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Review

Simultaneous carriage of multiple serotypes of Group B *Streptococcus*: Systematic review and meta-analysis

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ABSTRACT

Background: Epidemiological studies evaluating the distribution of Group B *Streptococcus* (GBS) serotypes are crucial for serotype-specific vaccine development and post-licensure surveillance. However, there is a paucity of data about the prevalence of simultaneous carriage of multiple serotypes.
Methods: We conducted a systematic review of three databases (Medline, Embase, PubMed) to identify studies reporting GBS serotype co-carriage at the same anatomical site (multiple serotypes in one sample) or different anatomical sites (paired samples from one individual with different serotypes). We conducted a random-effects meta-analysis to evaluate the prevalence of co-carriage.
Results: 18 articles met the inclusion criteria, representing at least 12,968 samples from 14 countries. In a random-effects meta-analysis, we identified that 10 % (95 % CI: 4–19) of the positive samples taken from one anatomical site have more than one serotype, and 11 % (95 % CI: 5–20) of positive participants with samples taken from two anatomical sites carried different serotypes. When reported, the number of serotypes simultaneously carried ranged from 1 to 4. The serotypes most often associated with co-carriage are III (20.3 %), V (20.3 %) and Ia (19.5 %).
Conclusion: This systematic review demonstrates that co-carriage is a minor but definite phenomenon, but the data are too limited to give a precise picture of the current epidemiology. Co-colonisation detection needs to be taken into consideration in the design and methods of future GBS carriage surveillance studies to estimate and evaluate the potential for serotype replacement once vaccines are introduced.

Contents

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1. Introduction

Streptococcus agalactiae – also known as Group B *Streptococcus* (GBS) – is a commensal bacterium of the intestinal and genital flora, occasionally found in the throat and urethra. GBS is a leading cause of mortality and morbidity among neonates and young infants [1]. Thanks to the introduction of intrapartum antibiotic prophylaxis (IAP), the incidence of GBS disease has been much reduced. However, IAP coverage is not optimal, even in good screening settings, and it has no impact on preterm, stillbirths and acquisition of GBS after the first few days of life [2]. The bacterium may also cause invasive disease in pregnant women, the elderly, immunocompromised individuals, and adults with underlying health conditions [2]. Together with the current efforts to control the emergence of antimicrobial resistance, these are strong motivators to find another solution to combat GBS disease that does not include antibiotics [2]. One such solution is immunisation of pregnant women. Several maternal vaccines are currently under development. The most advanced candidate targets the capsular polysaccharide antigen of six of the ten known serotypes [3].

Epidemiological studies evaluating the worldwide distribution of GBS serotypes are crucial for serotype-specific vaccine development and post-licensure surveillance [2]. However, most studies serotype a single colony per clinical sample, which may introduce a bias towards the predominant and easiest-to-pick isolate rather than giving details about all the potential carried isolates [4,5]. There is a paucity of data about the prevalence of the carriage of multiple serotypes simultaneously. Knowledge about the possibility and frequency of co-carriage is needed to predict the risk of serotype replacement and potential horizontal gene transfer. This is specifically important for the genes leading to capsular switching, as a capsular polysaccharide vaccine might put a selective pressure on virulent strains to evade vaccine coverage [6,7]. Both phenomena have been observed after the introduction of the pneumococcal capsular polysaccharide vaccine [8].

In this regard, we undertook a systematic review of human GBS co-carriage, defined as the simultaneous carriage of multiple serotypes of GBS at one or multiple anatomical sites of a human individual, in the published literature up to November 2021.

