The role of *Ureaplasma* spp. in the development of non-gonococcal urethritis and infertility among men.

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Summary

Ureaplasma spp. are a genus of bacteria for which two human associated species exist; *Ureaplasma urealyticum* and *Ureaplasma parvum*. Their definition as a pathogen in the context of non-gonococcal urethritis and infertility among males remains highly controversial, largely due to historically high isolation rates of these bacteria from the urethra of seemingly healthy men. This review summarises the emerging evidence suggesting a true pathogenic role of these bacteria under specific conditions, which we term as risk factors. We examine the historical, clinical and experimental studies which support a causal role for *Ureaplasma* spp in the development of NGU as well as some of the proposed mechanisms behind the association of *Ureaplasma* spp. and the development of infertility. Finally, we discuss the potential for developing a case-by-case risk-based approach towards the management of men who present with seemingly idiopathic NGU, but are positive for *Ureaplasma* spp.

Keywords: *Ureaplasma parvum*, *Ureaplasma urealyticum*, non-gonococcal urethritis, infertility

Introduction

Non-gonococcal urethritis (NGU) is a leading sexually acquired condition among men. It is defined by inflammation of the urethra in the absence of *Neisseria gonorrhoeae* and includes signs and symptoms such as penile discharge, dysuria as well as irritation inside and around the urethra. *Chlamydia trachomatis* has long been regarded as the predominant infectious agent among patients suffering from NGU, with 20 – 50% of individuals being positive for the pathogen, whereas more recently, *Mycoplasma genitalium* has achieved recognition as a pathogen and may be isolated from 10-30% of NGU patients (1). Although *C. trachomatis* and *M. genitalium* account for many cases of NGU, of concern is the high prevalence of up to 45% of idiopathic NGU cases, in which classic pathogens are not identified (2).

A leading candidate to fill the void presented by this idiopathic condition among NGU patients are bacteria from the genus *Ureaplasma*. The first documented isolation of these bacteria were from male patients experiencing NGU (3). Many reports have followed up this observation with a view to gather evidence to support the idea of *Ureaplasma* spp. being an aetiological agent of NGU, but the combination of inconsistencies in reporting, study design and high prevalence of between 5 – 15% among healthy males aged 16 – 44 years has prevented the acknowledgment of these organisms as true pathogens in the context of genito-urinary medicine (GUM) (4). For these reasons, the idea of *Ureaplasma* spp. as GUM pathogen remains controversial among GUM practitioners. Additionally, the potential role of *Ureaplasma* spp. as agents with a causal role in male infertility has been debated. Many of the recognised GUM pathogens, such as *N. gonorrhoeae* and *C. trachomatis*, have been implicated in complications such as male infertility, but more work is required to gain a clear understanding on the implications associated with a failure to clear *Ureaplasma* spp. from the urethra (5).

In this review we present an update from the current literature to discuss the potential of *Ureaplasma* spp. as a risk factor for male genital tract infections with specific reference to NGU and infertility. In the context of NGU we present the arguments for and against a role for these bacteria in disease development with a focus on some of the unique risk factors which have been overlooked historically. Increasing interest has focused towards *Ureaplasma* spp. and their potential role in the development of male infertility. We discuss the clinical evidence as well as the proposed mechanisms which have been neglected when taking into account markers for infertility. Finally, the potential therapeutic considerations are evaluated and we discuss the potential for risk-based screening approaches as an effective means to manage patients with seemingly idiopathic NGU in the face of growing concerns over antimicrobial resistance among GUM pathogens.

Background biology of *Ureaplasma* spp.

Ureaplasma spp. are recognised as some of the smallest self-replicating, free-living microorganisms. They are a unique genera of bacteria due to their essential requirement for urea in the synthesis of ATP, with further defining characteristics shared with the closely related mycoplasmas, including a low G+C genomic content, lack of a peptidoglycan containing cell wall and requiring cholesterol for growth (6, 7).

Ureaplasmas were first isolated from male NGU patients in 1954 and due to the tiny colony size upon agar plates, these bacteria were originally referred to as 'T-Strain' or 'tiny mycoplasmas' (3). Following the establishment of the essential requirement for urea, the genus *Ureaplasma* was adopted (8). A single species of human-associated *Ureaplasma* were initially recognised, *Ureaplasma urealyticum*, which was further sub-divided into two biovars. The nomenclature of *U. urealyticum* for describing all human-associated isolates was embedded until the work by Roberston *et al*. in 2002, which made substantial contribution to redefining these bacteria into two antigenically distinct human associated species (9). These were defined as *U. urealyticum* and *Ureaplasma parvum*. The two species are divided into fourteen serovars with serovars 1, 3, 6 and 14 being assigned to *U. parvum* with the remaining serovars 2, 4-5 and 7-13 being defined as *U. urealyticum*.

Numerous clinical manifestations have been associated with *Ureaplasma* spp. Among the most notable is the role of *Ureaplasma* spp. in adverse pregnancy outcomes such as chorioamnionitis and preterm premature rupture of membranes leading to preterm birth (10, 11). The subsequent colonisation of *Ureaplasma* spp. within the lungs of premature neonates has been associated with a 7.9-fold increased risk of bronchopulmonary dysplasia, 3.3-fold increased risk of intraventricular hemorrhage and a 2.5-fold increased risk of necrotising enterocolitis (12). In adults, attention has been drawn towards the development of an atypical hyperammonaemia in which lung transplant patients, and potentially kidney transplant patients, have increased serum ammonia levels as a result of systemic *Ureaplasma* spp. infection (13-15). If left untreated with antibiotics, such increased serum ammonia levels can lead to delirium, cerebral oedema and eventual fatality.

