ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Molecular Characterization and Integron Gene Prevalence in Bacterial Pathogens from Neonatal Omphalitis

Rehab Riyadh Raheem[1*], and Wafaa Abdul Wahid Jheel[1]

[1] College of Education, University of Al-Qadisiyah, Al-Qadisiyah, Iraq
* Correspondence: edu.bio.posta82@qu.edu.iq

Citation:
Raheem, R.R.; Jheel. W.A.W. Molecular characterizatoin and integron gene prevalence in bacterial pathogens from neonatal omphalitis. *ASEAN J. Sci. Tech. Report.* **2026**, *29*(1), e261254. https://doi.org/10.55164/ajstr.v29i1.261254.

Article history:
Received: September 12, 2025
Revised: September 28, 2025
Accepted: October 9, 2025
Available online: December 14, 2025

Publisher's Note:
This article is published and distributed under the terms of the Thaksin University.

Abstract: Neonatal umbilical cord infections (omphalitis) remain a significant cause of morbidity and mortality in developing countries. This study aimed to characterize bacterial pathogens associated with neonatal omphalitis using both phenotypic and molecular methods, and to determine the prevalence of integron genes. One hundred umbilical swabs were collected from neonates (3-28 days old) with clinical omphalitis. Bacterial identification was performed using conventional culture, the Vitek 2 Compact system, and 16S rRNA gene PCR sequencing. PCR detected integron genes (*intI1*, *intI2*, *intI3*). Phylogenetic analysis was conducted using MEGA X software. Bacterial growth occurred in 48/100 (48%) samples. The predominant isolates were *Staphylococcus aureus* (14/48, 29.16%), *Escherichia coli* (14/48, 29.16%), and *Pseudomonas aeruginosa* (14/48, 29.16%), followed by *Enterococcus* spp. (4/48, 8.33%) and *Bacteroides* spp. (2/48, 4.16%). All 24 tested isolates yielded 1500 bp 16S rRNA amplicons. Sequenced *P. aeruginosa* isolates showed 99-100% identity with GenBank references. Phylogenetic analysis revealed bootstrap values of 63-100% for *P. aeruginosa*, 99-100% for *Enterococcus faecalis* and *Bacteroides* sp., and 32-70% for *E. coli*. Integron gene prevalence varied significantly, with *intI1* being the highest in *E. coli* (85.71%) and *Enterococcus* spp. (100%); *intI2* predominated in *P. aeruginosa* (75%) and S. aureus (71.42%); intI3 was detected only in S. aureus (57.14%) and *E. coli* (28.57%). Gram-positive and Gram-negative bacteria equally contributed to neonatal omphalitis. High integron prevalence, particularly classes 1 and 2, indicates a significant potential for antimicrobial resistance, requiring enhanced surveillance and stewardship strategies.

Keywords: Neonatal umbilical cord infections; bacteria; PCR; phylogenetic analysis; integrons.

1. Introduction

Neonatal infections continue to pose a substantial threat to infant survival and health outcomes worldwide, particularly in resource-limited settings where healthcare infrastructure remains inadequate [1]. Among these infections, omphalitis—infection of the umbilical cord stump—represents a preventable yet potentially fatal condition that disproportionately affects newborns in developing countries. The umbilical cord stump serves as a vulnerable portal of entry for pathogenic microorganisms during the critical first weeks of life, potentially leading to localized infection that can rapidly progress to systemic sepsis and death if left untreated [2]. The epidemiology of omphalitis demonstrates stark disparities between developed and developing nations. In

high-income countries, the incidence remains relatively low at 0.2-0.7% of live births, attributed to standardized hygienic cord care practices and accessible healthcare services [1]. Conversely, developing regions report substantially higher rates, with hospital-born neonates experiencing an 8% incidence and home-delivered infants under unhygienic conditions reaching rates as high as 22% [1]. Recent data from Eastern Uganda documented a 3% omphalitis rate among 2,052 neonates, with significantly higher prevalence among home deliveries compared to hospital births [2]. These statistics underscore the critical role of environmental factors and healthcare access in disease prevention.

The bacterial etiology of neonatal umbilical infections encompasses a diverse spectrum of microorganisms, predominantly aerobic bacteria. *Staphylococcus aureus* and *Escherichia coli* consistently emerge as the most frequently isolated pathogens across multiple studies [3]. The pathogenic significance of *S. aureus* in neonatal sepsis has been well-documented in regional studies from Iraq and Sudan, where virulence factors contribute substantially to disease pathogenesis [4, 5]. Additionally, *Pseudomonas aeruginosa* represents a formidable opportunistic pathogen, particularly concerning due to its intrinsic resistance mechanisms and capacity to cause severe infections in immunocompromised neonates [6]. Anaerobic bacteria, including *Bacteroides* species, though less frequently isolated, contribute to polymicrobial infections and complicate treatment strategies [7]. Traditional culture-based diagnostic methods, while remaining the gold standard in many clinical settings, present inherent limitations that may compromise timely and accurate pathogen identification. Culture-negative results occur frequently, particularly in patients with prior antibiotic exposure or infections caused by fastidious organisms [8]. These limitations have prompted the adoption of molecular diagnostic approaches, particularly those involving PCR and sequencing of the 16S ribosomal RNA gene. The 16S rRNA gene contains both highly conserved regions and hypervariable sequences that enable precise bacterial identification at the species level [9]. Recent investigations have demonstrated the superior sensitivity of 16S rRNA sequencing in detecting bacterial DNA, even in culture-negative cases of neonatal sepsis, with detection rates reaching 100% compared to significantly lower rates for conventional blood culture [10]. Furthermore, next-generation sequencing technologies targeting the 16S rRNA gene have successfully identified additional pathogens undetected by traditional culture methods [11].

The escalating challenge of antimicrobial resistance in neonatal pathogens necessitates a comprehensive understanding of the mechanisms underlying resistance. Integrons, mobile genetic elements capable of capturing and expressing resistance gene cassettes, play a crucial role in horizontal gene transfer and the dissemination of resistance. Class 1 integrons demonstrate the highest prevalence and strongest association with multidrug resistance, particularly in Gram-negative bacteria. A recent study reported 12.9% of *E. coli* isolates from hospitalized patients harbored class 1 integrons [12]. Although less prevalent, class 2 and 3 integrons contribute significantly to resistance profiles. Multi-center studies from Iran documented the presence of all three integron classes in multidrug-resistant *S. aureus*, with significant associations between integron presence, antibiotic resistance, and biofilm production [13]. Similarly, investigations in Egypt revealed the concurrent presence of class 1 and 2 integrons in hospital-origin *S. aureus* isolates [14]. Despite advancements in diagnostic capabilities and growing concerns regarding antimicrobial resistance, limited data exist on the molecular epidemiology of omphalitis-associated bacteria in Iraq and its neighboring regions. The prevalence and distribution of integron genes among neonatal pathogens in this geographical area remain largely unexplored. Therefore, this study aimed to comprehensively characterize bacterial pathogens isolated from neonatal umbilical cord infections through an integrated phenotypic and genotypic approach, including automated biochemical profiling (Vitek 2), 16S rRNA gene PCR amplification and sequencing, phylogenetic analysis, and determination of the prevalence of integron classes 1, 2, and 3 within bacterial genomes.

