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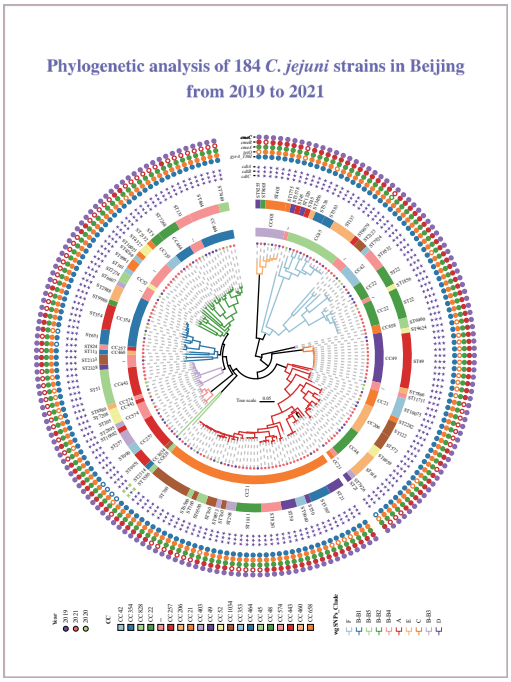

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Cover Image: adapted from the article titled "Genomic Analysis and Antimicrobial Resistance of *Campylobacter jejuni* Isolated from Diarrheal Patients — Beijing Municipality, China, 2019–2021" (Figure 1, Page 430).

Preplanned Studies

Pregnancy Loss in Relation to the Risks of Female-Specific Cancers in a Population-Based Cohort and Mendelian Randomization Study — China, 2004–2017

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Summary

What is already known about this topic?

Limited evidence exists regarding the relationship between pregnancy loss and female-specific cancers within the Chinese population from prospective cohort studies.

What is added by this report?

Terminations were associated with a 13% lower risk of endometrial cancer, whereas stillbirths were related to an 18% higher risk of cervical cancer. Rural residents with a history of pregnancy loss experienced a 19% and 38% increased risk of breast and cervical cancers, respectively, compared to their urban counterparts. Moreover, a positive graded relationship between live births and pregnancy loss on cervical cancer was observed.

What are the implications for public health practice?

This study has significant implications for identifying women at an increased risk for breast and genital cancers and contributes to the development of effective public health strategies for female cancer prevention. Future research on reproductive history, particularly in rural areas, should be given priority in efforts to improve female cancer screening and early detection.

Breast cancer (BC) is the most common cancer and the leading cause of cancer-related deaths among females globally (*1*). Cervical and ovarian cancer also contribute to significant burdens of cancer-related disability and mortality (*2*). Consequently, identifying at-risk groups is crucial for the early detection of breast and gynecological malignancies. Pregnancy loss (PL) is a prevalent occurrence worldwide, with approximately 2.6 million stillbirths and over 20% of pregnancies ending in spontaneous abortions in 2015 (*3–4*). Termination has reached a global prevalence of 3.5% among women of reproductive age between 2010–2014 (*5*). Reproductive factors, including pregnancy and PL, have been repeatedly associated with breast and gynecologic cancers, yielding inconclusive results. Earlier studies demonstrated that compared to nulliparous women, parous women exhibited lower long-term risks of breast cancer and endometrial cancer (*6–7*). However, a recent individual-level meta-analysis indicated that the risk of breast cancer increased for women with higher parity (*8*). Regarding PL, previous observational studies proposed its association with female-specific cancer (*9–10*), while a recent nationwide cohort study in Denmark contradicted any correlation between pregnancy loss and subsequent cancer development (*11*). Additionally, there is limited evidence addressing this topic within the Chinese population. Considering that the reproductive patterns of Chinese women have changed significantly over the past few decades, a proper understanding of the link between PL and female cancer risk is pivotal in China. Thus, this large cohort study, consisting of 298,008 participants, provides the most comprehensive assessment of the associations between PL and long-term female-specific cancer risk among Chinese women between 2004 and 2017.

In this study, we utilized a large female sample from the China Kadoorie Biobank (CKB) study, which is an ongoing nationwide cohort study involving 512,891 individuals (59% women) recruited at baseline between 2004 and 2008 and followed up for morbidity and mortality at ten regional study sites (Licang, Nangang, Meilan, Wuzhong, Liubei, Pengzhou, Maijixiang, Huixian, Tongxiang, and Liuyang) across China. After excluding participants with a cancer history (n=1,160), insufficient information on reproductive history (n=47), and nullipara (n=2,857), a total of 298,008 women were included in the final

analyses. Reproductive history, encompassing the number of pregnancies, parities, spontaneous miscarriages, terminations, and stillbirths, was assessed using a structured questionnaire. Time to first onset of breast cancer (C50), cervical cancer (C53), endometrial cancer (C54.1), and ovarian cancer (C56) was recorded through linkage to local disease and death registers, health insurance databases, residential records, and the registries of the China's Disease Surveillance Points system based on ICD-10 codes (International Classification of Diseases, 10th Revision). Follow-up person-years were calculated from the date of baseline enrollment to either the date of cancer, death, loss to follow-up, or December 31, 2017, whichever occurred first.

Cox proportional hazards (PH) models were conducted to estimate hazard ratios (*HR*s) and 95% confidence intervals (*CI*s) for cancer in relation to PL. Cox models were stratified by age-at-risk (five-year intervals) and regions, and HRs were adjusted for body mass index (BMI), socioeconomic status, alcohol consumption, smoking, physical activity, sleep quality, comorbidity, age at menarche, oral contraceptive use, and gynecological operation history. A two-sample Mendelian randomization (MR) analysis, adhering to the three assumptions (independence, relevance, and exclusion restriction), was further executed to estimate potential causal relationships. Instrumental variables [i.e., single nucleotide polymorphism (SNPs)] of three exposure traits (miscarriages, terminations, and stillbirths) were obtained from the genome-wide association studies (GWAS) catalog (https://www.ebi.ac.uk/gwas/). Summary GWAS data on cancer traits (i.e., breast, cervical, endometrial, and ovarian cancers) were acquired from the BioBank Japan.

The random-effects inverse-variance weighted (IVW) method and a series of sensitivity analyses, including MR-Egger regression, weighted-median, simple-mode, and weighted-mode, were employed for MR analyses. Horizontal pleiotropy was assessed by the MR-Egger intercept. All *P* values were two-sided, and statistical significance was set at the <0.05 level. The aforementioned analyses were performed using R4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). The study's flow chart is presented in Figure 1.

In the sample of 298,008 women, 9.1% reported a history of miscarriage, 52.5% reported termination, and 5.7% reported stillbirth. Participant characteristics across the number of PLs are shown in Table 1. After a median follow-up of 9.2 years, 1,997 new cases of female breast cancer (incidence rate: 0.7‰), 950 cervical cancer cases (incidence rate: 0.3‰), 338 endometrial cancer cases (incidence rate: 0.1‰), and 377 ovarian cancer cases (incidence rate: 0.1‰) were observed. A 13% decreased risk of endometrial cancer per additional termination (*HR*=0.87, 95% *CI*: 0.78–0.98, *P*=0.019) and an 18% increased risk of

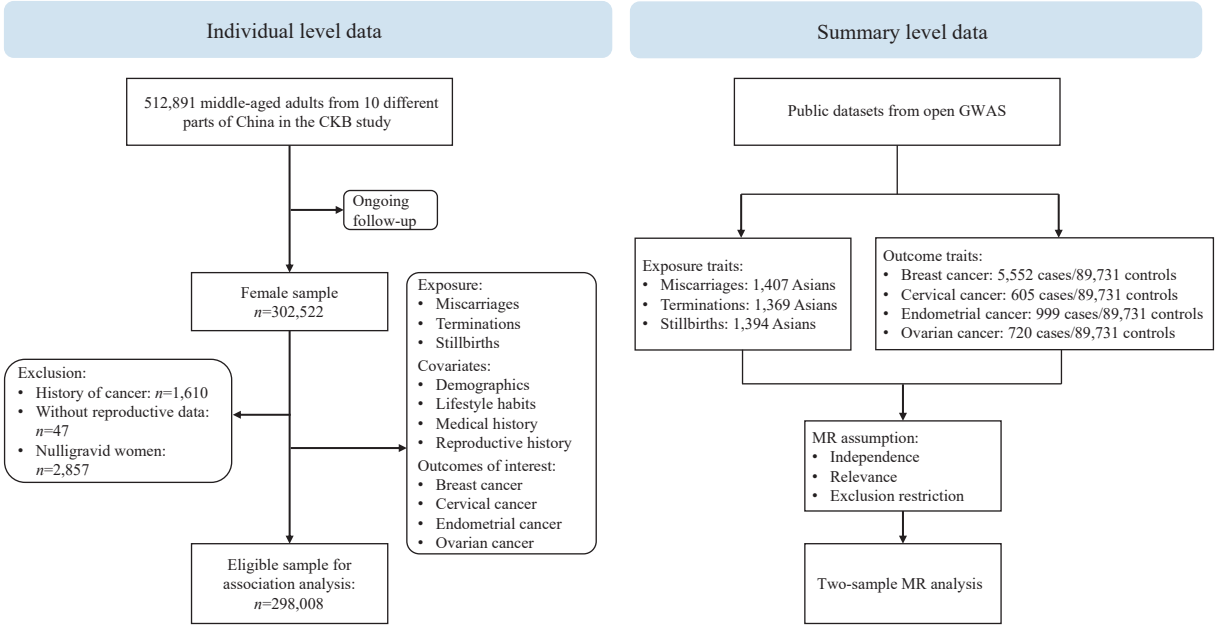

FIGURE 1. Flow chart of the present study.
Abbreviation: CKB=China Kadoorie Biobank; GWAS=genome-wide association studies; MR=Mendelian randomization.

TABLE 1. Baseline characteristics of participants by number of miscarriages, terminations, and stillbirths (*n*=298,008).