Historically the link between *Ureaplasma* spp. and development of NGU, as well as infertility, prostatitis and epididymitis among men, has been inconsistent. The reason for this, however, is certainly not from a lack of studies examining potential associations between *Ureaplasma* spp. and NGU (2, 16-21). Rather, the lack of conclusive evidence may reflect the complex interaction between host and pathogen, as discussed later, combined with the high prevalence of *Ureaplasma* spp. among control groups which suggests they are innocent bystanders present at time of screening. Although *Ureaplasma* spp. were isolated approximately 30 years prior to *Mycoplasma genitalium*, the latter has

risen to prominent pathogen status more rapidly, and new guidelines for its management are now in place in the UK (3, 22, 23) .

The proinflammatory potential of *Ureaplasma* spp.

Human volunteer experiments with *Ureaplasma* spp. infection of the urethra

To demonstrate the pathogenicity of *Ureaplasma* spp., several investigators have undertaken human participant experiments (24, 25). The first such experiment by Jänsch in 1972 identified a polymorphonuclear (PMN) response following inoculation with an unknown and poorly defined *Ureaplasma* spp. (24). Although the experiment was poorly designed and controlled for, this gave an initial insight into the inflammatory nature of a human infection with *Ureaplasma* spp. A second more defined experiment was conducted with two human participants. The first participant received an intra-urethral inoculation of a clinically relevant titre of a low passage clinical isolate of *U. urealyticum* serovar 5 isolated from a patient experiencing NGU in which no other organisms were present (25). The participant subsequently developed symptoms of dysuria and signs of urethritis in the form of a PMN response. The serum recovered from the volunteer demonstrated sero-conversion with high titres of specific antibodies. Upon administration of tetracycline, both signs and symptoms resolved. The second participant received an alternative isolate of *U. urealyticum* serovar 5 isolated from a second patient presenting with NGU. Again, signs and symptoms ensued, but upon administration of tetracycline signs, such as urinary threads, persisted in the absence of viable cultures. Seminal samples collected post-antibiotic treatment indicated that the *U. urealyticum* had disseminated suggesting potential involvement of the prostate and highlighting the potential adverse sequelae associated with exposure to *Ureaplasma* spp. Although such experiments are ethically questionable by today's standards, these studies provided initial evidence that exposure of the male urethra to clinically relevant titres of *U. urealyticum* has the capacity to elicit a PMN immune response in the presence of symptoms which reflects those seen among NGU patients. It should be noted, however, that what a 'clinically relevant titre' of *U. urealyticum* is was not defined in this study and is a notable limitation for interpretation of these data.

Animal models of *Ureaplasma* spp.-induced urethritis

Due to substantial ethical implications of human volunteer studies, investigators turned to model urethral infection caused by *Ureaplasma* spp. utilizing animal models. Like that of *Neisseria gonorrhoeae*, *Ureaplasma* spp. are host-specific, resulting in an early reliance upon chimpanzee models due to the close ancestry with humans. Initial experiments with intra-urethral inoculation saw rapid multiplication of the bacteria within the urethra, but in the absence of a PMN response (26). A possible explanation for this lack of immune response was suggested to be a loss of virulence from *in*

vitro passage. To examine this hypothesis a second study was conducted with a larger number of chimpanzees (27). The inoculum for this study consisted of *Ureaplasma* spp. from men with NGU resuspended in a transport media which was directly inoculated into the chimpanzees via intra-urethral inoculation. Unlike the first study, a substantial PMN response was noted in conjunction with an increase in *Ureaplasma* spp. titre. For reasons which are unknown, the species of *Ureaplasma* which was inoculated during this study was not determined.

Due to the lack of availability of chimpanzee models, investigators have moved to murine models to investigate colonisation of the genital tract (28-30). Although many of these models have relied upon female mice and vaginal colonisation, due to the physiology of the male mouse urethra, an inflammatory response as characterised by increased TNF-α and PMN recruitment have been described. A key confounding variable has been the essential requirement to pre-treat the mice with estradiol to allow for colonisation to establish. This requirement for estradiol is likely due to suppression of the innate immune system, but the presence of estradiol binding proteins as seen in other pathogens are yet to be ruled out (31, 32).

In vitro cell line models of *Ureaplasma* spp.-induced inflammation

The difficulty in assessing *Ureaplasma* spp. infection of the urethra has resulted in a reductionist approach utilising specific cell lines in isolation *in vitro* to look at cytokine responses. Some studies have focused on immune cells such as THP-1 monocytes, phorbol myristate acetate (PMA) differentiated macrophages and primary human macrophages derived from lung fluid which were then stimulated with *U. urealyticum* serovar 8 (33). In all cell types examined, stimulation with *U. urealyticum* resulted in a dose-dependent increase in levels of IL-6 and TNF-α at both the mRNA and protein level. However, it should be noted that studies examining cytokine expression in relation to stimulation by *Ureaplasma* spp. tend to examine a single bacterial isolate and therefore do not give a true representation of the diversity of stimulating properties of *Ureaplasma* spp. It has been suggested that the predominant antigen found on the surface of *Ureaplasma* spp., known as the multiple banded antigen (MBA), may account for differences in inflammatory response (34). Sweeny *et al*., noted that the size and number of MBA repeats had an effect upon the levels of IL-8 which is a primary chemoattractant of PMNs such as neutrophils (34). Although many of these studies were generalised for the immunogenic properties of *Ureaplasma* spp. they provide evidence for the inflammatory potential for these bacteria. An obvious limitation of these studies is the lack of consideration towards the complexities of a full biological system such as adaptive immune response, the presence of other microorganisms which may permit infection as well as the response to chronic exposure over time.

Risk factors linked with the development of *Ureaplasma* spp. associated NGU

Ureaplasma spp. can be detected in genital samples from men with NGU as well as healthy controls, which has fuelled much of the controversy surrounding the role of *Ureaplasma* spp. in NGU (Table 1). Much of this historic evidence may now be questioned due to developments in the reclassification of *Ureaplasma* spp., better techniques for species differentiation, fully quantitative reporting of sample titre, as well as a better understanding of patient sexual histories. A proposed overview of the natural history of *Ureaplasma* spp. taking into account these risk factors is presented in Figure 1.

