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Long-term surveillance of group B *Streptococcus* strains isolated from infection and colonization in pregnant women and newborns

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Abstract

Introduction. Group B *Streptococcus* (GBS) remains the leading cause of bacterial neonatal infections worldwide, despite the spread of recommendations on vaginal screening and antibiotic prophylaxis.

Hypothesis/Gap Statement. There is a need to evaluate the potential changes in GBS epidemiology over time following the introduction of such guidelines.

Aim. Our aim was to perform a descriptive analysis of the epidemiological characteristics of GBS by conducting a long-term surveillance of strains isolated between 2000 and 2018, using molecular typing methods.

Methodology. A total of 121 invasive strains, responsible for maternal infections (20 strains), fetal infections (8 strains) and neonatal infections (93 strains), were included in the study, representing all the invasive isolates during the period; in addition, 384 colonization strains isolated from vaginal or newborn samples were randomly selected. The 505 strains were characterized by capsular polysaccharide (CPS) type multiplex PCR assay and the clonal complex (CC) was assigned using a single nucleotide polymorphism PCR assay. Antibiotic susceptibility was also determined.

Results. CPS types III (32.1% of the strains), Ia (24.6%) and V (19%) were the most prevalent. The five main CCs observed were CC1 (26.3% of the strains), CC17 (22.2%), CC19 (16.2%), CC23 (15.8%) and CC10 (13.9%). Neonatal invasive GBS diseases were predominantly due to CC17 isolates (46.3% of the strains), which mainly express CPS type III (87.5%), with a very high prevalence in late-onset diseases (76.2%).

Conclusion. Between 2000 and 2018, we observed a decrease in the proportion of CC1 strains, which mainly express CPS type V, and an increase in the proportion of CC23 strains, mainly expressing CPS type Ia. Conversely, there was no significant change in the proportion of strains resistant to macrolides, lincosamides or tetracyclines. The two molecular techniques used in our study provide almost as much information as classical serotyping and multilocus sequence typing, but are quicker, easy to perform, and avoid long sequencing and analysis steps.

INTRODUCTION

Streptococcus agalactiae, known as group B *Streptococcus* (GBS), emerged in humans at the end of the 1960s and rapidly became a major pathogen involved in maternofetal and neonatal infectious diseases [1–4]. However, GBS is also a commensal inhabitant of urogenital and digestive tracts, colonizing approximately 15–25% of women. The colonization of pregnant women varies depending on the country: about 11% in Asia, which is less than in other regions of the world, such as in North America (22%),

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Keywords: colonization; group B *Streptococcus*; infection; neonatal.

Abbreviations: CDC, centers for disease control and prevention; CPS, capsular polysaccharide; EOD, early onset disease; GBS, group B *Streptococcus*; LOD, late-onset disease; MLSb, macrolides, lincosamides, streptogramin B; MLST, multilocus sequence typing; SNP, single nucleotide polymorphism.

A supplementary table is available with the online version of this article.

in Eastern Europe (20.8%) or in Africa (18.2%) [5–9]. Transmission of GBS from colonized mothers to newborns occurs in approximately 50–70% of cases in the absence of antibiotic prophylaxis. Within these cases, newborns develop invasive disease in 1–2% of cases [1, 10]. Neonatal infections are characterized by two different syndromes: early onset disease (EOD), which occurs during the first week of life, and late-onset disease (LOD), which occurs between the seventh day and 3 months of life.

One of the most important virulence factors of GBS is the polysaccharide capsule. Its composition differs in terms of the proportion of glucose, galactose, *N*-acetylglucosamine and sialic acid according to the serotype [11]. The capsule plays an important role in attachment to target cells through terminal sialic acid found in specific serotypes and allows GBS to escape the host immune response [12, 13]. Characterization of the capsular polysaccharides (CPSs) has enabled the description of 10 serotypes, named Ia, Ib, and II to IX [14]. The most common techniques to determine the CPS type are immunodiffusion tests and latex agglutination [15]. More highly discriminative typing methods have been recently developed. Currently, multilocus sequence typing (MLST) based on the sequencing of seven housekeeping genes is considered the reference technique for epidemiological studies [16]. These molecular methods, especially MLST, have identified clones particularly involved in invasive diseases. Thus, clonal complex CC17, which includes ST17 *sensu stricto* and other related sequence types, is recognized as a highly virulent clone involved in neonatal diseases. CC19 and CC23 contain both colonization and invasive strains and are genetically more diverse. Conversely, CC1 and CC10 are mainly composed of colonization strains [16–20].