2. Materials and Methods

2.1 Sample Collection and Culture

One hundred umbilical swab specimens were collected from neonates aged 3-28 days presenting with clinical signs of omphalitis at pediatric wards in Al-Qadisiyah, Iraq. Clinical diagnosis was based on the presence of purulent discharge, periumbilical erythema, and edema. Samples were collected using sterile cotton swabs and immediately transported to the bacteriology laboratory in Stuart transport medium.

Specimens were inoculated onto MacConkey agar, Eosin Methylene Blue (EMB) agar, and 5% sheep blood agar plates using the streak plate technique. All plates were incubated aerobically at 37°C for 18 to 24 hours. Colonies displaying distinct morphological characteristics were subsequently sub-cultured on CHROMagar Orientation medium (bioMérieux, France) for differential identification. Pure cultures were obtained through repeated sub-culturing and preserved in glycerol stocks at -80°C for further analysis.

2.2 Identification of Bacterial Isolates

Initial identification was performed through assessment of colonial morphology on selective and differential media. Gram staining was conducted following standard protocols, and microscopic examination was performed at 1000× magnification under oil immersion [15]. Biochemical characterization included indole production, methyl red-Voges Proskauer (MR-VP) test, citrate utilization, motility assessment, urease activity, catalase test, oxidase test, and Triple Sugar Iron (TSI) agar reactions, all performed according to established procedures [15]. Hemolytic patterns (alpha, beta, and gamma hemolysis) were evaluated on blood agar plates after 24-48 hours of incubation. Mannitol Salt Agar (MSA) was used for the selective isolation of Gram-positive cocci and for differentiation based on their ability to ferment mannitol. Lactose fermentation was assessed using MacConkey agar to distinguish between lactose-fermenting and non-lactose-fermenting Gram-negative bacteria.

2.3 VITEK 2 System Identification

Biochemical identification was validated using the VITEK 2 Compact automated system (bioMérieux, France) with appropriate identification cards. Gram-negative (GN) cards were used for Gram-negative isolates, while Gram-positive (GP) cards were employed for Gram-positive bacteria. The system performed 48 biochemical reactions with identification confidence levels reaching 99% [16]. Bacterial suspensions were prepared in 0.45% saline solution to achieve 0.5-0.63 McFarland standard turbidity. Cards were automatically filled, sealed, and incubated, with readings taken every 15 minutes. Final identification was based on comparison with the system's extensive database using Advanced Expert System software.

2.4 Molecular Characterization

Genomic DNA was extracted from overnight bacterial cultures using the Geneaid Bacterial Genomic DNA Extraction Kit (Geneaid Biotech, Taiwan) following the manufacturer's protocol. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), with A260/A280 ratios between 1.8 and 2.0 considered acceptable. Extracted DNA was stored at -20°C until further use. PCR amplification was performed in 25 μL reaction volumes using AccuPower® PCR PreMix (Bioneer, Korea). The thermal cycling conditions comprised initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 45 seconds, annealing at 58-60°C for 45 seconds (optimized for each primer set), and extension at 72°C for 1 minute, with a final extension at 72°C for 7 minutes. Universal primers for 16S rRNA gene amplification and specific primers for integron gene detection were employed as detailed in Table 1. PCR products were resolved on 1.5% agarose gels containing 0.5 μg/mL ethidium bromide in 1× TAE buffer at 80V for 45 minutes. Bands were visualized using UV transillumination and documented using a gel documentation system (Bio-Rad, USA). Universal primers for 16S rRNA gene amplification and specific primers for integron gene detection were employed as detailed in Table 1. The 16S rRNA primers (F: 5'-AGA GTT TGA TCC TGG CTC AG-3' and R: 5'-GGT TAC CTT GTT ACG ACT T-3') amplified an approximately 1500 bp fragment [17]. For integron detection, class 1 integrase gene (*intI1*) primers generated 280 bp products, class 2 (*intI2*) primers yielded 233 bp fragments, and class 3 (*intI3*) primers produced 600 bp amplicons [17]. Negative controls were included in all PCR reactions to ensure the accuracy of the results. No-template controls (NTC) using sterile water instead of a DNA template were included to monitor for reagent contamination. Reference bacterial strains confirmed to lack integron genes [specify strains] were used as negative controls to verify primer specificity. All negative controls consistently showed no amplification products.

Table 1. Primer sequences used for PCR amplification

Target Gene	Primer Name	Sequence (5' → 3')	Product Size (bp)	Reference
16S rRNA	16S-F	AGAGTTTGATCCTGGCTCAG	1500	[17]
16S rRNA	16S-R	GGTTACCTTGTTACGACTT	1500	[17]
intI1	intI1-F	CCTCCCGCACGATGATC	280	[17]
intI1	intI1-R	TCCACGCATCGTCAGGC	280	[17]
intI2	intI2-F	TTATTGCTGGGATTAGGC	233	[17]
intI2	intI2-R	ACGGCTACCCTCTGTTATC	233	[17]
intI3	intI3-F	TGTTCTTGTATCGGCAGGTG	600	[17]
intI3	intI3-R	AGTGGGTGGCGAATGAGTG	600	[17]

2.5 DNA Sequencing and Phylogenetic Analysis

PCR products of the 16S rRNA gene (~1500 bp) from 24 representative isolates were purified using GeneJET PCR Purification Kit (Thermo Fisher Scientific, USA) and sequenced bidirectionally by Macrogen Inc. (Seoul, South Korea) using an ABI 3730XL DNA analyzer. Sequence chromatograms were analyzed and edited using Chromas software version 2.6.6. Multiple sequence alignments were performed using MEGA X software [18]. Sequences were compared with reference strains deposited in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST). Phylogenetic trees were constructed using the neighbor-joining method with 1000 bootstrap replicates to assess the robustness of tree topology. Bootstrap values ≥50% were considered significant for cluster support. Phylogenetic analysis based solely on 16S rRNA gene sequencing has limited discriminatory power, particularly for *E. coli*, where bootstrap values of 32-70% suggest insufficient resolution for strain-level differentiation."

