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Ureaplasma in neonatal gastric fluid contributing to bronchopulmonary dysplasia

Longli Yan[1], Hua Deng[2], Jia Chen[1], Ying Liu[1], Shunyan Duan[1], Zhu Wang[1], Lanlan Du[1], Shu Liang[1], Linli Xu[3], Di Zhong[1], Weiwei Gao[1*] and Liang Zhang[2*]

Abstract

Background The association between the presence of common pathogens in the maternal cervicovaginal tract as well as neonatal gastric fluid and adverse outcomes in preterm newborns remains uncertain.

Methods Cervicovaginal swabs were collected from 98 mothers, and gastric fluid specimens were obtained from 121 premature infants with gestational ages of ≤ 32 weeks within 24 h of birth. Thirteen pathogens were tested using suspension microarray. Neonatal outcomes were monitored until either death or discharge.

Results Ureaplasma was the predominant species identified in both maternal cervicovaginal swabs and neonatal gastric fluid. Preterm newborns with *Ureaplasma* in gastric fluid at birth exhibited a smaller gestational age ($P < 0.001$), a lower 1-min Apgar score ($P = 0.01$), an increased requirement for pulmonary surfactant ($P = 0.029$), and a higher incidence of bronchopulmonary dysplasia (BPD) ($P = 0.02$) compared to those who tested negative for *Ureaplasma*. Similarly, pregnant women with *Ureaplasma* colonization in the genital tract were more likely to deliver babies with a smaller gestational age ($P = 0.002$), a higher rate of tracheal intubation after birth ($P = 0.013$), a lower proportion of small for gestational age (SGA) infants ($P = 0.018$), and an increased occurrence of BPD ($P = 0.048$) than mothers without the agent. Furthermore, the presence of *Ureaplasma* in the gastric fluid of premature infants was identified as a risk factor for BPD, with an odds ratio (OR) of up to 6, alongside gestational age and SGA as independent predictors of BPD.

Conclusions These findings suggest that antenatal exposure to *Ureaplasma* is correlated with the occurrence of BPD in premature infants, which has potential clinical implications.

Keywords Bronchoplumonary dysplasia, Premature infants, Cervicovaginal swab, Gastric fluid, *Ureaplasma*

*Correspondence:
Weiwei Gao
mgbbbear@qq.com
Liang Zhang
zhangliang1999@tsinghua.org.cn
[1]Neonatology Department, Guangdong Women and Children Hospital & Guangdong Neonatal ICU Medical Quality Control Center, Guangzhou 511400, China
[2]Maternal and Child Health Research Institute, Translational Medicine Center, Guangdong Women and Children Hospital, 521 Xing Nan Road, Guangzhou 511400, China
[3]Department of Obstetrics, Guangdong Women and Children Hospital, Guangzhou 511400, China

Introduction

Bronchopulmonary dysplasia (BPD) is a chronic lung disease predominantly affecting premature infants. It is associated with complications such as pulmonary hypertension, extrauterine growth retardation, and neurodevelopmental delays [1, 2], which diminish survival rates and quality of life in affected children. In recent years, the survival rate of premature infants has significantly improved due to an increase in high-risk pregnancies and advancements in neonatal care; however, this has been accompanied by a rise in the incidence of BPD. The pathogenesis of BPD is believed to be linked to factors such as infection, inflammation, oxygen toxicity, and the degree of prematurity [3]. Prenatal and postnatal inflammatory processes or infections can adversely affect lung development, thereby predisposing infants to the development of BPD [4].

Research has demonstrated that lung inflammation induced by respiratory bacteria significantly contributes to BPD [5]. Advances in detection methodologies have revealed that intrauterine infections resulting from ascending pathogens in the reproductive tract account for at least 40% of preterm births. *Ureaplasma* is among the most frequently identified pathogenic microorganisms in the placenta of preterm births [6]. Lohmann et al. reported an association between the colonization of Gram-negative rods and *Ureaplasma* with the incidence of BPD [7]. Additionally, another study indicated that newborns with BPD exhibited elevated levels of the phylum *Proteobacteria* [8]. These findings underscore the increasing acknowledgment of the role of pathogenic microorganisms in the pathogenesis of BPD. However, the specific pathogen exerting the most significant influence on the occurrence of BPD remains unclear. *Escherichia coli* (Ecoli) and *Streptococcus agalactiae* are commonly recognized as prevalent pathogens responsible for intrauterine infections, while *Chlamydia* and *Mycoplasma* have been implicated less frequently in this context [9, 10]. Consequently, it is particularly important to identify the pathogens associated with perinatal infections in premature infants to facilitate targeted interventions aimed at substantially reducing the incidence of BPD.