Various strategies have been developed to prevent neonatal GBS infections. In the absence of an efficient vaccine, one strategy relies on administrating antibiotic prophylaxis based on risk factors. Thus, pregnant women receive β-lactams in cases of (i) labour before 37 weeks of pregnancy, (ii) rupture of membranes for more than 12 h, (iii) a maternal temperature >38 °C during labour. This strategy is favoured in the UK, New Zealand and some Australian maternity units [21]. Another strategy is based on pre-partum vaginal screening, followed by antibiotic prophylaxis during labour. In the USA, the Centers for Disease Control and Prevention (CDC) published the first guidelines for the prevention of perinatal group B streptococcal disease in 1996, which recommend the use of one of the two prevention methods. In 2002, new CDC guidelines focused more on universal prenatal GBS screening. CDC and American Society for Microbiology (ASM) guidelines were later published in 2010 and 2020, respectively, indicating algorithms, antibiotic regimens and laboratory methods, but did not challenge the supremacy of the screening approach [22, 23]. A number of European countries have also updated their guidelines to recommend a screening-based prevention method [21]. In France, guidelines for antenatal prevention of GBS infection were published in September 2001 and recommend vaginal screening during the last month of pregnancy [24]. Systemic antibiotic prophylaxis with penicillin G (benzylpenicillin) or aminopenicillin (amoxicillin or ampicillin) is administrated in cases of positive GBS vaginal screening, whereas macrolides or lincosamides can be used in cases of allergy to β-lactams. Antibiotic prophylaxis is also administrated if a neonatal GBS infection occurred during a previous pregnancy or if a GBS bacteriuria or a GBS carriage was reported at any time during the current pregnancy. Overall, these recommendations have led to a significant decrease in GBS EOD in developed countries, such as in the USA, where the incidence has dropped from 1.7 cases per 1000 live births in the early 1990s to 0.19 cases today [6, 22, 25, 26]. However, antibiotic prophylaxis had no impact on the incidence of LOD, which remained unchanged, with an estimated 0.33 cases per 1000 live births. In addition, antibiotics may have an unfavourable impact on the newborn microbiota and can potentially lead to the emergence of antibiotic resistance [26].

In this study, we aimed to highlight potential changes of GBS epidemiology by characterizing with molecular typing methods a collection of strains involved in infection and colonization of pregnant women and newborns over a long period of nearly 20 years.

METHODS

Bacterial strains

From January 2000 to December 2018, 18458 GBS strains were isolated at the University Hospital of Tours, France. Among them, 384 strains were randomly selected from vaginal samples of pregnant women (183 strains), gastric fluids of asymptomatic neonates (191 strains) and placental culture without clinical issues (10 strains) to represent one or two colonization strains per month. In addition, all the 121 invasive strains recovered during the same period from maternal infections (20 strains), fetal infections (8 strains) and neonatal infections (93 strains) were studied; they all came from blood culture, cerebrospinal fluid, joint fluid, gastric fluid collected from newborns or placental culture from women with evident signs of infection. Thus, 505 strains were analysed in this study. For complete information about the strains, see Table S1 (available with the online version of this article).

Antibiotic susceptibility

Antibiotic susceptibility was systematically assessed for all the strains with the routine techniques used in the hospital laboratory. To avoid bias due to changes in techniques and because our collection does not contain any β-lactam-, glycopeptide- or fluoroquinolone-resistant strains, we performed new antibiotic-susceptibility tests for the 505 strains solely for macrolides, lincosamides (erythromycin and lincomycin) and tetracycline. Antibiotic susceptibility was determined by diffusion in agar medium according to CASFM/EUCAST (Comité de l'antibiogramme de la Société Française de Microbiologie/European Committee on

Antimicrobial Susceptibility Testing) recommendations [27]. Incubations were performed on MH-F medium (Mueller–Hinton medium +5% horse-blood +20 mg β-NAD l^{-1}) with a 0.5 McFarland suspension for 24 h at 35 °C.

The phenotype of macrolide-resistant strains was precisely determined. The constitutive MLSb (macrolides, lincosamides, streptogramin B) phenotype was attributed to strains with high resistance to erythromycin (diameter <21 mm) and lincomycin (diameter <21 mm). An inducible MLSb phenotype was attributed to strains with resistance to erythromycin and susceptibility to lincomycin, with antagonism between the two discs. Strains with this phenotype were considered lincomycin resistant. An efflux mechanism was attributed to strains with resistance to erythromycin and susceptibility to clindamycin without antagonism between the two discs.

DNA extraction

Strains were stored at −80 °C. After a 24 h incubation at 35 °C, a bacterial suspension in sterile water from isolated colonies (1.5 McFarland) was prepared and 50 µl mutanolysine (Sigma Aldrich; 1000 U ml^{-1}) was added to 500 µl bacterial suspension. The mixture was incubated at 56 °C for 1 h and then 10 min at 100 °C before stopping the reaction by placing the samples on ice. After 3 min of centrifugation (15000 ***g***), extracted DNA was collected and stored at 4 °C (for immediate use) or stored at −20 °C (for later use).

Molecular capsular typing

GBS strains were typed using the molecular capsular method described by Imperi *et al.* and PCR conditions were performed as previously described [28]. Briefly, the multiplex PCR was based on the use of 19 primers targeting the genes from the *cps* region involved in CPS synthesis. After amplification, the products were separated by gel electrophoresis to visualize the amplicon combinations and determine the molecular serotype. The primers *cpsL*-F and *cpsL*-R target part of a gene found in all GBS strains (*cpsL*). Thus, the amplicon of *cpsL* was used as an internal control of the reaction. Other primers target regions (of genes) of the *cps* locus that differ between strains.

