

MLST Application in Analysis of Relationship between Group B Streptococcus Resistance Gene and Induction Resistance in Shanghai, China

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Research

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Abstract

Background

With the gradual severe bacterial resistance and slow development of antibiotics, drug-resistant bacterial strains are widely distributed and have become a serious public health problem. Group B Streptococcus (GBS) which cause Group B strep-related disease is the major cause of severe infection in newborns. However, Clindamycin resistance of GBS induced by Erythromycin is emerging and become important clinical concerns today.

Methods

A retrospective study was conducted on the drug resistance analysis of GBS strains isolated from Obstetrics and Gynecology Hospital from Jan 2016 to Dec 2017. The clinical and microbiological data including patient demographics, antimicrobial susceptibility testing, relative distribution drug resistance-associated genes *mefA* & *ermB* to Erythromycin, and multilocus sequence typing (MLST typing) were collected and analyzed. The Kirby-Bauer and VITEK2-compact were used to perform the susceptibility testing. The double disk diffusion method (D-test) was used for the detection of inducible clindamycin resistance. MLST was employed to identify sequence types of these strains. Polymerase Chain Reaction (PCR) was conducted to detect the drug resistance genes *mefA* & *ermB* to Erythromycin.

Results

A total of 1021 strains were cultured and isolated from 31894 specimens. Erythromycin and clindamycin resistance was 53.6% (547/1021) and 50.1 % (512/1021), respectively, in which 74.4% (407/547) had harbored constitutive macrolide, lincosamide and streptogramin B resistance (cMLS B), 45.0% (63/140) were inducible MLS B (iMLS B). Additionally, MLST identified 12 different ST types including a new ST type ST1072 in 63 iMLS B GBS strains and the dominant STs were ST12 (30.1%) and ST19 (25.4%). The resistance ratio of ST19 to Levofloxacin (75.0%) was higher than that of other ST types. The relevance resistance ratio of *mefA* and *ermB* was respectively 27.0% and 41.3% among 63 GBS isolates.

Conclusion

Our study not only demonstrated a genetic diversity in iMLS B GBS in Shanghai through the analysis of MLST typing and resistance genes, but also found that there exist different distribution patterns of resistance and related resistance genes between different ST types. These findings would provide theoretical support for clinical prevention and treatment of resistant iMLS B GBS infection.

1 Introduction

Streptococcus agalactiae (Group B streptococcus; GBS) is the main cause of early-onset neonatal sepsis and meningitis, and is also an important cause of severe invasive infections in pregnant women, immunocompromised adults and the elderly around the world [1, 2]. Importantly, GBS disease was also

associated with significant morbidity and mortality, especially are the leading cause of life-threatening neonatal bacterial infections in developed countries [3, 4]. Since then, the researches about GBS received extensive attention, and it was still under investigation as well in epidemiology aspects as in genetic background [5]. US Center for Disease Control and Prevention(CDC)respectively published and revised GBS prevention & treatment guidance in 1996, 2002 and 2010 [6–8]. In China, the GBS colonization rate of pregnant women were various in different regions, which was 8.2% in Guangdong, 4.2%-5.2% in Nanjing, 6.7% in Beijing and 9.0%-10.0% in Zhejiang Province and Shanghai [9–11].

In recent years, with GBS prenatal screening and intrapartum anti-GBS therapy of pregnant woman, especially in some developed regions, there has been a sharp decline in the proportion of neonatal GBS-induced bloodstream infections or serious complications in China[10, 12]. However, due to the prevalence of multi-drug resistance of GBS, the resistance rate of erythromycin and clindamycin in GBS increased seriously [13, 14]. Inducible type of MLS_B resistance (iMLS_B) and cMLS_B phenotypes were distinguished by D-test as detailed by the Clinical & Laboratory Standards Institute (CLSI)[15]. According to the statistics collected from Grade III hospitals published by Chinese Antimicrobial Resistance Surveillance System in 2017, the drug resistance rate of GBS to Erythromycin and Clindamycin was over 60% and 50% respectively, which seriously affected the therapeutic effect in special groups who suffered from GBS infection. There are plenty of resistance gene types relating to GBS macrolide antibiotic resistance. Studies showed that the erythromycin ribosomal methylase encoded with ermB and macrolide antibiotic resistance induced by efflux pump encoded by mefA were popular in Asia [10].

In addition to the severe situation of drug resistance, GBS infection is bound to become a substantial risk to the obstetric diseases associated to pregnant women and newborn children [16]. Additionally, there were significant differences in drug resistance, molecular and serotype of GBS between diseases categories, specimen types or regions [17, 18]. MLST of GBS isolates from different countries indicated that only limited numbers including ST1, ST10, ST17, ST19 and ST23 were associated with colonizing or invasive isolates [19]. Among these STs, ST17 is a hypervirulent clone, mainly associated with invasive diseases in newborns, whereas ST19 can cause invasive diseases in both newborns and adults [20, 21]. According to MLST analysis, the majority of the invasive GBS diseases in infants from urban areas in southern China belong to the genotype ST17 or ST17-like genotype[22]. cMLS_B resistance (46%) mediated by the ermB gene was significantly associated with the Guelma isolates belonged to ST23 in France [23]. The molecular epidemiological study of GBS in Ireland showed that the isolates could be divided into 5 main clonal cluster (CC), among which CC1, CC17 and CC23 accounted for 67.2%[24]. In Iceland, the decline of serotype III was reflected in a decrease of clonal complexes CC17 and CC19 that included most serotype III isolates. On the other hand, the increase in frequency of CC1 was caused by ST1 and ST196[25].

