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Streptococcus pyogenes in Neonates and Postpartum Women: First Report on Prevalence, Resistance, *emm* Typing, and Risk Factors in Khyber Pakhtunkhwa

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Abstract

Streptococcus pyogenes is a significant pathogen in postpartum women and neonates. This study aimed to determine its prevalence, antibiotic susceptibility, clinical features, and associated risk factors in tertiary care hospitals in Khyber Pakhtunkhwa, Pakistan. A total of 384 clinical samples were collected from postpartum women (n = 192) and neonates (n = 192) in maternity wards. *S. pyogenes* isolates were identified using standard microbiological methods, and antibiotic susceptibility was assessed via the Kirby–Bauer disk diffusion assay. The *emm* typing was performed through PCR and sequencing. Clinical features and risk factors were analyzed statistically. The overall prevalence of *S. pyogenes* was 14.3% (55/384), with 16.7% in postpartum women and 11.9% in neonates. Isolates exhibited high sensitivity to β-lactams (penicillin ≥ 95%, ampicillin ≥ 91%) but moderate resistance to cephalosporins (cefepime ~12%) and macrolides (erythromycin 23.5–29.0%). Fluoroquinolones and tetracyclines showed the highest resistance rates (ciprofloxacin 38.7–43.5%, tetracycline 32.1–37.5%). Molecular typing revealed diverse *emm* types, with *emm*44, *emm*77, and *emm*12 being predominant. Fever and sepsis were common, with postpartum women experiencing more wound infections (33.3%) and neonates exhibiting respiratory distress (55.6%). Significant risk factors included prolonged labor (> 18 hours, $p = 0.030$) and premature rupture of membranes ($p = 0.039$) in mothers, preterm birth ($p = 0.013$), and neonatal resuscitation ($p = 0.028$) in neonates. The study highlights a substantial burden of *S. pyogenes* infections and increasing antibiotic resistance. Enhanced surveillance, antibiotic stewardship, and targeted infection control strategies are crucial to mitigating morbidity and mortality in these high-risk groups.

Keywords: *Streptococcus pyogenes*, postpartum infections, neonatal infections, antibiotic resistance, *emm* typing, risk factors

Introduction

Streptococcus pyogenes (Group A Streptococcus, GAS) is a major human pathogen responsible for a broad spectrum of infections, ranging from mild conditions such as pharyngitis and impetigo to severe invasive diseases, including necrotizing fasciitis and toxic shock syndrome (Cohen-Poradosu and Kasper 2007). While global child mortality has declined significantly, from 9.8 million in 2000 to 5.2 million in 2019, infectious diseases remain a major burden, particularly in low- and middle-income countries (LMICs) (Fleischmann-Struzek et al. 2018). Sepsis alone accounts for approximately 3 million neonatal and 1.2 million childhood cases annually, leading to 2.3 million deaths, with maternal sepsis being the third leading cause of maternal mortality worldwide (Say et al. 2014; Hariri et al. 2021).

Postpartum women and neonates are particularly susceptible to *S. pyogenes* infections due to immu-

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nological susceptibility and potential vertical spread during childbirth (Sherwood et al. 2022). Despite developments in maternal and neonatal care, GAS remains one of the key causes of puerperal sepsis and neonatal infections, contributing to substantial morbidity and mortality, particularly in resource-limited settings in low-income countries (Donders et al. 2021). Postpartum women have a 20-fold increased risk of having invasive *S. pyogenes* infections in comparison to non-pregnant women (Hassan et al. 2011), with invasive GAS (iGAS) occurring in 1 in 50 postpartum cases, carrying a mortality rate of around 3.5%. However, the incidence of neonatal GAS infections is not well documented in developing countries. Recent studies have reported an increasing burden of GAS infections in children in India and Pakistan (Haggar et al. 2012; Zafar et al. 2016).

The emergence of multidrug-resistant *S. pyogenes* strains has become a growing concern globally. Resistance to β-lactams, macrolides, lincosamides, and tetracyclines usually complicates treatments and underscores ongoing surveillance (Assafi et al. 2023; Cortés-Penfield and Ryder 2023; Shah et al. 2023). Molecular epidemiology studies highlight significant genetic diversity among *S. pyogenes* strains, with *emm* typing serving as a key tool for tracking transmission dynamics and evaluation of virulence potential in vulnerable populations, including children (Bonomo et al. 2025; Hall et al. 2025). The predominance of specific *emm* types varies geographically and demands region-specific surveillance to guide public health interventions (Weerasekara et al. 2024).

Several factors contribute to the risk of *S. pyogenes* infections in postpartum women and neonates, including prolonged labor, premature rupture of membranes in postpartum women, inadequate prenatal care, and neonatal resuscitation in neonates (Song et al. 2024; Hazelhorst et al. 2025). However, data on the prevalence, antimicrobial resistance, and molecular characteristics of *S. pyogenes* in postpartum women and neonates in Pakistan remain inadequate. Khyber Pakhtunkhwa (KP), a remote province of Pakistan having significant maternal and neonatal health challenges, remains largely unexplored in this regard because of limited healthcare infrastructure, low socioeconomic conditions, and inadequate disease control practices.

Given the increasing burden of GAS infections and the lack of systematic investigations in maternal and neonatal health settings in Pakistan, this study aims to determine the prevalence, antibiotic susceptibility, and *emm* typing of *S. pyogenes* isolates from postpartum women and neonates in tertiary care hospitals in KP. Findings from this study will provide crucial insights into GAS's epidemiology and resistance patterns, aiding in evidence-based clinical management and infection control strategies.

Experimental

Materials and Methods

Study design and setting. This cross-sectional study was conducted from January 2024 to December 2024 in the maternity units of 3 major tertiary care hospitals in Khyber Pakhtunkhwa (KP), Pakistan. KP Province is the smallest province of Pakistan, covering 74,521 km² with a population of 40.85 million as per the 2023 census (https://pwdkp.gov.pk/page/sec_message). The province shares borders with Punjab province to the southeast, Baluchistan province to the south, Gilgit-Baltistan to the northeast, Afghanistan to the northwest, and the former Federally Administered Tribal Areas (FATA) to the west. Sampling was conducted at major tertiary care hospitals in Peshawar, the capital of KP: Hayatabad Medical Complex, Khyber Teaching Hospital, and Lady Reading Hospital (Fig. 1).