Taken together, specific microbial species may play critical roles in the etiology of BPD in newborns. To test this hypothesis, we investigated the influence of maternal and neonatal microbiota on perinatal outcomes, including BPD, by analyzing 13 prevalent microbial agents in the gastric fluid of premature infants and the maternal cervicovaginal tract.

Materials and methods

Study population and sample Preparation

Preterm infants admitted to the neonatal department of Guangdong Women and Children Hospital between March 2021 and March 2022 were enrolled in this study. The inclusion criteria comprised: gestational age of ≤ 32 weeks; delivery in the obstetrical department; and hospitalization in the neonatal department of the study hospital. The exclusion criteria included: presence of congenital abnormalities; transfer of infants to or from other units or institutions; and failure to complete pathogen detection within 24 h. Gastric fluid samples were collected from preterm infants within 2 h of birth and prior to the first feeding. Maternal cervicovaginal swabs were obtained 2 h before delivery. Pathogen detection was performed on preterm infants and their mothers within 24 h of admission. The study protocol received approval from the Institutional Ethics Board of Guangdong Women and Children Hospital, China (No. 202101097). Written informed consent to participate was obtained from the parents or legal guardians of any participant under the age of 16.

Data collection

Demographic and clinical data were obtained from electronic medical records, encompassing (1) basic information on premature infants, including gestational age, birth weight, SGA status, sex, mode of delivery, Apgar scores at 1 min and 5 min, and the administration of postnatal steroids; (2) maternal pregnancy factors, such as histological chorioamnionitis, premature rupture of membranes (PROM), and the use of antenatal steroids; (3) hospitalization parameters, including the duration of invasive mechanical ventilation and the utilization of pulmonary surfactant; and (4) preterm complications, the occurrence of BPD, necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), and other conditions.

Parameters and definitions

Preterm PROM is defined as the rupture of membranes occurring 18 h prior to delivery. Histological chorioamnionitis is diagnosed when placental tissues demonstrate neutrophilic infiltration and inflammation. The degree and progression of chorioamnionitis are staged/graded using indicators such as the location, density, and degeneration of polymorphonuclear leukocytes.

BPD is characterized by the requirement for supplemental oxygen or mechanical ventilation at 36 weeks of postmenstrual age (PMA). The severity of BPD is classified as mild, moderate, or severe based on the presence of oxygen dependency at 36 weeks of PMA, in accordance with the National Institutes of Health consensus definition [11]. IVH is diagnosed through ultrasound imaging,

while NEC is staged according to Bell's criteria. Patent ductus arteriosus (PDA), confirmed via echocardiography, is defined by the presence of hemodynamically significant PDA that necessitates pharmacotherapy, surgical ligation, or transcatheter occlusion. Indications for tracheal intubation include an inability to maintain target oxygenation despite non-invasive ventilation with an oxygen concentration exceeding 40%, severe apnea unresponsive to medical treatment, gastrointestinal conditions that preclude non-invasive ventilation, persistent metabolic acidosis, and hemodynamic instability. Indications for the administration of pulmonary surfactant in preterm newborns include those requiring immediate intubation at birth and those exhibiting respiratory distress syndrome (RDS) that persists despite non-invasive ventilation with an oxygen concentration of more than 30%.

Microorganism detection

Nucleic acids were isolated from maternal cervicovaginal swabs and neonatal gastric fluid using a magnetic-bead extraction kit (Bright-Innovation Biomed, Shunde, China), following the manufacturer's protocol, and were subsequently stored at -20 ℃ until use.

To investigate common organisms that are vertically transmitted from mothers to newborns, we selected 13 target pathogens for the construction of an array aimed at perinatal intrauterine infection analysis, allowing for a single test to detect all 13 pathogens. The selected agents include *Chlamydia trachomatis* (CT), Ecoli, *Group B Streptococcus* (GBS), *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH), *Neisseria gonorrhoeae* (NG), *Ureaplasma parvum* (UP), *Ureaplasma urealyticum* (UU), human parvovirus B19 (B19), Cytomegalovirus (CMV), Enterovirus (EnV), Herpes simplex virus type II (HSV-2), and Rubella virus (RBV). In previous studies [12, 13], we built an in-house oligonucleotide suspension microarray to identify a variety of respiratory pathogens using Luminex xMAP technology. Given the differences in the targeted agents, the relevant primers and probes for the "perinatal intrauterine infection array" must be appropriately modified. Nevertheless, the fundamental experimental process remains consistent, encompassing probe design, probe attachment to microspheres, multiplex PCR, and molecular hybridization.