The study on multiple-resistant isolates can provide guidance in the case of treatment failure to complication induced by early-onset GBS infection through utilizing prophylactic antibiotic [26, 27]. To date, the research of iMLS_B GBS, which was of great significance for the prevention of infectious diseases and the treatment of severe complications to child-bearing age women and newborn babies, is still limited

in China. Moreover, the analysis of GBS resistance also showed a great necessity to the treatment for recurrent episodes of gynecologic inflammation and spontaneous abortion [28, 29]. As one of famous gynecologic & obstetrics hospitals in Asia and the world, Obstetrics and Gynecology Hospital affiliated to Fudan University has a larger proportion of high-risk pregnant women all over the country. In this study, the clinical features, drug resistance, drug resistance gene and MLST of iMLS_B GBS were analyzed, which would provide basic data for clinical prevention and treatment of GBS resistance.

2 Methods

2.1 Bacterial isolates and culture

1021 strains of GBS were collected from 31894 specimens from patients hospitalized in Obstetrics and Gynecology Hospital of Fudan University from Jan, 2016 to Dec, 2017. All endocervical swab samples were originated from patients who had the clinical manifestation of urogenital tract inflammation. After gathering specimen of cervical secretion, and eliminating trichomonas and candida infection by routine examination, all isolates were cultured for GBS on blood agar plates with 5% sheep blood 24 hours at 37 °C. For neonatal patients, the isolates were originated from the blood culture specimen. The isolated colonies after 24 hours culture was identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics Inc, Massachusetts, USA). In our study, all the strains' information can be timely identified during or after data collection. This study was performed in accordance with human subject protocols approved by the Ethics Committee (Ethics Approval Number: KYY-2016-22) of Obstetrics and Gynecology Hospital of Fudan University.

2.2 Antimicrobial susceptibility testing and determination of Minimum Inhibitory Concentration

For the antimicrobial susceptibility characterization, VITEK2-compact full-automatic bacterial susceptibility instrument and relevant bacterial susceptibility identification cards (bioMérieux Corporate in France) were used for detection and antibiotic susceptibility testing[30]. *Streptococcus pneumoniae* ATCC49619 was used as the reference strain. Inducible type of MLS_B resistance (iMLS_B) and cMLS_B phenotypes were distinguished by D-test with Mueller-Hinton agar with 5% of sheep blood (Shanghai Kehua Biological Co., Ltd., China) as detailed by CLSI. The susceptibility results were interpreted and analyzed based on M100-S27 document of CLSI breakpoints.

2.3 Multilocus Sequence Typing

Furthermore, for serotype and genotype determination, all GBS positive cultures were genetically characterized by MLST as described previously[31]. Briefly, the bacterial isolates were grown in their preferred medium and pelleted. 7 housekeeping genes of *adhP*, *pheS*, *atr*, *glnA*, *sdhA*, *glck*, *tkt* in GBS were amplified by PCR [32]. The amplification system and sequence primers were determined by MLST (<http://www.mlst.net/>). The total volume of reaction system was 25 μL, including 17 μL of ddH₂O, 2.5 μL of 10 × PCR buffer, 0.5 μL of dNTP, 0.25 μL of Taq DNA Polymerase, 1.5 μL of magnesium ion (25 mM), 1

μL for upstream primer and downstream primer each and 1.25 μL of template DNA. The loop condition: pre-denaturation at 94°C for 5 min, denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec, extension at 72°C for 1 min, and 30 cycles in total. The amplification product was analyzed by 1% of agarose gel electrophoresis. DNA extraction kit was purchased from Sangon Biotech (Shanghai) Co., Ltd. and the sequencing was performed by Beijing Genomics Institute (BGI, Beijing) Co., Ltd. The results were compared with the MLST databases online (<http://pubmlst.org/sagalactiae/>) to determine genotypes and ST types.

2.4 Determination of antimicrobial resistance gene markers by PCR

To investigate the drug resistance associated genes, PCR was carried out to detect the drug resistance genes *mefA* and *ermB* to GBS strains (positive to D-inhibition zone trial). The primer sets of the drug resistance locus were as follows: (*mefA*-f: AGTATCATTAACTACTAGTGC, *mefA*-r: TTCTTCTGGTACTAAAAGTGC); (*ermB*-f: GAAAAGGTACTCAACCAAATA, *ermB*-r: AGTAACGGTACTTAAATTGTTTAC). PCR reaction system and amplification analysis of the were conducted as described previously[33].

