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REVIEW ARTICLE

Ureaplasma species and preterm birth: current perspectives

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ABSTRACT

Preterm birth is the leading cause of neonatal morbidity and mortality worldwide, and the human *Ureaplasma* species are most frequently isolated from the amniotic fluid and placenta in these cases. Ureaplasma colonisation is associated with infertility, stillbirth, histologic chorioamnionitis, and neonatal morbidities, including congenital pneumonia, bronchopulmonary dysplasia, meningitis and perinatal death. The human *Ureaplasma* spp. are separated into *Ureaplasma urealyticum* and *Ureaplasma parvum* with 14 known serotypes. The small genome has several genes, which code for surface proteins; most significantly the Multiple Banded Antigen (MBA) where an antigenic C-terminal domain elicits a host antibody response. Other genes code for various virulence factors such as IgA protease and urease. *Ureaplasma* spp. infection is diagnosed by culture and polymerase chain reaction (PCR) and commercial assays are available to improve turnaround time. Microbroth dilution assays are routinely used to test antimicrobial susceptibility of clinical *Ureaplasma* spp. especially against doxycycline, azithromycin, ofloxacin and josamycin. Resistance to macrolides, fluoroquinolones and tetracyclines has been reported. A concise review of *Ureaplasma* spp. and their role in pregnancy outcomes, especially preterm birth, offers insight into the early diagnosis and appropriate antibiotic therapy to prevent long-term complications of *Ureaplasma* spp. infections.

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1. Introduction

The role of *Ureaplasma* spp. in human disease has been the focus of much research as these bacteria are commonly isolated as part of the normal genital tract flora. Two species, *Ureaplasma parvum* and *Ureaplasma urealyticum,* have been found to infect humans (Waites, Schelonka, et al. 2009; Gwee and Curtis 2014). While *U. parvum* is more commonly implicated in clinical disease, *U. urealyticum* is frequently seen in urogenital infection (Deguchi et al. 2004; Chang-Tai et al. 2011).

Published studies indicate that *Ureaplasma* spp. have been implicated in poor pregnancy outcome such as; spontaneous preterm labour, preterm premature rupture of foetal membranes (PPROM) and clinical chorioamnionitis (Watts et al. 1992; Yoon et al. 2003; Witt et al. 2005). Their presence in the lower genital tract has been linked to the presence of increased, matrix metallo-proteinases, prostaglandins and cytokines, which are associated with precipitation of preterm Labour and PPROM (Crouse et al. 1998; Li et al. 2000; Kacerovsky et al. 2013). The vertical transmission rate of *Ureaplasma* spp. may vary from 18 to 88% according to different studies. Neonates may be infected either by intrauterine infection or infection during labour–intrapartum transmission (Schelonka and Waites 2007). Preterm birth (<37 weeks of gestation) is a significant cause of neonatal morbidity and mortality globally. Prevention of preterm labour is therefore a significant priority in obstetric and perinatal research.

Neonates may be colonized in the respiratory and urogenital tracts due to the ability of *Ureaplasma* spp. to adhere to epithelial cells. The rate of colonization of the respiratory tract increases with prolonged duration of ruptured amniotic membranes, suggesting that *Ureaplasma* spp. gain entry by ascending infection (Witt et al. 2005). Foetal or neonatal *Ureaplasma* infections have been associated with a multitude of adverse outcomes including pneumonia, chronic lung disease, cerebral white matter lesions, cerebral palsy and death (Crouse et al. 1993; Kasper et al. 2011; Olomu et al. 2009).

Horner et al. (2018) recently published a review advocating against routine screening or testing of

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individuals for *Ureaplasma* spp. or *Mycoplasma hominis* and implying that treatment of 'commensal' *Ureaplasma* may in fact drive antimicrobial resistance of 'true' pathogens. In contrast, the abovementioned roles of *Ureaplasma* spp. and associated disease pathogenesis surely motivates continued research to gain better insight into effective prevention and treatment protocols.

2. Taxonomy of *Ureaplasma* spp

Human Ureaplasmas, discovered in 1954, are tiny microorganisms which were initially thought to belong to the genus Mycoplasma due to their resemblance. They are usually found in the mouth, upper respiratory tract and the urogenital tract of both men and women (Cassell et al. 1993). Ureaplasmas are frequently associated with urogenital diseases and adverse pregnancy outcomes including preterm birth and neonatal respiratory diseases (Kim et al. 2003; Waites et al. 2005).

Ureaplasma spp. belongs to the family *Mycoplasmataceae,* class *Mollicutes*, order *Mycoplasmatales*. They produce small colonies (7–15 μm diameter) and metabolize urea and not arginine or glucose. Ureaplasmas have evolved by degenerative evolution to lack a peptidoglycan cell wall and are therefore pleomorphic bacteria with sizes varying between 0.2 and 0.3 μm (Glass et al. 2000). Antibiotics such as beta-lactams are not effective as they lack a cell wall. Due to their small genome, they have limited biosynthetic capabilities, and require serum supplemented enriched growth medium for growth *in vitro* (Waites and Taylor-Robinson 2007).

