

Correlation between anti-*Proteus* antibodies and isolation rates of *Proteus mirabilis* in rheumatoid arthritis Iraqi patients

*Suzan Y. J., **Khudhair AL-Badri, ***Rashid AL-Muslih
and **** Jasim T. AL-Khafaji

*Department of clinical laboratory sciences, College of Pharmacy, University of AL-Mustansriyah.

**College of Medicine, University of Baghdad

*** Department of Biology, College of Sciences University of Baghdad

****Teaching Laboratories in the medical city

E-mail: suzanyousif78@yahoo.com

Abstract:

Rheumatoid arthritis (RA) is a chronic inflammatory polyarthritic disease associated with remissions and exacerbations and characteristic genetic, clinical, pathological, and immunological features. The present study was designed to examine the evidence linking *Proteus mirabilis* to RA in some Iraqi patients. The study was carried out on 70 Iraqi RA patients, during the period from March 2010 to March 2011. For purposes of comparison, 10 of Systemic Lupus Erythematosus (SLE) patients and 10 of apparently healthy subjects were involved as a control groups. After bacterial isolation and identification, Enzyme linked Immuno sorbent Assay (ELISA) technique has been applied for estimation of antibacterial antibodies.

Results of this study revealed that out of 70 urine samples of RA patients, *E.coli* was present in 12.9%, followed by *P. mirabilis* which present in 7.1%. Regarding SLE and healthy control group, urine samples were negative from any bacterial strains except *E. coli*, which present in about 10% of each group. Frequency of *Proteus mirabilis* in RA group was highly significant when compared with that of other groups ($p < 0.01$). All rheumatoid arthritis patients and control groups were tested by ELISA technique for detection of IgM, IgG and IgA antibodies specific to *Proteus mirabilis*. Results demonstrated that the levels of these antibodies were elevated in the sera of RA patients with a high significant degree, in comparison with control groups ($p < 0.01$). The same sera were tested for anti- *E. coli* antibodies and the results showed that there was no significant difference in the IgM and IgG antibodies level to *E. coli* in RA patients when compared with control groups ($p > 0.05$).

In conclusion, these findings suggested a possible association between infection with *Proteus mirabilis* and rheumatoid arthritis. The elevated levels of *Proteus mirabilis* antibodies within RA sera could be helpful in the identification of those patients during early stages of the disease.

Key words: Rheumatoid arthritis, *Proteus mirabilis*.

العلاقة بين الاجسام الضادة لبكتريا *proteus* ونسبة عزلها في المرضى العراقيين المصابين بالتهاب المفاصل الرثوي

سوزان يوسف جاسم*, خضير البدرى**, رشيد المصلح*** وجاسم الخفاجي****

* فرع العلوم المخبرية السريرية، كلية الصيدلة، الجامعة المستنصرية

**كلية الطب، جامعة بغداد

***قسم علوم الحياة، كلية العلوم، جامعة بغداد

****المختبرات التعليمية، مدينة الطب

الخلاصة:

التهاب المفاصل الرثوي هو مرض مزمن يصيب المفاصل ويمر بأطوار متناوبة من الشفاء والانتكاس حيث ان له صفات جينية، سريرية، مرضية، ومناعية مميزة. صممت الدراسة الحالية لفحص دلائل ارتباط بكتريا الـ *Proteus mirabilis* بمرض التهاب المفاصل الرثوي.

أجريت الدراسة على 70 مريضاً عراقياً مصاباً بمرض التهاب المفاصل الرثوي للفترة من اذار 2010- اذار 2011. ولغراض المقارنة فقد شملت الدراسة ايضا على 10 مرضى مصابين بداء الذئبة الحُمَامِيَّة المَجْمُوعِيَّة و 10 اشخاص اصحاء ظاهرياً. بعد عزل وتشخيص البكتريا طبق اختبار الانزيم المرتبط الممتز للتحري عن الاجسام المضادة للبكتريا المعزولة. من مجموع 70 عينة ادرار عائدة لمرضى التهاب المفاصل الرثوي عزلت بكتريا الـ *E. coli* بنسبة 12.9% والـ *Proteus mirabilis* بنسبة 7.1%، في حين كانت النسب المناظرة في مجاميع السيطرة معدومة لكل انواع البكتريا باستثناء بكتريا الـ *E. coli* التي ظهرت في 10% من عينات الادرار لكلا المجموعتين. كانت نسبة عزل بكتريا الـ *Proteus mirabilis* في مرضى التهاب المفاصل الرثوي مرتفعة معنوياً اذا ما قورنت نسبتها مع المجاميع الاخرى ($p < 0.01$).