2.5 Statistical Analysis

The results of antimicrobial susceptibility testing were analyzed by WHONET5.6 statistical software. BioNumerics 7.5(Applied Maths, Belgium)analysis was conducted to investigate the affinity of these isolates. Minimal Spanning Trees is a subset of the edges of a connected, edge-weighted directed graph that connects all the vertices together. Data were presented as mean values with SD or median with interquartile range when appropriate. Statistical significance was defined at a P value of < 0.05.

3 Results

3.1 Patient demographic characteristics

In total, 31894 patients were investigated from Obstetrics and Gynecology Hospital affiliated to Fudan University between Jan, 2016 and Dec, 2017. 1021 strains of GBS were compiled in Table 1 (Positive detection rate 3.20%), including 717 strains isolated from female genitourinary tract in gynecology, 286 strains in obstetrics and 18 strains from blood cultures specimens isolated from newborn baby within one week postpartum in neonatology. The GBS relevance ratios of cervical secretions & uterine cavity contents in Obstetrics (8.82%/2.14%) are obviously higher than those of in Gynecology (0.67%/0.67%). The detection rate of GBS in obstetric blood culture (0.54%) was also significantly higher than that in gynecological blood culture (0.08%). The detection rate among different ages and samples in Gynecology & Obstetrics departments showed the statistical significance($P \leq 0.01$). The maximum number of GBS participants GBS detection came from the age group 21–40 (25333/31849, 79.54%). The highest rate of GBS detection came from the group with age 41–60 (3.15%).

Table 1

Detection Rate of Streptococcus Agalactiae in Genitourinary Tract among Different Ages in Gynecology and Obstetrics

Age	Totals	Gynecology			Obstetrics		
		Cases	Strains	Detection Rate(%)	Cases	Strains	Detection Rate(%)
≤ 20	1011	746	15	2.01	265	5	1.89
21–40	25333	15017	434	2.89	10316	261	2.53
41–60	4901	4140	170	4.11	761	24	3.15
≥ 61	649	649	18	2.77	0	0	0.00
Total	31894	20552	637	3.10	11342	266	2.35

3.2 Antimicrobial susceptibility testing

Antimicrobial resistance profiles of isolates were shown in Table 2. The drug resistance rate and MIC values of GBS to 7 commonly used antimicrobial agents were tested and profiled. None of the 1021 strains were resistant to penicillin, vancomycin or linezolid. Resistance rates to Erythromycin, Clindamycin, Levofloxacin and Nitrofurantoin was 53.6%, 50.1%, 34.7% and 7.5%, and intermediate rate of them was 16.9%, 6.8%, 5.1% and 5.4% respectively. Among 1021 strains, 547 isolates were resistant to Erythromycin, 140 isolates susceptible or intermediate to Clindamycin. Further, 63 positive isolates through D-test were also found (positive rate: 45.0%).

Table 2

Susceptibility result of 1021 strains for seven types of antibiotics

Antimicrobial Agents	Susceptible Rate(%)	Intermediate Rate(%)	Resistant Rate(%)	MIC50(mg/L)	MIC90 (mg/L)	MIC Scope(mg/L)
Penicillin	100	0	0	0.125	0.125	0.12–0.12
Erythromycin	29.7	16.7	53.6	1	8	0.25-10
Clindamycin	43.0	6.8	50.2	0.25	8	0.25-8
Levofloxacin	60.2	5.1	34.7	1	8	0.25-8
Nitrofurantoin	87.1	5.4	7.5	16	16	16–256
Vancomycin	100	0	0	0.5	0.5	0.5–0.5
Linezolid	100	0	0	2	2	0.5-2

For 63 D-test positive strains in Table 3, the drug resistance rate and relevant MIC values indicated that no isolate was resistant to Penicillin, Vancomycin, Linezolid and Nitrofurantoin, but showed resistance rate to Levofloxacin was 27.0%, sensitive to other antibiotics, and intermediate rate to Clindamycin, Levofloxacin

and Nitrofurantoin was 11.1%, 4.8% and 9.5%, respectively. The iMLS_B GBS resistance rate of Levofloxacin (27.0%) and Nitrofurantoin (0%) was generally lower than the average value of all GBS isolates (Levofloxacin 34.7%, Nitrofurantoin 7.5%). MIC50 and MIC90 of Penicillin, Vancomycin, Linezolid and Levofloxacin was unchangeable compared with that of all GBS isolates, except MIC90 of Clindamycin decreased from 8 mg/L to 0.25 mg/L and Nitrofurantoin decreased from 16 mg/L to 256 mg/L.

