Changing Epidemiology of Group B *Streptococcus* Susceptibility to Fluoroquinolones and Aminoglycosides in France

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Group B *Streptococcus* (GBS) is the leading cause of neonatal invasive infections and an emerging pathogen in the elderly. Our objectives were to describe the evolution of GBS resistance to antibiotics in France and to investigate the emergence of fluoroquinolone (FQ)-resistant isolates. A total of 8,757 unrelated GBS isolates were collected and tested for antibiotic susceptibility from 2007 to 2014 according to EUCAST recommendations. All isolates were susceptible to penicillin G, amoxicillin, and vancomycin. Resistance to macrolides decreased from 47.0% to 30.0%, whereas high-level resistance to aminoglycosides, especially amikacin, increased from 6.4% to 8.8% and 24 isolates (0.3%) were highly resistant to gentamicin. FQ resistance gradually increased from 0.2% in 2007 ($n = 1$) to 1.5% in 2014 ($n = 18$, $P < 0.01$). Capsular polysaccharide (CPS) genotyping, multilocus sequence typing, and sequencing of the quinolone resistance-determining region (QRDR) showed that GBS isolates of sequence type 19 (ST-19) CPS type V were largely overrepresented in FQ-resistant isolates ($n = 30$, 45.5%). All 30 strains displayed the same QRDR mutations and were often associated with cross-resistance to macrolides (93.3%) and gentamicin (30%). In conclusion, we report the rise of FQ- and aminoglycoside-resistant GBS in France over an 8-year study period, an evolution likely linked to the clonal expansion of ST-19 CPS V-resistant isolates. This study emphasizes the need for a continuous surveillance of GBS epidemiology and antibiotic susceptibility.

Group B *Streptococcus* (*Streptococcus agalactiae*; GBS) is a Gram-positive encapsulated bacterium colonizing the intestinal and vaginal tracts of 10 to 30% of healthy adults. This commensal bacterium is also the leading cause of neonatal invasive infections (bacteremia and meningitis) in industrialized countries and a pathogen of growing importance in the elderly, especially in people suffering from underlying diseases such as diabetes or cancer (1–5). The mortality rate of neonatal infections reaches approximately 10%, and up to 40% of infants who survive meningitis are left with permanent neurologic sequelae, making the global burden of GBS infections significant (2, 5, 6). Epidemiological studies based on capsular polysaccharide (CPS) typing revealed that GBS isolates are not similarly represented among colonizing and infectious strains. Among the 10 serotypes described to date (Ia, Ib, and II to IX), CPS types Ia, II, III, and V are almost equally prevalent among colonizing strains and are responsible for 70% of adult invasive infections worldwide (3–5, 7, 8). Conversely, CPS type III has been identified since the 1970s as strongly associated with neonatal infections and is currently responsible for more than 60% of these (1–3, 7).

Penicillins or aminopenicillins are recommended as the first-line antibiotics against GBS infections (9). These antibiotics remain highly active, even though strains with reduced susceptibility were recently described in Asia and North America (10–12). Macrolides and clindamycin represent the second-line antibiotics and are usually administered in cases of allergy to beta-lactams. However, increased macrolide resistance among GBS isolates, especially in CPS type V isolates largely represented in adult infections, can lead to therapeutic issues (3, 4, 7, 13). Therefore, fluoroquinolones (FQ), especially levofloxacin (LVX), moxifloxacin (MXF), and gatifloxacin, which exhibit improved Gram-positive activity compared to other FQ such as norfloxacin and ciprofloxacin,

emerged as an important alternative for the treatment of adult GBS infections (14, 15). FQ inhibit bacterial growth primarily by binding to enzymes involved in DNA replication, the DNA gyrase and DNA topoisomerase IV encoded by the *gyr* and *par* genes, respectively. FQ resistance mechanisms relying on specific mutations found in a region of the *par* and *gyr* genes called the quinolone resistance-determining region (QRDR) have been reported in Gram-positive bacteria, mainly staphylococci, pneumococci, and enterococci (16). In GBS, FQ resistance emerged in the early 2000s and continues to increase worldwide, with rates reaching up to 40% in Asian countries (8, 17–21). Resistance is conferred by point mutations in the QRDR, mostly at codon position 81 in *gyrA* and position 79 or 83 in *parC*, through a stepwise process (22, 23). Epidemiological studies of GBS resistance to FQ in Europe are still scarce, but resistance seems to remain rare, with rates below 5% in Italy and with only one strain described in France in 2008 (24, 25).

