No diagnostic utility of antibody patterns against *Klebsiella pneumoniae* capsular serotypes in patients with axial spondyloarthritis vs. patients with non-specific low back pain: a cross-sectional study

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**Objectives:** To investigate whether antibody response patterns against *Klebsiella pneumoniae* capsular serotypes can discriminate patients with axial spondyloarthritis (axSpA) from patients with non-specific low back pain (LBP).

**Method:** Immunoglobulin (Ig)G and IgA antibodies against *K. pneumoniae* capsular serotypes K2, K26, K36, and K50 were measured, and antibody seropositivity compared between groups and analysed for patient correlation in five different groups: (a) 96 patients fulfilling the Assessment of SpondyloArthritis International Society (ASAS) classification criteria for axSpA; (b) 38 patients with either a positive magnetic resonance imaging (MRI) scan as defined by ASAS or a positive human leucocyte antigen (HLA)-B27 status plus one clinical SpA feature, characterized as ‘non-axSpA’; (c) 82 non-specific LBP patients; (d) 40 healthy blood donors and (e) 43 patients with diagnosed ankylosing spondylitis (AS) served as the negative and positive control groups.

**Results:** There was no difference in IgG and IgA seropositivity against all serotypes between the axSpA, non-axSpA, and LBP groups. No significant correlations were found between anti-*Klebsiella* antibodies and age, gender, HLA-B27, or high-sensitivity C-reactive protein (hsCRP). IgG seropositivity against K50 was more frequent in AS (25.6%) than in axSpA (13.5%, p < 0.05). axSpA patients with radiographic sacroiliitis and AS controls concordantly had the highest frequency of seropositivity for ≥ 2 serotypes (21%).

**Conclusions:** The antibody patterns against *K. pneumoniae* serotypes K2, K26, K36, and K50 did not discriminate between early axSpA and non-specific LBP.
been investigated (9), and based on numerous reports of elevated levels of antibodies against *K. pneumoniae* in AS patients compared to patients with other diseases or to healthy individuals (10), it has been hypothesized that AS may reflect an autoimmune consequence of repeated *Klebsiella* infections (11). A phenomenon called epitope spreading is observed in rheumatoid arthritis (RA), in which antibodies against cyclic citrullinated peptides (anti-CCP), a characteristic biomarker for established RA, are accumulated and can be detected in a pre-clinical RA phase (12, 13). Furthermore, it has been suggested that a pre-AS condition might be identified by the presence of anti-*Klebsiella* antibodies in combination with other SpA-related features (11). However, the diagnostic utility of *Klebsiella* serology has been challenged by a study in which no evidence for AS-specific serological responses to *K. pneumoniae* was found (14).

*K. pneumoniae* are encapsulated by a polysaccharide-rich capsule, and capsular antigens have been shown to be essential for the virulence of *Klebsiella* (15). Based on the capsular antigens, *Klebsiella* species are classified into 77 different capsular serotypes (16, 17). It has been suggested that immune responses against the specific *K. pneumoniae* serotypes K2, K26, K36, and K50 are clinically important as these serotypes evoked higher antibody levels in HLA-B27-positive AS patients, compared with all other *K. pneumoniae* serotypes (18–20). Others have found that K2, being prevalent in the UK (21), may also be clinically relevant in AS patients. For these reasons, the four serotypes, K2, K26, K36, and K50 were included in this study. The purpose of this study was to investigate the hypothesis that antibodies against *K. pneumoniae* may play a role in the SpA pathogenesis. We aimed to evaluate whether IgG and IgA antibody response patterns related to exposure to capsular antigens from *K. pneumoniae* serotypes K2, K26, K36, and K50 could assist in discriminating axSpA patients from patients with LBP.