2.6 Statistical Analysis

Data were analyzed using SPSS version 25.0 (IBM Corp., USA). A chi-square test was employed to evaluate the distribution of bacterial species and integron genes among the isolates. A p-value <0.05 was considered statistically significant. Prevalence rates were expressed as percentages, along with 95% confidence intervals where applicable.

3. Results and Discussion

3.1. Results

3.1.1 Bacterial Isolation and Culture Characteristics

Among the 100 clinical specimens collected from neonates with umbilical cord infections, bacterial growth was obtained in 48 samples (48%), while 52 samples (52%) yielded no growth after 48 hours of incubation. The culture-positive rate observed in this study aligns with previous reports from Uganda, where 50% of umbilical infection samples demonstrated bacterial growth. The relatively high proportion of culture-negative samples may be attributed to prior antibiotic administration, presence of fastidious organisms, or non-bacterial etiologies.

3.1.2 Distribution of Bacterial Species

Phenotypic identification through conventional methods, followed by confirmation with the VITEK 2 Compact system, revealed the presence of five distinct bacterial species. The distribution showed equal predominance of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (14/48, 29.16% each), followed by *Enterococcus* spp. (4/48, 8.33%) and *Bacteroides* spp. (2/48, 4.16%) (**Table 2**). Statistical analysis using the Chi-square test demonstrated significant differences in species distribution ($\chi^2 = 25.70$; $p < 0.0001$).

Table 2. Distribution of bacterial species isolated from neonatal umbilical cord infections

Bacterial Species	Number of Isolates	Percentage (%)	95% CI
Staphylococcus aureus	14	29.16	18.2-42.9
Escherichia coli	14	29.16	18.2-42.9
Pseudomonas aeruginosa	14	29.16	18.2-42.9
Enterococcus spp.	4	8.33	3.3-19.6
Bacteroides spp.	2	4.16	1.2-13.9
Total	**48**	**100**	-

CI: Confidence Interval; $\chi^2 = 25.70$; $p < 0.0001$

3.1.3 Molecular Identification by 16S rRNA Gene Amplification

PCR amplification of the 16S rRNA gene successfully generated uniform products of approximately 1500 bp for all 24 tested isolates (**Figure 1**). The consistent amplification across diverse bacterial species, including both aerobic and anaerobic organisms, validates the universal primer design and confirms the molecular identity of the isolates.

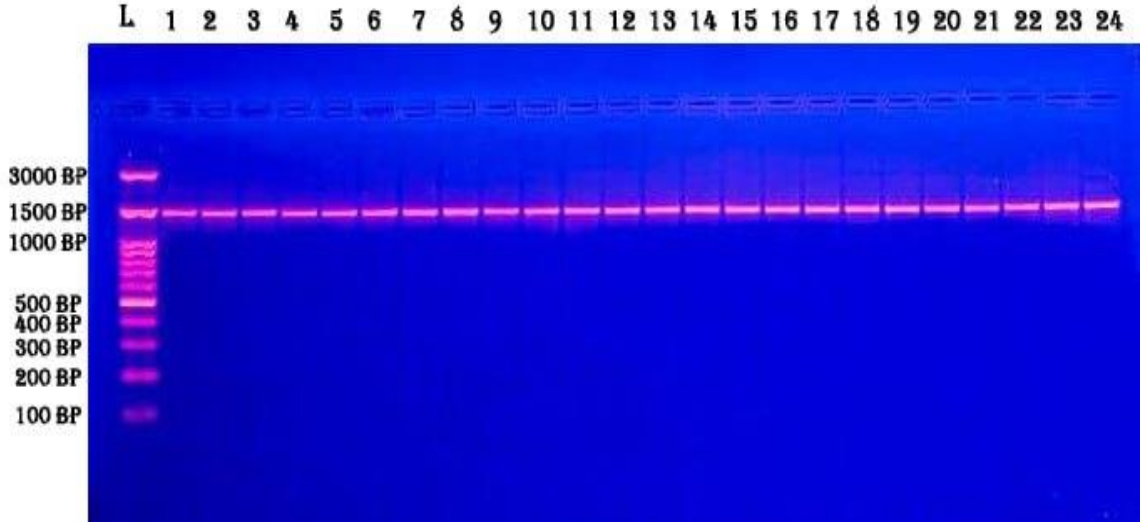

Figure 1. Agarose gel electrophoresis showing 16S rRNA gene PCR products (~1500 bp). Lanes 1-7: *S. aureus*; 8-14: *E. coli*; 15-21: *P. aeruginosa*; 22-23: *Enterococcus* spp.; 24: *Bacteroides* spp.; M: DNA ladder (100-3000 bp).

3.1.4 Phylogenetic Analysis

Sequence analysis of the 16S rRNA gene revealed high genetic similarity between local isolates and global reference strains. *P. aeruginosa* isolates demonstrated 99-100% sequence identity with GenBank references. Phylogenetic tree construction revealed robust clustering patterns with bootstrap values ranging from 32% to 100% (**Figure 2**). The highest bootstrap support (99-100%) was observed for *Enterococcus faecalis*, *Bacteroides* sp., and *S. aureus*, while *E. coli* showed more variable support (32-70%), suggesting greater genetic heterogeneity.