Risk factor 1: The species of *Ureaplasma* present within the urethra.

Until 2002 human ureaplasmas were recognised as a single species subdivided into two biovars. The result of phenotypic and genotypic analysis later saw the official recognition of two-independent species, *U. parvum* and *U. urealyticum* (9). This absence of species differentiation meant that studies prior to 2002 solely reported results as *U. urealyticum* and therefore may have over represented this species among clinical samples from both cases as well as control groups. The legacy of the original nomenclature is still evident today, with publications still referring to *U. urealyticum* or just *Ureaplasma* spp., and may be partially attributed to the use of culture-based rapid diagnostic kits which are commercially available (35). Over time, studies have begun to differentiate the *Ureaplasma* spp. found into the respective species, with some studies identifying the presence of *U. urealyticum* more often among NGU patients than controls, whereas *U. parvum* represented the inverse of this (17, 21, 36-39). In many of these studies, patient numbers in both NGU and control groups were seen as low and therefore lacked the power to confidently associate *U. urealyticum* with NGU. For this reason, Zhang *et al*. performed a meta-analysis which included seven eligible case-control studies encompassing 1507 NGU patients and 1223 controls from four separate continents (40). The findings identified that *U. urealyticum* was more prevalent among NGU patients than controls and *U. parvum* was significantly more associated with the control group than those with NGU. This analysis gave significant weighting towards the idea that *U. urealyticum* is the most commonly associated species of *Ureaplasma* among NGU patients and presents as a substantial risk factor for development of disease, although some studies have found the inverse result (41).

Due to the link between species and disease, it is essential that future studies differentiate *Ureaplasma* spp. from clinical samples using molecular methods to the species level to aid in epidemiological studies which will either support or refute the role of these bacteria in the

development of NGU. One of the limiting factors inhibiting this is the use of culture-based rapid diagnostic tests available commercially, which are able to yield semi-quantitative data with regards to titres with in a sample, as well as an indication of antibiotic susceptibility, but they are of limited diagnostic use in the instance of NGU due to the failure to differentiate between species (35). If reference or research facilities are accessible, molecular based techniques are available which can differentiate the two species based on the size of amplicons generated following PCR targeting the 5' region of the *mba* gene or with real-time based molecular probes (42-44). Additionally, multiplex molecular assays are also commercially available and may play an important role in the species-level identification of *Ureaplasma* spp., alongside more traditional STI pathogens. As discussed below, the presence of

Risk factor 2: The sexual history of the patient

It would be very simplistic to assume that the species of *Ureaplasma* was the sole differential which accounts for NGU among men and the inconsistency in reporting in previous cases. A fascinating insight most likely relating to the immune response of the host and the transition from pathogen to commensal has instead been indicated. For many STIs the risk of symptomatic disease is proportional to the number of sexual partners (36). In contrary to this, the relationship between *Ureaplasma* spp. and number of sexual partners is inversely correlated (17, 45). Some of the pioneering work in this area was identified by Wetmore *et al.*, who examined 329 patients with NGU, defined as $\geq$ 5 PMNs per high-powered field and/or visible discharge (17). In this study, two control groups were also assessed, consisting of 191 attendees to a Sexually Transmitted Disease (STD) clinic and 193 patients who were attending the emergency department who did not have NGU. Upon initial analysis, *U. urealyticum* was only marginally associated with NGU compared with the STD control group (adjusted odds ratio 1.6) or the emergency department group (adjusted odds ratio 1.7), but when the analysis considered the number of sexual partners the adjusted odds ratio rose to 2.9 for the STD group and 3.2 for the emergency department group when focusing on less than ten vaginal partners. This association was even greater when the number of vaginal partners was restricted to less than five with an adjusted odds ratio increasing to 6.2 and 5.2, respectively. When the same analysis was performed on patients positive for *U. parvum*, there was no association among any group, adding further weighting to the argument to differentiate the species of *Ureaplasma*. A similar finding was noted by Frolund *et al.*, who examined a Danish cohort of 211 NGU patients and 73 asymptomatic controls (45). Again, a similar finding was observed with the increase in number of sexual partners being associated with a reduced likelihood of disease.

These studies suggest that *Ureaplasma* spp. infections resulting in NGU are associated with patients with fewer sexual contacts. At first, this may seem counter-intuitive, considering other sexually transmitted infections are positively associated with the number of sexual contacts and therefore represents a significant risk factor. The scenario with *Ureaplasma* spp. suggests a significant role for the adaptive immune system in the presentation of disease. Early work by Brown *et al.*, examined the serological response to *Ureaplasma* spp. among NGU patients in acute and convalescent serum (46). They noted that a change in antibody titre was identified in 68% of patients in which greater than 80% saw a change in IgM titre suggesting an active infection. When examining titres of IgG and IgA, the immunoglobulins responsible for protective immunity, only 10% of patients had an increase in titre. When the data was stratified by prior NGU or not there was no significant difference in IgG levels in either acute or convalescent serum, whereas prior NGU accounted for a greater IgA response. These data suggest that some patients gained protective immunity following previous NGU, whereas others did not. This ability to develop protective immunity may account for why some individuals do not develop NGU on future re-exposure, whereas others may.

