

Case Studies

PENICILLIN-RESISTANT BETA-HAEMOLYTIC GROUP B *STREPTOCOCCUS* ISOLATED FROM A URINARY TRACT INFECTION IN A NON-PREGNANT ADULT

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ABSTRACT

Streptococcus agalactiae (Group B Streptococcus, GBS) is an established cause of urinary tract infections (UTIs) and is typically susceptible to penicillin. However, although rare, resistance to beta-lactam antibiotics is increasingly reported and may complicate treatment in adults. Early identification of resistant strains is essential to guide appropriate antimicrobial therapy. We report a case of a 54-year-old non-pregnant woman with diabetes mellitus (DM) who presented with symptomatic UTI. Urine culture yielded beta-haemolytic GBS at $>10^5$ CFU/mL. Initial empirical treatment with amoxicillin-clavulanate was ineffective. Antimicrobial susceptibility testing (AST) demonstrated resistance to penicillin. The isolate was identified as *S. agalactiae* by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), and susceptibility testing was performed in accordance with the latest Clinical and Laboratory Standards Institute (CLSI) guidelines. Following the susceptibility results, therapy was switched to intravenous ceftriaxone, resulting in complete clinical resolution of symptoms. This case highlights the clinical importance of emerging beta-lactam resistance in GBS urinary infections in non-pregnant adults and emphasises the significance of culture-directed therapy. Although phenotypic resistance was confirmed, the absence of molecular characterisation warrants cautious interpretation given the rarity of penicillin-resistant GBS. Continued surveillance is necessary to monitor resistance trends and guide treatment strategies.

KEYWORDS: *Streptococcus agalactiae*, Group B Streptococcus, urinary tract infection, penicillin resistance, antimicrobial resistance

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INTRODUCTION

Group B Streptococcus (GBS), or *S. agalactiae*, is a Gram-positive, beta-haemolytic bacterium historically recognised as a primary cause of neonatal and perinatal infections (1). It is a facultative Gram-positive coccus that colonises the gastrointestinal and genitourinary tracts. While it is well known for neonatal infections, it has emerged as a significant pathogen in adults, particularly those with comorbidities. Beyond its well-established role in obstetric populations, GBS has increasingly been identified as an important pathogen causing infections in non-pregnant adults, especially among the elderly and individuals with chronic conditions such as diabetes mellitus and immunosuppression (2). In this demographic, GBS is implicated in a broad spectrum of diseases ranging from bloodstream infections and skin and soft tissue infections to urinary tract infections (UTIs) (3).

Although GBS accounts for a smaller proportion of UTIs compared with Gram-negative organisms such as *Escherichia coli*, it is increasingly identified in patients with predisposing factors such as diabetes, urinary tract obstruction, and advanced age; recent epidemiological studies have demonstrated a growing clinical significance of GBS-associated UTIs in adults, with isolation rates reported between 1% and 5% of positive urine cultures, particularly in female patients with predisposing factors such as diabetes mellitus, urinary tract obstruction, and immunosuppression (4,5). Molecular investigations have further revealed that certain GBS strains harbour adaptations that allow survival and persistence in the urinary tract milieu, indicating a unique pathogenic capacity in this niche (4,6). In non-pregnant adults, GBS-associated UTIs are often linked to underlying conditions such as diabetes mellitus, urinary tract abnormalities, or advanced age. These infections may present similarly to typical UTIs but are often under-recognised due to the predominance of Gram-negative uropathogens (7–9).

However, recent phenotypic and molecular studies have documented increasing macrolide and lincosamide resistance and, more rarely, reduced susceptibility to beta-lactam antibiotics associated with alterations in penicillin-binding proteins (10–13). Accurate organism identification and antimicrobial susceptibility testing according to established standards are therefore essential to guide appropriate therapy (14). Given the rarity yet growing recognition of penicillin-resistant GBS outside perinatal settings, documenting these cases is vital to inform antimicrobial stewardship and public health surveillance strategies.