Clinical and laboratory data collection. Definitions established by the World Health Organization (WHO) were used for categorizing neonates (0–27 days), infants (0–1 year), and the postpartum period (up to 42 days after birth) (Sherwood et al. 2022). Demographic and clinical data were collected from the patient's medical records in maternity units, and structured questionnaires were administered to postpartum women or legal guardians of neonates. *S. pyogenes* infection was defined by the existence of at least one of the following clinical criteria: respiratory distress requiring mechanical ventilation, hemodynamic instability requiring fluid resuscitation, or Vaso-pressive amines, seizures, or admission to the ICU. The collected data included maternal age, gestational history, delivery mode (natural childbirth or cesarean), neonatal birth weight, Apgar score, and clinical symptoms. Laboratory parameters like complete blood count (CBC), C-reactive protein (CRP), and blood culture results were also recorded. Each hospital provided hospitalization reports to the first author, who recorded initial clinical and laboratory data with the help of a trained doctor, along with clinical outcomes such as length of

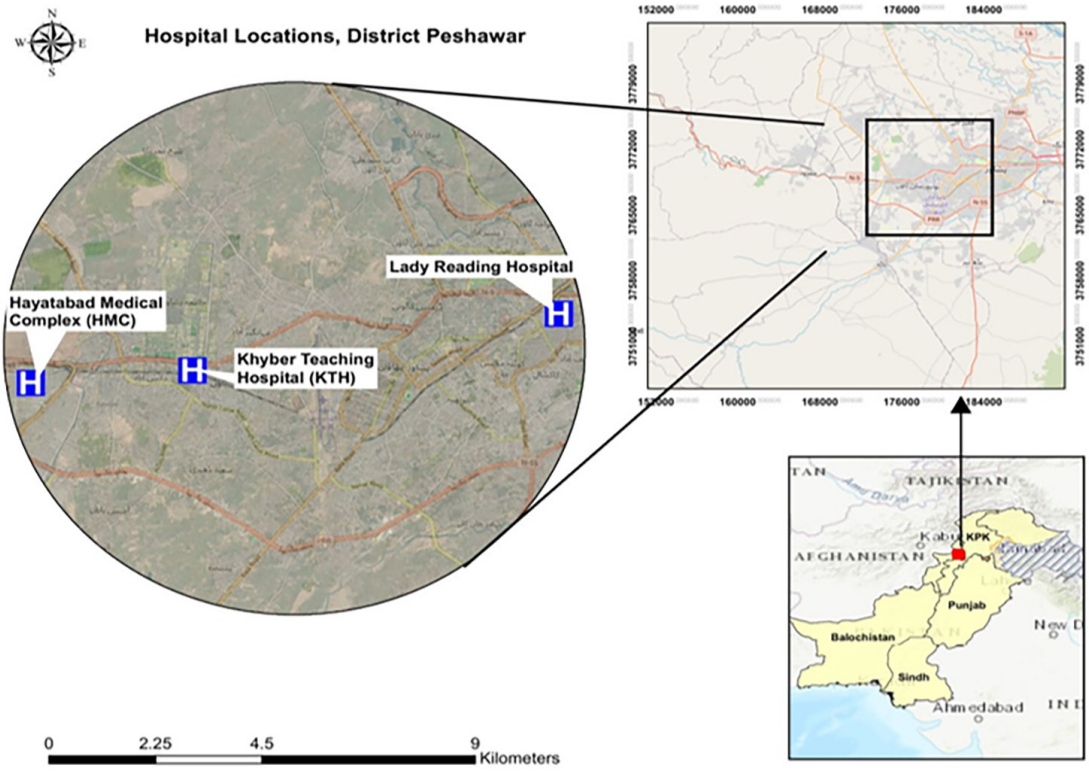

Fig. 1. Map of Khyber Pakhtunkhwa, Pakistan, highlighting the locations of hospitals where *Streptococcus pyogenes* samples were collected. The hospitals included Hayatabad Medical Complex (HMC), Lady Reading Hospital (LRH), and Khyber Teaching Hospital (KTH) in Peshawar.

stay, complications, and ICU admissions, using a standardized anonymized case report form.

Sample collection and processing. Sample size was calculated as described previously (Naing et al. 2006). A total of 384 clinical samples were collected, including 192 samples from postpartum women and 192 from neonates. Sample types included vaginal swabs, blood, and wound swabs from postpartum women, while blood, nasopharyngeal swabs, and cerebrospinal fluid (CSF) were collected from neonates. All samples were aseptically collected using sterile cotton swabs and transported to the Medical Microbiology Laboratory at KUST in Amies transport medium within two hours of collection.

Microbiological identification of *S. pyogenes*. Samples were cultured on blood agar supplemented with 5% sheep blood and incubated at 37°C in a CO_2 incubator (5% CO_2) for 24–48 hours. Colonies exhibiting β-hemolysis were subjected to Gram staining, catalase testing, and bacitracin susceptibility testing for preliminary identification. Confirmation was performed using the pyrrolidonyl arylamidase (PYR) test and latex agglutination for Lancefield Group A carbohydrate antigen detection (Oxoid, USA).

Antibiotic susceptibility testing. The antibiotic susceptibility of *S. pyogenes* isolates was assessed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar supplemented with 5% sheep blood. The following antibiotics were tested: penicillin (10 U), ampicillin (10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefepime (30 µg), meropenem (10 µg), erythromycin (15 µg), clindamycin (2 µg), azithromycin (15 µg), tetracycline (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), and vancomycin (30 µg). Results were interpreted following Clinical and Laboratory Standards Institute guidelines (CLSI 2023).