Characteristic	Total	Miscarriages			Terminations			Stillbirths		
		0	1	≥2	0	1	≥2	0	1	≥2
Sample, %		90.9	7.2	1.9	47.5	27.8	24.7	94.3	4.4	1.3
Rural residents, %	55.7	54.3	68.5	72.8	70.0	44.8	40.3	54.8	67.5	77.9
Age, years	51.4±10.5	51.2±10.4	53.7±11.0	55.1±11.6	52.4±10.9	50.9±10.2	50.1±9.7	51.0±10.3	56.7±10.5	62.3±9.5
BMI, kg/m^2	23.8±3.5	23.8±3.4	23.7±3.5	23.6±3.6	23.6±3.5	23.9±3.3	24.1±3.4	23.9±3.4	23.4±3.5	22.8±3.6
High school and above, %	17.8	18.4	11.3	10.4	11.4	21.9	25.3	18.4	7.9	2.8
Manual worker, %	57.7	57.8	57.5	55.5	62.9	54.9	51.1	58.4	48.8	37.2
Income ≥20,000 CNY/year, %	40.7	41.7	32.4	27.3	33.1	49.4	45.7	41.0	37.9	30.8
High SES, %	32.4	33.6	21.8	18.9	21.5	40.8	44.2	33.5	17.3	7.5
Ever drinker, %	36.4	36.5	34.9	35.2	30.7	38.5	44.9	37.2	23.9	17.6
Ever smoker, %	5.1	5.0	5.8	6.5	4.7	4.7	6.1	5.0	6.2	5.8
Physical activity, MET-hour/day	20.5±12.8	20.6±12.8	19.6±12.5	18.6±11.9	20.9±12.8	20.5±13.0	19.6±12.4	20.7±12.8	17.6±11.5	14.3±9.8
Sleep disturbance, %	18.8	18.5	22.2	22.7	18.8	18.3	19.5	18.5	22.5	27.1
NCD history, %	33.7	33.4	37.0	38.5	32.6	34.1	35.4	33.3	40.1	43.4
Age at menarche, years	15.4±2.0	15.6±2.0	15.6±2.0	15.6±2.0	15.6±2.0	15.3±1.9	15.2±1.9	15.4±2.0	15.9±2.0	15.9±2.0
Oral contraceptive use, %	9.9	10.1	8.0	7.2	4.6	12.0	17.8	10.1	6.7	5.5
Gynecological operation history, %	5.4	5.4	4.9	4.8	4.5	5.8	6.6	5.5	4.6	3.7
Number of live births	2.2±1.3	2.2±1.3	2.7±1.5	2.9±1.7	2.6±1.4	2.0±1.2	1.8±1.1	2.2±1.3	2.8±1.4	3.3±1.5

Abbreviation: BMI=body mass index; CNY=Chinese Yuan; SES=socioeconomic status; MET=metabolic equivalent; NCD=noncommunicable disease.

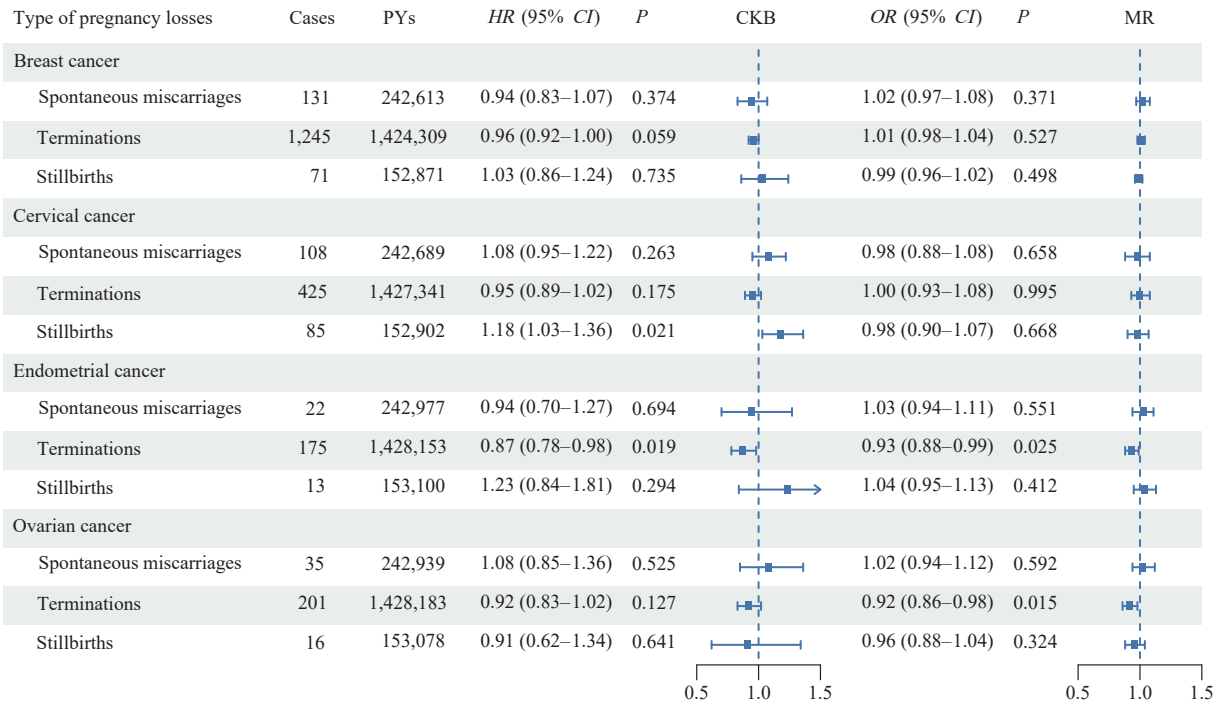

FIGURE 2. Associations between pregnancy losses and female-specific cancers by CKB and MR in Asian population.
Abbreviation: PYs=person-years; *HR*=hazard ratio; *CI*=confidence interval; CKB=China Kadoorie Biobank; MR=Mendelian randomization.

cervical cancer per additional stillbirth (*HR*=1.18, 95% *CI*: 1.03–1.36, *P*=0.021) were identified (Figure 2).

When stratified by living region, rural residents with a termination history had a 19% higher risk of breast cancer (*HR*=1.19, 95% *CI*: 1.02–1.40, $P_{heterogeneity}$<0.001) compared to urban residents. Furthermore, rural residents with a miscarriage history faced a 38% increased risk of cervical cancer (*HR*=1.38, 95% *CI*: 1.11–1.73, $P_{heterogeneity}$<0.001). In the joint-relationship analysis, a significant graded relationship between live birth and pregnancy loss on cervical cancer was observed. Among women having 3 or more live births, adjusted *HR*s were 4.93 (95% *CI*: 3.76–6.45) for those without miscarriage, 5.32 (3.63–7.79) for those with one miscarriage, and 6.99 (4.06–12.03) for those with more than two miscarriages.

Details on exposure-associated SNPs for MR analyses are shown in Table 2. The numbers of included SNPs ranged from 23 to 41, and the proportions of variance explained by SNPs (R^2) varied from 6.28% to 9.82%. Most traits displayed a considerable weak instrument bias (F-stat <10). The power to detect a significant effect size [odds ratio (*OR*)=0.8/1.2] in our MR analyses was highest for termination on breast cancer (power=99%), and lowest for stillbirth on cervical cancer (power=28%). In the MR analysis, terminations were found to be causally associated with a 7% lower risk of endometrial cancer (*OR*=0.93, 95% *CI*: 0.88–0.99, *P*=0.025) and an 8% lower risk of ovarian cancer (*OR*=0.92, 95% *CI*: 0.86–0.98, *P*=0.015) (Figure 2). MR-Egger intercepts indicated low levels of horizontal pleiotropy among all selected SNPs (*P*>0.05) (Supplementary Table S1, available in http://weekly.chinacdc.cn). Sensitivity analysis results are presented in Supplementary Figure S1 (available in http://weekly.chinacdc.cn).

DISCUSSION

This extensive cohort study, consisting of 298,008 participants, offers a comprehensive evaluation of the associations between pregnancy loss and long-term female-specific cancer risks in Chinese women. Termination was found to be linked with a reduced risk of endometrial cancer, whereas stillbirth was correlated with an increased risk of cervical cancer. A Mendelian randomization analysis in individuals of Asian ancestry was conducted to further assess the causal relationships between these factors.

The findings of this study align closely with previous research conducted in other countries. For example, a cross-national study demonstrated that pregnancies ending in either spontaneous or induced abortion did not increase the risk of female breast cancer [relative risk (*RR*)=0.98, 95% *CI*: 0.92–1.04] (*12*).

TABLE 2. Details of the instruments used as proxy pregnancy loss on female-specific cancer risk.

Phenotypes	Total population	SNPs (*n*)	F-stat	R^2 (%)	Power (%)
Breast cancer					
Spontaneous miscarriages		23	4.03	6.28	94
Terminations	95,283	32	4.18	9.11	99
Stillbirths		34	2.71	6.34	95
Cervical cancer					
Spontaneous miscarriages		29	4.29	8.29	29
Terminations	90,336	35	4.15	9.82	35
Stillbirths		41	2.71	7.60	28
Endometrial cancer					
Spontaneous miscarriages		29	4.29	8.29	42
Terminations	90,730	35	4.15	9.82	50
Stillbirths		41	2.71	7.60	41
Ovarian cancer					
Spontaneous miscarriages		29	4.29	8.29	33
Terminations	90,451	35	4.15	9.82	39
Stillbirths		41	2.71	7.60	31

Abbreviation: SNP=single nucleotide polymorphism.

Additionally, a nationwide register-based study with 57,347,622 person-years of follow-up revealed that pregnancy termination was associated with a significantly decreased risk of endometrial cancer (*RR*=0.53, 95% *CI*: 0.45–0.64) (*9*).

A potential explanation for the reduced risk of endometrial cancer associated with pregnancy termination may be that the termination process contributes to the removal of endometrial cells, particularly those in the early stages of malignant transformation (*13*). Another conceivable mechanism involves the early gestational effect occurring in the initial weeks post-conception, wherein the rapid increase of the progesterone-to-estrogen ratio enhances the endometrial system (*9*). In terms of the increased risk of cervical cancer linked to stillbirth, the underlying mechanism remains uncertain. Nonetheless, evidence has demonstrated that recurrent pregnancy loss is associated with aberrant progesterone receptors, which may subsequently contribute to the dysfunction of tumor suppressors in uterine cancer cells and further induce uterine tumors (*14*).

Our study revealed an intriguing dose-response relationship between the number of PLs and live births in relation to female cancer risk. As the number of live births increased, a higher risk of female cancer was observed in women with multiple PLs. Although parity, serving as a proxy for women's fecundity, may indicate a healthy endocrine and endometrial system, completed pregnancies could still stimulate the growth of cells that are in the early stages of malignant transformation (*15*).

Previous research has demonstrated that social determinants, such as geographical location, socioeconomic status, and education level, significantly influence the incidence and progression of female cancers. Particularly in rural areas, female residents may face financial constraints, poor sanitation, and inadequate medical care due to their relatively low socioeconomic status. As a result, they may be less aware of the importance of regular medical check-ups and treatments for cancer prevention and management (*16*). Furthermore, rural Chinese women often begin childbearing at a younger age and continue to be sexually active for an extended duration (*17*). Consequently, they may experience increased exposure to unidentified risk factors associated with the development of gynecological malignancies.

The primary strength of this study lies in its comprehensive examination of the associations between three types of pregnancy loss and the long-term risk of female malignancy in a large-scale prospective cohort in China. Additionally, a two-sample Mendelian randomization analysis was utilized to further confirm these causal relationships. Nevertheless, our study faces several limitations. First, reproductive history data were self-reported, which may be subject to recall and reporting bias. Second, some residual confounders (e.g., environmental factors and pregnancy-induced conditions) related to pregnancy loss and female oncology were not adjusted for. Third, data regarding the number, consecutiveness, and intervals of pregnancy losses were unavailable, limiting our ability to gain an in-depth understanding of the underlying mechanisms linking incomplete pregnancy and female malignancy. Finally, the current study was unable to perform an MR interaction analysis due to the unavailability of individual-level genotype data.