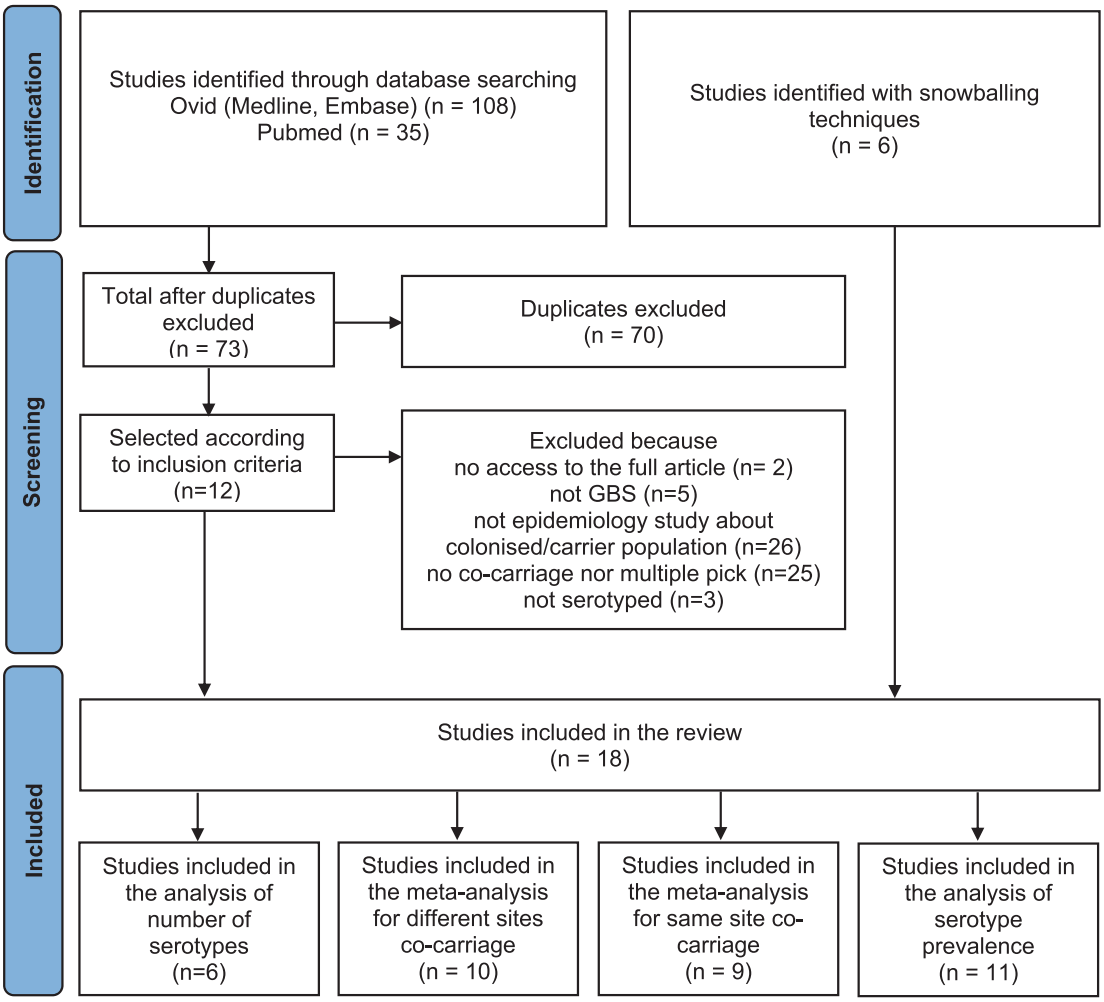

Fig. 1. Flow diagram of the data search and included studies.

Table 1
Abstracted data from the included studies. *Total samples or paired samples* represents the number of samples (or participants, if the co-carriage has only been reported per participant) or pairs of samples taken from the population and tested for GBS carriage. In the case of same sample analysis, anatomical sites separated by commas indicate that all these sites were serotyped for multiple carriage, while anatomical sites separated by OR indicate that co-carriage was reported per participant without disclosing which sample was concerned. *Total positive samples or paired samples* represents either the number of positive samples/participants (with the possibility of including multiple samples from the same individual at the same or different visits) or the number of pairs of samples from the same individual, with at least one positive sample and all of the positive samples serotyped (with the possibility of including the same sample in multiple pairs within the same individual if more than two anatomical sites are investigated). NR: Non-reported; yr: years.

