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A large prospective study of group B Streptococcus carriage in pregnant women in Morocco: Prevalence, serotype distribution, antimicrobial resistance and virulence determinants

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Abstract

Background*:* Group B streptococcus (GBS) is a commensal bacterium that can cause severe infection in neonates. Vaginal colonization with GBS is a risk factor for mother-to-child transmission and, therefore, its monitoring is essential for prevention strategies. Epidemiological data on maternal GBS carriage and the characterization of recovered isolates in North African regions are scarce, mainly due to the lack of systematic GBS screening in pregnant women. Maternal vaccination based on the use of various capsular polysaccharides (CPS) remains a promising strategy for the control of GBS infection. Therefore, studying the distribution of CPS is of paramount importance to ensure vaccine efficacy in diverse and large populations. This investigation aims to provide the first large-scale epidemiological analysis in pregnant women in Morocco, regarding prevalence of GBS carriage and the main characteristics of circulating strains.

Methods: GBS screening was conducted between April 2021 and July 2024 on 1710 pregnant women at 35-40 weeks of gestation. Capsular serotyping was performed using multiplex PCR. Disk diffusion test was applied to evaluate antimicrobial susceptibility. Resistance genes and key virulence genes were detected by PCRs.

Results: The prevalence of colonization was 9.9%. The serotypes Ib, IV, II, V, Ia, and III were more prevalent, accounting for 19.8%, 17.1%, 14.3%, 13.7%, 12.3%, and 7.5% respectively. All GBS isolates were found to be susceptible to penicillin. Resistance to tetracycline was observed in 98.8% of strains, with a significant correlation to the presence of *tetM* gene. 6.4% of isolates were clindamycin resistant and 14.1% were erythromycin resistant, with a significant correlation to the *ermB* gene. In addition, *mefA*

gene was detected in 16.6% of erythromycin-resistant isolates. Regarding virulence genes, *scpB* was present in all tested isolates and *bac, fbsA,* and *cylB* were found in more than 70.0% of tested isolates, whereas *rib* gene was detected in 37.5% of tested isolates.

Conclusion*:* The serotype distribution indicates that a candidate vaccine based on hexavalent capsular polysaccharides (Ia, Ib, II, III, IV, and V) would prevent 85.0% of cases. Resistance to macrolides (erythromycin 14.1%) and lincosamides (clindamycin 6.4%), which are used as alternative therapies, was observed in a total of 20.5% of strains. This highlights the need for sustained epidemiological surveillance of GBS in the region.

Trial registration: Clinical trial number: not applicable.

***Keywords*:** Group B *Streptococcus*, maternal carriage, capsular polysaccharides serotype, antibiotic resistance, virulence factors

Background

Group B Streptococcus or *Streptococcus agalactiae* is a commensal bacterium of genital and digestive tracts that can cause maternal-fetal and neonatal infections (1). It is among the leading causes of severe perinatal infections occurring through vertical transmission from mother to newborn (2). Nowadays, the risk of infection for mothers and newborns is greatly reduced in countries that have implemented GBS screening during the last trimester of pregnancy, combined with intrapartum antibiotic prophylaxis (IAP) (3). This approach has particularly contributed to prevent neonatal early-onset disease which occurs during the first week of life (4). However, IAP does not provide effective prevention against late-onset disease occurring after the first week, hence the need for a maternal GBS vaccine as an alternative preventive strategy (5,6). Maternal GBS vaccination is being considered a promising strategy to prevent infection both in pregnant women and neonates through passive immunity (7). Therefore,

the implementation of GBS vaccines in low- and middle-income countries is considered a global health priority by the WHO (8). Vaccine candidates are currently in advanced clinical trials, among which GBS capsular polysaccharides seem promising in providing effective protective immunity (9). GBS can be classified into 10 different serotypes, Ia, Ib, II-IX, based on their specific CPS structure. The distribution of GBS serotypes exhibiting distinct virulence properties varies across different regions of the world (10). In addition to the capsular polysaccharides, several factors such as surface-associated proteins and enzymes contribute to the virulence of GBS. Among these factors, the *bac* gene encoding the β C protein, which is known for its role in adhesion to epithelial host cells and immune evasion through binding to IgA and complement components; the *fbsA* gene that encodes the fibrinogen-binding protein A, which facilitates fibrinogen binding and contributes to bacterial adhesion; the *rib* gene encoding the Rib surface protein, which is associated with resistance to opsonophagocytosis; the *cylB* gene, the product of which is essential for hemolysin synthesis; and finally, the *scpB* gene encoding the C5a peptidase that disrupts complement-mediated immunity. Giving their important role in virulence, these factors are considered relevant targets for vaccine development (11,12).