In conclusion, this study is the first large-scale nationwide cohort investigation in China to uncover the relationship between pregnancy loss and female-specific cancers among Chinese women. The utilization of Mendelian randomization analysis further strengthens the causal evidence supporting these findings. The results offer valuable insights into the link between reproductive history and the development of cancer, as well as guidance for identifying high-risk groups for female-specific cancers. This research contributes significantly to public health practices aimed at preventing and detecting these cancers early. Further examination of reproductive history, particularly in rural regions, is essential in determining accurate risk stratification, which will prove beneficial for developing screening and prevention strategies regarding female-specific cancers.

Conflicts of interest: No conflicts of interest.

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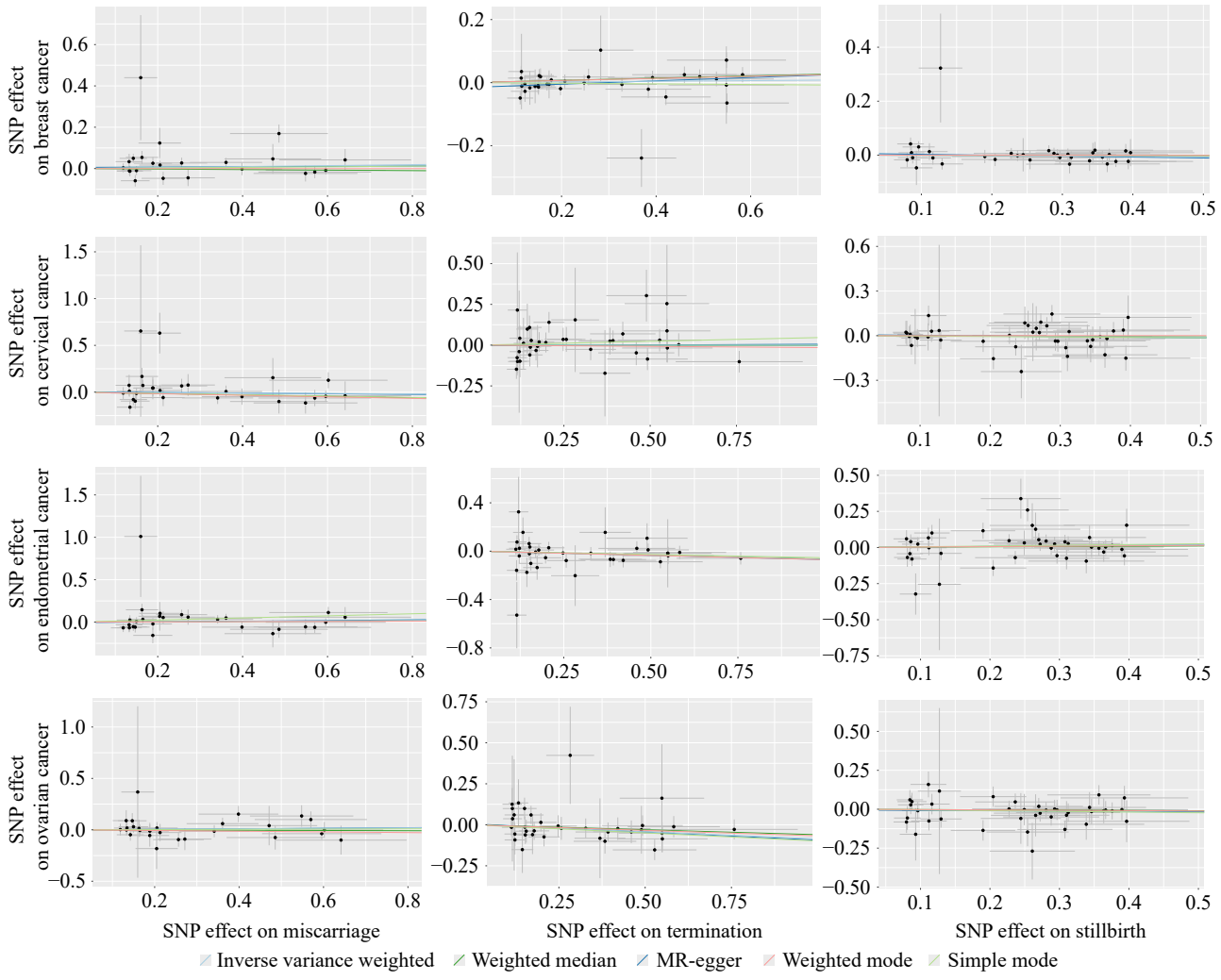
SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Results of MR using the inverse-variance weighted method.

Phenotypes	Effect size		Heterogeneity			Pleiotropy		
	OR (95% *CI*)	*P*	Q-stat	*P*	I^2 (%)	MR-egger intercept	MR-egger *P*	MR-PRESSO global test-*P*
Breast cancer								
Spontaneous miscarriages	1.02 (0.97–1.08)	0.371	42.24	0.006	47.92	0.01	0.731	0.175
Terminations	1.01 (0.98–1.04)	0.527	38.16	0.176	18.75	−0.02	0.150	0.152
Stillbirths	0.99 (0.96–1.02)	0.498	18.67	0.971	0.00	0.01	0.561	0.982
Cervical cancer								
Spontaneous miscarriages	0.98 (0.88–1.08)	0.658	30.57	0.245	14.94	<0.01	0.896	0.323
Terminations	1.00 (0.93–1.08)	0.995	31.97	0.567	0.00	<0.00	0.878	0.538
Stillbirths	0.98 (0.90–1.07)	0.668	36.66	0.577	0.00	0.01	0.827	0.534
Endometrial cancer								
Spontaneous miscarriages	1.03 (0.94–1.11)	0.551	31.49	0.210	17.44	−0.01	0.814	0.227
Terminations	0.93 (0.88–0.99)	0.025	31.26	0.602	0.00	0.00	0.948	0.656
Stillbirths	1.04 (0.95–1.13)	0.412	60.27	0.016	35.29	<0.00	0.989	0.388
Ovarian cancer								
Spontaneous miscarriages	1.02 (0.94–1.12)	0.592	16.57	0.922	0.00	<0.00	0.974	0.893
Terminations	0.92 (0.86–0.98)	0.015	20.80	0.963	0.00	0.01	0.822	0.962
Stillbirths	0.96 (0.88–1.04)	0.324	33.23	0.730	0.00	−0.01	0.846	0.732

Abbreviation: *OR*=odds ratio; *CI*=confidence interval; MR=Mendelian randomization; PRESSO=pleiotropy residual sum and outlier.



SUPPLEMENTARY FIGURE S1. Scatter plot displaying the impact of each SNP on various female cancer types for each phenotype.
Abbreviation: MR=Mendelian randomization; SNP=single nucleotide polymorphism.

Preplanned Studies

Survey on Immunization Services for Children with Medical Conditions — China, 2022

Yong Huang[1,2]; Yudan Song[2]; Junhong Li[2]; Yamin Wang[2]; Xiang Zeng[2,3,4]; Chao Ma[2,#]; Zundong Yin[2]

Summary

What is already known about this topic?

Children with medical conditions frequently experience under-immunization. Ensuring high-quality immunization services is crucial for enhancing vaccination coverage levels; nevertheless, the state of immunization service provision for children with medical conditions in China remains unclear.

What is added by this report?

Immunization support for children with medical conditions in China demonstrates considerable variability and may be inadequate. Primary obstacles to the provision of immunization services include an absence of comprehensive vaccination recommendations and assessment guidelines for specific medical conditions, as well as inconsistencies among vaccine recommendations, package inserts, and expert consensus statements pertaining to the vaccination of children with medical conditions.

What are the implications for public health practice?

The examination of provincial practices in providing immunization services for children with medical conditions, as well as understanding the barriers faced by National Immunization Program providers in administering vaccinations, can contribute to the improvement of immunization services for this population in China.

Children with medical conditions are defined as those possessing specific physiological or disease states that may increase their risk of infection or exacerbate the severity of vaccine-preventable diseases. Such conditions can also impact the safety and effectiveness of vaccinations, often necessitating evaluations prior to immunization (*1*). A strategic objective of Immunization Agenda 2030 (IA2030) is to extend immunization services to "zero dose" and under-immunized children, ensuring that all children receive full benefits from vaccines (*2*).

In China, vaccination rates for the National Immunization Program (NIP) vaccines have reached 99% among age-eligible children (*3*). However, delayed and missed vaccinations are common among children with medical conditions for various reasons. These factors include a lack of awareness of vaccine-preventable diseases, uncertainty surrounding vaccination safety, restrictions stated in vaccine package inserts, difficulties in assessing medical condition severity, misperceptions regarding contraindications and precautions, and barriers related to operations and systems (*1*).

Immunization services are critical for maintaining and enhancing high vaccination coverage levels. Nevertheless, the current state of immunization service support for children with medical conditions in China remains unclear. In order to address this knowledge gap and lay the groundwork for potential improvements in immunization services, we conducted a study examining immunization service patterns for children with medical conditions across 31 provincial-level administrative divisions (PLADs) in China.

Between August 3 and 16, 2022, we conducted a cross-sectional, questionnaire-based survey targeting provincial-level CDC immunization program departments responsible for implementing immunization services for children with medical conditions in China. Questionnaires were electronically disseminated and collected, including questions about relevant immunization policies, development of recommendations or expert consensus statements, utilization of vaccination evaluation clinics, the structure and processes of such clinics, and the availability of relevant training programs. Additionally, we inquired about perceived barriers to and urgent demands for the provision of immunization services. The question addressing urgent demands featured eight items ranked from zero to seven, with higher scores indicating higher demand. Definitions and meanings of each question were clarified through online face-to-face interviews.

The 31 PLADs surveyed were divided into 3 distinct socioeconomic regions in China: Eastern (Beijing, Tianjin, Hebei, Liaoning, Shanghai, Jiangsu, Zhejiang, Fujian, Shandong, Guangdong, and Hainan), Central (Shanxi, Jilin, Heilongjiang, Anhui, Jiangxi, Henan, Hubei, and Hunan), and Western (Inner Mongolia, Guangxi, Chongqing, Sichuan, Guizhou, Yunnan, Tibet, Shaanxi, Gansu, Qinghai, Ningxia, and Xinjiang). Categorical variables are presented as frequencies and proportions. Analyses were conducted using R software (version 4.1.3, R Foundation for Statistical Computing, Vienna, Austria).

All 31 PLADs completed the survey. Table 1 shows specific immunization services support for children with medical conditions by region. In general, supporting services were more numerous in eastern PLADs than in central and western PLADs, especially in the use of expert consensus statements and use of vaccination evaluation clinics for certain medical conditions. There were 74 vaccination evaluation clinics in pediatric hospitals nationwide, distributed in 16 PLADs.

TABLE 1. Support for immunization services among children with medical conditions by region, China, 2022.

Indicator	Eastern (11 PLADs)	Central (8 PLADs)	Western (12 PLADs)	Nationwide (31 PLADs)	*P* value**
Local vaccination recommendations*, *n* (%)	1 (9.1)	0 (0.0)	0 (0.0)	1 (3.2)	>0.999
Local expert consensus statements†, *n* (%)	5 (45.5)	1 (12.5)	0 (0.0)	6 (19.4)	0.009
Specialized training programs§, *n* (%)	5 (45.5)	3 (37.5)	2 (16.7)	10 (32.3)	0.353
Presence of vaccination evaluation clinics¶, *n* (%)	9 (81.8)	2 (25.0)	5 (41.6)	16 (51.6)	0.036
Number of vaccination evaluation clinics, *n*	56	6	12	74	–
Defined structures and processes for evaluation clinics, *n* (%)	2 (18.2)	0 (0.0)	2 (16.7)	4 (12.9)	0.530

Abbreviation: PLADs=provincial-level administrative divisions; NIP=National Immunization Program.