أظهرت نتائج التحري عن اعداد بكتريا الـ *Proteus mirabilis* (IgM, IgG, IgA) ارتفاعاً معنوياً عالياً في مستوى جميع الاضداد مقارنة مع مجاميع السيطرة ($p < 0.01$)، فضلاً عن إن ايجابية اعداد الـ *Proteus mirabilis* كانت مرتفعة معنوياً في مصول مرضى التهاب المفاصل الرثوي مقارنة مع مجاميع الدراسة الاخرى ($p < 0.01$). في ضوء النتائج اعلاه يمكننا الاستنتاج بان هنالك علاقة بين بكتريا الـ *Proteus mirabilis* ومرض التهاب المفاصل الرثوي حيث ان ارتفاع اعداد الـ *Proteus mirabilis* في مصول الاشخاص المصابين بهذا المرض يمكن ان يساعد في تشخيص هؤلاء المرضى خلال المراحل المبكرة منه.

Introduction:

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease that primarily affects the synovium, with local symptoms of joint swelling, pain and morning stiffness, inflammation, and subsequent cartilage damage with joint destruction^[1,2].

Rheumatoid arthritis considered as an immune-mediated disease that could possibly be triggered by an environmental factors in a genetically susceptible individuals^[3].

Numerous studies have demonstrated that some viruses, bacteria, and some other microorganisms might be related with the pathogenesis of RA through their previous or chronic infection^[4,5,6].

Many clinical and laboratory methods used to find the role of microorganisms in the pathogenesis of rheumatoid arthritis. During the last three decades, serological studies were carried out by different independent groups on microbial antibodies against various microbial antigens in patients with RA and other rheumatic diseases. One study showed that certain microorganisms could be detected in the blood of patients with RA^[6], while some investigators showed that the antibody titers for certain

microorganisms in the serum of patients with RA are higher than those in the control group^[7]. The high titers of anti-*Proteus* antibodies in RA patients appeared to be specific because there was no such elevation in antibodies against other microorganisms^[8].

Previous clinical studies support the suggestion that there is a link between RA and urinary tract infection (UTI) mainly caused by *Proteus mirabilis*. Patients with RA were reported to have a higher frequency of recurrent urinary tract infection^[9]. Urine samples from RA patients yield higher isolation rates of *Proteus mirabilis* than patients with osteoarthritis or healthy controls^[10]. Rheumatoid arthritis patients had higher levels of antibodies against *Proteus mirabilis* in their urine when compared to those of healthy subjects^[11].

The present study was designed to examine the evidence linking *Proteus mirabilis* to RA in some Iraqi patients

Materials and Methods:

This randomized clinical study was carried out on (70) Iraqi RA patients who were referred to the consultant clinic at the department of Rheumatology, Baghdad Teaching Hospital. For purposes of

comparison, 10 of Systemic Lupus Erythematosus (SLE) patients (from the department of Rheumatology) and 10 of apparently healthy subjects (from the National Center of Blood Transfusion), were involved as a control groups.

Samples:

Ten milliliters (10 ml) of venous blood were collected from patients as well as controls. Serum was separated and kept at deep freeze (-20° C) to be used for different investigations.

The midstream urine was collected into sterile wide-mouth container from patients as well as controls. General urine examination was carried out for the detection of UTI and cultured into appropriate media for bacterial isolation.

Bacterial isolation and identification:

The collected samples were streaked on MacConkey agar and blood agar plate. Plates were incubated at 37°C for 24 hrs [8]. The isolates were identified depending on the colonial characteristics on the culture media, microscopic examination and biochemical tests according to Bergey's manual of systematic bacteriology [9] and methods mentioned in other literature reviews [14].

Enzyme immunoassay for estimation of bacterial antibodies:

Antibacterial antibodies were estimated by using ELISA kit especially prepared for this purpose depending on methods mentioned in previous literature reviews [15,16,17].

Statistical analysis:

Chi-Square test (χ^2), Analysis of variance (ANOVA) and Least Significant Difference (LSD) were used to accept or reject the statistical hypotheses [18].