Around 20 million pregnant women across the world were colonized by GBS (7). Despite its clinical significance, data on maternal GBS carriage remain limited in several low-and-middle-income regions, including North Africa (13). Given the lack of routine GBS screening in pregnant mothers, available data are often limited, and mainly concern studies on small

cohorts and of short duration (14,15). The more recent investigation in Morocco was conducted in 2018 in a public maternal tertiary level hospital in Rabat on 350 pregnant women over a period of 5 months. The study revealed a prevalence of GBS vaginal carriage of 8.0% with a predominance of serotypes II, V, and III (14). The need therefore exists for further investigations to provide accurate rate of maternal colonization and serotype distribution on a large cohort to better evaluate the risk of GBS congenital transmission. In this study, we conducted a prospective study in pregnant women during 3 years with the aim to assess vaginal GBS carriage and to analyze the characteristics of recovered isolates regarding capsular serotypes, antibiotic susceptibility profile, and virulence factors.

Methods

Cohort recruitment

This prospective study was conducted from April 2021 to July 2024 at the maternity unit of Ibn Rochd University Hospital Center- Abderrahim El Harrouchi Hospital of Casablanca, Morocco. It is a reference center for monitoring maternal pregnancy receiving a large flow of pregnant women in the region. Pregnant women were recruited upon routine clinical examinations, and screened for GBS vaginal colonization during the third trimester (35-40 weeks) of gestation. The inclusion criteria were parturient aged over 18 who were admitted to the hospital for labor and who have not received antibiotic therapy during 15 days preceding the screening.

Sample collection and isolation of GBS strains

A vaginal swab was performed according to the recommendations of the Centers for Disease Control and Prevention (CDC, 2010) guidelines (16). The enrichment step was not applied; the samples were cultured directly for 18-24 hours at 37°C in ready-to-use Columbia agar medium with nalidixic acid (15 µg/ml) and colistin (10 µg/ml), supplemented with 5% sheep blood agar medium (bioMérieux, Marcy-l'Étoile, France) (17). In case of detection of beta-hemolytic colonies, biochemical identification was carried out using the API® 20 Strep system (bioMérieux, Marcy-l'Étoile, France), and specific identification of group B streptococcus was performed using latex agglutination reactions of Lancefield serogroups (Thermo Scientific™ Oxoid™ Streptococcal Grouping Kit using Latex Agglutination, Basingstoke, United Kingdom) according to the manufacturer's instructions. Recovered isolates were stored at -80°C in brain heart infusion broth containing 20% glycerol until use.

Phenotypic characterization of antimicrobial susceptibility

The colonies were sub-cultured onto fresh blood agar and incubated for 24h in 5% CO_2 at 37°C for antibiotic susceptibility testing, using disk diffusion method on Mueller-Hinton agar supplemented with horse blood (bioMérieux, Marcy-l'Étoile, France) according to the European Committee on Antimicrobial Susceptibility Testing EUCAST/CA-SFM 2021 guidelines (18). The following antibiotics (Thermo Scientific™ Oxoid™, Basingstoke, United Kingdom) were tested: penicillin G, gentamicin, erythromycin, clindamycin, tetracycline, tigecycline, linezolid, norfloxacin, rifampicin, chloramphenicol, and trimethoprim-sulfamethoxazole.

Penicillin MICs were determined in part (n=60) of recovered isolates using E-test strips (bioMérieux, Marcy-l'Étoile, France) following EUCAST/CA-SFM 2021 recommendations.

Capsular polysaccharides typing and molecular characterization of isolates

DNA extraction was performed from fresh bacteria culture using Canvax HigherPurity™ Bacterial Genomic DNA Isolation kit (Canvax Biotech, S.L., Cordoba, Spain) according to the manufacturer's instructions. Extracted DNA was stored at -20□C until use. Capsular polysaccharides serotyping was investigated using multiplex PCR and the housekeeping genes *recA* or 16SrRNA were used as internal control of the amplification in non-typeable isolates (19,20). For genotypic characterization of antimicrobial resistance, we used conventional PCR to identify relevant genes for erythromycin and tetracycline as previously described (17,21). The corresponding primers are listed in Table 1.