Assignment of the clonal complex

The method described by Honsa *et al.* was used to assign the clonal complex to the GBS strains [29]. This method is based on an allele-specific real-time PCR assay that explores four single nucleotide polymorphisms (SNPs) located in three of the seven housekeeping genes used in the MLST method: glutamine synthetase (*glnA36* and *glnA429*); glucose kinase (*glcK180*); and alcohol dehydrogenase (*adhP111*). Two or three PCR reactions were performed with specific primers of each allele for each SNP. The primer pair that matches the allele of the strain amplifies a fragment that reaches the fluorescence corresponding to the cycle threshold (C_t) earlier (with a smaller C_t), while other primers amplify fragments that reach the fluorescence threshold with a higher C_t because of a mismatch with the nucleotide sequence. The combination of the four nucleotides determines the SNP profile and allows definition of the clonal complex of the strain.

RESULTS

Origin of the strains

In total, 76.9% (93/121) of the invasive strains were isolated from patients with neonatal disease. Strains responsible for EOD represented 54.8% (51/93) and strains responsible for LOD represented 45.2% (42/93). The other invasive strains were isolated from in utero fetal deaths following chorioamnionitis (8 strains) or from maternal infections (20 strains). Considering exclusively perinatal infections, which include both neonatal infections and in utero deaths, 50.5% (51/101) were isolated from cultures associated with EOD, 41.6% (42/101) from cultures associated with LOD and 7.9% (8/101) from cultures associated with in utero fetal deaths.

Prevalence of the CPS type according to the origin of the strains

The CPS type was determined for most of the 505 strains. More than 80% of the invasive strains were represented by two CPS types: CPS type III (57%, 69/121) and CPS type Ia (24.8%, 30/121). The remaining invasive strains expressed CPS types V, II or Ib, but in a lower proportion (Fig. 1). LOD strains were almost exclusively from CPS type III (85.7%, 36/42), and CPS type Ia (7.1%, 3/42). Considering EOD, CPS type III (49%, 25/51) was less represented than in LOD, whereas CPS type Ia (29.4%, 15/51) was more prevalent; other CPS types were found in nearly 10% of cases, such as CPS type II (11.8%, 6/51) and CPS type V (9.8%, 5/51). Conversely, colonization strains were more equally distributed within the main CPS types: 24.5% of strains (94/384) for CPS type Ia, 24.2% (93/384) for CPS type III and 22.7%(87/384) for CPS type V (Fig. 1).

Change in the prevalence of the CPS types from 2000 to 2018

As the French recommendations were published at the end of 2001 and to allow sufficient time to observe potential epidemiological changes, we distinguished two periods: the first between 2000 and 2004, and the second between 2005 and 2018. Considering

