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Molecular characterization and antimicrobial susceptibility of hemolytic *Streptococcus agalactiae* from post-menopausal women

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ABSTRACT

Purpose: Streptococcus agalactiae (Group B streptococcus, GBS) is increasingly recognized as a pathogen in adult populations, including the elderly. Appropriate treatment involves antibiotics. An alternative to this strategy would be the administration of a polysaccharide vaccine therefore the capsular serotypes and molecular characterization of circulating strains needs to be known. Few studies have been conducted in this population.

Methods: One hundred and seven GBS isolates collected from vagino-rectal swabs from 600 post-menopausal women were analysed for their capsular type, antimicrobial resistance and genetic relatedness (multilocus sequence typing, MLST).

Results: The colonization rate was 17.8%. Capsular type III was predominant (34.6%), followed by type V (22.4%). The most frequent sequence type (ST) was 19 (23.3%), followed by 23 (18.7%), 1 (16.8%) and 17 (12.1%). Isolates were assembled into three phylogenetic groups from ST-19, ST-23 and ST-17 founders. All isolates were susceptible to penicillin, whereas resistance to erythromycin and clindamycin was recorded in 23.4% and 20.6% of isolates, respectively.

Conclusions: In our setting, the GBS colonization rate in postmenopausal women is similar to that reported in others populations studied. The population structure of these isolates is highly diverse and contains different STs. These data can contribute to the future development of a polysaccharide vaccine for preventing GBS infection in older adults.

1. Introduction

Streptococcus agalactiae (group B streptococci, GBS) is the most common etiologic agent of neonatal sepsis [1]. Severe GBS infections are increasingly recognized in adults, mainly in the elderly and in individuals compromised by underlying medical conditions, with reported incidence rates ranging from 4.4 to 23 cases per 100,000 adults [2–5].

The incidence is higher in patients over the age of 60 years. Primary bacteraemia is the most frequent form of invasive GBS disease, followed by skin and soft-tissue infection, pneumonia and urinary tract infections [3–5]. The prevalence of colonization reported among healthy elderly adults (20–25%) is similar to that among women of child-bearing age [3,6].

The incidence of neonatal early onset GBS infections has nose-dived since the generalized use of intrapartum antibiotic prophylaxis in GBS-colonized pregnant women [7]. In contrast, there are no strategies to prevent GBS infection in infants aged over 7 days (late-onset GBS disease) or in adult patients [8], and several studies have reported an increase in the rate of GBS invasive infections among adults over the past few years [2,9,10].

Abbreviations: GBS, group B streptococcus; MLST, multilocus sequence typing; ST, sequence type; CC, clonal complex.

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The most promising approach for the prevention of GBS-induced invasive disease is the development of an effective vaccine to prevent infection not only in neonates but also in the elderly [4,11,12].

Numerous studies have analyzed the variability of colonization by GBS, but most have focused on pregnant women [13], and only a few have investigated the prevalence of colonization among non-pregnant adults [3,6]. Investigation of the distribution of capsular serotypes and sequence types (STs) among GBS infection strains in neonates and pregnant women has revealed strong differences between these populations, suggesting that some GBS lineages are more prone to cause disease in neonates than in the women. Fewer data are available on the serotypes and MLSTs of strains colonizing the elderly and causing infections in this population. The objective of this study was to assess the GBS colonization rate and GBS antibiotic resistance profile in post-menopausal women in our area, using capsular serotyping and MLST to study possible correlations between capsular types and clonal complexes of GBS colonizing isolates.

2. Methods

The study was approved by the ethics committee of Virgen de las Nieves University Hospital in Granada (Southern Spain) in April 2010. Sample size calculation was performed to get a 3% accuracy, with a confidence interval 95% and assuming a prevalence of colonization GBS in adults 15% [14] Six hundred consecutive post-menopausal women attending the Emergency Department of the hospital for non-infectious conditions between March 2011 and February 2012 were enrolled in the study (none of them showed clinical signs of infection by *S. agalactiae*). The mean (SD) age was 65.4 (range: 55–70) years. One vagino-rectal swab was collected from each patient with informed consent, placed in amies transport medium and kept at room temperature. Within 12 h, swabs were inoculated onto a plate of Granada agar prepared in our laboratory. Haemolytic GBS strains were detected as orange–red colonies after 48 h of anaerobic incubation [15].