Molecular analysis for *emm* typing. Genomic DNA was extracted from *S. pyogenes* isolates using the phenol-chloroform method (Miller et al. 1988). The *emm* typing was performed by amplifying the *emm* gene using previously described primers in a thermal cycler (Bio-Rad, USA), followed by commercial sequencing (Macrogen, Korea) with the forward primer CDC1 forward (5′-TATT(C/G)GCTTAGAAAATTAA-3′) and CDC3 reverse (5′TTCTTCAAGCTCTTTGTT-3′). The PCR reaction was carried out in a total volume of 25 µl, containing 12.5 µl of 2× PCR master mix (Thermo Fisher Scientific Inc., USA), 1.0 µM of each primer,

Table I

Prevalence of *Streptococcus pyogenes* among postpartum women and neonates.

Group	Total samples	Positive cases	Prevalence (%)	Male cases	Female cases	χ^2 Value	*p*-Value
Postpartum women	192	32	16.7%	–	32	1.52	0.217
Neonates	192	23	11.9%	12	11		
Total	384	55	14.3%	12	43		

Chi-square (χ^2) value = 1.06, $p = 0.304$ (not statistically significant)

1 µl of template DNA (~50 ng), and nuclease-free water to the final volume. PCR amplification was carried out with initial denaturation at 94°C for 1 minute followed by 30 cycles of 94°C for 15 seconds, 47°C for 30 seconds, and 72°C for 1 minute and 25 seconds, with final extension at 72°C for 7 minutes (Frost et al. 2020) The resulting sequences were analyzed for homology using the BLAST search tool (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm). *emm* Types were assigned based on the Centers for Disease Control and Prevention (CDC) criteria, where sequences exhibiting ≥ 95% identity to the first 160 bases of a reference *emm* gene were classified accordingly (http://www.cdc.gov/ncidod/biotech/strep/assigning.htm).

Quality control. Structured questionnaires were designed and translated into Urdu and Pashto for better comprehension. All laboratory materials and media were sterilized according to standard protocols. Each antibiotic susceptibility test and minimum inhibitory concentration (MIC) determination was conducted in duplicate, with consistent results obtained. If discrepancies occurred, a third independent replicate was performed. DNA extraction and PCR assays included positive and negative controls, with *S. pyogenes* ATCC® 49399™ used as the reference strain.

Statistical analysis. Data analysis was performed using IBM SPSS Statistics for Windows v24.0 (IBM Corp., USA). The prevalence of *S. pyogenes* infection was calculated as the percentage of positive cases among total samples. The chi-square (χ^2) test was used to compare prevalence rates between postpartum women and neonates, while logistic regression was applied to assess risk factors associated with infection. Antibiotic susceptibility results were presented as percentages of sensitive and resistant isolates. A *p*-value < 0.05 was considered statistically significant.

Results

Prevalence of *S. pyogenes* in postpartum women and neonates. A total of 384 clinical samples were collected from postpartum women and neonates admitted at maternity units of tertiary care hospitals in Khyber Pakhtunkhwa, Pakistan. The overall prevalence of *S. pyogenes* was 14.3% (55/384). The prevalence was 16.7% (32/192) in postpartum women, while in neonates, it was 11.9% (23/192). The distribution of positive cases among neonates was 52.2% (12/23) in males and 47.8% (11/23) in females. Statistical analysis using the chi-square (χ^2) test showed no significant difference in prevalence between postpartum women and neonates ($\chi^2 = 1.52$, $p = 0.217$), indicating a relatively uniform burden of *S. pyogenes* infections across the two groups (Table I).

Antibiotic sensitivity patterns of *S. pyogenes* isolates. Fig. 2 represents the antibiotic susceptibility patterns of *S. pyogenes* isolates from postpartum women (n = 32) and neonates (n = 23). The results showed high sensitivity of *S. pyogenes* isolates to β-lactam antibiotics, with penicillin and ampicillin showing over 90% efficacy in both groups. Among cephalosporins, cefepime showed a 12.0% resistance rate in postpartum women and 13.1% in neonates. The isolates showed 100% sensitivity towards meropenem and vancomycin in both groups. Resistance to macrolides (erythromycin: postpartum women 23.5%, neonates 29.0%) and clindamycin (15.6%, neonates 21.7%) was observed. Ciprofloxacin resistance rate was 38.7% in postpartum women and 43.5% in neonates, while tetracycline resistance was 32.1% and 37.5%, respectively.

***emm* Genotype diversity.** The distribution of *emm* types among *S. pyogenes* isolates in our study revealed significant diversity. As shown in Fig. 3A, a wide range

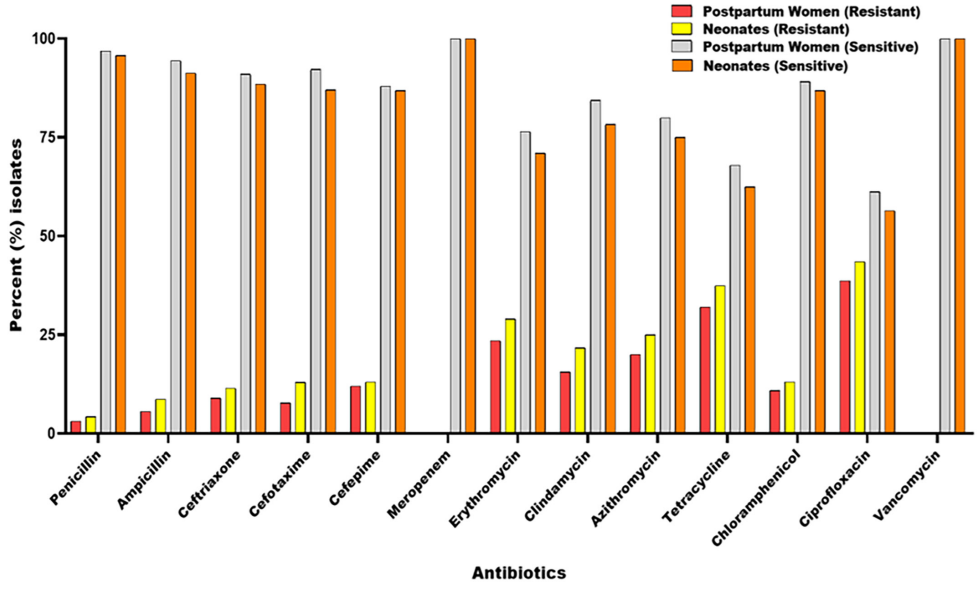

Fig. 2. Antibiotic resistance and sensitivity patterns in bacterial isolates from postpartum women and neonates. Bars represent the percentage of resistant (red, yellow) and sensitive (gray, orange) isolates across different antibiotics.