Table 1 : Primers used for the amplification of resistance genes

Gene	Primer sequence (5'□3')	Amplicon size (bp)	Annealing temperature (□C)	Reference
tetM-F	GTGGAGTACTACATTTACGAG	359	55	(21)
tetM-R	GAAGCGGATCACTATCTGAG			
tetO-F	GCGGAACATTGCATTTGAGGG	538	55	(21)
tetO-R	CTCTATGGACAACCCGACAGAAC			
tetT-F	CAGTGGGAATATAAGGACACGTC	644	55	(21)
tetT-R	CAAGCCTTCTCTACAGCATC			
int-Tn-F	GATGGTATTGATGTTGTAGG	528	55	(21)
int-Tn-R	GGTCTATATTGACAAGACG			
ermB-F	GGTAAAGGGCATTTAACGAC	454	55	(21)
ermB-R	CGATATTCTCGATTGACCCA			
mefA-F	AGTATCATTAATCACTAGTGC	328	55	(21)
mefA-R	TTCTTCTGGTACTAAAAGTGG			

bp: base pairs, F: Forward, R: Reverse

Detection of GBS virulence factors was carried out on isolates recovered during 2024 (n=40), by conventional PCR assays according to Bobadilla et al. 2021 (22). The specific primers used for each gene are listed in Table 2. Amplification products were analyzed by agarose gel electrophoresis using 6× gel loading dye (GDSBIO, Guangzhou, China). The identity of the amplified fragments was confirmed by DNA sequencing. Representatives of the amplified DNA fragments were sequenced using Illumina next-generation sequencing workflow (Novogene, UK). The search for nucleotide comparison was performed using BLASTn, which resulted in 99% identity with 98% coverage and an e-value cutoff of 10^{-7}.

Table 2 : Primers used to amplify virulence-associated genes

Gene	Primer sequence (5'□3')	Amplicon size (bp)	Annealing temperature (□C)	Reference
bac-F	TGTAAAGGACGATAGTGTGAAGA	530	50	(22)
bac-R	CATTTGTGATTCCCTTTTGC			
rib-F	CAGGAAGTGCTGTTACGTTAAAC	369	50	(22)
rib-R	CGTCCCATTTAGGGTCTTCC			
scpB-F	AGCCATATGCTGCGATCTCT	198	58	(22)
scpB-R	GGGTTGAACCAAGTGTGCTT			
cylB-F	GGGCTGCAGGTATTATCGAA	176	58	(22)
cylB-R	ATTTCCACCAAAAGCAAACG			
fbsA-F	TGTAGCTAATGGACCGATGTT	156	58	(22)
fbsA-R	TTTTCATTGCGTCTCAAACC			

bp: base pairs, F: Forward, R: Reverse

Statistical analysis

The statistical analyses were performed using R software version 4.4.2 □R Core Team, 2024□. The chi-square test and Fisher exact test were used to

examine the association and the difference between variables. Significance was defined as *p*-value < 0.05.

Results

1. Demographic characteristics of the study cohort

A total of 1710 healthy pregnant women were recruited for this study from 2021 to 2024. The characteristics of recruited pregnant mothers are listed in Table 3. The average age of the enrolled participants is 30 years (standard deviation (SD) 6.74; range 18-47 years), while gestational age at the time of clinical examination ranged from 35 to 40 weeks. Nearly half of the screened pregnant women were pauciparous [47.89% (819/1710)].

2. Prevalence of maternal GBS carriage

Out of 1710 vaginal samples, 170 were positive for GBS, indicating a prevalence of colonization of 9.9% (95% CI: 8.6-11.5%). The prevalence of colonization remained relatively stable over the three-year study period, ranging from 9.1% to 10.5% with no significant change over time (*p*=0.991). The distribution of GBS carriage was assessed based on demographic and obstetric data. As shown in Table 3, no significant effect of age, gestational age or parity was observed on GBS carriage (P>0.05).

Table 3 : Prevalence of Group B Streptococcus carriage according to demographic and obstetric data. A *p*-value <0.05 is considered statistically significant.