Results:

Bacterial Isolation:

Out of 70 urine samples of RA patients, *E.coli* was present in 12.9%, followed by *P. mirabilis* which present in 7.1%, while *Proteus vulgaris* and *Klebsiella* were present in 1.4% of those patients. Regarding SLE and HC groups,

Date of acceptance: 20-4-2014

urine samples were negative from any bacterial strains except *E. coli*, which present in about 10% of each group, as demonstrated in figure-1.

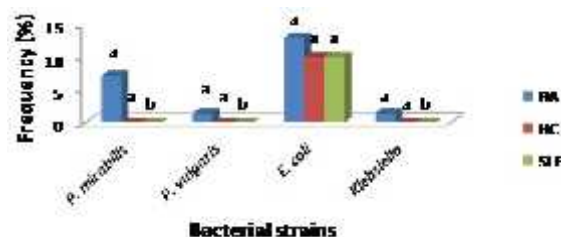


Figure-1: Frequency of bacterial strains among studied groups.

RA= rheumatoid arthritis, SLE= systemic lupus erythematosus, HC= healthy control. Results with non identical superscripts (a, b) within different studied groups were considered as a highly significant difference ($p<0.01$).

Antibodies levels to *P. mirabilis* among sera of the studied groups:

Figure-2 show that the frequency of *P. mirabilis* antibodies for RA patients sera was significantly higher than those of control groups ($p<0.01$).

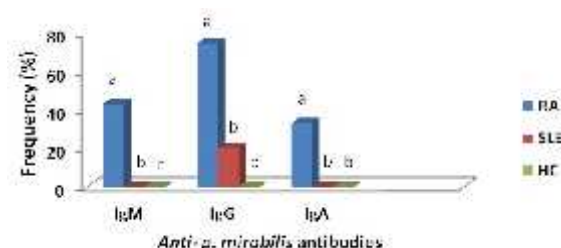


Figure-2: Frequency of anti-*proteus* antibodies among sera of the studied groups.

RA= rheumatoid arthritis, SLE= systemic lupus erythematosus, HC= healthy control. Results with non identical superscripts (a, b, c) within different studied groups were considered as a highly significant difference ($p<0.01$).

Table-1 document that the levels of IgM, IgG and IgA to *P. mirabilis* (0.37 ± 0.1 , 0.76 ± 0.3 and 0.22 ± 0.1 , respectively) were elevated in the sera of RA patients with a highly significant degree in comparison with HC and SLE groups ($p<0.01$). However, the levels of

IgG antibodies were significantly higher ($p<0.01$) than those of IgM and IgA antibodies

Table- 1: Mean distribution of OD value for IgM, IgG and IgA antibodies to *P. mirabilis* among studied groups.

Studied groups	N	Mean of OD value \pm SEM		
		IgM	IgG	IgA
RA	70	0.37 \pm 0.1 ^a	0.76 \pm 0.3 ^{a*}	0.22 \pm 0.1 ^a
SLE	10	0.09 \pm 0.01 ^b	0.26 \pm 0.12 ^b	0.06 \pm 0.12 ^b
HC	10	0.07 \pm 0.05 ^b	0.21 \pm 0.05 ^b	0.09 \pm 0.06 ^b

SEM: standard error of mean Results with non identical superscripts (a,b) were considered as a highly significant difference among the studied groups ($p<0.01$).

*= highly significant difference within the same group ($p<0.01$).

To investigate the relationship between IgM, IgG and IgA antibodies to *p. mirabilis* and IgM, IgG and IgA antibodies to *E. coli*, the same sera were tested for IgM, IgG and IgA to *E. coli*.

The results showed that there was no significant difference in the IgM and

IgG antibodies level to *E. coli* in RA patients when compared with control groups ($p>0.05$), while IgA antibodies level was elevated significantly in RA patients when compared with control groups ($p<0.05$), as present in table-2.

Table -2: Mean distribution of OD value for IgM, IgG and IgA antibodies to *E. coli* among studied groups.