Risk factor 3: The bacterial load of *Ureaplasma* spp. within the male urethra

The third significant risk factor linking *U. urealyticum*, and in some cases *U. parvum*, to development of NGU is bacterial load within the urethra. As mentioned previously, *in vitro* stimulation on monocytic cell lines with *U. urealyticum* resulted in a dose-dependent response between the *U. urealyticum* titre added and mRNA and cytokine production for the pro-inflammatory cytokines IL-6 and TNF-α (33). This *in vitro* evidence is supported by clinical findings from a number of studies (45, 47, 48). Frolund *et al*., reported that in the presence of *U. urealyticum* at concentrations of $\geq$ 1.3 x 10^3 genome equivalents/ml of urine, corresponding to approximately 1 x 10^3 bacteria/ml, there was a significant association with development of NGU (45). This figure was similar to earlier papers which looked at cut off points for bacterial load for both *U. urealyticum* and *U. parvum* (47, 48). It is important to note that the study by Deguchi *et al*., reported that 83% of subjects which were positive for *U. parvum* had less than 5 x 10^3 bacteria/ml urine of which 80 % had less than 12.5 leukocytes/ml (48). This gives further weighting to the idea that *U. parvum* are less pro-inflammatory, but in situations in which high titres of *U. parvum* are present they have the capacity to generate an inflammatory response. An observation by Frolund *et al*., ties in Risk Factor 2 (sexual history of the patient) with Risk Factor 3 (bacterial load) (48). Analysis of their cohort identified that as the number of vaginal sexual partners increased, the load of *Ureaplasma* isolated decreased with a predicted drop by 2.2 % with each additional sexual partner. It is conceivable that the host immune response which develops due to multiple exposures to *Ureaplasma* spp. may have a direct impact on keeping the titre of *Ureaplasma*

spp. low and therefore under the threshold to mount a significant proinflammatory response as would result in a PMN response, but these findings needs to be expanded by future studies.

The data from studies presented here suggest that a simple qualitative result would not be enough to predict a causal relationship between the presence of *Ureaplasma* spp. and NGU, a factor which has been overlooked by previous studies. From these data it may be possible to develop an objectively determined titre of *Ureaplasma* spp. which clinical laboratories could use to differentiate between causality or association.

Impact of *Ureaplasma* spp. on male fertility

The link between sexually transmitted pathogens and a negative impact on fertility in females is well established. In males, however, such a link is less defined, yet it has been controversially suggested by some that *Ureaplasma* spp. may be associated with male infertility (Table 2). The chronic and often asymptomatic carriage of *Ureaplasma* spp. may therefore have important implications on the development and progression of infertility among men. In this section we will discuss the studies which have contributed to this argument and examine the clinical and mechanistic studies which contribute towards the argument for a causal role of *Ureaplasma* spp. in impaired male fertility.

Clinical studies associating *Ureaplasma* spp. with infertility

As discussed, *Ureaplasma* spp. can be found in the male urethra among seemingly healthy individuals, but have also been isolated from expressed prostatic secretions, urine following prostatic massage and prostate tissue (49-53). This colonisation therefore permits a source to contaminate semen during ejaculation and serve as means to impact on male fertility.

Many studies have examined the clinical association between the presence of *Ureaplasma* spp. in men who are infertile compared with a control group of men without signs of infertility (53-57). In one relatively small study of 100 infertile and 100 control individuals the authors identified 12% of infertile men as *Ureaplasma* spp. positive by PCR compared with only 3% of fertile men (53). The individuals which were *Ureaplasma* spp. positive and infertile had significantly lower volumes of seminal fluid, lower concentrations of sperm cells, and higher levels of sperm cells with abnormal morphology when compared to the *Ureaplasma* spp.-negative infertile patients. Of significance, was the finding that *U. urealyticum* in semen of infertile men was found to be higher (9%) than in healthy controls (1%) whereas the presence of *U. parvum* was 3% in the infertile group and 2% in healthy men suggesting *U. parvum* may not have a causal role in infertility in this patient group. In a similar fate to previous NGU studies, many have neglected to differentiate between species and in one instance examined in excess of 19,000 samples which although identified a significant negative impact of *Ureaplasma* spp.

on semen quality, further power may have been afforded if species-level discrimination had been conducted (57, 58). To further investigate the species-specific association on infertility, a meta-analysis was conducted which examined 14 studies comprising 611 cases and 506 controls (56). These studies suggested an association between *U. urealyticum* and a negative impact on fertility, whereas there was little evidence for a role of *U. parvum*, which draws a parallel to that of NGU patients, as discussed previously.

Proposed mechanisms of *Ureaplasma* spp. associated infertility

It is important to discuss the proposed mechanisms behind these observations of *Ureaplasma* spp. contributing to infertility amongst men (Figure 2). Sexually transmitted pathogens are known to affect sperm quality by reducing motility, negatively effecting sperm morphology and inducing apoptosis (5). Several mechanisms have been proposed to account for infertility in men because of *Ureaplasma* spp. colonisation. These include direct binding of *Ureaplasma* spp. to spermatozoa, which may impede swimming motility (59-62); to production of toxic metabolites, which can damage spermatozoa membranes and result in DNA fragmentation (54, 63); as well as the host generation of cross-reactive antibodies between *Ureaplasma* spp. and sperm surface proteins (64-66).

The work by Potts *et al.* identified 17 out of 50 chronic prostatitis patients to be positive for *Ureaplasma* spp., in which the levels of reactive oxygen species (ROS) were significantly higher among the *Ureaplasma*-positive infertile patients compared with the *Ureaplasma*-negative infertile patients and control group (63). ROS have the potential to induce lipid peroxidation, therefore compromising the integrity of sperm membranes and leading to impaired fertilization capabilities. Of interest was the finding that only one out of seventeen of the positive samples in which ROS were elevated showed signs of leukocytospermia suggesting that in some cases traditional signs of prostatitis, such as leukocytospermia, may not be indicative of *Ureaplasma* spp. infection. The potential for *Ureaplasma* spp. to contribute to lipid peroxidization through generation of ROS, as well as malondialdehyde formation was further developed and stratified by either *U. urealyticum* or *U. parvum* (54). ROS levels, malondialdehyde, and DNA fragmentation, were all significantly higher in *U. urealyticum* positive samples compared with *U. parvum*. The high levels of ROS could therefore result in DNA fragmentation and subsequent apoptosis (67).

