60 min) and requiring a regular NSAID for control of symptoms; (c) active disease—prolonged EMS (> 60 min), or worsening back pain over the previous 2–3 weeks, or the presence of iritis, synovitis or significant pain on sacroiliac compression.

Acute-phase response: determination of ESR and CRP

Venous blood samples were taken from patients 1–2 days prior to sending the stool sample. ESR and CRP were not measured in controls. The ESR was determined by the Westergren method and the serum CRP by fluorescence polarization immunoassay (TDx System, Abbott Laboratories).

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TABLE I
Characterization of patients providing faecal sample

<table>
<thead>
<tr>
<th>Patients</th>
<th>No.</th>
<th>AS</th>
<th>IBD</th>
<th>ReA</th>
<th>PSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>82</td>
<td>67</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Male (M)</td>
<td>66</td>
<td>54</td>
<td>1</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Female (F)</td>
<td>16</td>
<td>13</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Mean age (range) (yr)</td>
<td>42 (24–71)</td>
<td>37 (20–45)</td>
<td>39 (22–56)</td>
<td>41 (31–49)</td>
<td></td>
</tr>
</tbody>
</table>

*AS, ankylosing spondylitis; IBD, inflammatory bowel disease; ReA, reactive arthritis; PSA, psoriatic spondyloarthritis.

Identification of colonies

Any mucoid colonies consisting of Gram-negative bacilli were subcultured onto MacConkey agar and then formally identified by API 20E strips. Non-mucoid colonies consisting of Gram-negative bacilli were subcultured onto BGA, incubated overnight and the plate examined for colour. A yellow–green colour indicated the presence of β-glucuronidase and that the bacterium was almost certainly *E. coli*. If no colour appeared, formal identification was by API 20E strips. The *Yersinia* plates were examined daily for 7 days for red bull's eye colonies. Gram stains were made of suspect colonies and Gram-negative bacilli identified by API 20E.

Statistical methods

Comparisons of the frequencies of bacterial species isolated from patient and control populations were made by χ² tests with Yates’ correction. Associations between disease activity, ESR and CRP were tested by Spearman rank correlations. Correlations between disease activity and isolation of *Klebsiella* were determined by Mann–Whitney analysis. Correlations between the HLA-B16 antigen status and the presence of *Klebsiella* were determined by χ² tests with Yates’ correction.

RESULTS

None of the patients (all were out-patients) had eaten hospital food. All 36 controls, except one vegetarian, ate a mixed diet. Hospital food was eaten 1–3 times per week by five controls.

Growth on inositol agar

Among stool samples from patients, 8/82 (9.8%) did not show any growth on inositol (<500 organisms/g faeces) and 74 samples grew a total of 93 different organisms (Table II). Stool samples from controls (36) grew a total of 57 different bacteria (Table II).

The range of bacterial species identified was similar in patients and controls. Enterococci were more

TABLE II
Bacteria isolated from patients’ and controls’ stools cultured on inositol agar

<table>
<thead>
<tr>
<th>Species</th>
<th>All patients</th>
<th>AS = 67 (%)</th>
<th>IBD = 4</th>
<th>ReA = 6</th>
<th>PSA = 5</th>
<th>Controls</th>
<th>All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>69 (84)</td>
<td>59</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>15 (42)</td>
<td>30 (82)</td>
</tr>
<tr>
<td>Enterococci</td>
<td>13 (16)</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>4 (5)</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2 (6)</td>
<td>1 (3)</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>3 (4)</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 (14)</td>
<td>1 (3)</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>1 (1)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
<td>1 (3)</td>
</tr>
<tr>
<td><em>Citrobacter diversus</em></td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Kleveera</em></td>
<td>1 (1)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td><em>Aeromonas hydrophilia</em></td>
<td>1 (1)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (0)</td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td>1 (1)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td><em>Salmonella group C</em></td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td><em>Yersinia</em></td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>57</td>
</tr>
</tbody>
</table>