Demographic and clinical factors	Positive vaginal swabs (n=170)	Negative vaginal swabs (n=1540)	Total (n=1710)	*p*-value (chi-square test)

Age (years)				
< 24	40	354	394	
25 – 31	54	526	580	0.728
32 – 38	49	460	509	
> 39	27	200	227	
Gestational age (weeks of gestation)				
	30	211	241	0.315
35 – 36	71	709	780	
37 – 38	69	620	689	
>39				
Parity				
Primiparous	54	455	509	
Pauciparous	87	732	819	0.284
Multiparous	25	277	302	
Grand multiparity	4	76	80	

3. Capsular serotype distribution in GBS isolates

Diverse distribution of serotypes among GBS isolates was observed (Table 4). Examples of multiplex PCR data are shown in supplementary figure S1. The most prevalent serotype was Ib, accounting for 19.8% of the isolates, followed by IV **(**17.1%), and II (14.3%). Other notable serotypes included V (13.7%), Ia (12.3%), and III (7.5%). Less frequent serotypes were VI and VII, each representing 5.4% of isolates. No isolates belonging to serotype VIII were detected and 4.1% of the isolates were classified as non-typeable (NT).

Table 4 : Serotype distribution among GBS isolates (n=146/170, p<0.001), Chi-square test significant value $p<0.05$

Serotype	**Ia**	**Ib**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**NT**
No of isolates (%)	**18 (12.3)**	**29 (19.8)**	**21 (14.3)**	**11 (7.5)**	**25 (17.1)**	**20 (13.7)**	**8 (5.4)**	**8 (5.4)**	**0**	**6 (4.1)**

4. Antimicrobial susceptibility of GBS isolates

All GBS isolates were tested with a panel of antibiotics. 100% (n=170) of GBS isolates were susceptible to penicillin G, linezolid, rifampicin, chloramphenicol, and trimethoprim-sulfamethoxazole, while 99.4% (n=169) and 90.0% (n=153) were susceptible to tigecycline and norfloxacin, respectively. In contrast, 98.8% were resistant to tetracycline, while 14.1% (n=24) and 6.5% (n=11) exhibited resistance to erythromycin and clindamycin, respectively. Of note is that all erythromycin-susceptible isolates were susceptible to clindamycin. Next, we examined whether resistance profile to tetracycline and erythromycin were linked to given serotypes (Table 5). For tetracycline, there was no significant association between serotype distribution and resistance pattern ($p = 0.630$). However, it showed a trend toward significance in erythromycin resistance across serotypes (p=0.051).

Penicillin MICs were determined in part (n=60/170) of isolates using E-test method. They were all within susceptible range. The MIC range was 0.023–0.0645 µg/mL, with MIC_{50} and MIC_{90} of 0.0645 µg/mL. Distribution analysis showed that 58.3% (n=35) of isolates had MIC of 0.0645 µg/mL, 23.3% (n=14) had 0.047 µg/mL, 10.0% (n=6) had 0.032 µg/mL, and 8.3% (n=5) had 0.023 µg/mL.

Table 5 : Tetracycline and erythromycin resistance of GBS serotypes. A p-value <0.05 is considered statistically significant.

Serotype	Tetracycline resistance (No. of isolates)	*p*-value (chi-square test)	Erythromycin resistance (No. of isolates)	*p*-value (Fisher exact test)
Ia (n=18)	18 (100%)		6 (33.3%)	
Ib (n=29)	29 (100%)	0.630	3 (10.3%)	0.051
II (n=21)	20 (95.2%)		1 (4.7%)	

III (n=11)	11 (100%)	4 (36.3%)
IV (n=25)	25 (100%)	2 (8.0%)
V (n=20)	19 (95.0%)	4 (20.0%)
VI (n=8)	8 (100%)	2 (25.0%)
VII (n=8)	8 (100%)	0 (0%)
VIII (n=0)	-	-
NT (n=6)	6 (100%)	2 (33.3%)