1	Study	Country of collection	Year of collection	Type of co-carriage	Population (age)	Anatomical site(s)	Total samples or paired samples	Total positive samples or paired samples	Co-carriage events	Bias assessment score
2	Anthony et al., 1978 [13]	The USA	1973–1976	paired samples	Pregnant women (13–44 yr)	cervix, urethra	1488	NR	5	5.5/9
3	Anthony et al., 1981 [14]	The USA	1979–1980	paired samples	Pregnant women (NR)	genitals, rectum	295	64	1	5.5/9
4	Anthony et al., 1981 [14]	The USA	1979–1980	paired samples	Pregnant women (NR)	rectum, stool	135	33	1	5.5/9
5	Anthony et al., 1981[14]	The USA	1979–1980	paired samples	Pregnant women (NR)	genitals, stool	135	37	1	5.5/9
6	Ferrieri et al., 2004 [15]	The USA	1998–2000	paired samples	Non-pregnant women (18–30 yr)	vagina, rectum	NR	102	18	4/6
7	Whitney et al., 2004 [16]	Thailand, The Philippines, Zimbabwe, Myanmar, Ireland, the USA	1999–2001	paired samples	Pregnant women (23–31 yr)	cervix, vagina, urine	1308	128	1	6.5/9
8	Taylor et al., 2007 [17]	Australia	2003–2005	paired samples	Pregnant and non-pregnant women (18–50 yr)	vagina, anus	374	70	12	5.5/9
9	El Aila et al., 2009 [18]	Belgium	2007	paired samples	Pregnant women (NR)	vagina, rectum	150	36	4	3.5/6
10	Palmeiro et al., 2010 [19]	Brazil	2006–2008	paired samples	Pregnant women and healthy patients (0->64 yr)	rectum, urethra	NR	NR	1	3/6
11	Slotved et al., 2017 [20]	Ghana	2012–2013	paired samples	Pregnant women (<20–>30 yr)	vagina, rectum	400	107	1	8/9
12	To et al., 2021 [5]	The Gambia	2014	paired samples	Women post-delivery (>18 yr) and infants (0–89 days)	rectovaginal, breastmilk, nasopharyngeal, rectal	NR	NR	12	4/6
13	Furfaro et al., 2019 [21]	Australia	2015–2017	paired samples	Pregnant women (16–50 yr)	vagina, rectum	1381	337	35	8/9
14	Jisuvei et al., 2020 [22]	Kenya	2017	paired samples	Pregnant women (<25–>36)	vagina, rectum	288	53	30	7.5/9
15	Maurer et al., 1979[23]	The USA	NR	paired samples	Children (0–14 yr)	throat, anus, vagina	415	47	2	6.5/9
16	Hoogkamp-Korstanje et al., 1982 [24]	The Netherlands	NR	paired samples	Pregnant women (NR)	vagina, cervix, rectum	762	106	24	3/9
17	Anthony et al., 1978 [13]	The USA	1973–1976	same sample	Pregnant women (13–44 yr)	cervix OR urethra	1488	NR	4	5.5/9
18	Anthony et al., 1981 [14]	The USA	1979–1980	same sample	Pregnant women (NR)	stool, rectal, genitals	743	134	2	5.5/9
19	Ferrieri et al., 2004 [15]	The USA	1998–2000	same sample	Non-pregnant women (18–30 yr)	vagina OR rectum	NR	102	4	4/6
20	Taylor et al., 2007 [17]	Australia	2003–2005	same sample	Pregnant and non-pregnant women (18–50 yr)	vagina, anus	374	92	15	5.5/9
21	El Aila et al., 2009 [18]	Belgium	2007	same sample	Pregnant women (NR)	vagina OR rectum	150	36	11	3.5/6
22	Khatami et al., 2019 [4]	The USA	2010–2012	same sample	Non-pregnant women (18–55 yr)	vagina	433	91	6	4/6
23	Foster-Nyarko et al., 2016 [25]	The Gambia	2011–2012	same sample	Infants (2 months)	nasopharynx	1170	NR	2	6.5/9
24	Jisuvei et al., 2020 [22]	Kenya	2017	same sample	Pregnant women (<25–>36)	vagina OR rectum	292	53	7	7.5/9
25	Baker et al., 1976 [26]	The USA	NR	same sample	Non-pregnant women (NR)	vagina	210	79	4	3.5/6
26	Pérez-Ruiz et al., 2004 [27]	Spain	2001–2002	same (including rectovaginal)	Pregnant women (NR)	vagina-rectum	NR	30	1	3/6
27	To et al., 2021 [5]	The Gambia	2014	same (including rectovaginal)	Women post-delivery (>18 yr) and infants (0–89 days)	rectovaginal, breastmilk, nasopharyngeal, rectal	NR	96	31	4/6
28	Foxman et al., 2006 [1]	The USA	2001	unclear	Young adults (17–28 yr)	urine, rectum, vagina	977	NR	1	5.5/9