*Two weeks prior to providing a stool sample for this study, this control had vomiting and diarrhoea for 1–2 days. He was asymptomatic at the time of stool collection. The Public Health Department was notified and the likely source of the *Salmonella* gastroenteritis traced to a contaminated hamburger.
frequently isolated in the control population (15/36, 42%) than in the patient population (13/82, 16%). This was highly significant ($\chi^2 = 9.13, P < 0.01$). Among stool samples from patients, 7/82 (8.5%) grew *Klebsiella*, mostly from AS patients, compared with 7/36 (19.4%) of samples from controls (Table II). This did not represent a significant difference ($\chi^2 = 1.90$). Of the seven controls in whose faeces *Klebsiella* isolates were found, two ate hospital food once or twice per week.

**Growth on Yersinia selective agar**

*Yersinia* were not isolated from any of the patients’ stool samples. *Yersinia enterocolitica* was isolated from the stool of a control individual who had experienced some abdominal discomfort 2 weeks prior to providing a stool specimen. The discomfort then became more severe and on presentation to his general practitioner a stool sample sent to the routine microbiology laboratory also grew *Y. enterocolitica*.

**Disease activity, acute-phase response and *Klebsiella* isolation in AS patients**

The results of the clinical disease activity, ESR and CRP for the 67 AS patients are recorded in Table III. Because of the small numbers of patients in the IBD, ReA and PSA categories, these subgroups were not subjected to statistical analysis.

Clinical disease activity correlated significantly with both ESR and CRP ($P < 0.01$), but did not correlate with the isolation of *Klebsiella* (Table III). There were no correlations between *Klebsiella* isolation and ESR or CRP.

**DISCUSSION**

**Bacterial species isolated from patients and controls**

There was no major difference in the range of bacteria identified from the stools of healthy individuals and AS patients. By making dilutions of the stool specimen, we ensured that a minimum growth of 500 organisms/g faeces would be detected. Similar results have been reported [10] in the only other study using this methodology.

Enterococci were isolated significantly more frequently from the stools of controls (15/36, 42%) than from patients (13/52, 16%). Enterococci form part of the faecal flora of most healthy adults and although they are potentially pathogenic they have relatively low virulence [15]. They may cause urinary tract infections, particularly in hospitalized patients, and endocarditis. Enterococci are relatively resistant to environmental conditions and so the quicker arrival of stool specimens from the controls than from patients is unlikely to explain the higher isolation rate of enterococci in controls. We cannot explain the disparity in the isolation of enterococci from the two populations, but we do not believe that this has any clinical significance.

In most previous surveys of stool bacteria in patients with AS, only the prevalence of *Klebsiella* was sought [7, 8, 12]. In others, faecal samples were also examined for *Enterobacter* and *Yersinia* [4, 5, 9]. In the only other survey which examined for a wider range of organisms, scanty numbers of *Citrobacter, Proteus* and *Alkaligenes* were detected, and *Salmonella, Shigella* and *Yersinia* were not isolated [6].

The range of bacteria identified from patients with IBD, ReA and PSA was similar to that from AS patients.

**Influence of diet on *Klebsiella* carriage**

All types of hospital food, particularly salads and cold meat, may become contaminated with *Klebsiella* organisms. In the present study, none of the patients ate hospital food and *Klebsiella* was isolated in 7/82 (8.5%). By contrast, 5/36 (13.9%) of the controls ate hospital food at least once per week and *Klebsiella* was isolated from the stool samples of two of these.

**Klebsiella isolation**

There was no increased isolation of *Klebsiella* from the faecal samples of AS patients than from controls. This agrees with many studies [7–10, 12], but disagrees with the results of others [4–6]. Indeed, in the present work, *Klebsiella* were isolated less frequently from patients’ stools (8.5%) than samples from controls (19.4%), but this difference did not reach statistical significance. Because of the wide geographical area of patient recruitment, stool specimens were sent by post and therefore were 24–48 h old at the time of processing. By contrast, delivery of specimens from controls was within 3 h of defaecation. The delay in arrival, and possible lower temperature exposure of samples in the post, might explain why eight patients’ samples did not grow any bacteria on inositol, relatively fewer species of bacteria were isolated from patients’ stools, and the isolation rate for *Klebsiella* in patients’ specimens was lower than that of healthy individuals.