In this context, we present a rare case of confirmed penicillin-resistant beta-haemolytic GBS isolated from a urinary tract infection in a non-pregnant adult, emphasising the importance of culture-directed therapy and resistance monitoring in adult GBS infections.

CASE DETAILS

A 54-year-old nulliparous woman with a known pelvic-ureteric junction (PUJ) stricture presented with a five-day history of dysuria, increased urinary frequency, and suprapubic discomfort. Her medical history included well-controlled type 2 DM and hypertension. She denied recent antibiotic use or prior urinary tract infections. On examination, she was afebrile with stable vital signs. Mild suprapubic tenderness was noted, with no flank pain. Urinalysis showed pyuria (>10 white blood cells per high-power field) and positive leukocyte esterase. Urine culture yielded beta-haemolytic Group B Streptococcus (GBS) at $>10^5$ CFU/mL. On culture, grey beta-haemolytic colonies were observed on blood agar (Figure 1), while CLED agar demonstrated small, yellow, convex colonies consistent with GBS morphology (Figure 2). The organism was identified as *S. agalactiae* by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS), with a log score of 2.01,

indicating high-confidence species-level identification according to the manufacturer's criteria (Figure 3). However, initial susceptibility testing demonstrated reduced inhibition zones for penicillin, erythromycin, and clindamycin (6 mm each). Confirmatory MIC testing yielded a penicillin MIC of 6 µg/mL consistent with resistance according to CLSI M100, 35th edition (2025), breakpoints (14) (Table 1 and Figure 4). The patient was initially treated empirically with oral amoxicillin-clavulanate, but symptoms persisted after three days. Following susceptibility results, treatment was changed to intravenous ceftriaxone, resulting in complete resolution of symptoms within one week. No adverse drug reactions were observed. At four-week follow-up, there was no recurrence.

METHODOLOGY

Urinalysis and Microscopy: A midstream urine specimen collected as part of routine clinical care was submitted to the microbiology laboratory in a sterile, leak-proof container. The specimen was processed on the same day according to the laboratory's routine diagnostic procedures. It underwent urinalysis, microscopy, quantitative culture, organism identification, and antimicrobial susceptibility testing. The resulting bacterial isolate was archived and subsequently used for retrospective laboratory characterization. For microscopy, the urine specimen was examined for pyuria, while leukocyte esterase was assessed using standard urine dipstick analysis according to the manufacturer's instructions.

Quantitative Bacterial Culture: For culture, urine was inoculated using a calibrated sterile loop to allow semi-quantitative colony counting. A measured volume of urine was streaked onto 5% sheep blood agar and cystine-lactose-electrolyte-deficient (CLED) agar plates. The plates were incubated aerobically at 35–37°C for 18–24 h. After incubation, plates were examined for colony morphology, haemolysis, purity of growth, and colony count.

Organism Identification (MALDI-TOF MS): Bacterial identification was performed using matrix-assisted laser desorption ionisation–time of flight mass spectrometry (MALDI-TOF MS). Briefly, a fresh bacterial colony was smeared directly onto a reusable stainless steel MALDI target plate. The sample spot was then overlaid with 1 µL of matrix solution of α -cyano-4-hydroxycinnamic acid (HCCA) in 50% acetonitrile and 2.5% trifluoroacetic acid and allowed to air-dry at room temperature to facilitate co-crystallisation. Mass spectra were acquired on a Microflex LT MALDI-TOF MS instrument (Bruker Daltonics, Bremen, Germany) in linear positive ion mode, covering a mass-to-charge ratio (m/z) range of 2,000 to 20,000 Da. Each acquired spectrum was the sum of 40 laser shots into 6 distinct, randomly selected spots on a target spot of the MALDI plate. The generated spectra were analysed using MALDI Biotyper software version 3.1 against the locally installed MBT Compass Library. Identifications were validated utilising the manufacturer's standard log score criteria: a score of ≥ 2.00 was accepted for high-confidence identification at the species level; a score between 1.70 and 1.99 was accepted for reliable identification at the genus level; and a score < 1.70 was considered unreliable.