5. Molecular characterizations of GBS isolates

Identification of antimicrobial resistance genes

To assess the molecular basis of the antibiotic resistance profile, we performed PCR tests to identify relevant genes potentially involved. Target genes were selected based on previous studies that highlighted their epidemiological significance in different geographic regions (21,23). Among the various *tet* genes tested, the *tetM* gene was detected in 100% of tetracycline-resistant isolates, while *tetT* was identified in 7.5%, and *tetO* was not detected in any of resistant isolates. Additionally, the *intTn* gene, known for its association with transposon-mediated resistance was detected in 80.0% of isolates. Regarding erythromycin, we tested erythromycin resistance methylases, *ermB* gene, involved in reducing the ability of macrolide antibiotics to bind the ribosome, together with macrolide efflux gene A (*mefA*). *ermB* and *mefA* were identified in 75.0% and 16.6% of erythromycin-resistant isolates, respectively. However, three (12.5%) of the erythromycin-resistant isolates did not carry any of these genes. For clindamycin-resistant strains, 72.7% of these isolates carried the ermB gene. Representative examples of identified genes are presented in supplementary figure S2.

Identification of key virulence genes

We specifically analyzed isolates recovered during the latter study period of the study to provide a current, an up-to-date representation of the virulence profile of circulating strains. The distribution of serotypes was as following: Ib (n=11; 27.5%), II (n=7; 17.5%), III (n=7; 17,5%), IV (n=5; 12.5%), Ia (n=4; 10.0%), V (n=4; 10.0%), VI (n=1; 2.5%), and VII (n=1; 2.5%). Notably, these strains cover the six most frequent serotypes that were identified among all tested GBS isolates. As shown in Table 6, we found varying frequencies of virulence factors: The *bac* gene was found in 70.0% of isolates. The *fbsA* gene was detected in 90.0% of strains. The *rib* gene was identified in 37.5%, while the *cyIB* gene was detected in 87.5% of isolates. Furthermore, the *scpB* gene was present in all GBS isolates. We observed significant difference in the distribution of virulence genes among the strains, with some genes being detected at higher frequencies in some isolates compared to others ($p<0.001$). These results highlight the potential virulence of circulating GBS strains.

Table 6 : Distribution of virulence genes among GBS strains (n=40)

Virulence factors	**Number of positive isolates**	**Percentage (%)**
bac	28/40	70.0%
fbsA	36/40	90.0%
rib	15/40	37.5%
cyIB	35/40	87.5%
scpB	40/40	100%

Next, we examined whether the distribution of virulence genes vary among the six most predominant GBS serotypes (Figure 1). The results show that *scpB* was present in 100% of isolates across all serotypes: Ia (n=4/4), Ib (n=11/11), II (n=7/7), III (n=7/7), IV (n=5/5), and V (n=4/4). The *cyIB* was detected in 75.0% to 100% of GBS isolates: Ia (n=3/4), Ib (n= 9/11), II (n=6/7), III (n=7/7), IV (n=5/5), and V (n=3/4). The *bac* gene was more frequent in serotypes Ia (n= 4 /4), Ib (n=11/11), and III (n= 6 /7), than in IV (n= 1/5) and V (n= 1/4), while the *rib* gene was predominantly found in serotypes III (n= 7/7) and Ia (n= 3/4). This indicates substantial differences in the presence of virulence genes among the six most frequent circulating GBS serotypes.

