Association between rheumatoid arthritis and urinary tract infections caused by *Proteus spp.*: a review

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ABSTRACT: Rheumatoid arthritis (RA) is a systemic and arthritic autoimmune disease that affects millions of people worldwide. The World Health Organization has reinforced its concerns regarding RA morbidity. Characterized by inflammation of the joints which can lead to the destruction of the periarticular tissue, causing pain and joint deformities. During the last four decades, scientific data has suggested that urinary tract infections (UTI) caused by *Proteus* spp. have a key role in the aetiopathogenesis of RA. Here, we performed a qualitative systematic review of the available literature regarding the association between RA and UTI caused by *Proteus spp.* in a worldwide perspective. We selected four studies that either show increased isolation of *Proteus* spp. in the urine of patients with RA or show elevated levels of anti-*Proteus* antibodies in the serum of RA patients, of the population of different countries from three continents, always comparing with healthy and/or non-RA disease controls. This work reinforces the evidence linking *Proteus* spp. to RA, from the recurrent sub-clinical *Proteus* UTIs to the full development of RA. Antimicrobial susceptibility testing guided therapy must be considered crucial to ensure therapeutic success and prevent or minimize the occurrence of RA associated with UTI and future research should be performed with the aim to access if the usage of antibiotics against *Proteus spp.* from the urinary tract could be implemented as adjuvant therapies to treat RA. Additionally, due to the fact that no research regarding the described association has been performed in Portugal, we suggest the development of a future research project, to access if the Portuguese population follows the trend of the countries referred to in this review.

Keywords: Rheumatoid arthritis; Urinary tract infections; Proteus spp.; Shared epitope

Associação entre artrite reumatoide e infeções do trato urinário causadas por *Proteus spp.*: revisão

RESUMO: A artrite reumatoide (AR) é uma doença crónica, inflamatória e autoimune que afeta milhões de cidadãos a nível mundial. A Organização Mundial da Saúde reforçou as suas preocupações relativamente à morbilidade da AR, caracterizada pela inflamação das articulações, que pode conduzir à destruição do tecido articular e periarticular, provocando dor e deformação das articulações. Durante as últimas quatro décadas, estudos sugeriram que infeções do trato urinário (ITU) provocadas por *Proteus spp.* possam apresentar um importante papel na patogénese da AR. Deste modo, realizou-se uma revisão sistemática qualitativa da literatura disponível sobre a associação entre AR e ITU causadas por *Proteus spp.* assente numa perspetiva mundial. Foram selecionados quatro estudos que mostram a existência de um aumento do isolamento de *Proteus spp.* na urina de pacientes com AR ou que mostram a presença de níveis elevados de anticorpos anti-*Proteus* no soro de pacientes com AR da população de diferentes países de três continentes, comparando sempre com pacientes saudáveis, ou seja, indivíduos que não possuam AR. Esta revisão reforça a existência de uma associação entre *Proteus spp.* e AR, desde a ocorrência de ITU recorrentes até ao total desenvolvimento de AR. A terapêutica guiada com auxílio de testes de suscetibilidade antimicrobiana deve ser considerada crucial para garantir o sucesso terapêutico e prevenir ou minimizar a ocorrência de AR associada à ITU e futuros estudos devem ser implementados com o

objetivo de avaliar a utilização de antibióticos contra *Proteus spp.* como uma potencial terapêutica para a AR. Adicionalmente, devido ao facto de, até ao momento, não ter sido realizado nenhum estudo sobre a associação analisada nesta revisão em Portugal, sugere-se o desenvolvimento de um futuro projeto de investigação, de modo a ser possível avaliar se a população portuguesa segue a tendência dos restantes países incluídos na presente revisão.

Palavras-chave: Artrite reumatoide; Infeções do trato urinário; Proteus spp.; Epítopo partilhado

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease[1-3] which makes the immune system improperly attack healthy cells in the organism, mainly affecting peripheral joints[4]. RA is characterized by small joint inflammation, causing damage to the articular tissue[2]. Thus, it is classified as a disabling disease that usually causes pain and deformation. The World Health Organization indicates that in a period of 10 years after the onset of the disease, at least 50% of patients living in developed countries will not be able to maintain a full-time job[5].

RA therapy is mainly focused on reducing joint inflammation because if it disappears quickly, its progression rate will be reduced, and patients' physical function can be improved to the maximum without additional drawbacks[3]. To accomplish this, the main drugs used for the treatment of RA are disease-modifying antirheumatic drugs which reduce structural damage progression; non-steroidal anti-inflammatory drugs while reducing pain and stiffness and improving physical function, do not interfere with joint damage; and glucocorticoids, which offer rapid symptomatic effects, but are associated with serious long-term adverse effects[3]. These include accelerated cardiovascular disease, insulin resistance, and altered bone metabolism[6].

Worldwide, different research groups and scientific data have correlated a higher isolation rate of *Proteus spp.* in urine samples from patients with RA in the respective population, which has been suggested to have a key role in the aetiopathogenesis of RA[7-9]. However, no data is available regarding RA association with UTI caused by *Proteus spp.* in the Portuguese population.