Human *Ureaplasmas* are divided into *U. parvum* and *U. urealyticum* and 14 serovars. *U. parvum* includes serovars 1, 3, 6 and 14 while *U. urealyticum* includes serovars 2, 4, 5, 7–13 (Robertson et al. 2002; Kong and Gilbert 2004; Schelonka and Waites 2007).

Various subtyping methods have been developed to investigate epidemiology of *Ureaplasma* spp. These include traditional PCR for species/serovar identification based on sequencing of 16S rRNA, MBA and urease genes, pulsed field gel electrophoresis, restriction fragment length polymorphisms and real-time PCR (Kong et al. 2000; Mallard et al. 2005; Xiao et al. 2010; Xiao et al. 2011; Dando et al. 2014). Multilocus sequence typing (MLST) of *Ureaplasma* spp. adds value to exploring the epidemiology and clinical diversity of the species. Zhang et al. developed a MLST scheme with four housekeeping genes (*ftsH, rpL22, valS* and *thrS*) and subsequently added two virulence genes (*ureG* and *mba-np1*) for an expanded MLST (eMLST) with improved discrimination and higher level of resolution for subtyping *Ureaplasma* spp. MLST is considered more specific, sensitive and reproducible than other molecular subtyping methods (Zhang et al. 2014a, 2014b).

3. *Ureaplasma* spp.–genome

The *Ureaplasma* spp. 'pan genome' contains 1020 genes coding for proteins, which includes the 'core genome' of which 515 genes are universally conserved among all serovars. *U. urealyticum* possesses a slightly larger genome (0.84–0.95 Mbp) than *U. parvum* (0.75–0.78 Mbp), however they are amongst the smallest self-replicating organisms. All *Ureaplasma* strains have two rRNA operons and tRNA coding genes, together with an average of 608 genes for *U. parvum* serovars and 664 genes for *U. urealyticum* serovars. There are slight variations in some clinical strains i.e. SV3F4 from Japan, with 571 predicted coding DNA sequences, 6 rRNA and 30 tRNA genes (Tully and Taylor-Robinson 1986; Paralanov et al. 2012; Wu et al. 2014). The *Ureaplasma* genome codes for several virulence factors such as surface proteins and lipoproteins. The most frequently studied is the *mba* gene encoding the Multiple Banded Antigen (MBA), which is unique to *Ureaplasma* spp. where the antigenic C-terminal domain elicits a host antibody response during infection. The *Ureaplasma* spp. genome may also contain remnants of transposases, integrase recombinase genes and some phage associated protein genes. The *tet*M gene, in serovar 9, has been identified as originating from a Tn916 transposon, conferring tetracycline resistance (Zheng et al. 1995; Paralanov et al. 2012; Kokkayil and Dhawan 2015).

4. *Ureaplasma* spp. and infections of the genital tract

Ureaplasma spp. are considered a part of normal genital flora and have an average colonisation rate of 40–80% (Cassell et al. 1993). However, *Ureaplasma* spp, are associated in a causal manner with gynecological diseases such as infertility, non-gonococcal urethritis and prostatitis (Chang-Tai et al. 2011). They may be detected in the lower urogenital tract of both healthy and diseased individuals and are therefore considered opportunistic pathogens.

The role of *Ureaplasma* spp. in bacterial vaginosis (BV) remains unclear, although present in a large proportion (62–97%) of patients with BV. The rate of vaginal colonisation ranges from 8.5 to 77.5% and is dependent on sexual activity with a higher incidence

seen in individuals having multiple sexual partners (Taylor-Robinson 1996).

Increasing frequency of *Ureaplasma* spp. infections have been reported in HIV infected patients. Genital Mycoplasmas, such as *M. genitalium* and *M. hominis,* have previously been noted as potential 'co factors' in AIDS pathogenesis as they act synergistically with the HIV virus exacerbating the syndrome. *Ureaplasma* spp. may also act as a co factor in HIV infected patients, though this is currently unverified (Ghosh et al. 2013).

Ureaplasma spp. lower genital tract colonisation has been suggested to cause infertility. In their study, Gupta et al. (2009) determined the presence of *Ureaplasma urealyticum* in the lower genital tract of 32% of infertile women. *Ureaplasma* spp. has also been isolated from the fallopian tubes of women with pelvic inflammatory disease, a known cause of infertility. A recent study by Zhou et al. (2018) detected the prevalence of *Ureaplasma* spp. in semen samples from infertile men. They reported a significantly higher prevalence of *Ureaplasma* in infertile men, compared to fertile men, and that *U. parvum* is more prevalent and pathogenic with regard to sperm motility, than *U. urealyticum*. In contrast, Knox et al. (2003) found that *U. parvum* serovar 6 and *U. urealyticum* were most prevalent in washed semen samples from men attending an IVF facility. They described an association between significantly higher concentrations and more adherent ureaplasmas with fewer non-motile sperm and increased sperm motility.

5. *Ureaplasma* spp. and adverse outcomes in pregnancy

Spontaneous preterm birth (PTB) (<32 weeks gestation) is thought to be due to chorioamnionitis in 25 − 40% of cases. The most common pathogens are the human *Ureaplasma* spp.–*U. urealyticum, U. parvum* and *Mycoplasma hominis* (Goldenberg et al. 2008; Prince et al. 2016; Sweeney et al. 2016).