GBS colonies were sub-cultured in plates of Columbia blood for capsular typing and MLST. The capsular type of the strains was determined by multiplex PCR assays for types Ia to VIII [16] and type IX [17].

The ST was determined by MLST, seven housekeeping genes were PCR-amplified using oligonucleotide primers [18] using the *S. agalactiae* MLST database (http://pubmlst.org/sagalactiae). The e-BURST v.3 programme http://eburst.mlst.net) was used to assign the isolates to a clonal complex. The BURST algorithm first identifies mutually exclusive groups of related genotypes in the population (multilocus sequence typing [MLST] database), and attempts to identify the founding genotype (sequence type or ST) of each group. The algorithm then predicts the descent from the predicted founding genotype to the other genotypes in the group, displaying the output as a radial diagram centered on the predicted founding genotype.

The definition of the group can be changed, but the default eBURST setting is to identify groups of related STs using the most stringent (conservative) definition, according to which all members assigned to the same group share identical alleles at 6 of the 7 loci with at least one other member of the group.

The study results were compared with the *S. agalactiae* MLST database (http://pubmlst.org/sagalactiae). Antimicrobial susceptibility testing was performed by disc diffusion on agar plates of Muller-Hinton blood 5% following Clinical and Laboratory Standards Institute (CLSI) [19] indications; the antibiotic discs tested were penicillin 10 μg, erythromycin 15 μg and clindamycin 2 μg. (Biomérieux, Suecia)

Table 1
Capsular serotype distribution in our sample of postmenopausal women.

Capsular serotype distribution		
Serotype	*n*	%
Ia	22	20.6
Ib	4	3.7
II	15	14.0
III	37	34.6
IV	4	3.7
V	24	22.4
NT[a]	1	0.9
Total	107	100.0

[a] NT = non-typeable.

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3. Results

GBS were recovered from 107 (17.8%) out of the 600 vaginal-rectal specimens studied. The distribution of capsular types is shown in Table 1.

The most frequent types were Ia, III and V, which together accounted for 77.6% of strains. None of the isolates belonged to types VI, VII, VIII or IX. In one case (0.9%), the serotype of the strain could not be determined. The proportion of strains non-typeable by serological methods was not determined in our study, which exclusively used molecular capsular typing methods.

As shown in Table 2, the most frequent STs were 19, 23, 17 and 1, which together accounted for 76% of strains. In two cases, the ST of the strain was not be determined because no PCR products could be obtained from any of the seven alleles. The most frequent combinations of capsular type and ST were III-19 (14.9%), Ia-23 (14.9%) and V-1 (14%).

A clonal complex is a set of STs that are all believed to be descended from the same founding genotype. Using the stringent group definition described above (6/7 shared alleles), isolates in the group defined by eBURST are considered to belong to a single clonal complex. The e-BURST analysis grouped all isolates collected into three groups or clonal complexes (Figs. 1–3) whose founders were ST-19 (CC19), ST-23 (CC23) and ST-17 (CC17)., The CC19 complex comprised four subgroups founders: ST-1, ST-8, ST-12, ST-28. The CC17 complex comprised one subgroup founder: ST-2. No subgroups founders were found for CC23 (Figs. 1–3).

Regarding antibiotic susceptibility, all strains were sensitive to penicillin. The resistance rates of the isolates against erythromycin and clindamycin were 23.4% ($n = 25$) and 20.6% ($n = 20$), respectively. D-tests detected clindamycin induction in one case (0.9%). No direct relationship was found between a particular serotype or ST and resistance to macrolides.

4. Discussion

There has been little research on the prevalence of GBS colonization in post-menopausal women, which was found to be 17.8% in the present study, slightly higher than the prevalence of 15.9% detected in pregnant women in the same area between 2009 and 2011 [20].

The distribution of capsular types was similar in both population groups, with serotype III being the most frequent [20]. However, in comparison to the distribution generally reported in colonized pregnant women in Europe, we found a higher percentage of strains belonging to serotype V and a lower percentage belonging to serotype Ia in our population of post-menopausal women [21–23]. Serotype IV accounted for 3.7% of the strains in our study,

Table 2
Distribution of capsular serotypes and sequence types in our postmenopausal population.