Some studies have suggested the presence of *Ureaplasma* spp. in seminal fluid has no real impact on semen quality (68, 69). One possibility is that the mechanism associated with infertility is one which cannot be identified by classic markers of infertility, but may impact on the interaction between the

sperm and egg. P34H is a key membrane bound protein, which is essential for sperm-zona pellucida interactions. P34H is incorporated into membranes covering the acrosomal cap as it transits across the epididymis and therefore can serve as a marker for epididymal function (67). In the *Ureaplasma* spp. positive group, levels of P34H were significantly lower than that of control groups, as determined by western blot and immunofluorescence imaging, which identified 38% of sperm with P34H in the *Ureaplasma* spp. positive group compared with 73% in the control group. These data suggest a potential impact of chronic asymptomatic infection of the epididymis. The acrosomal cap also contains the enzyme hyaluronidase (HYD) which is essential for the sperm to penetrate the egg. In the *Ureaplasma* spp. positive group, levels of HYD activity were significantly different between the infertile *Ureaplasma* spp. positive group as well as the infertile *Ureaplasma* spp. negative group and fertile controls (67). By reducing the activity of HYD the likelihood of successful sperm penetration into the egg is therefore reduced.

An alternative mechanism to *Ureaplasma*-related infertility is the development of cross-reactive antibodies to human sperm membrane proteins following exposure to *Ureaplasma* spp. (64-66). Shi demonstrated that antibodies raised against the UreG protein of *Ureaplasma* spp. were able to cross-react with human nuclear autoantigenic sperm protein (NASP). A higher titre of anti-UreG antibody was found in the sera of infertile men compared with fertile controls. In an *in vitro* fertility assay, sperm which had been pre-treated with anti-UreG antibodies had significantly lower binding and fusion to eggs compared with non-treated controls.

The evidence presented here suggests that the impact of *Ureaplasma* spp. on male infertility is similar to that described for NGU, although unlike in the context of NGU the effect of bacterial titre has yet to be investigated. The lack of species differentiation has hindered studies, but association as well as mechanistic studies are pointing towards a potential for *U. urealyticum* as the primary *Ureaplasma* spp. associated with infertility in men. Furthermore, some of the traditional markers for infertility may not indicate a causal role for *Ureaplasma* spp. in male infertility.

Treatment of genital tract infections in men caused by *Ureaplasma* spp.

A position statement from the European STI Guidelines Editorial Board states that routine testing of asymptomatic or symptomatic men for the presence of *Ureaplasma* spp. is not recommended, however, one of the key messages the authors made states that *Ureaplasma urealyticum* in high bacterial loads can cause a small proportion of male NGU (3-11% of NGU cases) (4). The authors also noted that NGU caused by *U. urealyticum* was more likely to develop in younger men and men with fewer lifetime sexual partners. They highlight that there is a paucity of well-designed large controlled studies which investigate the role of *Ureaplasma* spp. with STI syndromes and NGU.

In the light of mounting evidence of a causal role for *Ureaplasma* spp. in the development of NGU and male infertility, the question remains whether we should treat individuals who are *Ureaplasma* spp. positive with symptoms. Currently, a Position Statement from the European STI Guidelines Editorial Board does not recommend routine testing or treatment of either asymptomatic or symptomatic men for any *Ureaplasma* spp., however this Position Statement also suggests that *U. urealyticum* is causal in up to 11% of NGU cases which contradicts this recommendation (4). The evidence presented in this review suggests that in a subset of men with symptoms of NGU and the absence of other aetiological factors, a risk-based approach could be used to guide treatment of these patients. For example, symptomatic NGU patients, with the absence of other sexually transmitted infections, younger age, low number of partners and high titres of *U. urealyticum* may benefit from treatment. Currently it is clinically difficult to implement a risk-based approach in countries such as the UK as sexual healthcare settings do not widely test for *Ureaplasma* spp. and when done it almost never involves differentiation of *U. urealyticum* and *U. parvum* or determination of bacterial load.

Currently guidelines set out by the British Association for Sexual Health and HIV (BASHH) suggest treatment of a first episode of NGU with a 7 day course of doxycycline 100mg twice daily or if contraindicated, azithromycin 1g STAT followed by 500mg once daily for two days, or ofloxacin 200mg twice daily, or 400mg once daily, for 7 days (70). With recurrent episodes of NGU, where re-infection has been excluded, the recommended first line regimen is azithromycin 1g STAT then 500mg once daily for the next two days, plus metronidazole 400mg twice daily for five days. If symptoms still persist, treatment with moxifloxacin 400mg once daily for 10-14 days, plus metronidazole 400mg twice daily for five days is the recommended regimen. It is considered reasonable to provide epidemiological treatment to the partners of men with NGU using the same antimicrobial regimen that resulted in cure in the index case. In practice, if *Ureaplasma* spp. were present and responsible for symptoms of NGU, the first-line treatment recommended (a short course of doxycycline 100mg bd for 7 days) would be adequate to treat it in the UK at the moment in light of low levels of *tet(M)* mediated doxycycline resistance among these organisms (71). Any decision to treat would need to be carefully weighed up with the risk of inappropriate prescribing. Antibiotic resistance, in particular azithromycin resistance, among recognised GUM pathogens such as *M. genitalium* and *N. gonorrhoeae* is of growing concern (72, 73).

