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Antimicrobial therapies for early-onset group B streptococcal sepsis: insights from an Italian Multicenter Study

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Background and objective: Group B *Streptococcus* (GBS) remains a leading cause of early onset sepsis (EOS, the disease presenting from 0 to 6 days of life). We reviewed empirical and definitive antimicrobial therapies administered to treat newborns with confirmed GBS-EOS.

Design: This is a multicentre study based on data from an Italian prospective surveillance program for GBS-EOS covering 35 birthing centres (from January 1st, 2003 to December 31st, 2024).

Method: Population included full-term (⩾37 weeks' gestation) and preterm (<37 weeks' gestation) neonates with blood and/or cerebrospinal fluid culture positive for GBS within the first 72 h of life. Adequacy of empiric, definitive therapy and duration of antibiotic courses were defined according to NICE and AAP guidelines. Adequacy of empiric therapy was defined as administration of penicillin (or ampicillin), with an aminoglycoside (±a third-generation cephalosporin), within the first 48–72 h following blood culture collection. Adequacy of definitive neonatal therapy (or de-escalation) was defined as administration of penicillin (or ampicillin) after 48–72 h of empiric therapy.

Results: There were 967,054 live births and 200 cases of GBS-EOS, of which 43 (21.5%) were preterm and 157 (78.5%) were full-term; 35 (17.5%) out of 200 had no signs of illness (17.5%), whereas 165 (82.5%) developed signs of illness (56 of them -28.0%- had severe disease). Fourteen (7.0%) died (1 of 14 was full-term; the remaining 13 were preterm newborns under 34 weeks' gestation). Based on the available information, antibiotics were adequate in 106/137 (77.4%) empiric and 48/119 (40.3%) definitive therapies. Duration of antibiotic courses did not differ between severe (median 10 days, IQR 8.0–14.0) and non-severe cases (median 10 days, IQR 10.0–12.5; $p = 0.68$). Antibiotic treatments lasted ⩾15 days in 34 (20.1%) out of 169 cases with available information.

Conclusion: Deviations from international recommendations in antimicrobial therapies for GBS-EOS were substantial critical. Our findings underscore the importance of timely antimicrobial de-escalation and the need to avoid excessively prolonged courses of antimicrobials.

Antimicrobial patterns in neonatal group B *Streptococcus* late-onset disease: a report from the Emilia-Romagna Region

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Background and objective: Group B *Streptococcus* (GBS) remains a leading cause of late-onset sepsis (LOS, from 7 to 89 days of life). We reviewed the appropriateness and duration of antibiotic courses in relation to the severity of GBS-LOS.

Design: This is a retrospective, multicenter, area-based study conducted (from January 1st, 2003 to December 31st, 2022) in 14 neonatal and pediatric units in Emilia-Romagna, Italy.

Method: Infants aged 4–90 days with GBS positive blood or CSF culture were enrolled. The disease severity was defined by the clinician. Empiric therapy was defined as appropriate when administered within the first 72–96 h following blood culture collection, while definitive therapy (or de-escalation) was defined as appropriate when administered using only penicillin (or ampicillin).

Results: There were 211 cases of GBS-LOD, of which 90/211 (42.7%) were preterm and 121/211 (57.3%) were full-term, with a median gestational age of 38 weeks. 26/211 (12.3%) had meningitis. Based on the available information, the infection was mild-to-moderate in 127/209 (60.8%) and severe in 82/209 (39.2%). Median duration of antibiotic courses was 14 days (for cases with available information). Empiric therapy was appropriate in 141/211 cases (66.8%), inappropriate in 58/211 (27.5%), and not assessable in 9/211 cases (4.3%). Definitive therapy was appropriate in 63/211 (29.9%), inappropriate in 124/211 (58.8%), and not assessable in 21/211 (9.9%). In 3/211 cases (1.4%), no antibiotic therapy was administered. In severe infections, definitive therapy was appropriate in 19/63 (30.2%); in mild-moderate infections, it was appropriate in 44/63 (69.8%).

Conclusion: Empiric therapy was appropriate in most cases, while definitive therapy was appropriate in less than 1/3 of cases, both in the general population and in severe infections.

Neonatal group B *Streptococcus* disease in the Netherlands, 1987–2024: epidemiology and vaccine coverage

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Summary: Molecular epidemiology and potential vaccine coverage of neonatal GBS disease was assessed in the Netherlands from 1987 till 2024. Neonatal GBS disease is still increasing in the Netherlands and different vaccines would potentially cover most cases.