Table 3

Susceptibility result of 63 positive GBS isolates through D-inhibition zone trial for seven types of antibiotics

Antimicrobial Agents	Susceptible Rate(%)	Intermediate Rate(%)	Resistant Rate(%)	MIC50(mg/L)	MIC90 (mg/L)	MIC Scope(mg/L)
Penicillin	100	0	0	0.125	0.125	0.12–0.12
Erythromycin	0	0	100	8	8	8–8
Clindamycin	88.9	11.1	0	0.25	0.25	0.25-1
Levofloxacin	68.2	4.8	27.0	1	8	0.25-8
Nitrofurantoin	90.5	9.5	0	16	16	16–64
Vancomycin	100	0	0	0.5	0.5	0.5–0.5
Linezolid	100	0	0	2	2	0.5-2

3.3 Multilocus Sequence Typing Analysis

All 63 iMLS_B GBS strains were typed by MLST (in Fig. 1). MLST analysis identified 12 STs and showed high degrees of genetic diversity. Among these STs, the most frequently detected was ST12 (20/63, 31.75%), followed by ST19 (16/6, 325.40%), ST27 (7/63, 11.11%), ST17 (5/63, 7.96%) and other ST strains, etc. A new ST type (ST1072) (adhp16,phes1atr4glnA2sdhA2glck3tk2) was found and uploaded onto MLST database (<http://www.mlst.net/>) in this work. Based on BioNumerics 7.5 (Applied Maths, Belgium) analysis, 29 isolates (46.03%) were confirmed belonged to the main clonal cluster (CC) 19, which contained 16 ST19 strains, 7 ST 27, 3 ST335, 2 ST28 and 1 ST885. 26 isolates (41.27%) originated from CC10 containing 20 ST12, 3 ST 3 strains, 2 ST10 and 1 ST4 strain. Few of them (8/63, 12.70%) were from other ST types with diffused distribution. Minimal Spanning Trees for these 63 GBS strains was conducted to analyze the evolutionary relationships. We found that genotypes ST27, ST28 and ST335 differ from ST19 in only one allele, showing a relatively close relationship. ST885 differs from ST19 in two alleles, and the other genotypes are far from ST19, while all other isolates exhibited distant genetic relationships.

3.4 Resistance patterns against various antimicrobial agents

For the resistance patterns of these 63 iMLSB GBS strains, none of the 63 iMLSB GBS were resistant to penicillin, Vancomycin, Linezolid, Clindamycin and Nitrofurantoin. Intermediate rate of iMLSB_B GBS to Nitrofurantoin was 9.5%. As shown in Fig. 2, among these 63 iMLSB_B GBS, 7 strains were intermediate (11.11%) and 56 strains susceptible (88.89%) to Clindamycin, 17 strains were resistant (26.98%), 3 strains were intermediate (4.76%) and others were susceptible to Levofloxacin. ST3 and ST4 were susceptible to Levofloxacin and Cefotaxime; ST10 was resistant (66.67%) to Levofloxacin; ST17 was susceptible to Levofloxacin as well. ST12 was susceptible to Levofloxacin and ST19 was resistant (75.0%) to Levofloxacin, which was obviously higher than that of other types (10.62%). In collection, statistical data obtained in this study was higher than the average level of whole strains (34.71%) and 63 strains (27.0%) resistant to Levofloxacin.

3.5 The correlation between MLST typing Analysis and drug resistance gene *mefA* and *ermB*

MefA and/or *ermB* gene are recognized as drug-resistant related genes for iMLSB GBS strains. Here, in addition to studying the relationship between ST typing and drug-resistant phenotypes, we investigated the correlation between STs and drug resistance gene, *mefA* and/or *ermB* genes of the 63 iMLSB_B GBS. As shown in Table 4, 17 strains of 63 GBS isolates harbour resistance gene *mefA*, 26 strains with resistance gene *ermB* and only 4 strains contained the both. There are 20 GBS isolates with ST12 and 19 strains of them were detected to contain resistance gene *ermB*. 16 strains of ST19 didn't contain *ermB*.

Table 4

Correlation between MLST typing and drug resistance 63 positive GBS isolates through double disk diffusion method (D test)

Gene Type		mefA (+) ermB (+)	mefA (+) ermB (-)	mefA(-) ermB(+)	mefA (-) ermB(-)	Totals(%)
CC10(26/41.27)	ST3	0	0	1	2	3(4.76)
	ST4	0	0	0	1	1(1.59)
	ST10	0	0	0	2	2(3.17)
	ST12	2	0	17	1	20(31.75)
CC19(29/46.03)	ST19	0	8	0	8	16(25.40)
	ST27	0	1	2	4	7(11.11)
	ST28	0	2	0	0	2(3.17)
	ST335	0	1	0	2	3(4.76)
	ST885	0	0	0	1	1(1.59)
Others(8/12.70)	ST17	2	0	2	1	5(7.94)
	ST23	0	1	0	1	2(3.17)
	ST1072	0	0	0	1	1(1.59)
Totals(%)		4(6.3)	13(20.6)	22(34.9)	24(38.1)	63(100.0)

4 Discussion

The U.S. CDC revised GBS prevention and cure guideline in 2010 and advised to collect vaginal and rectal swab from all pregnant women with 35–37 weeks' gestation for GBS screening[6]. By screening for GBS colonization during childbirth and using antibiotics prophylactic medication for pregnant women in labor with GBS colonization, severe complications caused by early-onset neonatal GBS were greatly decreased [6, 34–36]. In recent years, GBS screening has been carried out in China, especially in developed regions such as Beijing and Shanghai where GBS screening policies are widely implemented. Notably, it was the big limitations on antibiotic range selected for infection prevention to those women who are high-risk and susceptible to Penicillin due to increased resistance to iMLSB GBS. CDC recommends using Vancomycin for such population, however, Vancomycin has a potential damage to animal embryos. GBS vaccines against non-pregnant adults can effectively prevent bacteremia, however, such research has not been applied to clinical treatment in China[37]. Besides, as is known to all, China is a developing country with large population base. Now the two-child policy has been gradually liberalized and plenty of child-bearing age women are facing with the possibility of another pregnancy[38]. Therefore, the drug resistance, MLST

typing and drug resistance gene analysis of iMLSB GBS are of great significance in clinical diseases such as urogenital tract inflammation and unexplained abortion in Chinese women of reproductive age.