* Local vaccination recommendations represent the official guidelines, which NIP providers must adhere to when vaccinating children with medical conditions.

† Local expert consensus statements, developed by expert teams, are not considered official standards. However, they serve as a foundation for NIP providers to enhance their scientific understanding of vaccination necessity, as well as to investigate the safety and efficacy of vaccinations in children with medical conditions.

§ Specialized training programs aim to enhance the vaccination of children with medical conditions by offering education on fundamental knowledge, professional skills, and relevant case studies.

¶ Vaccination evaluation clinics have been established to provide counseling and assessment for children with medical conditions, addressing safety concerns and the necessity of vaccination.

** The comparison of indicator rates between regions was conducted using Fisher's exact test, as the small sample size of one cell (<5) necessitated this statistical approach.

"–" means data not available.

TABLE 2. Barriers to delivery of immunization services for children with medical conditions by region, China, 2022.

Barrier	Eastern (11 PLADs)	Central (8 PLADs)	Western (12 PLADs)	Nationwide (31 PLADs)	*P* value*
Lack of detailed vaccination recommendations for certain conditions, *n* (%)	9 (81.8)	7 (87.5)	7 (58.3)	23 (74.2)	0.339
Lack of standardized procedures for evaluation of specific conditions, *n* (%)	7 (63.6)	7 (87.5)	9 (75.0)	23 (74.2)	0.451
Inconsistency between official recommendations, vaccine package inserts, and expert consensus statements, *n* (%)	10 (90.9)	3 (37.5)	6 (50.0)	19 (61.3)	0.041
Insufficient authority of vaccination expert consensus statements, *n* (%)	6 (54.5)	4 (50.0)	7 (58.3)	17 (54.8)	>0.999
Insufficient official policy support, *n* (%)	3 (27.3)	3 (37.5)	3 (25.0)	9 (29.0)	0.887
Unwillingness to vaccinate children with medical conditions due to fear of adverse events, *n* (%)	2 (18.2)	2 (25.0)	6 (50.0)	10 (32.3)	0.239
Sustainability of services in vaccination evaluation clinics due to high operational costs, *n* (%)	4 (36.4)	2 (25.0)	4 (33.3)	10 (32.3)	0.897

* The rates for the barriers between regions were compared using Fisher's exact test, due to the small sample size of one cell (<5).

Table 2 shows barriers to immunization service provision as perceived by PLADs. More than half of the PLADs identified the following barriers: lack of comprehensive vaccination recommendations for specific medical conditions (74.2%); absence of standardized procedures to assess the appropriateness of vaccination in certain medical conditions (74.2%); inconsistencies between official recommendations, vaccine package inserts, and published expert consensus statements (61.3%); and limited authority of expert consensus statements (54.8%).

Table 3 shows scores and rankings of urgent demands for immunization services for children with medical conditions. All three regions indicated that the top priority is to develop detailed official vaccination recommendations for children with medical conditions.

DISCUSSION

This study revealed that the provision of immunization services varies throughout China and might not be adequate to guarantee that children with medical conditions receive the recommended vaccinations. Incomplete vaccination can increase the vulnerability of these children to vaccine-preventable diseases. While significant efforts are needed at a national level, some PLADs have already addressed this issue and have explored suitable immunization service models for children with medical conditions (*4*–*5*). To facilitate effective service provision, it is crucial to identify the barriers and unmet needs, as well as to implement measures that enhance vaccination coverage for children with medical conditions.

Immunization service support for children with medical conditions varies across regions, revealing inconsistencies in the development and implementation of local recommendations, expert consensus statements, training programs, and vaccination evaluation clinics. Although progress has been observed in eastern PLADs, this is expected to enhance protection for children with medical conditions against vaccine-preventable diseases in the long run (*6*). Nonetheless, the limited-service support in central and western PLADs may indicate a lack of sufficient child health resources. Taking into account the practices and experiences from leading PLADs could potentially improve immunization service capacity in other regions.

This study discovered that the majority of provincial-level CDCs placed a higher importance on enhancing official vaccination recommendations for immunizing children with medical conditions. This preference outweighed the development of expert consensus statements, which would cover a broader range of conditions and operational details. The inclination towards official recommendations is primarily due to the fact that expert consensus statements are not recognized as official documents under the Vaccine Administration Law of the People's Republic of China. Consequently, these statements may vary depending on the expert teams involved, leading to a lack of confidence among healthcare workers (*7*).

Vaccination recommendations for prevalent childhood medical conditions, including prematurity, low birth weight, allergic predisposition, immune system dysfunction, congenital diseases, and congenital infections, have been outlined in the 2021 version of China's national immunization schedule (*8*). As more data emerge from pertinent vaccine effectiveness and safety studies, national recommendations ought to encompass additional medical conditions. Providing training on these updated recommendations and

TABLE 3. Demands for immunization services support for children with medical conditions by region, China, 2022.

Demand	Eastern		Central		Western		Nationwide	
	Score	Rank	Score	Rank	Score	Rank	Score	Rank
Detailed official vaccination recommendations	6.00	1	5.50	1	5.75	1	5.77	1
Technical guidelines for vaccination evaluation	4.73	2	4.00	3	4.42	2	4.42	2
Official policy support	4.00	3	4.50	2	3.25	3	3.84	3
Use of vaccination evaluation clinics for certain medical conditions	3.55	4	2.50	5	3.25	3	3.16	4
Developing Standard Operating Procedures for Counseling, Vaccination, and Managing Adverse Events	2.82	5	3.13	4	2.75	5	2.87	5
Establishing a multidisciplinary consultation team	2.73	6	1.63	7	2.08	6	2.19	6
Training with case studies for healthcare workers	1.27	7	1.75	6	2.00	7	1.68	7
Developing public education materials	0.64	8	0.63	8	0.67	8	0.65	8

technical guidelines is crucial for successful implementation.

Inconsistencies between vaccine package inserts and official recommendations have the potential to cause confusion among healthcare workers and concern parents. These inconsistencies were identified by provincial CDCs in our survey as significant barriers to vaccinating children with medical conditions. Similar discrepancies have been observed in other countries (*9*). Factors contributing to these discrepancies, which are important considerations for provincial CDCs, include varying disease burdens in children with medical conditions, differing risk and benefit estimates, vaccine characteristics, and parental and public acceptance (*9*). It is essential that all stakeholders collaborate to develop a well-coordinated immunization policy for potentially off-label vaccine recommendations.

This study discovered that 50% of PLADs have implemented vaccination evaluation clinics within pediatric hospitals to assess the appropriateness of vaccinations for children who have medical conditions that are challenging for NIP providers in community healthcare centers to evaluate. In other nations, children with medical conditions are typically assessed and vaccinated by NIP providers rather than specialist physicians, resulting in vaccination rates similar to those among healthy children (*10*). Variations in methodology may be related to a hesitancy among Chinese NIP providers to administer vaccinations to children with medical conditions (*11*), which may be influenced by unfamiliarity with certain medical conditions, insufficient knowledge regarding vaccine safety and efficacy in specific medical cases, and an absence of official, detailed guidelines for particular conditions (*12*). Although vaccination evaluation clinics positively impact the intentions of concerned parents to vaccinate their children (*5*), it is crucial to comprehend and overcome the barriers preventing NIP providers from recommending and administering vaccinations to children with medical conditions.

To address ongoing and emerging challenges in enhancing immunization services for children with medical conditions, several steps should be taken. These include monitoring vaccination coverage among children with medical conditions, expanding current national vaccination recommendations, developing evaluation procedure guidelines, addressing barriers experienced by immunization service providers, creating targeted educational tools for the public, and advocating for changes in immunization policies to safeguard providers.

The current study presents several limitations. While the survey encompassed 31 PLADs in China, it may not adequately represent the perceptions of CDCs at the prefecture and county levels, nor the NIP providers and management operating within vaccination clinics. Additionally, the study did not incorporate the viewpoints of parents concerned about vaccinations. Future research should endeavor to assess the opinions and perspectives of these vital stakeholders in this field.

In conclusion, this study identified challenges related to the provision of immunization services for children with medical conditions. Furthermore, it highlighted potential opportunities to address these challenges, ultimately aiming to enhance vaccination coverage and protect children with medical conditions from vaccine-preventable diseases.

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Vital Surveillances

Genomic Analysis and Antimicrobial Resistance of *Campylobacter jejuni* Isolated from Diarrheal Patients — Beijing Municipality, China, 2019–2021

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ABSTRACT

Introduction: *Campylobacter jejuni (C. jejuni*) is the leading cause of human bacterial gastroenteritis worldwide and has a major impact on global public health. The objective of the present study was to conduct whole genome sequencing (WGS) to determine the genetic diversity, virulence factors, and determinants of antimicrobial resistance of *C. jejuni* during a 3-year surveillance period in Beijing, China.

Methods: A total of 184 clinical isolates were obtained from sentinel hospital surveillance between 2019 and 2021. Antimicrobial susceptibility testing was conducted using the agar dilution method. WGS was employed to characterize the 184 *C. jejuni* strains.

Results: Multilocus sequence typing analysis revealed high genetic diversity among the 184 *C. jejuni* strains, identifying 71 sequence types (STs) and 19 clonal complexes (CCs). The most prevalent ST was ST760 (6.5%), and the most common CC was CC21 (24.5%), consisting of 11 STs. High resistance rates were observed for ciprofloxacin (76.6%), nalidixic acid (76.1%), and tetracycline (71.2%). A total of 77 *C. jejuni* isolates (41.8%) exhibited multidrug resistance with 43 resistance patterns. Virulome analysis disclosed the differential distribution of virulence factors related to adherence, colonization, chemotaxis, as well as lipo-oligosaccharide and capsular polysaccharide biosynthesis. Resistome analysis demonstrated widespread resistance to quinolones and tetracycline, but low rates of macrolides resistance. The phylogeny, based on whole genome single nucleotide polymorphisms, indicated a high degree of clonality and grouped the *C. jejuni* strains into six clades. Closely related isolates that were part of a genetic cluster mostly shared a homogenous clonal complex.

Conclusions: The present study emphasizes the rising resistance to quinolones and tetracycline, as well as the virulence potential and diverse genotypes identified among *C. jejuni* strains isolated from diarrheal patients in Beijing.

Campylobacter jejuni (*C. jejuni*) is a prevalent foodborne zoonotic pathogen and has been identified as the primary cause of human gastroenteritis globally (*1*–*2*). Besides gastrointestinal illness, *C. jejuni* is also associated with autoimmune conditions such as Guillain–Barré syndrome and Miller Fisher syndrome (*1*,*3*). Although infections caused by *C. jejuni* are often self-limiting and typically resolve within a few days without antibiotic intervention, effective antimicrobial treatment is essential for immunocompromised patients or severe cases of the disease. The rapid increase in antimicrobial resistance (AMR) among *C. jejuni* strains has become a critical public health concern. Consequently, the World Health Organization has listed fluoroquinolone-resistant *Campylobacter* spp. as one of the six high-priority antimicrobial-resistant pathogens posing the greatest threat to human health (*4*).