There is poor correlation between clinical and histological chorioamnionitis and interuterine infection (IUI) is frequently not diagnosed prior to the onset of preterm labour (PTL). At this stage, the chorioamnionitis is ill established, as is the foetal inflammatory response syndrome (FIRS) and tocolysis is generally ineffective and may even be harmful. Identifying pregnant women at risk of infection-associated PTB, especially early enough for therapeutic intervention, would be a significant advancement in preventing PTB (Ireland and Keelan 2014; Prince et al. 2016). Other reasons for preterm birth are medical indications or interventions such as pre-eclampsia or eclampsia, and intrauterine growth restriction.

This is in contrast to spontaneous preterm labour and preterm premature rupture of the foetal membranes (PPROM)–together called spontaneous preterm births which, as stated previously, may be due to infection, inflammation, vascular disease, and over distension of the uterus. There are various risk factors for spontaneous preterm birth, these include; prior preterm birth, low maternal body-mass index, black race and periodontal disease (CDC 2016). The strongest predictors of spontaneous preterm birth include a raised cervical-vaginal foetal fibronectin concentration and short cervical length (Goldenberg et al. 2000).

Chorioamnionitis (CAM), an inflammatory condition of foetal membranes, is casual in pathologies of complicated pregnancies such as premature rupture of membranes (PROM), spontaneous PTB, funisitis (inflammation of the umbilical cord), FIRS, and foetal death. Although CAM has multiple aetiologies, it is most commonly caused by microbial invasion of the amniotic cavity (MIAC). *U. urealyticum, U. parvum* and *Mycoplasma hominis* are most frequently isolated from the amniotic fluid and placenta in cases of histologic and clinical chorioamnionitis and in association with spontaneous PTL and PROM (Goldenberg et al. 2008; Prince et al. 2016; Sweeney et al. 2016). A recent study by de Goffau et al. (2019) used a metagenomic approach to detect bacterial DNA in the placenta in association with preterm birth, pre-eclampsia or delivery of small for gestational age (SGA) neonates, and found a significant association of *Ureaplasma* spp. with preterm birth. However, this was in contrast to their main conclusion where they found no evidence for the existence of a placental microbiome and no significant relationship between bacterial placental infection and the risk of preterm birth, pre-eclampsia and SGA.

Ureaplasma spp. colonisation may result in preterm labour through the production of cytokines which initiate contractions. Figure 1 shows pathways where bacteria, including *Ureaplasma* spp., might enter the intra-amniotic cavity and elicit an inflammatory response, thereby initiating preterm labour during pregnancy (Sweeney et al. 2017). *Ureaplasma* spp. are also associated with synergistic introduction of other bacteria into the amniotic cavity resulting in inflammation, spontaneous abortion, foetal intrauterine growth restriction and chorioamnionitis, (Kim et al. 2003; Redline 2006; Taylor-Robinson 2007). *Ureaplasma* spp vaginal colonisation does not reliably predict preterm labour, however, when present in the amniotic fluid or placenta there is an increased risk. Women whose cervices are