Rheumatoid arthritis state of the art

RA is not a rare disease as its frequency varies between 0.5-1.5% of individuals in industrialized countries. For example, the prevalence of RA in the Portuguese population ranges more specifically from 0.8 to 1.5%. The overall occurrence of RA is two to four times higher in women than in men. The peak incidence in women is after menopause but people of all ages can develop the disease including teenagers and adults during the most productive years of their life (between 20 and 40 years of age)[1,5].

The cause underlying the dysregulation of the immune system associated with RA is unknown. However, research in this area points to some of the risk factors that can increase the likelihood of developing the disease, which includes age (RA can begin at any age, but the likelihood increases with age), sex (new cases of RA are typically two-to-three times higher in women than men), smoking (cigarette smoking increases a person's risk of developing RA and can make the disease worse), obesity (being obese can increase the risk of developing RA) and genetics/inherited traits (people born with specific genes are more likely to develop RA)[2].

This last risk factor is probably the most important one. The HLA (human leukocyte antigen) class II genes can also make arthritis worse. The risk of RA may be highest when people with these genes are exposed to environmental factors like smoking or when a person is obese[2].

It is known that there is a strong genetic predisposition for RA. It occurs predominantly in individuals with the HLA-DR1 or HLA-DR4 haplotype (approximately 90% of patients with severe RA have been found with this type of haplotype). The common denominator in HLA-DR4 and HLA-DR1 individuals is a highly immunogenic sequence due to the charged amino acids. This sequence (EQRRAA) has been called the 'RA susceptibility sequence' or 'shared epitope' and has been found in several HLA groups that are frequently observed in RA patients[7,9].

Patients diagnosed with RA have an increased risk of developing infections, being the majority originated in the respiratory tract (pneumonia), comparing to patients without RA[10]. The reasons associated with this increased risk of infection are multifactorial and include the immune disorder underlying the disease process, immunosuppressive medication for the treatment of RA, and other immune-compromising comorbidities. However, it is suggested that there is a trend according to which RA patients appear to be hospitalized as a result of urinary tract infections (UTI) as commonly as from pneumonia[4,11].

Based on previous studies, it has been reported that there is a higher incidence of UTI in patients with RA[11]. A UTI is a microbial infection that can affect any part of the urinary tract including the kidneys, ureters, bladder, and urethra[12]. It can be categorized anatomically. If it is in the bladder it is called cystitis; which is the most common clinical manifestation of UTI; if there is renal involvement it is called pyelonephritis. Cystitis and pyelonephritis can both be subcategorized, into being either an uncomplicated or complicated infection[12-13]. UTI affects women more than man given the proximity of the female urethra to the anus and vagina, and the fact that it is much shorter than males urethra which allows microorganisms to reach the bladder more easily[14]. Most UTIs are caused by the *Escherichia coli (E. coli)*, but it is also common to find other microorganisms that cause UTI, namely *Klebsiella pneumoniae, Proteus mirabilis* (*P. mirabilis*), *Enterococcus*

faecalis, and *Staphylococcus saprophyticus*[15]. In women who are not pregnant, diagnostic of UTI include dysuria, urgency, and frequency of micturition, pyuria, and the presence of high numbers of bacteria in the urine[13]. The most common factors associated with UTI include diabetes, pregnancy, renal failure, urinary tract obstruction, immunosuppression, and functional or anatomic abnormalities of the urinary tract[12]. Interestingly, previous studies have also demonstrated a higher isolation rate of *Proteus* bacteria in urine samples from patients with RA[7-8].

Proteus spp. infections and treatment

Proteus spp. bacteria were first described in 1885 by Gustav Hauser, who had revealed their feature of intensive swarming growth. Currently, the genus is divided into *Proteus mirabilis*, *Proteus vulgaris*, *Proteus penneri, Proteus hauseri*, and three unnamed genomospecies 4, 5, and 6 and consists of 80 O-antigenic serogroups[16]. However, the most common species to cause infection and consequently the most studied ones are *P. mirabilis* and *P. vulgaris*.

Proteus spp. are Gram-negative rod-shaped microorganism, well-known for its urease production and its distinctive swarming behaviour characterized by the development of concentric rings of growth that are formed as cyclic events of swarmer cell differentiation, swarming migration, and cellular differentiation is repeated during colony translocation across a surface producing the so-called bull's-eye colony. *Proteus spp*. belongs to the class *Gammaproteobacteria* and has long been recognized as a member of the order *Enterobacteriales*, family *Enterobacteriaceae*[17-18]. *Proteus spp*. can cause symptomatic infections of the urinary tract including cystitis and pyelonephritis and is present in cases of asymptomatic bacteriuria, particularly in the elderly and patients with type 2 diabetes. It is currently acknowledged that the main risk factors for *P. mirabilis* UTI are anatomical and/or functional abnormality in the urinary tract and/or the presence of a catheter and can cause the formation of urinary stones. It is thought that the majority of UTI caused by *P. mirabilis* result from the ascension of bacteria from the gastrointestinal tract while others are due to person-to-person transmission, particularly in healthcare settings. In addition to UTI whereas *P. mirabilis* causes between 1-10% of all diagnosed infections, this species can also cause infection in the respiratory tract, eye, ear, nose, skin, throat, and wounds[19].