In this study, 1021 isolates collected from 31894 patients were received systematic analysis, of which 8.82% of GBS detection was found in Obstetrics of cervix secretion higher than the detection rate of other sample types, so in Obstetrics patients, GBS monitoring is extremely important and necessary in our country. The detection rate of 1021 strains of GBS among different ages in Gynecology & Obstetrics departments showed statistical significance ($P \leq 0.01$). Among the patients with obvious local clinical symptoms in this study, the detection rate of gynaecological GBS was 3.49% and that of obstetrical GBS was 2.68%, which was much lower than that of healthy women. The maximum number of GBS detection came from the group with Cervix secretion and Age 21–40. The highest rate of GBS detection came from the group with Cervix secretion and Age 41–60. This might be related to the features of GBS, the change of sex hormone levels and the decrease of immunity in these groups. With the China's family planning policy opening, the increase of parturient women over Age 40 also may brought the growth of GBS detection in Obstetrics for Age 41–60 group compared with those in previous years.

For the resistance patterns of GBS in this study, none of the 1021 strains were resistant to Penicillin, Vancomycin or Linezolid, which was similar to the most of the other countries and regions[8, 39]. By statistically analyzing the resistance patterns of strains in this study, among 493 strains resistant to Erythromycin, 84% of them were resistant to Clindamycin and this was greater than the percentage in Italy (53%). The resistance to Erythromycin, Clindamycin and Levofloxacin conformed to bacterial resistance surveillance data published by Shanghai [10]. The drug resistance rate of Erythromycin was higher than the reported data in Italy (15%), Brazil (19.3%) [40], the United States (38%-41.9%) [41], France (35.3%) [42], and South Korea (51.8%) [43]etc. The resistance to Clindamycin was also larger than the values of most states & regions, for example, Brazil (13.3%) and Africa (17.2%) [40, 44]. However, it was a bit lower than the rate in South Korea (55.4%) and this may attribute to its social structure & medical level [43]. There were 140 isolates susceptible or intermediate to Clindamycin, including 63 iMLSB_B GBS and positive rate of D-test reached 45.0%, which was greatly improved by comparing with the positive rate (21.7%) through D-test during 2009–2012 reported in Shanghai [45]. It was much higher than the rate in Brazil (20%) and Africa (17.4%) [44, 46]. Understanding the distribution of GBS infection and drug resistance is of great significance for guiding rational drug use in clinical practice, especially for the rational drug use of iMLSB GBS strain. Therefore, it is imperative to strengthen the monitoring of GBS resistance.

In this study, largest number of iMLSB GBS isolates was CC19 and the maximum relating to inflammation of pregnancy women in Obstetrics was CC19. The highest incidence of cervical inflammation in Gynecology was CC19 and adverse pregnancy outcomes in Birth Control Department was ST12. In other states and regions, CC19, CC1, CC10 and CC17 were the most in Romania from Eastern Europe and CC23, CC19 and CC17 were mainly detected in Poland from Central Europe [47, 48]. Like Iran, the most common clonal complexes were CC19 and CC10 [39, 49]. CC17 was the main GBS strains to induce invasive diseases of newborn babies in Beijing, China [11]. At the same time, CC17 was predominant in GBS neonatal infections in France [42, 50]. CC17 strains cause both neonatal and adult invasive infections

which cluster tightly in a phylogenetic tree, signifying that they are derived from the same genetic pool in Canada [51]. The research to capsular polysaccharide on virulent strain's surface had important implications for the development of GBS vaccine [52].

Multiple resistance isolates severely threaten the public health due to the pressure selection of antibiotics. Among 63 iMLSB GBS isolates, the drug resistance rate of ST19 to Levofloxacin (75.0%) was higher than that of other types, while ST12 was susceptible to Levofloxacin. Similarly, the predominant genotype of the levofloxacin-resistant isolates was ST19 in Taiwan [53]. This rate was close to that of ST19 from non-genitourinary tract specimens reported in Shanghai (76.9%). and lower than III/ ST19 resistance to Levofloxacin in Beijing (> 90%) [54]. What's different is that ST19 was reported as the isolates with lower susceptibility to Penicillin in Japan [55]. Those strains not susceptible to Penicillin were usually resistant to macrolides and fluoroquinolone antibiotics. The drug resistance rate of CC10 to Levofloxacin (7.69%) was obviously lower than Korean [56]. We found the distinct gaps and genetic diversity exist in the drug resistance and relevant resistance genes with different ST types. These data suggested that the epidemiological investigation of GBS MLST in Shanghai has important clinical reference.