Background: Group B *Streptococcus* (GBS) is the leading causes of neonatal early and late onset sepsis and meningitis. In the Netherlands the incidence of neonatal GBS disease is increasing despite the implementation of risk factor based intrapartum antibiotic prophylaxis in 1998. The molecular epidemiology and potential vaccine coverage of neonatal GBS disease was assessed in the Netherlands over 37 years.

Method: A nationwide surveillance study in the Netherlands including neonates age 0–89 days with GBS culture positive sepsis or meningitis between July 1987 and June 2024. Cases were identified through the Netherlands Reference Laboratory of Bacterial Meningitis. GBS serotype was determined by latex agglutination. Whole genome sequencing results were used to determine the presence of Alpha-like- protein (Alp) genes. Linear and logistic regression were used for statistical analysis. Vaccines GBS6 (serotype Ia, Ib, II, III, IV and V), GBS-NN (Alp genes AlphaC and Rib) and GBS-NN2 (Alp genes AlphaC, Rib, Alp1 and Alp2/3) were analysed.

Results: A total of 2212 episodes in 2180 patients were identified; 1307 early onset disease (0–6 days) and 905 late onset disease episodes (LOD,

7–89 days). Meningitis was associated with LOD ($p < 0.001$). The mean annual incidence of GBS disease was 0.33 per 1000 livebirths and significant increased over time from 0.19 in 1987 to 0.57 in 2023 ($p < 0.001$) due to an increase in sepsis cases. There was a shift in the distribution of serotypes causing GBS disease, with an increase in the proportion of serotype Ia (9% (25/295) in the first decade versus 17% (160/954) in the last decade) and a decrease in the proportion of serotype Ib and III (9% vs 4% and 69% vs 60%, $p < 0.01$). Of all cases, 2095 (97%) of 2163 would be covered by the GBS6 vaccine and this percentage did not change over investigated time. 96% of sepsis and 98% of meningitis cases would be covered. 1638 (74%) of 2212 isolates were sequenced and WGS showed an Alp gene in all sequenced isolates. The Rib gene was most commonly present (64%), followed by Alp1 (17%) and AlphaC (13%). Rib was associated with meningitis ($p < 0.001$). The GBS-NN vaccine would potentially cover 78% of cases; 73% of sepsis cases and 87% of meningitis cases. The GBS-NN2 vaccine would cover 99% of cases.

Conclusion: Neonatal GBS disease is still increasing in the Netherlands. The GBS-6 and GBS-NN2 vaccines would potentially cover most cases.

The experiences of parents and caregivers of infants who develop late-onset Group B Strep infections

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Background and objective: Late-onset GBS infections (LOGBS) account for ~40% of iGBS and are not currently preventable. Little is known about the impact on families, before, during and after diagnosis. This study aimed to explore the experiences of parents and caregivers of infants developed LOGBS.

Design: A qualitative interview study, informed by a Patient and Public Involvement (PPI) group of parents and caregivers with lived experience of LOGBS.

Method: Participants were recruited to 5 online PPI workshops via adverts posted on social media for GBS charities, pregnancy and parenting groups. These aimed to confirm the need for the research and inform the study design. Individual online qualitative interviews are underway.

Results: Six mothers of infants who developed LOGBS in the past 5 years, and two health professionals (midwife, neonatologist, 5–10 years' experience) took part in PPI workshops. Parents ranged in parity, area deprivation (3–9 IMD) and time since infection (1–4 years). Four mothers' infants had made a full recovery, whereas two had long-term complications. Findings confirmed the need for the research; parents described difficult experiences and emotions that had lasting impact within and beyond the immediate family. In particular, they described how a lack of information, communication and support compounded these impacts. Health professionals wanted more training on LOGBS and how best to communicate with parents. Participants also informed the interview study design by widening the eligibility criteria (from 5 to 10 years post infection) and recommending snowball sampling to recruit other caregivers. A total of 14 parents and caregivers (10 mothers, 3 fathers, 1 grandparent) have participated in interviews to date. Findings from early analysis will be shared.

Conclusion: LOGBS infections have a lasting impact on parents, caregivers and families, even when babies 'fully recover'. More information, communication and support is needed to fortify families.

Clinical evaluation of the Xpert® Xpress GBS (Cepheid) test performed by midwives as a point-of-care test, University Hospital of Liège, Belgium: preliminary results

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Background and objective: Neonatal disease caused by Group B *Streptococcus* (GBS) is primarily prevented through intrapartum antibiotic

prophylaxis (IAP) to the mother based on a positive antenatal screening or the presence of risk factors. However, the ideal method for identifying candidates for IAP should be an intrapartum test that accurately reflects the GBS colonization status at delivery. This study aims to evaluate the performance of the Xpert® Xpress GBS test (XGBS; Cepheid), performed by midwives on intrapartum vaginal-rectal samples, as a point-of-care test (POCT) and to compare the performances with the current strategy based on antenatal culture screening in Belgium.