Capsular serotype and sequence type									
		Capsular serotype (% on total)							Total (%)
		Ia	1b	II	III	IV	V	NT	
Sequence type	No ST	–	–	–	2 (1.9)	–	–	–	2 (1.9)
	1	2 (1.9)	–	–	–	–	**15 (14)**[a]	1 (0.9)	18 (16.8)
	8	–	4 (3.7)	–	–	–	–	–	4 (3.7)
	12	–	–	2 (1.9)	–	–	–	–	2 (1.9)
	17	–	–	–	13	–	–	–	13 (12.1)
	19	–	–	2 (1.9)	**16 (14.9)**[a]	–	7 (6.5)	–	25 (23.3)
	21	–	–	2 (1.9)	–	–	–	–	2 (1.9)
	22	–	–	7 (6.5)	–	–	–	–	7 (6.5)
	23	**16 (14.9)**[a]	–	–	–	2 (1.9)	2 (1.9)	–	20 (18.7)
	28	–	–	2 (1.9)	–	–	–	–	2 (1.9)
	42	–	–	–	2 (1.9)	–	–	–	2 (1.9)
	144	4 (3.7)	–	–	–	–	–	–	4 (3.7)
	179	–	–	–	2 (1.9)	–	–	–	2 (1.9)
	196	–	–	–	–	2 (1.9)	–	–	2 (1.9)
	520	–	–	–	2 (1.9)	–	–	–	2 (1.9)
Total		22 (20.6)	4 (3.7)	15 (14)	37 (34.6)	4 (3.7)	24 (22.4)	1 (0.9)	107 (100)

[a] Boldface numbers refer to the most frequent ST-capsular serotype combinations.

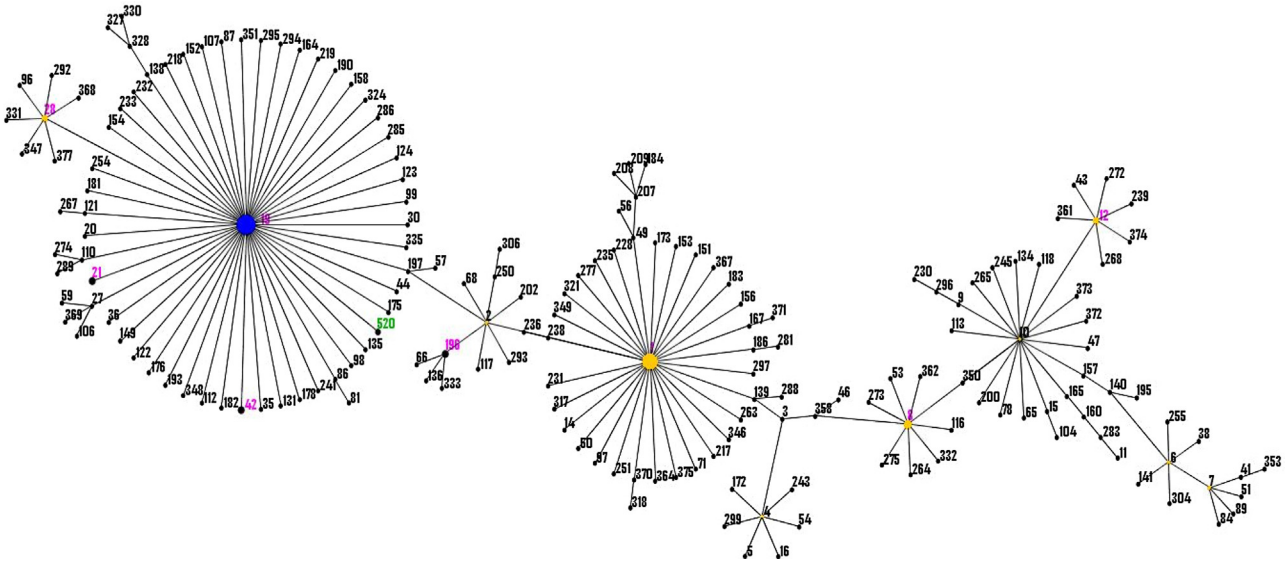

Fig. 1. Clonal complex 19.
Primary founder, Subgroup founder. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
STfrom strains included in MLST and our own databases, ST from strains included only in our own database
, ST from strains included only in MLST.

confirming the emergence of this serotype as reported elsewhere [24]. The epidemiological distribution of *S. agalactiae* serotypes can vary depending on several factor, including the geographical region, profile of the population being studied and source of the bacterial isolates.