Currently, no specific recommendations regarding therapeutic approaches are available for complicated- or catheter-associated UTI, whereas antibiotic treatment is based on the clinical situation and the clinician's discretion in accordance with laboratory diagnostics and antimicrobial susceptibility testing (AST). On the other hand, for acute uncomplicated cystitis, it is recommended double-strength trimethoprim-sulfamethoxazole (SXT) and in cases of local SXT resistance (above 10-20%), antibiotic therapy can include fluoroquinolones, nitrofurantoin, or fosfomycin, nevertheless, resistance may appear for all indicated antibiotics and consequently, AST guided therapy is of foremost importance to ensure therapeutic success[19].

Proteus spp. and RA aetiopathogenesis

Recently, Ebringer and Rashid have advocated that UTI caused by *Proteus spp*. are responsible for triggering RA in a healthy individual[9,20]. Through genetic, biological, and immunological studies, it was concluded that RA is a disease produced by autoantibodies against joint tissue that were evoked by UTI caused by *Proteus* spp. microbes. This concept is because a bacterial sequence ESRRAL found in *Proteus* haemolysin cross-reacts with the 'shared epitope' found in the HLA-DR1 and HLA-DR4 molecules. Thus, antibodies to *Proteus* haemolysin will bind to cells containing such HLA antigens and in the presence of complement cause cytopathic damage which will lead to inflammation. On the other hand, there is the IRRET sequence found in *Proteus* urease resembles or cross-reacts with the LRREI sequence present in type XI collagen, a component of hyaline cartilage that is found especially in the small joints of hands and feet. Thus, antibodies to *Proteus* urease will bind to tissues containing collagen XI, namely hyaline cartilage, and in presence of complement cause cytopathic damage which will lead to synovial inflammation[9,20].

Several studies have been performed in various countries around the world, some of which were already mentioned and/or were used for the elaboration of this review. However, to date no investigation study that seeks to demonstrate the association described in this article has been conducted in the Portuguese population.

In this context, the main objective of this review is to gather the important information of four selected studies, about the association between RA and UTI caused by *Proteus spp*. We intend that this could be used as base work for the performance of a study in our country.

Materials and methods

An exhaustive search was performed for papers available in scientific databases reporting EDCs mixtures effects and associated mechanisms. The articles presented and discussed were obtained using diverse scientific databases such as PubMed and Google Scholar and using the following keywords: 'Rheumatoid arthritis', '*Proteus*', 'UTI', and 'population'.

Inclusion and exclusion criteria were established before the bibliographic search. Studies published in languages other than English were considered only if an abstract was available. Additionally, for the study selection, we used the following criteria: studies performed only in human beings from a range of 20 years to the moment of this review, to include data from the past two decades, and that represents the population of the country where the study was performed.

The main limitations that we encountered when performing the study selection were the fact that most of them were not recent (the majority was published before 2010), the selected studies did not provide all their results, mainly the ones obtained from statistical analysis and there was a reduced number of studies that used urine samples as their main biological investigation element.

For the elaboration of this review, we used 4 articles in which we sought to analyze and relate their information about RA, UTI, and *Proteus spp*. These articles correspond to studies that were carried out in the United Kingdom[7], Greece[21], Japan, Finland[22], USA and Canada[23] and are summarised in the Appendix.

Worldwide evidence of *Proteus spp*. role in the aetiopathogenesis of RA

In this review, we will describe the scientific evidence regarding the association between RA and UTI caused by *Proteus spp*. through a global perspective, more precisely from studies performed in Europe, Asia, and North America.

In Europe, studies were carried out in the United Kingdom, Greece, and Finland. Regarding the United Kingdom one, the RA patient group consisted of 76 known local patients who attended the Rheumatology outpatient clinic, and the control group comprised 48 healthy individuals without RA[7]. Concerning the Greek population, the authors studied 63 patients diagnosed with RA who belonged to the outpatient clinic of the rheumatology department and a healthy control group of 38 individuals[21]. About the Finnish population, 129 serum samples were obtained, from which 72 belonged to patients with early RA (less than 12 months since disease diagnosis) and 27 samples that belonged to patients with advanced RA. All of these, attended the Second Department of Medicine, University of Helsinki and fulfilled the American College of Rheumatology criteria, and 30 healthy control individuals attending the blood bank in Helsinki[22].

Regarding the Asian Continent, in Japan, the studied population was formed by individuals from two different cities, namely Tokyo and Otsu. The authors obtained serum samples from 30 RA patients that were attending the rheumatology department, Juntendo University in Tokyo, as well as 23 samples taken from healthy individuals. The remaining serum samples were collected from 30 RA patients from Otsu[22].

Regarding the study conducted in North America, a cohort of 246 patients with inflammatory arthritis for less than one year involving one or more swollen joints was enrolled into an early synovitis study at the National Institute of Arthritis and Musculoskeletal and Skin Diseases, the patients were evaluated clinically and serologically at the initial visit, and later at six weeks, six months and one year. The American College of Rheumatology criteria for RA was applied to each member of the cohort based on the clinical and laboratory data obtained[23].

Europe

United Kingdom

In this study, a fresh midstream specimen of urine and a serum sample was collected from each patient. Bacteriological analysis of urine was performed where the presence of ≥ 10^4 colonies/ml of urine of one colony type was recorded as significant bacteriuria and < 10^4 colonies/ml of urine as no significant bacteriuria or as no growth[7].