**Method**

**Study population**

The study was based on patients from the Spines of Southern Denmark cohort, comprising 1037 patients, 18–40 years of age, referred to the Spine Centre of Southern Denmark between March 2011 and October 2013 with LBP (22). A flowchart describing the patient inclusion is provided in Figure 1. In a first screening step, all patients were examined with magnetic resonance imaging (MRI) of the SIJs, screened by questionnaires (22) for clinical axSpA features (23). Furthermore, high-sensitivity C-reactive protein (hsCRP) analysis and HLA-B27 genotyping was performed. According to the Assessment of SpondyloArthritis international Society (ASAS) classification criteria for axSpA, patients were grouped as axSpA according to ASAS criteria, or non-axSpA, defined as patients not meeting ASAS classification criteria for axSpA with either ‘positive’ MRI without additional axSpA features or positive HLA-B27 and one axSpA feature. Groups of LBP patients, a blood donors from the local blood bank, and AS patients were also included.

![Figure 1. Patient inclusion. Patients from the Spines of Southern Denmark cohort (22), 18–40 years of age with low back pain (LBP) > 3 months, a 'positive' MR image according to the ASAS criteria for axSpA or positive HLA-B27 with ≥ 1 axSpA feature were referred to axSpA evaluation by rheumatologists. Patients were grouped as axSpA according to ASAS criteria, or non-axSpA, defined as patients not meeting ASAS classification criteria for axSpA with either ‘positive’ MRI without additional axSpA features or positive HLA-B27 and one axSpA feature. Groups of LBP patients, a blood donors from the local blood bank, and AS patients were also included.](www.scandjrheumatol.dk)
axSpA, the screened clinical axSpA features comprised inflammatory back pain, good response to non-steroidal anti-inflammatory drugs (NSAIDs), family history of SpA, HLA-B27 positivity, elevated CRP, and extraspinal manifestations such as arthritis, heel enthesitis, uveitis, dactylitis, psoriasis, and inflammatory bowel disease (15). The second step of patient assessment was clinical examination by a board-certified rheumatologist [Departments of Rheumatology at Hospital Lillebælt, Vejle (AGL, LHH) or King Christian 10th Hospital for Rheumatic Diseases, Gråsten (OH)]. To minimize the risk that the screening step may have missed patients with early or atypical axSpA, the referral criteria to rheumatologist examination was broadened and also included patients with LBP ≥ 3 months who either fulfilled the ASAS definition for a positive MRI scan (2), or were HLA-B27 positive with at least one concomitant clinical axSpA feature. The rheumatologists classified the patients into (1) axSpA according to ASAS criteria; (2a) ‘positive’ MRI according to the ASAS definition but no additional clinical axSpA features, or (2b) positive HLA-B27 and one clinical axSpA feature. The latter two groups were pooled and called the non-axSpA group. A group of patients with non-specific LBP without axSpA-related features was included as a control group. In the initial screening, six of these individuals met the initial rheumatologist referral criteria and were referred to the rheumatologist. After rheumatological examination they did not meet the criteria for the axSpA and non-axSpA groups. The remaining 76 subjects in the LBP group comprised a random sample from the non-specific LBP group.

Serum samples from two additional groups were included: a ‘negative’ control group comprising a random sample of female and male blood donors (BDs), 18–40 years of age, recruited from the Blood Bank at Odense University Hospital, and a ‘positive’ control group consisting of patients diagnosed with AS, 18–41 years of age, at secondary rheumatology referral centres at Vejle, Esbjerg, and Odense University Hospitals confirmed by one of the authors (HLM). The characteristics of the groups are presented in Table 1.