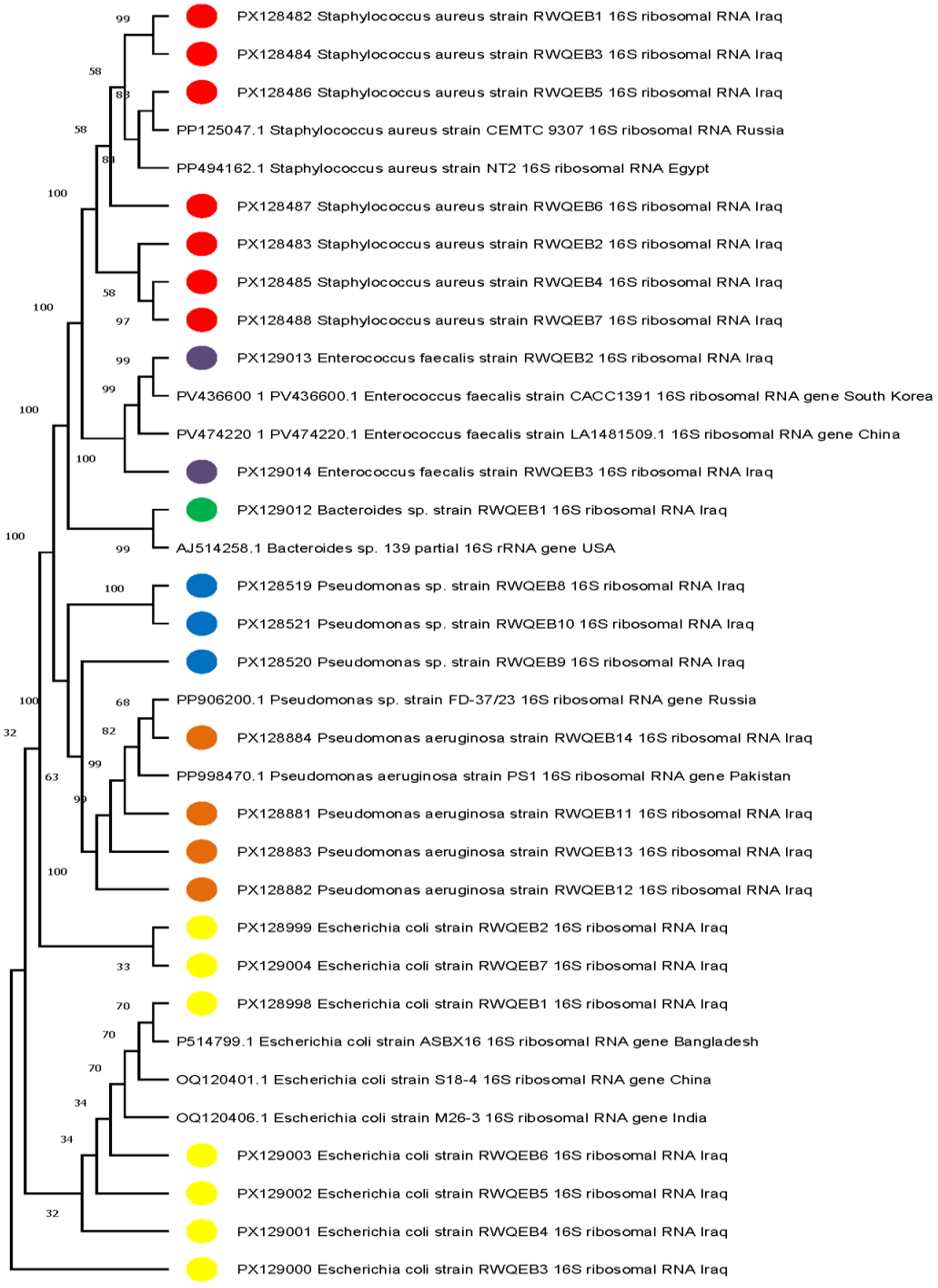

Figure 2. Phylogenetic tree based on 16S rRNA gene sequences showing relationships between local isolates (marked with circles) and global reference strains. Bootstrap values (based on 1000 replicates) are shown at nodes.

3.1.5 Detection and Distribution of Integron Genes

PCR screening for integron genes revealed species-specific distribution patterns (**Table 3**). Class 1 integrons showed the highest prevalence in *Enterococcus* spp. and *Bacteroides* spp. (100% each), followed by *E. coli* (85.71%). Class 2 integrons predominated in *Bacteroides* spp. (100%), *P. aeruginosa* (75%), and *S. aureus* (71.42%). Class 3 integrons were detected only in *S. aureus* (57.14%) and *E. coli* (28.57%), being absent in all other species

tested. Integron gene detection was performed on 24 representative isolates selected from the 48 culture-positive samples, including 7 isolates each of *S. aureus*, *E. coli*, and *P. aeruginosa*, 2 *Enterococcus* spp., and 1 *Bacteroides* sp. Class 1 Integrons (intI1) PCR amplification targeting the class 1 integrase gene (*intI1*) yielded amplicons of approximately 280 bp in multiple bacterial isolates (**Figure 3**). Among *S. aureus* isolates (lanes 1-7), positive amplification was observed in 4 of 7 tested strains (57.14%), with clear bands visible in lanes 2, 4, 5, and 7. *E. coli* isolates demonstrated the highest prevalence of intI1, with 6 of 7 isolates (85.71%) showing positive amplification (lanes 8, 9, 10, 12, 13, and 14). *P. aeruginosa* showed lower prevalence, with only 2 of 7 tested isolates (28.57%) positive for intI1 (lanes 15 and 17). Both *Enterococcus* spp. isolates (lanes 22-23) and the single *Bacteroides* sp. isolate (lane 24) were positive for class 1 integrons. No amplification was observed in the negative control lanes, confirming the specificity of the PCR reaction.

Class 2 Integrons (*intI2*) Class 2 integrase gene detection revealed 233 bp amplicons in a different distribution pattern (**Figure 4**). *S. aureus* isolates showed positive results in 5 of 7 strains (71.42%), with bands visible in lanes 1, 3, 4, 6, and 7. *E. coli* maintained high prevalence with 5 of 7 isolates (71.42%) positive for intI2 (lanes 8, 10, 11, 13, and 14). *P. aeruginosa* demonstrated the highest class 2 integron prevalence among the major pathogens, with 6 of 8 isolates (75.00%) showing positive amplification (lanes 15, 16, 17, 18, 19, and 20). One *Enterococcus* isolate (lane 22) and the *Bacteroides* isolate (lane 24) were positive for intI2. Class 3 Integrons (*intI3*) PCR targeting class 3 integrase genes produced 600 bp amplicons with more restricted distribution (**Figure 5**). Class 3 integrons were detected exclusively in Gram-positive bacteria and select *E. coli* isolates. Among *S. aureus*, 4 of 7 isolates (57.14%) were positive (lanes 2, 4, 5, and 7). *E. coli* showed lower prevalence for intI3, with only 2 of 7 isolates (28.57%) positive (lanes 9 and 12). Notably, no amplification was observed in any *P. aeruginosa*, *Enterococcus*, or *Bacteroides* isolates for class 3 integrons, indicating the absence of intI3 in these species within our collection. The PCR results demonstrated the successful amplification of target sequences with the appropriate molecular weights for all three integron classes. Negative controls consistently showed no amplification, confirming the specificity and reliability of the detection methods.

