Abstract

Objective: To analyse macrolide resistance and molecular characteristics of Bordetella pertussis clinical isolates from western China, and to explore the relationship between macrolide-resistance and genotypes.

Methods: Susceptibilities of B. pertussis clinical isolates to erythromycin, azithromycin and clarithromycin were determined by epsilometer test (E-test). Isolated strains were sequenced to ascertain the presence of the 23S rRNA gene A2047G mutation. Strains were typed using multilocus antigen sequence typing, multilocus variable-number tandem-repeat analysis (MLVA) and pulsed-field gel electrophoresis (PFGE).

Results: Of 58 B. pertussis strains isolated in this study, 46 were macrolide-resistant and 12 were macrolide sensitive. All macrolide-resistant strains carried the A2047G mutation and were the prn1/ptxP1/ptxA1/fim3-1/fim2-1 genotype; the MLVA types were MT195 (19/58), MT55 (13/58) and MT104 (14/58), and the PFGE profiles were classified into BpSR23 (17/58) and BpFINR9 (29/58) types. None of the macrolide-sensitive strains carried the A2047G mutation; genotypes were (prn9 or prn2)/ptxP3/ptxA1/fim3-1/fim2-1, and all were MT27. PFGE profiles differed from the macrolide-resistant strains.

Conclusions: B. pertussis clinical isolates from western China were severely resistant to macrolides. Genotypes differed between macrolide-resistant and macrolide-sensitive strains, and there may be a correlation between acquisition of macrolide resistance and changes in specific molecular types.
**Introduction**

Pertussis is an acute respiratory disease caused by *Bordetella pertussis* that mainly affects infants. Macrolide antibiotics, such as erythromycin, have always been the first choice for preventing and treating pertussis. During the past decade, erythromycin-resistant pertussis strains have been reported in many countries around the world, but resistance remains rare in Europe and the USA. In China, since erythromycin-resistant *B. pertussis* strains were first identified in the Shandong province in 2011, high rates of erythromycin-resistant isolates have been identified in several large cities: 87.5% (14/16) in Xi’an (2012–2013); 57.4% (81/141) in Shanghai (2016–2017); and 94.44% (17/18) in Tianjin (2013–2018). High rates of resistance have also been reported in other areas of China, and the resistance may be related to an adenine (A) to guanine (G) mutation at site 2047 in the 23S rRNA gene. Moreover, a previous study showed that pertussis strains are also resistant to macrolides other than erythromycin, such as azithromycin and clarithromycin.

Adaptive changes in vaccine-associated genes of circulating pertussis strains have been reported in many countries over the past 10 years. For example, the main current pertussis toxin (ptx)A allele is *ptxA1*, which differs from the vaccine strain that harbours the *ptxA2* allele. In addition, the *B. pertussis* multilocus variable-number tandem-repeat analysis (MLVA)-type MT27 strain has gradually replaced the MT83 vaccine strain across Europe. However, there are few published investigations in this field regarding China.

The macrolide-resistance phenotypes of *B. pertussis* have been shown to be associated with their genotypes. For example, a study from Shanghai demonstrated that the *ptx* promotor (*ptxP*)1 strains were significantly more resistant to erythromycin than the *ptxP3* strains. Although a large amount of data on macrolide resistance for *B. pertussis* have been published in China, most relate to the east of China, including Beijing, Shanghai, and Shenzhen, and few studies are from western China. The aim of the present study was to explore macrolide susceptibility in *B. pertussis* clinical isolates from the west of China, to investigate the correlation between susceptibility and *B. pertussis* genotype, and to compare genotypes between isolated *B. pertussis* strains with different macrolide-resistant phenotypes. Clinical *B. pertussis* isolates were collected and analysed for susceptibility to three macrolide antibiotics using the epilometer (E)-test and were assessed for the presence of the A2047G mutation in the 23S rRNA gene. Genotyping was performed using three methods: multilocus antigen sequence typing (MAST); multilocus variable-number tandem-repeat analysis (MLVA); and pulsed-field gel electrophoresis (PFGE).