Design: This prospective study, initiated in April 2024, still ongoing, includes women admitted to give birth in the obstetrical department, University Hospital of Liège, who gave their informed consent for the collection of an intrapartum vaginal-rectal sample for the XGBS test.

Method: Vaginal-rectal samples were collected using a double-swab device. One swab was used immediately by midwives for the XGBS test on a GeneXpert system present on-site, and the second one was sent to the microbiology laboratory for an enriched culture (Lim broth + selective media). When discordance between XGBS and culture results, another PCR test was performed from the Lim broth. In addition to antenatal screening results, intrapartum XGBS results, if positive, were considered to decide on IAP administration.

Results: Between April 30, 2024, and April 1st, 2025, 482 patients were enrolled in the study, with 20 excluded due to PCR results in error (4.1%), mainly occurring during the first 3 months of the study. The intrapartum GBS colonization rate was 16.2%. Compared to intrapartum culture, the XGBS test showed 89.5% sensitivity and 98.2% specificity. Compared to intrapartum culture, the antenatal screening culture showed 77.1% sensitivity and 95.6% specificity: 33 patients changed their GBS-colonization status (16 negative-patients became positive, 17 positive-patients became negative).

Conclusion: These preliminary results already indicate that, easily performed by midwives, within 30–42 min, the XGBS test shows good performances when compared to intrapartum culture. However, midwives demonstrated full proficiency only after a few months, highlighting the importance of continuous training to ensure accurate results. Validation continues by including more patients and completing control PCRs. Overall, intrapartum testing proved its superiority to antenatal screening in identifying candidates for IAP.

Funding
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Pharmacokinetics of intrapartum benzylpenicillin: insights into candidate regimens to prevent early onset neonatal Group B *Streptococcus* disease

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Background: Early onset neonatal Group B Streptococcal disease (EOGBS) accounts for significant global morbidity and mortality.[1] Intrapartum prophylaxis with benzylpenicillin is advised for women at high-risk of having a baby affected by EOGBS.[2] Altered pharmacokinetics with pregnancy-related physiology can result in suboptimal drug exposure. We aimed to describe the intrapartum pharmacokinetics of benzylpenicillin and identify regimens for optimal drug exposure to prevent EOGBS.

Methods: We conducted a prospective cohort study at Liverpool Women's Hospital. Pregnant women with risk factors for having a baby with EOGBS received IV benzylpenicillin 3 g at the onset of labour and 1.5 g 4 hourly until delivery (standard care, SC).[2] Benzylpenicillin concentrations in plasma and umbilical cord were quantified. Population pharmacokinetic modelling was performed, regimens optimising drug exposure were determined using Monte-Carlo simulations.

Results: Benzylpenicillin pharmacokinetics were adequately described by a three-compartment. Simulations showed that benzylpenicillin 2.4 g followed by 1.2 g 4 hourly results in adequate

drug exposure with free drug minimum concentration (fCmin) > epidemiological cutoff value for penicillin minimum inhibitory concentrations of 0.125 mg/L in more than 90% of the simulated population. Similar exposure was achieved with the SC. Simulations of continuous infusions of benzylpenicillin resulted in higher proportions of pharmacodynamic target attainment when compared to intermittent infusions using the same total dosage.

Conclusion: There is potential to reduce drug burden and consider the advantages of portable continuous benzylpenicillin infusions to avoid bolus administrations during labour. Evaluation of regimens on clinical isolates is required to confirm non-inferiority to SC.

References

1. Madrid L, Seale AC, Kohli-Lynch M, et al. Infant group B streptococcal disease incidence and serotypes worldwide: systematic review and meta-analyses. *Clin Infect Dis* 2017; 65(suppl_2): S160–S172.
2. Huges R, Brocklehurst P, Steer P, et al. Prevention of early-onset neonatal group B streptococcal disease. *BJOG: Int J Obstetrics Gynaecol* 2017; 124(12): e280–e305.

A survey on Italian early-onset sepsis prevention policies

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Introduction: Early-onset sepsis (EOS) remains a leading cause of neonatal morbidity and mortality. Currently, no Italian national guidelines exist for management of infants at risk for EOS, and the approach across different Italian centers is not well known.

Objectives: To investigate the management practices implemented at neonatal centers across Northern, Central, and Southern Italy.

Methods: In 2024–2025, we administered a questionnaire to all Italian neonatal centers via email. Non-responding centers were contacted by phone.