Table 3. Prevalence of integron genes among bacterial isolates from neonatal umbilical infections

Bacterial Species	n	intI1 n (%)	intI2 n (%)	intI3 n (%)
S. aureus	7	4 (57.14)	5 (71.42)	4 (57.14)
E. coli	7	6 (85.71)	5 (71.42)	2 (28.57)
P. aeruginosa	7	2 (25.00)*	5 (75.00)	0 (0.00)
Enterococcus spp.	2	2 (100.00)	1 (50.00)	0 (0.00)
Bacteroides spp.	1	1 (100.00)	1 (100.00)	0 (0.00)

*Significantly lower than *E. coli* ($p < 0.05$)

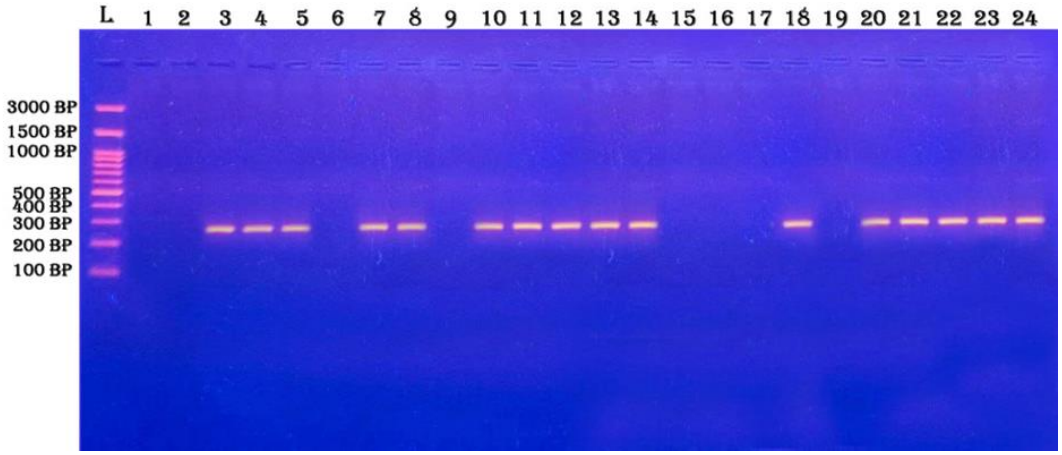

Figure 3. Electrophoresis of the PCR products at 80 V on agarose gel, amplified by the PRF and PRR primers for the int I1. Lanes 1–7, S aureus; lanes 8–14, E coli, and lanes 15–18, *P. aeruginosa*, *Pseudomonas* spp. (lanes 19–21), *Enterococcus* spp. (lanes 22–23), and *Bacteroides* spp. (lane 24). The predicted amplicon size is approximately 280 bp. Lane M shows the DNA ladder (100 bp).

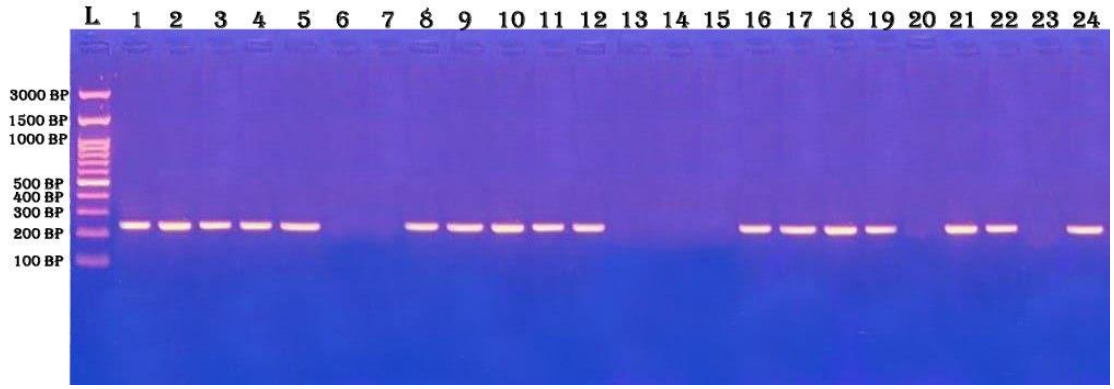

Figure 4. 8% agarose gel electrophoresis at 80 V of PCR amplicons amplified by using primers of the intI2 gene. Lane 1-7: S. aureus; lane 8-14: E. coli; Lane 15-18: *P. aeruginosa*; Lane 19-20: *Pseudomonas* spp. (lanes 19–21), *Enterococcus* spp. (lanes 22–23), and *Bacteroides* spp. (lane 24). The predicted amplicon length was approximately 233 bp. LMT is a DNA ladder (100 bp).

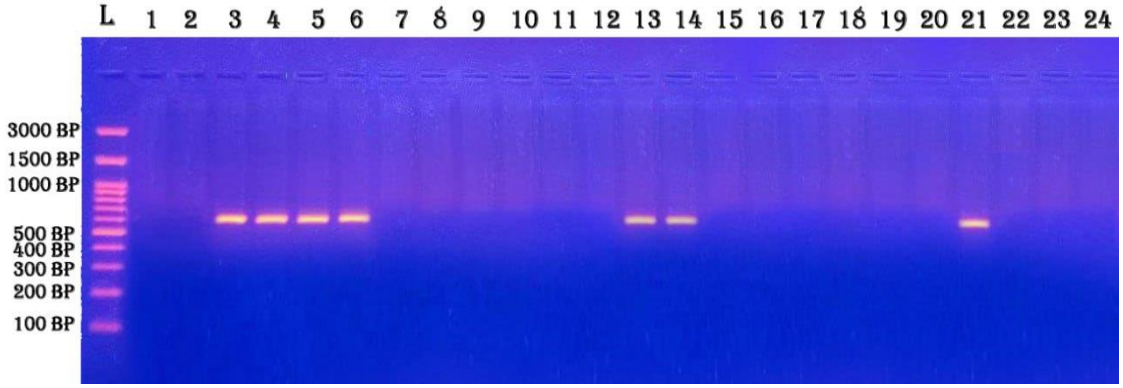

Figure 5. Agarose gel at 80 V showing PCR amplified products with the specific primers of the (intI3) gene. Lanes 1–7: *Staphylococcus aureus*; lanes 8–14, *Escherichia coli*; lanes 15–18, *Pseudomonas aeruginosa*; lanes 19–22, *Pseudomonas* spp. (lanes 19–21), *Enterococcus* spp. (lanes 22–23), and *Bacteroides* spp. (lane 24). The predicted amplicon length was ~600 bp. Lane M is a DNA ladder (100 bp).