Whole genome sequencing (WGS) has the capacity to generate vast amounts of precise data rapidly, which can subsequently be utilized for species identification, typing, phylogenetic analyses, and determining virulence and resistance characteristics (*5*). WGS is progressively utilized as the foremost approach for foodborne pathogen surveillance in public health laboratories, supplanting prior conventional typing methods.

Recently, *Campylobacter* spp. has emerged as the most prevalent bacteria causing human diarrhea in Beijing, China, with an alarmingly high burden on human health (*6*). Only a few reports have analyzed the genetic diversity and AMR profiles of clinical strains of *Campylobacter* in Beijing (*6*–*7*), and such studies have lacked the resolution provided by WGS-based typing methods. Therefore, larger genomic

epidemiology studies are needed to elucidate the molecular characteristics of *Campylobacter* strains circulating in Beijing and more broadly in China.

In this investigation, a total of 184 *C. jejuni* strains were collected from patients with diarrhea during active surveillance in Beijing throughout a three-year period (2019–2021). This study presents a comprehensive genomic analysis, examining the genetic diversity, virulence potential, and AMR profiles of the isolated strains.

METHODS

Sample Collection and *Campylobacter* Isolation

Hospital-based active surveillance of *Campylobacter* has been conducted since 2010 in Beijing, China. A *Campylobacter* isolation kit incorporating a membrane filter method (ZC-CAMPY-002, Sinova Biotechnology Co., Ltd., Qingdao, China) was employed to isolate *Campylobacter*. In brief, a 1 mL stool specimen suspension was transferred into 4 mL of enrichment medium provided in the kit. The enriched suspension was incubated at 37 ℃ for 24 hours under microaerophilic conditions, consisting of 5% O_2, 10% CO_2, and 85% N_2. Approximately 300 μL of the enriched culture was spotted onto the membrane filter surface and then pasted onto both Karmali and Columbia agar plates. The plates were subsequently incubated in a microaerophilic atmosphere at 37 ℃ for 48 hours. Suspected colonies were picked and identified using a real-time PCR assay targeting the *hipO* gene of *C. jejuni*. From 2019 to 2021, 184 *C. jejuni* isolates were obtained from this surveillance program.

Antimicrobial Susceptibility Testing (AST)

The minimum inhibitory concentration (MIC) of all *C. jejuni* isolates was determined using the agar dilution method recommended by the Clinical and Laboratory Standards Institute document (CLSI M45-P). Six classes of 11 antimicrobial agents (Sinova Biotechnology Co., Ltd., Qingdao, China) were used for AST: erythromycin (ERY), azithromycin (AZI), nalidixic acid (NAL), ciprofloxacin (CIP), gentamicin (GEN), streptomycin (STR), chloramphenicol (CHL), florfenicol (FLO), tetracycline (TET), telithromycin (TEL), and clindamycin (CLI). The breakpoints for resistance used in this study were based on standards used in the National Antimicrobial Resistance Monitoring System (NARMS), except for ERY, CIP, and TET, which were based on CLSI guidelines. The following MIC values were determined for *C. jejuni*: ERY ≥32 μg/mL, AZI ≥1 μg/mL, NAL ≥32 μg/mL, CIP ≥4 μg/mL, GEN ≥4 μg/mL, STR ≥16 μg/mL, CHL ≥32 μg/mL, FLO ≥8 μg/mL, TET ≥16 μg/mL, TEL ≥8 μg/mL, and CLI ≥1 μg/mL. *C. jejuni* ATCC 33,560 was included in the test as a quality control strain. Multidrug resistance was defined as resistance to at least three classes of antimicrobials in this study.

WGS and Genomic Analysis

DNA was extracted utilizing a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The quantification of the extracted genomic DNA (gDNA) was determined through agarose gel electrophoresis and fluorometric analysis (Qubit 2.0). WGS was performed on an Illumina PE150 platform with 100× coverage (Novogene Technology Co., Ltd., Beijing, China). Raw sequencing data were assessed for quality, trimmed, and subsequently assembled *de novo* into a draft genome sequence using the SPAdes 3.13 software.

Multilocus sequence typing (MLST) was conducted utilizing WGS data in accordance with the *Campylobacter* PubMLST scheme (https://pubmlst.org/). STs and CCs were identified for each isolate. AMR genes were analyzed using the NCBI AMRFinderPlus tool 3.1.1b (https://ftp.ncbi.nlm.nih.gov/pathogen/Antimicrobial_resistance/AMRFinder/). Virulence genes were detected via the virulent factors of pathogenic bacteria (VFDB) database (http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi). Whole genome single nucleotide polymorphisms (wgSNPs) analysis was performed on all draft genomes with the reference strain NCTC 11168 (GenBank ID: NC_002163.1) using the parsnp software. Finally, the phylogenetic tree and heatmap of resistance and virulence genes were visualized using the ChiPlot tool (https://www.chiplot.online/).

RESULTS

Diversity of MLST Genotype STs and CCs

MLST analysis of the 184 *C. jejuni* isolates produced 71 distinct STs, categorized into 19 CCs and unassigned (not belonging to any known CC) categories (Supplementary Table S1, available in https://weekly.chinacdc.cn/). Out of these, 23 isolates belonged to 16 unassigned STs. The most prevalent STs were ST760 (6.5%), followed by ST49 (6.0%) and ST22 (5.4%). Thirty STs were represented by only

one isolate each. The most common CC, CC21 (24.5%), consisted of 11 STs: ST21, ST50, ST298, ST760, ST1811, ST3597, ST6500, ST8261, ST9873, ST9960, and ST10075. The second most frequent CC, CC45, comprised six STs and 13 isolates (7.1%). The remaining CCs included relatively few STs or a small number of strains.

AST

In the present study, 13 *C. jejuni* isolates (7.1%) demonstrated susceptibility to all 11 tested antimicrobials. The highest recorded resistance rates were observed for CIP (76.6%), with NAL (76.1%) and TET (71.2%) following closely behind. Conversely, resistance rates for ERY, TEL, and CHL were relatively low at 3.8%, 7.6%, and 8.2% respectively. The resistance rates for other antimicrobials included FLO (27.3%), CLI (18.5%), STR (11.4%), and both AZI and GEN (10.9%) (Table 1).

Regarding multidrug resistance, 77 *C. jejuni* isolates (41.8%) were resistant to three or more classes of

TABLE 1. MIC distribution and AMR phenotype and genotype in 184 *C. jejuni* strains.

Class	Antibiotic agent	MIC (μg/mL)												Resistant strains
	AMR gene	Breakpoint	Range	≤0.25	0.5	1	2	4	8	16	32	64	>64	*n* (%)
Macrolides	ERY	≥32	0.5–64		84	69	14	7	1	2	1	2	4	7 (3.8)
	*50S rRNA*_L22_A103V													0
	23S rRNA_A2075G												1	1
	cmeABC											2	3	5
	AZI	≥1	0.5–64		164	5	2	6	1	1	1	2	2	20 (10.9)
	*50S rRNA*_L22_A103V							1						1
	23S rRNA_A2075G												1	1
	cmeABC					3	2	4	1	1	1	1	2	15
Quinolones	NAL	≥32	0.5–64		3	19	6	4	8	4	5	36	99	140 (76.1)
	*gyrA*_T86I										5	34	98	137
	cmeABC										3	21	58	82
	CIP	≥4	0.5–64		17	15	11	5	26	34	41	22	13	141 (76.6)
	*gyrA*_T86I							5	26	34	39	22	12	138
	cmeABC							3	25	27	11	11	11	88
Aminoglycosides	GEN	≥4	0.5–64		144	13	7	4	0	3	2	3	8	20 (10.9)
	aph(3')–IIIa							2			1	1	2	6
	aph(2'')–If									1	1	3		5
	aac(6')–Ie/aph(2'')–Ia							2					1	3
	STR	≥16	0.5–64		104	23	23	4	9	6	4	5	6	21 (11.4)
	ant(6)-Ia									1	1			2
	aadE											1		1
Tetracyclines	TET	≥16	0.5–64		28	12	6	3	4	4	7	37	83	131 (71.2)
	tetO									3	7	35	78	123
	cmeABC									4	5	23	46	78
Phenicols	CHL	≥32	0.5–64		16	27	25	43	21	37	12	1	2	15 (8.2)
	FLO	≥8	0.5–64		25	24	50	35	30	8	8	4		50 (27.3)
Ketolides	TEL	≥8	0.25–32	45	33	30	50	12	4	3	3	4		14 (7.6)
Lincosamides	CLI	≥1	0.25–32	107	43	16	4	4	1	4	1	4		34 (18.5)

Abbreviation: MIC=minimum inhibitory concentration; AMR=antimicrobial resistance; *C. jejuni*=*Campylobacter jejuni*; ERY=erythromycin; AZI=azithromycin; NAL=nalidixic acid; CIP=ciprofloxacin; GEN=gentamicin; STR=streptomycin; TET=tetracycline; CHL=chloramphenicol; FLO=florfenicol; TEL=telithromycin; CLI=clindamycin.

* The gray shade indicates resistance strains and red numbers indicate the number of resistance strains.

antimicrobials and showed 43 different resistance patterns. Among these, the predominant resistance pattern was NAL-CIP-TET-FLO (12.0%). Three isolates were resistant to at least 10 antimicrobials (Supplementary Figure S1, available in https://weekly.chinacdc.cn/).

Detection of Antimicrobial Resistance Genes

Genomic analysis revealed a total of 29 AMR determinants among 184 *C. jejuni* isolates. These included 26 acquired AMR genes and three resistance-conferring point mutations (Table 2). The *gyrA*_T86I point mutation, associated with resistance to quinolones, was the most prevalent, identified in 96.2% of the isolates. The *tetO* gene, conferring resistance to tetracycline, was also highly prevalent, found in 82.6% of isolates.

A variety of 11 known bla_{OXA} variants were detected in 147 strains (79.9%), with $bla_{OXA\text{-}193}$, $bla_{OXA\text{-}461}$, and $bla_{OXA\text{-}591}$ being the most prevalent (40.2%,

TABLE 2. Distribution of AMR and virulence genes in 184 *C. jejuni* strains.