Results: Data were collected from 226 centers (127 level 1 centers and 99 level 2 centers). In 2023, the number of live births (LBs) among participating centres was 268,095. Over 90% of centers reported using a protocol for the management of term and late preterm neonates at risk for EOS. Among these, 48% adopt serial clinical evaluations, 20% follow the CDC 2010 algorithm, 13% apply the Early-Onset Sepsis Risk Calculator, 7% use the NICE 2021 protocol. The remaining 12% of centers use unspecified algorithms. In nearly all cases (98%), vaginal-rectal swabs for Group B *Streptococcus* (GBS) screening are performed between the 35th and 37th weeks of gestation. Intrapartum antibiotic prophylaxis (IAP) is administered in 37% of women with unknown GBS status and with no risk factors for EOS. The most commonly used antibiotic for IAP is ampicillin (>75% of cases). IAP is considered adequate in 66% of centers if administered at least 4 h before delivery, and in 15% if given at least 2 h before. Nel 18% dei casi la IAP is considered complete with the administration of at least 2 doses of antibiotic before delivery. A total of 2% of the responding centers believe that there is no "temporal" or "quantitative" criterion to define a complete or incomplete IAP, but the clinical conditions of the newborn at birth demonstrate whether the prophylaxis was sufficient or not. Asymptomatic neonates born to mothers with chorioamnionitis undergo in most centers (65%) laboratory tests and, among these, the vast majority (70%) receive antibiotics while awaiting culture results. In the case of asymptomatic neonates born to mothers with a negative GBS swab and prolonged rupture of membranes (>18 h), a minority of centers (27%) perform diagnostic tests, and 58% of them administer antibiotics while awaiting culture results. Finally, for asymptomatic neonates born to GBS-colonized mothers with inadequate IAP, a minority of centers (42%) perform diagnostic tests, and a non-negligible proportion (75% of them) administer antibiotics while awaiting culture results.

Conclusion: Data from this survey provide valuable insights into the current practices for preventing EOS across neonatal centers in Italy. The majority of centers follow a protocol based on serial clinical observation. However, even in asymptomatic neonates, a significant proportion of centers combine clinical observation with laboratory testing. Abnormal laboratory markers lead to the administration of antibiotics (while awaiting blood culture results) even in asymptomatic neonates. These findings underscore the need for standardized national guidelines to optimize EOS prevention strategies and ensure uniform care across the country. Further data collection and analysis will be essential to refine these practices and develop evidence-based recommendations for all Italian neonatal centers.

Capsular type distribution, virulence determinants and antimicrobial susceptibility of Group B *Streptococcus* isolated from newborns

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Introduction: *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) is the most common cause of infections in the first 3 months of life. Vertical transmission from GBS-colonized mothers to their newborns during pregnancy and delivery can cause serious infections, such as sepsis, pneumonia, and meningitis, which are the leading cause of neonatal morbidity and mortality worldwide. The pathogenicity of GBS is complex and multifactorial, including virulence factors, clonal complex, and antimicrobial resistance. Therefore, expanding our knowledge through phenotypic and molecular assays to identify and differentiate isolates within the neonatal population will be essential for future GBS interventions.

Objective: This study investigated the genetic characteristics, virulence factors involved in colonization and invasion, as well as antimicrobial susceptibility of GBS strains isolated from newborns.

Method: Umbilical swabs were collected from newborns up to 24 h old at the Carmela Dutra Maternity Hospital, Rio de Janeiro, Brazil. We evaluated the type of birth, ethnicity, capsular types, virulence genes, multilocus sequence typing, and antibiotic resistance. This project was approved by the Ethics Committee of the State Secretariat of Rio de Janeiro, receiving the number CAAE 51317415.8.3001.5259.

Results: Among 77 samples collected, 15 were identified as GBS, where 95% of births were by vaginal delivery. Among the neonates colonized by GBS, 73.3% were black, 20% were white, and 6.7% was indigenous. Three capsular types were detected, with type V being the most frequent, followed by types Ia and II (Figure 1(a)–(c)).

The isolates belonged to 4 clonal complexes (CCs); CC23 and CC17 were the most frequent with 46.7% and 26.7%, respectively. CC19 and CC452 were also identified with 13.3% each. In addition, 11 different STs were verified. Virulence genes were detected, where *iag* and *lmb* genes were found in all GBS strains (Figure 2(a) and (b)).

Resistance to tetracycline (80%), levofloxacin (13.3%), chloramphenicol (13.3%), ofloxacin (13.3%), cotrimoxazole (33.3%) were verified in GBS strains (Table 1).

Conclusion: Our data revealed variation in capsular type, virulence genes, clonal complex and antimicrobial resistance in neonates colonized with GBS strains. Furthermore, they provided important information on the pathogenic potential of GBS strains isolated from colonized neonates, and that continued epidemiological surveillance is needed to provide insights into the dynamics of GBS pathogenesis, particularly in newborns.