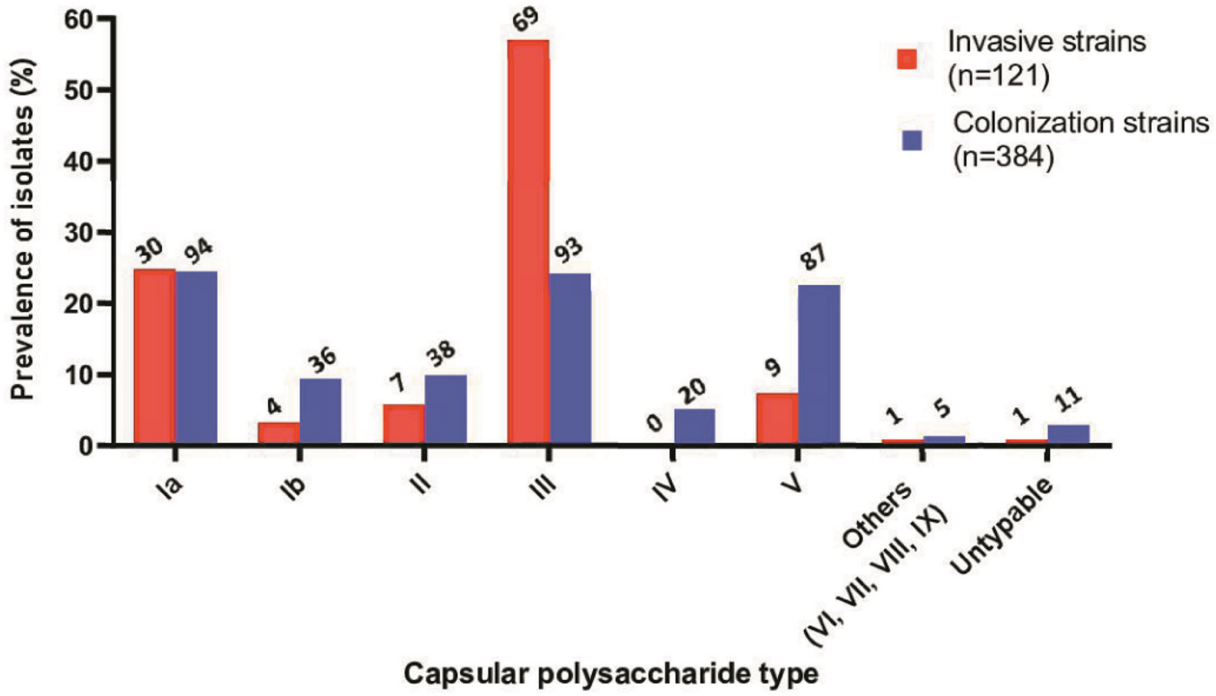

Fig. 1. Prevalence (%) of the CPS types according to strain. The invasive strains (*n*=121) are shown in red, and the colonization strains (*n*=384) in blue. The numbers of isolates are indicated above the bars.

invasive strains, CPS type III remained the most prevalent over time. A trend showing a decrease of CPS type V and an increase of CPS Ia was observed without statistically significant difference because the effective sample size was too small in the 2000–2004 period (*n*=10) (Fig. 2a, b). Considering colonization strains, we observed a decrease in the prevalence of CPS type V and, to a lesser extent, CPS type IV, while during the same period, there was an increase in the prevalence of CPS type Ia (Fig. 2c, d).

Prevalence of the clonal complexes according to the origin of the strains

We were able to determine the clonal complex of nearly all strains, except for six colonization strains and one invasive strain. Colonization strains were overrepresented in CC1 strains (28.6%, 110/384), while the four other main clonal complexes were found in nearly the same proportion: CC19 (18.2%, 70/384), CC23 (16.9%, 65/384), CC10 (16.1%, 62/384) and CC17 (14.6%, 56/384) (Fig. 3).

The distribution of clonal complexes in invasive strains was different. Unlike colonization strains, invasive strains were over-represented in CC17 isolates (50.4%, 61/121). We also found strains belonging to CC1 (17.4%, 21/121) and, less frequently, to CC23 (14.9%, 18/121), CC19 (9.1%, 11/121) and CC10 (8.3%,10/121).

Distinguishing between EOD and LOD provided additional information. LOD strains overwhelmingly belonged to CC17 (81.0%, 34/42); with CC1 (11.9%, 5/42) and CC19 (9.5%, 4/42) making up the rest. EOD clonal complexes were more diverse. CC17 was less prevalent (37.3%, 19/51), whereas approximately a quarter of EOD strains belonged to CC23 (25.5%, 13/51) and 17.6% to CC1 (9/51), with a smaller number of strains belonging to CC10 (11.8%, 6/51) and CC19 (7.8%, 4/51).

Change in the prevalence of the clonal complexes over time

Considering invasive strains, CC17 remained the most prevalent over time. A trend showing a decrease in CC10 and an increased occurrence of other clonal complexes (CC1, CC19, CC23) could be observed, but because of the small effective sample size in the 2000–2004 period (*n*=10), no statistically significant difference was observed (Fig. 4a, b). Considering colonization strains, the evolution of the clonal complexes over time showed a decrease in the prevalence of CC1 and an increase in that of CC23, whereas the prevalence of the other main clonal complexes remained stable (Fig. 4c, d).

Correlation between CPS types and clonal complexes

Our results show several correlations between the CPS types and clonal complex. First, some clonal complexes showed a good correlation with one specific CPS type, such as CC17 and CC23 mainly expressing CPS type III (87.5%, 98/112) and CPS type