Antimicrobial Susceptibility Testing (AST): Initial antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion susceptibility test according to the laboratory's standard operating procedure. Because the initial AST reported penicillin resistance, confirmatory antimicrobial susceptibility testing was performed using the gradient diffusion strip in accordance with the Clinical and Laboratory Standards Institute (CLSI) M100, 35th edition (2025) (14). The panel was incubated aerobically at 35–37°C for 18–24 h. The MIC was read at the point where the edge of the inhibition

ellipse intersected the MIC scale on the gradient diffusion strip. MIC results were interpreted according to CLSI M100, 35th edition (2025), breakpoints (14).

Reference Laboratory Confirmation: The isolate was subsequently referred to the Institute for Medical Research, Malaysia, for further confirmation. Repeat phenotypic identification and antimicrobial susceptibility testing were performed in accordance with routine reference laboratory protocols. The organism identity and susceptibility profile were confirmed based on concordant phenotypic findings. No molecular methods were employed for confirmation. Specifically, 16S rRNA gene sequencing and whole genome sequencing (WGS) were not performed, and no molecular characterisation of antimicrobial resistance mechanisms was undertaken.

Table 1. Antimicrobial susceptibility profile of *S. agalactiae* isolate

Antibiotic	Method Used	Zone diameter (mm) / MIC (µg/mL)	Interpretation (CLSI M100, 2025)
Penicillin	Disk diffusion	6 mm	Resistant (R)
Erythromycin	Disk diffusion	6 mm	Resistant (R)
Clindamycin	Disk diffusion	6 mm	Resistant (R)
Penicillin	Gradient diffusion strip	6 µg/mL	Resistant (R)



Figure 1. Blood agar culture showing grey, beta-haemolytic colonies (urine isolate)