**Table 1. Characteristics of the study groups.**

|           | axSpA          | non-axSpA       | LBP            | BD              | AS              |
|-----------|----------------|-----------------|----------------|-----------------|-----------------|
| Male, n (%) | 36 (37.5)      | 16 (42.1)       | 43 (52.4)      | 23 (57.5)       | 34 (79.1)       |
| Age (years), median (IQR) | 31 (27–35)      | 34 (29–36)      | 32 (26–36)     | 25 (24–29)      | 37 (33–39)      |
| HLA-B27 positive, n (%) | 35 (36.5)       | 6 (15.8)        | 15 (18.3)      | nd*             | 42 (97.7)       |
| hsCRP, median (IQR) | 1.65 (0.6–6.1)  | 0.5 (0.2–2.0)   | 0.85 (0.2–2.8) | nd              | 2.1 (1–7)       |
| Sacroiliitis MRI positive, n (%) | 86 (89.6)       | 32 (84.2)       | 0 (0)          | nd              | nd              |
| mNYc positive, n (%) | 12 (12.5)       | nd              | nd             | nd              | 43 (100)        |

axSpA, Axial spondyloarthritis; LBP, lower back pain; BD, blood donor; AS, ankylosing spondylitis; IQR, interquartile range; HLA, human leucocyte antigen; hsCRP, high-sensitivity C-reactive protein; MRI, magnetic resonance imaging; mNYc, modified New York criteria; nd, not determined.

* HLA-B27 not performed.
† mNYc were met if both readers independently (not by consensus) classified the pelvic radiographs as positive. Statistically significance is noted as: different from LBP group: * p < 0.01, † p < 0.001; different from BD group: ‡ p < 0.01, † p < 0.001; different from non-axSpA: § p < 0.01; different from AS group: ‡ p < 0.01, † p < 0.001.

**Imaging**

MRI of the SIJs and spine was performed by the Department of Radiology, Hospital Lillebælt, Middelfart according to a previously described MRI protocol (24). One of three radiologists with long-standing expertise in imaging in SpA (AGJ, AZ, NE), blinded to patient identifiers and clinical characteristics, except for age and gender, assessed the MRI scans. The presence of sacroiliitis was noted according to the ASAS definition of a positive MR image (2). The inter- and intra-observer agreement of different types of lesions has previously been tested (24). Patients with axSpA according to the rheumatologists’ expert opinion had pelvic radiographs performed. The pelvic radiographs were centrally anonymized and randomized and independently evaluated by two blinded readers (a musculoskeletal radiologist and a rheumatologist with experience in imaging in SpA); and 12 axSpA patients met the modified New York criteria (mNYc) (25) by both readers (26).

**Ethics**

The study was approved by the local Human Research Ethics Committee (project no. S-20110029) and the Danish Data Protection Agency (project no. 2008-58-0035), and was conducted according to the Declaration of Helsinki II. All study participants were enrolled after informed written and oral consent was obtained.

**Routine laboratory tests**

Samples from the axSpA, non-axSpA, and LBP groups were analysed for HLA-B27 (EuroImmun Microarray, Lübeck, Germany) and levels of hsCRP (ABX pentra 400, Horiba, Arkay, Japan), according
to the manufacturers’ instructions. The upper limit of hsCRP considered within normal is ≤ 6 mg/L.

**Preparation of capsular extracts from K. pneumoniae**

Preparation of the capsular extracts from *K. pneumoniae* is described in the Supplementary Material.

**Enzyme-linked immunosorbent assay (ELISA)**

A previously published ELISA protocol by Tiwana et al was followed (27). In brief, polystyrene flat-bottom 96-well microtitre plates (Nunc, Roskilde, Denmark) were coated with 100 μL/well of capsular antigen solution and incubated overnight at 4°C. Plates were washed four times with 200 μL washing solution [phosphate-buffered saline (PBS)–0.05% Tween 20] and 300 μL of blocking solution [0.1% bovine serum albumin (BSA) (Sigma, USA), 0.05% Tween 20 in PBS] was added to each well. Plates were covered and incubated for 1 h at 37°C. Excess blocking solution was removed and 200 μL of serum samples diluted 1:200 in PBS-Tween was added in duplicates, and plates incubated for 2 h at 37°C. Plates were washed four times with washing solution, and 200 μL of 1:10000 horseradish peroxidase (HRP)-conjugated mouse antihuman IgG or IgA (Abcam, USA) antibody was added to each well. Plates were covered and incubated for 1.5 h at 37°C, and washed four times with washing solution. Finally, 100 μL of 3,3′,5,5′-tetramethylbenzidine (TMB; Thermo Fisher Scientific, Waltham, MA, USA) was added to each well, incubated for 20 min in the dark until sufficient colour development, and reactions were stopped with 100 μL of 2 M sulfuric acid (H₂SO₄). Optical density (OD) was measured at 450 nm using a multilabel microplate reader (Victor™X4 2030, PerkinElmer, Waltham, MA, USA). To diminish intra-assay variation, serum samples from each patient were measured in adjacent wells. To diminish inter-assay variation, two positive controls 1:100 1 mg/mL human IgG or IgA (Sigma) and two uncoated wells were included. All assays were performed blinded.