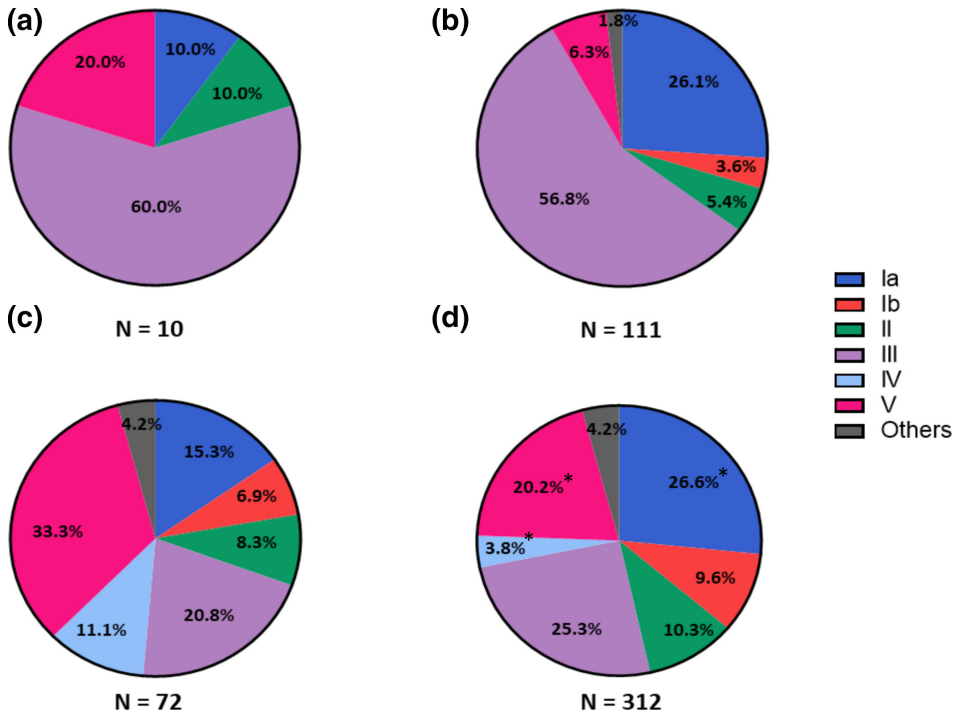

Fig. 2. Evolution over time of the repartition of the GBS CPS types for invasive and colonization strains. The distribution of invasive strains is shown: (a) for the 2000–2004 period and (b) for the 2005–2018 period. The distribution of colonization strains is shown: (c) for the 2000–2004 period and (d) for the 2005–2018 period. Statistically significant differences are observed concerning CPS types Ia, IV and V prevalence for colonization strains between the two time-periods (*Z-score >1.96).

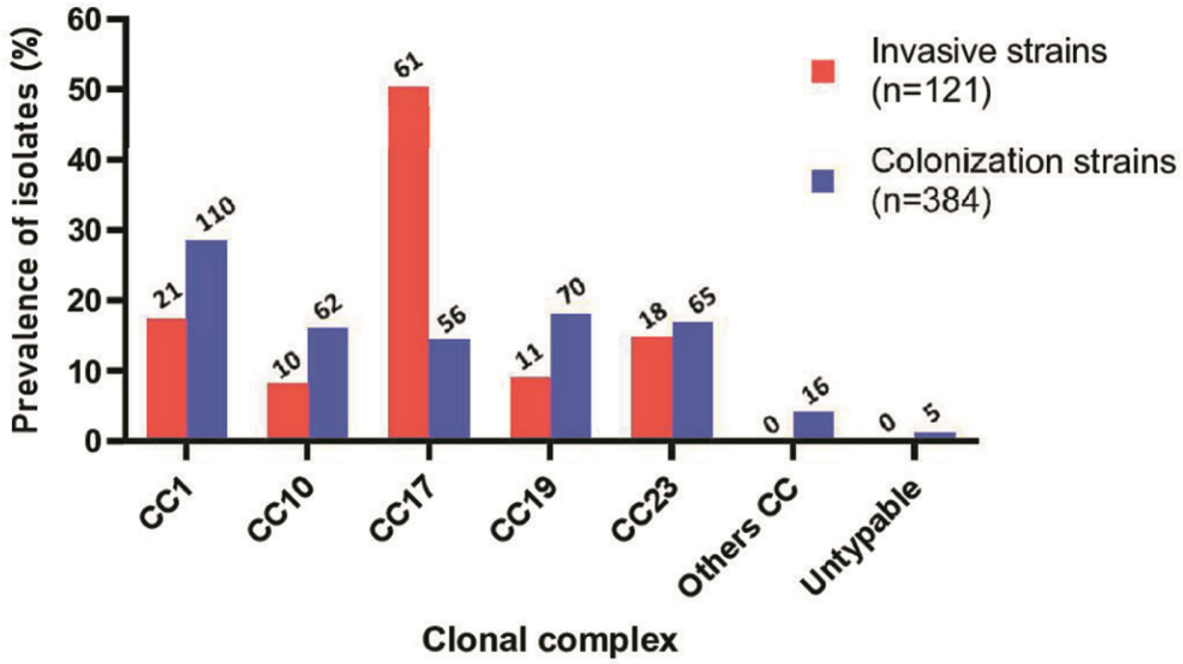

Fig. 3. Prevalence (%) of the clonal complexes according to strain. The invasive strains (n=121) are shown in red, and the colonization strains (n=384) in blue. The numbers of isolates are indicated above the bars.

