THE ROLE OF *UREAPLASMA UREALYTICUM* IN ADVERSE PREGNANCY OUTCOME

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Published 1997:

Australian and New Zealand Journal of Obstetrics and Gynaecology. **37:**45-51.

Statement of Joint Authorship.

ABSTRACT

We investigated *Ureaplasma urealyticum* genital tract colonisation rates in an Australian population to determine whether colonisation was associated with adverse pregnancy outcome. Women attending an antenatal clinic were evaluated for lower genital tract colonisation at their first antenatal visit (162 women) and at 28 weeks gestation (120 women). Placentas from 92 women were cultured. *U. urealyticum* was the predominant isolate from the lower (57.4%) and upper (17.4%) genital tract in this population of pregnant women. *U. urealyticum* was a persistent coloniser during mid-trimester of pregnancy (in 88% of women colonised) whereas *M. hominis, G. vaginalis,* and Group B streptococcus were present as transient flora of the lower genital tract. Lower genital tract colonisation during pregnancy was not directly associated with adverse pregnancy outcome. However preterm delivery in afebrile, asymptomatic women, could possibly be associated with chorioamnionitis (four of 16 preterm births). Screening of women with a history of preterm birth may prevent upper genital tract infections and preterm delivery.

INTRODUCTION

Ureaplasma urealyticum is the potentially pathogenic microorganism most frequently isolated from the lower and upper genital tract of women (1,2). However its role in invasive infections remains controversial. Many studies have shown that lower genital tract colonisation with *U. urealyticum* is not directly correlated with adverse pregnancy outcome. In a multicentre study by Carey *et al* (1), 4,934 women were evaluated for vaginal colonisation between 23-26 weeks, and *U. urealyticum* was found not to be directly associated with preterm rupture of membranes, preterm labour or preterm birth. Similarly cervical infections in 1,365 women at their first antenatal visit did not predict low birth-weight, abortion, stillbirth, preterm birth and preterm prolonged rupture of membranes (3). Whilst McGregor *et al* (4) found that women with bacterial vaginosis had an increased risk of preterm labour, and *Mycoplasma hominis* was also shown to be associated with preterm labour and preterm birth, their data did not demonstrate a relationship between *U. urealyticum* colonisation and adverse pregnancy outcome. Polk *et al* (5) showed an association between preterm birth and *Chlamydia trachomatis* and *M. hominis* colonisation; however, again there was no association with *U. urealyticum.* Others however have demonstrated a link between *U. urealyticum* and preterm birth and preterm labour (6,7). *U. urealyticum* and other organisms found in bacterial vaginosis have been shown to be associated with an increased risk of preterm labour (6). In an Australian population of 786 women, McDonald *et al* (7) found that women carrying *Gardnerella vaginalis* (16%) or *U. urealyticum* (34%) during their mid-trimester had nearly twice the risk of preterm birth; however, in this study tests were not performed for *C. trachomatis.*

U. urealyticum is a significant cause of chorioamnionitis frequently with the sequelae of adverse pregnancy outcome and perinatal morbidity and mortality (2,8). *U. urealyticum* is the organism predominantly isolated from newborns with chronic lung disease (9) and meningitis (in some populations) (10). Only a small proportion of women colonised with *U. urealyticum* in the lower genital tract develop chorioamnionitis. It remains to be determined if upper genital tract infection is due to maternal factors, infections ascending from the lower genital tract, the individual pathogenicity of the ureaplasma isolates, or a combination of these factors.

This present study was conducted to: (i) establish the *U. urealyticum* colonisation rates and the duration of lower genital tract colonisation during pregnancy in an Australian population (ii) determine the demographic and behavioural characteristics associated with *U. urealyticum* colonisation (iii) determine if either lower or upper genital tract colonisation with *U. urealyticum* was associated with adverse pregnancy outcome (iv) identify factors associated with preterm delivery.

MATERIALS AND METHODS

Population

From August to December, 1993, 162 women attending the Toowoomba Base Hospital local public antenatal clinic for their first antenatal visit (8-25 weeks) were enrolled in this prospective study. Informed consent was obtained from all participants and the study was approved by the Ethics Committees of the University of Southern Queensland and the Darling Downs Regional Health Authority. Upon enrolment participants were interviewed and completed a standard questionnaire in order to provide demographic and behavioural information and a medical history of previous deliveries and of past genital tract infections.