The measurement of rheumatoid factor (RF) was performed by agglutination with IgG-coated latex particles in 35 blood samples obtained from the RA patient group and 27 blood samples from the healthy control group. The presence of agglutination indicated that the serum had an RF content ≥ 20 IU/ml. RF was determinable in 29 of 35 sera from the RA patients with values of 50-1140 IU/ml and it was not detectable in any of the 27 sera from the control patients' group.

Bacteriological analysis of urine revealed that *P. mirabilis* was the most frequently found microorganism in the infected urines of the RA group. It was found in purity in 13 (52%) of the infected urine specimens of the RA patients, almost twice as frequently as *E. coli*, the second most frequently microorganism found in the RA patients infected urine[7].

P. mirabilis was found in significant numbers in two RA patients infected urines and it was found in non-significant numbers in 11 RA patients infected urines whereas in the healthy control group this microorganism was not found in significant numbers in any of the 48 urines samples and it was identified in non-significant numbers in only one control group infected urine[7].

Regarding the analysis of serum and urine by enzyme-linked immunosorbent assay (ELISA), only 21 serum samples belonging to the RA group corresponded to those of the control group in terms of age and gender, and these were selected for ELISA studies. The IgM, IgG, and IgA levels to *P. mirabilis* in the sera of the RA patients were extremely significantly or very significantly higher compared to those of the control group (Table 1)[7].

Table 1. Mean ELISA values of different classes of antibody to P. Mirabilis in the serum of RA and control patients

	RA patients		Control patients		
	No. of samples	ELISA±SD	No. of samples	ELISA±SD	*P*-value
IgM	21	0.28±0.17	21	0.17±0.04	0.0063
IgG	21	0.25±0.11	21	0.14±0.09	0.0010
IgA	21	0.25±0.13	21	0.08±0.06	<0.0001

The antibody levels to *P. mirabilis* in the urine from the same 21 matched RA patients and 21 control subjects were also determined by ELISA. The IgM, IgG, and IgA levels to *P. mirabilis* in the urine of the RA patients were found to be extremely significantly raised (Table 2)[7].

Table 2. Mean ELISA values of different classes of antibody to P. Mirabilis in urine of RA and control patients

	RA patients		Control patients		
	No. of samples	ELISA±SD	No. of samples	ELISA±SD	*P*-value
IgM	21	0.23±0.10	21	0.10±0.05	<0.0001
IgG	21	0.27±0.13	21	0.11±0.06	<0.0001
IgA	21	0.17±0.05	21	0.12±0.02	0.0001

Greece

In the study conducted in the Greek population, blood samples from 63 patients with RA and 38 healthy controls were collected, from which the determination of RF and the levels of antibodies against *P. mirabilis* was performed. The titer of RF was assessed using laser nephelometry and the anti-*Proteus* antibodies were determined by ELISA and using three synthetic amino acid peptides – homologous sequences with *P. mirabilis* haemolysin (HpmB), *P. mirabilis* urease C (UreC), both being cross-reactive with human tissues and *P. mirabilis* urease F (UreF), which is non-cross-reactive[21].

The results showed that patients with RA presented elevated levels of antibodies against the synthetic peptides from *P. mirabilis* enzymes when compared to healthy controls (Figure 1).

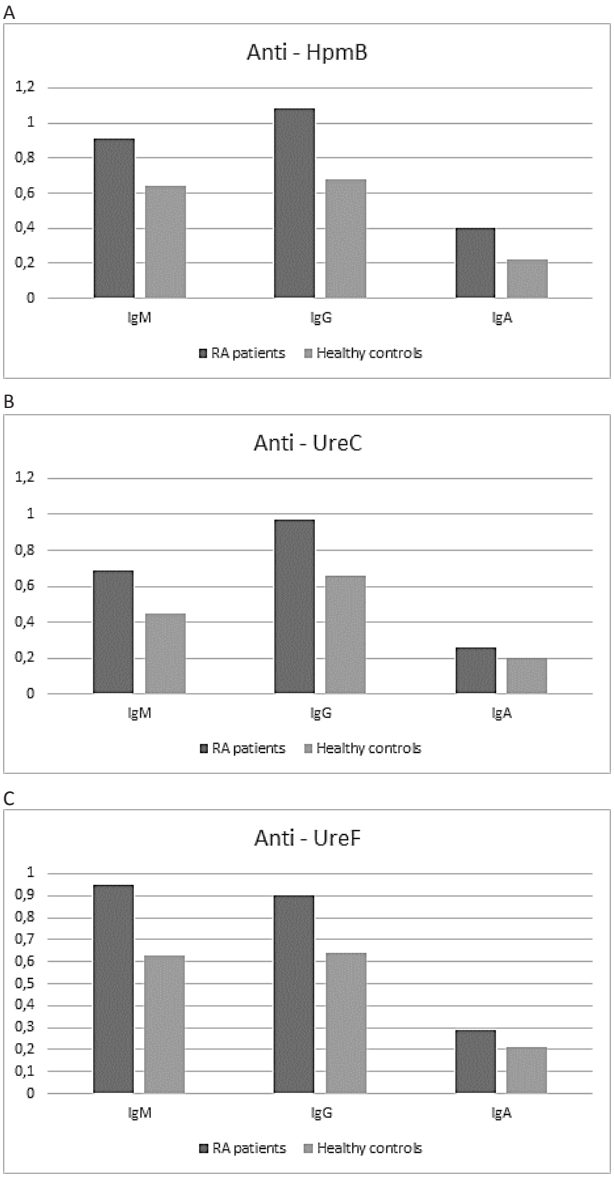

Figure 1. Graphic representation of IgM, IgG, and IgA antibodies against (A) HpmB, (B) UreC, and (C) UreF Proteus peptide in RA patients and healthy controls.