**Materials and methods**

**Bacterial isolates**

Nasopharyngeal swabs (NPSs) were collected from 1200 patients, aged <1 years, hospitalized with suspected pertussis infection at
Xi’an Children’s Hospital, Lianhu District, Xi’an, China, between 2018 and 2020. Samples were collected from infants who met the Chinese diagnostic criteria clinical case definition for pertussis, i.e., cough lasting at least two weeks and/or with one of the following: paroxysms of coughing, and/or inspiratory whoop and/or post-tussive vomiting and/or iterative cyanosis and apnoea. Bacterial DNA was extracted from the NPSs using the QIAamp DNA Mini Kit (Qiagen, Dusseldorf, Germany), according to the manufacturer’s instructions, and subjected to real-time polymerase chain reaction (PCR) to detect the presence of IS481 and ptxP using Double Nucleic acid Test kit for Bordetella pertussis (Mabsky Biotech, Shenzhen, China) and QuantStudio5 Real-Time PCR System (ThermoFisher Scientific, Waltham, MA, USA), according to the manufacturer’s instructions. The NPSs positive for both IS481 and ptxP were cultured on charcoal agar (Oxoid, Hampshire, UK) plates supplemented with 15% sheep blood, at 37°C for 3–5 days. A total of 58 isolates were positive for culture, biochemical testing, and slide agglutination with specific anti-sera to B. pertussis (BD Biosciences, Sparks, MD, USA). Strains were stored at –70°C with 30% glycerine for later use.

This study was approved by the Human Ethics Committee of Xi’an Centre for Disease Control and Prevention, and was conducted in accordance with the Declaration of Helsinki. The samples used in this study were non-invasive throat swab specimens and were collected for the sole purpose of the present study. Written informed consent for study inclusion and preparation of the manuscript was obtained from each participant’s parents or legal guardian prior to the study.

Antimicrobial susceptibility testing and mutation site detection

Minimum inhibitory concentrations (MICs) of three macrolide antibiotics (erythromycin, azithromycin and clarithromycin) were determined via E-test. Briefly, previously frozen bacterial samples were thawed and cultured for 72 h at 36°C on charcoal agar plates containing 10% sheep blood. Suspensions of B. pertussis isolates, equivalent to a 0.5 McFarland standard, were then prepared in sterile saline and inoculated onto Bordet-Gengou agar (BD, Franklin Lakes, NJ, USA) containing 10% sheep blood and cultured at 36°C with antibiotic concentrations ranging from 0.016 to 256 mg/L, according to a previously published method.12 MICs and inhibition zone sizes were measured at day 5 of incubation. Antibiotic-free plates were inoculated to check for growth and purity, and E-tests using sulfamethoxazole/trimethoprim (BioMérieux, Marcy l’Etoile, France), a common alternative antibiotic for macrolide-resistant pertussis strains, were also conducted as above. Reference strains of B. pertussis (ATCC9797) and Staphylococcus aureus (ATCC29213) served as controls. The Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) do not yet provide breakpoint criteria for B. pertussis antimicrobial susceptibility. Therefore, the Standardized interpretation criteria of CLSI breakpoints for fastidious organisms of the Haemophilus species, Aggregatibacter species, Cardiobacterium hominis, Eikenella corrodens and Kingella species (HACEK) group were referenced,13 and the MIC50, MIC90 and MIC ranges were reported.

The 23S rRNA gene was amplified in DNA from each of the 58 isolates using PCR with the following primers (1505F: GGCACGAGCGAGCAAGTCTC; and 2128R: TCTGGCGACTCGAGTTCTGC), according to a previously published protocol.5 PCR products were then sequenced via the Sanger (dideoxy chain-termination) method to ascertain the presence of the A2047G mutation, using a second-generation sequencer (ABI3730XL;
ThermoFisher). The sequencing results were compared against the standard X68323 sequence and the allele of the GenBank Chinese vaccine strain (accession No. CP002695). Mutant and sensitive strains preserved in the laboratory were used as controls.