Background absorbance was removed by subtracting OD values from the uncoated wells from mean OD values of the samples. A sample was considered positive against a serotype if the OD unit was greater than the 95th percentile of the BDs. For categorical variables, the χ² test or Fisher’s exact test was used. Spearman’s rank order with Bonferroni correction for multiple testing was used for correlation analysis between antibody response and continuous patient characteristics, and for correlation analysis between antibody response and categorical patient characteristics the Mann–Whitney rank sum test was used. P-values < 0.05 were considered statistically significant.

**Results**

**Characteristics of the study groups**

The characteristics of the 299 study subjects in the study are presented in Table 1. Of these, 96 patients fulfilled the ASAS axSpA criteria, 38 patients had non-axSpA, and 82 subjects had non-specific LBP with either a positive or negative HLA-B27, but without a positive MRI scan according to ASAS or any clinical SpA features, 40 subjects were BDs, and 43 subjects had AS according to the mNYc.

Female gender was more common in the axSpA and non-axSpA groups than in the LBP and BD groups with a significant difference between the axSpA and BD groups. There was no significant difference between median ages in the three patient groups; however, subjects in the three patient groups were significantly older than in the BD group (p < 0.001). The prevalence of HLA-B27-positive individuals was significantly higher in the axSpA group than in the non-axSpA (p < 0.01) and LBP (p < 0.01) groups. The HLA-B27 prevalence in the BD group was expected to be similar to the prevalence among Danish blood donors (8.86%) (personal communication, T Barington, Department of Clinical Immunology, Odense University Hospital). The proportion of HLA-B27-positive individuals in the LBP group (18.3%) was considered as a consequence of the recruitment strategy.

**Antibodies against capsular extracts of K. pneumoniae serotypes**

The percentages of patients in each group with IgG antibody response against *K. pneumoniae* serotypes K2, K26, K36, and K50 are presented in Figure 2A. No significant differences were found in the IgG antibody responses against K2, K26, K36, or K50, in the axSpA, non-axSpA, and LBP groups. The percentage of individuals with IgG seropositivity for all serotypes was higher in the AS group than in the BD group. In addition, the percentage of seropositive AS patients (25.6%) against K50 was higher compared to the
axSpA group (13.5%, p < 0.05). Regarding the antibody response against IgA, no patient group showed increased percentages of seropositivity compared to the BD group, for any of the serotypes (data not shown).

The percentages of individuals showing simultaneous IgG recognition against ≤1 and ≥2 serotypes are illustrated in Figure 2B. Polymicrobial events (i.e. seropositivity for ≥2 serotypes) were observed in an increased percentage of AS patients (20.9%) compared to BDs (7.5%, p < 0.01).

The axSpA group was stratified according to the mNYc and the polymicrobial events per subgroup were compared to the AS control group as illustrated in Figure 3. Both AS controls and axSpA patients with radiographic sacroiliitis had an increased percentage of individuals recognizing several serotypes (20.9% and 21.4%, respectively) compared to the axSpA mNYc negative subgroup (13.4%).