of *emm* types was identified, with varying prevalence among isolates. The most frequently detected *emm* types included *emm*44, *emm*77, *emm*12, *emm*1, *emm*3, *emm*28, and *emm*87, indicating their dominance in the studied population. In Fig. 3B, the most common *emm* types are highlighted, demonstrating that *emm*44, *emm*77, and *emm*12 accounted for a substantial proportion of isolates. Among these types, *emm*44, *emm*77, and *emm*12 showed the highest resistance frequencies to different antibiotics, with overall resistance rates of 19.27%, 18.75%, and 17.71%, respectively. In particular, *emm*44 showed the highest resistance to ciprofloxacin (n = 8), followed by high frequencies for tetracycline (n = 7), erythromycin (n = 6), and azithromycin (n = 5), highlighting its multidrug-resistant potential followed by *emm*1 and *emm*3 contributing 16.15% and 13.54% of the total resistance burden, respectively.

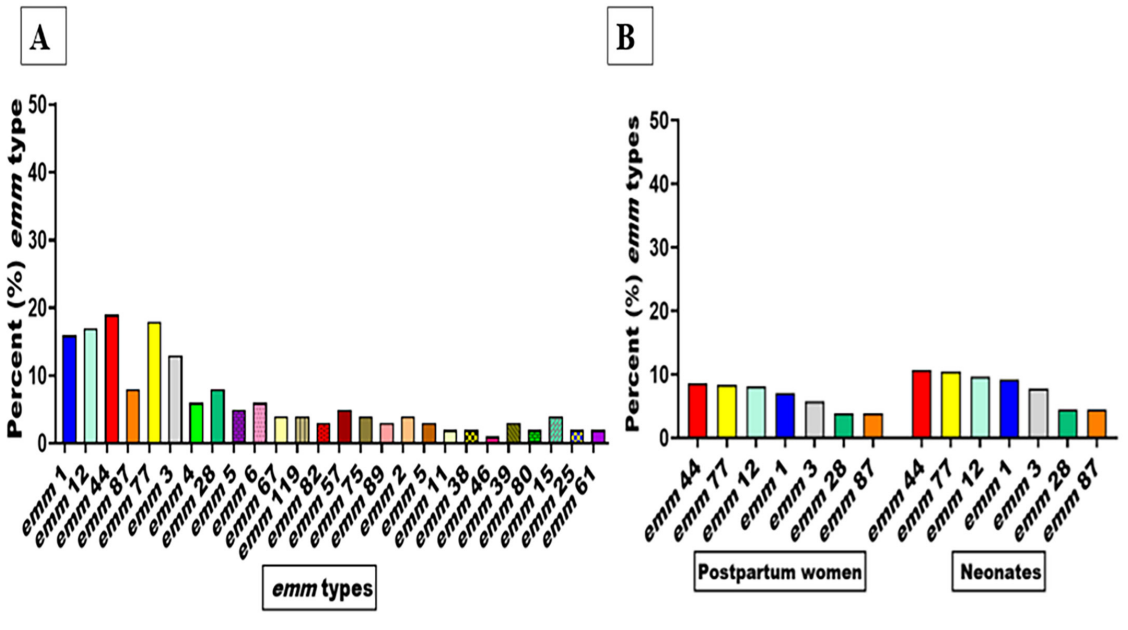

Fig. 3. Distribution of *emm* types of *Streptococcus pyogenes*.
A) Percentage of different *emm* types among isolates; B) the most common *emm* types in postpartum women and neonates identified in the study population. Various colors and patterns indicate distinct *emm* types.

Clinical Features of *S. pyogenes* Infection in Postpartum Women and Neonates. Table II presents the clinical features of *S. pyogenes* infection that vary between postpartum women and neonates. Among postpartum women, the most common symptoms were fever > 38°C (75.0%), sepsis (50.0%), and bacteremia (positive blood culture) (58.3%), indicating systemic infection. Other notable clinical signs included hypotension (20.8%), tachycardia (25.0%), and abdominal pain (29.2%), suggestive of a severe inflammatory response. In neonates, the most frequently observed symptoms were fever > 38°C (66.7%), respiratory distress (55.6%), and positive blood culture (66.7%), highlighting the severity of systemic involvement in neonatal cases. Additional clinical features, including lethargy (50.0%), vomiting (44.4%), and hypoglycemia (38.9%), were common in infected neonates, indicating potential metabolic disturbances. Symptoms such as cyanosis (16.7%) and feeding difficulties (33.3%) further highlighted the critical nature of neonatal infections. Statistical analysis revealed no significant difference in fever prevalence between postpartum women and neonates ($\chi^2 = 0.34$, $p = 0.56$). Postpartum women exhibited wound infections (33.3%, all secondary to cesarean delivery or episiotomy), while neonates had no surgical-site infections.