Accurately elevated levels of IgM, IgG, and IgA antibodies against haemolysin *Proteus* peptide (anti-HpmB), against UreC *Proteus* peptide (anti-UreC), and against UreF *Proteus* peptide (anti-UreF) when RA patients were compared to healthy controls (Table 3)[21].

Table 3. p-value obtained when levels of IgM, IgG, and IgA antibodies against each peptide (HpmB, UreC, and UreF) were compared between RA patients and healthy controls

	anti-HpmB	anti-UreC	anti-UreF
IgM	p=0.005	p=0.007	p=0.007
IgG	p=0.001	p=0.002	p=0.001
IgA	p=0.003	p=0.001	p=0.001

Regarding RF titers, a significant correlation was observed between RF levels and IgM antibodies against UreC *Proteus* peptide and between RF levels and between RF levels and IgG antibodies against UreF *Proteus* peptide[21].

Finland

In the study conducted in Finland, isotypic determination of antibacterial antibodies and determination of levels of isotypic antipeptide antibodies, against EQRRAA and ESRRAL, was performed. Antibacterial and antipeptide antibody levels were investigated using the indirect immunofluorescence and ELISA methods, respectively[22].

Regarding the isotypic determination of antibacterial and antipeptide antibody levels in the Finnish population, elevated levels of IgG and IgM anti-*Proteus* antibodies were found in patients with early and advanced RA when compared to the Finnish healthy controls (Table 4). Likewise, significant elevations

Table 4. Statistical results which show significant relation between titers of IgG and IgM classes of immunoglobulins anti-Proteus from early RA (ERA) patients and advanced RA (ARA) patients when compared to healthy controls in Finland

	ERA	ARA
IgG	t=4.10, p<0.001	t=10.76, p<0.001
IgM	t=2.78, p<0.01	t=3.85, p<0.001

were observed among the IgG class of antibodies to EQRRAA and ESRRAL peptides in the same groups of RA patients also when comparing to Finnish healthy subjects (Table 5)[22].

Table 5. Statistical results which show significant relation between titers of IgG class of immunoglobulins against EQRRAA and ESRRAL peptides from early RA (ERA) patients and advanced RA (ARA) patients when compared to healthy controls in Finland

	EQRRAA		ESRRAL	
	ERA	ARA	ERA	ARA
IgG	t=3.15, p<0.005	t=8.17, p<0.001	t=3.71, p<0.001	t=8.05, p<0.001

Asia

Japan

In the study conducted in the Japanese population, in a similar way as the Finnish study, the isotypic determination of antibacterial antibodies and determination of levels of isotypic antipeptide antibodies, against EQRRAA and ESRRAL, was performed[22].

The isotypic determination of antibacterial antibodies in the Japanese population showed that titers of IgG, IgM, and IgA classes of immunoglobulins anti-*Proteus* from RA patients were significantly elevated when compared to the Japanese healthy controls (Table 6)[22].

Table 6. Statistical results which show significant relation between titers of IgG, IgM, and IgA classes of immunoglobulins anti-Proteus from RA patients when compared to healthy controls in Japan

	Japan
IgG	t=9.63, p<0.001
IgM	t=2.78, p<0.01
IgA	t=3.46, p<0.005

Regarding the levels of isotypic antipeptide antibodies, significant elevations were mainly observed among the IgG class of antibodies to EQRRAA in RA patients when compared to Japanese healthy subjects. When the determination of other isotypes was carried out, increased antibody titers against EQRRAA or ESRRAL peptides were observed only among the IgM class of immunoglobulins in the RA patients group (Table 7)[22].

Table 7. Statistical results which show significant relation between titers of IgG and IgM classes of immunoglobulins against EQRRA and ESRRAL peptides from RA patients when compared to healthy controls in Japan

	EQRRAA	ESRRAL
IgG	t=7.68, p<0.001	t=6.76, p<0.001
IgM	t=2.92, p<0.01	-

When total IgM, IgG, and IgA antibody levels against *P. mirabilis* were compared with antibodies to EQRRAA and ESRRAL peptides among Finnish and Japanese patients with RA, significant correlations were observed (Table 8)[22].

Table 8. Statistical results which show significant relation between total titers of IgG, IgM, and IgA classes of immunoglobulins anti-*Proteus* from Finnish and Japanese patients an Anti-EQRRAA and Anti-ESRRAL

	Anti-*Proteus*
Anti-EQRRAA	r=0.79, p<0.001
Anti-ESRRAL	r= 0.86, p<0.001

North America

USA and Canada

In this study, the serum samples taken at the patients' initial visit were assayed by ELISA for antibodies to *P. mirabilis*, more specifically, antibodies against eleven strains of *P. mirabilis* representing the 11 commonest O serotypes of strains associated with a *P. mirabilis* UTI[23].