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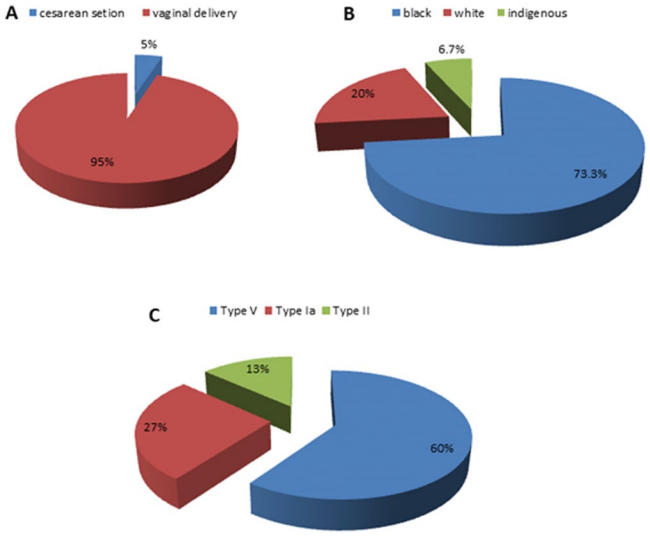

Figure 1. GBS isolates in the study population. Baby delivery by cesarean section or vaginal delivery (a). distribution of GBS by skin color (b) and capsular type (c).

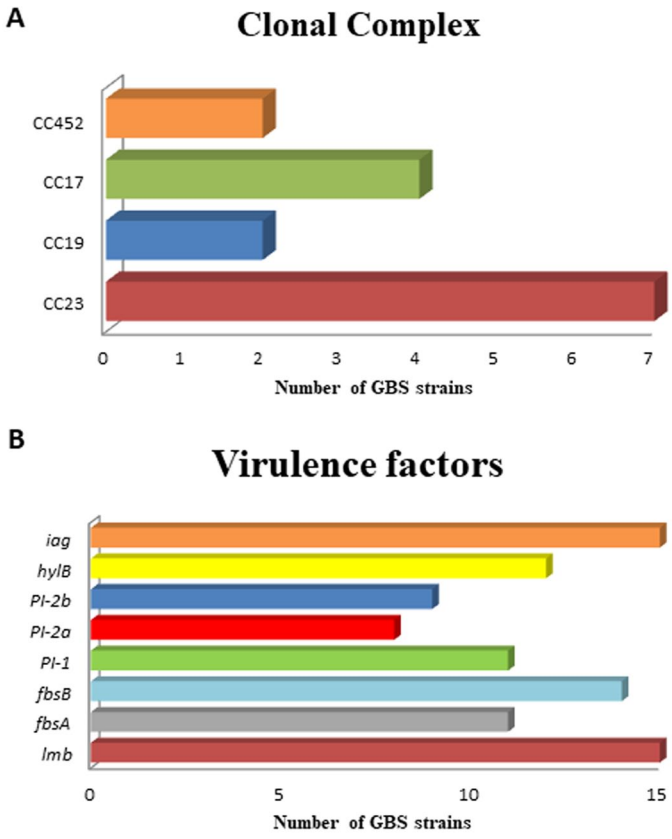

Figure 2. Molecular and virulence characteristics of GBS strains, Clonal complexes (a) and virulence factor genes (b).

Table 1. Antimicrobial susceptibility profiles for GBS isolates.

GBS strains	Antimicrobial resistance														
	VAN	CLIN	LEV	PEN	TET	AZI	CHL	ERY	CF	LIN	NOR	CIP	OFL	PEF	COT
N1801	S	S	S	S	**R**	S	S	S	S	S	S	S	S	S	**R**
N1901	S	S	S	S	**I**	S	S	S	S	S	S	S	S	S	S
N1902	S	S	S	S	**R**	S	S	S	S	S	S	S	S	S	S
N1903	S	S	S	S	**R**	S	S	S	S	S	S	S	S	S	S
N1905	S	S	**R**	S	**R**	S	**R**	S	S	S	S	S	**R**	S	S
N1906	S	S	**R**	S	**R**	S	**R**	S	S	S	S	S	**R**	S	S
N1910	S	S	S	S	**R**	S	S	S	S	S	S	S	S	S	S
N1912	S	S	S	S	**I**	S	S	S	S	S	S	S	S	S	S
N1913	S	S	S	S	**R**	S	S	S	S	S	S	S	S	S	S
N1915	S	S	S	S	**R**	S	S	S	S	S	S	S	S	S	**R**
N1916	S	S	S	S	**I**	S	S	S	S	S	S	S	S	S	**R**
N1918	S	S	S	S	**R**	S	S	S	S	S	S	S	S	S	**I**
N1919	S	S	S	S	**R**	S	S	S	S	S	S	S	S	S	**R**
N1921	S	S	S	S	**R**	S	S	S	S	S	S	S	S	S	S
N1922	S	S	S	S	**R**	S	S	S	S	S	S	S	S	S	**R**