**Multilocus antigen sequence typing**

Multilocus antigen sequence typing was performed for the following antigenic genes: pertactin (prn), ptxP, ptxA, serotype 2 fimbrial subunit (fim2) and serotype 3 fimbrial subunit (fim3), according to the methods described previously.\(^{14,15}\) The alleles mentioned above were determined by sequencing and compared against sequences at the public databases for molecular typing and microbial genome diversity (https://pubmlst.org/). The *B. pertussis* standard strain ATCC 9797 and reference strain 13038 with known alleles, maintained in the present authors’ laboratory, were included as positive controls.

**Multilocus variable-number tandem-repeat analysis**

For MLVA, the variable number of tandem repeats (VNTRs) in six loci (VNTR1, VNTR3a, VNTR3b, VNTR4, VNTR5, and VNTR6) was determined as described previously.\(^{16,17}\) After the allele of every VNTR locus was obtained, the MLVA types (MTs) were assigned to the reference database at http://www.mlva.net/. The *B. pertussis* standard strain ATCC 9797 (18323) with known MT was included as a reference strain in each run.

**Pulsed-field gel electrophoresis**

Isolates were analysed according to the standardized recommendations for typing *B. pertussis* using *XbaI* as a restriction enzyme, as previously described.\(^{18}\) Briefly, the PFGE groups were defined as distinct DNA band patterns if they differed by at least one band. The procedures included bacterial embedding, bacterial lysis, cleaning gel blocks, DNA enzymatic digestion using *XbaI* (Qiagen), sample addition and PFGE (Bio-Rad, Hercules, CA, USA) for 18 h, and image acquisition and analysis using a ThermoFisher gel electrophoresis imager. The nomenclature was based on profiles already defined for Finland (BpFINR), Sweden (BpSR) and China (BpCHR). Furthermore, isolates with new profiles were designated as BPX01XA001 and BPX01XA002, respectively, in the series. The *B. pertussis* standard strain ATCC 9797 and *Salmonella* standard strain H9812 were included as reference strains. BioNumerics software, version 7.6 (www.Bionumerics.com) was used for cluster analysis of the electrophoresis band.

**Results**

**Antimicrobial susceptibility testing and mutation site detection**

The E-test results showed that 44 of the 58 isolates (isolate codes 01–27, 29, 32–44, and 46–48) were resistant to erythromycin, azithromycin and clarithromycin (MIC ≥256 mg/L), and two isolates (isolate codes 28 and 45) were resistant to erythromycin and azithromycin (MIC >256 mg/L) but sensitive to clarithromycin (MIC <0.016 mg/L). A total of 12 isolates (isolate codes 30, 31, and 49–58) were sensitive to erythromycin, azithromycin and clarithromycin (MIC ≤0.032 mg/L). The A2047G mutation was detected in 46 macrolide-resistant strains (isolate codes 01–29 and 32–48), while no A2047G mutation was found in the 12 sensitive strains. The MIC50 was >256 mg/L and the MIC90 was >256 mg/L in all macrolide-resistant strains. In addition, all the isolates were sensitive to sulfamethoxazole/trimethoprim (MIC range, 0.002–0.008 mg/L). Results of
antibiotic susceptibility and mutation site detection are summarized in Table 1.