Table 1 Pathogen analysis of maternal cervicovaginal swabs and neonatal gastric fluid

	Cervicovaginal swabs (*N* = 98, n and %)	Gastric fluid (*N* = 121, n and %)
Negative	35 (35.7)	74 (61.2)
Ureaplasma parvum	33 (33.7)	27 (22.3)
Ureaplasma urealyticum	11 (11.2)	7 (5.8)
Escherichia coli	29 (29.6)	17 (14.1)
Cytomegalovirus	11 (11.2)	1 (0.8)
Sterptococcus agalactiae	5 (5.1)	1 (0.8)
Mycoplasma genitalium	1 (1.0)	0 (0)

Statistical analysis

Statistical analyses were conducted using SPSS version 24.0. Data are presented as means with standard deviations, or as counts (percentages). For normally distributed data, one-way ANOVA was employed, and the chi-squared test or Fisher's exact test was utilized for comparison of frequencies. Candidate variables that were clinically associated with BPD or exhibited a P value of less than 0.05 in univariate analyses were included in multivariate logistic regression models. The significance threshold for all tests was set at $P < 0.05$ (two-sided).

Results

Pathogens identified in maternal cervicovaginal swabs and neonatal gastric fluid

Between March 2021 and March 2022, a total of 98 mothers and 121 premature infants with gestational ages of ≤ 32 weeks, including 23 pairs of twins, participated in this study. As shown in Tables 1 and 35 mothers (35.7%) tested negative in cervicovaginal swabs. Among the 13 pathogens identified using the suspension array, at least one pathogen was detected in 64.3% of the mothers. Notably, 44.9% of the mothers tested positive for *Ureaplasma* spp. with 33.7% positive for UP and 11.2% for UU. Ecoli was detected in 29 mothers (29.6%). Of the 121 premature infants, 28.1% (22.3% UP and 5.8% UU) tested positive for *Ureaplasma* spp. in gastric fluid, followed by 14.1% for Ecoli. One infant exhibited co-colonization with both UU and UP. Consequently, the estimated rate of *Ureaplasma* vertical transmission from mothers to infants was approximately 63% (28.1%/44.9%) in this study. Overall, both sample types yielded similar findings regarding the primary pathogens, with *Ureaplasma* and Ecoli being the most frequently identified agents. UP was the predominant pathogen detected in maternal cervicovaginal swabs and neonatal gastric aspirates.

Maternal cervicovaginal *Ureaplasma* colonization linked to decreased gestational ages and SGA proportion, as well as elevated rates of neonatal endotracheal intubation

In this study, microbiological examination of maternal samples classified cervicovaginal swabs into four groups: group 1 (negative for microorganisms), group 2 (*Ureaplasma* spp. only), group 3 (*Ureaplasma* spp. in conjunction with other microorganisms), and group 4 (other microorganisms identified). Although UP and UU were analyzed separately, they were combined as *Ureaplasma* spp. for the purposes of this analysis due to the limited sample size. Table 2 presents the perinatal clinical

Table 2 Correlation of pathogens identified in maternal cervicovaginal swabs with neonatal clinical characteristics

	Group 1	Group 2	*P* value	Group 3	*P* value	Group 4	*P* value
	U-/O-, *n* = 40	U+/O-, *n* = 29		U+/O+, *n* = 24		U-/O+, *n* = 28	
GA (weeks)	29.89 ± 1.54	28.67 ± 1.51	0.002	29.20 ± 1.75	n.s	29.14 ± 1.64	n.s
BW (kg)	1.24 ± 0.25	1.16 ± 0.31	n.s	1.13 ± 0.26	n.s	1.22 ± 0.26	n.s
Gender							
Female	16	13	n.s	11	n.s	12	n.s
Male	24	16	n.s	13	n.s	16	n.s
Surfactant (any dose)	20 (50.0%)	16 (55.2%)	n.s	15 (62.5%)	n.s	16 (57.1%)	n.s
1-min Apgar score	7.97 ± 0.97	7.58 ± 1.80	n.s	7.29 ± 1.89	n.s	7.57 ± 1.42	n.s
5-min Apgar score	9.05 ± 0.50	8.75 ± 1.02	n.s	8.89 ± 0.95	n.s	8.9 ± 1.00	n.s
Natural delivery, n (%)	21 (52.5%)	17 (58.6%)	n.s	19 (79.1%)	n.s	19 (67.8%)	n.s
Time of PROM (d)	2.56 ± 6.89	0.90 ± 1.68	n.s	4.90 ± 12.58	n.s	3.17 ± 10.45	n.s
Chorioamnionitis, n (%)	5 (12.5)	7 (24.1)	n.s	8 (33.3)	0.045	9 (32.1)	0.049
Antenatal steroids, n (%)	26 (65.0)	23 (79.3)	n.s	18 (75.0)	n.s	24 (85.7)	n.s
Postnatal steroids, n (%)	3 (7.5)	0 (0)	n.s	3 (12.5)	n.s	1 (3.6)	n.s
SGA, n (%)	12 (30.0)	2 (6.9)	0.018	0 (0)	0.003	3 (10.7)	n.s
Tracheal intubation n (%)	12 (30.0%)	18 (62.1%)	0.013	14 (58.3%)	0.036	18 (64.3%)	0.007
Invasive mechanical ventilation time (h)	193.74 ± 371.04	115.04 ± 162.44	n.s	162.05 ± 205.69	n.s	107.72 ± 155.15	n.s