2. Methods

2.1. Definitions

Studies reporting at least one case of serotype co-carriage, defined as the simultaneous carriage of multiple serotypes of GBS at one or multiple anatomical sites of a human individual within a population of asymptomatic GBS-positive individuals were included, irrespectively of the sample type, culture, serotyping techniques, population type and size.

2.2. Search strategy

The published literature dated from 1946 up to the 2nd of November 2021 was searched using the Medline (1946–2021), Embase (1974–2021), and PubMed (1976–2021) databases with detailed search terms (Supplementary Material S1). The relevant articles were searched using snowballing techniques to identify additional related references. Abstracts were screened using the Rayyan software [9] by answering sequentially the questions (1) and (2), full-text articles were then screened to answer (3) and (4).

(1) Is the study about GBS?
(2) Does the study investigate multiple clinical samples from a population carrying GBS asymptomatically?
(3) Are co-carriage of multiple strains or multiple colony-picks mentioned in the study?
(4) Are the strains serotyped?

Data from the published studies and correspondence with their authors were abstracted into an Excel sheet by two independent reviewers (CB and MS), disagreements were resolved through discussion and with other reviewers (KLD and SL). Data are reported using the PRISMA guidelines [10].

2.3. Quality assessment

Each study was scored independently by two reviewers (CB and MS) according to questions adapted from the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Prevalence Studies [11]. Questions 1, 2 and 9 were not relevant for studies whose primary aim was not prevalence; thus, these studies were scored out of six while prevalence studies were scored out of nine. Each positive answer scores one point. Unclear or negative answers score zero points.

2.4. Data analysis

The data for the prevalence of co-carriage with two, three or four serotypes were collected from studies designed to identify more than two serotypes. Studies with more than two colony picks or reporting more than two serotypes carriage were included. The data for the serotype distribution in co-carriage events were collected from the studies giving a detailed composition of each combination. The data for the meta-analyses of co-carriage prevalence were collected from studies whose design could have had identified co-carriage at the same or different anatomical site. The data were analysed in RStudio 1.4.1106 with the *meta* 4.19–1 and *metafor* 3.0–2 packages. After double-arcsine transformation, a random-effects model with Der Simonian and Laired method was conducted to weigh the proportions, as described elsewhere [12]. Results are reported as means with 95 % confidence intervals.

3. Results

3.1. General characteristics

Out of the 79 identified studies, 18 met the inclusion criteria (Fig. 1), representing more than 12,968 samples from various populations, including pregnant and non-pregnant women, neonates, children, female and male adults and from 14 different countries, screened between 1973 and 2017 (Table 1). Each study was assessed for bias. The scores are reported in Table 1, and the details of each score are reported in Supplementary Material S2. The scores rank from 3 to 4 out of 6 for studies whose primary endpoint was not prevalence and from 5.5 to 8 out of 9 for prevalence studies (Table 1). The main weaknesses were low sample size and suboptimal serotyping methods, as not all ten serotypes were tested (Supplementary Material S2).

3.2. Number of co-carried isolates

The number of co-carried serotypes observed goes from two to four. However, some studies only refer to “more than one” or “different” serotypes in the same sample or individual. In the studies designed to identify more than two serotypes, co-carriage of two serotypes is more common than three or four (Table 2). One study found that three colony picks enable the accurate identification of all serotypes present for 91.1 % of the screened samples [15].

3.3. Prevalence of co-carriage at the same and different anatomical sites

In a random-effects meta-analysis of the studies reporting the incidence of co-carriage, 10 % (95 % CI: 4–19) of the positive samples/participants had more than one serotype at the same anatomical site. 11 % (95 % CI: 5–20) of participants with samples taken from two anatomical sites and at least one of these samples being positive carried different serotypes (Fig. 2A). The meta-analyses of same site co-carriage in pregnant women versus non-pregnant

Table 2
Prevalence of co-carriage with two, three or four serotypes in studies designed to identify more than two serotypes. Studies with more than two colony picks or reporting more than two serotypes carriage were included. NR: Non-reported.