Vancomycin (VAN); clindamycin (CLIN); levofloxacin (LEV); penicillin (PEN); tetracycline (TET); azithromycin (AZI); chloramphenicol (CHL); erythromycin (ERY); ceftriaxone (CF); linezolid (LIN); norfloxacin (NOR); ciprofloxacin (CIP); ofloxacin (OFL); pefloxacin (PEF); cotrimoxazole (COT); sensitive (S); intermediary (I); resistant (R).

Development of next generation sequencing technology for the prevention of neonatal meningitis

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Introduction: Bacterial meningitis presents significant diagnostic challenges in neonates, with *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) as the predominant causative agent. GBS colonises the rectal and vaginal regions of approximately 25%–30% of healthy pregnant women and can be transmitted to new borns before, during, or after birth, leading to early-onset (⩽7 days) or late-onset (1 week–3 months) disease. The absence of routine screening in the UK contrasts with the CDC-recommended testing at 35–37 weeks of pregnancy in the USA, highlighting a critical gap in early detection strategies. The gold- standard enriched culture medium (ECM)-based diagnostic method requires 2–3 days for definitive results, which delays timely intervention.

Objective: To develop and evaluate a rapid clinical metagenomic pipeline using nanopore sequencing for the detection of GBS and antimicrobial resistance (AMR) genes directly from swabs of pregnant women, benchmarked against established microbiological and molecular diagnostics.

Methods: A total of 91 clinical and reference molecular standard swab samples were processed for host DNA depletion and microbial enrichment, followed by multiple displacement amplification (MDA), and sequencing using the Oxford Nanopore rapid barcoding workflow. Pathogen detection, virulence, and AMR gene profiling were performed using custom bioinformatics pipelines. Assay performance was compared to qPCR and

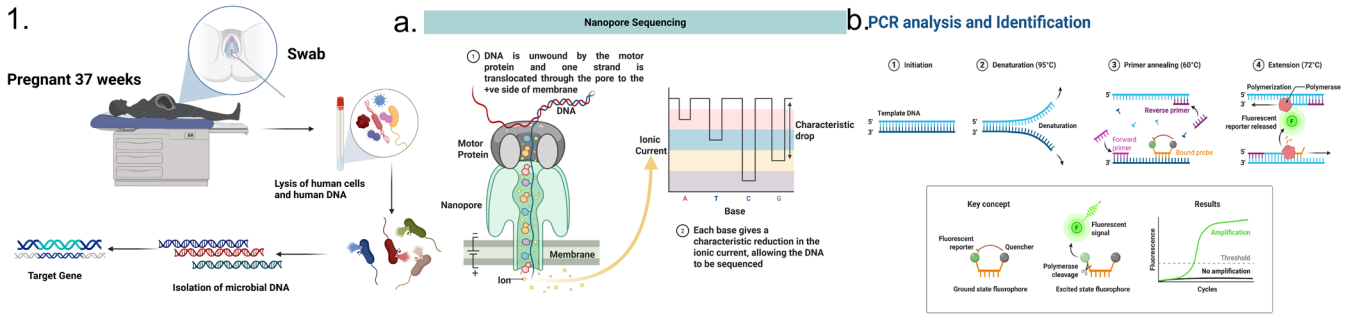

Figure 1. Workflow for Detection and Identification of Microbial DNA in Pregnant Women Using Nanopore Sequencing and PCR. (1) A vaginal swab is collected from a pregnant woman at 37 weeks of gestation. Following sample collection, host cells are lysed to remove human DNA, and microbial DNA is isolated. Isothermal amplification is performed to enrich microbial DNA targets. (a) In the Nanopore sequencing workflow, DNA strands are passed through a protein nanopore, enabling real-time sequencing based on characteristic changes in electrical current as nucleotides pass through. (b) in parallel, PCR analysis involves denaturation, primer annealing, and extension steps, during which fluorescence-based detection enables quantification and identification of specific microbial gene cfb of GBS through cycle threshold (Ct) values. Created using Biorender.com.