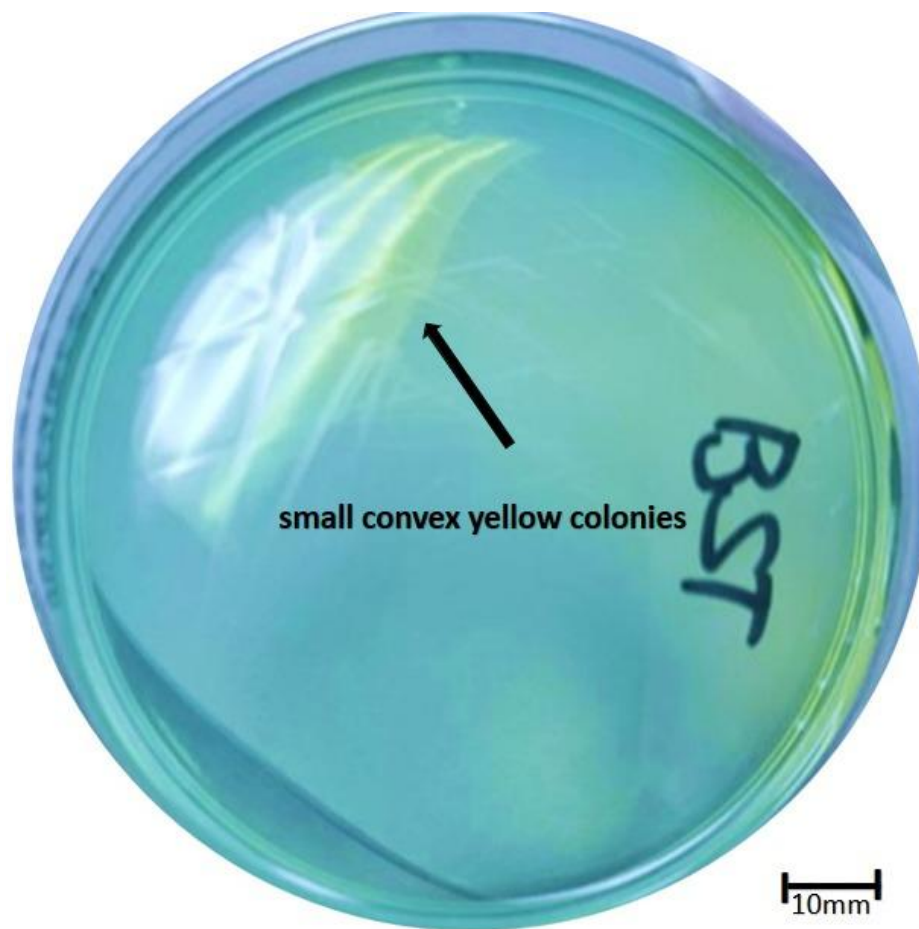


Figure 2. CLED (Cystine–Lactose–Electrolyte-Deficient) agar showing small yellow convex colonies (arrow) consistent with GBS

Result Overview

Sample Name	Sample ID	Organism (best match)	Score Value	Organism (second-best match)	Score Value
<u>B7</u> (+++)(A)	U.9796 (standard)	Streptococcus agalactiae	<u>2.01</u>	Streptococcus agalactiae	<u>1.99</u>

Figure 3. Exported machine-generated MALDI-TOF MS identification report showing identification of *S. agalactiae* using Bruker Microflex LT system with a log score of 2.01, consistent with high-confidence species-level identification according to the manufacturer's criteria