Summary

The role in which *Ureaplasma* spp. play in the development of genitourinary medicine-related infections is still a controversial area for many, but there is mounting evidence that these bacteria, especially *U. urealyticum*, have a causative role in infection under very specific conditions. *Ureaplasma* spp. are by no means a leading cause of NGU, but this is not to say that they do not contribute to cases which are currently classified as idiopathic; as such these patients are no less deserving of attention or correct management. Furthermore, the role of *Ureaplasma* spp. in the development of infertility among men is beginning to be recognised, but further work exploring the mechanism, as well as appropriate criteria for identifying patients with *Ureaplasma* spp.-induced infertility is required.

Ureaplasma spp. have a proven proinflammatory capacity in cell lines, animal and human models of disease, but the species of *Ureaplasma* spp., the sexual history of the patient and titre of bacteria present all appear to be key risk factors for the development of disease. A large prospective case-controlled study considering the species and load of *Ureaplasma*, presence of other microorganisms, number of PMNs as a marker of inflammation and number of sexual partners will be crucial to confirm or refute the role of *Ureaplasma* spp. in the development of NGU in men. If a clear link is identified, then current qualitative diagnostic methods may not be appropriate for determining a causal role for *Ureaplasma* spp. in cases of NGU. In the meantime, in light of the evidence presented in this review, we recommend that among cases of symptomatic NGU, in which classic aetiological agents have been ruled out, a risk-based approach taking into account patients with a younger age, low number of partners and high-titres of *U. urealyticum* should be considered for treatment.

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Figure Legends

Figure 1. Proposed natural history of *U. urealyticum* urethral infection in men following initial exposure. A hypothetical scenario in an immunologically naïve male when exposed to *U. urealyticum* for the first time. The lack of prior exposure to *U. urealyticum* results in an increased bacterial titre and subsequent polymorphonuclear neutrophil influx (signs of infection) with accompanying symptoms. Depending on the adaptive immunological response to *U. urealyticum*, the infection may clear without intervention or result in persistent urethral colonisation. With an increase in number of sexual contacts the presence of an adaptive immune response is able to keep the titre of any newly acquired *U. urealyticum* below the threshold which results in inflammation. In the absence of an adaptive immune response signs and symptoms may be present again. Persistent urethral colonisation may result in a commensal-like association with the host, accounting for the high prevalence among healthy individuals, or alternatively may result in the factors which are associated with the development of infertility.

Figure 2. Mechanisms associated with *Ureaplasma* spp.-induced infertility in men. A number of mechanisms have been proposed to account for the clinical observational studies showing decreased fertility among men who experience urethral colonisation with *Ureaplasma* spp. These include 1. Cross-reactivity of host generated antibodies against the UreG protein of *Ureaplasma* spp. to the autoantigenic sperm protein. 2. Generation of toxic compounds such as reactive oxygen species (ROS) which contributes to lipid peroxidation, DNA fragmentation and subsequent apoptosis. 3. Direct binding to spermatozoa which may result in reduced motility. 4. Reduced incorporation of P34H and hyaluronidase activity in the acrosomal cap, which may reduce the capacity of spermatozoa to penetrate the egg.

Author biographies

Dr Michael Beeton

Dr Beeton is a Lecturer in Medical Microbiology at Cardiff Metropolitan University and has been working on ureaplasmas for over 10 years. He obtained his PhD from Cardiff University, School of Medicine, in 2009 where his research focused on the incidence and molecular mechanisms of antibiotic resistance among *Ureaplasma* spp. isolated form preterm neonates. With an extensive publication history with regards to Ureaplasma and infectious disease he currently sits on the Executive Committee for the European Society of Clinical Microbiology and Infectious Diseases Study Group for Mycoplasma and Chlamydia Infections (ESGMAC). Furthermore, with an interest in sexually transmitted infections he represents the Microbiology Society on the Public Health England External Advisory Group on Sexual Health, Reproductive Health and HIV. His current research interests are focused on developing rapid diagnostic tests for *Ureaplasma* as well as understanding the immune response to *Ureaplasma* infections.

Dr Matthew Payne

Dr Payne received his PhD from the University of Queensland in 2007. Since then he has conducted microbiological research at Kings College London (London, UK), La Trobe University (Melbourne, Australia) and currently at the University of Western Australia (Perth, Australia). He is a molecular microbiologist whose research is focused on the microbiology of the perinatal period. He has specific interests in the perinatal microbiome, in particular, use of microbial biomarkers to predict women at high risk of preterm birth, and developing methods to accurately define microbial communities in low biomass samples. Dr Payne has specific interests in Ureaplasmas and Group B Streptococcus (GBS) as pathogens, as well as the protective role of vaginal *Lactobacillus* spp. in pregnancy. He also has a significant interest in the use of bacteriophages as antimicrobial agents, particularly for use in the perinatal period for removal of GBS as an alternative to intrapartum antibiotic prophylaxis.

Dr Lucy Jones

Dr Jones is an Associate Specialist in Sexual Health at Cwm Taf University Health Board and an Honorary lecturer at Cardiff University School of Medicine. She is Chief Investigator on four clinical studies in the field of sexual health and has a specialist interest in non-gonococcal urethritis, recurrent

683 vaginitis and antimicrobial resistance. She is Secretary to the British Association of Sexual Health and
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685 University before returning to live and work in Wales.