Risk factors and laboratory features associated with *S. pyogenes* infection. Table III presents the risk factors and laboratory findings associated with *S. pyogenes* infection in postpartum women and neonates. Among maternal risk factors, prolonged labor (> 18 hours) (adjusted OR: 2.6, 95% CI: 1.2–6.0, $p = 0.030$), premature rupture of membranes (adjusted OR: 2.1, 95% CI: 1.0–4.6, $p = 0.039$), poor socioeconomic status (adjusted OR: 2.3, 95% CI: 1.2–4.6, $p = 0.027$), and limited prenatal care (< 3 visits) (adjusted OR: 2.4, 95% CI: 1.2–5.0, $p = 0.036$) were significantly associated with infection. Similarly, among neonates, preterm birth (< 37 weeks) (adjusted OR: 3.0, 95% CI: 1.4–6.7, $p = 0.013$) and neonatal resuscitation (adjusted OR: 2.6, 95% CI: 1.2–5.7, $p = 0.028$) showed strong associations. Laboratory findings revealed a higher prevalence of positive blood cultures for *S. pyogenes* among infected individuals (adjusted OR: 1.8, 95% CI: 0.9–3.7, $p = 0.054$), though borderline significant. Other hematological markers, including elevated CRP, leukocytosis, thrombocytopenia, and anemia, exhibited weaker or nonsignificant associa-

Table II
Clinical manifestations of *Streptococcus pyogenes* infection in postpartum women and neonates in Khyber Pakhtunkhwa.

Clinical Feature	Postpartum women (n = 32)	Neonates (n = 23)	χ^2 Value	*p*–Value
Fever (> 38°C)	24 (75.0%)	16 (66.7%)	0.34	0.56
Hypothermia (< 36°C)	4 (12.5%)	6 (27.8%)	1.98	0.16
Sepsis	16 (50.0%)	12 (55.6%)	0.16	0.69
Meningitis	6 (16.7%)	8 (33.3%)	2.18	0.14
Wound infection	10 (33.3%)	–	–	–
Bacteremia	18 (58.3%)	16 (66.7%)	0.37	0.54
Respiratory distress	–	12 (55.6%)	–	–
Jaundice	–	8 (33.3%)	–	–
Hypotension	7 (20.8%)	5 (22.2%)	0.01	0.92
Tachycardia	8 (25.0%)	9 (38.9%)	1.13	0.29
Multi-organ dysfunction	4 (12.5%)	3 (11.1%)	0.02	0.88
Vomiting	8 (25.0%)	10 (44.4%)	2.25	0.13
Hypoglycemia	6 (16.7%)	9 (38.9%)	3.73	0.05
Lethargy	7 (20.8%)	12 (50.0%)	5.46	0.02
Rash	6 (16.7%)	6 (27.8%)	0.96	0.33
Abdominal pain	10 (29.2%)	–	–	–
Feeding difficulties	–	8 (33.3%)	–	–
Cyanosis	–	4 (16.7%)	–	–

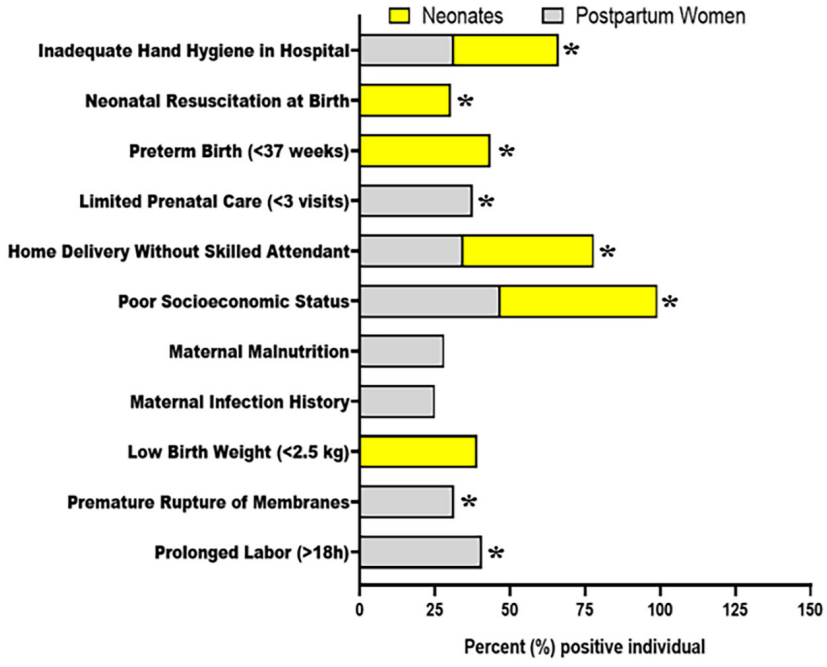

Fig. 4. Risk factors associated with neonatal and postpartum maternal infections. Neonatal-associated factors are highlighted in yellow, whereas postpartum maternal-associated factors are shown in gray. * – indicates statistical significance ($p < 0.05$)

Table III
Risk factors and laboratory findings associated with *Streptococcus pyogenes* infection.