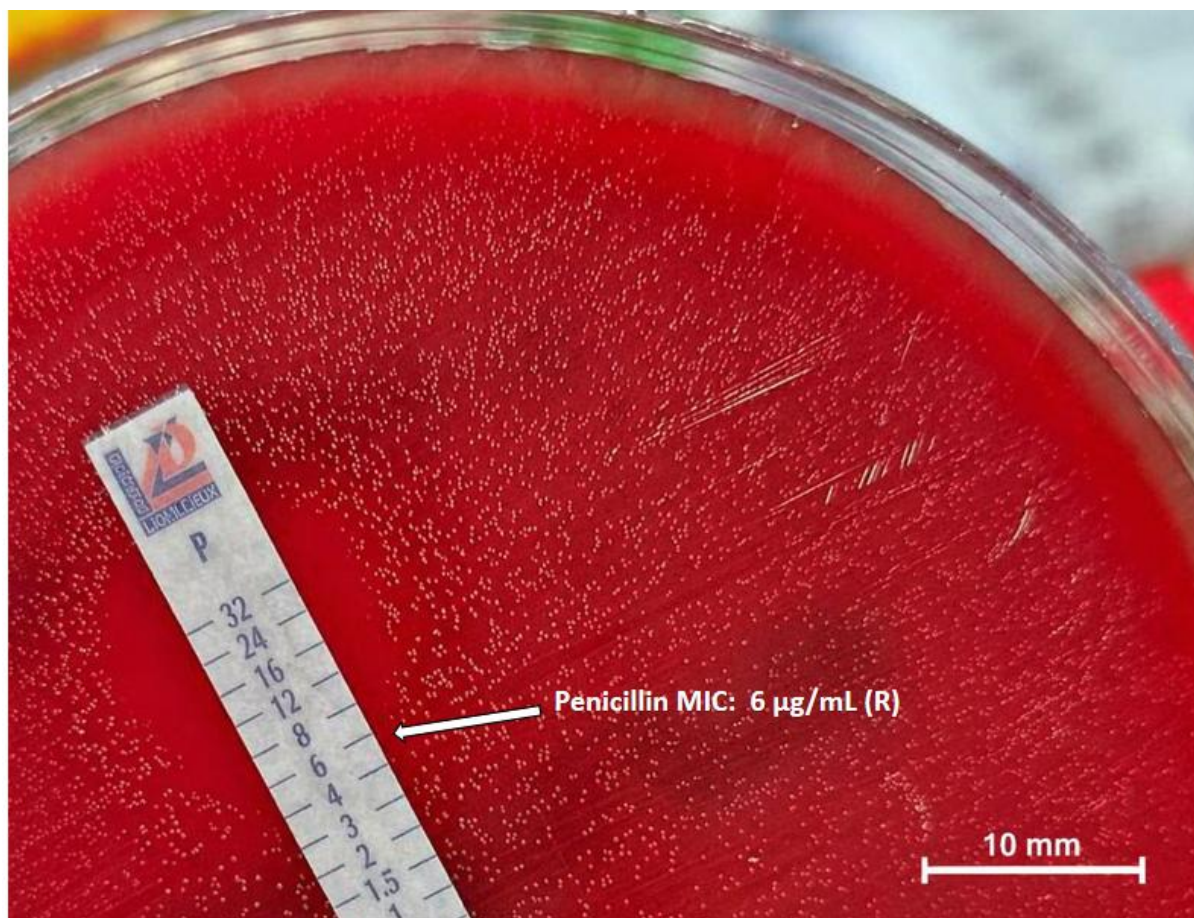


Figure 4. Up-close image of gradient diffusion strip showing a penicillin minimum inhibitory concentration (MIC) of 6 µg/mL, interpreted as resistant according to CLSI M100, 35th edition (2025) breakpoints (14)

DISCUSSION

S. agalactiae (Group B Streptococcus, GBS) is a key pathogen in perinatal infections and is increasingly recognised as an invasive organism in non-pregnant adults, particularly those with diabetes or structural urinary tract abnormalities. GBS epidemiology has shifted, with rising colonisation and infection rates in older and immunocompromised individuals, contributing to a growing burden of urinary tract infections, soft tissue infections, and bacteraemia (15,16).

Penicillin and aminopenicillins have traditionally been highly effective against GBS. However, increasing resistance to macrolides and lincosamides has been well documented, and reports of resistance to beta-lactam antibiotics are emerging (17,18). Although still rare, these findings are clinically noteworthy as they may compromise standard empirical therapy.

Resistance in GBS has been associated with alterations in penicillin-binding proteins (PBPs), reducing affinity for beta-lactam antibiotics. In addition, macrolide and lincosamide resistance is often mediated by the MLSB phenotype, which can confer cross-resistance to multiple antimicrobial classes (19–21). Reports of human- and aquatic-derived strains exhibiting multidrug-resistant (MDR) GBS, including

resistance to penicillin, erythromycin, clindamycin, and fluoroquinolones, further highlight the need for continued surveillance and antimicrobial stewardship (20).

In this case, the patient had underlying diabetes and a structural urinary abnormality, both recognised risk factors for GBS infection. The failure of initial empirical therapy with amoxicillin-clavulanate highlights the importance of performing susceptibility testing, particularly in atypical presentations or when clinical response is suboptimal. The subsequent clinical response to ceftriaxone highlights the value of culture-directed therapy.

The major limitation of this study is the absence of molecular confirmation of antimicrobial resistance mechanisms. Phenotypic antimicrobial susceptibility testing using an Oxoid™ gradient diffusion strip (Thermo Fisher Scientific) was performed in accordance with CLSI M100, 35th edition (2025) (14), and demonstrated a penicillin minimum inhibitory concentration (MIC) within the resistant range. No molecular methods were used to validate this finding. Specifically, 16S rRNA gene sequencing, whole-genome sequencing (WGS), and targeted analysis of mutations in penicillin-binding protein (PBP) genes were not performed. Reduced beta-lactam susceptibility in GBS has been associated with alterations in penicillin-binding proteins that reduce beta-lactam affinity (10,11,13).

This limitation is particularly important given that penicillin-resistant *Streptococcus agalactiae* remains exceptionally rare (12). Phenotypic resistance alone may be subject to variability or misclassification due to technical factors, heteroresistance, or testing variability, and without molecular confirmation, the underlying genetic basis of resistance cannot be established (11,17). As a result, the designation of this isolate as penicillin-resistant should be interpreted with caution.