Here, the evolution of GBS antibiotic susceptibility was studied using a collection of 8,757 isolates collected over an 8-year study

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period (2007 to 2014) in France. Molecular characterization of FQ-resistant isolates (CPS genotyping, sequence typing [ST], resistance gene detection, QRDR sequencing) was performed.

MATERIALS AND METHODS

Bacterial isolates. GBS isolates were collected and tested for antibiotic susceptibility between 2007 and 2014. The collection comprises strains isolated at the Bacteriology Laboratory of the Cochin Hospital and strains sent by correspondents located throughout the national territory on a voluntary basis to the National Reference Centre for Streptococci (CNR-Strep; <https://cnr-strep.fr>), which receives and characterizes invasive GBS isolates. GBS strains were considered responsible for invasive infection when they were isolated from a normally sterile site (blood, cerebrospinal fluid [CSF], joint, bone, synovial fluid, or pleural fluid) and as colonizing or carriage isolates when they were isolated from a non-normally sterile site (skin, mucosal surfaces, urine, gastric fluid) without being associated with an infection. All strains were identified as GBS on the basis of colony morphology, Lancefield grouping with type B antisera, and, since 2011, by mass spectrometry MALDI-TOF (matrix-assisted laser desorption-ionization time of flight) (Bruker) according to the manufacturer's recommendations. CPS genotyping and multilocus sequence typing (MLST) were performed as previously described (26, 27). The STs were determined by using the *S. agalactiae* MLST website (<http://pubmlst.org/sagalactiae/>). Isolates were grouped into clonal complexes (CCs) by eBURST, with the group definition relaxed to six out of seven shared alleles.

Antimicrobial susceptibility tests. Antibiotic resistance profiles were determined by disk diffusion for beta-lactams, erythromycin, clindamycin, tetracycline, vancomycin, and levofloxacin (LVX) according to the European Committee on Antimicrobial Susceptibility Testing guidelines (<http://www.eucast.org>) and for kanamycin and gentamicin according to the Committee on Antimicrobial Susceptibility Testing guidelines of the French Society for Microbiology (<http://www.sfm-microbiologie.org/UserFiles/files/casfm/CASFM2013vjuin.pdf>). A double-disk agar diffusion test (D-test) was performed by placing erythromycin and clindamycin 12 mm apart edge to edge to detect the iMLS_B (inducible macrolide-lincosamides-streptogramins B) phenotype. Isolates were defined highly resistant to amikacin and gentamicin when the kanamycin inhibition diameter (disk content, 1,000 µg) was less than 14 mm and when the gentamicin inhibition diameter (disk content, 500 µg) was less than 17 mm, respectively. High-level gentamicin resistance and FQ susceptibility were confirmed by MIC measurements for gentamicin, LVX, and MXF by using Etest methods (bioMérieux, Marcy l'Etoile, France). Isolates were defined highly resistant to gentamicin when the MIC was greater than 128 mg/liter, not susceptible to FQ when the LVX MIC was greater than 1 mg/liter, and resistant to FQ when the LVX MIC was greater than 2 mg/liter.

Detection of resistance genes and QRDR mutations. Detection of macrolide and tetracycline resistance genes was performed by multiplex PCR as previously described (28). The *erm(T)* resistance determinant was detected as described previously (29). Primers used for the detection of aminoglycoside resistance genes were GenF 5'-TGTTGCATTTAGTCTTTCAT-3' and GenR 5'-GGAGTAAAGGAATTGGTACAAG-3' for the *aac(6')Ie-aph(2'')Ia* gene and KanF 5'-GGGAGGCCTTTAAATACTGTAG-3' and KanR 5'-TCTGAGGCCTAAACAATTCATCC-3' for the *aphA-3* gene (30, 31). Identification of FQ resistance-associated mutations was performed after amplification and sequencing of the QRDR of *gyrA* and *parC* genes using primers *gyrA*_F 5'-CGAGTTTTATCGATTA CGCC-3', *gyrA*_R 5'-TCACCAAGGCACCAAGTAGG-3', *parC*_F 5'-AAGGGATTTCGCAATCTGC-3', and *parC*_R 5'-TCCTGAATKATAGC GCCAG-3' (20).