Antibodies to a panel of possible arthritogenic organisms (*E. coli, Chlamydia trachomatis, Salmonella typhi, Shigella flexneri, Campylobacter jejuni, Yersinia enterocolitica*, and parvovirus B19) were also measured by ELISA. T[23].

Levels of anti-*Proteus* IgG, IgM, and IgA antibodies in the RF-negative and RF-positive RA patients' group were determined by ELISA at their initial visit to the National Institutes of Health. It was not observed no significant differences between the means of serum anti-*Proteus* IgG antibodies in both groups. In contrast, the means of serum anti-*Proteus* IgM and IgA antibody levels were both significantly higher in the RF-positive RA group compared to the RF-negative RA group[23].

Moderate correlations between anti-*Proteus* IgA, IgM, and IgG antibody levels with total serum IgA, IgM, and IgG levels and between the titre of IgM antibodies to *P. mirabilis* and the RF titre were found. A weak correlation between RF titre and IgA anti-*Proteus* antibodies levels and a no correlation between IgG anti-*Proteus* antibody level and the RF titre were also found (Table 9)[23].

Table 9. Correlation coefficients between anti-Proteus IgA, IgM, and IgG antibody levels and total serum IgA, IgM, IgG antibody levels and RF

	Anti-Proteus IgA	Anti-Proteus IgM	Anti-Proteus IgG
Total IgA	0.46	-	-
Total IgA	-	0.45	-
Total IgA	-	-	0.28
RF	0.21	0.46	0.04

Due to the correlation between IgM anti-*Proteus* antibody levels and RF, the authors decided to examine whether the IgM RF was causing a false-positive result in the *Proteus* assay. Therefore, serum from 12 patients with the highest levels of RF who also had high anti-*Proteus* IgM antibody titres was selected, and RF was removed. In the 10 serum samples belonging to the patients with the highest levels of RF who also had high anti-*Proteus* IgM antibody titres, it was not observed a significant drop in anti-*Proteus* IgM titre after removal of RF[23].

Regarding the analysis of the presence of antibodies to other potential arthritogenic pathogens, including *C. trachomatis, C. jejuni, S. typhi, S. flexneri, Y. enterocolitica*, and Parvovirus, no significant differences in the frequency of antibody presence or levels were observed for any of the anti-pathogen antibodies listed[23].

Discussion

Rheumatoid arthritis is a chronic autoimmune inflammatory disease associated with high morbidity rates which are major concerns for the World Health Organization[5]. Relevantly, for the past four decades, UTI caused by *Proteus spp.* has been suggested as a key player in the aetiopathogenesis of RA, and this correlation has been documented in populations of different countries from Europe, Asia, and North America being an association based in either serum or urine samples, all with significant results[7,9,24].

In a study conducted in the population of the United Kingdom named A UK MULTICENTRE STUDY OF THE ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL PATHOGENS CAUSING URINARY TRACT INFECTIONS where the main goal was to determine the prevalence of UTI pathogens, it was found that *E. coli* was the predominant one found in more than 50% of all studied urine samples, followed by *Enterococcus faecalis*, *Klebsiella pneumoniae* and *P. mirabilis*[25]. This means that in the general population of the same country *Proteus spp.* are only the fourth most common UTI-causing pathogen while in RA patients these are the most prevalent ones. Also, in the study involving Japanese and Finnish subjects, the results showed that were found, in serum samples, more antibodies against *P. mirabilis* when compared with antibodies against *E. coli* and *Serratia marcescens*[22]**.** A Japanese study named NATIONWIDE SURVEILLANCE OF BACTERIAL PATHOGENS FROM PATIENTS WITH ACUTE UNCOMPLICATED CYSTITIS CONDUCTED BY THE JAPANESE SURVEILLANCE COMMITTEE DURING 2009 AND 2010: ANTIMICROBIAL SUSCEPTIBILITY OF *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS SAPROPHYTICUS*, which used 364 urine samples, showed that the prevalence of *Proteus spp.* in patients with acute uncomplicated cystitis was 0.3%[26]. As well as the study NATIONWIDE SURVEY OF ANTIBACTERIAL ACTIVITY AGAINST CLINICAL ISOLATES FROM URINARY TRACT INFECTIONS IN JAPAN (2008), in which 1,312 urine samples from patients with acute uncomplicated cystitis showed that *P. mirabilis* had a prevalence of 1.6% and in 994 samples from patients with complicated cystitis, *P. mirabilis* had a prevalence of 2.4%[27].

Following the same trend, two different studies conducted in North America, more specifically in USA[28] and Canada[29] showed that *P. mirabilis* is, in both, the fifth most common pathogen found in urine samples from community-onset UTI.