Microbiology

Specimen collection

A speculum examination was performed at the first antenatal visit (8-25 weeks) and separate endocervical samples were collected for: Papanicolaou smear; *Chlamydia* EIA test, Vidas ^{Tm} (Vitek/bioMerieux, Hazelwood, MO) (swab placed in transport media); Ureaplasma/Mycoplasma culture; bacteriological screen (swab placed in charcoal transport media); and a smear for Gram staining. A further specimen was collected from the posterior fornix of the high vagina for Ureaplasma/Mycoplasma culture. The Ureaplasma swabs were: (i) inoculated immediately onto Mycoplasma A8 agar medium (modified) (Becton Dickinson, Brisbane, Australia) and (ii) also vortexed and expressed into Shepard U9B liquid broth (11) (then held at 4^0 C until transported to the laboratory). After each clinic, specimens were transferred to the laboratory and processed immediately. Specimens were also collected at 28 weeks' gestation. Endocervical specimens and a vaginal specimen were collected by speculum examination for a bacteriological screen and for Ureaplasma/Mycoplasma culture. In addition a low vaginal swab and a rectal swab were collected for isolation of Group B Streptococcus.

U. urealyticum and *M. hominis* isolation

A8 agar plates were incubated at 37^0C, under 95% N_2 and 5% CO_2 atmosphere. Plates were examined microscopically after 2 days and ureaplasma and/or mycoplasma colonies were excised on an agar block, subcultured in U9B broth and stored at -70^0C for future testing. Four serial 10-fold dilutions were made of the U9B broth culture and 2 aliquots (0.5 mL) of the remaining original specimen were frozen at -70^0C. Ureaplasma growth was detected and quantitated by an alkaline shift and subsequent colour change in
the media. Broths were subcultured onto A8 agar and into a further U9B broth just as the pH change began. Broths showing no colour change were routinely subcultured onto A8 agar after 2 days.

Chlamydia trachomatis detection

All samples were tested using the *Chlamydia* EIA, VIDAS ^{Tm} (Vitek/bioMerieux, Hazelwood, MO). Positive results were confirmed by DFA (Cellab).

Bacteriology

All swabs from charcoal transport medium were inoculated routinely onto Horse Blood agar, MacConkey agar (BBL, Cockeysville, Md), Sabouraud dextrose agar with chloromycetin, and half Chocolate Agar/ New York plate (Oxoid, Australia) for the isolation of bacterial and yeast pathogens. A wet preparation in saline was examined for the presence of motile trichomonads and the swab was then incubated in a thioglycollate broth for the detection of anaerobic bacteria. All plates were incubated at 37^0C under 5% CO_2 atmosphere. Twenty eight-week low vaginal and rectal swabs were inoculated onto Streptococcus selective media (Oxoid, Australia) and Horse Blood agar. The Gram stains were examined for the presence of *G. vaginalis* and *Mobiluncus* species morphotypes and other bacteria and yeasts, the presence of 'clue' cells and for the quantitation of leucocytes. Slides were evaluated by two independent readers who were unaware of culture results.

Placenta culture and histology

After delivery, placentas from 93 participants were available for culture and histological examination. The chorionic surface of the placenta was sterilised by burning alcohol (2). Using aseptic technique, a block of villous tissue was excised, inoculated onto an A8 agar plate, a Horse Blood agar plate, and a half Chocolate Agar/ New York plate (Oxoid, Australia) and then placed into U9B broth. Three serial 10-fold dilutions were made of the U9B broth. Broths were subcultured at the beginning of the pH change or after 2 days incubation. The placenta was then fixed for histological examination. Histological examination of the placenta was performed in eight of 16 preterm births and 8 of 129 (placenta culture positive) term births. The placenta was sliced and macroscopically abnormal areas were sectioned for histological examination of both the maternal and foetal surface. Macroscopically abnormal areas of the cord and membranes were also sectioned. Chorioamnionitis was diagnosed by the presence of an acute inflammatory cell response in the membranes.

Statistical methods

The data involved in this prospective study involved mainly tests of equality of proportions. These were performed using the chi-squared test or Fisher exact test as appropriate.