Statistical analyses. Statistical analyses were performed using Fisher's exact test for categorical variables. A *P* value ≤0.05 was considered significant.

RESULTS

Evolution of GBS antibiotic susceptibility from 2007 to 2014. A total of 8,757 unrelated GBS isolates were collected and tested for antibiotic susceptibility over the 8-year study period. A total of 7,110 (81.2%) GBS strains were isolated from samples sent to the bacteriological laboratory of the Cochin Hospital for routine clinical care, and 1,647 (18.8%) were collected by the CNR-Strep (Table 1). The number of strains analyzed each year, approximately 1,000 at the hospital laboratory and 200 at the CNR-Strep, did not vary during the study period, except in 2007, when it was 50% lower (data not shown). The clinical origins of the 8,757 isolates are presented in Table 1. Overall, the Cochin Hospital collection mainly comprises carriage strains (73.9%), whereas the CNR-Strep collection is composed almost exclusively of strains responsible for invasive infections (97.0%). Carriage strains (*n* = 5,302; 60.6%) were mainly isolated from vaginal samples (*n* = 4,795; 90%) and neonatal birth samples (*n* = 467; 9%), essentially represented by gastric fluids and ear swabs.

All isolates were susceptible to penicillin G, amoxicillin, and vancomycin. Resistance to tetracycline remained stable during the study period (*n* = 7,579, 86.5%; and data not shown). Resistance to macrolides decreased from 47.0% in 2007 (*n* = 248 out of 528) to 30.0% in 2014 (*n* = 365 out of 1,217; *P* < 0.001) (Fig. 1). Resistance rates were similar between colonizing isolates (36.7%) and strains responsible for infection (35.5%), but the rate was significantly lower in strains responsible for neonatal and infant infections (22.0%, *P* < 0.001) (Table 2). Cross-resistance to lincosamides (MLS_B phenotype) was observed in 68.6% of macrolide-resistant isolates and decreased in parallel with resistance to macrolides, from 27.3% in 2007 (*n* = 144) to 21.6% (*n* = 263) in 2014 (*P* < 0.01) (Table 2 and data not shown). High-level resistance to aminoglycosides, especially amikacin, increased from 6.4% in 2007 (*n* = 34 out of 528) to 8.8% in 2014 (*n* = 107 out of 1,217; *P* = 0.05) (Fig. 1). This trend was observed in both adult and neonatal isolates, mainly in colonizing isolates (Fig. 1). As for macrolides, rates of resistance to amikacin were similar between colonizing isolates (6.4%) and strains responsible for infection (6.7%), but the rate was significantly lower in strains responsible for neonatal and infant infections (4.9%, *P* < 0.05) (Table 2).

Importantly, a total of 24 strains, of which 8 were isolated in 2014, displayed high-level gentamicin resistance (HLGR), confirmed by the detection of the *aac(6')Ie-aph(2'')Ia* determinant which encodes the bifunctional aminoglycoside-inactivating enzyme with 6'-acetyltransferase and 2''-phosphotransferase activities. Although the number of isolates is too small for a statistical analysis, with HLGR strains representing only 0.3% of the bacterial population, our data clearly indicate a rise of HLGR GBS in France, starting from 0.1% in 2008 and reaching 0.7% in 2014 (Fig. 1). Besides, 70.8% (*n* = 17) and 54.2% of HLGR isolates (*n* = 13) were also resistant to macrolides and FQ, respectively.

Increased FQ resistance in GBS isolates from 2007 to 2014. Decreased susceptibility to FQ was routinely screened using a disk of LVX according to EUCAST recommendations and confirmed by MIC measurements for LVX and MXF. Of the 8,757 GBS isolates, 66 were found not susceptible to LVX over the 8-year study period. LVX nonsusceptibility, defined as an LVX MIC of >1 mg/liter, gradually increased, starting from 0.2% in 2007 (1 isolate) and 0.4% in 2008 (*n* = 5 out of 1,214) to 1.5% (*n* = 18) in 2014 (*P* < 0.01) (Fig. 1). The vast majority of LVX-nonsusceptible