Genes	Class	Gene	No. of isolates (%)
Resistance genes	Quinolones	*gyrA*_T86I	177 (96.2)
	Tetracyclines	*tetO*	152 (82.6)
	ß-Lactams	Any of the following	147 (79.9)
		$bla_{OXA\text{-}193}$	74 (40.2)
		$bla_{OXA\text{-}461}$	19 (10.3)
		$bla_{OXA\text{-}591}$	17 (9.2)
		$bla_{OXA\text{-}184}$	12 (6.5)
		$bla_{OXA\text{-}460}$	9 (4.9)
		$bla_{OXA\text{-}592}$	7 (3.8)
		$bla_{OXA\text{-}583}$	3 (1.6)
		$bla_{OXA\text{-}631}$	3 (1.6)
		$bla_{OXA\text{-}465}$	1 (0.5)
		$bla_{OXA\text{-}594}$	1 (0.5)
		bla_{EC}	1 (0.5)
	Macrolides	Any of the following	37 (20.1)
		*50S rRNA*_L22_A103V	35 (19.0)
		23S rRNA_A2075G	2 (1.1)
	Aminoglycosides	Any of the following	67 (36.4)
		aph(3')-IIIa	18 (9.8)
		ant(6)-Ia	14 (7.6)
		sat4	12 (6.5)
		aph(2'')-If	9 (4.9)
		aac(6')-Ie/aph(2'')-Ia	5 (2.7)
		aadE	4 (2.2)
		aad9	1 (0.5)
	Phenicols	*catA13*	2 (1.1)
	Arsenics	*arsP*	95 (51.6)
		acr3	73 (39.7)
	Lincosamides	*lnuC*	6 (3.3)
	Multidrug efflux pump	*cmeA*	183 (99.5)
		cmeB	114 (62.0)
		cmeC	184 (100.0)
		cmeABC	114 (62.0)

TABLE 2. (Continued)

Genes	Class	Gene	No. of isolates (%)
Virulence genes	Invasion	*ciaB; ciaC; flhA; flhB; fliP; fliQ; fliR;*	184 (100.0)
		flaC	182 (98.9)
	Adhesion	*cadF; jlpA; pebA;*	184 (100.0)
		porA	112 (60.9)
	Toxin	*cdtB*	184 (100.0)
		cdtA	182 (98.9)
		cdtC	182 (98.9)
	Chemotaxis	*cheA; cheV; cheW; cheY;*	184 (100.0)
	Motility	*flgG; flgH; fliF; fliM;*	184 (100.0)
		fliY	183 (99.5)
		flgI	183 (99.5)
		flgE	181 (98.4)
		fliK	178 (96.7)
		flaB	22 (12.0)
		flaA	21 (11.4)
	LOS	*hldD; waaC;*	184 (100.0)
		htrB	183 (99.5)
		gmhA	66 (35.9)
		hldE	55 (29.9)
		cstIII	41 (22.3)
		neuA	41 (22.3)
		neuB	41 (22.3)
		neuC	41 (22.3)
	CPS	*kpsS*	183 (99.5)
		kpsD	183 (99.5)
		kpsE	177 (96.2)
		Cj1417c	158 (85.9)
		Cj1419c	158 (85.9)
		Cj1420c	154 (83.7)
		waaF	130 (70.7)
		kpsF	71 (38.6)
		kpsT	36 (19.6)

Abbreviation: AMR=antimicrobial resistance; *C. jejuni*=*Campylobacter jejuni*; LOS=lipo-oligosaccharide; CPS=capsule polysaccharide.

10.3%, and 9.2%, respectively). In contrast, a much smaller fraction of strains harbored resistance markers for other antimicrobials. Resistance to macrolides via two point mutations, *50S rRNA*_L22_A103V and *23S rRNA_A2075G*, was observed in 19.0% and 1.1% of isolates, respectively.

Additionally, seven genes for resistance to aminoglycosides (i.e., *aph(3')-IIIa*, *ant(6)-Ia*, *sat4*, *aph(2'')-If*, *aac(6')-Ie/aph(2'')-Ia*, *aadE*, and *aad9*) were detected in fewer than 10% of the isolates. High prevalence of arsenic resistance genes, *arsP* and *acr3*, was observed. Notably, the *cmeABC* operon, encoding a multidrug efflux pump and consisting of *cmeA*, *cmeB*, and *cmeC* genes, was present in 62% of *C. jejuni* isolates. The prevalence of remaining resistance genes was no greater than 3.3%. Detailed prevalence rates of the aforementioned AMR genes can be found in Table 2.

Correlation of Phenotypic and Genotypic Resistance

Of the seven strains exhibiting ERY-resistant phenotypes, one strain carried the *A2075G* mutation

in the *23S rRNA* gene, and five strains contained the *cmeABC* multi-efflux pump operon gene (Table 1). Among the 20 strains with AZI-resistant phenotypes, one displayed the *23S rRNA_A2075G* mutation, another exhibited the *50S rRNA*_L22_A103V mutation, and fifteen possessed the *cmeABC* operon. These two isolates with the *23S rRNA_A2075G* point mutation demonstrated high-level resistance to macrolides (ERY, AZI MIC >64 μg/mL).

Out of 141 CIP-resistant strains, 97.9% (*n*=138) presented the *gyrA*_T86I mutation, and 62.4% (*n*=88) carried the *cmeABC* operon. Interestingly, the *gyrA*_T86I point mutation was present across the entire range of CIP resistance (MIC 4–64 μg/mL). Of the 131 TET-resistant strains, 93.9% (*n*=123) harbored the *tetO* gene. Moreover, 59.5% (*n*=78) of the TET-resistant strains contained the *cmeABC* operon.

Detection of Virulence Factors

Virulome analysis identified 47 virulence genes across 184 *C. jejuni* isolates. These genes were classified into seven categories based on their roles in pathogenesis/colonization (Table 2). The prevalence rates of cell invasion-associated genes included: *ciaB*, *ciaC*, *flhA*, *flhB*, *flip*, *fliQ*, and *fliR* (all at 100%), and *flaC* (98.9%). Adherence and colonization-related genes demonstrated prevalence rates of *cadF*, *jlpA*, *pebA* (all at 100%), and *porA* (60.9%). The cytolethal distending toxin (CDT) genes encoded by the *cdtABC* operon showed prevalence rates of *cdtB* (100%) and *cdtA* and *cdtC* (both at 98.9%). All 184 isolates contained chemotaxis-associated genes: *cheA*, *cheV*, *cheW*, and *cheY*. Genes involved in motility displayed prevalence rates of: *flgG*, *flgH*, *fliF*, *fliM* (all at 100%), *flgI*, *fliY* (both at 99.5%), *flgE* (98.4%), *fliK* (96.7%), *flaB* (12.0%), and *flaA* (11.4%). Moreover, the analysis identified various genes implicated in the biosynthesis of lipo-oligosaccharide (LOS) and capsule polysaccharide (CPS). Percentages of all detected virulence genes categorized by function are outlined in Table 2.

Phylogenetic Analysis and Heatmap of AMR and Virulence Genes

The wgSNP phylogenetic analysis of 184 *C. jejuni* strains identified six major clades, designated A–F (Figure 1), with no association between the lineages and the years of isolation. Clade A consisted of 62 strains from CC21, CC206, or CC48, while the largest clade, clade B, included 68 isolates forming five sub-clusters (B1–B5) with diverse CCs as follows: B1: CC257, CC354, CC460; B2: CC52, CC353, CC464; B3: CC443, CC574; B4: CC257; B5: CC828, CC1034. Notably, CC257 appeared in both B1 and B4, indicating the differentiation and expansion of clonal groups. Lineages C, D, and E each contained unique independent CCs, specifically CC49, CC58, and CC403, respectively. Additionally, 33 strains from CC22, CC42, or CC45 formed a single lineage, lineage F.

The heatmap of virulence genes revealed that all strains were divided into two clusters related to CC, based on the distribution of virulence factors. Strains from CC21 exhibited a wide distribution of virulence determinants, particularly those related to LOS (Figure 2A). CC354 and CC257 strains possessed fewer CPS genes compared to other strains. Although no correlation was found between the observed AMR genes and their distribution of CCs on the heatmap (Figure 2B), interestingly, 12 isolates from CC49 displayed the least resistance genes and were sensitive to most antimicrobial agents.

DISCUSSION

In recent decades, the dramatic increase in AMR in *Campylobacter* worldwide has prompted research on the prevalence and molecular determinants of resistance. In this study, *C. jejuni* isolates exhibited a high rate of resistance to certain antimicrobials (CIP, NAL, and TET) and a low rate of resistance to ERY, which concurs with findings previously reported in China (*8*) and elsewhere (*9–12*). The high percentage of quinolone-resistant *C. jejuni* strains (97.9%) with point mutation *gyrA*_T86I was frequently observed, which contributed to the high-level resistance to quinolones (*9–10*,*12*). The *tetO* gene was detected in 93.9% of TET-resistant *C. jejuni* isolates, which also corroborates previous studies (*9–10*,*12*). The two markers of AMR to macrolides, *50S rRNA*_L22_A103V and *23S rRNA_A2075G*, were detected at low prevalence (19.0% and 1.1%, respectively) among *Campylobacter* isolates. Notably, *C. jejuni* isolates (62.0%) harbored the *cmeABC* operon, which encodes a member of the resistance-nodulation cell division superfamily of multidrug efflux transporters and functions synergistically with other mechanisms to contribute to resistance to quinolones, macrolides, and tetracyclines (*9–10*). In this study, three strains with phenotypic resistance to