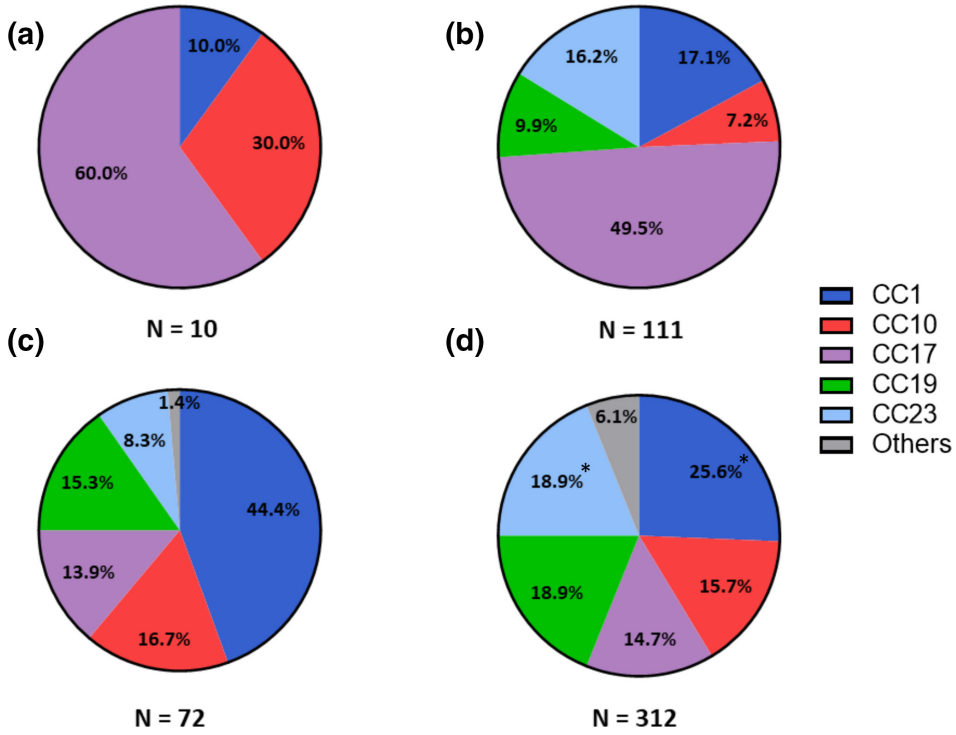

Fig. 4. Evolution over time of the repartition of the GBS clonal complexes for invasive and colonization strains. The distribution of invasive strains is shown: (a) for the 2000–2004 period and (b) for the 2005–2018 period. The distribution of colonization strains is shown: (c) for the 2000–2004 period and (d) for the 2005–2018 period. Statistically significant differences are observed concerning CC1 and CC23 prevalence for colonization strains between the two time-periods (*Z-score >1.96).

Ia (78.8%, 63/80), respectively (Table 1). Second, some clonal complexes expressed a more predominant CPS type, such as CPS type V for CC1 (54.9%, 73/133), CPS type Ib for CC10 (42.9%, 30/70) and CPS type III for CC19 (52.4%, 43/82), with other CPS types representing approximately half of the strains.

Antibiotic resistance

Overall, we found tetracycline resistance for 83% (419/505) of all strains, erythromycin resistance for 27.9% (141/505) and lincomycin resistance for 23.8% (120/505). We observed three antibiotic susceptibility phenotypes: 58,6% (296/505) of strains were

Table 1. Distribution of the GBS strains within clonal complexes and CPS types

CPS type	CC1	CC10	CC17	CC19	CC23	Others	Total
Ia	18	15	7	8	65	10	123
Ib	7	31	0	1	1	0	40
II	5	18	0	14	5	3	45
III	2	1	105	41	9	4	162
IV	16	0	3	0	0	1	20
V	74	4	0	15	1	2	96
VI	2	0	0	0	0	0	2
VII	1	0	0	0	0	0	1
VIII	0	0	0	0	0	0	0
IX	0	3	0	0	0	0	3
Untypable	5	1	2	2	2	1	13
Total	130	73	117	81	83	21	505