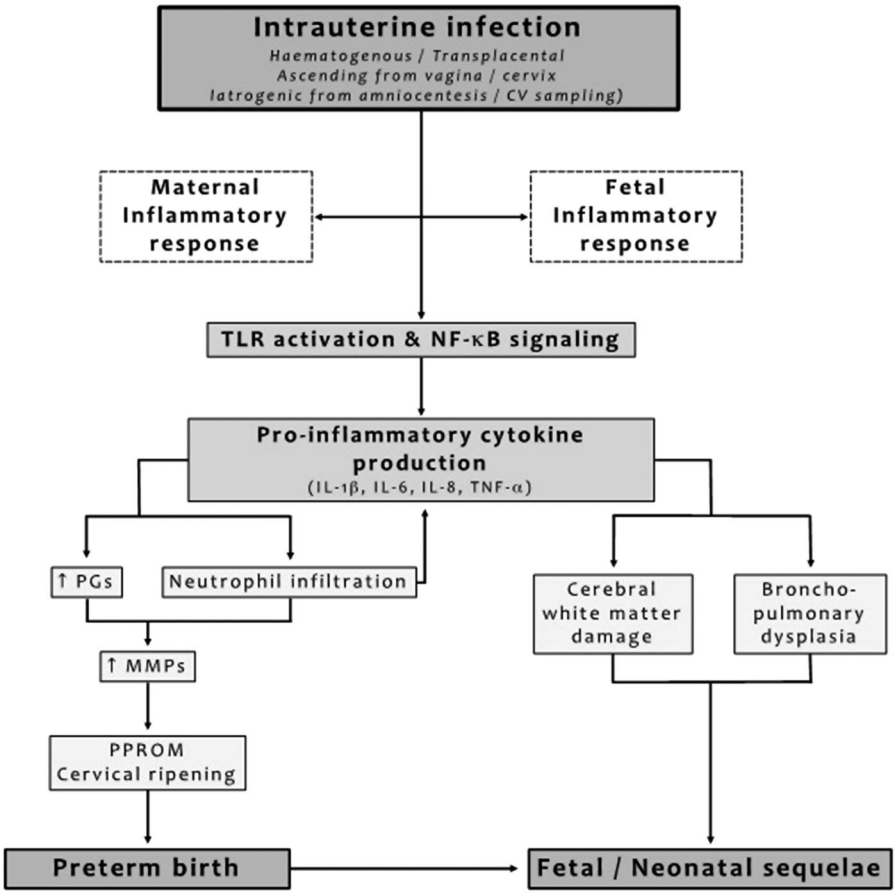

Figure 1. Intrauterine infection and inflammatory mediated preterm birth. Microbial invasion of the intra-amniotic cavity activates cytokine production which stimulates prostaglandin production and neutrophil infiltration, leading to the synthesis of matrix metalloproteinases and cervical ripening. TLRs, expressed by the chorioamnion, detect pathogen associated molecular patterns which is critical in the initiation of inflammatory mediated preterm birth. CV, chorionic villous; IL, interleukin; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa B; PG, prostaglandin; PPROM, preterm premature rupture of membranes; TLR, Toll-like receptor; TNF, tumour necrosis factor (Sweeney et al. 2017).

Ureaplasma spp. culture positive are more likely to develop complications, during pregnancy, than those who are culture negative (Waites, Schelonka, et al. 2009). Presence of *Ureaplasma* spp. in the preterm (<28 weeks) placental parenchyma is associated with an increased risk of maternal and foetal inflammation and PTL (Olomu et al. 2009).

In women with PPROM, *Ureaplasma* spp. was the bacteria most commonly identified in the amniotic fluid by using PCR amplification of 16S ribosomal DNA (DiGiulio 2012). Rittenschober-Böhm et al. (2019) reported a significantly increased risk for spontaneous PTB at extremely low (<28 weeks) and very low (<32 weeks) gestational age associated with vaginal *Ureaplasma parvum* serovar 3 colonisation and not for serovar 1 nor serovar 6.

Namba et al. (2010) carried out a study to confirm the prevalence of placental *Ureaplasma* spp. in preterm delivery, and whether there is an association between *Ureaplasma* spp. and chorioamnionitis. *Ureaplasma* spp. and *M. hominis* were shown to specifically recognize host cell surface glycolipids (sulfogalactoglycerolipid and the sphingolipid counterpart, sulfogalactosyl ceramide), which have been implicated in sperm-egg interactions. This glycolipid-receptor binding may relate to the reproductive pathogenesis of these organisms.

PPROM is often complicated by MIAC and IUI, causing development of histological chorioamnionitis. MIAC is predominantly caused by *Ureaplasma* spp. and complicates approximately 25–40% of cases of PPROM, depending on gestational age, ethnicity, and method of detection (Jacobsson et al. 2003; Kacerovsky et al. 2012). MIAC may precede the PPROM, allowing access of the organism into the amniotic cavity.

The presence of *Ureaplasma* spp. in the amniotic fluid or choriodecidual tissue stimulates the production of cytokines by activating the decidua and foetal membranes. Prostaglandins are then synthesized and released, stimulating uterine contractions. Activation and chemotaxis of neutrophils is initiated and the

synthesis and release of metalloproteases attacks and remodels cervical and chorioamnionic membrane collagen. This leads to cervical ripening, PROM and initiation of preterm labour (Olomu et al. 2009).

Kacerovsky et al. (2013) used multiplex xMAP technology (a bead-based multiplex immunoassay) to assess protein amniotic fluid profiles and determine the magnitude of the *Ureaplasma* spp associated inflammatory response. Increased interleukin (IL)−6, IL-8, IL-10, macrophage inflammatory protein-1, granulocyte macrophage colony stimulating factor, brain-derived neurotropic factor, matrix metalloproteinase-9 and monocyte chemotactic protein-1 in the amniotic fluid was associated with the presence of *Ureaplasma* spp. Preterm labour is ultimately initiated by the generalized inflammation in the choriodecidua and amnion, resulting in production of pro-inflammatory mediators such as IL-1β, IL-6 and prostaglandins.

Triantafilou et al. (2013) investigated the role of Toll-like receptors (TLRs) in the cytokine response during *Ureaplasma* associated inflammation of the amniotic epithelium. Their study, using a human cell line, suggested that the inflammatory response is mediated by the synergic activation of multiple TLRs including TLR2, TLR6 and TLR9. The *Ureaplasma* lipoprotein MBA triggers a response in TLRs 2 and 6, whereas TLR9 is the main initiator of inflammation once the *Ureaplasmas* are intracellular.

Studies in animal models with inoculation of *Ureaplasma* spp. into the intrauterine cavity have helped our understanding of the pathogenesis of chorioamnionitis and preterm birth associated with *Ureaplasma* infection. Novy et al. (2009) demonstrated that inoculation of *U. parvum* into the intrauterine cavity resulted in an inflammatory response and was responsible for chorioamnionitis, preterm birth and foetal pneumonia in rhesus macaques, a nonhuman primate model. This is in contrast to a more recent study by Senthamaraikannan et al. (2016) who found no significant association of *U. parvum* infection with chorioamnionitis and only modest inflammation in the foetal lungs of rhesus macaques. More recently, Pavlidis et al. (2020) used a mouse model to show ascending infection of *Ureaplasma parvum* is associated with preterm birth. The study reports an increase in preterm birth from 13% to 28% following vaginal colonisation with Ureaplasma and upregulation of pro-inflammatory cytokines, aligning with the human clinical response. Their results highlight the importance of the cervical epithelium as a barrier against ascending infection and is unique compared to other preterm induced murine models in that the majority of the preterm pups were live-born instead of stillborn.