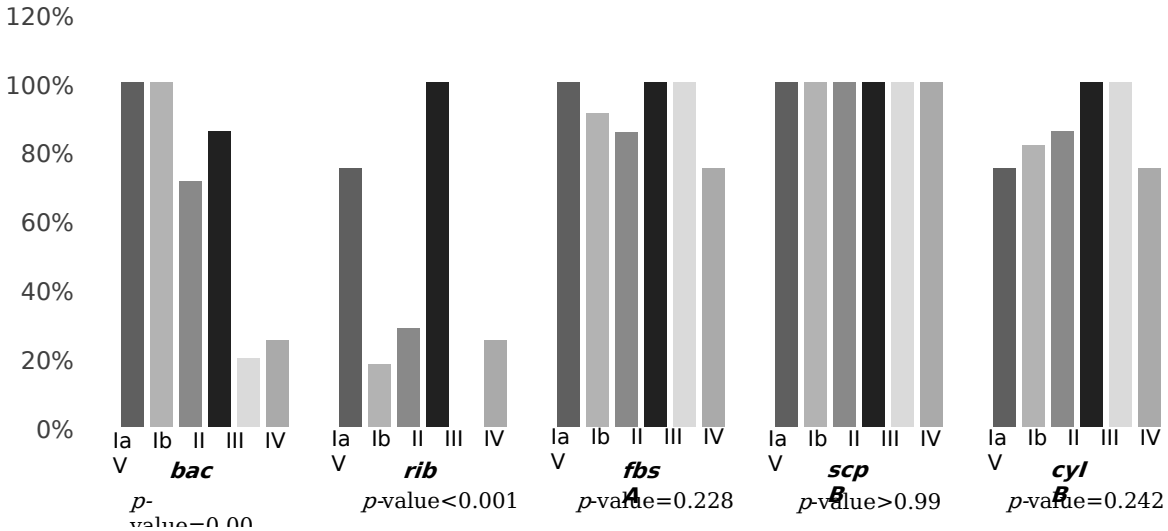

Figure 1 Distribution of virulence genes within GBS serotypes (n=40). *ScpB* and *cyIB* genes are carried by the majority of serotypes with *scpB* present in 100% of isolates across all serotypes and cyIB is detected in 75.0% to 100% of them. The *bac* gene was more frequent in Ia, Ib, and III (100%, 100% and 85.7% respectively), with lower presence in IV and V isolates (20.0% and 25.0% respectively). While *rib* gene is more frequently present in serotype III (100%).

Discussion

Group B streptococcus remains a major health issue for the mother-newborn dyad worldwide. This is particularly true in many developing countries, given the lack of systematic screening of pregnant women and the implementation of preventive treatments in the perinatal period. To our knowledge, this is the first study in a North African country, Morocco, conducted on a large cohort of pregnant women and which provides a comprehensive assessment of the GBS carriage rate, the distribution of capsular serotypes, the antibiotic resistance profile, and the identification of genes potentially involved in antimicrobial resistance and in virulence.

The prevalence of vaginal GBS carriage in our study was 9.9%. This is slightly higher compared to 8.0% reported in a previous investigation performed in another region, Rabat, in 2013 on 350 pregnant women over a period of 5 months (14). Earlier study carried out in 2012, in the region of Marrakech, including 240 pregnant women, showed a vaginal carriage of 3.3% (15). Regarding studies in other North African countries, a Tunisian study reported a colonization rate of 27.0% among pregnant women at 35–37 weeks of gestation (24). In Egypt, a prevalence of 27.4% was reported in a cohort including both pregnant and non-pregnant women, while another study reported a prevalence of 26.5% specifically among pregnant women (25,26). This difference in GBS carriage between regions of the same country or different countries can be attributed to number of parameters including geographic and demographic factors, clinical practice, sampling methodology, and diagnosis methods. Furthermore, it is likely that the cohort size and duration of the study as well as the gestation period may explain such a discrepancy. The

prevalence of GBS colonization remains, however, lower compared to other regions of Africa, such as West Africa (33.7%) South Africa (37%), and East Africa (21.3%) (13,27–29).

Our study provides an update regarding CPS typing of circulating GBS in Morocco. This is important in the context of current investigations aiming to develop CPS-based GBS vaccine. Vaccine candidates such as hexavalent GBS, which targets six major capsular serotypes (Ia, Ib, II, III, IV, and V), are under clinical trials (5). The inclusion of geographically specific data is therefore critical for global vaccine efficacy (30). Variant frequencies of serotypes were found among recovered isolates with the predominance of serotype Ib followed by IV, II, V, Ia, and III, representing 85% of circulating strains. Thus, the potential coverage of an hexavalent (Ia, Ib, II, III, IV, and V) GBS conjugate vaccine candidate would be about 85% (31). Although serotypes Ia, III, and V are predominantly found in several studies, a notable increase in the frequency of serotype Ib is observed (13). Indeed, Ib has been progressively recognized as an emerging serotype in both maternal and neonatal infections. Previous studies have shown that serotype Ib strains are often associated with specific clonal complexes (CC10 and CC12), characterized by distinct antimicrobial resistance profiles and invasive potential (32,33). Regarding Morocco, a previous study performed in pregnant women in Rabat, indicated that serotypes V, II, and III were most frequent (14). This difference could be attributed to the smaller cohort size and shorter study period in that study, but also to genetic diversity. However, the prevalent serotypes found in our study were comparable to those reported in sub-Saharan Africa (34). In North

Africa, several studies also reported varying frequencies of GBS serotypes. In Egypt, serotype V was the most prevalent among isolates, followed by serotypes II, III, and Ia (25). The predominance of serotypes V, II, and III has been also reported in a cohort study concerning both Algerian and French isolates (35). The Regional variability in serotype frequency can also be influenced by environmental and genetic factors (36). Hence the need for continuous monitoring of the evolution of the epidemiological situation in the North African region compared to sub-Saharan Africa.