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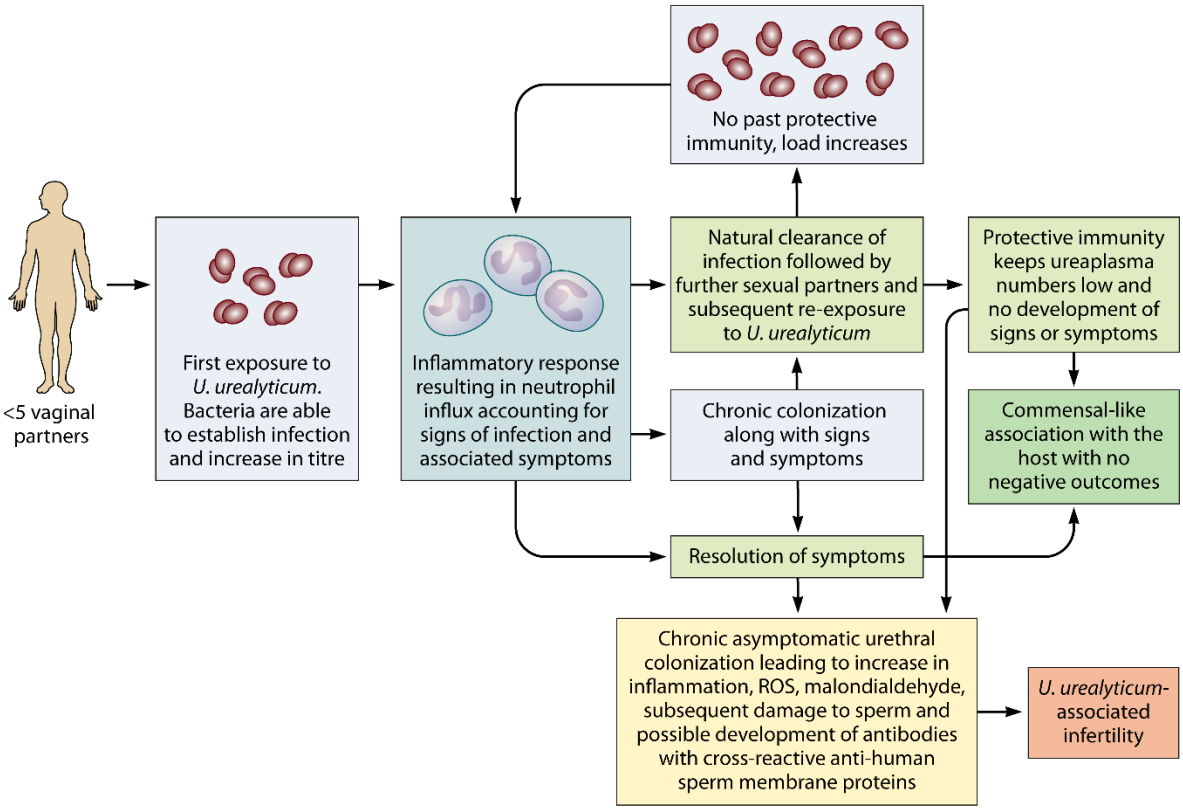

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691 Figure 1.

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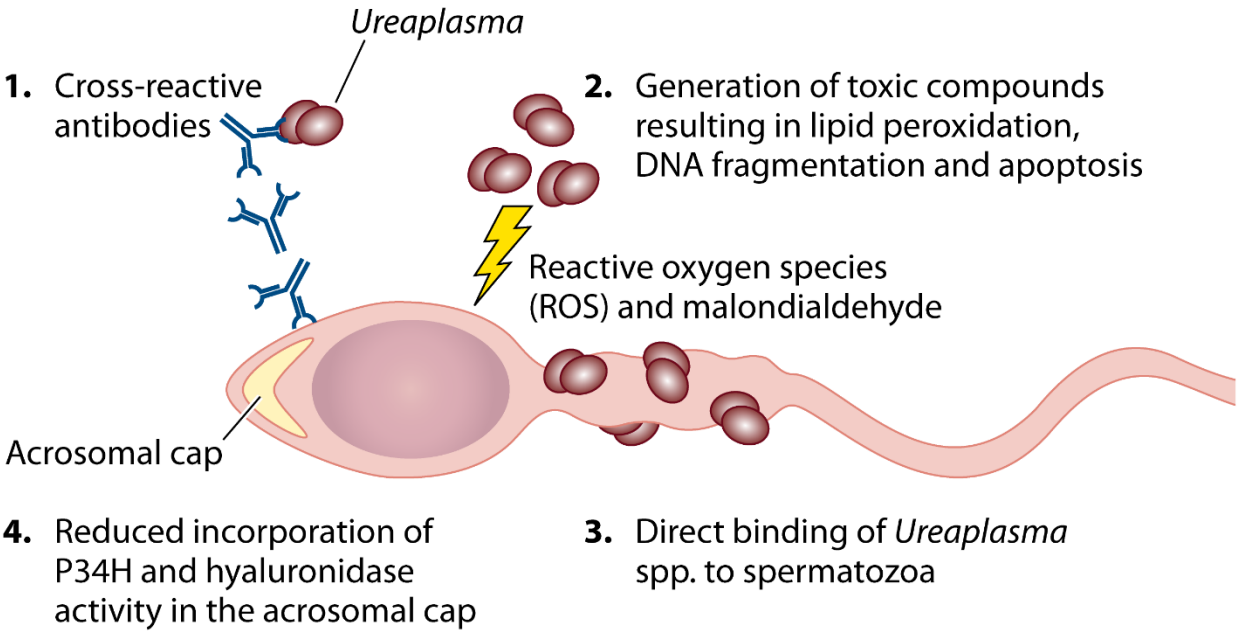

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694 Figure 2.