**Table 1.** Antibiotic susceptibility and presence of A2047G 23S rRNA mutation in 58 *Bordetella pertussis* isolates.

| Isolate code | A2047G | Erythromycin | Azithromycin | Clarithromycin | Sulfamethoxazole/trimethoprim |
|--------------|--------|--------------|--------------|----------------|-------------------------------|
| 02–11, 13–15, 17–27, 29, 32–34, 36–37, 39–44, 46, 48 | G      | >256         | >256         | >256          | 0.006                         |
| 12, 16, 35, 47 | G      | >256         | >256         | 128           | 0.006                         |
| 01, 38      | G      | >256         | 128          | 128           | 0.004                         |
| 28, 45      | G      | >256         | >256         | <0.016        | 0.002                         |
| 30–31, 49–55| A      | <0.016       | <0.016       | 0.023         | 0.008                         |
| 56          | A      | <0.016       | 0.032        | 0.023         | 0.004                         |
| 57–58       | A      | <0.016       | 0.032        | 0.023         | 0.004                         |
| MIC range, mg/L |       | <0.016–>256 | <0.016–>256 | <0.016–>256  | 0.002–0.008                   |
| MIC50, mg/L  |        | >256         | >256         | >256          | 0.004                         |
| MIC90, mg/L  |        | >256         | >256         | >256          | 0.006                         |

MIC, minimum inhibitory concentration (evaluated by epsilometer test).

**MAST types of B. pertussis isolates and the relationship with macrolide resistance**

All isolates in the current study harboured the *ptxA1/fim2-1/fim3-1* alleles. Three different *prn* alleles were detected: *prn1, prn2*, and *prn9*; and there were two *ptxP* alleles: *ptxP1* and *ptxP3*. Three antigenic genotypes were found to be present in the isolates according to the different *prn* and *ptxP* alleles: *prn1/prxP1/prxA1/fim3-1/fim2-1, prn9/prxP3/prxA1/fim3-1/fim2-1*, and *prn2/prxP3/prxA1/fim3-1/fim2-1*. The antigenic genotypes of all the macrolide-resistant strains were *prn1/prxP1/prxA1/fim3-1/fim2-1* (46/58 [79.3%]), while the sensitive strains were identified as (*prn9 or prn2/prxP3/prxA1/fim3-1/fim2-1* (12/58 [20.7%]). The results suggested that the antigenic genotypes of macrolide-resistant *B. pertussis* are different from those of macrolide-sensitive *B. pertussis*. The macrolide susceptibility and genotypic distribution of the 58 *B. pertussis* isolates are summarized in Table 2.

**MLVA types of B. pertussis isolates and the relationship with macrolide resistance**

For MLVA, four different MTs were obtained from the 58 strains: MT195 (19 strains [32.8%]), MT104 (14 [24.1%]), MT55 (13 [22.4%]) and MT27 (12 [20.7%]). Of note, MT195, MT104 and MT55 were detected from macrolide-resistant strains, while the 12 susceptible strains were identified as MT27, suggesting that MLVA types of macrolide-resistant *B. pertussis* may be radically different from macrolide-sensitive isolates.

**PFGE profiles of B. pertussis isolates and the relationship with macrolide resistance**

Eight different PFGE profiles (BpFINR9, BpSR23, BpCHR23, BpCHR16, BPX01X001, BPX01XA002, BPX01XA003, and BPX01XA005) were retrieved (Table 2). The two most common PFGE profiles were BpFINR9 (29 strains [50.0%]) and BpSR23 (17 strains [29.3%]), and these
were both obtained from the macrolide-resistant strains. The remaining PFGE profiles were identified in the 12 macrolide-susceptible strains, suggesting that PFGE type may differ between macrolide-resistant and macrolide-sensitive strains of *B. pertussis*. A dendrogram of PFGE profiles and associated molecular characteristics in the 58 *B. pertussis* isolates is shown in Figure 1.