RESULTS

Population

The majority of the participants were Caucasian 136 (84%), 17 were Aboriginals (10.5%) and seven were of Asian descent (4.3%). The mean maternal age was 24.9

± 5.2, years with a range from 15 to 42 years. The mean gestational age at enrolment was 14.9 ± 3.6 weeks. The mean parity was 2.4 ± 1.6.

Lower genital tract flora

Table 1 shows the recovery rates of microorganisms from lower genital tract specimens collected from 162 women during the first antenatal visit and from 120 women who were retested at 28 weeks' gestation.

TABLE 1. 'Microbiological screen' of pregnant women

		1st visit (14.9 ± 3.6 weeks)	28 weeks
Microorganism		Carriage (n=162) n (%)	Carriage (n=120) n (%)
Ureaplasma urealyticum (UU)	endocervix	70 (43.2)	60 (50)
	vagina	90 (55.6)	62 (51.7)
	either	93 (57.4)	63 (52.5)
Mycoplasma hominis (MH)[1]	endocervix	9 (5.6)	5 (4.1)
	vagina	11 (6.7)	4 (3.3)
	either	12 (7.4)	6 (5)
Gardnerella vaginalis, (GV)	endocervix	8 (4.9)	5 (4.2)
Group B *Streptococci* (GBS)		4 (2.5) endocervix	20 (16.7)low vaginal, rectal 5 (4.1) endocervix
Chlamydia trachomatis (CT)		6 (3.7)	NT
Candida albicans		8 (4.9)	10 (8.3)
Trichomonas vaginalis		1 (0.6)	NT
GV + UU		4 (2.5)	3 (2.5)
GV + UU + MH		1 (0.6)	1 (0.8)
GBS + UU		2 (1.2)	7 (5.8)
CT + UU		4 (2.5)	-
CT + UU + MH		1 (0.6)	-
GV + CT + UU		1 (0.6)	-
GBS + GV		-	1 (0.8)

1=always found in association with U. urealyticum, NT=not tested

Of the organisms investigated in this study *U. urealyticum* was the most prevalent coloniser of the lower genital tract in the second (57%) and third trimester (53%) of pregnancy. Overall 5.6% of women were colonised with *M. hominis* and this is a much lower colonisation rate than reported in overseas studies (24 - 39.9%) (4-6). Isolation rates for *G. vaginalis* (4.6%) and *C. trachomatis* (3.7%) were also very low, although a more recent study of *C. trachomatis* detection in our population has shown a low sensitivity of VIDAS Tm EIA, compared to PCR when testing asymptomatic carriers (12). Polymicrobial colonisation was present in 26 women (16%). As shown in Table 2, in this Australian population *U. urealyticum* was isolated more frequently (p=0.03) from Aboriginal women (82%) than from Caucasian (59%) or Asian women (28%). There was a higher *U. urealyticum* colonisation rate in smokers (67.2%, p=0.03) and in women who had previously used non-barrier contraceptives (57.9%, p=0.03). *U. urealyticum* colonisation was significantly associated with several 'risk' factors: (i) single marital status (p<0.01); (ii) more than 1 sexual partner in the previous 12 months (p=0.01); and (iii)

commencement of sexual activity at less than 18 years of age ($p<0.01$). There were no significant associations between *U. urealyticum* and either *C. trachomatis,* or *G. vaginalis,* or *M. hominis* or Group B streptococcus.

TABLE 2. Demographic and behavioural characteristics of women colonised with *U. urealyticum* (UU) in the lower genital tract

Characteristic	Number of women	Endocervical Samples % UU culture positive	p value	High Vaginal Samples % UU culture positive	p value
Race-Aboriginal	17	64.7	0.05	82.4	0.03
-Caucasian	136	58.8		55.1	
-Asian	7	14		28.6	
Single	77	55.84	0.001	75	.0001
Married	85	30.5		40	
1 sexual partner in past year	146	41.1	0.08	53.4	0.01
>1 sexual partner in past year	12	66.7		91.7	
Began sexual activity age ≤18	127	48.8	0.001	60.6	0.04
Began sexual activity age >18	30	16.7		40.0	
Non-barrier contraception	133	43.6	0.15	57.9	0.03
Barrier contraception	19	26.3		31.6	
Education <Grade 12	96	46.8	0.07	61.5	0.04
Completed Grade 12	33	50		51.5	
Tertiary	33	65		48	
Alcohol consumption- Yes	23	43.5	0.94	52.2	0.64
- No	138	42.8		57.3	
Marijuana use - Yes	5	60	0.43	80	0.28
- No	156	42.3		55.8	
Smoking - Yes	61	50.8	0.11	67.2	0.03
- No	100	38		50	

Not all of the 162 women were retested at 28 weeks. These defaulters either (i) elected not to have further lower genital tract specimens collected, (ii) failed to attend the antenatal clinic or (iii) clinicians advised against collection of specimens when other obstetric maternal risk factors were present.