Parameter	Postpartum women (%) (n = 32)	Neonates (%) (n = 23)	Crude OR (95% CI)	Adjusted OR (95% CI)	*p*-Value
Risk factors					
Prolonged labor (> 18h)	13 (40.6%)	–	2.9 (1.3–6.4)	2.6 (1.2–6.0)	0.030
Premature rupture of membranes	10 (31.3%)	–	2.3 (1.1–4.8)	2.1 (1.0–4.6)	0.039
Low birth weight (< 2.5 kg)	–	9 (39.1%)	1.4 (0.6–3.0)	1.2 (0.5–2.8)	0.315
Maternal infection history	8 (25.0%)	–	1.8 (0.9–3.5)	1.6 (0.8–3.2)	0.092
Maternal malnutrition	9 (28.1%)	–	1.4 (0.7–2.8)	1.3 (0.6–2.7)	0.256
Poor socioeconomic status	15 (46.9%)	12 (52.2%)	2.6 (1.4–5.1)	2.3 (1.2–4.6)	0.027
Home delivery without skilled attendant	11 (34.4%)	10 (43.5%)	3.0 (1.5–6.0)	2.7 (1.3–5.7)	0.020
Limited prenatal care (< 3 visits)	12 (37.5%)	–	2.7 (1.3–5.5)	2.4 (1.2–5.0)	0.036
Preterm birth (< 37 weeks)	–	10 (43.5%)	3.3 (1.6–7.1)	3.0 (1.4–6.7)	0.013
Neonatal resuscitation at birth	–	7 (30.4%)	2.9 (1.4–6.1)	2.6 (1.2–5.7)	0.028
Inadequate hand hygiene in hospital	10 (31.3%)	8 (34.8%)	2.8 (1.3–6.0)	2.4 (1.1–5.2)	0.034
Laboratory features					
Elevated CRP (> 10 mg/l)	20 (62.5%)	15 (65.2%)	1.2 (0.6–2.3)	1.1 (0.5–2.2)	0.984
Leukocytosis (WBC > 12,000/µl)	18 (56.3%)	13 (56.5%)	1.4 (0.7–2.7)	1.2 (0.6–2.4)	0.874
Thrombocytopenia (< 150,000/µl)	6 (18.8%)	8 (34.8%)	1.7 (0.8–3.5)	1.5 (0.7–3.1)	0.365
Anemia (Hb < 10 g/dl)	10 (31.3%)	9 (39.1%)	1.5 (0.7–3.1)	1.4 (0.6–2.9)	0.710
Positive Blood Culture for *S. pyogenes*	18 (56.3%)	15 (65.2%)	2.0 (1.1–3.9)	1.8 (0.9–3.7)	0.054

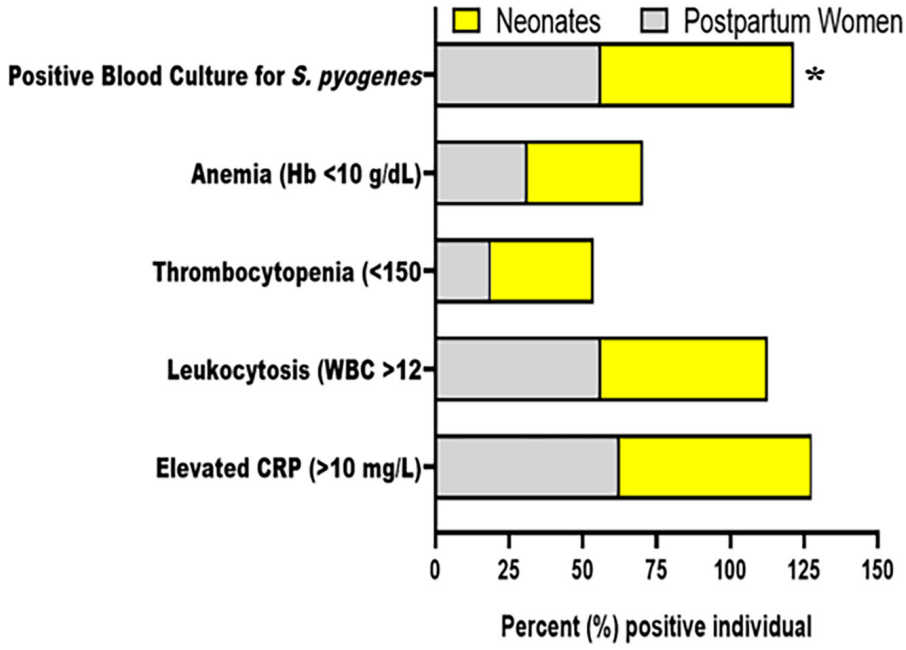

Fig. 5. Clinical and laboratory findings in neonates and postpartum women with *S. pyogenes* infections. Neonatal-associated cases are shown in yellow, whereas postpartum maternal cases are depicted in gray. * – indicates statistical significance ($p < 0.05$)

tions ($p > 0.05$). Low birth weight (adjusted OR: 1.2, 95% CI: 0.5–2.8, $p = 0.315$) and maternal malnutrition (adjusted OR: 1.3, 95% CI: 0.6–2.7, $p = 0.256$) were not significantly correlated (Fig. 5).

Discussion

The present study explored the prevalence, antibiotic susceptibility, clinical manifestations, and risk factors associated with *S. pyogenes* infections in postpartum women and neonates in tertiary care hospitals in Khyber Pakhtunkhwa, Pakistan. The overall prevalence of *S. pyogenes* was found to be 14.3%, with postpartum women showing a slightly higher prevalence (16.7%) than neonates (11.9%). These findings are in accordance with previous studies reporting prevalence rates of *S. pyogenes* ranging from 2–18% in similar healthcare settings (Leonard et al. 2019; Sherwood et al. 2022), including rural India (12–15%) (Haggar et al. 2012) and Karachi, Pakistan (11.17%) (Zafar et al. 2022), suggesting a consistent burden in low-resource regions.

Our findings confirmed that *S. pyogenes* remains susceptible to β-lactam antibiotics, with penicillin showing ≥ 95% and ampicillin ≥ 91% susceptibility rates. However, the detection of penicillin-resistant isolates, although infrequent, is a growing concern. These findings agree with reports from neighboring India, where Berwal et al. (2019) reported 8% penicillin resistance. Similarly, Ahmad et al. (2024) and Zafar et al. (2016) documented increasing minimum inhibitory concentrations (MICs) in Pakistan, raising early warning signals despite apparent laboratory susceptibility. Akram et al. (2021) also reported a 13.1% resistance rate to amoxicillin. These findings suggest that while *pbp2x* mutations remain rare in KP (potentially due to restrictive penicillin use in obstetric practice). Regional surveillance for this emerging resistance is imperative to maintain penicillin's clinical utility as first-line therapy. Nonetheless, as reported by Musser et al. (2020) from Europe, the spread of *pbp2x*-associated reduced β-lactam susceptibility across different regions highlights the critical need for ongoing molecular surveillance in our area.