Studied groups	N	Mean of OD value \pm SEM		
		IgM	IgG	IgA
RA	70	0.05 \pm 0.02 ^a	0.28 \pm 0.1 ^a	0.39 \pm 0.1 ^{a*}
SLE	10	0.03 \pm 0.09 ^a	0.27 \pm 0.12 ^a	0.22 \pm 0.12 ^b
HC	10	0.02 \pm 0.05 ^a	0.30 \pm 0.05 ^a	0.18 \pm 0.06 ^b

SEM: standard error of mean RA= rheumatoid arthritis, SLE= systemic lupus erythematosus, HC= healthy control.

Results with non identical superscripts (a, b) were considered as a significant difference among the studied groups ($p<0.05$).

*= significant difference within the same group ($p<0.05$).

Table- 3: Mean distribution of OD value for IgM, IgG, and IgA antibodies to *P. mirabilis* and *E. coli* among sera that give positive results for *P. mirabilis* antibodies.

Studied groups		Mean of OD value \pm SEM					
		N	IgM	N	IgG	N	IgA
RA	<i>P. mirabilis</i>	30	0.51 \pm 0.4 ^ψ	52	0.82 \pm 0.1 ^{aψ*}	23	0.35 \pm 0.2 ^ψ
	<i>E. coli</i>		0.03 \pm 0.02		0.10 \pm 0.03		0.16 \pm 0.01
SLE	<i>P. mirabilis</i>	0	-	2	0.28 \pm 0.1 ^{b*}	0	-
	<i>E. coli</i>		-		0.22 \pm 0.12		-
HC	<i>P. mirabilis</i>	0	-	0	-	0	-
	<i>E. coli</i>		-		-		-

SEM: Standard error of mean RA= rheumatoid arthritis, SLE= systemic lupus erythematosus, HC= healthy control.

Results with non identical superscripts (a, b) were considered as a highly significant difference ($p < 0.01$) among the studied groups.

ψ= highly significant as compared with *E. coli* within the same group ($p < 0.01$).

*= highly significant as compared with other Ig-isotypes within the same group ($p < 0.01$).

Discussion:

In a total of 70 RA patients, *E. coli* was present as a single causative pathogen in 11.4% of patients, while the corresponding percent of frequency for *P. mirabilis* was 5.7%. Additionally, 1.4% of patients showed mixed infection of *E. coli* and *P. mirabilis*.

Results of the present study demonstrated that *P. mirabilis* isolated in frequency of about 7.1% from RA patients, and there is a highly significant difference when compared with that of other groups ($p < 0.01$). These results were consistent with previous study which recorded that *P. mirabilis* was the principal causative agent of UTI in RA patients [19]. Another study recorded that urine samples from RA patients yield higher isolation rates of *P. mirabilis* than patients with osteoarthritis or healthy control subjects [10]. Various microbiological and immunological data results support the suggestion that there is a link between RA and UTI mainly caused by *P. mirabilis* [3, 11, 20].

In the current study, *P. mirabilis* isolated from the urine of RA patients in a percent of 7.1%, as demonstrated in figure 1, while the results of antibodies levels to *P. mirabilis* indicate the presence of high frequency of *P. mirabilis* specific

antibodies among the sera of RA patients, as shown in figure-2. This may attribute to the incorrect information history that taken from the patients who received treatment when the urine samples were taken from them or due to the previous infection, which could explain the high frequencies of IgG anti-*P. mirabilis* antibodies among the sera of RA patients. These results not agree with findings of many other studies which denoted the presence of positive correlation between anti-*Proteus* antibody levels in serum samples and the number of *Proteus* colony-forming units obtained from urine specimens of RA patients [21, 22].

The current study demonstrated high titers of IgG anti-*Proteus* antibodies in sera of RA patients compared to control groups using ELISA technique. The difference was statistically highly significant ($p < 0.01$) (table-1). These results were comparable to many other studies which found that the RA patients under studies showed a significant elevation in IgG anti-*Proteus* antibodies compared to control groups [8, 23, 24].

Certain studies detected a different results, were they found that RA patients showed a significant elevation in IgM anti-*Proteus* antibodies in sera of RA patients compared to control groups ($p < 0.05$) [16, 17].

The difference in the types of anti-*Proteus* antibodies attributed to the duration of the disease. The detection of IgG anti-*Proteus* antibodies indicate a previous infection because IgG has a long half-life in the serum (about month), while the detection of IgM anti-*Proteus* antibodies indicate a recent infection because IgM has a short half-life in the serum (about week) compared with IgG^[16].