Significantly this study documents the duration of lower genital tract colonisation in pregnancy (Table 3). Of those women colonised with *U. urealyticum ,* 88% (59/67) were colonised at the first antenatal visit and also at 28 weeks. Whilst the isolation rates of *M. hominis, G. vaginalis* were very low, it appears that these organisms and Group B streptococcus were present in the lower genital tract as transient flora. The higher total incidence of Group B streptococcus at 28 weeks is due to the additional, routine collection of specimens from the low vagina and rectum at that time. All women who tested positive for *C. trachomatis* at their first antenatal visit were treated. Two of these women were positive for both *C. trachomatis* and *U. urealyticum* and were treated with erythromycin until tests for *C. trachomatis* were negative. *U. urealyticum* was detected in both these women again at 28 weeks.

TABLE 3. Duration of colonisation of the lower genital tract

Colonising microorganism	Total number of women colonised (n=120)	Colonised at 1st antenatal visit only (n)	Colonised at 28 weeks only (n)	Colonised at both 1st visit and 28 weeks n (%)
U. urealyticum	67	4	4	59 (88)
M. hominis	8	2	3	3 (37.5)
G. vaginalis	10	5	5	0
Group B	19 (total)	0	16	3 (15.8)
streptococcus	5 (endocervix)	0	3	2 (40)

Pregnancy outcome

There were 16 (11%) preterm deliveries (<37 weeks), one miscarriage, one blighted ovum, one intrauterine foetal death at 15 weeks' gestation and 129 (87%) term deliveries. Delivery details were not obtainable for 14 participants. Twenty three (15.9%) women experienced preterm labour and five (3.4%) women had preterm rupture of membranes. Twelve (8.3%) low birth-weight infants (<2,500g) and two (1.3%) very low birth-weight infants (<1,500g) were delivered. A comparison of the rates of colonisation of *U. urealyticum* in women who delivered at term and those who delivered preterm (Table 4) demonstrated no significant difference in the colonisation rates. However it is unusual that the colonisation rates of *U. urealyticum* were lower in those women who delivered preterm (42.5%), compared to those who delivered at term (52.5%). The presence of *U. urealyticum* in the endocervix or the vagina was not significantly associated with adverse pregnancy

outcome. The isolation of *C. trachomatis, G. vaginalis, M. hominis,* and Group B streptococcus from the lower genital tract was also not significantly associated with preterm delivery.

TABLE 4. Lower genital tract colonisation with *U. urealyticum* at the first antenatal visit (12-20 weeks) and at 28 weeks-a comparison of women who delivered preterm and those who delivered at term

Time of antenatal visit	Preterm deliveries % endocervix / % vagina colonisation	Term deliveries % endocervix / % vagina colonisation
1st antenatal visit[1] (14.9± 3.6 weeks)	37.5 / 43.8	42.6 / 59.7
28 weeks [2]	44.4 / 44.4	51.9 / 55.8

[1] n=145, [2] n=113

Placenta culture and histology

Of the preterm placentas 44% (4 of 9) were 'culture positive' compared to 18% (15 of 83) of the term placentas (p=0.07). *U. urealyticum* 16 of 92 (17.4%) and *M. hominis* 4 of 92 (4.3%) were the organisms isolated most frequently from placentas. Whilst not all placentas were examined histologically it is interesting to note that the histology results revealed: (i) evidence of ascending infection in three of eight culture positive term placentas (culture negative term placentas were not examined) and three of eight preterm placentas; (ii) chorioamnionitis in four women (4 of 8) who delivered preterm and one woman who delivered at term (1 of 8 culture positive placentas). Not all preterm placentas were available for culture and histology as some women were transferred to a major teaching hospital for delivery. Due to the small numbers these outcomes were not statistically analysed.