TABLE 1 Clinical origins of the 8,757 GBS isolates analyzed during the 8-year study-period

No. (%) of isolates ^a											
Source of sample	Hospital					CNR-Strep				Total, <i>n</i> = 8,757 (100%)	
	Adults (nonpregnant), <i>n</i> = 1,740 (19.9%)	Pregnant women, <i>n</i> = 4,807 (54.9%)	Children, <i>n</i> = 32 (0.4%)	Neonates and infants, <i>n</i> = 531 (6.1%)	Subtotal, <i>n</i> = 7,110 (81.2%)	Adults (nonpregnant), <i>n</i> = 893 (10.2%)	Pregnant women, <i>n</i> = 116 (1.3%)	Children, <i>n</i> = 10 (0.1%)	Neonates and infants, <i>n</i> = 628 (7.2%)		Subtotal, <i>n</i> = 1,647 (18.8%)
Infection	1,740 (100.0)	20 (0.4)	19 (59.4)	78 (14.7)	1,857 (26.1)	893 (100.0)	82 (70.7)	10 (100.0)	613 (97.6)	1,598 (97.0)	3,455 (39.5)
Urine	1,152 (66.2)		11 (34.4)	10 (1.9)	1,173 (16.5)	21 (2.4)	2 (1.7)	1 (10.0)	2 (0.3)	26 (1.6)	1,199 (13.7)
Blood	30 (1.7)	6 (0.1)		10 (1.9)	46 (0.6)	591 (66.2)	25 (21.6)	5 (50.0)	332 (52.9)	953 (57.9)	999 (11.4)
Skin and soft tissue	219 (12.6)		5 (15.6)	1 (0.2)	225 (3.2)	64 (7.2)		1 (10.0)	1 (0.2)	66 (4.0)	291 (3.3)
Cerebrospinal fluid	1 (0.1)			2 (0.4)	3 (0.1)	44 (4.9)		1 (10.0)	222 (35.4)	267 (16.2)	270 (3.1)
Respiratory tract	139 (8.0)		1 (3.1)	44 (8.3)	184 (2.6)	10 (1.1)	1 (0.9)	1 (10.0)	43 (6.8)	55 (3.3)	239 (2.7)
Bone and joint	29 (1.7)				29 (0.4)	123 (13.8)		1 (10.0)	11 (1.8)	135 (8.2)	164 (1.9)
Genital tract	60 (3.4)	13 (0.3)			73 (1.0)	3 (0.3)	53 (45.7)			56 (3.4)	129 (1.5)
Digestive tract	24 (1.4)				24 (0.3)	8 (0.9)				8 (0.5)	32 (0.4)
Others	86 (4.9)	1 (0.02)	2 (6.3)	11 (2.1)	100 (1.4)	29 (3.2)	1 (0.9)		2 (0.3)	32 (1.9)	132 (1.5)
Carriage		4,787 (99.6)	13 (40.6)	453 (85.3)	5,253 (73.9)		34 (29.3)		15 (2.4)	49 (3.0)	5,302 (60.5)

^a Percentages in parentheses in boxheads indicate the proportion of isolates relative to the total number of isolates for all groups. Percentages in parentheses in the table body indicate the proportion of isolates relative to the total number of isolates for each group. Children were 1 to 15 years old, while neonates and infants were 0 to 364 days old.

FIG 1 GBS antibiotic resistance over the 8-year study period. Resistance to erythromycin, amikacin, levofloxacin and gentamicin of the 5,302 GBS colonizing isolates (solid line) and the 3,455 GBS isolates responsible for infection (dashed line) collected and routinely tested from 2007 to 2014 is shown. Filled triangles, erythromycin; filled squares, amikacin; filled diamonds, levofloxacin; filled circles, gentamicin.