Ureaplasma spp. can undergo vertical transmission from mother to child either perinatally or *in utero* (Biernat-Sudolska et al. 2006; Pinna et al. 2006). The vertical transmission rate approaches 90% in neonates less than 1 kg when the mother is colonized with *Ureaplasma* spp. as these premature infants appear to have a higher risk of infection. Infection may occur via exposure of the foetus to ascending *Ureaplasma* spp. intra-uterine infection (IUI), by placental haematogenous dissemination and passage through the vagina during birth. This exposure may result in colonisation of neonatal skin, mucosal membranes, respiratory tract, and occasionally disseminate into the central nervous system and bloodstream (Schelonka and Waites, 2007). The reasons why commensal *Ureaplasma* spp. cause IUI leading to preterm birth in some women, but not others, may be complex and multifactorial in nature.

In spontaneously aborted foetuses, stillborn and premature newborns, it has been found that *Ureaplasma* spp. has been isolated more frequently than from induced abortion foetuses or healthy full-term infants. In these cases, *Ureaplasma* spp have also been isolated from neonatal internal organs and therefore infection is not necessarily exclusively due to superficial contamination (Taylor-Robinson 1996).

Persistence and virulence of *Ureaplasma* spp. may be augmented by their ability to form a biofilm. Pandelidis et al. (2013) confirmed in a study, that clinical isolates of *Ureaplasma* spp. can form biofilms *in vitro* thereby contributing to their persistence resulting in chronic inflammation.

6. *Ureaplasma* spp. and diseases in the newborn

The most common cause of perinatal morbidity and mortality in preterm neonates is respiratory disease. Investigations as early as the 1970s, where *Ureaplasma* spp were isolated from the lungs of stillborn infants with pneumonitis, suggest the potential for these organisms to play a role in neonatal respiratory disease. Ureaplasma infection may occur either *in utero* or perinatally in prematurely born infants and can trigger a vigorous pro-inflammatory response in neonatal lungs, increasing risk for developing bronchopulmonary dysplasia (BPD) (Schelonka and Waites 2007).

Ureaplasma infection detected in the amniotic fluid, chorioamnion and lungs of neonates with an acute inflammatory response, is evidence that *Ureaplasma* may cause congenital pneumonia. Further evidence may be a specific neonatal IgM response, changes shown on radiographs of neonatal pneumonia, or

detection of microorganisms in the lung tissue either by immunofluorescence or electron microscopy. Severe neonatal pneumonia may be caused by *Ureaplasma* associated bacteraemia (Schelonka and Waites 2007).

Although commonly isolated from respiratory secretions, *Ureaplasma* spp. has also been isolated from the cord blood, neonatal blood, gastric aspirates, lungs, cerebrospinal fluid and brain tissue, indicating that it may cause bacteraemia and systemic infection (Sung 2010). Mycoplasmal meningitis is more common in very low birth weight (VLBW) infants than in those born at full term. A study by Waites, Schelonka, et al. (2009), showed that, of 100 preterm infants, Ureaplasma were isolated from cerebrospinal fluid of 8 babies undergoing treatment for suspected sepsis or hydrocephalus. Several other studies have also identified *Ureaplasma* spp. and *M. hominis* as potential etiologic agents of neonatal cerebrospinal fluid infection. *Ureaplasma* spp. may also cause central nervous system inflammation in preterm neonates depending upon factors such as serovar pathogenicity, host susceptibility and vulnerability of the CNS at low gestational age (Glaser and Speer 2015).

By interfering with normal retinal vascularisation, *Ureaplasma* spp. infection may cause retinopathy of prematurity (ROP) in preterm neonates. Ozdemýr et al. (2012) confirmed that neonatal colonisation with *U. urealyticum* is associated with severe ROP which may be treated with laser ablation surgery.

7. Pathogenesis

Ureaplasma spp. adherence has been reported in various human cells such as urethral epithelial cells, spermatozoa and mucosal surfaces by means of cytadherence proteins expressed on their cell surface. Five virulence factors have been identified as contributing to the evolution of disease pathogenesis and virulence mechanisms of *Ureaplasma* spp. These include the MBA, phospholipases A and C, IgA protease, and the urease gene.

The multiple banded antigen (MBA) protein, encoded by the *mba* gene, is unique to ureaplasmas and is not homologous to any other prokaryotes. During infection, the MBA protein is recognized by the host and increases cytokines production by activating NFκB via Toll like receptors 1, 2 and 6. The MBA protein comprises a signal peptide, conserved N-terminal transmembrane domain and a surface exposed C-terminal variable domain. The C-terminal domain is composed of multiple repeating units with serovar-specific and cross-reactive epitopes and undergoes antigenic phase- and size-variation (Zheng et al. 1995; Dando et al. 2014). Size variation in *mba*/MBA may be due to slipped-strand mispairing and may be the means whereby *Ureaplasma* spp. evade the host immune response during gestation, allowing the development of chronic asymptomatic infection, as investigated in a sheep model (Dando et al. 2014).