The largest difference in strains between pregnant and post-menopausal women in our setting was in the MLST distribution, with the former showing a greater ST diversity and three new sequence types, i.e., 539–541 double-locus variants from ST-17 (CC17), ST-366 (CC23) and ST-233 (CC19), respectively (DEVANI project, unpublished data).

The ST distribution in the present group of post-menopausal women population was highly similar to that reported by other authors in different populations [18,25,26]. The most frequent ST obtained in both pregnant and post-menopausal women is ST-23 associated with serotype Ia, while it is frequently associated with ST17-III in pregnant women and is implicated in neonatal disease, being described as a GBS hyper-virulent clone [25,27,28]. In the present post-menopausal women, the most frequent associations were with III-ST19 and V-ST1, which are characterized as colonizer strains [18,29].

Molecular characterization studies of strains from non-pregnant [30] and pregnant [27] women in other European countries found that the most frequent ST was ST-19. This was the second most frequent type isolated in the present study after ST-23 and has been described as a colonizer and associated with invasive disease [18,31]. In our setting, ST 23 has been implicated in the aetiology of neonatal meningitis (Liébana-Martos, personal communication, 2010).

Some authors have reported an increase in capsular type IV, which belongs to ST complex CC17 and has demonstrated increasing resistance to macrolides [24,27]. In our study, this serotype

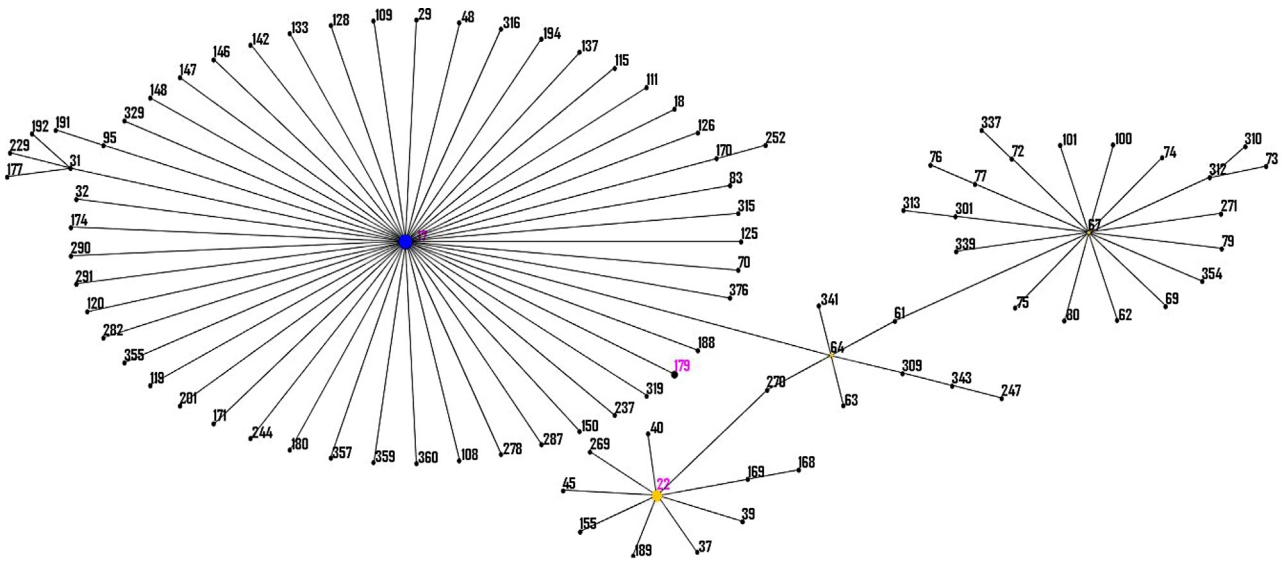

Fig. 2. Clonal complex 17.

Primary founder, Subgroup founder. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

STfrom strains included in MLST and our own databases, ST from strains included only in our own database, ST from strains included only in MLST.