GBS isolates (87.9%, *n* = 58) were considered carriage strains or responsible for noninvasive infections and were isolated from vaginal (86.2%, *n* = 50), urinary tract (12.1%, *n* = 7), and birth (1.7%, *n* = 1) samples. Eight LVX-nonsusceptible GBS strains (12.1%) were responsible for invasive infections, of which 7 were in adults and 1 in a neonate. Adult strains were isolated from blood (*n* = 2), CSF (*n* = 1), bone and joint (*n* = 1), skin and soft tissue (*n* = 1), and the lower respiratory tract (*n* = 2). The neonatal strain was isolated from CSF and was responsible for late-onset meningitis at 2 months of life. Overall, the FQ resistance rate was higher among carriage isolates than infectious isolates (0.94% versus 0.46%, *P* < 0.01) (Fig. 1). However, resistance seems to increase in parallel in both groups, especially since 2011, rising from 0.6% to 1.9% in 2014 for carriage strains and from 0.5% to 0.7% for strains responsible for infection (Fig. 1).

Almost all the LVX-nonsusceptible isolates were highly resistant to LVX (93.9%, *n* = 62), with MICs of ≥ 32 mg/liter, and 4 (6.1%) exhibited decreased susceptibility, with MICs of 2 mg/liter. These last strains were susceptible to MXF, with MICs of ≤ 0.5 mg/liter, whereas the others were found resistant, with MICs ranging from 2 to 6 mg/liter. All 62 LVX- and MXF-resistant isolates had previously described DNA point mutations with amino acid substitutions in both *gyrA* and *parC*, including an S81L substitution in the product of *gyrA* (*n* = 62, 100%) and S79F (*n* = 46, 74.2%), S79Y (*n* = 15, 24.2%), or D83Y (*n* = 1, 1.6%) in the product of *parC* (Table 3). The four GBS strains susceptible to MXF with decreased susceptibility to LVX (MICs between 1 and 2 mg/liter) exhibited amino acid substitutions only in *ParC* (S79F, *n* = 2; S79Y, *n* = 1; D83Y, *n* = 1).

Importantly, cross-resistance to antibiotics among FQ-resistant GBS isolates was extremely high compared to that of the 8,691 non-FQ-resistant GBS isolates, with 66.7% with resistance to macrolides (*n* = 44; *P* < 0.001), 30.3% with high-level resistance to amikacin (*n* = 20; *P* < 0.001), and 19.7% with high-level resistance to gentamicin (*n* = 13; *P* < 0.001) (Tables 2 and 4).

Prevalence of ST-19 CPS type V isolates among FQ-resistant GBS isolates. The molecular typing of the 66 LVX-nonsusceptible

TABLE 2 Antibiotic resistance of the 8,757 GBS isolates analyzed during the 8-year study period

Antibiotic	No. (%) of resistant isolates ^a									Total, <i>n</i> = 8,757
	Adults			Children			Neonates and infants			
	Carriage, <i>n</i> = 4,821 (55.1%)	Infection, <i>n</i> = 2,735 (31.2%)	Subtotal, <i>n</i> = 7,556 (86.3%)	Carriage, <i>n</i> = 13 (0.1%)	Infection, <i>n</i> = 29 (0.3%)	Subtotal, <i>n</i> = 42 (0.5%)	Carriage, <i>n</i> = 468 (5.3%)	Infection, <i>n</i> = 691 (7.9%)	Subtotal, <i>n</i> = 1,159 (13.2%)	
Erythromycin	1,759 (36.5)	1,064 (38.9)	2,823 (37.4)	7 (53.8)	12 (41.4)	19 (45.2)	179 (38.2)	152 (22.0)	331 (28.6)	3,173 (36.2)
Clindamycin	1,188 (24.6)	751 (27.5)	1,939 (25.7)	6 (46.2)	7 (24.1)	13 (31.0)	120 (25.6)	105 (15.2)	225 (19.4)	2,177 (26.3)
Amikacin	308 (6.4)	195 (7.1)	503 (6.7)	1 (7.7)	2 (6.9)	3 (7.1)	32 (6.8)	34 (4.9)	66 (5.7)	572 (6.5)
Gentamicin	19 (0.4)	4 (0.1)	23 (0.3)	0	0	0	0	1 (0.1)	1 (0.1)	24 (0.3)
Levofloxacin	49 (1.0)	15 (0.5)	64 (0.8)	0	0	0	1 (0.2)	1 (0.1)	2 (0.2)	66 (0.8)
Tetracycline	4,221 (87.6)	2,312 (84.5)	6,533 (86.5)	12 (92.3)	25 (86.2)	37 (88.1)	405 (86.5)	604 (87.4)	1,009 (87.1)	7,579 (86.5)

^a Percentages in parentheses in the boxheads indicate the proportion of isolates relative to the total number of isolates for all groups. Percentages in parentheses in the table body indicate the proportion of resistant isolates relative to the total number of isolates for each group. Children were 1 to 15 years old, while neonates and infants were 0 to 364 days old.