In the current study, the levels of IgM and IgG anti-*E.coli* were comparable among the studied groups, only the IgA anti-*E.coli* being higher in the RA group compared with control groups. These findings were consistent with the previous studies^[8,26]. Senior *et al.* previously reported a slight elevation of antibodies to *E. coli* in RA patients but the differences did not reach statistical significance^[16].

The *p. mirabilis* positive sera reveal significant elevation in IgM, IgG and IgA antibodies to *p. mirabilis* in comparison with IgM, IgG and IgA antibodies to *E. coli* ($p < 0.01$) (table 2 and 3); while the levels of IgM, IgG and IgA antibodies to *p. mirabilis* in all serum samples (nine), which taken from RA patients that have positive culture for *E. coli*, were lower than the cut-off value for *p. mirabilis* antibodies.

Epidemiological studies reported an association between *P. mirabilis* seropositivity and rheumatoid arthritis^[11,27]. During the last three decades, many serological studies were carried out by different independent groups on microbial antibodies against various microbial antigens in patients with RA and other rheumatic diseases. Antibodies against *P. mirabilis* were found to be significantly elevated among a total of 1375- RA patients, but not in other diseases or healthy subject controls, from more than 15 different countries^[12]. The high titers of anti- *p. mirabilis* antibodies in RA patients would appear to be specific because there was no such elevation in antibodies against 27 other microbial

agents (*Klebsiella*, *E.coli*, *Yersinia*, *Salmonella*, *Chlamydia*, *Shigella*, *Pseudomonas*, *Serratia*, *Campylobacter*, and normal bowel flora species)^[28].

In conclusion the results of the present study demonstrated a possible association between infection with *P. mirabilis* and rheumatoid arthritis, but how the bacteria or the antibodies or both contribute to the disease process is not clear.

References:

- 1- Ruzickova, S.; Cimburek, Z. and Moravcova, T. Citrullinated peptide-specific CD19+B cells are present in synovial tissue and peripheral blood of patients with rheumatoid arthritis. *Arthritis Res. ther.* 2005. Vol. 7(1). Pp: 149.
- 2- Goronzy, J. and Weyand, E. Developments in the scientific understanding of rheumatoid arthritis. *Arthritis Res. Ther.* 2009. Vol. 11. Pp: 249.
- 3- Ebringer, A. and Rashid, T. Rheumatoid arthritis is an autoimmune disease triggered by *Proteus* urinary tract infection. *Clin. Develop. Immunol.* 2006. Vol. 13. Pp: 41-8.
- 4- Ford, D. K. One syndrome-many infectious agents. *J. Rheumatol.* 1987. Vol. 14. Pp: 650-2.
- 5- Tingle, A. J. One-infectious agent-many syndromes. *J. Rheumatol.* 1987. Vol. 14. Pp: 653-5.
- 6- Kempell, k.; Cax, C. and Hurle. M. Reverse transcriptase-PCR analysis of bacterial rRNA for detection and characterization of bacterial species in arthritis synovial tissue. *Infect. Immun.* 2000. Vol. 68. Pp:6012-26.
- 7- Krause, A.; Kamradt, T. and Burmester, G.R. . Potential infectious agents in the induction of arthritides. *Curr. Opin. Rheumatol.* 1996. Vol. 8. Pp: 203-9.
- 8- Subair, H.; Tiwana, H. and Fielder, M. Elevation in anti-*Proteus* antibodies in patients with rheumatoid arthritis from Bermuda and England. *J.Rheumatol.* 1995. Vol. 22. Pp: 1825-8.
- 9- Tishler, M.; Caspi, D.; Aimgo, Y.; Segal, R. and Yaron, M. Increased incidence of