1	Study	Colony picks (average)	Samples with 2 serotypes (%)	Samples with 3 serotypes (%)	Samples with 4 serotypes (%)	Total positive samples analysed
2	Baker et al., 1976	5	4 (5)	0 (0)	0 (0)	79
3	Ferrieri et al., 2004	10	20 (20)	2 (2)	0 (0)	102
4	Khatami et al., 2019	NR	5 (5)	1 (1)	0 (0)	91
5	Pérez-Ruiz et al., 2004	15	1 (3)	0 (0)	0 (0)	30
6	Taylor, 2006	NR	13 (14)	2 (2)	0 (0)	92
7	To et al., 2021	10	18 (19)	4 (4)	1 (1)	96

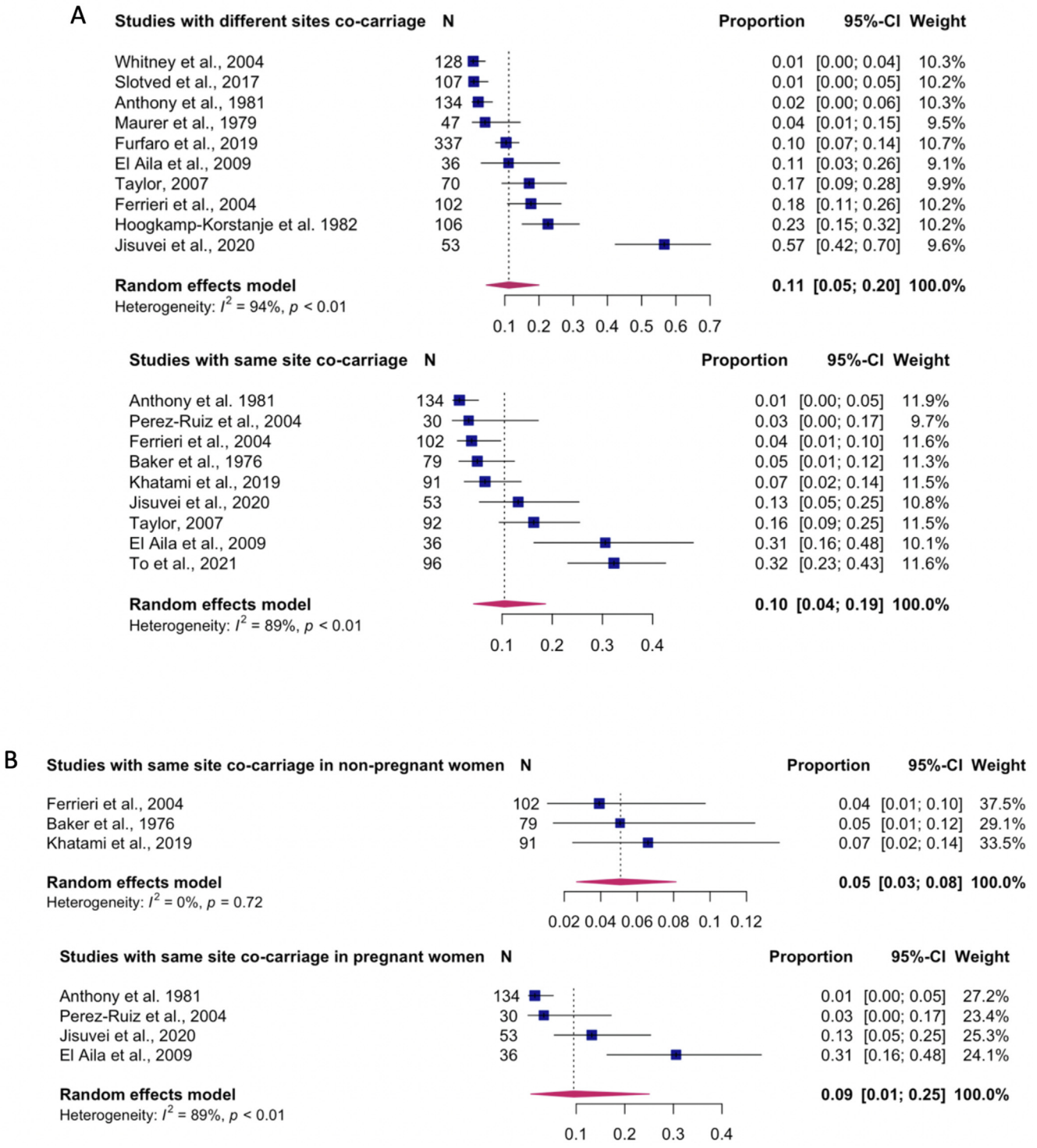

Fig. 2. A. Meta-analyses of the proportion of same and different site(s) co-carriage. Same site co-carriage proportion is defined as the number of samples/participants from which more than one serotype was recovered at the same anatomical site, among all positive samples/participants (N) identified during the study. Different sites co-carriage proportion is defined as the number of pairs of clinical samples taken simultaneously from the same individual at different anatomical sites and that retrieve discordant serotypes, among the total number of individuals who have given multiple samples with at least one of them being positive (N). B. Meta-analyses of the proportion of same site co-carriage defined as the number of samples/participants from which more than one serotype was recovered at the same anatomical site, among all positive samples/participants (N) in non-pregnant versus pregnant women. Random-effect models were used to weigh the studies.

women were unable to show a significant difference in prevalence (Fig. 2B). Moreover, the data we have do not allow to conclude on differences between male and female.

It is to be noted that Pérez-Ruiz and colleagues [27] and To and colleagues [5] found one and three rectovaginal swabs, respectively, each with two serotypes, which were counted as same site carriage in order not to bias the proportions. Considering that the measure of heterogeneity I^2 is high, a bias score was determined for each study (Table 1), but no study was excluded if the incidence data were available.