Considering the results reported in this review, the United Kingdom is the only country that presents results based on urine samples. The bacteriological analysis of urine revealed that 96% of samples from the control group were sterile (46 of 48 urines) and 25 (33%) of RA patients who appeared to be healthy without clinical symptoms of UTI had infected urine. This could not be explained by differences between gender or age in both groups because the proportions of the males and females in both groups were very similar (81.8% females and 80% males in the RA group and 80.9% female and 83% male in the control group)[7]. *P. mirabilis* was the most frequent organism encountered in the RA patient's urine. It was found in 13 of the 25 urines with microbial growth (52%), almost twice as frequent as *E. coli* that was found in seven of the 25 urines with microbial growth belonging to RA patients. In the urines from the control group, only two of them showed microbial growth, one for *P. mirabilis* and one for *E. coli.* In the majority of the infected urines by *P. mirabilis* belonging to the RA group, the bacteria were found in low numbers to constitute significant bacteriuria, supporting the fact that none of them had clinical symptoms of UTI[7]. At the time of publication of this study, some researchers gave support to the hypothesis that RA may have developed because of a subclinical UTI with a small number of *P. mirabilis* bacteria which provides a frequent or prolonged exposure to *P. mirabilis* antigens. Nowadays this association is well established by other authors and what was back then a hypothesis, new discoveries have shown this is now a reality[9,20]. The levels of IgM, IgG, IgA to *P. mirabilis* were significantly increased in the RA group when compared to the control group[7]. This finding had significant importance at that time because it was one of the first studies to show elevated levels of anti-*Proteus* antibodies in the serum.

Results from three different studies where the levels of IgG, IgM, and IgA immunoglobulins against *P. mirabilis* in serum samples were tested, showed a significant correlation between the presence of those immunoglobulins against *P. mirabilis* and the diagnosis of RA. In the study conducted in Greece, the specific antibody classes IgM, IgG, and IgA against the three synthetic peptides HpmB and UreC which are cross-reactive, and UreF which is non-cross-reactive were measured. The increased levels from RA patients, when compared with healthy controls, is an indicator that *P. mirabilis* could be an important factor for the development of RA[21]**.** These results, despite different biologic samples, confirm the higher prevalence of *Proteus* microbes in RA patients.

Furthermore, the IgM class of immunoglobulins against *P. mirabilis* showed significantly elevated levels in RA patients when compared to control groups. This is showed in Japan, Finland, Greece, the USA, and Canada[7,21-23]. This class of immunoglobulins against the EQRRA peptide was also elevated in Japanese RA patients[22]. This strong correlation agrees with the presence of an acute reaction against *P. mirabilis* expressed by the elevation of IgM antibodies[21,23].

On the other hand, the levels of IgG antibodies against *P. mirabilis*, in serum, was significantly elevated in the Japanese population. It was also raised in Finnish patients with early and advanced RA. Also, in both countries, the levels of IgG against EQRRAA and ESRRAL peptides (the 'shared epitopes') showed a significant increase[22]. In Greek RA patients, when compared with the healthy controls, a significant statistical correlation among IgG antibodies against the three synthetic peptides, cross-reactive and non-cross-reactive was observed. This class of antibody remains for a long time in serum which demonstrates the chronicity of an infection caused by *P. mirabilis*[21]. In the USA and Canada populations, it was not observed a significant difference because in this study the patients were in an early stage of the disease and this class of immunoglobulin doesn't reflect acute changes[23].

Moreover, regarding the IgA class of immunoglobulins, interesting observations can be made. The investigators from the study conducted in Japan and Finland failed to detect increased IgA antibodies to *P. mirabilis* in Finnish RA patients, indicating that these results suggest the involvement of an upper UTI, which usually evokes IgM and IgG antibodies[22].

Contrarily to these, the study conducted in the Greek population showed that these RA patients presented increased

levels of the IgA class of anti-*Proteus* antibodies, concluding that the infection from *P. mirabilis* exists and evolves on the mucosal surface of the urinary tract[21].

In a study published in 1994, before both referred above, investigators studied the human immune response in the urinary tract, detecting antibody levels in both serum and urine samples from either lower or upper UTI. In this study the IgA class of immunoglobulins had a simultaneous occurrence in serum and in urine, presenting higher levels than both the other studied classes (IgG and IgM)[30]. Another study about the IgA class affirms that these antibodies are not deficient in complicated UTI, but in contrast, a low mucosal IgA level might be a host factor that predisposes a limited group of women to have recurrent UTI[31].

With all these results it can be said that IgA antibodies do not give clear indications of the occurrence of UTI in RA patients meaning that other factors should be investigated to confirm that anti-*Proteus* antibodies are evoked by a UTI and not by an infection on another mucosal surface.

Additionally, the evaluation of the RF also suggested a significant correlation between levels of RF and IgG and IgM antibodies against UreC and UreF, in the study from Greece. RF is a disease activity marker and decreases with treatment. In this study the authors suggested that this correlation may be due to *P. mirabilis,* contributing to the production of RF. Most patients were receiving treatment which may affect both antibodies and RF levels[21].

In the study conducted in the USA and Canada, it was shown that the RF-positive subgroup of RA patients was associated with elevated levels of anti-*P. mirabilis* IgM and IgA antibodies. The authors proved that this association was not due to a result of a false-positive anti-*P. mirabilis* antibody analysis[23]. These findings indicate that *Proteus* antibodies can be associated with markers of disease severity, in this case, RF, in patients with RA.

The results discussed here supports the evidence that the molecular mimicry or cross-reactivity mechanisms between self-tissue and *P. mirabilis* is a strong justification for the development of classic RA[21]. From the immunoblot analysis performed in the United Kingdom study, it was not possible to find a *P. mirabilis* antigen that was reactive in all the RA sera and unreactive in the control sera. As already mentioned, at the time of publication the authors theorized that two antigens may have significant importance. These consist of the *P. mirabilis* haemolysin-associated protein HpmB and the *P. mirabilis* urease due to their polypeptide sequences (ESRRAL and IRRET respectively) being similar in molecular structure to the amino acid motifs QRRAA and LRREI that are associated respectively with RA and type XI collagen, has already mentioned in this review[7,9].