GBS isolates revealed a large predominance of CPS type V isolates (*n* = 34, 51.5%), followed by CPS type III (*n* = 19, 28.8%) and CPS type Ib (*n* = 11, 16.7%) (Table 3). Apart from studies based on CPS typing, epidemiological studies by multilocus sequence typing (MLST) identified 5 major clonal complexes (CCs) associated with human colonization and infections. These five CCs, namely, CC-1, CC-10, CC-17, CC-19, and CC-23, are highly prevalent worldwide in various GBS collections (32, 33). Strikingly, in our collection of FQ-resistant isolates, CPS types III and V belonged almost exclusively to CC-19 (*n* = 32, 94.1%, and *n* = 18, 94.7%, respectively), whereas isolates of CPS type Ib were predominantly of CC-10 (*n* = 10, 90.9%) (Table 3). Overall, CC-19, mainly composed of ST-19 isolates (*n* = 45, 90%), represented 75.8% (*n* = 50) of FQ-resistant isolates, followed by CC-10 (*n* = 12, 18.2%). The other CCs (CC-1, CC-8, CC-23) were largely underrepresented (4 isolates). The number and proportion of ST-19 CPS V isolates among FQ-resistant isolates increased drastically since 2012, as they represented 61.4% of all FQ-resistant

strains isolated between 2012 and 2014 (*n* = 27 out of 44) compared to 13.6% between 2007 and 2011 (*n* = 3 out of 22, *P* < 0.001) (Fig. 2). In contrast, CPS III strains of ST-19 and other STs were stable over the study period and never represented more than 4 isolates per year.

Arguments for the clonal expansion of a few FQ-resistant GBS isolates. Interestingly, strains containing identical ParC amino acid substitutions were associated with a limited number of CPS types and CCs and displayed similar antibiotic resistance phenotypes, arguing for a clonal expansion of a few isolates (Table 3). The S79F mutation was found in all CPS Ib CC-10 strains and CPS V CC-19 strains. In contrast, strains of CPS III CC-19 mainly carried the S79Y mutation (77.8%, *n* = 14 out of 18). Cross-resistance among FQ-resistant GBS isolates was especially important in ST-19 CPS V strains, for which resistance to macrolides, amikacin, and gentamicin reached 93.3%, 43.3%, and 30%, respectively, compared to 44.4% (*n* = 16), 19.4% (*n* = 7), and 11.1% (*n* = 4) for the other isolates, respectively (Table 3). Resis-

TABLE 3 Molecular characteristics of the 66 FQ-nonsusceptible GBS isolates^a

CPS	Clonal complex	Total no. (%) of isolates	No. (%) of isolates expressing indicated antibiotic resistance determinant									No. (%) of isolates with indicated aa substitution			
			<i>erm</i> (A)	<i>erm</i> (B)	<i>mef</i> (A/E)	<i>erm</i> (T)	<i>aphA</i> -3	<i>aac</i> (6') <i>Ie</i> - <i>aph</i> (2'') <i>Ia</i>	<i>tet</i> (M)	<i>tet</i> (M) + <i>tet</i> (L)	<i>tet</i> (O) + <i>tet</i> (L)	GyrA S81L	ParC		
													S79F	S79Y	D83Y
Ia	CC-23	1 (1.5)			1				1			1			1
Ib	CC-8	1 (1.5)					1		1					1	
	CC-10	10 (15.2)				1 (10.0)			1 (10.0)			10 (100)	10 (100)		
II	CC-1	1 (1.5)	1						1				1		
III	CC-10	1 (1.5)							1			1		1	
	CC-19	18 (27.3)	3 (16.7)	5 (27.8)	3 (16.7)		4 (22.2)	3 (16.7)	11 (61.1)	2 (11.1)	4 (22.2)	18 (100)	4 (22.2)	14 (77.8)	
	ST-19 ^b	15 (22.7)		5 (33.3)	3 (20)		4 (26.7)	3 (20)	8 (53.3)	2 (13.3)	4 (26.7)	15 (100)	1 (6.7)	14 (93.3)	
V	CC-1	1 (1.5)	1						1			1	1		
	CC-10	1 (1.5)							1						1
	CC-19	32 (48.5)	25 (78.1)	3 (9.4)	1 (3.1)		15 (46.9)	10 (31.3)	32 (100)			31 (96.9)	32 (100)		
	ST-19 ^b	30 (45.5)	24 (80.0)	3 (10.0)	1 (3.3)		13 (43.3)	9 (30.0)	30 (100)			30 (100)	30 (100)		
Total		66 (100)	30 (45.5)	8 (12.1)	5 (7.6)	1 (1.5)	20 (30.3)	13 (19.7)	50 (75.8)	2 (3.0)	4 (6.1)	62 (93.9)	48 (72.7)	16 (24.2)	2 (3.0)