Preterm birth

In this population, as would be expected, preterm birth was significantly associated with maternal risk factors of: smoking (p=0.012), cervical incompetence (p<0.0001), polyhydramnios (p<0.01), production of anti-Kell antibodies (p<0.01) and in women expecting twins (p<0.0001). A history of prior preterm birth (p<0.0001) and of a previous low birth-weight infant (p<0.01) was also more common in those women who delivered preterm. For 10 of 16 women who delivered preterm it was their second or subsequent pregnancy. Five of these 10 women had previously delivered preterm. Three of the five women (with previous preterm and present preterm delivery) were colonised in the lower genital tract with *U. urealyticum.* One other of these five women (and her partner) had been treated with tetracycline prior to conception until cultures for *U. urealyticum* were negative.

Identification of causes of preterm deliveries

Of those women who delivered preterm 11 of 16 (68.8%) had one or more maternal obstetric risk factors as compared to 15 of 129 (11.6%) of women who delivered at term. By comparing the lower genital tract microbiological findings and the maternal obstetric risk factors of women who delivered preterm (Table 5) it is apparent that eight of the 16 preterm deliveries (Preterm Patient Nos 3, 5, 8, 9, 12, 13, 15, 16) can be attributed to maternal risk factors. In seven of the remaining eight preterm deliveries, infectious agents could possibly be a contributing (causal) factor. Five of the 16 women who delivered preterm were Aboriginal or had Aboriginal partners and four of these women were colonised with *U. urealyticum,* whilst of the remaining 11 women only three were colonised with *U. urealyticum* (p=0.064). This result is based on a small sample and suggests that further investigation of the relationship between race, the incidence of *U. urealyticum* colonisation in Aboriginals and its involvement in preterm deliveries is warranted.

Chorioamnionitis was diagnosed histologically in four women: Patient No 2 had preterm prolonged rupture of membranes (>1 week) and a positive placenta culture for *U. urealyticum,* with evidence of ascending infection: Patient No 4 had a positive placenta culture for *G. vaginalis* and *U. urealyticum* and Patient No 10 had a positive placenta culture for *U. urealyticum.* In these three women *U. urealyticum* was also isolated from the lower genital tract during the pregnancy. Of interest two of these three patients were Aboriginal (Patients No 2 and 4), and two patients had Aboriginal partners (Patients No 2 and 10). Preterm Patient No 14 delivered twins at 23 weeks, and postmortem histology of the infants and placentas reported extreme prematurity, acute chorioamnionitis and an ascending infection. The placenta was fixed and microbiological culture of the tissue was not performed. The pregnancy histories for another three women (Patients No 6, 7, 11) suggested that an infectious agent may be implicated in the preterm birth. Therefore of these 16 preterm deliveries an infectious agent may be implicated in at least four (25%).

DISCUSSION

This study confirms that whilst *U. urealyticum* is the most prevalent microorganism isolated from the lower and upper genital tracts, its mere presence in the lower genital tract during pregnancy is not always associated with adverse pregnancy outcome. However this small sample showed that most women with *U. urealyticum* culture positive placentas and histological evidence of an upper genital tract infection, delivered preterm. Colonisation rates and demographic and behavioural characteristics of women colonised with *U. urealyticum* in this population are similar to those reported in overseas studies (1,3,4). Total rates of colonisation with *U. urealyticum* in this population were higher (57.4%) than those reported in another Australian population (34.8%) (7) even though both studies surveyed women attending public hospital antenatal clinics.

Microorganisms establish various symbiotic relationships within the lower genital tract. By comparing microbiological findings from lower genital tract samples collected at the first antenatal visit and again at 28 weeks we have shown that *M. hominis, G. vaginalis,* and Group B streptococcus can be considered as transient flora of the lower genital tract whereas *U. urealyticum* is generally present (88% of colonised women) from mid-trimester to early third trimester as a constant or persistent coloniser of the lower genital tract. McDonald *et al* (13) showed that *G. vaginalis, U. urealyticum* and *M. hominis* commonly persisted between mid-trimester and labour. It is not possible to ascertain whether *U. urealyticum* is constantly present in the lower genital tract or if it is perhaps repeatedly introduced from an endogenous source such as the gastrointestinal tract, or from sexual partner(s). Subtyping of the isolates present in the lower genital tract at the first antenatal visit and those present at 28 weeks (in the same women) may further elucidate the nature of colonisation.