- urinary tract infection in patients with rheumatoid arthritis and secondary Sjogren syndrome. *Ann. Rheum. Dis.* 1992. Vol. 51. Pp: 601-6.
- 10- Ebringer, A.; Wilson, C. and Ahmadi, K. Rheumatoid arthritis as a reactive arthritis to *Proteus* infection: Prospects for therapy. In: Machtey, I., Progress in rheumatology, sixth international seminar on the treatment of rheumatic diseases. 1993. pp: 77-83.
 - 11- Senior, B.W.; Anderson, G.A. and Morley, K.D. Evidence that patients with rheumatoid arthritis have asymptomatic non-significant *Proteus mirabilis* bacteriuria more frequently than healthy controls. *J. Infect.* 1999. Vol. 38. Pp: 99-106.
 - 12- Forbes, B. A.; Sahn, D. F. and Wissfeld, A. S. Infections of the urinary tract. In: Diagnostic Microbiology. 1998. 10th ed. Mosby, USA. Pp: 355-61.
 - 13- Garrity, G. M.; Brenner, D. J. and Krieg, N. R. Bergeys manual of Systematic Bacteriology. 2005. 2nd ed. East Lansing, USA.
 - 14- MacFaddin, J. F. Biochemical tests for identification of medical bacteria. 2000. 3rd ed. Lippincott Williams and Wilkins, London.
 - 15- Tijssen, P. Laboratory techniques in biochemistry and molecular biology. In: Practice and theory of enzyme immunoassay. 1985. 15. Pp: 358-6.
 - 16- Senior, B. W.; McBride, P. D. and Morley, K. D. The detection of raised levels of IgM to *Proteus mirabilis* in sera from patients with rheumatoid arthritis. *J. Med. Microbiol.* 1995. Vol. 43. Pp: 176-84.
 - 17- Newkirk, M. M.; Goldbach-Mansky, R. and Senior, B. W. Elevated levels of IgM and IgA antibodies to *Proteus mirabilis* and IgM antibodies to *Escherichia coli* are associated with early rheumatoid factor (RF)-positive rheumatoid arthritis. *Rheumatol.* 2005. Vol. 44. Pp: 1433-41.
 - 18- Daniel, W. W. biostatistics: A foundation for analysis in the health sciences. 2009. 10th ed.
 - 19- AL-Sheikh, S. K. Studying the causal relationship between cytokines, systemic immunity and urinary tract infection in autoimmune disease. 2011. Ph. D. thesis. College of Science for Women. University of Baghdad.
 - 20- Foxman, B.; Barlow, R. and Darcy, H. Urinary tract infection: Self reported incidence and associated costs. *Ann. Epidemiol.* 2000. Vol. 10. Pp: 509-15.
 - 21- Thakore, A.; Wilson, C.; Isenberg, D. Correlation between anti-*Proteus* antibodies and isolation rates of *P. mirabilis* in rheumatoid arthritis. *Rheumatol. Int.* 1997. Vol. 16. Pp: 187-9.
 - 22- Ebringer, A.; Rashid, T. and Wilson, C. Rheumatoid arthritis: proposal for the use of anti-microbial therapy in early cases. *J. Rheumatol.* 2003. Vol. 32. Pp: 2-11.
 - 23- Yoshi, T.; Harmale, T.; Sinsuke, H.; Junichi, N.; Mark, F.; Clyde, W.; Sukhvinder, B. and Ebringer, A. Antibodies to *Klebsiella*, *Proteus* and HLA-B27 peptides in Japanese patients with ankylosing spondylitis and rheumatoid arthritis. *Br. J. Rheumatol.* 1995. Vol. 31. Pp: 241-5.
 - 24- Ushakova, M. A.; Muravijuv, U. V.; Vaneeva N.P. The *p.mirabilis* antibody levels in patients with rheumatoid arthritis. *Rheumatol.* 2000. Vol. 14. Pp: 78.
 - 25- Wilson, C.; Ebringer, A. and Ahmadi, K. Shared amino acid sequences between major histocompatibility complex class II glycoproteins, type XI collagen and *P.mirabilis* in rheumatoid arthritis. *Ann. Rheum. Dis.* 1995. Vol. 54. Pp: 216-20.
 - 26- Fielder, M.; Tiwana, H.; Youinou, P. The specificity of the anti-*Proteus* antibody response in tissue-typed rheumatoid arthritis (RA) patients. *Rheumatol. Int.* 1995. Vol. 15. Pp: 79-82.
 - 27- Blankenberg, S. H.; Fielder, M.; Feltkamp, T. E. Antibodies to *Proteus mirabilis* in Dutch patients with rheumatoid arthritis. *J. Rheumatol.* 1998. Vol. 25. Pp: 743-7.
 - 28 - Rashid, T. and Ebringer, A. Rheumatoid arthritis is linked to *Proteus* -the evidence. *Clin. Rheumatol.* 2006. Vol. 26. Pp: 1036-43.