BW: birth weight; GA: gestational age; Group 1: negative microorganism testing; Group 2: only *Ureaplasma* spp. identified; Group 3: positive testing for *Ureaplasma* spp. along with other microorganisms; Group 4: other microbes identified; O: other pathogens except for *Ureaplasma* spp.; PROM: premature rupture of membranes; SGA: small for gestational age; U: *Ureaplasma* spp

n.s: non-significant

P values applied to comparison with Group 1, respectively

Table 3 Correlation between pathogens identified in maternal cervicovaginal swabs and neonatal clinical outcomes

	Group 1	Group 2	*P* value	Group 3	*P* value	Group 4	*P* value
	U-/O-, *n* = 40	U+/O-, *n* = 29		U+/O+, *n* = 24		U-/O+, *n* = 28	
BPD, n (%)	11 (27.5%)	15 (51.7%)	0.048	13 (54.2%)	0.033	13 (46.4%)	n.s
--Moderate	1/11 (9.1%)	3/15 (20.0%)		0/13 (0%)		3/13 (23.1%)	
--Severe	1/11 (9.1%)	6/15 (40.0%)		9/13 (69.2%)		4/13 (30.8%)	
RDS, n (%)	28 (70.0%)	25 (86.2%)	n.s	19 (79.2%)	n.s	22 (78.6%)	n.s
-- RDS ≥ grade 3	4/28 (14.2%)	4/15 (26.7%)		6/19 (31.6%)		8/22 (36.4%)	
NEC, n (%)	0 (0%)	2(6.9%)	n.s	5 (20.8%)	0.003	4 (14.3%)	0.014
ROP, n (%)	7 (17.5%)	6 (20.7%)	n.s	4 (16.7%)	n.s	8 (28.6%)	n.s
ICH, n (%)	7 (17.5%)	5 (17.2%)	n.s	5 (20.8%)	n.s	6 (21.4%)	n.s
Sepsis, n (%)	0 (0)	0 (0)	n.s	2 (8.3%)	n.s	6 (21.4%)	0.003
PDA requiring treatment, n (%)	4 (10.0%)	1 (3.4%)	n.s	1 (4.2%)	n.s	0 (0%)	n.s
Mortality, n (%)	2 (5.0%)	5 (17.2%)	n.s	4 (16.7%)	n.s	3 (10.7%)	n.s

BPD: bronchoplumonary dysplasia; Group 1: negative microorganism testing; Group 2: only *Ureaplasma* spp. identified; Group 3: positive testing for *Ureaplasma* spp. along with other microorganisms; Group 4: other microbes identified; ICH: Intracranial hemorrhage; NEC: necrotizing enterocolitis; O: other pathogens except for *Ureaplasma* spp; RDS: Respiratory distress syndrome; ROP: Retinopathy of prematurity; PDA: Patent ductus arteriosus; U: *Ureaplasma* spp

n.s: non-significant

P values applied to comparison with Group 1, respectively

data for each group. Infants born to mothers colonized with *Ureaplasma* exhibited a significantly younger gestational age ($P = 0.002$), a smaller proportion of SGA infants ($P = 0.018$), and a higher rate of endotracheal intubation ($P = 0.013$) compared to controls in group 1. Furthermore, the presence of other microorganisms (group 4) in the maternal genital tract was also linked to an increased rate of neonatal endotracheal intubation ($P = 0.007$). Additionally, group 3 (*Ureaplasma* plus other organisms) demonstrated a significantly higher rate of intubation and a lower rate of SGA ($P = 0.036$ and $P = 0.003$, respectively).

Positive maternal cervicovaginal swab for *Ureaplasma* related to bronchopulmonary dysplasia

Table 3 indicates that the presence of *Ureaplasma* exclusively in maternal cervicovaginal swabs is associated with BPD morbidity in preterm infants ($P = 0.048$). When *Ureaplasma* is present alongside other microbial species, the association becomes even more pronounced ($P = 0.033$). In addition, other microorganisms found in the maternal genital tract were linked to neonatal complications, including NEC and sepsis ($P < 0.05$).