Resistance to erythromycin in neonatal invasive GBS has been reported worldwide, and the resistance to erythromycin from GBS strains was mostly mediated by *ermB*, *ermA* and *mef*. In the 63 erythromycin resistant strains, 26.98% of the strains contained the *mefA* gene, and 41.27% of the strains contained the *mefB* resistance gene, while only 6.34% shared the *mefA* and *mefB* genes together. This was close to *ermB* relevance ratio for Erythromycin resistant GBS strains in Suzhou (47.1%), and similar to the result of the relevance ratio of *mefA* in Beijing (27%), while the rate of *ermB* in Beijing (94.6%). In Africa, the relevance ratio of *mefA* was merely 3.4% but *ermB* was 55%. In South Korea, there was low relevance ratio of *mefA* (3.4%) and high rate of *ermB* (82.8%). A high ratio of *ermB* (83.1%) also existed in strains not susceptible to Erythromycin in Taiwan. In Portugal, the relevance ratio of *ermB* was 25.9% in GBS resistant to Erythromycin. According to the statistics reported by Federal University of Brazil, no *mefA* was found and only few *ermB* (27.8%) were detected in GBS resistant to Erythromycin .

The relevance ratio of *mefA* and *ermB* was 27.0% and 41.3% respectively in this study and only 4 strains included the both genotypes, which was close to *ermB* relevance ratio (47.1%) and largely different to *mefA* relevance ratio (0%) for Erythromycin resistant GBS strains separated from urine specimen in Suzhou, China[57, 58]. In general, Erythromycin resistance induced by efflux pump was negative in D-test. However, 27% of induced resistant strains included *mefA* and was mostly concentrated on CC19 in this research. These isolates might spontaneously mutate from induced resistance to structural resistance and continue to show high resistance to Erythromycin. Treatment with clindamycin may induce antibiotic strain resistance, even leading to treatment failure. Therefore, the further study of CC19 spontaneously-mutated isolates could be regarded as the important supplement to GBS resistance system and gave the guidance on rational use of antibiotics for clinicians to avoid the occurrence of drug resistance.

In general, Erythromycin resistance induced by efflux pump was negative in D-test. However, induced resistant strains included *mefA* and *ermB* were mostly concentrated on CC19 in this research. Such

isolates might spontaneously mutate from induced resistance into structural resistance during the course of treatment and continually expressed high resistance to Erythromycin. Previous research indicated that resistance induced by Clindamycin was almost caused by ribosomal RNA methylation modification[59]. By detecting gene *ermB* related to ribosome methylation commonly found in China, 18 strains of 19 GBS isolates with ST12 were detected to contain *ermB* and 16 strains with ST19 cannot be verified to contain *ermB*. 58.7% of such isolates did not include *ermB* and most of them still concentrated on CC19 in this study. In additions, only 3 of 7 alleles were different and there was close affinity between the new genotype ST1072 and ST19. Therefore, the further study of CC19 spontaneously-mutated isolates could be regarded as the important supplement to GBS resistance system and gave the guidance on rational use of antibiotics for clinicians to avoid the occurrence of drug resistance. So it should explore more methods and researches about CC19 gene group of induced resistant isolates in local region. It may provide a key piece of information about the GBS resistance system study in China.

5 Conclusion

Our study systematically explored the distribution, patient cohort, the STs, antimicrobial susceptibility phenotype and resistance genes distribution of GBS strains detected from patients hospitalized in Obstetrics and Gynecology Hospital of Fudan University. Patient demographic characteristics showed that the maximum number of GBS participants GBS detection came from the age group of 21–40. Resistance patterns presented that the situation of drug resistance is serious. Regional differences were apparently reflected into the distribution of Erythromycin resistance genes. The relevance ratio of *mefA* and *ermB* in China was obviously larger than those in other states and regions. MLST analysis identified 12 STs and showed high degrees of genetic diversity, and ST12 was the most frequently detected. The correlation between MLST typing analysis and drug resistance gene distribution showed that ST12 has a certain correlation with the resistance gene *ermB*.

Declarations

Ethics approval and consent to participate

The present study conformed to the principles of the Declaration of Helsinki. Approval was obtained from the Research Ethics Committee of the Obstetrics & Gynecology Hospital of Fudan University (approval number: Kyy2016-22), and written consent was obtained from all participants in this study.

Consent for publication

Consent to publish is obtained from all the individuals whose data contained in this manuscript.

Availability of data and material

The data and material presented in the article are available and is applicable for sharing.