The relationship between antepartum lower genital tract colonisation and adverse pregnancy outcome has previously been studied (1-7), however the design of these studies varies considerably particularly with respect to the types of microorganisms isolated and the consideration of maternal risk factors (other than aetiological agents) associated with adverse pregnancy outcome. Conflicting results have been obtained and it has not been possible to formulate a demographic and microbiological profile of women at risk of an adverse pregnancy outcome. Our results confirm that antepartum lower genital tract cultures for *U. urealyticum, M. hominis, G. vaginalis, C. trachomatis* and Group B streptococcus, in 'isolation' are not useful for predicting preterm labour, preterm birth nor low birth-weight infants. However *U. urealyticum* is the most common, potentially pathogenic coloniser in both the lower and upper genital tract of pregnant women. Whilst colonisation of the lower genital tract is not predictive of an adverse pregnancy outcome, in this study upper genital tract infections due to *U. urealyticum* occurred only in women with lower genital tract colonisation. Moreover, histological examination of both term and preterm placentas reported evidence of ascending infection and chorioamnionitis, which supports an ascending route of invasive infection. Sampling of the upper genital tract of pregnant women is an invasive procedure with inherent risks, and therefore further investigations of lower genital tract microorganisms and their ability to cause invasive, ascending infections is warranted.

The results of this study strongly suggested that four of 16 preterm births which occurred in afebrile, asymptomatic women were as a result of acute chorioamnionitis (three of these in the absence of preterm prolonged rupture of membranes). *U. urealyticum* alone (two cases), and *U. urealyticum* and *G. vaginalis* (one case) were isolated from these placenta cultures. No culture results were available for the fourth case. Associations of chorioamnionitis and intrauterine infection have previously been documented (2,8). These infections may be asymptomatic (2) and so the problem remains in identifying women with intact membranes and asymptomatic upper genital tract infections. It may be as suggested by Cassell *et al* (14) that *U. urealyticum* is present in the endometrium at the time of implantation and subsequently infects amniotic fluid and the placental membranes resulting ultimately in an adverse pregnancy outcome. Alternatively, (i) there may be a sub-population of women colonised in the lower genital tract who possess maternal risk factors which facilitate ascending microorganisms, (ii) some ureaplasmas present in the lower genital tract may be more pathogenic than others, and capable of ascending and causing upper genital tract infections or (iii) pathogenicity may be due to a combination of these factors.

The pathogenicity of the ureaplasmas may be dependent on antigenic variation within the species. Studies have sought to evaluate the pathogenicity of the different serovars of *U. urealyticum*. However Zheng *et al* (15) concluded that the invasiveness was not limited to a few particular serotypes among the 14 serovars of *U. urealyticum.* Other methods of subtyping the ureaplasmas have been reported and these are also being used to investigate the pathogenicity of different clinical isolates (16). There is still a need to develop alternate molecular subtyping methods for ureaplasmas with the ultimate aim of using these methods to identify clinical isolates with a greater potential for producing invasive infections. Meanwhile, antepartum lower genital tract microbiological screening of pregnant women in order to predict adverse pregnancy outcome is not useful. By comparison routine culture of all amniotic fluids collected by amniocentesis as well as culture and histology of all placentas from preterm births may reveal the true incidence of adverse pregnancy outcome due to infectious agents. With thorough microbiological antepartum screening of women who have a history of a prior preterm birth and subsequent appropriate antibiotic management, it may be possible to prolong the gestation of women at risk of preterm delivery or to prevent upper genital tract infections and adverse pregnancy outcome.

ACKNOWLEDGMENTS

We wish to thank Norelle White and all the staff of the antenatal clinic and maternity ward, scientists and technicians of the microbiology and histology departments of the Toowoomba Pathology Laboratory and technical staff from the University of Southern Queensland for their assistance with this project. Special thanks to: Dr Roger Guard, Director of Pathology, Toowoomba Base Hospital for performing histological examinations and for the provision of workspace and complementary bacteriological testing for this project; and to Simstat of the University of Southern Queensland for assisting in the statistical analysis of the data.
This work was supported by grants from Darling Downs Unilink Pty Ltd and the Faculty of Sciences, University of Southern Queensland.

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