Our analysis of antibiotic susceptibility patterns provides an up-to-date overview of the current situation in light of the global emergence of drug resistance worldwide, such in parts of Asia and Africa (37,38). All isolates were susceptible to penicillin, the drug of choice used as standard first-line treatment. However, high rate of resistance was observed for tetracycline (98.8%), while it was lower for erythromycin (14.1%) and clindamycin (6.4%). These findings align with global trends, where high resistance rates to tetracyclines and macrolides has been documented in different African countries (39,40) as well as in Europe (41) and in South America (42). Regarding macrolides and lincosamides that are used as alternative therapy in cases of allergy to beta-lactams, we observed a slightly higher rate (14.1%) of resistance to erythromycin compared to the most recent study conducted in Morocco reporting a resistance rate of 9.0% (14). However, the rate of clindamycin resistance (6.4%) was comparable to that previously reported (6.0%) (13). This remains, however, lower compared to other countries like in Ethiopia (26.5% for erythromycin and 21.4% for clindamycin) and Brazil (70.8% for erythromycin and 58.3% for

clindamycin) (39,42). It should be noted that our testing did not include the D-test, hence limiting the interpretation of clindamycin resistance. This should be considered in future studies for accurate phenotypic characterization.

Several factors may contribute to the emergence and spread of such drug resistance, including the inappropriate and widespread use of antibiotic treatments in pregnant mothers as well as in livestock and the horizontal transfer of resistance genes (43). We found *tetM* in all the tetracycline resistant isolates and *intTn* in 80.0% of them. However, *tetT* gene was found at low frequency (7.5%). Similar high frequency of *tetM* was reported in 2015 on GBS isolates in a cohort study from Algeria and Marseille, France (35). This seems to be also the case in other regions like in Europe (92.0%), South America (92.5%) and South Africa (95.8%) (41,42,44). *TetO* was not detected in any of our isolates, while lower rates were observed in other regions such as in South Africa (1.3%), France (3.8%) and Brazil (10.4%) (37–39).

Regarding erythromycin resistance, we identified *ermB* in 75.0% of GBS erythromycin-resistant isolates. This finding is similar to that reported (73.5%) on GBS isolates in a cohort study from Algeria and France (35), but remains higher compared to other regions like in Brazil (48.2%) (42). Whereas for clindamycin-resistant strains, 72.7% of these isolates carried the *ermB* gene. However, discordances between phenotype and genotype were observed. For instance, a few isolates carrying *ermB* (n=2) remained susceptible to clindamycin, which could be explained by lack of gene expression, presence of genetic mutations affecting functionality, or

regulatory mechanisms (17). Similar findings were reported in Southern Africa (17). In our study, *mefA* was detected at lower rates (16.6%) compared to *ermB* (75.0%). The same trend has been reported in France (41). Whereas, in South Africa *ermB* was found in 55.0% of isolates and *mefA* gene in 3.4% (45). Notably, 12.5% of erythromycin-resistant isolates did not harbor any of the genes studied. This phenotype may be related to other genes that were not investigated here such as *ermA*, *erm(T), erm(TR)* or *erm(A/TR)* (15, 16, 33, 34). Therefore, it would be of value to include in future studies other relevant genes to provide a more comprehensive overview on macrolide resistance. Noteworthy, the high prevalence of *tetM* and *ermB* genes has largely been attributed to their association with conjugative transposons such as the Tn916-Tn1545 family, which facilitate their horizontal transfer (46,47). Such mechanisms contribute to the dissemination and increased prevalence of macrolides, lincosamides, and tetracycline resistance (48). A number of studies have shown that resistance to erythromycin is not evenly distributed, with the highest rates observed in serotype III, Ia and V isolates, while lower rates were found in serotype IV (49–52). Our study revealed that erythromycin resistance follows a similar pattern, being more common in serotype III (36.3%), while serotype IV exhibited lower resistance levels (8.0%). This could be related to the association of serotype III with multiple gene transfer events (51,53). Serotype III is considered highly virulent accounting more frequently for neonatal early onset diseases (9,13). The distribution of serotype III in our study is quite low (7.5%), yet, when exploring the distribution of virulence