The *Klebsiella* isolation rate of 19.4% in control samples in this study is comparable with those of other studies, e.g. 13% in Arizona [6] and 22.2% in Brazil [10], is lower than that in two English surveys, 38% and 37% [4, 5], and considerably lower than that in a Dutch investigation (60%) [12]. Apart from possible geographical variations in *Klebsiella* prevalence and differences in culture methods, these control populations differed widely from each other. In the two studies by Ebringer et al. [4, 5], the control groups included both healthy individuals and convalescent hospital in-patients. These in-patients, although they

<table>
<thead>
<tr>
<th>Disease activity</th>
<th>No. of patients</th>
<th>Mean (s.d.) ESR (mm/h)</th>
<th>Mean (s.d.) CRP (mg/dl)</th>
<th>No. (%) of Klebsiella positive samples in each disease activity category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive</td>
<td>30</td>
<td>20 (20)</td>
<td>1.6 (1.8)</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Probably active</td>
<td>29</td>
<td>27 (21)</td>
<td>1.8 (1.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Active</td>
<td>8</td>
<td>55 (33)</td>
<td>7.0 (7.6)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td></td>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disease activity</th>
<th>No. of patients</th>
<th>Mean (s.d.) ESR (mm/h)</th>
<th>Mean (s.d.) CRP (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive</td>
<td>30</td>
<td>20 (20)</td>
<td>1.6 (1.8)</td>
</tr>
<tr>
<td>Probably active</td>
<td>29</td>
<td>27 (21)</td>
<td>1.8 (1.8)</td>
</tr>
<tr>
<td>Active</td>
<td>8</td>
<td>55 (33)</td>
<td>7.0 (7.6)</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE III**

Clinical disease activity, ESR, CRP and *Klebsiella* isolation rate in primary AS patients
did not have arthritis, were in a hospital environment and thus were not strictly comparable with the patient group who were all out-patients. Other control populations have comprised hospital out-patients without arthritis or sacroiliitis [6], individuals with mechanical back pain [9], healthy relatives of patients attending general practitioner clinics [10] and rheumatology out-patients with a variety of inflammatory arthritis and gout [12].

The *Klebsiella* isolation rate in AS patients varies between 8.5% in the current work and 27% [7, 8], 32% [10], 38% [4], 40% [6] and 54% [12]. In the only other Scottish study [11] (which did not include a control population), an isolation rate of *Klebsiella* of 17.6% was found in AS patients.

*Klebsiella*, disease activity and acute-phase response

Of the seven patients from whom *Klebsiella* was isolated, six had AS and one had ReA. In these patients, there was no relationship between its presence and clinical disease activity. There was also no association between *Klebsiella* carriage and the presence of a raised ESR or CRP. Increased isolation of *Klebsiella* has been reported in active AS [4, 5] using the same criteria for disease activity as in the present study and also in another study using different criteria [6]. Ebringer et al. [5] concluded that a positive *Klebsiella* culture in a patient with inactive disease could be a harbinger of active disease 2–3 months later, but the validity of this hypothesis remains controversial.

Although the results of *Klebsiella* isolation in active disease have been variable, there is agreement in the literature that AS patients with inactive disease do not have increased carriage of *Klebsiella*. Indeed, many normal individuals carry faecal *Klebsiella*.

**HLA-B27 antigen status**

It has previously been suggested that the postulated link between *Klebsiella* carriage and AS might only occur in HLA-B27-positive patients [4]. In this study, the HLA-B27 antigen status was known in five out of the seven patients from whom *Klebsiella* was isolated: four were HLA-B27 positive and one was HLA-B27 negative. Overall, *Klebsiella* was isolated in 4/45 (9%) of patients known to carry the HLA-B27 antigen and in 1/8 (12%) of those known to be HLA-B27 negative, suggesting that there is no significant difference in the *Klebsiella* isolation rates between the two groups.