**Discussion**

Erythromycin has been the preferred antibiotic for treatment and prevention of pertussis, but resistance to erythromycin has been reported in many countries, particularly China. Clinically isolated *B. pertussis* has previously been reported as not only resistant to erythromycin, but also to other macrolides, such as azithromycin and clarithromycin. In the present study, resistance rates of the strains were 79.3% (46/58) to erythromycin and azithromycin and 75.9% (44/58) to clarithromycin, indicating a relatively high rate of macrolide-resistance in *B. pertussis* strains in China. Despite no standard procedure for antimicrobial susceptibility testing of *B. pertussis*, the present macrolide resistance rates are considered to be reliable because all macrolide-resistant isolates showed erythromycin MICs ≥128 mg/L. The A2047G 23S rRNA mutation was detected in macrolide-resistant strains, while the susceptible strains were not found to carry the mutation. The present results are consistent with previous research and provide further evidence that the A2047G mutation may be the main mechanism of *B. pertussis* resistance to macrolide antibiotics.

Of note, two of the isolates in the present study (isolate codes 28 and 45) were resistant to erythromycin and azithromycin (MIC >256 mg/L), but sensitive to clarithromycin (MIC <0.016 mg/L). The A2047G mutation is thought to be the main mechanism of *B. pertussis* resistance

| MIC (mg/L) | EM | AZ | CH | A2047G | prn | ptxP | ptxA | fim2 | fim3 | MT | PFGE profile | Frequency (n, %) |
|-----------|----|----|----|--------|-----|------|------|------|------|----|--------------|-----------------|
| >256      | ≥128 | ≥128 | G   | 1 | 1 | 1 | 1 | 1 | 195 | BpFINR9 | 15 (25.9%) |
| >256      | >256 | ≥128 | G   | 1 | 1 | 1 | 1 | 1 | 195 | BpSR23 | 4 (6.9%) |
| >256      | >256 | >256 | G   | 1 | 1 | 1 | 1 | 1 | 104 | BpFINR9 | 2 (3.4%) |
| >256      | ≥128 | ≥128 | G   | 1 | 1 | 1 | 1 | 1 | 104 | BpSR23 | 10 (17.2%) |
| >256      | >256 | ≥128 | G   | 1 | 1 | 1 | 1 | 1 | 55  | BpFINR9 | 11 (19.0%) |
| >256      | >256 | >256 | G   | 1 | 1 | 1 | 1 | 1 | 55  | BpSR23 | 2 (3.4%) |
| >256      | >0.016 | >0.016 | G   | 1 | 1 | 1 | 1 | 1 | 104 | BpFINR9 | 1 (1.7%) |
| >256      | >0.016 | >0.016 | G   | 1 | 1 | 1 | 1 | 1 | 104 | BpSR23 | 1 (1.7%) |
| <0.016    | ≤0.032 | 0.023 | A   | 2 | 3 | 1 | 1 | 1 | 27  | BpCHR23 | 2 (3.4%) |
| <0.016    | <0.016 | 0.023 | A   | 2 | 3 | 1 | 1 | 1 | 27  | BpCHR16 | 1 (1.7%) |
| <0.016    | <0.016 | <0.016 | A   | 9 | 3 | 1 | 1 | 1 | 27  | BPX01XA001 | 2 (3.4%) |
| <0.016    | <0.016 | <0.016 | A   | 9 | 3 | 1 | 1 | 1 | 27  | BPX01XA002 | 1 (1.7%) |
| <0.016    | <0.016 | <0.016 | A   | 9 | 3 | 1 | 1 | 1 | 27  | BPX01XA003 | 3 (5.2%) |
| <0.016    | <0.016 | <0.016 | A   | 9 | 3 | 1 | 1 | 1 | 27  | BPX01XA004 | 3 (5.2%) |