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Authors' contributions

Jing Gao composed the manuscript and analyzed the data. Yisheng Chen, Yiqian Peng, Nanyan Jiang and Ying Zhang provided the samples and collected the clinical data. Chunmei Ying designed and coordinated the study. All the authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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Figures

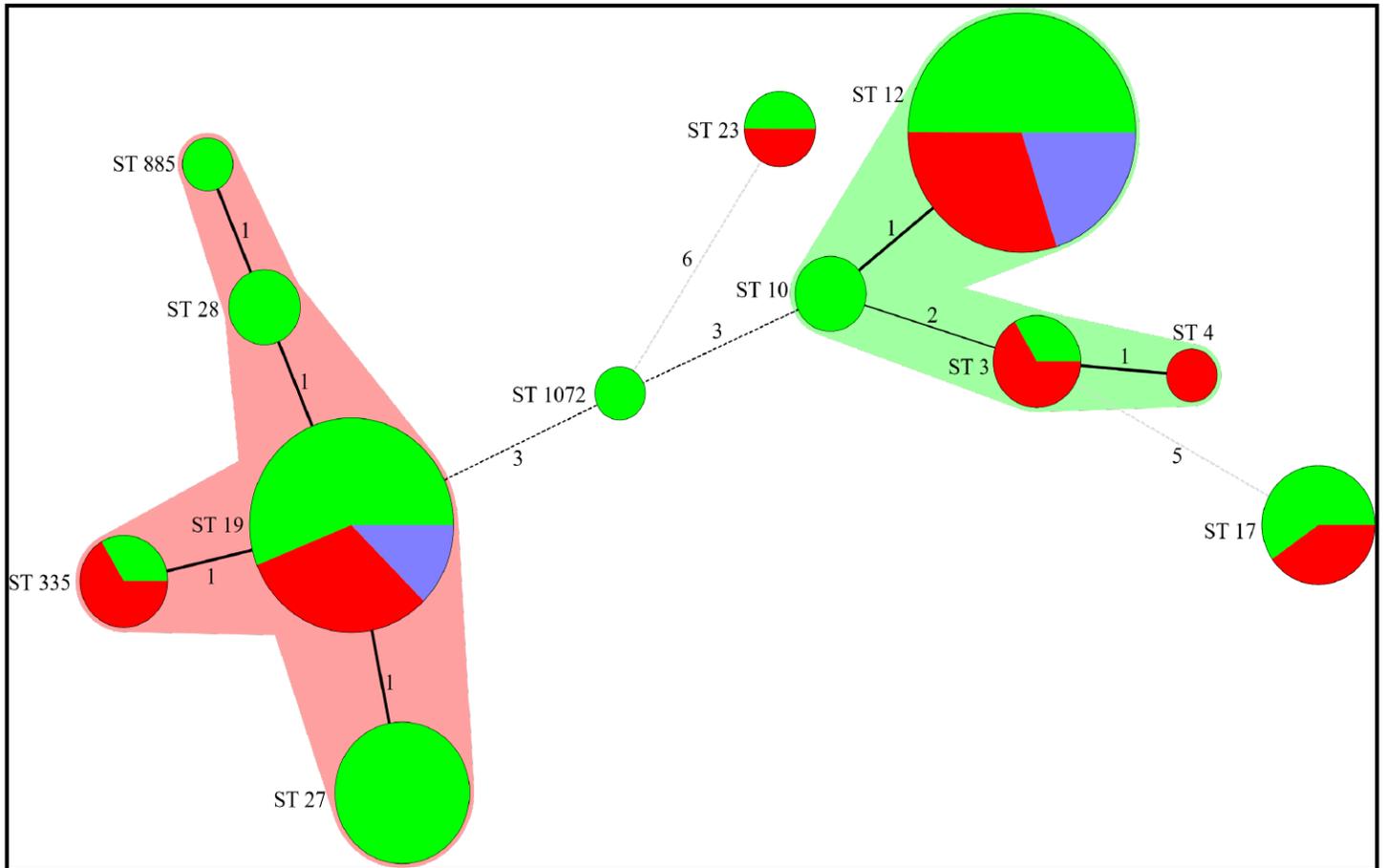


Figure 2

Minimal Spanning Trees of GBS strains in different STs. The values on the lines among different ST types indicate different numbers of 7 alleles; the line length shows the far or close affinity; the circle size expresses the strain quantity of corresponding ST type; various colors represent different clinical departments, green is Gynecology, red is Obstetrics and purple is Family Planning.