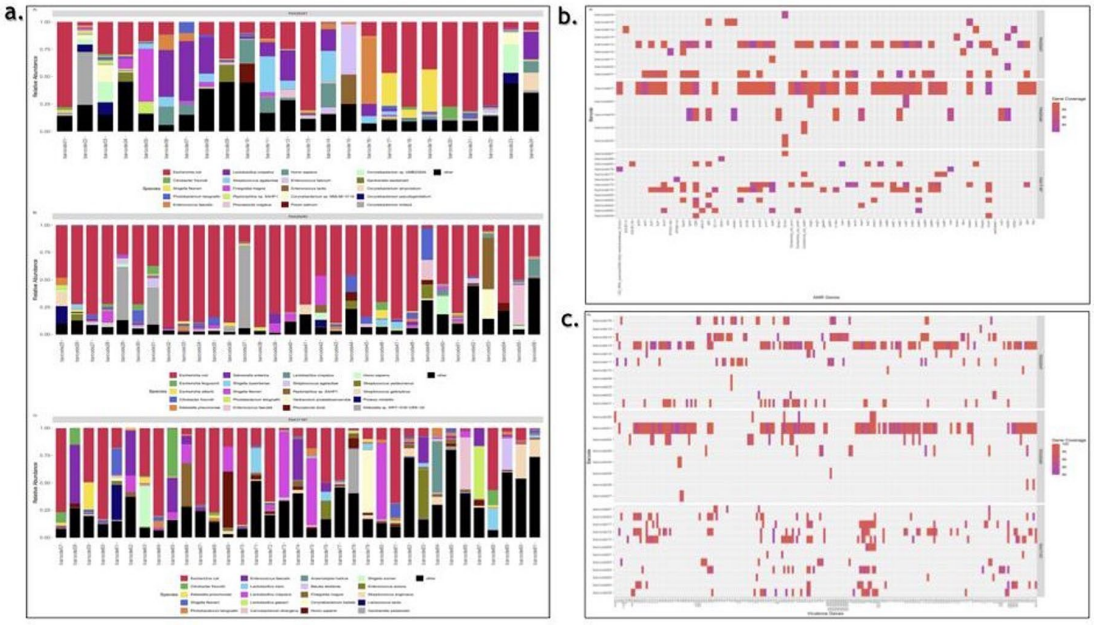

Figure 2. Taxonomic and genomic landscape of vaginal microbiota in pregnant women. (a) A species-level examination of microbial distributions in the three MinION flow cells of 91 clinical and reference molecular standard swab samples under Q8L50 provides critical insights into pathogenicity and public health applications. (b) The CARD AMR Profile presented here offers an in-depth examination of the antimicrobial resistance genes present in 27 distinct samples. (c) Heatmap of virulence gene profiles across samples, showing the presence and distribution of virulence-associated genes.