Phospholipases are present in many organisms as catabolic enzymes responsible for phospholipid metabolism or they may act as virulence factors for migration through host membranes. Phospholipases result in pathogenesis by producing compounds which destabilize or degrade host cell membranes and may contribute clinically to preterm labour by the production of arachidonic acid and subsequently prostaglandins (Paralanov et al. 2012). Although endogenous phospholipase activity has been recorded in *Ureaplasma* spp. (specifically serovars 3, 4 and 8), full genome analysis could not identify genes with any homology to known phospholipase genes. A study by Paralanov et al. 2012, using two assays to detect phospholipase activity in Ureaplasmas, could not detect either phospholipase A or C in *U. parvum* serovar 3 nor *U. urealyticum* serovar 8. When Paralanov et al. repeated the DeSilva and Quinn experiments exactly as originally outlined, they failed to detect phospholipase C, and together with the lack of any homologue detected via extensive metagenomic analysis, it appears that phospholipases do not play a role as virulence factors for *Ureaplasma* spp (DeSilva and Quinn 1999; Glass et al. 2000).

IgA proteases degrade host IgA antibodies, thus allowing microorganisms to evade host immune Defence mechanisms. Similar to phospholipases, endogenous IgA proteases have been detected in *Ureaplasma* spp, however, genes encoding these proteins have not been identified during examination of the genomes of multiple serovars (Waites, Schelonka, et al. 2009; Dando et al. 2014). A study by Arfi et al. (2016) provides evidence that *Ureaplasma* spp. contain genes encoding an IgG binding protein and IgG serine protease which were previously identified within *Mycoplasma mycoides* subsp. *capri*. Further studies are required to determine their potential as a virulence factor in *Ureaplasma* spp.

In 1966, it was discovered that *Ureaplasma* spp have the unique ability to hydrolyse urea for the production of ATP. During infection, urease metabolism results in an increase in ammonia which alters the pH of amniotic- and foetal lung fluids, and even in the absence of inflammation, may lead to lung damage. In patients who have undergone lung transplants and have *Ureaplasma* spp. infection, this process may also lead to

an uncharacteristically high ammonia level within the blood, known as hyperammonemia. When treated for Ureaplasma infection, their syndromes subsequently resolved. A relapse occurred in one case, and it was later identified that the patient was colonized with an antimicrobial-resistant *Ureaplasma* spp. strain (Bharat et al. 2015). The urease activity of *Ureaplasma* spp. may result in further tissue damage by causing pH changes within the amniotic fluid and foetal and adult lungs.

8. Laboratory testing of *Ureaplasma* spp

8.1. Detection methods

Ureaplasma spp. can be detected from specimens obtained via urine, endometrial tissue biopsy and urethral/endocervical swabs. Dacron, calcium alginate and polyester swabs on plastic shafts are used for specimen collection. Since *Ureaplasma* spp. lack a cell wall and are therefore extremely sensitive to desiccation and heat, it is essential to use a specialized transport medium to maintain specimen integrity. Specimens should be transported to the laboratory immediately, however, if this is not possible they should be refrigerated until testing occurs (Waites and Taylor-Robinson 2007).

Culturing *Ureaplasma* spp. is difficult as these fastidious microorganisms require serum, metabolic substrates and growth factors for isolation. Specialized media such as SP4-, Shepard's 10 B- and PPLO- broth and agar may be used to culture *Ureaplasma* spp. Since growth of *Ureaplasma* spp. does not yield turbidity in broth, phenol red is added as a pH indicator and growth is measured by a colour change. In order to prevent contamination of such enriched media, antibiotics, like penicillin G, and antifungals, like nystatin, may be added during culture of *Ureaplasma* spp.

Culture and detection of *Mycoplasma* spp. and *Ureaplasma* spp. have been simplified due to the development of diagnostic kits and commercially prepared media. These media include arginine broth for culturing *M. hominis* and U9 broth for *Ureaplasma* spp. [Bio-Rad]. Diagnostic kits include: MycoIST2 (bioMerieux), Mycoview (Ivagen) and MycoDuo and SIR Antibiogram (Bio-Rad) for identification and antibiotic susceptibility testing. These commercial products require extensive quality control testing and their limitations should also be considered (Duffy and Waites 2008).

As culture of *Ureaplasma* spp. is often difficult, molecular methods of detection may be of value. Nelson et al. (1998), established PCR to be a more sensitive detection method than culture, especially if analysed rapidly after specimen collection. In neonates that are infected at birth, the concentration of the organism would be low at day zero, increasing over time, thus rapid and sensitive PCR assays for early diagnosis would be of tremendous benefit in antibiotic treatment practices.

Real time PCR assays are invaluable for the simultaneous detection of *Ureaplasma* spp and species determination in the clinical setting (Yi et al. 2005). Cao et al. (2007), have established two RT-PCR Taqman assays for the quantitative detection of *Ureaplasma* spp. with a detection rate higher than that of conventional PCR and traditional culture methods.

Conventional PCR assays target sequences of the 16S rRNA gene and the 16S rRNA–23S rRNA intergenic spacer regions for species determination, and genes for MBA and urease for species differentiation (Blanchard, 1990; Robertson et al. 1993; Cordova et al. 2000). Real time PCR assays usually target the MBA or urease genes to determine the bacterial load, which is a valuable clinical indication of infection (Waites et al. 2012).