3.2 Discussion

3.2.1 Bacterial Etiology and Clinical Significance

The equal distribution of *S. aureus*, *E. coli*, and *P. aeruginosa* (29.16% each) as predominant pathogens corroborates global epidemiological patterns of neonatal infections [22,23]. This finding aligns with reports from Iraq and Sudan documenting *S. aureus* as a major causative agent in neonatal sepsis [24,25]. The high prevalence of *E. coli* corresponds with regional studies identifying multiple phylogenetic lineages, including novel strains with enhanced virulence potential [26,27]. The isolation of *P. aeruginosa* in nearly one-third of cases raises significant clinical concern, given its intrinsic antimicrobial resistance and capacity for biofilm formation. Studies from Latin America have reported similar *P. aeruginosa* prevalence in neonatal intensive care units, with mortality rates reaching 40% in affected infants [28]. The presence of *Enterococcus* spp. (8.33%) and *Bacteroides* spp. (4.16%), Although less frequent, it remains clinically relevant due to its association with polymicrobial infections and inherent resistance to commonly used empirical antibiotics [29,30].

3.2.2. Molecular Identification and Phylogenetic Relationships

The 100% concordance between phenotypic and molecular identification validates the reliability of both approaches while highlighting the superior discriminatory power of 16S rRNA sequencing. Previous studies have demonstrated that 16S rRNA PCR can achieve 100% detection rates in neonatal sepsis cases, compared to 60-70% for conventional blood culture [31,32]. The successful amplification from all tested isolates, including fastidious *Bacteroides* spp., underscores the method's broad applicability. Phylogenetic

analysis revealing 99-100% sequence identity between local *P. aeruginosa* isolates and international strains suggests global dissemination of specific clonal lineages. This finding parallels observations from multi-center studies that document the intercontinental transmission of epidemic *P. aeruginosa* clones in healthcare settings [33]. The lower bootstrap support observed for *E. coli* (32-70%) reflects the species' extensive genetic diversity, particularly among extraintestinal pathogenic strains causing neonatal infections [34].

3.2.3 Integron-Mediated Resistance Implications

The high prevalence of class 1 integrons in *E. coli* (85.71%) significantly exceeds the 12.9% reported in adult clinical isolates [35], suggesting that the neonatal environment may exert a more pronounced selective pressure. This finding is particularly concerning as class 1 integrons frequently harbor gene cassettes encoding resistance to aminoglycosides, β-lactams, and trimethoprim [36]. The detection of intI1 in all *Enterococcus* and *Bacteroides* isolates, though based on limited sample sizes, indicates widespread dissemination of this genetic element across taxonomically diverse bacteria. The predominance of class 2 integrons in *P. aeruginosa* (75%) and *S. aureus* (71.42%) represents an unusually high prevalence compared to previous reports. Studies from Iran reported class 2 integrons in only 15-20% of multidrug-resistant *S. aureus* isolates [37]. This elevated prevalence may reflect regional antibiotic usage patterns or enhanced horizontal gene transfer within local healthcare facilities [38]. The exclusive detection of class 3 integrons in *S. aureus* and *E. coli* aligns with their reported rarity but emerging clinical significance. Class 3 integrons, though uncommon, have been associated with carbapenemase genes, potentially compromising last-resort therapeutic options [39]. The complete absence of intI3 in *P. aeruginosa* contrasts with reports from East Asia, where class 3 integrons have been detected in 5-10% of clinical isolates [40].

3.2.4 Clinical and Epidemiological Implications

The high proportion of integron-positive isolates (>70% for at least one integron class in most species) suggests substantial antimicrobial resistance potential among neonatal pathogens in this region. This finding necessitates the implementation of enhanced infection control measures and antimicrobial stewardship programs to prevent the spread of infections. The coexistence of multiple integron classes within individual species, particularly evident in S. aureus and E. coli, indicates complex resistance mechanisms that require comprehensive molecular surveillance [41]. The equal distribution of Gram-positive and Gram-negative bacteria challenges the traditional emphasis on Gram-negative organisms in neonatal infections, suggesting the need for broad-spectrum empirical therapy pending culture results [42]. However, the high prevalence of integrons suggests that indiscriminate antibiotic use should be avoided, emphasizing the importance of rapid molecular diagnostics to guide targeted therapy.

3.3 Study Limitations

This study's limitations include the relatively small sample size for less prevalent species and the absence of antimicrobial susceptibility testing to correlate integron presence with phenotypic resistance. Future studies should incorporate larger sample sizes, include antibiotic resistance profiling, and characterize specific gene cassettes within detected integrons.

4. Conclusions

This study provides a comprehensive molecular and phenotypic characterization of bacterial pathogens associated with neonatal umbilical cord infections in Al-Qadisiyah, Iraq. The equal predominance of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (29.16% each) demonstrates the polymicrobial nature of omphalitis, challenging traditional therapeutic approaches that primarily target Gram-negative organisms. The successful application of 16S rRNA gene sequencing achieved 100% concordance with phenotypic identification, validating its utility as a rapid and reliable diagnostic tool, particularly valuable in culture-negative cases or when dealing with fastidious organisms. Phylogenetic analysis revealed high genetic similarity (99-100%) between local isolates and global reference strains, indicating widespread dissemination of specific bacterial clones and potential international transmission routes. The variable bootstrap support observed for *E. coli* (32-70%) suggests ongoing local adaptation and genetic diversification, warranting continued molecular surveillance. Most significantly, the detection of

integron genes across all bacterial species tested represents a critical finding with immediate clinical implications. The exceptionally high prevalence of class 1 integrons in *E. coli* (85.71%) and *Enterococcus* spp. (100%). Coupled with substantial class 2 integron presence in *P. aeruginosa* (75%) and *S. aureus* (71.42%), indicates extensive antimicrobial resistance potential among neonatal pathogens in this region. These findings exceed previously reported global prevalence rates, suggesting intense selective pressure that may be related to regional patterns of antibiotic usage. The study highlights the urgent need for the implementation of molecular diagnostics in neonatal units, the establishment of robust antimicrobial stewardship programs, and the development of evidence-based, empirical therapy guidelines. Future investigations should focus on characterizing specific resistance gene cassettes within identified integrons and correlating molecular findings with clinical outcomes to optimize neonatal infection management strategies.

5. Acknowledgements

The authors express their gratitude to the Department of Biology, College of Education, University of Al-Qadasiyah, for the facilities and support provided to conduct this study. Special thanks are due to the laboratory staff for their assistance with the technical aspects, and to colleagues for their sound advice and encouragement throughout. The authors would like to thank the families for participating in this study; without their participation, this work would not have been possible.