^a The number, and proportion (%) if relevant, of isolates belonging to each CPS and clonal complex is indicated. For each CPS-CC or CPS-ST combination, the number of isolates expressing antibiotic resistance determinants and the amino acid (aa) substitutions identified in GyrA and ParC are indicated.

^b ST-19 isolates identified among CC-19 isolates.

TABLE 4 Antibiotic resistance of GBS isolates from vaginal carriage and of FQ-nonsusceptible GBS isolates according to CPS type^a

Antibiotic	No. (%) of resistant GBS isolates													P value ^b		
	From vaginal carriage (n = 308)								FQ nonsusceptible (n = 66)							
	Ia ^c	Ib	II	III	IV	V	VI + VII + NT	Total	Ib	III	V	Others	Total	p ^{CPSIb}	p ^{CPSIII}	p ^{CPSV}
ERY	19 (31.1)	8 (42.1)	11 (25.6)	23 (28.4)	6 (33.3)	49 (63.6)	2 (22.2)	118 (38.3)	1 (9.1)	11 (57.9)	29 (85.3)	2 (100)	43 (65.2)	<0.01	<0.05	
AMK	1 (1.6)	1 (5.3)		10 (12.3)	1 (5.6)	4 (5.2)	1 (11.1)	18 (5.8)	1 (9.1)	4 (21.1)	15 (44.1)		20 (30.3)	NS	<0.001	
GEN	1 (1.6)			1 (1.2)			1 (11.1)	3 (1.0)		3 (15.8)	10 (29.4)		13 (19.7)	<0.05	<0.001	
TET	54 (88.5)	16 (84.2)	38 (88.4)	71 (87.7)	12 (66.7)	66 (85.7)	1 (11.1)	258 (83.8)	2 (18.2)	18 (94.7)	34 (100)	2 (100)	56 (84.8)	NS	<0.05	
Total	61 (19.8)	19 (6.2)	43 (14)	81 (26.3)	18 (5.8)	77 (25.0)	9 (2.9)	308 (100)	11 (16.7)	19 (28.8)	34 (51.5)	2 (3.0)	66 (100)	<0.01	NS	<0.001

^a ERY, erythromycin; AMK, amikacin; GEN, gentamicin; TET, tetracycline; NT, nontypeable; Others, CPS Ia (one strain) and CPS II (one strain); NS, not significant.
^b The P values were generated to compare antibiotic resistances between vaginal commensal GBS isolates and FQ-nonsusceptible GBS strains for CPS Ib (p^{CPSIb}), CPS III (p^{CPSIII}), and CPS V (p^{CPSV}).
^c CPS type.

tance to macrolides was associated with the presence of the *erm*(A) gene (85.7%, n = 24 out of 28), in contrast to the other strains, where the proportions of the presence of *erm*(A), *erm*(B), and *mef*(A/E) were 37.5% (n = 6), 31.3% (n = 5), and 25% (n = 4), respectively.