MIC, minimum inhibitory concentration (evaluated by epsilometer test); EM, erythromycin; AZ, azithromycin; CH, clarithromycin; MT, multilocus variable-number tandem-repeat analysis (MLVA) type; PFGE, pulsed-field gel electrophoresis; prn, pertactin; ptxP, pertussis toxin promoter; ptxA, pertussis toxin A; fim2, serotype 2 fimbrial subunit; fim3, serotype 3 fimbrial subunit.
to macrolides, however, the possible mechanism causing differences in resistance among erythromycin, azithromycin and clarithromycin remains unknown. We speculate that the possible mechanism may include the following: (1) In 1991, a study reported a pattern of resistance mediated by an efflux system that resulted in *Streptococcus pneumoniae* being resistant to macrolides but sensitive to clindamycin. The two *B. pertussis* strains in the present study may also have such a resistance pattern, and this warrants further investigation in subsequent experiments; (2) The clindamycin resistance gene ‘*lnu*’ has been found to exist in streptococcus strains, and the nucleotide transferase encoded by this gene can inactivate

Figure 1. Dendrogram of the association between pulsed-field gel electrophoresis (PFGE) profiles and molecular characteristics of 58 *Bordetella pertussis* isolates. All isolates carried the *ptxA1/fim2-1/fim3-1* alleles. The 9797 susceptible isolate is a standard reference strain. Prn, pertactin; ptxP, pertussis toxin promotor; MT, multilocus variable-number tandem-repeat analysis (MLVA) type; ptxA, pertussis toxin A; fim2, serotype 2 fimbrial subunit; fim3, serotype 3 fimbrial subunit.
clindamycin, resulting in streptococcus with erythromycin resistance and clindamycin sensitivity. Whether there is a similar clarithromycin resistance gene in B. pertussis isolates is currently unknown and also requires further study; and (3) The removable methylase genes, *erm* (mainly *ermA* and *ermB*) and efflux genes, *mef* (mainly *mefA* and *mefE*) play an important role in the mechanism of macrolide antibiotic resistance. Comprehensive screening of *erm* and *mef* genes may help uncover the unique resistance mechanism of the two *B. pertussis* strains isolated in the present study. The present authors aim to conduct further in-depth research into these three possible mechanisms to fully characterise the underlying processes involved in macrolide resistance but clarithromycin sensitivity in the two isolated strains.

The strains used for pertussis vaccine production in China include the P3slO strain, the 18323 strain and the CS strain. The P3slO and 18323 strains have been used for whole cell pertussis vaccine (wP) production, while the CS strain has been used for acellular pertussis vaccine (aP). Prior to 2012, both wP and aP were in use, however, since 2012, the aP vaccine has been used exclusively, due to side effects of whole cell vaccines, and all children have been vaccinated with aP. Large-scale pertussis vaccine manufacturers in China include Chengdu Institute of Biological Products (Chengdu), Wuhan Institute of Biological Products (Wuhan) and Yunan Watson Biological Company (Kunming). In the present study, all isolates carried *ptxA1*. However, the sustained rise of whooping cough began in 2013, suggesting that the replacement of *ptxA2* by *ptxA1* may not be related to the rising incidence of pertussis in China.

In the present study, all the *prn1* strains were resistant to macrolides, while *prn9* and *prn2* strains were macrolide-sensitive. In addition, all the *ptxP1* strains were resistant to macrolides, while *ptxP3* strains were susceptible. This may explain why there is no prevalence of macrolide-resistant strains in Europe, as the prevalent strain is *ptxP3*. The results suggest that the acquisition of macrolide resistance in *B. pertussis* may be related to the change of antigen-related genes, especially *ptxP* and *prn* genes. At present, there is no systematic report on the resistance mechanism of *B. pertussis* to macrolides, with the mutation of A to G at site 2047 of the 23S rRNA gene being the only unified understanding of macrolide resistance in *B. pertussis* strains. Fu et al. speculated that the *B. pertussis* strain carrying *prn1*/*ptxP1* has been prevalent in the population for a long time and acquired resistance during the screening of macrolides, while the *ptxP3* strains emerged gradually after the popularization of the vaccine in China, and was sensitive to macrolides due to short exposure time. In addition, antibiotic misuse is known to be widespread in China, and macrolide misuse may provide favourable conditions for the transmission of erythromycin-resistant pertussis strains and may have caused the limited spread of *ptxP3* type strains (prevalent in erythromycin-susceptible strains) in China. In the present study, the *prn1* and *ptxP1* genes always appeared at the same time, accompanied by the A2047G mutation and macrolide resistance. The potential existence of a mechanism in such an association requires further study. Possible resistance mechanisms of pathogens to macrolide antibiotics include active efflux, target change (gene mutation or
methylation) and drug inactivation. Comprehensive screening of the resistance mechanism above will contribute to elucidating the possible cause of macrolide resistance in prn1/ptxP1 isolates.