Ureaplasma in neonatal gastric fluid associated with various adverse clinical phenotypes

Similarly, the pathogen analysis results of neonatal gastric aspirate were categorized into four groups to rule out potential interactions between *Ureaplasma* and other microorganisms concerning clinical outcomes in premature infants (Table 4). Preterm infants who tested positive solely for *Ureaplasma* in gastric fluid had a younger gestational age ($P<0.001$), a lower 1-min Apgar score ($P=0.01$), and a higher incidence of pulmonary surfactant administration ($P=0.029$) compared to those who tested negative. When *Ureaplasma* was present alongside other microbes (group 3), it was associated with several clinical parameters, including gestational age ($P=0.002$), duration of PROM ($P=0.002$), hours of invasive mechanical ventilation ($P=0.02$), incidence of chorioamnionitis ($P=0.028$), and need for tracheal intubation ($P=0.044$). However, it did not correlate with any adverse clinical characteristics when other organisms (group 4) were detected in the neonatal gastric samples. This finding suggests that *Ureaplasma* plays a specific role in the pathogenesis of BPD and not just colonization with any of these 13 organisms.

Positive neonatal gastric aspirate for *Ureaplasma* associated with Bronchoplumonary dysplasia

As indicated in Table 5, premature infants with simplex *Ureaplasma* detected in gastric fluid had a significantly higher incidence of BPD, including moderate and severe forms, compared to those without *Ureaplasma* ($P=0.02$). Notably, the presence of other microbial agents (group 4) or the co-occurrence of *Ureaplasma* with other microbes (group 3) did not demonstrate a significant impact on the risk of BPD ($P>0.05$). Additionally, when *Ureaplasma* colonization was combined with other pathogens, it was associated with an increased incidence of neonatal sepsis and mortality ($P<0.05$).

Independent predictors of bronchopulmonary dysplasia

Variables that were clinically relevant to BPD or demonstrated a significance level of less than 0.05 in the initial univariate analysis were incorporated into multivariate logistic regression models. The results indicated that neonatal *Ureaplasma* exposure, gestational age, and SGA status were independent risk factors for BPD in premature infants born at or below 32 weeks of gestation ($P<0.05$). More importantly, neonates with *Ureaplasma* in gastric fluid exhibited a risk of developing BPD that was up to six times greater than those without such exposure (Table 6).

Discussion

In recent years, the survival rate of premature infants has substantially improved due to an increase in the number of high-risk mothers and advancements in treatment technologies. However, this progress has also led to a rising incidence of BPD [14]. Intrauterine inflammation is a primary contributor to premature birth [15], and inflammatory lung lesions represent a critical risk factor in the pathogenesis of BPD, disrupting normal lung

Table 4 Correlation between clinical characteristics and pathogens identified in neonatal gastric fluid

	Group 1	Group 2	*P* value	Group 3	*P* value	Group 4	*P* value
	U-/O-, *n* = 74	U+/O-, *n* = 26		U+/O+, *n* = 7		U-/O+, *n* = 14	
GA (weeks)	29.80 ± 1.62	28.35 ± 1.40	< 0.001	27.84 ± 1.73	0.002	29.22 ± 1.02	n.s
BW (kg)	1.22 ± 0.28	1.10 ± 0.25	n.s	1.09 ± 0.24	n.s	1.30 ± 0.24	n.s
Gender							
Female	30	13	n.s	2	n.s	7	n.s
Male	44	13	n.s	5	n.s	7	n.s
Surfactant (any dose) n (%)	44 (59.5%)	22 (84.6%)	0.029	6 (85.7%)	n.s	7 (50.0%)	n.s
1-min Apgar score	7.85 ± 1.24	6.96 ± 2.18	0.01	7.57 ± 1.27	n.s	7.78 ± 1.18	n.s
5-min Apgar score	8.94 ± 0.87	8.50 ± 1.44	n.s	9.28 ± 0.48	n.s	8.92 ± 1.26	n.s
Natural delivery, n (%)	34 (45.9%)	15 (57.7%)	n.s	5 (71.4%)	n.s	10 (71.4%)	n.s
Time of PROM (d)	2.65 ± 8.27	1.24 ± 1.82	n.s	13.07 ± 21.23	0.002	1.07 ± 2.89	n.s
Chorioamnionitis, n (%)	15 (20.3)	8 (30.8)	n.s	4 (57.1)	0.028	1 (7.1)	n.s
Antenatal steroids, n (%)	57 (77.0)	23 (88.5)	n.s	6 (85.7)	n.s	11 (78.6)	n.s
Postnatal steroids, n (%)	5 (6.8)	0 (0)	n.s	2 (28.6)	0.05	1 (7.1)	n.s
SGA, n (%)	10 (13.5)	1 (3.8)	n.s	0 (0)	n.s	0 (0)	n.s
Tracheal intubation n (%)	34 (45.9%)	15 (57.7%)	n.s	6 (85.7%)	0.044	8 (57.1%)	n.s
Invasive mechanical ventilation time (h)	88.28 ± 148.81	104.79 ± 172.14	n.s	309.00 ± 193.14	0.02	128.00 ± 178.24	n.s