Taken together, our data strongly suggest that a clonal expansion of a few FQ-resistant isolates is responsible for the increased rate of FQ resistance in GBS isolates since 2012. Our collection is largely composed of vaginal carriage strains (55.1% of total, n = 4,821, and 71.2% of FQ-resistant GBS isolates, n = 47). To confirm our hypothesis, we compared antibiotic resistances and CPS type distributions between FQ-resistant isolates and a total of 308 FQ-susceptible isolates collected from vaginal samples between 2013 and 2014 (Table 4). While CPS type distribution in the FQ-susceptible group was very similar to that reported in other countries, CPS types Ib and V were clearly overrepresented in the FQ-resistant group (16.7% versus 6.2%, P < 0.01, and 51.5% versus 25%, P < 0.001, respectively) (5, 34). As expected, cross-resistance to other antibiotics in GBS isolates of CPS types III and V was also significantly more frequent in FQ-resistant than FQ-susceptible isolates (Table 4).

DISCUSSION

We analyzed the evolution of GBS antibiotic susceptibility in a significant collection of isolates over an 8-year study period and showed that resistance to aminoglycosides and FQ is steadily increasing, more markedly in carriage strains. The GBS collections

studied here come from two different sources, which can be considered representative of GBS colonizing isolates in the Paris area (Cochin Hospital) and of circulating invasive GBS isolates in France (CNR-Strep). We found that resistance levels to macrolides, aminoglycosides, and FQ were lower in isolates responsible for neonatal and infant infections than in colonizing and adults isolates. This difference might be linked to the particular epidemiology of GBS neonatal infections, where CPS type III is usually predominant, whereas CPS type V, reported to be more frequently resistant to antibiotics and especially macrolides, is classically underrepresented (3, 4, 7, 13). Nevertheless, in contrast to other studies which report an increase in GBS resistance to macrolides until 2010, macrolide resistance seems to stabilize in France, in both invasive and colonizing strains, with rates similar to those currently observed worldwide (3, 13).

Importantly, whereas HLGR has been rarely reported in beta-hemolytic streptococci, in particular in GBS, with only two reports published over the last 25 years in Europe, we describe the emergence of HLGR in GBS over the 8-year study period (35, 36). The HLGR isolates display a high proportion of cross-resistance to macrolides and FQ, confirming the possible issue of multidrug resistance in GBS (12). This observation, together with other studies performed worldwide, might have significant clinical implications, mainly for the treatment of endocarditis and meningitis for which a combination of a beta-lactam and an aminoglycoside is frequently recommended.

Resistance to FQ reached 1.5% in 2014, a rate similar to that of neighboring countries, such as Italy (24). The vast majority of FQ-resistant isolates were not responsible for infection, and none of them belonged to the hypervirulent CC-17 lineage, reported to be responsible for neonatal infections worldwide (2, 37, 38). However, we showed a trend for an increase in both carriage strains and strains responsible for infection, which has to be confirmed in future studies. The molecular characterization of FQ-resistant isolates and their comparison to circulating GBS isolates strongly suggest a clonal expansion of FQ-resistant ST-19 CPS V isolates. As for HLGR GBS, cross-resistance to macrolides and aminoglycosides among FQ-resistant GBS isolates was extremely important in ST-19 CPS V strains. In other countries, especially in Asia, FQ resistance has been mainly associated with CC-10 and CC-19 isolates, which displayed, in each study, identical QRDR mutations and similar resistance patterns, also suggesting a clonal expansion of resistant isolates (8, 17, 18). The differences in the distribution of CPS types, CCs, and QRDR mutations between geographical

FIG 2 Molecular typing of FQ-nonsusceptible GBS isolates over the 8-year study period. CPS genotyping and MLST were performed on the 66 FQ-nonsusceptible GBS isolates.

areas suggest that FQ resistance is acquired through independent mutations. However, one cannot exclude the possible selection, fixation, and worldwide dissemination of highly epidemic multidrug-resistant GBS clones through the extensive use of FQ, as the extensive use of tetracyclines specifically selected GBS clones infecting humans in the 1970s (32).

In conclusion, this work highlights the rise of FQ- and aminoglycoside-resistant GBS and the emergence of HLGR GBS in France, associated with the expansion of ST-19 CPS V-resistant isolates. Although FQ and gentamicin resistance rates remain low, the continuous increase observed over the 8-year study period, especially in the last 3 years, the probable clonal expansion, and the substantial level of cross-resistance among FQ-resistant isolates raise concerns about GBS antibiotic susceptibility in the future. Together with worldwide reports on emerging multidrug-resistant GBS isolates, this work reinforces the need for a continuous surveillance of GBS epidemiology and antibiotic susceptibility.

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