Correlation between antibody response patterns and patient characteristics

No statistically significant association was found between the percentage of IgG and IgA antibody positive individuals in the groups and patient characteristics (age, gender, HLA-B27 status, hsCRP, and radiographic SIJ lesions according to mNYc) across the groups (data not shown).

Discussion

The clinical relevance of *K. pneumoniae* in axSpA is supported by reports of increased antibody response patterns against this microorganism. However, in this cohort comprising patients classified as having axSpA according to ASAS criteria, non-axSpA and non-specific LBP patients, a difference in the IgG and IgA antibody response pattern against any of the *K. pneumoniae* serotypes in the three patient groups could not be shown, indicating that axSpA cannot be discriminated from non-specific LBP by this method.

The control group of AS patients included increased percentages of IgG seropositive individuals against all serotypes. A previous evaluation of all 77 capsular serotypes showed that HLA-B27-positive AS patients had significantly higher IgG antibody levels against K26, K36, and K50 (18). In addition, others confirmed that a significant percentage of AS patients were IgG positive against K36 (27). We found that AS patients recognized K2, as demonstrated previously by Tiwana et al, although their recognizing antibody was of the IgA isotype (27) and not of the IgG isotype as shown in our study. However, the comparison of all 77 serotypes demonstrated that antibodies towards all other 74 serotypes (including K2) were either absent or undetectable in less than 10% of all serum samples in their study (18).

In accordance with previous reports (18, 19), our results indicate that the anti-*Klebsiella* antibody response in AS patients is predominantly of the IgG and not the IgA isotype. This finding contradicts a previous study which reported elevated IgA antibody levels (27). This discrepancy may be due to different prevalence patterns of *K. pneumoniae* spp. across different geographic regions. For example, the most frequent serotypes isolated from the general population in Japan are K1, K2,
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1 K7, K10, K33, and K43 (28) whereas the most prevalent serotypes in the UK are K2, K3, and K21 (21). To our knowledge, the prevalence of the various K. pneumoniae serotypes has not been determined in Denmark, thus we relied on the findings from our neighbouring countries in the choice of serotypes.

In accordance with an increased IgG seropositivity in the AS control group, axSpA patients with radiographic sacroiliitis showed higher IgG seropositivity than the mNYc group (Figure 3); however, statistical significance was not reached. This might be because this is a subanalysis on the axSpA group, and accordingly there is a marked reduction in group size, and the statistics should be considered accordingly.

axSpA has a multifactorial pathogenesis involving both a genetic predisposition and environmental triggers and develops in some individuals due to an infection. In SpA patients, the most prevalent genetic predisposition is HLA-B27. In a recent review it was suggested that K. pneumoniae is the most likely microbe causative of AS (29). According to this review, the organism’s ability to resemble HLA-B27 and spinal collagens is brought up as a possible explanation for the mode of action. More specifically, Klebsiella-related antibodies are considered to interact with HLA-B27, which is present in different forms in articular synovial tissues, at least when regarding the involvement in inflammatory processes (9, 29). In our study, the IgG anti-Klebsiella antibody response pattern of the axSpA group did not reach the immune response pattern in the AS group. Thus, our findings do not support the hypothesis of the axSpA group being a K. pneumoniae ReA or pre-AS as hypothesized previously (10, 11).

However, when analysing polymicrobial events, a trend possibly supporting the phenomenon of spreading could be noted. Based on these results it could be argued that our cohort and eventually patients diagnosed as axSpA differed significantly from the AS patients. Furthermore, the results also lead to a consideration of the patient inclusion. There is no doubt that the patient groups in the axSpA and AS groups are different. First, the axSpA group for this study was derived from a back pain cohort and the AS control group from Departments of Rheumatology. When considering the large frequency of females in the present axSpA group compared to other SpA cohorts in which the frequency of males is much more dominant (30), the difference becomes clear. Furthermore, the differences could also be due to the low prevalence of HLA-B27-positive individuals in the axSpA group compared to the high prevalence (80–95%) in AS patients (31), as a positive HLA-B27 status is a part of the proposed criteria of the pre-AS hypothesis (10, 11). Finally, Ebinger et al suggested that an elevated antibody response against K. pneumoniae can only be detected in active disease states when inflammation is present in AS patients (11), and as the axSpA group in this cohort represented the disease in the early course, similar antibody profiles were not found.