Regarding the results obtained from the research performed in Japan and Finland, when total levels of IgM, IgG, and IgA immunoglobulins against *P. mirabilis* were compared with anti-EQRRAA and anti-ESRRAL, strong correlations were observed[22]. Cross-reactive epitopes, like the IRRET sequence, could be responsible for the production of cross-reactive antibodies, which bind to the homologous amino acid LRREI found in type XI collagen, a component from hyaline cartilage found in synovial joints[9,22].

Therefore, individuals who get infected with *P. mirabilis* will produce antibodies against this microbe. Due to the presence of the 'shared epitopes' on self-tissue, these antibodies will bind to the articular tissue with cross-reactive antigens evoked by *P. mirabilis*. This immune response leads to the release of more antigens with the consequent increase in the production of autoantibodies by the organism. It is important to emphasize the crucial role of laboratory diagnosis of *Proteus spp*. infections and antimicrobial susceptibility testing guided therapy to ensure therapeutic success and prevent or minimize the occurrence of RA associated with UTI.

Conclusion

The association between RA and UTI caused by *Proteus spp*. has been investigated in depth in different countries worldwide. The studies analyzed in this work were conducted in countries from three different continents and have demonstrated that the association between this autoimmune disease and *Proteus spp*. in its populations is a global phenomenon.

Through microbiological and serological methods, authors identified higher isolation rates of *Proteus spp*. in urine samples from UTI patients and elevated levels of antibodies against *Proteus spp.* in the serum of RA patients when comparing with healthy or more specifically, non-RA controls, meaning that *Proteus* bacteria causes an immune response on RA patients. Data presented in this work report compelling evidence of the link between this microbe and RA, from recurrent sub-clinical *Proteus* UTIs to the full development of RA supporting the already referred theory, that RA is an autoimmune disease produced by autoantibodies against joint tissues which can be enhanced by upper urinary tract infections caused by *Proteus spp*.

Future research

As previously mentioned, no research regarding the association between RA and *Proteus spp.* has been performed in the Portuguese population. Therefore, considering that the results discussed here to emphasize the need for further studies, we suggest the development of a future research project that will be able to access if the Portuguese population follows the trend of other European countries like the ones referred in this work, in which patients with RA develop UTI caused by *Proteus spp*. more frequently or have elevated levels of anti-*Proteus* antibodies in their serum.

Another line of research that can be explored is related to RA therapeutic strategies. As already stated, RA therapy is mainly focused on reducing joint inflammation however, with the knowledge regarding the association described in this review, studies should be carried out to assess if the usage of *Proteus* sensitive antibiotics together with the currently used medical treatments, or even the immunization of susceptible individuals with vaccines from *Proteus* bacterial cell components, can increase and improve the therapeutic effectiveness of this disease.

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Conflito de interesses

Os autores declaram não ter quaisquer conflitos de interesse.

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Appendix. Summary of the selected articles for the elaboration of this review

Country (reference)	Study subjects	Biological sample	Analysed parameters	Relevant results
United Kingdom[7]	UK: RA – 76 HC – 48	Serum: RA – 36 HC – 27 Urine: RA – 76 HC – 48	Rheumatoid factor; Bacteriology analysis of urine; IgM, IgG, and IgA anti-Proteus levels in the sera; IgM, IgG, and IgA anti-*Proteus* levels in urine	Higher isolation of *Proteus mirabilis* in urine samples of RA patients when compared with healthy controls. IgM, IgG, and IgA levels to *Proteus mirabilis* in the sera of the RA patients was significantly higher compared to those of the control group.
Greece[21]	RA – 63 HC – 308	Serum	IgG, IgM, IgA against HpmB, UreC and UreF peptides and Rheumatoid factor.	Patients with RA presented elevated levels of antibodies (IgG, IgM, and IgA) against the synthetic peptides from *P. mirabilis* enzymes, when compared to healthy controls. It was observed a significant correlation between class specific antibodies against all three Proteus peptides, between RF levels and IgM antibodies against UreC *Proteus* peptide and also between RF levels and IgG antibodies against UreF Proteus peptide.
Finland and Japan[22]	Finland: ERA – 72 ARA – 27 HC – 30 Japan: RA – 60 HC – 23	Serum	IgG, IgM, and IgA against *Proteus*, EQRRAA and ESRRAL peptides	Elevated levels of *Proteus* IgG and IgM antibodies were found in the Finnish and Japanese patients with early and advanced RA compared to the healthy controls; Elevated levels of IgG antibodies to EQRRAA and ESRRAL peptides in the Finnish and Japanese patients with early and advanced RA compared to healthy controls.
USA and Canada[23]	246	Serum	Antibodies to *Proteus mirabilis* (the 11 commonest O serotypes of strains associated with a *P. mirabilis* UTI)	Anti-IgA and IgM *P. mirabilis*-specific serum antibody titres are elevated in patients with RF-positive RA. *Proteus* antibodies associate with markers of disease severity only in patients with RA. No significant differences were seen in the IgG, IgM antibody responses to *E. coli* for any patient group. Differences in antibody responses to other potential arthritogenic pathogens were not seen in the different disease groups.