Four MTs were obtained in the present study: MT195, MT104, MT55 and MT27. The rate of MT27 strain isolation has been increasing continuously in Europe over the past 20 years, and more than 80% of isolates were reported to be MT27 in recent years. However, the rate of MT27 strain detection remains relatively low in China, and a total of 12 such strains (20.7%) were detected in the present study. Substantial heterogeneity of MLVA types has been identified among erythromycin-resistant and erythromycin-sensitive pertussis isolates. In the present study, MT195, MT104, and MT55 were detected from macrolide-resistant strains, while MT27 was identified from susceptible strains. The results corroborate the opinion that the acquisition of macrolide-resistance may be associated with change of MLVA types.

A certain correlation has been shown to exist between the drug-resistance phenotype and the PFGE genotype in Salmonella enterica. In a bioinformatics analysis, macrolide-resistant pertussis isolates were shown to mainly belong to the Va, Vb and Vc sub-lineages below the lineage V. Furthermore, erythromycin-resistant lineages I, II, and III have been reported, which were similar to the sub-lineages Va, Vb, and Vc above. The present results were consistent with those of the above studies: PFGE profiles BpFINR9 and BpSR23 were obtained from macrolide-resistant pertussis strains, while susceptible strains were identified as having different profiles (Figure 1). The results suggest that the high macrolide resistance rate may due to the horizontal spread of resistant clones in China.

The high macrolide resistance rate in the present study suggests that antibiotic resistance in pertussis strains should be carefully monitored. In addition, the MICs of macrolides to ptxP1 strains were significantly higher than those to ptxP3 strains, and the MICs to other MT strains were also higher than to MT27 strains, suggesting that the molecular characteristics of pertussis strains should also be noted. All macrolide-resistant strains in the present study carried the A2017G mutation, the ptxP1 and prn1 alleles, the MLVA types MT195, MT55 and MT104, and the PFGE profiles were classified into BpSR23 and BpFINR9 types. However, no A2017G mutations were found in any of the macrolide-sensitive strains, whose genotypes were (prn9 or prn2)/ptxP1/MT27, and PFGE profiles were different from the macrolide-resistant strains. These results suggest that the acquisition of macrolide resistance may be associated with changes in specific molecular characteristics.

In conclusion, as outbreaks and drug resistance of B. pertussis have been reported in many parts of the world, there have been numerous in-depth studies on the genomic diversity of pertussis strains, however, there are few published reports regarding B. pertussis in China. Strains in the present study were collected mainly from the western region of China, including Shaanxi, Gansu and Qinghai. The data published here highlight potential inadequacies of B. pertussis vaccination, and B. pertussis genomic diversity, in western China, and provide a reference value for the importance of improving the pertussis vaccine in China. However, the present results may be limited by the relatively small number of strains investigated, and future studies should include an expanded range and volume of sampling to verify the conclusions. In addition, comprehensive screening for possible mechanisms of macrolide resistance, such as dynamic efflux, target change and drug inactivation, should be conducted to further clarify the connection between...
the acquisition of macrolide resistance in B. pertussis and change of genotypes.

Author contributions
ZJS wrote the manuscript; ZDQ revised the manuscript; ZJS, ZDQ, WXQ, LH, and WXG collected the data; and WXQ, LH, and WXG analysed the data. All authors read and approved the final manuscript.

Declaration of conflicting Interest
The Authors declare that there is no conflict of interest.

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