BW: birth weight; GA: gestational age; Group 1: negative microorganism testing; Group 2: only *Ureaplasma* spp. identified; Group 3: positive testing for *Ureaplasma* spp. along with other microorganisms; Group 4: other microbes identified; O: other pathogens except for *Ureaplasma* spp.; PROM: premature rupture of membranes; SGA: small for gestational age; U: *Ureaplasma* spp

n.s: non-significant

P values applied to comparison with Group 1, respectively

Table 5 Correlation between clinical outcomes and pathogens identified in neonatal gastric fluid

	Group 1	Group 2	*P* value	Group 3	*P* value	Group 4	*P* value
	U-/O-, *n* = 74	U+/O-, *n* = 26		U+/O+, *n* = 7		U-/O+, *n* = 14	
BPD, n (%)	25 (33.8%)	16 (61.5%)	0.02	4 (57.1%)	n.s	7 (50.0%)	n.s
--Moderate	2/25 (8.0%)	4/16 (25.0%)		0/4 (0%)		2/14 (14.2%)	
--Severe	7/25 (28.0%)	7/16 (43.8%)		3/7 (42.8%)		2/14 (14.2%)	
RDS, n (%)	57 (77.0%)	20 (76.9%)	n.s	6 (85.7%)	n.s	11 (78.5%)	n.s
-- RDS ≥ grade 3	11 (14.8%)	2 (7.6%)		5 (71.4%)		4 (28.5%)	
NEC, n (%)	7 (9.5%)	2 (7.7%)	n.s	2 (28.5%)	n.s	0 (0%)	n.s
ROP, n (%)	14 (18.9%)	6 (23.1%)	n.s	1 (14.3%)	n.s	6 (42.9%)	n.s
ICH, n (%)	14 (18.9%)	3 (11.5%)	n.s	2 (28.6%)	n.s	4 (28.6%)	n.s
Sepsis, n (%)	1 (1.4%)	0 (0%)	n.s	2 (28.6%)	0.019	5 (35.7%)	< 0.001
PDA requiring treatment, n (%)	4 (5.4%)	2 (7.7%)	n.s	0/7 (0%)	n.s	0 (0%)	n.s
Mortality, n (%)	6 (8.1%)	2 (7.7%)	n.s	4 (57.1%)	< 0.001	2 (14.3%)	n.s

BPD: bronchoplumonary dysplasia; Group 1: negative microorganism testing; Group 2: only *Ureaplasma* spp. identified; Group 3: positive testing for *Ureaplasma* spp. along with other microorganisms; Group 4: other microbes identified; ICH: Intracranial hemorrhage; NEC: necrotizing enterocolitis; O: other pathogens except for *Ureaplasma* spp.; RDS: Respiratory distress syndrome; ROP: Retinopathy of prematurity; PDA: Patent ductus arteriosus; U: *Ureaplasma* spp

n.s: non-significant

P values applied to comparison with Group 1, respectively

Table 6 Predictor variables for bronchopulmonary dysplasia

Variables	Univariate analysis		Multivariate analysis	
	P value	OR (95% CI)	*P* value	Adjusted OR (95% CI)
Gestational age (weeks)	0.001	0.447 (0.318–0.627)	0.002	0.490 (0.312–0.770)
Invasive mechanical ventilation time (h)	0.001	1.007 (1.003–1.010)	0.069	1.005 (1.000-1.010)
Chorioamnionitis	0.365	1.553 (0.599–4.026)	0.457	0.596 (0.153–2.329)
Antenatal steroids	0.067	0.394 (0.146–1.067)	0.068	0.301 (0.083–1.095)
Postnatal steroids	0.168	3.191 (0.613–16.603)	0.707	0.621 (0.052–7.395)
Small for gestational age (SGA)	0.529	1.532 (0.406–5.778)	0.042	5.898 (1.065–32.663)
Ureaplasma in maternal cervicovaginal tract	0.612	1.222 (0.563–2.650)	0.226	0.458 (0.129–1.622)
Ureaplasma in neonatal gastric fluid	0.004	4.219 (1.602–11.107)	0.020	6.127 (1.326–28.312)

OR: odds ratio; CI: confidence interval

development in premature neonates and exacerbating lung tissue injury [4, 16]. Therefore, it is essential to identify the pathogens that contribute to the pathological changes of BPD.

In this study, *Ureaplasma* emerged as the most prevalent pathogen detected in both maternal cervicovaginal swabs and gastric aspirate from premature infants born at or before 32 weeks of gestation. Here, neonatal gastric fluid samples were used for pathogen detection since they are more readily available compared to airway secretions due to the need for routine gastric tube insertion after birth in extremely premature newborns. This methodology was also used in a recent study in which gastric fluid samples were collected from 47 neonates to detect *Ureaplasma*. All three extremely low-birth-weight infants with *Ureaplasma* developed BPD [17].