**Faecal sampling and culture methods**

The method of faecal sampling has differed between previous studies. Fresh faeces delivered or posted by subjects, or collected by investigators, have been most commonly employed. Rectal swabs are less messy to collect than faecal samples and are probably more acceptable to subjects.

Analysis of stool samples of patients and controls was carried out during the same period to avoid any seasonal variation in faecal flora which might have biased the results.

Different culture methods have been used. MacConkey–inositol–carbenicillin (MIC) agar was developed [16] as a nutrient medium selective for the isolation of *Klebsiella* from faeces. About 97–99% of *Klebsiella* strains and only 0–1% of *E. coli* strains are able to ferment inositol and appear as pink colonies. Most strains of *E. coli* are susceptible to carbenicillin, so the medium is selective for *Klebsiella*. This concentration of carbenicillin (100 mg/ml) will, however, prevent the growth of 10–15% of *Klebsiella* strains (depending on the geographical area), so some investigators have reduced the concentration of carbenicillin to 10 mg/ml. Because of the risk of missing susceptible strains, van Kregten et al. [12], who found particularly high *Klebsiella* isolation rates in Dutch patients (60%) and controls (54%), used Simmons citrate agar (SCA) with 1% inositol but without antibiotics.

A comparative study of 15 methods to isolate *Klebsiella* from faeces was made using different types and combinations of media [17]. The solid media producing the highest isolation rates were MIC and SCA, and the highest isolation rates overall were obtained by subculturing citrate broth to MIC agar and SCA.

Most culture techniques used in the studies of faecal flora in AS have employed a differential medium containing inositol and standard identification methods including API 20E strips.

In the present study, a medium selective for the growth of *Klebsiella* was not chosen as one of our objectives was to determine whether other organisms were more prevalent in the faecal flora of AS patients compared with healthy individuals. The medium chosen was inositol-containing nutrient agar with a neutral red indicator. This allows the growth of many other bacterial species, but it allows the easy identification of inositol fermenters such as *Klebsiella*. It was calculated that the detection rate was 500 organisms/g faeces. Carbenicillin was not incorporated to avoid missing carbenicillin-sensitive *Klebsiella* strains.

By these methods, no major difference was found between the faecal flora of patients with AS and healthy subjects.

**Yersinia isolation**

The absence of *Yersinia* in the patient group in the present study is in agreement with others. The optimum conditions for isolation of *Yersinia* are considered to be enrichment culture using phosphate-buffered saline (pH 7) at 4°C for 3 weeks followed by subculture and incubation at 35°C for 24–48 h [18]. In our hospital microbiology department, faecal specimens suspected of harbouring *Y. enterocolitica* are routinely subcultured onto YSA and incubated at 37°C for 48 h. If no growth occurs, the sample is regarded as being negative for *Yersinia* organisms. Prolonged cold incubation is not performed as it is considered that any clinically significant growth will be detected under the conditions employed. This practice agrees with the conclusions by
Pai et al. [18] that cold enrichment is not indicated for the culture of *Y. enterocolitica* from diarrhoeal stools. Cold enrichment methods do, however, considerably increase the sensitivity of *Y. enterocolitica* isolation in convalescent and asymptomatic subjects. The present study could be criticized for not using cold enrichment methods for the isolation of *yersinia*. The culture conditions employed, however, did succeed in isolating *Y. enterocolitica* from the stool sample of a control who had had abdominal pain 2 weeks prior to providing a faecal sample.

In summary, we have found no increase in the isolation rate of *Klebsiella* or other proposed arthritogenic organism from faecal samples of patients with spondyloarthropathy compared with healthy controls. In those patients in whom *Klebsiella* was identified, there was no relationship between its presence and clinical disease activity or between its presence and the ESR or CRP. The particular strength of this study is that by using standard techniques, a whole range of aerobic bacteria have been investigated in addition to *Klebsiella*.

**References**