In addition to β-lactams, our study identified moderate resistance levels to cephalosporins – 12.0% among postpartum women and 13.1% among neonates. While not alarmingly high, these figures urge caution when selecting empirical therapies, particularly for vulnerable populations. Our results align with regional data, including reports of up to 49.3% cefixime resistance in

Pakistan (Rizwan et al. 2016), cefixime (17.6%) and ceftriaxone (8.1%) resistance in Iran (Khademi et al. 2021). In the US, Vannice et al. (2020) reported GAS clinical isolates with elevated ampicillin, amoxicillin, and cefotaxime MICs conferred by a *pbp2x* mutation. A resistance rate of 1.5 % to penicillin has been reported from China (Li et al. 2016). Together, these patterns emphasize the urgent need for robust antimicrobial stewardship programs, especially in maternal and neonatal healthcare settings, to safeguard the efficacy of these critical antibiotics in maternal-neonatal care.

In the present study, macrolide resistance among *S. pyogenes* isolates to erythromycin was observed in 23.5% of postpartum women and 29% of neonates, and azithromycin resistance rates were 20% and 25%, respectively. Although concerning, these rates are slightly lower than the 30.5% erythromycin resistance previously reported from Mardan, Pakistan (Akram et al. 2021). Our findings are broadly comparable to resistance levels documented in India (18.1%; Khandekar and Dangre-Mudey 2019) and somewhat higher than those reported in Turkey (9.4%; Ünübol et al. 2025) but lower than the 31.8% resistance described in Ethiopia (Gebre et al. 2024). Macrolide resistance in *S. pyogenes* primarily arises through the macrolide-lincosamide-streptogramin B resistance mechanism, typically mediated by *erm* genes (leading to methylation of the 23S rRNA target site) or *mef* genes (resulting in active efflux of the drug) (Leclercq and Courvalin 1991). These genetic mechanisms confer resistance to macrolides and may impact susceptibility to lincosamides and streptogramins, complicating therapeutic options. Importantly, the widespread and empirical misuse of macrolides, particularly azithromycin, for treating respiratory infections and pharyngitis in both adults and children in Pakistan (Bilal et al. 2021). This likely exerts significant selective pressure favoring the emergence and spread of resistant strains. Based on the studies of resistance rates using the *ermB* and *mefA* genes, resistance rates have been reported in other regions, such as Brazil (15.4%; Arêas et al. 2014), Spain (8.7%; Villalón et al. 2023), and the US (up to 19%; Li et al. 2023), showing the global relevance of this trend.

Our findings of moderate clindamycin resistance (15.6% in postpartum women and 21.7% in neonates) further pose therapeutic challenges, as clindamycin is often considered an alternative agent for macrolide-resistant infections. Overall, these results emphasize the urgent need for antimicrobial stewardship efforts focused on rational macrolide use and ongoing surveillance of resistance patterns to preserve the efficacy of these critical agents.

We observed high resistance rates to fluoroquinolones (ciprofloxacin: 38.7% in postpartum women, 43.5% in neonates) and tetracycline (32.1% in postpartum women, 37.5% in neonates). These findings align with the increasing resistance trends reported across South Asia, where tetracycline resistance has reached 51% and levofloxacin resistance 8.9% (Khandekar and Dangre-Mudey 2019), and in Europe, with Greece reporting 40.8% resistance to tetracycline, 18.8% to clindamycin, and 2% to levofloxacin (Meletis et al. 2023).

All isolates in our study were fully susceptible to carbapenems (meropenem) and glycopeptides (vancomycin), supporting their continued reliability for managing severe or multidrug-resistant *S. pyogenes* infections. Our findings are in agreement with Camara et al. (2013), who also reported 100% susceptibility to vancomycin, while Gashaw et al. (2025) from Ethiopia noted slightly lower sensitivity (93.87%). Additionally, Sakata et al. (2013) demonstrated potent in vitro activity of carbapenems against *S. pyogenes*, with minimal inhibitory concentrations of 0.008 μg/ml for meropenem, highlighting their superior antimicrobial profiles. The regional differences in susceptibility could partly be attributed to antibiotic usage patterns; in Pakistan, vancomycin is infrequently used in pediatric practice, and carbapenems are typically reserved for only the most severe infections, potentially preserving their efficacy.

To our knowledge, this is the first study in KP to characterize *emm* type distribution patterns in neonates and postpartum women. Among the 30 *emm* types identified in the study, predominant types (*emm*44, *emm*77, *emm*12) differ from Karachi, Pakistan (*emm*68/104/1/39; Khan et al. 2020) and Chinese (*emm*1/12; Yu et al. 2021) strains, suggesting geographic variability. These geographical differences in *emm* types could be due to variations in host immunity, climate, healthcare infrastructure and practices, or regional antibiotic usage patterns (Xiang et al. 2023). The distribution of *emm* types among both neonates and postpartum women, particularly the overlapping prevalence of *emm*44 (48% in neonates, 52% in mothers) followed by *emm*77, *emm* 12, *emm*1, *emm*3, *emm*28, and *emm*27 with varying frequencies, suggests the possibility of vertical transmission during delivery, a route supported by previous studies linking with invasive neonatal infections and toxic shock-like syndrome (Bonomo et al. 2025). The vertical transmission pattern was not directly evaluated in the present study.

Future molecular analyses comparing mother-infant transmission could explain transmission dynamics. In this study, *emm* types common in both groups indicate a highly virulent feature of these types affecting any age group as reported previously (Gergova et al. 2024). Notably, *emm*12 and reported in the study are linked to pharyngitis disease in both pediatric and adult populations including women as reported previously (Koutouzi et al. 2015). *emm*12 and *emm*1 are found to be commonly associated with both pharyngitis and severe infections in the pediatric population in Spain and China (Yu et al. 2021; Ramírez de Arellano et al. 2024). The presence of *emm*3 and *emm*28 in the present study, which are recognized for their association with streptococcal toxic shock syndrome and necrotizing fasciitis, suggests a high pathogenic potential in the regional strains (Imöhl et al. 2010). The immature pediatric immune system and the epidemiological differences in the *emm*-types detected between the age groups may explain the differences (Zachariadou et al. 2014).