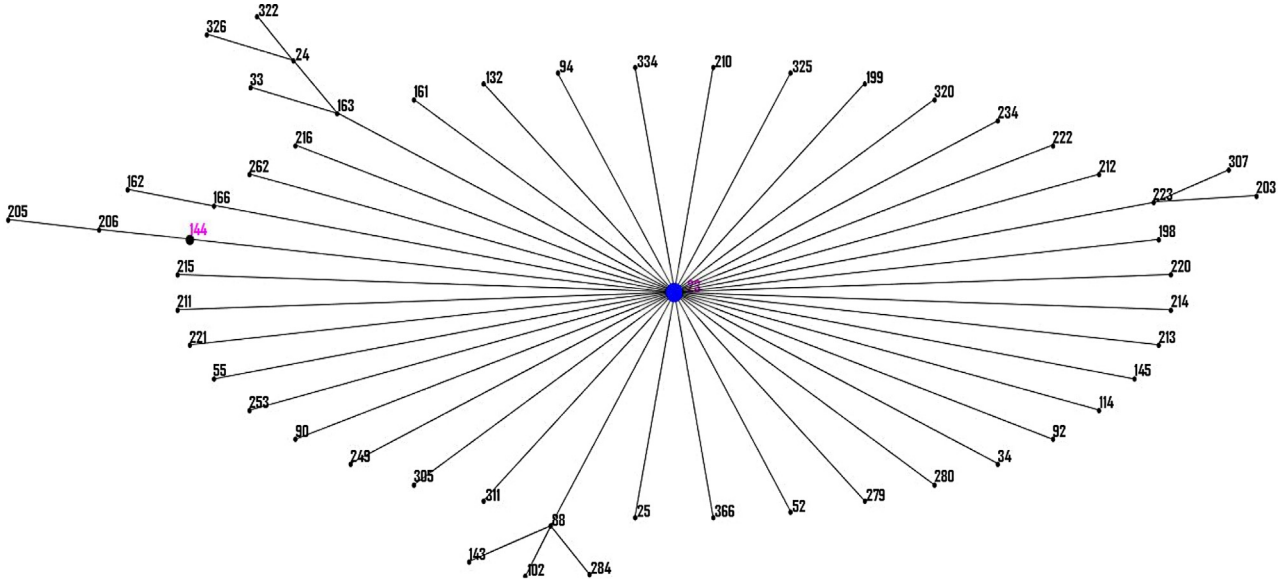

Fig. 3. Clonal complex 23.

Primary founder Subgroup founder. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

STfrom strains included in MLST and our own databases, ST from strains included only in our own database, ST from strains included only in MLST database.

was detected in four (3.7%) of the isolates (although two of them belonged to ST-23 and not to CC17), which also showed a high rate of resistance to macrolides.

Resistance rates in other European countries have ranged between 4% and 69% to erythromycin and between 2% and 14% to clindamycin [21,27,30,32]. In the present study, the rates of resistance to erythromycin and clindamycin were 23.4% and 20.6%, respectively, which were higher than previously observed among pregnant women in our setting [20]. This may be in part influenced by the higher percentage of strains with serotype V, which carries higher rates of antimicrobial resistance [33]. This serotype, associated with ST-1, is one of the most frequently isolated in our region and, although usually described as a colonizer, is also an etiological pathogen for adult infections, e.g., prosthetic joint infections [9,34].

Epidemiological surveillance of GBS strains and their phenotypic and genotypic characteristics is important to predict the spread of especially virulent clones.

The prevalence of GBS colonization in the post-menopausal women in our study is similar to that previously observed in women of child-bearing age. However, most of the serotypes and STs in samples from the former are associated more with colonization than with invasive disease, whereas a high proportion of isolates in the pregnant women in our area are associated with invasive disease (e.g., the hypervirulent clone ST17-III). Nevertheless, ST-23, which was the most frequent among our post-menopausal women, has also been associated with infection ([25,31], Liébana-Martos, personal communication 2010). Moreover, associated co-morbidities in this age group can predispose these women to GBS-cauzed disease. This fact, together with the increase in serotype V isolates, which are frequently associated with high antibiotic resistance rates, underlines the importance of monitoring this wide group of patients.

Finally, knowledge of the distribution of capsular types and MLSTs among post-menopausal women can contribute to the development of a polysaccharide vaccine for preventing GBS infection in older adults. The need for a GBS vaccine is therefore of utmost importance, although various questions must be addressed before the final development this vaccine. Will the vaccine completely replace standard-of-care antibiotics? Which vaccine formula/structure would be most effective (e.g., 3-valent or 5-valent conjugate vaccine). What is the optimal vaccination strategy? Would it be effective the same vaccine for pregnant women and adults?

Conflicts of interest

The authors declare no conflict of interest.

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