genes according to capsular serotypes, we observed that *rib* gene was mainly present in serotype III, in accordance with previous studies (22,52). Noteworthy, studies on virulence gene profiling remain limited in Africa compared to Europe and Asia where investigations have reported diversity of virulence factor distribution (17,22,23,54). Our investigation on the profiling of key virulence factors involved in adhesion, invasion, and immune evasion revealed variable gene distribution with *scpB* being detected in all GBS isolates (100%), while *fbsA, cyIB, bac,* and *rib* genes were detected in 90.0%, 87.5%, 70.0%, and 37.5%, respectively. In addition to the hexavalent CPS-based vaccine, protein-based vaccine candidates such as GBS-NN vaccine which is based on the N-terminal domains of Rib and AlphaC surface proteins, were also shown to be promising (55,56). Consequently, the study of the distribution of the *rib* gene among circulating GBS strains has important clinical relevance.

Taken together, our investigation, the first to be conducted in a large cohort in North Africa, fills an important gap related to currently circulating GBS serotypes and the molecular characteristics of vaginal colonizing strains in Morocco regarding drug resistance and virulence gene distribution.

The present study has however some limitations. First, although conducted in one of the country's major university hospitals, which receives a large flow of patients from the region, the investigation remains monocentric reflecting a single geographic region and therefore may not reflect the epidemiology of GBS in other regions. Second, the serotyping was performed on the majority, but not in all recovered isolates. Third, the

analysis of virulence factors was performed only on isolates recovered during the latter study period, with the aim to provide an up-to-date current representation of the virulence profile of the circulating strains. Finally, penicillin MIC values were determined in a subset of isolates and the macrolide susceptibility tests did not include testing for the inducible MLSB phenotype, hence limiting the accurate characterization of clindamycin resistance. Further studies in the region of North Africa integrating molecular and phenotypic approaches are needed to confirm and expand our findings.

Conclusion

This study represents the most comprehensive investigation of GBS maternal colonization in Morocco, providing important epidemiological data on a large cohort over a long period. Our findings stressed the need of implementing systematic screening of pregnant women for better control of perinatal diseases and for continuous monitoring of serotype prevalence and antimicrobial resistance. Furthermore, our data add valuable insights to the global understanding of GBS epidemiology in North of Africa, particularly in the context of vaccine development, by identifying the predominant serotypes and their associated virulence factors.

Abbreviations

GBS: Group B Streptococcus; CPS: Capsular polysaccharides; IAP: intrapartum antibiotic prophylaxis; CDC: Centers for Disease Control and Prevention; CNA: Columbia Nalidixic Acid agar; MIC: Minimum Inhibitory Concentration; DNA: Deoxyribonucleic acid; PCR: Polymerase chain reaction; bp: base pairs; SD: Standard Deviation; CI: Confidence interval; NT: Non typeable

Declarations:

Ethics approval and consent to participate

The study was performed under ethical principles in accordance with the Declaration of Helsinki. The protocol was approved by the Committee for Research and Ethics of Ibn Rochd University Hospital Center (N 21-20) and by the National Commission of the Control and protection of Personal Data (N° A-RS-248/2021). Written informed consent was obtained from all participants after explaining study outlines and objectives.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are available in the Figshare repository, DOI: https://doi.org/10.6084/m9.figshare.30632678. BEN-TALEB H et al., 2025, DOI: 10.6084/m9.figshare.30632678 (57).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HB performed the laboratory work, analyzed data and wrote the original draft of the manuscript. NA and AC provided help with laboratory work. KN helped in the implementation of the study and in the guidance of the laboratory work. AL and AB provided clinical data. KM provided assistance in the implementation of the study. RSo, RD and CM contributed to the laboratory work and to the analysis of data. PB and TH contributed to the guidance of the study and to the analysis of data. HL provided guidance of the study. RSa coordinated the project and supervised the study. MC designed and coordinated the project, contributed to the analysis of data and reviewed and edited the final version. All authors reviewed and approved the manuscript content.

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