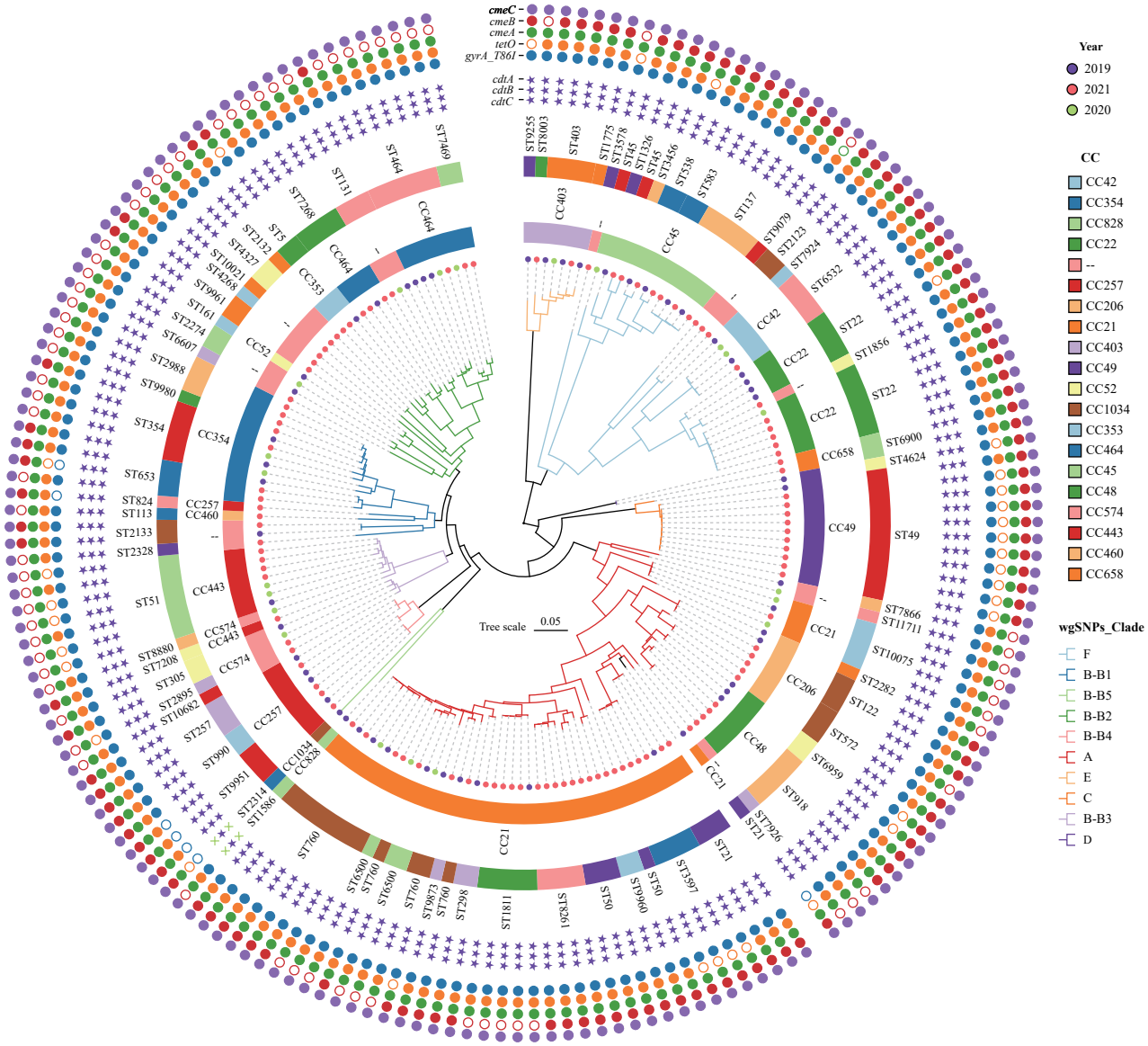

FIGURE 1. Phylogenetic analysis of 184 *C. jejuni* strains in Beijing from 2019 to 2021.
Note: The phylogenetic tree is based on wgSNPs analysis. CCs and STs are shown in colored rings for each strain, and tree branches are color coded to highlight *C. jejuni* strains from clades A, C, D, E, F, and sub-cluster of clade B (B1-B5). The isolation year of each strain is indicated by colored dots. Unassigned CC is denoted by two short lines (--). The presence of 5 main resistance genes is denoted by colored solid circles: *gyrA*_T86I (blue), *tetO* (orange), *cmeA* (green), *cmeB* (red) and *cmeC* (purple). The absence is indicated by hollow circles. The presence of toxin genes (*cdtA*, *cdtB* and *cdtC*) is denoted by purple star symbol and the absence is denoted by cross symbol.
Abbreviation: *C. jejuni=Campylobacter jejuni*; wgSNPs=whole genome single nucleotide polymorphisms; CC=clonal complex; ST=sequence type.

quinolones did not present the mutation in *gyrA*_T86I; however, the presence of the *cmeABC* operon was observed, indicating that the efflux pumps could be conferring resistance to quinolones in these three strains. Also, six ERY-resistant strains did not carry the mutation *A2075G* in the *23S rRNA* gene; however, the presence of the *cmeABC* operon was observed in five strains, which would likely be responsible for the resistance to ERY in strains that did not present the mutation in the *23S rRNA*. Finally, eight TET-resistant strains did not harbor the *tetO* gene; similarly, the presence of the *cmeABC* operon was observed in all eight strains, which would also be responsible for the resistance to TET. Taken together, these data contribute to our understanding of the *Campylobacter* resistome, which will support the development of AMR surveillance programs in China.

The mechanisms underlying the pathogenic

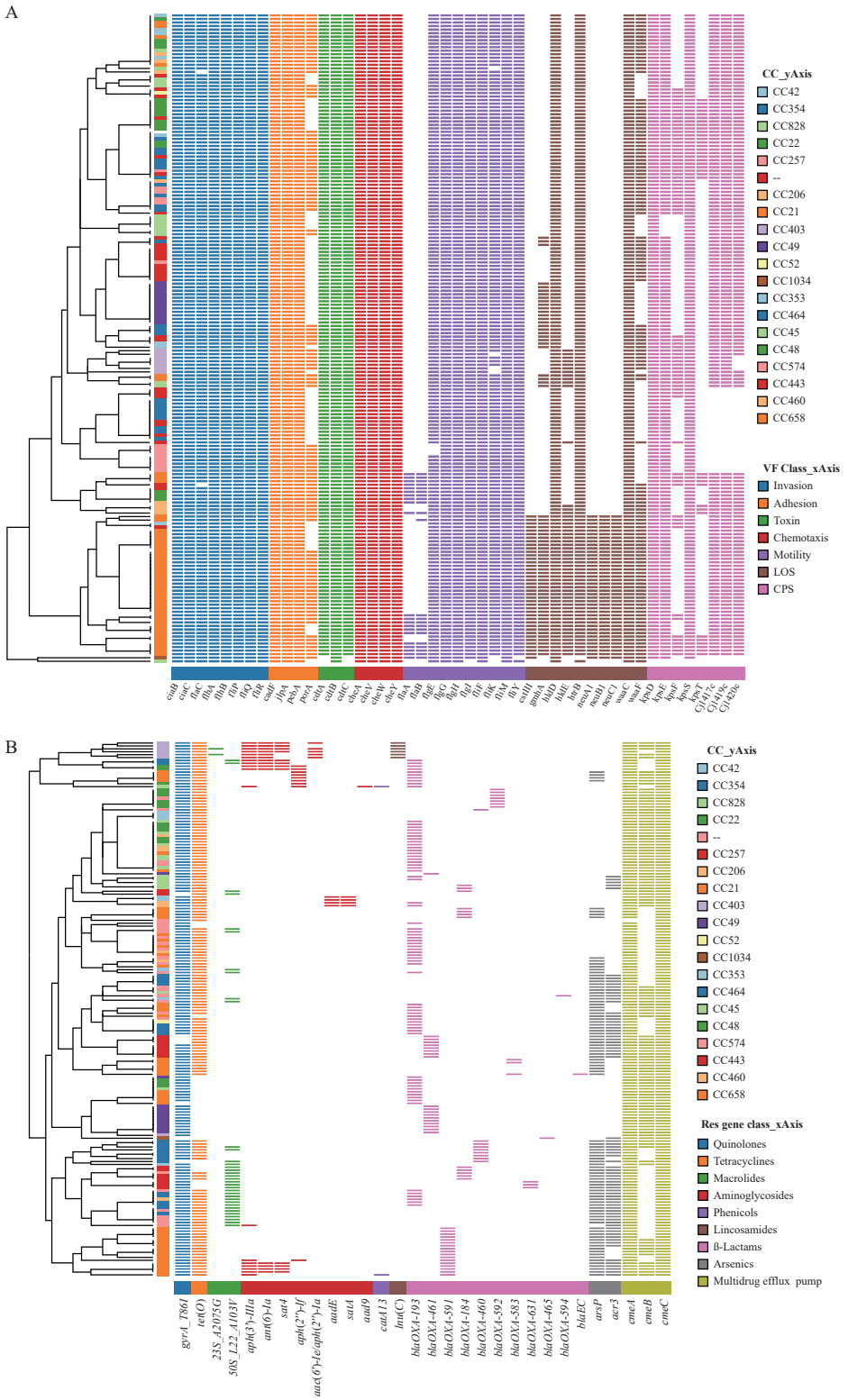

FIGURE 2. Annotation heatmap of virulence and AMR genes among 184 *C. jejuni* strains in Beijing from 2019 to 2021. (A) Virulence genes; (B) AMR genes.

Note: The color strips of Y-axis represent CCs corresponding to each strain. The color blocks of X-axis represent the categories of genes. Binary heatmaps show the presence and absence of virulence and AMR genes. Colored cells represent the presence of genes and white cells represent the absence of the genes. Unassigned CC is denoted by two short lines (--).

Abbreviation: AMR=antimicrobial resistance; *C. jejuni*=*Campylobacter jejuni*; CC=clonal complex; VF=virulence factors.

processes by which *Campylobacter* species cause diarrhea remain unclear (*1*). This study found that a large proportion of *Campylobacter* strains possessed genes linked to bacterial motility, invasion, adhesion, and chemotaxis to epithelial cells, all of which play vital roles in the onset of *Campylobacter* infection (*2*). These results support earlier research indicating that adherence, colonization, and invasion genes (e.g., *cadF*, *ciaB*, *ciaC*, *flaC*, *jlpA*, *pebA*, *porA*) are highly conserved among *C. jejuni* strains and present in the majority of clinical isolates (*13–14*), emphasizing the potential virulence of these *Campylobacter* strains in causing human infections.

Furthermore, virulence marker determinants *cdtABC*, which encode CDT and significantly contribute to diarrhea by interfering with the division and differentiation of intestinal crypt cells, were also detected in most examined isolates (>98.9%). Previous investigations have reported a high prevalence of CDT in *Campylobacter* strains isolated from patients experiencing life-threatening diarrhea (*10*,*14–15*). While the functions of individual LOS genes (*cstIII*, *gmhA*, *hldD*, *hldE*, *trB*, *neuA*, *neuB*, *neuC*, *waaC*, and *waaF*) have yet to be clearly established, prior studies have suggested that these genes are crucial for forming human ganglioside-like LOS structures capable of inducing Guillain–Barré syndrome (*3*). In this study, the majority of strains carried at least four genes associated with LOS biosynthesis.

This study emphasized the extensive diversity of clinical *C. jejuni* isolates circulating in Beijing. Our findings demonstrated that 184 *C. jejuni* strains were classified into 71 STs, with strains belonging to CC21 as the predominant group. CC21 is the largest and most widely distributed CC globally, representing 17.9% of all *C. jejuni* strains submitted to the PubMLST database. Numerous studies have reported varying major CCs of *Campylobacter* in different countries and regions, but CC21, CC45, CC48, and CC353 are consistently the predominant CCs among isolates in many investigations. The high prevalence of ST760 in our study was unexpected, given its scarce representation within the PubMLST database (0.033%, as of February 2023). ST760 was first reported in 2000 from a human gastroenteritis case in England. Subsequently, it spread to several neighboring European countries but not to North or South America. In Asia, there were four reports of ST760 in Jiangsu Province in China in 2006, but no reports in other regions of China or other Asian countries (based on the PubMLST database). These data highlight the wide-range transmission of these strains across regions and indicate that ST diversity varies among countries and regions.

Previous research has demonstrated that associations between clades and the presence of resistance and virulence determinants are prevalent, although not all clades within a phylogenetic tree are characterized by factors such as geographic distribution or year of isolation (*11*). To date, few studies have focused on the phylogenetic analysis of *Campylobacter* strains in Beijing. In this study, wgSNPs phylogenetic analysis revealed that the *C. jejuni* population from diarrheal patients in Beijing encompasses a wide range of lineages and genotypes, with strains belonging to the same ST and/or CC grouping together in distinct clusters. Although this study presents limited literature regarding virulence factors linked to specific CCs or STs, it emphasizes the virulence potential of the investigated *Campylobacter* isolates. Additionally, one limitation of this study is that all *Campylobacter* strains were isolated from human samples, with none originating from alternative sources such as food or animals.

In conclusion, this study represents one of the most extensive genomic analyses of *C. jejuni* in Beijing, offering valuable insights into the prevalence of virulence genes, AMR markers, as well as the phylogenetic relationships and circulating genotypes from 2019 to 2021. Moreover, we report the high resistance rates to quinolone and tetracycline alongside the low resistance rate to erythromycin among the *C. jejuni* strains identified in Beijing. This information proves crucial for the development of monitoring, control, and prevention strategies to address the growing concern of resistance posed by this pathogen.