Nevertheless, the isolate underwent repeat phenotypic testing, including confirmatory gradient diffusion strip and external validation at a reference laboratory with concordant results. While this supports the reliability of the observed resistance phenotype, the lack of molecular characterisation remains a significant limitation, and future studies incorporating molecular approaches are necessary to definitively confirm resistance mechanisms and contribute to the broader understanding of emerging beta-lactam resistance in GBS. This case adds to the growing evidence that antimicrobial resistance in GBS is evolving and warrants continued surveillance.

CONCLUSION

Although penicillin remains the cornerstone of treatment for GBS infections, emerging resistance highlights the need for vigilance in clinical practice. This case reinforces the need for clinicians to remain alert to the evolving landscape of GBS resistance, particularly considering emerging resistance profiles that may require adjustments in therapeutic approaches.

Supplementary Materials: Not applicable

Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Y.N.A.M.; Conceptualisation, data collection, manuscript drafting; Y.L.L.; Supervision, critical revision; S.D.G.S.; Laboratory analysis; S.B.; Data interpretation; H.E.H; External validation and review. All authors approved the final manuscript.

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Institutional Review Board Statement: This case report involved the retrospective laboratory characterisation of an archived bacterial isolate obtained during routine diagnostic testing. Relevant clinical information was retrospectively extracted from the patient's medical records and de-identified before inclusion in the manuscript. No additional specimen collection or clinical intervention was undertaken for research purposes. The study was registered under the National Medical Research Register (NMRR-ID-25-02857-ELN).

Informed Consent Statement: Written informed consent was obtained from the patient for the publication of this case report and its accompanying images. The patient received information in both English and Bahasa Malaysia and gave consent voluntarily.

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REFERENCES

1. Seale AC, Bianchi-Jassir F, Russell NJ, Kohli-Lynch M, Tann CJ, Hall J, et al. Estimates of the burden of group B streptococcal disease worldwide for pregnant women, stillbirths, and children. *Clin Infect Dis*. 2017;65(Suppl 2):S200-S219. doi: 10.1093/cid/cix664.
2. Raabe VN, Shane AL. Group B Streptococcus (*Streptococcus agalactiae*). *Microbiol Spectr*. 2019;7(2):10.1128/microbiolspec.GPP3-0007-2018. doi: 10.1128/microbiolspec.GPP3-0007-2018.
3. Sendi P, Johansson L, Norrby-Teglund A. Invasive group B streptococcal disease in non-pregnant adults: a review with emphasis on skin and soft-tissue infections. *Infection*. 2008;36(2):100–111. doi: 10.1007/s15010-007-7251-0.
4. Ulett KB, Benjamin WH Jr, Zhuo F, Xiao M, Kong F, Gilbert GL, et al. Diversity of group B Streptococcus serotypes causing urinary tract infection in adults. *J Clin Microbiol*. 2009;47(7):2055–2060. doi: 10.1128/JCM.00154-09.
5. Mongilardi N, Wilson BM, Bej TA, Briggs JM, Banks RE, Song S, et al. Group B Streptococcus in the urine in nonpregnant adults: disease or distraction? *Antimicrob Steward Healthc Epidemiol*. 2022;2(1):e131. doi: 10.1017/ash.2022.236.
6. Nguyen LM, Omage JI, Noble K, McNew KL, Moore DJ, Aronoff DM, et al. Group B streptococcal infection of the genitourinary tract in pregnant and non-pregnant patients with diabetes mellitus: an immunocompromised host or something more? *Am J Reprod Immunol*. 2021;86(6):e13501. doi: 10.1111/aji.13501.