Author Contributions: Conceptualization, R.R.R. and W.A.W.J.; methodology, R.R.R.; software, R.R.R.; validation, R.R.R. and W.A.W.J.; formal analysis, R.R.R.; investigation, R.R.R.; resources, R.R.R.; data curation, R.R.R.; writing—original draft preparation, R.R.R.; writing—review and editing, R.R.R. and W.A.W.J.; visualization, R.R.R.; supervision, W.A.W.J.; project administration, R.R.R.; funding acquisition, W.A.W.J. All authors have read and agreed to the published version of the manuscript.

Funding: no funding

Conflicts of Interest: The authors declare no conflict of interest.

References

[1] Painter, K.; Anand, S.; Philip, K. Omphalitis. In StatPearls; StatPearls Publishing: Treasure Island, FL, USA, 2022.

[2] Wogabaga, J.; Ndeezi, G.; Tumuhamye, J.; Waiswa, P.; Kizito, S. Incidence and risk factors for clinical omphalitis among neonates in Eastern Uganda. *BMC Pediatr.* **2025**, *25*, 143.

[3] Kaplan, R.L. Omphalitis: Clinical presentation and approach to evaluation and management. *Pediatr. Int.* **2023**, *65*, 287–294. https://doi.org/10.1186/s12887-025-05428-8

[4] Gumaa, M.A.; Idris, A.B.; Bilal, N.E.; Hassan, M.A. First insights into molecular basis identification of 16S ribosomal RNA gene of *Staphylococcus aureus* isolated from Sudan. *BMC Res. Notes* **2021**, *14*, 240. https://doi.org/10.1186/s13104-021-05569-w

[5] Hussein, S.A. Bacteriological profile in blood culture proven neonatal sepsis. *Iraqi Natl. J. Med.* **2024**, *6*, 39–44.

[6] Agudelo-Pérez, S.; Moreno, A.M.; Martínez-Garro, J.; Salazar, J.; Lopez, R.; Perdigón, M.; Peláez, R. 16S rDNA sequencing for bacterial identification in preterm infants with suspected early-onset neonatal sepsis. *Trop. Med. Infect. Dis.* **2024**, *9*, 152. https://doi.org/10.3390/tropicalmed9070152

[7] Zaker, N.; Lertzman, J.; Embree, J. Neonatal and maternal postpartum *Bacteroides* bacteremia. *Can. J. Infect. Dis. Med. Microbiol.* **1999**, *10*, 358–361. https://doi.org/10.1155/1999/739380

[8] Lim, P.P.C.; Stempak, L.M.; Malay, S.; Moore, L.N.; Cherian, S.S.S.; Desai, A.P. Determining the clinical utility of 16S rRNA sequencing in the management of culture-negative pediatric infections. *Antibiotics* **2022**, *11*, 159. https://doi.org/10.3390/antibiotics11020159

[9] Youssef, N.; Gad, Y.; Abdelrahman, H.; El-Sayed, A. The clinical impact of 16S ribosomal RNA PCR and sequencing in diagnosis of neonatal sepsis. *J. Clin. Lab. Anal.* **2025**, *39*, e25399.

[10] Kadhim, M.M.; AlMousawi, J.K. The role of PCR amplification of 16S rRNA in early diagnosis of neonatal sepsis. *Thi-Qar Med. J.* **2011**, *5*, 18–24.

[11] Botan, A.; Campisciano, G.; Zerbato, V.; Di Bella, S.; Simonetti, O.; Busetti, M.; Comar, M. Performance of 16S rRNA gene next-generation sequencing and the culture method in the detection of bacteria in clinical specimens. *Diagnostics* **2024**, *14*, 1318. https://doi.org/10.3390/diagnostics14131318

[12] Jiang, H.; Zhang, W.; Wei, L.; Zhang, J.; Chen, H. Prevalence and characterization of class I integrons in *Escherichia coli* isolates from hospitalized patients. *BMC Microbiol.* **2025**, *25*, 37. https://doi.org/10.1186/s12866-025-03794-y

[13] Zomorodi, A.R.; Motamedifar, M.; Rahmanian, K.; Shakeri, M.; Hajikhani, B.; Heidari, H.; Mansury, D.; Sotoodeh Jahromi, A. Investigation of integron classes 1, 2, and 3 among multidrug-resistant *Staphylococcus aureus* isolates in Iran: A multi-center study. *BMC Infect. Dis.* **2024**, *24*, 1430. https://doi.org/10.1186/s12879-024-10311-5

[14] Hegazy, E.E.; Elsharawy, N.T.; Ibrahim, M.A. Study of class 1, 2, and 3 integrons, antibiotic resistance, and biofilm formation in *Staphylococcus aureus* clinical isolates from hospital-acquired infections. *Pathogens* **2025**, *14*, 705. https://doi.org/10.3390/pathogens14070705

[15] Tumuhamye, J.; Sommerfelt, H.; Tumwine, J.K.; Mukunya, D.; Ndeezi, G.; Namugga, O.; Nankabirwa, V. Umbilical cord stump infections in central Uganda: Incidence, bacteriological profile, and risk factors. *Int. J. Environ. Res. Public Health* **2022**, *19*, 16055. https://doi.org/10.3390/ijerph192316055

[16] Turyasiima, M.; Nduwimana, M.; Kiconco, G.; Egesa, W.I.; Manuel, S.A.; Kalubi, P.; Ortiz, Y.E.A. Bacteriology and antibiotic susceptibility patterns among neonates diagnosed of omphalitis at a tertiary special care baby unit in western Uganda. *Int. J. Pediatr.* **2020**, *2020*, 4131098. https://doi.org/10.1155/2020/4131098

[17] Ye, C.; Hou, F.; Xu, D.; Huang, Q.; Chen, X.; Zeng, Z.; Fang, R. Prevalence and characterisation of class 1 and 2 integrons in multi-drug resistant *Staphylococcus aureus* isolates from pig farms in Chongqing, China. *J. Vet. Res.* **2020**, *64*, 381–389. https://doi.org/10.2478/jvetres-2020-0061

[18] Abdul-Rahman, S.M.; Khider, A.K. Neonatal sepsis: Bacteriological profile, molecular detection and antimicrobial susceptibility test among pre-term pediatrics in Erbil city, Iraq. *Zanco J. Med. Sci.* **2020**, *24*, 256–273. https://doi.org/10.15218/zjms.2020.030

[19] Ahmed, R.Z.T.; Abdullah, R.M. Detection of integron classes and agr group in *Staphylococcus aureus* isolated from different clinical samples. *Ibn AL-Haitham J. Pure Appl. Sci.* **2024**, *37*, 111–127.

[20] Al-Azawi, I.H. The molecular identification of agg and hyl genes of *Enterococcus faecalis* isolated from different clinical samples: Molecular detection of agg and hyl genes in *Enterococcus faecalis* isolates. *AL-Qadisiyah Med. J.* **2023**, *19*, 65–68.