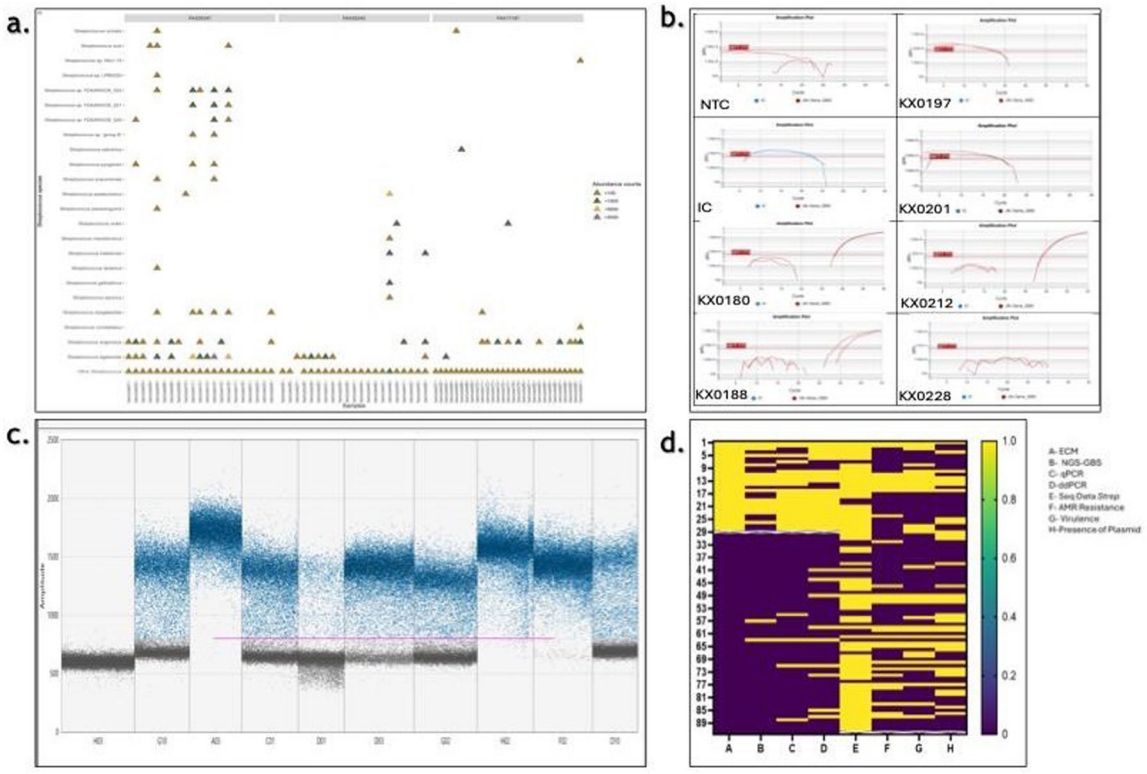

Figure 3. Multi-platform genomic and molecular characterisation of vaginal swab samples for group B streptococcus (GBS) detection. (a) The abundance of *Streptococcus* species across various samples provides insight into the microbial diversity and potential health implications associated with it. The figure indicates the presence and relative abundance of multiple *Streptococcus* species across a range of swab samples, using different colour-coded triangles to denote varying levels of abundance. (b) Representative qPCR plots across multiple samples, including negative and internal controls, highlighting positive amplification in GBS-positive samples and no amplification in GBS-negative samples. (c) Digital droplet PCR (ddPCR) scatter plot showing fluorescence amplitude for positive (blue) and negative (black) droplets, enabling absolute quantification of the target *cfb* gene across swab samples. (d) This heatmap visualises the presence (yellow) and absence (purple) of key genomic and molecular features across 91 *swab* samples. The x-axis represents various molecular and genomic detection categories, The y-axis corresponds to individual samples, highlighting variability in feature presence across different samples.

digital droplet PCR (ddPCR) targeting the *cfb* gene of Group B *Streptococcus* (GBS).

Results: Oxford Nanopore Technologies (ONT) whole genome/ metagenomic sequencing enabled the identification of key pathogens from 91 swab samples. Detected species included *Streptococcus agalactiae* (GBS), *Streptococcus pneumoniae*, *S. lutetiensis*, and *S. macedonicus*, revealing both clinically relevant infections and intra-genus diversity that is often undetectable by conventional methods. In comparative diagnostic performance, ONT outperformed both quantitative PCR (qPCR) and droplet digital PCR (ddPCR). ONT achieved a sensitivity of 87.10%, specificity of 100%, positive predictive value (PPV) of 100%, negative predictive value (NPV) of 93.75%, and an F1 score of 93.08%, with zero false positives and a diagnostic turnaround time of under 7 h. In contrast, qPCR and ddPCR demonstrated reduced sensitivity (78.57% and 82.14%) and specificity (96.67% and 90.00%), with ONT resolving all discrepant positives. Antimicrobial resistance (AMR) gene profiling using CARD, ResFinder, and NCBI AMR databases revealed high-prevalence resistance determinants, including *mecA*, *blaCTX- M*, *vanA*, *blaEC-19*, *tetM*, and *qnrB*. Resistance genes conferring reduced susceptibility to beta -lactam, macrolides, aminoglycosides, tetracyclines, and sulfonamides were

Table 1. Comparative diagnostic performance metrics of nanopore sequencing, qPCR, and ddPCR for microbial detection in vaginal swabs.

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	F1 Score (%)	False Positives
Nanopore Sequencing	87.1	100.0	100.0	93.75	93.08	0
qPCR	78.57	96.67	91.67	90.63	84.85	Discrepant true positives confirmed by sequencing
ddPCR	82.14	90.0	79.31	91.52	80.55	Discrepant true positives confirmed by sequencing

widely distributed, with sequence identity scores ranging from 85% to 95%. In parallel, ONT sequencing facilitated detection of virulence-associated genes such as *neuA* (capsular synthesis), *fimH*(adhesion), and *hcp1* (type VI secretion), along with resistance-bearing plasmids including pKPC-CAV1321. The co-occurrence of resistance and virulence elements within mobile genetic structures highlights the risk of horizontal gene transfer and reinforces the need for genomic AMR surveillance and precision-guided antimicrobial strategies in maternal and neonatal health.

Conclusion: This study demonstrates the clinical utility of a real-time, culture-independent nanopore metagenomic workflow for rapid GBS detection. The platform provides comparable or superior sensitivity and precision relative to qPCR and ddPCR, while offering additional insights into AMR and virulence genes. These findings support the integration of sequencing technologies into neonatal diagnostics to improve early detection and guide targeted antimicrobial therapy.

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