7. Francois Watkins LK, McGee L, Schrag SJ, Beall B, Jain JH, Pondo T, et al. Epidemiology of invasive Group B streptococcal infections among nonpregnant adults in the United States, 2008–2016. *JAMA Intern Med.* 2019;179(4):479–488. doi: 10.1001/jamainternmed.2018.7269.
8. Mohanty S, Purohit G, Rath S, Seth RK, Mohanty RR. Urinary tract infection due to group B *Streptococcus*: a case series from Eastern India. *Clin Case Rep.* 2021;9(10):e04885. doi: 10.1002/ccr3.4885.
9. Farley MM. Group B streptococcal disease in nonpregnant adults. *Clin Infect Dis.* 2001;33(4):556–561. doi: 10.1086/322696.
10. Nagano N, Nagano Y, Kimura K, Tamai K, Yanagisawa H, Arakawa Y. Genetic heterogeneity in *pbp* genes among clinically isolated group B streptococci with reduced penicillin susceptibility. *Antimicrob Agents Chemother.* 2008;52(12):4258–4267. doi: 10.1128/AAC.00596-08.
11. Kimura K, Nagano N, Nagano Y, Wachino JI, Suzuki S, Shibayama K, et al. Predominance of sequence type 1 group with serotype VI among group B streptococci with reduced penicillin susceptibility identified in Japan. *J Antimicrob Chemother.* 2011;66(11):2460–2464. doi: 10.1093/jac/dkr335.
12. Seki T, Kimura K, Reid ME, Miyazaki A, Banno H, Jin W, et al. High isolation rate of multidrug-resistant group B streptococci with reduced penicillin susceptibility in Japan. *J Antimicrob Chemother.* 2015;70(10):2725–2728. doi: 10.1093/jac/dkv203.
13. Kimura K, Suzuki S, Wachino JI, Kurokawa H, Yamane K, Shibata N, et al. First molecular characterization of group B streptococci with reduced penicillin susceptibility. *Antimicrob Agents Chemother.* 2008;52(8):2890–2897. doi: 10.1128/AAC.00185-08.
14. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 35th ed. CLSI supplement M100. Wayne (PA): Clinical and Laboratory Standards Institute; 2025.
15. Choi Y, Han HS, Chong GO, Le TM, Nguyen HDT, Lee OE, et al. Updates on Group B *Streptococcus* infection in the field of obstetrics and gynecology. *Microorganisms.* 2022;10(12):2398. doi: 10.3390/microorganisms10122398.
16. Edwards MS, Baker CJ. Group B streptococcal infections in elderly adults. *Clin Infect Dis.* 2005;41(6):839–847. doi: 10.1086/432804.
17. Hsu CY, Moradkasani S, Suliman M, Uthirapathy S, Zwamel AH, Hjazi A, et al. Global patterns of antibiotic resistance in group B *Streptococcus*: a systematic review and meta-analysis. *Front Microbiol.* 2025;16:1541524. doi: 10.3389/fmicb.2025.1541524.
18. Le Doare K, O'Driscoll M, Turner K, Seedat F, Russell NJ, Seale AC, et al. Intrapartum antibiotic chemoprophylaxis policies for the prevention of Group B streptococcal disease worldwide: systematic review. *Clin Infect Dis.* 2017;65(Suppl 2):S143–S151. doi: 10.1093/cid/cix654.
19. Decoster L, Frans J, Blanckaert H, Lagrou K, Verhaegen J. Antimicrobial susceptibility of group B streptococci collected in two Belgian hospitals. *Acta Clin Belg.* 2005;60(4):180–184. doi: 10.1179/acb.2005.032.
20. Li C, Sapugahawatte DN, Yang Y, Wong KT, Lo NWS, Ip M. Multidrug-resistant *Streptococcus agalactiae* strains found in human and fish with high penicillin and cefotaxime non-susceptibilities. *Microorganisms.* 2020;8(7):1055. doi: 10.3390/microorganisms8071055.
21. Petca A, Șandru F, Negoită S, Dumitrașcu MC, Dimcea DAM, Nedelcu T, et al. Antimicrobial resistance profile of Group B streptococci colonization in a sample population of pregnant women from Romania. *Microorganisms.* 2024;12(2):414. doi: 10.3390/microorganisms12020414.