In conclusion, individuals recruited from primary care with LBP for ≥3 months were IgG seropositive against K. pneumoniae, capsular serotypes K2, K26, K36, and K50 independent of their classification status by the ASAS criteria. Elevated IgG and IgA antibody responses against these serotypes may not be useful as discriminative markers between axSpA and non-specific LBP in early disease. However, the findings in the AS group support a possible role of capsular serotypes not only for K26, K36, and K50 serotypes but also for K2. Furthermore, our results suggest that repeated exposure to this microorganism might play a role in the pathogenesis of AS.

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Figure 3. Polymicrobial IgG antibody response patterns against K. pneumoniae serotypes in axial spondyloarthritis (axSpA) and ankylosing spondylitis (AS; n = 43). Percentage of mNYc positive and negative individuals in the axSpA group and AS control patients seropositive for ≤1 and ≥2 of the capsular serotypes K2, K26, K36, K50; mNYc-, not fulfilling modified New York criteria (n = 84); mNYc+, fulfilling modified New York criteria (n = 12).
References

1. Braun J, Sieper J. Ankylosing spondylitis. Lancet 2007;369:1379–90.
2. Rudwaleit M, Jurik AG, Hermann K-GA, Landewe R, van der Heijde D, Baraliakos X, et al. Defining active sacroiliitis on magnetic resonance imaging (MRI) for classification of axial spondyloarthritis: a consensual approach by the ASAS/OMERACT MRI group. Ann Rheum Dis 2009;68:1520–7.
3. Brown MA, Kennedy LG, MacGregor AJ, Darke C, Duncan E, Shatford JL, et al. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. Arthritis Rheum 1997;40:1823–8.
4. Dougados M, Baeten D. Spondyloarthritis. Lancet 2011;377:2127–37.
5. Pedersen OB, Svendsen AJ, Ejstrup L, Skytte A, Harris JR, Junker P. Ankylosing spondylitis in Danish and Norwegian twins: occurrence and the relative importance of genetic vs. environmental effects in disease causation. Scand J Rheumatol 2008;37:120–6.
6. Reveille JD. Genetics of spondyloarthritis–beyond the MHC. Nat Rev Rheumatol 2012;8:296–304.
7. Girschick HJ, Guilherme L, Inman RD, Latsch K, Rihl M, Sherer D. Pneumonia in the pathogenesis of familial ankylosing spondylitis. Ann Rheum Dis 1997;56:1520–6.
8. Ebringer A, Rashid T, Wilson C, Ptaszynska T, Fielder M. Ankylosing spondylitis and Klebsiella. London, England: Academic Press, 1984:113–64.
9. Girschick HJ, Guilherme L, Inman RD, Latsch K, Rihl M, Sherer D. Pneumonia in the pathogenesis of familial ankylosing spondylitis. Ann Rheum Dis 1997;56:1520–6.
10. Braun J, Sieper J. Ankylosing spondylitis. Lancet 2007;369:1379–90.
11. Reveille JD. Genetics of spondyloarthritis–beyond the MHC. Nat Rev Rheumatol 2012;8:296–304.
12. Sokolove J, Bromberg R, Deane KD, Lahey LJ, Derber LA, Chandra PE, et al. Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. PLoS ONE 2012;7:e35296.
13. van der Woude D, Rantapaa-Dahlqvist S, Ioan-Facsinay A, Onnefield C, Schwartz CM, Verpoort KN, et al. Epitope spreading of the anti-citrullinated protein antibody response occurs before disease onset and is associated with the disease course of early arthritis. Ann Rheum Dis 2010;69:1554–61.
14. Stone MA, Payne U, Schentag C, Rahman P, Pacheco-Tena C, Inman RD. Comparative immune responses to candidate arthritogenic bacteria do not confirm a dominant role for Klebsiella pneumonia in the pathogenesis of familial ankylosing spondylitis. Rheumatology (Oxford) 2004;43:148–55.