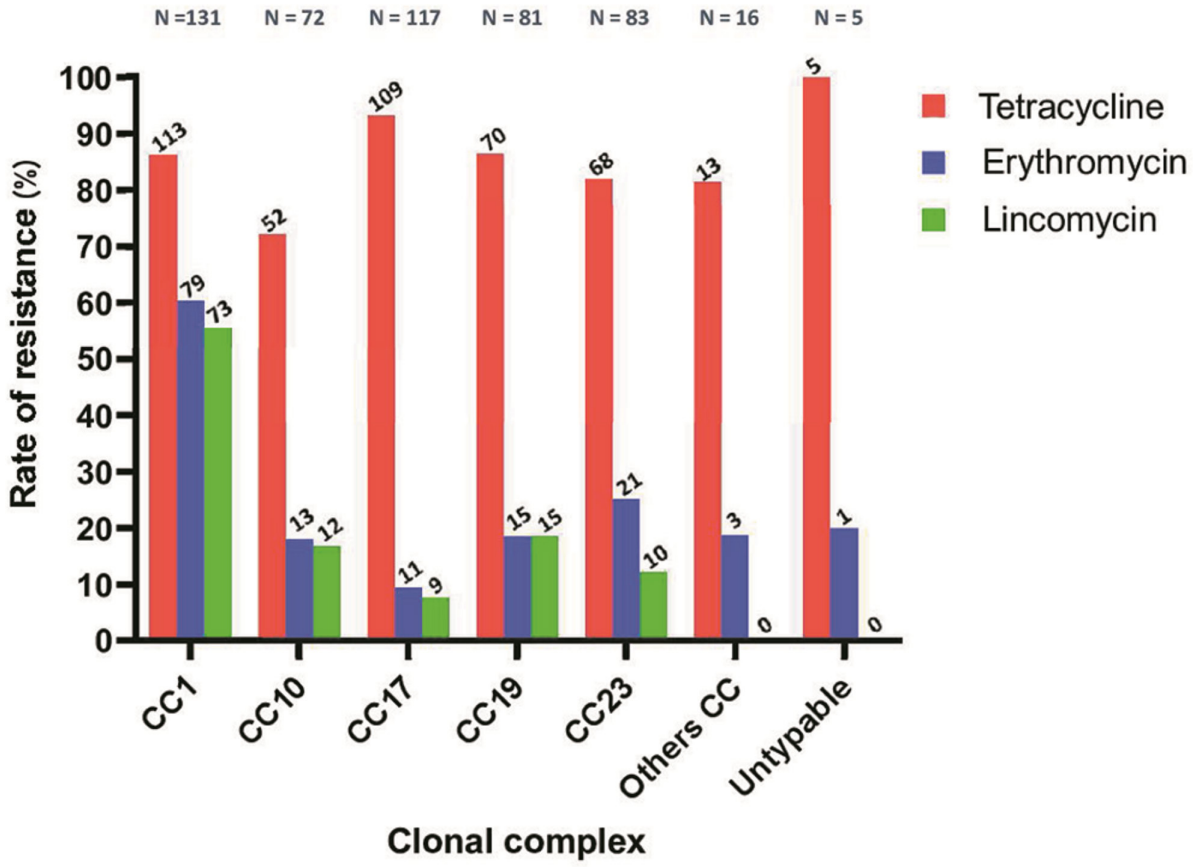

Fig. 5. Rate of antibiotic resistance (%) according to the clonal complex of the strains. The rate of tetracycline resistance is shown in red, that of erythromycin resistance in blue and that of lincomycin resistance in green. The numbers of isolates are indicated above the bars.

only resistant to tetracycline, 19,6% (99/505) were resistant to all three antibiotics tested and 12,3% (62/505) were susceptible to all three antibiotics tested. The other phenotypes represented <5% (<20/505) of strains. Macrolide and lincosamide resistance differed according to the origin of the strain, as colonization strains seemed to have a higher frequency of resistance: 28.9% (111/384) were resistant to erythromycin versus 24.8% (30/121) of invasive strains. However, no statistically significant difference was observed. For lincomycin, 25.3% (97/384) of colonization strains were resistant versus 19% (23/121) of invasive strains. Colonization strains expressed more frequently the MLSb mechanism for macrolide and lincosamide resistance. Strains susceptible to tetracycline were more frequent in EOD than in LOD. In terms of macrolide and lincosamide resistance, we found all the possible phenotypes for strains causing EOD versus strains responsible for LOD, which were mostly wild-type or inducible MLSb.

Antibiotic resistance and clonal complexes

We found differences between clonal complexes in terms of antibiotic resistance (Fig. 5). Overall, all clonal complexes exhibited a high-level of tetracycline resistance. More precisely, CC17 (93.2%, 109/117) and CC19 (86.4%,70/81) strains showed a high-level of resistance, whereas CC10 (72.2%, 52/72) strains were more susceptible. For macrolides and lincosamides, three categories could be distinguished. First, CC1, which mainly expressed CPS types IV and V, exhibited a higher resistance than other clonal complexes (erythromycin 60.3%, 79/131; lincomycin 55.7%, 73/131). Second, CC17 presented less resistance (erythromycin 9.4%, 11/117; lincomycin 7.7%, 9/117). Third, all the other clonal complexes exhibited an intermediate prevalence of resistance to macrolides and lincosamides (approximately 20%). MLSb mechanisms (inducible or constitutive) were the most frequently found among strains resistant to macrolides, except for CC23 strains, which expressed more frequently an efflux-mediated mechanism of resistance.

Change in antibiotic resistance over time

Our results showed no significant evolution of resistance to macrolides and lincosamides or tetracycline following establishment of the 2001 French recommendations. The level of resistance against tetracycline, erythromycin and lincomycin was relatively

stable over time, except during the last years, for which a slight decrease was observed. In addition, no significative difference was observed between invasive and colonization strains (data not shown).

DISCUSSION

GBS is still the leading bacterium responsible for invasive neonatal infections worldwide, especially for EOD, despite the publication of guidelines in many developed countries over the past 30 years. In France, recommendations for GBS screening were published in the early 2000s [24]. We evaluated the potential changes in GBS epidemiology by conducting a monocentric study over a long period (approximately 20 years) at the University Hospital of Tours, France. We decided to separate the study into two time-periods: the first representative of the situation before and during the establishment of the recommendations, and the second representative of the epidemiology after the potential impact of the recommendations. All invasive strains were selected in the study and one or two strains per month from colonized pregnant women or newborns were also randomly included, to be as exhaustive as possible.