The predominance of *emm*44, *emm*77, and *emm*12 in our study suggests potential links to pathogenicity and transmission dynamics in our region. Notably, *emm*44 and *emm*77 isolates exhibited higher resistance to macrolides, tetracyclines, and ciprofloxacin than other *emm* types. This aligns with global reports associating specific *emm* types with resistance genes (*ermB*, *mefA*) (Meletis et al. 2023; Ramírez de Arellano et al. 2024). Co-resistance to tetracyclines and macrolides in *S. pyogenes* often results from horizontally transferred genetic elements such as phages, transposons, and plasmids encoding resistance genes (*tetM*, *tetO*, *tetK*, *tetL*) (Villalón et al. 2023). Previous studies have shown that tetracycline resistance is associated with the *ermB* and *tetM* genes (Villalón et al. 2023). Fluoroquinolone resistance has been reported to increase in *S. pyogenes* in different countries post-2020, primarily due to mutations in the *parC* and *gyrA* topoisomerase genes (Gergova et al. 2024). Neonatal *emm*77 isolates showed higher fluoroquinolone resistance (43.5%) than postpartum women (38.7%), possibly reflecting NICU antibiotic use (Song et al. 2024).

The clinical presentations of *S. pyogenes* infections observed in this cohort align with the patterns reported in global literature, reflecting local healthcare infrastructure. Fever, sepsis, respiratory distress, and bacteremia (confirmed by positive blood cultures) were the important features among both postpartum women and neonates, which are consistent with findings from Sherwood et al. (2022) and Sokou et al. (2023), who emphasized the rapid progression and severity of invasive infections. Among the neonates, lethargy and hypoglycemia were prominent clinical signs, and this finding is consistent with Sokou et al. (2023), who reported lethargy, respiratory distress, and poor feeding as early clinical indicators of neonatal GAS infection. The hypoglycemia observed likely reflects systemic inflammatory disturbances, particularly IL-6-mediated gluconeogenesis suppression, as Wynn and Wong described (2010).

Our study identified significant predictors of GAS infection in KP province. Among postpartum women, prolonged labor (> 18 hours; $p = 0.030$), premature rupture of membranes ($p = 0.039$), poor socioeconomic status ($p = 0.027$), home deliveries without skilled attendants (34.4% of cases), and limited prenatal care (< 3 visits; $p = 0.036$) were strongly associated with infection. These findings align with global studies highlighting social determinants and delayed obstetric care as major contributors to puerperal sepsis (Say et al. 2014; Harris et al. 2023). Notably, while Cooper et al. (2023) reported healthcare-associated outbreaks in high-resource settings, our infections were predominantly community-acquired, reflecting systemic gaps in KP's maternal healthcare infrastructure. In neonates, preterm birth (< 37 weeks; $p = 0.013$) and resuscitation needs ($p = 0.028$) emerged as critical risk factors, consistent with the heightened vulnerability of preterm infants documented by Sokou et al. (2023) and Birrie et al. (2022). The frequent requirement for resuscitation in our cohort almost reflects perinatal care deficiencies, which are common in low-resource areas like KP.

Blood culture positivity (bacteremia) for *S. pyogenes* showed borderline significance ($p = 0.054$), emphasizing its diagnostic utility for invasive GAS infections (Sherwood et al. 2022). Elevated CRP, leukocytosis, thrombocytopenia, and anemia, but not statistically significant, and supported clinical suspicion of invasive bacterial disease. Early empirical antibiotic use may have tempered these laboratory responses.

Clinically, postpartum women presented with fever (> 38°C; 75%), sepsis (50%), and bacteremia (58.3%), while neonates exhibited respiratory distress (55.6%), lethargy (50%), and bacteremia (66.7%). These manifestations correlated strongly with the pathogenic *emm* types identified (*emm*1, *emm*3, *emm*28), which are historically linked to severe invasive disease (Creti et al. 2007)

Conclusions

The present study, conducted for the very first time in KP, showed a high burden of serious *S. pyogenes* infections affecting postpartum mothers and newborns, demonstrating the necessity to improve infection prevention strategies in perinatal healthcare units in low-resource settings. Continuous antibiotic surveillance and antibiotic stewardship operations are required to observe resistance patterns and modify treatment regimens. β-Lactams act as the primary first-line empirical treatment, yet medical staff should cautiously choose antibiotics due to macrolide resistance. Preventing *S. pyogenes* infections among high-risk populations requires strategic measures that combine enhanced prenatal healthcare with early diagnosis systems and infection prevention protocols inside hospitals. Large-scale surveillance, including community-based sampling in other cities of KP, is recommended for a more precise assessment of the disease burden. Future studies incorporating genomic analysis are critical to identifying the specific resistance causes and possible transmission dynamics.

Limitations

Certain limitations need to be acknowledged. Firstly, although the study included clinical samples from three major tertiary care hospitals of KP, the sample size may limit the generalizability of the present findings to the broader population of Khyber Pakhtunkhwa or other regions of Pakistan. secondly, molecular characterization of the underlying resistance genes was beyond the scope of this study.

Ethical statement

Ethical approval was granted by the Institutional Ethical Committee (Reference No: KUST/ethical committee/563/18/10/2022) of Kohat University of Science and Technology (KUST), Pakistan, as part of the principal author's doctoral research. Written informed consent was obtained from all participants or, in the case of neonates, from their parents or legal guardians.

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Author contributions

Conceptualization, methodology: Abdul Basit, Muhammad Qasim; data collection and processing: Abdul Basit; formal analysis and writing – original draft: Mubbashir Hussain, Abdul Rehman, Hassan Naveed; critical review and editing: Mubbashir Hussain, Abdul Rehman, Hassan Naveed, Taj Ali Khan, Madiha Fatima, Mian Mufarih Shah; supervision: Muhammad Qasim, Mubbashir Hussain; funding acquisition: Khalid J. Alzahrani, Khalaf F. Alsharif. All authors have read and approved the final version of the manuscript.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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