A study by Kikhney et al. (2017) demonstrated the combination of two molecular techniques in order to detect microorganisms in the placenta in cases of preterm birth. They used broad range 16S rRNA-gene PCR together with pan-bacterial probes (general and species/genera- specific) for fluorescence *in situ* hybridisation (FISH) to simultaneously identify and visualize microorganisms in placental tissue and correlate findings with incidence of chorioamnionitis.

Tissues such as the placenta often have a low microbial biomass, and as such may be challenging to analyse methodologically and statistically. Sequencing using 16 s rRNA could erroneously identify microorganisms due to the presence of non-endogenous transcripts, contamination from sample preparation or the presence of PCR and sequencing artefacts. It is therefore, essential to critically analyze data for microbiome studies (Leon et al. 2018).

8.2. Antimicrobial susceptibility testing

The Clinical Laboratory Standards Institute (CLSI) published guidelines in 2011 for methods of antimicrobial susceptibility testing for human Mycoplasmas. The publication provides guidelines for the implementation and quality control of antimicrobial susceptibility tests on *Mycoplasma* spp. and *Ureaplasma* spp. using agar and microbroth dilution assays (CLSI 2011).

The microbroth dilution test is the most economical, practical and frequently used method for antimicrobial susceptibility testing. In this method, a standardized inoculum of organisms (usually 10^4/mL) in broth

medium (with an indicator) is mixed with decreasing concentrations of antibiotics and then incubated. Susceptibility to the antibiotic, at a specific concentration, causes growth inhibition resulting in no change of colour. The minimum inhibitory concentration (MIC) is the lowest concentration of antibiotic to inhibit colour change at the time when the colour change in the antibiotic-free control has just occurred (Bebear et al. 2003).

Commercial antibiotic susceptibility tests such as the MycoIST 2 kit (bioMerieux) and SIR Mycoplasma kit (Biorad) consist of microwells with different concentrations of antibiotics including: clarithromycin, josamycin, erythromycin, azithromycin, clindamycin, pristinamycin, doxycycline, tetracycline and ofloxacin. The SIR Mycoplasma kit is used in preliminary antibiotic resistance screening has been validated by laboratories in France (Bebear et al. 2003; Degrange et al. 2008).

9. Treatment of infections caused by *Ureaplasma* spp

Owing to the lack of cell wall, antibiotics that target wall synthesis are ineffective against *Ureaplasma* spp. infection. The MLS (macrolide, lincosamide and streptograminin) group of antibiotics is frequently used to treat human mycoplasma and Ureaplasma infections, as well as ketolides, fluoroquinolones and tetracyclines. *Ureaplasma* spp. are inherently resistant to lincosamide antibiotics such as clindamycin (Pereyre et al. 2002; Lu et al. 2010).

Ureaplasma spp. infections during pregnancy and in neonates are most commonly treated with macrolides (Pereyre et al. 2007). For the treatment of respiratory tract infections, The MLSK (macrolide, lincosamide, streptograminin and ketolides) group of antibiotics are often prescribed, especially when fluoroquinolones or tetracyclines are contra-indicated, for instance during pregnancy or in neonates and children. First line treatment for neonatal respiratory tract infections is erythromycin for *Ureaplasma* spp. and josamycin for *M. hominis* (Waites et al. 2005). In clinical isolates, an investigational ketolide, CEM-101, showed potent growth inhibition of *Mycoplasma* and *Ureaplasma* spp. at a concentration of $\leq$0.5 μg/mL (Waites, Crabb, et al. 2009).

Solithromycin (Cempra Inc. Chapel Hill, NC, USA) is a novel fluoroketolide antibiotic and a fourth-generation macrolide which has been derived from clarithromycin. Solithromycin has been shown to be effective in treating infection-associated preterm delivery, including *Ureaplasma* spp. and *Mycoplasma* spp. Solithromycin may be used as a prophylactic in treating asymptomatic women with a high risk of preterm birth. and in women with PPROM and may also be effective in improving preterm neonatal outcomes by providing anti-inflammatory and antimicrobial benefit prior to delivery (Keelan et al. 2016).

During pregnancy, antimicrobial agents administered in cases of preterm rupture of the membranes (PROM) may extend gestation thereby decreasing risk of associated preterm complications and neonatal infection. Since fluoroquinolones and tetracyclines are contraindicated in pregnancy, macrolides are the most frequently prescribed antibiotics. However, erythromycin cannot effectively penetrate the amniotic cavity and hence may not fully eradicate intrauterine *Ureaplasma* spp. infection. Azithromycin treatment is as successful as erythromycin but has fewer side effects (Pitsouni et al. 2007).

The risk of increasing the rate of necrotising enterocolitis or late onset sepsis with prolonged antibiotic exposure emphasizes the need for caution when prescribing antibiotic treatment in neonates (Cotten et al. 2009).

10. Antimicrobial drug resistance

Antimicrobial drug resistance may arise from exposure to non-lethal concentrations or failure to comply with dosage recommendations. Resistance may occur via drug efflux pumps, target site modification or drug inactivation mediated by short peptides.