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Authors (year)	Reference number	Country of study	Patient group	Sample type	Number of participants	Method of Identification	Differentiation of *Ureaplasma* spp.	Key findings relating to *Ureaplasma* spp.
Frolund *et al.* (2016)	45	Sweden	Male patients attending STD clinic	First void urine	• 187 men with acute NGU • 24 men with chronic NGU • 73 men without NGU	Species specific qPCR	Yes	• Number of lifetime sexual partners was negatively associated with *U. urealyticum* load. • Urine containing *U. urealyticum* with > 1.3×10^3 genome equivalents/ml were associated with NGU.
Cox *et al.* (2016)	41	UK	Male patients attending a GUM clinic	Urine	• 75 men with NCNGU • 90 men without NCNGU	Species specific real-time PCR	Yes	• Significantly higher prevalence of *U. parvum* in the NCNGU group. • No association between *U. urealyticum* and NCNGU.
Khatib *et al.* (2015)	19	UK	Males attending an urban Sexual Health Clinic	Urine	• 83 men with urethritis	Multiplex PCR	Yes	• Only four patients were positive for *U. urealyticum.*
Deguchi *et al.* (2015)	48	Japan	Retrospective study of men attending urology clinic	First void urine	• 15 symptomatic men • 38 asymptomatic men	qPCR	Yes	• *U. parvum* load of $\geq 5 \times 10^3$ cells/ml were significantly associated with ≥ 12.5 leucocytes/µl of urine. • 83% of subjects had < 5×10^3 cells/ml suggesting a low bacterial load and lack of signs of inflammation.
Zhang N *et al.* (2014)	40	Multiple-countries	Meta-analysis	N/A	• 1507 men with NGU • 1223 men without NGU	N/A	Yes	• No significant difference between undifferentiated *Ureaplasma* spp. positive rate between NGU and control group. • When species was differentiated *U. urealyticum* was significantly associated with the NGU group whereas *U. parvum* was significantly associated with the control group.

Shimada *et al.* (2014)	47	Japan	Retrospective study of men attending urology clinic	First void urine	• 25 symptomatic • 26 asymptomatic	Species specific qPCR	Yes	• Bacterial load of *U. urealyticum* was positively correlated with NGU and number of leukocytes in urine.
Wetmore *et al.* (2011)	17	USA	Men attending STD clinic	Urine	• 329 men with NGU • Control Group 1 – 191 males without NGU attending a sexually transmitted disease clinic • Control Group 2 – 193 males attending emergency room without NGU	Culture	Yes	• *U. urealyticum* was associated with NGU. • Association was significantly stronger when analysing men with <10 vaginal partners. • Association was further strengthened when analysing men with <5 vaginal partners. • *U. parvum* was not associated with NGU.
Couldwell *et al.* (2010)	38	Australia	Men attending a sexual health clinic	First void urine	• 237 men with NGU • 268 controls	PCR	Yes	• *U. urealyticum* was significantly associated with NGU in the absence of another urethral pathogen.
Ondondo *et al.* (2010)	21	USA	Archived samples from heterosexual males attending STD clinic	Urine	• 119 men with NGU • 117 controls	PCR	Yes	• *U. urealyticum* was strongly associated with NGU. • This association was strongest in men <28 years of age. • *U. parvum* not associated with NGU.
Yu *et al.* (2008)	18	Hong Kong	Males attending a government sexually transmitted disease clinic	Urine	• 98 men with NGU • 235 controls	Real-time PCR targeting urease gene	No	• Neither *Ureaplasma* nor *M. genitalium* were associated with symptomatic NGU.

Bradshaw *et al.* (2006)	39	Australia	Men attending a sexual health clinic	First stream urine	• 329 men with NGU • 307 controls	PCR	Yes	• Neither *U. urealyticum* nor *U. parvum* were associated with NGU.
Povlsen *et al.* (2002)	36	Sweden	Men attending a sexual health clinic	Urethral swab	• 125 men with NGU • 205 without NGU	PCR	Yes	• No difference between NGU and non-NGU group if Ureaplasmas were not differentiated to the species level. • When differentiated, significantly more *U. urealyticum* were associated with males with NGU than those without.
Horner *et al.* (2001)	16	UK	Heterosexual men with NGU and control group	First pass urine	• 114 men with NGU • 64 without NGU	Culture	No	• Ureaplasmas were not associated with acute NGU. • Ureaplasmas were associated with NGU during follow-up. • Ureaplasmas were associated with chronic NGU.

Table 1. Published studies examining the relationship between *Ureaplasma* spp. and non-gonococcal urethritis. PCR = Polymerase Chain Reaction. NCNGU = Non-chlamydial non-gonococcal urethritis. N/A = not applicable.

Authors (year)	Reference	Country of study	Patient group	Sample type	Number of participants	Method of Identification	Species differentiated	Key findings relating to *Ureaplasma* spp.
Huang *et al.* (2016)	57	China	Men attending a reproductive centre	Semen	• 19,098 infertile men • 3,368 fertile men	Culture	No	• *Ureaplasma* spp. were significantly associated with infertility. • *Ureaplasma* spp. were significantly associated with reduced motility and normal forms compared with fertile controls.
Huang *et al.* (2015)	56	Multiple-countries	Meta-analysis	N/A	• 611 infertile men • 506 fertile men	N/A	Yes	• *U. urealyticum* was significantly associated with infertility. • *U. parvum* was not associated with infertility.
Zhang *et al.* (2014)	54	China	Men attending an infertility clinic	Semen	• 223 infertile men • 146 fertile men	Culture	Yes	• *U. urealyticum* was significantly associated with infertility compared with *U. parvum*. • Semen positive for *U. urealyticum* showed decreased concentration of spermatozoa and decreased motility.
Abusarah *et al.* (2013)	55	Jordan	Men attending a urology clinic	Semen and first void urine	• 93 infertile men • 70 fertile men	PCR	Yes	• Ureaplasmas were found more frequently among samples from infertile men (10.8%) vs fertile men (5.7%).
Zeighami *et al.* (2009)	53	Iran	Men attending an infertility centre	Semen	• 100 infertile men • 100 fertile controls	PCR	Yes	• Ureaplasmas were detected significantly more often in semen from infertile men compared with controls. • *U. urealyticum* was detected in 9% of infertile men vs 1% of control men. • *U. parvum* was detected in 3% of infertile men vs 2% of control men.

Table 2. Published studies examining the relationship between *Ureaplasma* spp. and male infertility. PCR = Polymerase Chain Reaction. N/A = not applicable.