Ureaplasma typically inhabits the human urogenital tract, with colonization rates in women of childbearing age ranging from 40 to 80% [18]. In the present study, *Ureaplasma* was detected in 44.9% of pregnant women and 28.1% of neonates. The rate of vertical transmission of *Ureaplasma* from mothers to children was found to be 63%, which falls within the previously reported range of 18–80% [19]. Although Table 3 indicates a marginal association between *Ureaplasma* identified in maternal cervicovaginal swabs and neonatal BPD ($P = 0.048$), further investigation is warranted. Our primary findings suggest that neonates colonized with *Ureaplasma* have a higher incidence of BPD and a younger gestational age. Moreover, further analysis revealed that the presence of *Ureaplasma* in neonates may increase the risk of developing BPD by up to 6-fold. It is important to note that, these are just associations because it is difficult to refer to cause and effect with this study design. As previously mentioned, group 2 contained a significantly higher proportion of extremely preterm infants, which is a critical predictor of adverse outcomes such as BPD. To clarify this potential confounder, we additionally conducted a more detailed breakdown of gestational ages. Overall, the baseline characteristics, including Apgar scores, appeared to be more evenly distributed across all groups after stratification. Meanwhile, the primary conclusion remains consistent: a relationship between *Ureaplasma* and BPD persists ($P = 0.027$). These results may

help understand the distribution of preterm infants and its influence on the outcomes. For the infants in group 2 with lower 1-minute Apgar scores, a logistic regression analysis was performed, which did not find the factor associated with BPD occurrence (OR = 0.865; 95% CI, 0.667–1.121; $P > 0.05$).

Numerous studies, reviews, and meta-analyses have examined the relationship between the presence of *Ureaplasma* and the risk of BPD, yet the conclusions remain controversial. Most early investigations reported no significant association between maternal *Ureaplasma* colonization and BPD incidence [20, 21]. However, recent research has suggested that maternal *Ureaplasma* colonization may not be an innocent bystander in the pathogenesis of neonatal BPD [22]. Our study revealed a borderline association between maternal *Ureaplasma* colonization and neonatal BPD ($P = 0.048$). The microorganisms detected in the female genital tract usually represent colonization. Only under specific conditions, such as cervical injury, will they facilitate *Ureaplasma* ascending infection and subsequent intrauterine inflammation [6]. Conversely, *Ureaplasma* existing in neonatal gastric fluid, originating from the maternal reproductive tract, may exert a direct influence on the development of BPD.

Previous studies investigating the relationship between neonatal respiratory *Ureaplasma* colonization and the development of BPD in premature infants have demonstrated a significant association between the two factors. Evaluations were performed either 28 days after birth or at 36 weeks of corrected gestational age. However, the sample size of the greatest reported effect among these studies was less than 100 infants [23]. In another study, although neonatal gastric fluid was used for pathogen detection, only UU, but not UP, could be detected via PCR. Moreover, UU colonization was found to be negatively correlated with BPD incidence [24]. In our study, the prevalence of UU in neonatal gastric fluid was lower than that of UP (5.8% vs. 22.3%). Therefore, focusing exclusively on UU in that assay may not accurately represent the association between *Ureaplasma* and BPD.

Preterm infants exposed solely to *Ureaplasma* had a higher incidence of BPD than the control group; however, this effect diminished when co-exposure to other microbial agents was considered. In this study, Ecoli accounted for the largest proportion of other pathogens, suggesting a potential protective effect on BPD development. In fact, this hypothesis has been explored in an animal model, where co-exposure to bacterial lipopolysaccharides and UP attenuated pulmonary neutrophil infiltration in sheep fetuses [25].

Since we found that *Ureaplasma*-positive premature infants had a higher BPD incidence than controls, this raises an interesting question regarding the potential impact of early *Ureaplasma* clearance on BPD occurrence and overall clinical outcomes. Previously, empiric antibiotic therapy for premature infants suspected of microbiological infections did not specifically target *Ureaplasma*. In recent years, macrolides such as azithromycin and clarithromycin have been increasingly used to treat *Ureaplasma* colonization and prevent BPD development [26, 27]. Despite this trend, there remains a lack of consensus on the choice of drugs, treatment course, and patient populations, largely due to conflicting evidence and contradictory findings [28, 29]. The majority of premature infants with *Ureaplasma* exposure in our study did not receive macrolides treatment, making it impossible to assess the impact of antibiotic intervention on BPD. Further research is warranted.