15. Podschen R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998;11:589–603.
16. Ørskov I, Fife-Asbury MA. New Klebsiella antigen K82 and the deletion of five of those previously assigned. Int J Syst Evol Microbiol 1997;27:386–7.
17. Ørskov I, Ørskov F. Serotyping of Klebsiella. In: Bergan T, editor. Methods in microbiology, Vol. 14. London, England: Academic Press, 1984:113–64.
18. Sahly H, Kokow J, Podschen R, Schaff M, Gross WL, Ullmann U. Comparison of the antibody responses to the 77 Klebsiella capsular types in ankylosing spondylitis and various rheumatic diseases. Infect Immun 1994;62:4838–43.
19. Sahly H, Podschen R, Sass R, Bröker B, Kekow J, Gross WL, et al. Serum antibodies to klebsiella capsular polysaccharides in ankylosing spondylitis. Arthritis Rheum 1994;37:754–9.
20. Sahly H, Podschen R, Ullmann U. Increased antibody responses to Klebsiella serotypes K26, K36, and K50 in patients with ankylosing spondylitis. Rheumatology (Oxford) 1999;38:481–2.
21. Gaston MA, Aylung-Smith BA, Pitt TL. New bacteriophage typing scheme for subdivision of the frequent capsular serotypes of Klebsiella spp. J Clin Microbiol 1987;25:1228–32.
22. Ambak B, Jensen T, Egund N, Zejden A, Horslev-Petersen K, Manniche C, et al. Prevalence of degenerative and spondyloarthritis-related magnetic resonance imaging findings in the spine and sacroiliac joints in patients with persistent low back pain. Eur Radiol 2016;26:1191–203.
23. Rudwaleit M, van der Heijde D, Landewe R, Listing J, Akko N, Brandt J, et al. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. Ann Rheum Dis 2009;68:777–83.
24. Ambak B, Jensen TS, Manniche C, Zejden A, Egund N, Jurik AG. Spondyloarthritis-related and degenerative MRI changes in the axial skeleton - an inter- and intra-observer agreement study. BMC Musculoskelet Disord 2013;14:274.
25. van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modifications of the New York criteria. Arthritis Rheum 1984;27:361–8.
26. Christiansen A, Hendricks O, Kuettel D, Horslev-Petersen K, Jurik A, Nielsen S, et al. Evaluation of sacroiliac joint radiographs in patients with chronic low back pain: is erosion the main driver of interreader disagreement? [abstract]. Arthritis Rheumatol 2015; 67 (suppl 10):3852–54.
27. Tiwana H, Walmsley RS, Wilson C, Yiannakou YJ, Ciclitira PJ, Wakefield AJ, et al. Characterization of the humoral immune response to Klebsiella species in inflammatory bowel disease and ankylosing spondylitis. Br J Rheumatol 1998;37:525–31.
28. Mori M, Ohta M, Agata N, Kido N, Arakawa Y, Ito H, et al. Identification of species and capsular types of Klebsiella clinical isolates, with special reference to Klebsiella planticola. Microbiol Immunol 1989;33:887–95.
29. Sahly T, Wilson C, Ebringer A. Raised incidence of ankylosing spondylitis among Inuit populations could be due to high HLA-B27 association and starch consumption. Rheumatol Int 2015;35:945–51.
30. Van Tubergen A. The changing clinical picture and epidemiology of spondyloarthritis. Nat Rev Rheumatol 2015;11:110–18.
31. Feldtkeller E, Khan MA, van der Heijde D, van der Linden S, Braun J. Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis. Rheumatol Int 2003;23:61–6.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Supplementary Material: Preparation of capsular extracts from K. pneumoniae.

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