Conflicts of interest: No conflicts of interest.

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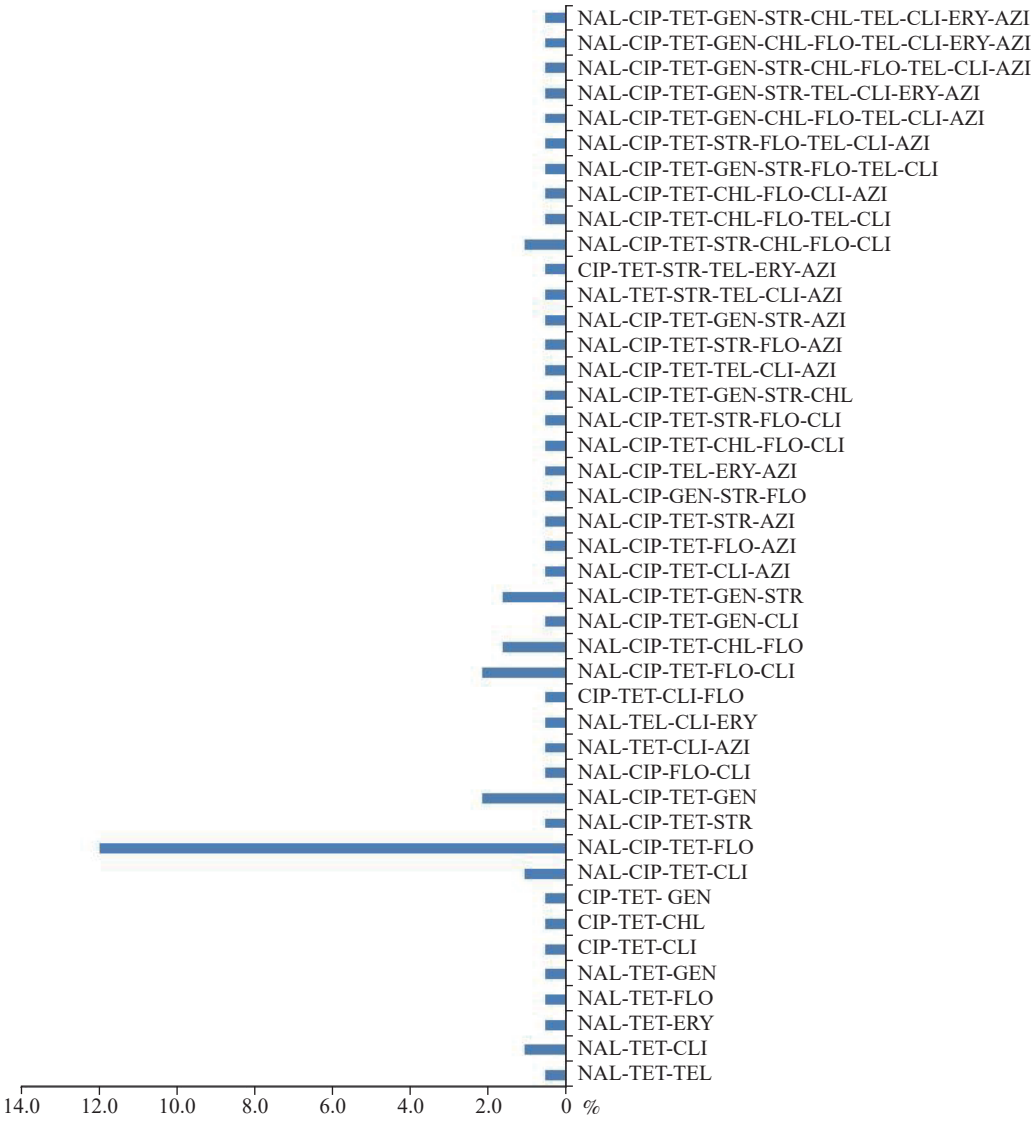
SUPPLEMENTARY TABLE S1. Distribution of CCs and STs in 184 *C. jejuni* strains.

CC type	ST type	No. of isolates (%)	CC type	ST type	No. of isolates (%)	CC type	ST type	No. of isolates (%)
CC21	ST21	4 (2.2)	CC52	ST161	1 (0.5)	CC574	ST305	2 (1.1)
	ST50	4 (2.2)	CC206	ST122	3 (1.6)		ST2895	1 (0.5)
	ST298	2 (1.1)		ST572	3 (1.6)		ST8880	1 (0.5)
	ST760	12 (6.5)		ST2282	1 (0.5)		ST10682	1 (0.5)
	ST1811	5 (2.7)	CC257	ST257	3 (1.6)	CC658	ST6900	2 (1.1)
	ST3597	4 (2.2)		ST824	1 (0.5)	CC828	ST1586	1 (0.5)
	ST6500	3 (1.6)		ST990	2 (1.1)	CC1034	ST2314	1 (0.5)
	ST8261	4 (2.2)		ST9951	3 (1.6)	unassigned	ST131	3 (1.6)
	ST9873	1 (0.5)	CC353	ST5	2 (1.1)		ST1856	1 (0.5)
	ST9960	2 (1.1)		ST2132	1 (0.5)		ST2123	2 (1.1)
	ST10075	4 (2.2)	CC354	ST354	5 (2.7)		ST2133	2 (1.1)
CC22	ST22	10 (5.4)		ST653	3 (1.6)		ST2274	2 (1.1)
CC42	ST6532	4 (2.2)		ST2988	3 (1.6)		ST2328	1 (0.5)
	ST7924	1 (0.5)		ST9980	1 (0.5)		ST3578	1 (0.5)
CC45	ST45	2 (1.1)	CC403	ST403	4 (2.2)		ST4268	1 (0.5)
	ST137	5 (2.7)		ST1775	1 (0.5)		ST4327	2 (1.1)
	ST538	2 (1.1)		ST8003	1 (0.5)		ST6607	1 (0.5)
	ST583	2 (1.1)		ST9255	1 (0.5)		ST7866	1 (0.5)
	ST1326	1 (0.5)	CC443	ST51	7 (3.8)		ST7926	1 (0.5)
	ST3456	1 (0.5)		ST7208	1 (0.5)		ST9079	1 (0.5)
CC48	ST918	5 (2.7)	CC460	ST113	1 (0.5)		ST9961	2 (1.1)
	ST6959	2 (1.1)	CC464	ST464	6 (3.3)		ST10021	1 (0.5)
CC49	ST49	11 (6.0)		ST7268	4 (2.2)		ST11711	1 (0.5)
	ST4624	1 (0.5)		ST7469	2 (1.1)			

Abbreviation: CC=clonal complex; ST=sequence type; *C. jejuni=Campylobacter jejuni*.



SUPPLEMENTARY FIGURE S1. MDR patterns of 184 *C. jejuni* strains to various antibiotic combinations.
Abbreviation: MDR=multidrug resistance; *C. jejuni*=*Campylobacter jejuni*.

Commentary

Atlas of Classified Disability in China: Spatial Statistics and Pattern

Ruitai Shao[1]

Disability, stemming from heredity, chronic illness or injury, constitutes a global public health concern, a human rights matter, and a significant obstacle for social development. In recent years, China has acknowledged the significance of disability prevention and control as an integral aspect of its health strategy, aiming to enhance the overall quality of life for its citizens. However, the evolving landscape of disability epidemiology remains inadequately understood, which substantially hinders precise prevention and control strategies and the optimization of resource distribution. Consequently, there is an urgent necessity for research that provides solid scientific evidence to effectively address this pressing issue.

Professors Xiaoying Zheng and Jinfeng Wang, along with their respective teams, have been extensively involved in epidemiological research on population health and disability. Specifically, they have cooperated across disciplines to investigate the spatial prevalence patterns of disability in China over the past few years. The comprehensive results from their research are presented in the 2023 monograph titled "The Atlas of Classified Disability in China: Spatial Statistics and Pattern," published by China Science Publishing & Media Ltd (*1*). This pioneering publication, organized into three sections — introduction, sequence map, and disability spectrum — represents the world's first inquiry into the incidence, distribution, and pattern atlas of disability within a national population. As an individual involved in non-communicable disease management at the World Health Organization headquarters, I highly endorse this book for its significant contributions in the following areas:

First, this monograph employs an interdisciplinary approach, combining medical and spatial attribution studies from traditional epidemiology and spatial epidemiology. This innovative method transcends the limitations of conventional analysis. The team identifies multiplicative interactions and linear relationships while pioneering the spatial nonlinear attribution and generalized interaction metric geographic detector model. By incorporating data from the national sample survey on disabled persons and relevant open-source materials, the team has generated the world's first country-level disability subtype spectrum for the entire population's lifespan, using data from 734 sampled counties in China. Through rigorous cross-checking, they have established a three-way distribution of disability levels at the county level, providing a comprehensive representation of disability levels, distribution, and pattern track pedigrees with optimal precision. Furthermore, building on previous research regarding gestational age developmental disorders, the team has advanced innovative research in the field of population health across the life course. This work has become a critical foundation for the trinity of "gestational age-aging process-old age" scientific identification of risk markers throughout the life cycle and serves as a technical reference for resource allocation.

Second, the monograph delivers a thorough examination of the spatiotemporal patterns within the disability spectrum, offering valuable insights for policymakers and researchers. The disabled population in China has experienced an increase due to the aging population and the prevalence of chronic diseases. Social transformation, accompanied by the overlapping effects of "disability aging" and "aging disability," has altered the patterns and composition of disabilities. In this monograph, disability is defined as one or more abnormalities in anatomical structure, or the loss of a specific organ or function (either physical or psychological) that impacts an individual's ability to engage in normal activities and fully participate in study, work, and community and social life. While congenital genetic factors contributing to disabilities have decreased, disabilities resulting from chronic diseases and injuries have increased. Furthermore, the incidence of hearing, speech, intellectual, and visual disabilities has declined, while neurodegenerative, psychiatric, and physical disabilities have experienced a

rise. This monograph can provide guidance for the development of disability prevention and control policies, assisting in refining the targeted prevention and control of disabilities in the future. Moreover, it presents an economical and effective reference framework for disability information management in China. The unbiased optimal global estimation derived from limited local sample survey data in this monograph prevents insufficient data mining or excessive resource usage for large-scale cross-sectional epidemiological surveys.

This monograph is primarily based on the theory of population health and the concept of the disability epidemic trajectory. Utilizing a multidisciplinary, multifaceted, and multidimensional approach, it integrates spatial analysis techniques with disability epidemiology to identify the prevalence patterns of population disabilities at the county level in China, as well as the range of various disability types across distinct spatial regions. This work serves as a foundation for examining the multidimensional and intricate factors underlying the levels, distribution, and patterns of disabilities within the entire population. The outcomes of this monograph not only reveal the levels, distribution, and patterns of disabilities but also demonstrate the integration of interdisciplinary knowledge and the spatial variability analysis of disability occurrence and development. Future research should encompass the spatial and temporal distribution of disabilities in developing countries, which could contribute to providing scientific references for the formulation and optimization of disability prevention and control policies.

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