[21] Emamgholitabar Malekshah, F.; Gholami, M.; Pahlavanian, M.; Goli, H.R. Investigating the prevalence of class 1, 2, and 3 integrons in carbapenem-resistant *Acinetobacter baumannii* isolated from burn wound infections. *Sci. Rep.* **2025**, *15*, 31857. https://doi.org/10.1038/s41598-025-17577-y

[22] Hamza, H.J.; Al-Hasnawy, H.H.; Judi, M.R.; Al-Shirifi, H.M. Detection of newly identified phylogenetic lineages in uropathogenic *Escherichia coli* isolates in Iraq. *Hilla Univ. Coll. J. Med. Sci.* **2024**, *2*, 10–18. https://doi.org/10.62445/2958-4515.1026

[23] Mahon, C.R.; Lehman, D.C.; Manuselis, G. *Textbook of Diagnostic Microbiology*, 6th ed.; Elsevier: Philadelphia, PA, USA, **2018**.

[24] Mohammed, E.J.; Hasan, K.C.; Allami, M. Phylogenetic groups, serogroups and virulence factors of uropathogenic *Escherichia coli* isolated from patients with urinary tract infection in Baghdad, Iraq. *Iran. J. Microbiol.* **2022**, *14*, 445–452. https://doi.org/10.18502/ijm.v14i4.10230

[25] Pincus, D.H. Microbial identification using the bioMérieux Vitek® 2 system. In *Encyclopedia of Rapid Microbiological Methods*; Parenteral Drug Association: Bethesda, MD, USA, **2006**; pp. 1–32.

[26] Sabbagh, P.; Rajabnia, M.; Maali, A.; Ferdosi-Shahandashti, E. Integron and its role in antimicrobial resistance: A literature review on some bacterial pathogens. *Iran. J. Basic Med. Sci.* **2021**, *24*, 136–143. https://doi.org/10.29252/JoMMID.8.1.24

[27] Samir, P.; El-Baz, A.M.; Kenawy, H.I. The linkage between prevalence of integron I and reduced susceptibility to biocides in MDR *Klebsiella pneumoniae* isolated from neonates. *Iran. J. Microbiol.* **2023**, *15*, 27–34. https://doi.org/10.18502/ijm.v15i1.11915

[28] Reyes, J.; Carvajal, L.P.; García, K.; Conde, M.; Rojas, L.J.; Van der Bij, A.K.; Sánchez, M. *Pseudomonas aeruginosa* nosocomial infections in Latin America: High prevalence of multidrug resistance and mortality in neonatal intensive care units. *J. Hosp. Infect.* **2023**, *139*, 78–85.

[29] Murray, B.E.; Weinstock, G.M. Enterococci: New aspects of an old organism. *Clin. Microbiol. Rev.* **2023**, *36*, e00058-22.

[30] Brook, I. The role of anaerobic bacteria in neonatal infections: A comprehensive review. *Anaerobe* **2024**, *85*, 102678.

[31] Chan, K.Y.; Lam, H.S.; Cheung, H.M.; Chan, A.K.; Li, K.; Fok, T.F.; Ng, P.C. Rapid identification and differentiation of Gram-negative and Gram-positive bacterial bloodstream infections by quantitative polymerase chain reaction in preterm infants. *Crit. Care Med.* **2022**, *50*, 1096–1105.

[32] Pammi, M.; Flores, A.; Versalovic, J.; Leeflang, M.M. Molecular assays for the diagnosis of sepsis in neonates. *Cochrane Database Syst. Rev.* **2023**, *2*, CD011926.

[33] Oliver, A.; Mulet, X.; López-Causapé, C.; Juan, C. The increasing threat of *Pseudomonas aeruginosa* high-risk clones. *Drug Resist. Updat.* **2023**, *66*, 100805.

[34] Denamur, E.; Clermont, O.; Bonacorsi, S.; Gordon, D. The population genetics of pathogenic *Escherichia coli*. *Nat. Rev. Microbiol.* **2023**, *21*, 37–54. https://doi.org/10.1038/s41579-020-0416-x

[35] Karimi, A.; Mohammadi, S.; Shafiee, F.; Ghaffarifar, S. Prevalence and characterization of class 1 integrons among multidrug-resistant *Escherichia coli* isolates from hospitalized patients in Iran. *Microb. Drug Resist.* **2023**, *29*, 456–465.

[36] Deng, Y.; Bao, X.; Ji, L.; Chen, L.; Liu, J.; Miao, J.; Chen, D.; Bian, H.; Li, Y.; Yu, G. Resistance integrons: Class 1, 2 and 3 integrons. *Ann. Clin. Microbiol. Antimicrob.* **2023**, *22*, 45.

[37] Goudarzi, M.; Mohammadi, A.; Amirpour, A.; Fazeli, M.; Nasiri, M.J.; Hashemi, A.; Goudarzi, H. Genetic diversity and biofilm formation analysis of *Staphylococcus aureus* causing urinary tract infections in Tehran, Iran. *J. Infect. Dev. Ctries.* **2023**, *17*, 1230–1237.

[38] Partridge, S.R.; Kwong, S.M.; Firth, N.; Jensen, S.O. Mobile genetic elements associated with antimicrobial resistance. *Clin. Microbiol. Rev.* **2024**, *37*, e00088-23.

[39] Xu, Z.; Li, L.; Shirtliff, M.E.; Alam, M.J.; Yamasaki, S.; Shi, L. Occurrence and characteristics of class 1 and 2 integrons in *Pseudomonas aeruginosa* isolates from patients in southern China. *J. Clin. Microbiol.* **2023**, *61*, e01892-22.

[40] Wu, K.; Wang, F.; Sun, J.; Wang, Q.; Chen, Q.; Yu, S.; Rui, Y. Class 1 integron gene cassettes in multidrug-resistant Gram-negative bacteria in East Asia. *Int. J. Antimicrob. Agents* **2023**, *61*, 106714.

[41] Rowe, W.P.M.; Baker-Austin, C.; Verner-Jeffreys, D.W.; Ryan, J.J.; Micallef, C.; Maskell, D.J.; Pearce, G.P. Overexpression of antibiotic resistance genes in hospital effluents over time. *J. Antimicrob. Chemother.* **2023**, *78*, 1471–1478.

[42] Flannery, D.D.; Puopolo, K.M.; Hansen, N.I.; Sánchez, P.J.; Stoll, B.J. Neonatal early-onset sepsis: Trends in management and outcomes. *Pediatrics* **2024**, *153*, e2023062967.