Retrograde *Ureaplasma* infection during pregnancy has been associated with adverse outcomes, including chorioamnionitis, PROM, and abortion [21]. Here, we observed no significant difference in the duration of PROM between the *Ureaplasma*-positive group and the control group. This finding may be attributed to factors such as serotype variation, bacterial titer, genetic background, and the host immune response to *Ureaplasma* exposure. As a secondary objective, we evaluated the incidence of other complications in preterm infants exposed to *Ureaplasma* and found no association with the development of NEC, septicemia, ROP, or intracranial hemorrhage (ICH), presumably because of the small sample size of this study or the low entry of *Ureaplasma* into the bloodstream. The lung microbiome is likely affected by the oral microbiome, which is in turn likely influenced by the gastric microbiome since premature neonates have a high incidence of gastroesophageal reflux disease. By sampling the gastric microbiome, we may be detecting bacteria that have access to the respiratory tract and could affect local inflammation and influence the development of BPD.

The etiology of BPD is complex and multifactorial, involving a range of prenatal and postnatal risk factors. Key contributors to the pathogenesis of BPD include maternal smoking, chorioamnionitis, pregnancy-induced hypertension, antenatal steroids, delivery mode, gestational age, intrauterine growth restriction, mechanical ventilation, ventilator-associated lung injury, oxygen toxicity, infection, nutritional deficiencies, birth weight, PDA, genetic susceptibility, and epigenetic modifications [30–39]. Among them, prematurity and low birth weight are the most significant predictors of BPD [40]. However, conflicting results and insufficient evidence regarding some proposed risk factors underscore the necessity for more rigorous research. Confounding remains a major concern in observational studies, and assessing its impact on the certainty of evidence for exposure effects has always been challenging [41]. Various methodologies have been proposed to prevent or control for potential

confounders during the study design or data analysis phases, including randomization, restriction, matching, stratification, multivariate regression, inverse probability weighting, propensity score analysis, and instrumental variable estimation [42, 43]. Each of these approaches has its own advantages and disadvantages, with the choice of method being contingent upon the specific study context. Notably, the performance and application of these approaches can differ across studies. For unknown confounders, control may be only partially effective, if not entirely ineffective. Sometimes, over-adjustment for certain variables may introduce bias [44]. In this complex multifactorial setting, it is essential to interpret study results correctly based on both statistical evidence and substantial clinical knowledge [45].

This study has several limitations. First, suspension array was used for the first time to detect multiple organisms in samples from mothers and newborns due to its unique features such as high throughput, low cost and rapid hybridization kinetics compared to other commonly employed methods [12, 46]. We validated this platform including a magnetic bead extraction kit by identifying the pathogens using qPCR in our lab and found that it has the same sensitivity as qPCR for UP, with a cycle threshold (Ct) of 35. Similarly, the sensitivity of suspension microarray for detecting other organisms was equivalent to that of qPCR, with a Ct range of 34–35. Second, only 13 pathogens were included in the suspension array platform, potentially overlooking other microbes, such as *Fusobacterium nucleatum* and *Gardnerella vaginalis*, which are associated with adverse pregnancy outcomes. Third, although over 100 neonatal gastric fluid samples were collected, the sample size for each category was insufficient for further analysis; specifically, there were only 27 UP cases and 7 UU subjects. Consequently, UU and UP were combined for analysis as *Ureaplasma* spp. to enhance statistical power. To ascertain whether UU or UP serves as an independent risk factor for the development of BPD, larger cohort studies are warranted. In addition, this is a single-center study conducted in Southern China, which may introduce bias and limit the generalizability of the findings to other populations. Finally, despite employing a logistic regression model to adjust for measured confounders, the potential for unmeasured or residual confounding, as well as other biases, cannot be entirely excluded.

Conclusions

In summary, we examined 13 common pathogens in maternal cervicovaginal swabs and neonatal gastric fluid using suspension array. Our pilot study indicate that *Ureaplasma* may be a risk factor for BPD in preterm newborns. It would be interesting to explore whether the eradication of colonized *Ureaplasma* could mitigate the morbidity of BPD in future research.

Abbreviations

BPD	Bronchoplumonary dysplasia
ICH	Intracranial hemorrhage
IVH	Intraventricular hemorrhage
NEC	Necrotizing enterocolitis
PDA	Patent ductus arteriosus
ROP	Retinopathy of prematurity
RDS	Respiratory distress syndrome
SGA	Small for gestational age
UP	Ureaplasma parvum
UU	Ureaplasma urealyticum

Supplementary Information

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Supplementary Material 1

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Not applicable.

Author contributions

WWG and LZ designed the study and edited the manuscript. LLY was a major contributor in writing the manuscript. JC, YL, LLX and SYD collected the clinical samples. HD performed the experiments. ZW, LLD and SL analyzed the data. DZ collected the references. All authors reviewed the final manuscript.

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Data availability

The original data are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Institutional Ethics Board of Guangdong Women and Children Hospital, China (No. 202101097). All procedures performed in studies involving humans were in accordance with the Declaration of Helsinki. Written informed consent to participate was obtained from the parents or legal guardians of any participant under the age of 16.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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