A

Combinations of 2 serotypes at the same site												
Ia	Ia											
Ib	2	Ib										
II	2		II									
III	6	2	3	III								
IV	4	1		2	IV							
V	4		6	6	4	V						
VI							VI					
VII						1		VII				
VIII				1		1			VIII			
IX										IX		
NT		1		1	2	1					NT	
Total	18	6	11	21	13	23	0	1	2	0	5	100

B

Combinations of >2 serotypes at the same site			
Ia	II	III	IV
NT	II	III	
Ia	Ib	V	
Ib	II	III	
Ia	Ib	III	
Ia	II	III	
Ia	II	V	
Ia	II	IV	
Ia	IV	V	

Total per serotype	
Ia	7
Ib	3
II	6
III	5
IV	3
V	3
VI	0
VII	0
VIII	0
IX	0
NT	1

C

		Ia	Ib	II	III	IV	V	VI	VII	VIII	IX	NT	Total
Same site	Number	25	9	17	26	16	26	0	1	2	0	6	128
	Percentage	19.5	7.0	13.3	20.3	12.5	20.3	0.0	0.8	1.6	0.0	4.7	100.0
All studies	Number	57	20	48	57	26	59	6	1	3	1	18	296
	Percentage	19.3	6.8	16.2	19.3	8.8	19.9	2.0	0.3	1.0	0.3	6.1	100.0

Fig. 3. Serotype distribution in co-carriage events. A. Combinations of two serotypes carriage and total occurrence per serotype. B. Combinations of more than two serotypes carriage and total occurrence per serotype. C. Summary of the serotype prevalence in co-carriage events, for same site co-carriage and all studies (same site, different sites and unclear). NT: non-typeable.

3.4. Prevalence of serotypes in co-carriage events

A review of the 59 cases of same site co-carriage with identified serotypes demonstrated that serotypes III and V are the most often co-carried (20.3 % each). This is followed by Ia (19.5 %), II (13.3 %), IV (12.5 %), and Ib (7.0 %). The most frequent combinations of two serotypes are Ia/III, III/V and II/V. Serotype Ia is the serotype most often associated with co-carriage of more than two serotypes (Fig. 3).