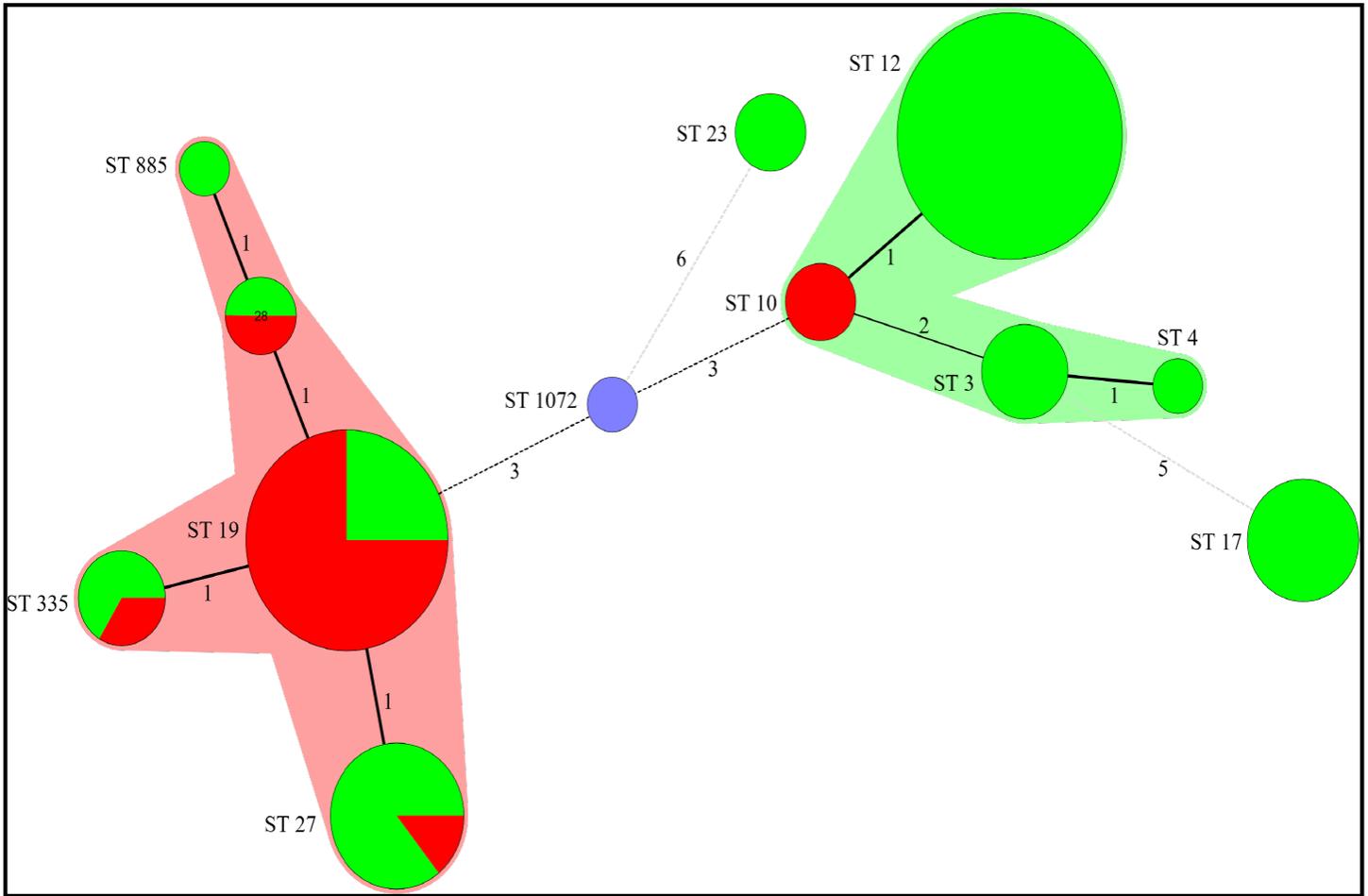
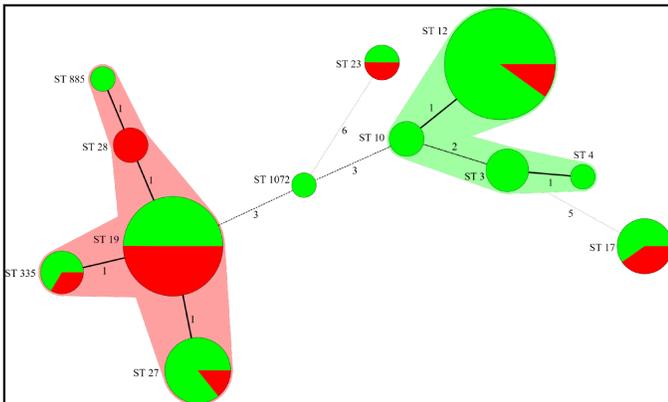


Figure 4

63 GBS strains showed significant differences in resistance phenotypes for Levofloxacin. The values on the lines among different ST types indicate different numbers of 7 alleles; the line length shows the far or close affinity; the circle size expresses the strain quantity of corresponding ST type; various colors represent the susceptibility gap of Levofloxacin, Green is Susceptibility, Purple is Intermediacy and Red is Resistance.

A



B

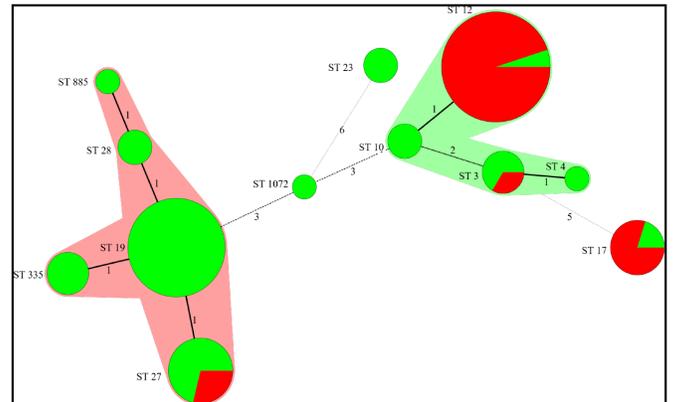


Figure 6

63 GBS strains showed significant differences in resistance genotypes. The values on the lines among different ST types indicate different numbers of 7 alleles; the line length shows the far or close affinity; the circle size expresses the strain quantity of corresponding ST type; Red is positive and Green is non-detected. A, For gene *mefA*, B, For gene *ermB*.