Genital Mycoplasmas are inherently resistant to beta lactams which target components of the cell wall. *Ureaplasma* spp. are innately resistant to lincosamides such as clindamycin (Lu et al. 2010) and Mycoplasmas show resistance to rifampicin, trimethoprim and sulphonamides. The presence of a single amino acid of the beta sub unit of RNA polymerase, at position 526, confers resistance to rifampicin (Taylor-Robinson and Bébéar 1997).

Resistance to macrolides is associated with mutations in the 23S rRNA gene, and ribosomal protein L4 and L22 genes. Tetracycline resistance is associated with the presence of the moveable *tetM* transposon (Beeton et al. 2009; Xiao et al. 2011; Sweeney et al. 2017). Resistance to macrolides may also occur via drug efflux pumps, associated with the macrolide streptogramin resistance (msr) genes, which export antimicrobials out of the bacterial cell. Previously, Lu et al. (2010) detected msr(A), msr(B), and msr(D) subtypes and proposed that they may be associated with *Ureaplasma* spp. resistance to lincosamides and/or macrolides (Dando et al. 2014). As suggested in a previous study, quinolone resistance is mainly as a result of mutation of

target enzyme-DNA helicase, especially residues 68–107, known as quinolones regions of drug-resistance (QRDR) (Duffy et al. 2000).

From the three leading classes of antibiotics (quinolones, tetracyclines and macrolides) active against *Ureaplasma* spp., resistance to tetracyclines may pose the most significant threat. Resistance to tetracyclines is conferred via the horizontal transfer of the Tn*916*-like transposable element which harbours the *tet*(M) gene and the potential to disseminate resistance within a population poses a great threat to the success of antibiotic therapy (Beeton et al. 2016).

Prior to 2006, fluoroquinolone resistance in *Ureaplasma* spp. had been suggested to be associated with mutations resulting in amino acid substitutions at Ser83Leu and Asp87Lys in *ParC* and Ala125Thr and Ala136Thr in *ParC* together with a triple substitution of Asp112Glu in *GyrA* protein (Bebear et al. 2000; Zhang et al. 2002; Bebear et al. 2003). However, Beeton et al. (2009), attributed these three mutations to serovar-specific polymorphisms and therefore were not considered to be a resistance phenotype. Only mutations in the 8-amino acid region of the most common *ParC* substitution (Ser83Leu) should be considered in association with quinolone resistance. Kawai et al. (2015) reported the first *in vitro* quinolone-resistant clinical strains of *Ureaplasma* spp. associated with an S83L mutation. They also identified a *parC* gene mutation at S83W and S84P, and a mutation at P462S in the *gyrB* gene, from perinatal specimens in Japan.

In a South African setting, Govender and Chalkley (2012) reported tetracycline and doxycycline resistance in clinical isolates of *Ureaplasma* spp. Characterisation of the *Int-Tn* gene of tetracycline-resistant strains revealed three varieties and indicated that transposons from different sources had undergone genome integration. *TetM* sequences from tetracycline-resistant strains were observed to be highly mosaic in structure. The finding of *tetM* regions and/or transposons with, or without, tetracycline resistance, in conjunction with *int-Tn* and *tetM* gene diversity, may verify that *Ureaplasma* spp. undergo extensive genetic exchange of transposon or resistance genes, with concomitant genomic remodelling.

Govender et al. (2012) reported *U. parvum* resistance to quinolones, erythromycin and azithromycin in South Africa. The study revealed a point mutation in *parC* (Pro57Leu) and two novel mutations in *parE* (Ile73Thr and a methionine insertion at codon 86) that were found in an ofloxacin-resistant strain. This highlights how *Ureaplasma* spp. adapt to develop resistance by acquiring, modifying and maintaining resistance genes located on transposons. Le Roux et al. (2013) conducted a study in Pretoria, South Africa and reported resistance to tetracycline and doxycycline. In a follow up study, Ngobeni et al. (2014) reported a significantly higher prevalence of *Ureaplasma* spp. in 2013 than 2012.

A report by Beeton and Spiller (2017) has focussed on the importance of scientific rigour during antimicrobial susceptibility testing of *Ureaplasma* spp. They conclude that commercial kits are no longer adequate for reporting on international antibiotic resistance trends and furthermore, that other methods are required to accurately attribute resistance to *Ureaplasma* spp. such as molecular identification of resistance mechanisms or CLSI-compliant methodologies.

11. Conclusion

Ureaplasma spp. colonisation is associated with adverse pregnancy outcomes such as preterm birth, stillbirth, histologic chorioamnionitis, and neonatal morbidities, such as congenital pneumonia, bronchopulmonary dysplasia, meningitis, and perinatal death. Due to their fastidious nature, *Ureaplasma* infections are difficult to identify and diagnose in a clinical environment, and as such, additional research would be of value in order to improve identification of infection and treatment of *in utero* inflammation, ultimately leading to improved pregnancy and neonatal outcomes. Continued research investigating the development of new-generation drugs or targeted therapies may lead to more effective treatment of *Ureaplasma* infections. A better understanding of *Ureaplasma* spp. and their role in pregnancy and preterm birth, will offer insight into the early diagnosis and effective use of antibiotic therapy to prevent long-term sequelae of *Ureaplasma* spp. infections.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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