4. Discussion

Our systematic review shows that more than one serotype carriage is a minor but definite phenomenon. According to our data, this would be the case in 10 % (95 % CI: 4–19) in case of same site co-carriage and 11 % (95 % CI: 5–20) in case of different sites co-carriage. Given the limitations of the available data in terms of reported numbers and serotypes, we believe this to be a minimum estimate and we advocate for improved surveillance to better understand this phenomenon.

With a commercialised serotype-specific vaccine covering only a subset of the more than 90 known serotypes, *Streptococcus pneumonia* is of interest [4,6,28]. Co-carriage with multiple pneumococcus serotypes is reported as common in children in low and middle-income countries [29]. In a longitudinal study conducted in Indonesian infants, 34.9 % of the positive infants in a total sample size of 198 participants showed multiple serotypes carriage [29]. Given that we also report multiple serotype carriage with GBS, the possibility of serotype replacement and serotype switching after vaccine introduction is to be considered. Indeed, the introduction of the PCV7 vaccine was followed by the replacement of vaccine strains by non-vaccine strains both among carriers and in disease [28]. Capsular switching is a regular occurrence among pneumococcus strains [8] and drives evasion in the context of vaccine-induced pressure. Capsular switching among GBS strains has also been documented [6].

Some of the studies of our review suggest that co-carriage may only be a transient phenomenon. Anthony and colleagues found that carriage of multiple GBS serotypes is never associated with chronicity [13]. Furfaro and colleagues observed that co-carriage occurs « significantly less than what would happen by chance », meaning that the presence of one GBS serotype decreases the chance of acquiring a second one [21]. Murad and colleagues were able to differentiate pneumococcus serotype replacement, stable co-colonisation, and short-term colonisation, which represent, respectively, 12.6 %, 4.8 %, and 4.8 % among all colonisation events, making stable co-colonisation a rare event [29]. Longitudinal studies evaluating GBS colonisation are lacking to confirm this trend. The capacity of certain colonising strains to compete with others is important due to its implication for vaccine-induced pressure on the ecology of GBS colonisation. In the present review, co-carriages with serotypes III, V and, Ia were the most common. They are also the most prevalent maternal colonising serotypes in most regions [30]. Further research is needed to evaluate if the co-carriage bias toward these three serotypes comes from their global prevalence or if, inversely, their prevalence is due to a capacity to out-compete other serotypes and thereby replace them.

Nonetheless, the incidence of co-carriage could be underestimated: anatomical sites, culture techniques, and protocol design may all impact upon the detection of GBS, and thus the detection of the serotypes. The results of our meta-analyses must be taken cautiously because different methods and protocols were used, which makes it difficult to aggregate the data, as demonstrated by our bias assessment score. Indeed, the optimal number of colony picks to identify all carried serotypes has not been universally defined. In addition, studies assessing different site co-carriage may underestimate the prevalence because only one isolate per swab is serotyped. Our knowledge and capacity to identify all ten serotypes have improved with time, therefore older studies might

also underestimate the co-carriage phenomenon. Refined techniques such as sweep-agglutination, microarray, and multiplex PCR benefitted the detection of pneumococcal serotype co-carriage [31]. Recently, a Random Amplified Polymorphic DNA (RAPD) PCR protocol has been developed by To and colleagues to quickly screen the presence of multiple strains in clinical samples [5].

Serotype distribution varies geographically and historically, and carriage may have different implications in different populations, for example, pregnant women versus non-pregnant adults [30,32]. Our analysis was not able to find a significant difference in the prevalence of co-carriage between pregnant and non-pregnant women. Disaggregated analysis by age, population and geography, restricted to recent sample collections would be relevant to evaluate the serotype co-carriage combinations in different contexts. Non-epidemiological studies that would focus on a few participants and multiple colonies serotyping within host would be informative but feasibility would limit its applicability to inform worldwide serotype carriage and thus vaccine development.

Our data demonstrate that multiple GBS serotypes are present in a small number of carriage samples. This should encourage the design of improved epidemiological studies, able to detect multiple serotypes per participant sample, to monitor serotype distribution and replacement in preparation for the introduction of a capsular polysaccharide-based specific vaccine.

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Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.11.024.

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