As the most contributive techniques for epidemiological studies are currently serotyping and MLST, we chose two recently described techniques that were reported to be as efficient and specific as the reference techniques for both serotyping and the determination of clonal complexes, but quicker, simpler and less expensive. The first is a molecular assay that allows determination of the CPS type in a one-step multiplex PCR, reported and successfully used by Imperi *et al.* [28]. This method allowed us to determine the CPS type, even if there was poor expression of the *cps* genes, and avoid the disadvantages of serotype determination using reference tests, such as immunodiffusion or latex agglutination, which suffer from a lack of standardization and sensitivity [30–32]. Still, some strains remained untypable by this method, probably because primers did not hybridize perfectly, due to a mutation of the nucleotide sequence. Similarly, we used a method that is faster than MLST for determination of the clonal complex. This method allowed us to assign a clonal complex to all but seven strains, which were probably part of rare clonal complexes or other singletons expressing different SNPs that were not described by Honsa *et al.* [29]. Thus, despite the inability to precisely determine sequence types, this technique is a good contributive method for the assignment of the clonal complex to numerous strains and avoids sequencing steps.

The particularity of the French recommendations is that they propose 'simple' GBS screening by culturing vaginal samples on blood agar media. At the University Hospital of Tours, this is performed on a GBS-specific chromogenic medium, resulting in a prevalence of 10% of GBS vaginal carriage (data not shown). Although the maternal prevalence of GBS is 18% worldwide [9], a recent review provided a reminder that GBS carriage varies depending on the world region ranging from 11% in Asia to more than 30% in Caribbean women. However, the low prevalence in our study appears to be associated more with the lack of sensitivity of the culture method used, and highlights the need to review the French recommendations and to possibly propose a screening strategy based on more sensitive techniques [33, 34]. Such molecular detection strategies were already proposed by an European consensus conference [35] but does not necessarily mean an immediate pre-partum PCR that may be difficult to set up in some maternity units.

Another aspect of GBS prevention is that delivering broad-spectrum antibiotics may lead to the emergence of antibiotic resistance. Such evolution has been described for other streptococci, such as *Streptococcus pneumoniae*, which has acquired reduced penicillin susceptibility, but more rarely for β-haemolytic streptococci, except for a few GBS strains first isolated in Japan 20 years ago. These strains expressed altered PBP2X genes, which encode one of the most important penicillin G targets [36], but comparative phylogenetic analyses demonstrated that this phenotype emerged independently in several strains by accumulating mutations in the PBP genes [37]. Unlike penicillins, macrolides or lincosamides are used less frequently in GBS prophylaxis and only in cases of penicillin allergy. Our study shows no variation in macrolide and lincosamide resistance over 20 years. In non-pregnant adults who are also exposed to macrolides or lincosamides, erythromycin and clindamycin resistance remained stable between 2007 and 2019 in France [38]. However, GBS resistance to macrolides and lincosamides appears to have increased in several other developed countries [39, 40], as well as in developing countries [41]. For example, such resistance increased in Canada following the first step of introducing GBS guidelines and coincided with a period of increasing macrolide use [42]. Consequently, macrolide-resistant GBS is now considered as a concerning public-health threat according to the 2019 CDC Antibiotic Resistance report [43]. However, the short duration of antibiotic prophylaxis is also probably the reason for a limited effect of GBS prophylaxis on antibiotic resistance, although a larger impact on the vaginal and digestive microbiota has not been evaluated.

Just as GBS carriage varies according to world region, there are also differences in serotype and clonal complex distributions. Our results show strains expressing mainly CPS types Ia, III and V in the same proportions found over the last decade in Western Europe [9]. Other CPS types, such as VI, VII, VIII and IX, were rarely or never observed in our study, but have been found in colonization strains of pregnant women in Asia [9]. Our study also highlights the correlation between CPS types and clonal complexes. Thus, CPS type III strains mainly belong to CC17 if they are invasive strains, especially LOD strains, or to CC19 if they are colonization strains [44]. Neonatal invasive GBS diseases were mainly represented by CC17 strains in our study, as reported by the French reference centre for streptococci and as also found in other developed countries [45, 46]. Concerning EOD, more diverse CPS types have been highlighted, regardless of the country. Indeed, we found more frequently CC1 and CC23, related to CPS types V and Ia, respectively, as reported by the French National Reference Center for Streptococci and in other French or American studies [47–49]. However, unlike antibiotic susceptibility, the distribution of CPS types and clonal complexes has evolved over the 20 year study period regarding

colonization strains. We observed a decrease in the prevalence of CC1 strains expressing CPS types V or IV, which have been replaced by CC23 strains expressing CPS type Ia. This begs the question of whether such evolution, already observed in Iceland between 1975 and 2014 [50], predicts similar changes in other countries. However, even if guidelines on GBS prevention could have an indirect effect by selecting resistant strains, the short duration of antibiotic prophylaxis, and the low usage of penicillin alternatives limit its influence in epidemiological changes. Indeed, despite CC1 strains being more frequently resistant to antibiotics, a decrease in their prevalence was observed. Including strains from other centres may confirm the changes we observed.

In summary, our study, although monocentric, provides a large amount of epidemiological data thanks to a long-term survey of strains from a university hospital. It highlights the capacity of quick and ready to use molecular techniques, even though the use of whole-genome sequencing is increasing nowadays. It also interrogates about the sensitivity of vaginal screening methods, the need to update prevention guidelines, and the relative insensitivity of macrolides against GBS, especially because they do not cross the placental barrier.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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