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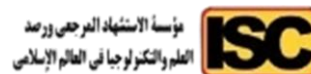
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Study of the evolutionary origin and virulence factors of bacterial species causing umbilical cord infections in newborns

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Abstract:

The umbilical cord is a vital physiological structure that connects the fetus to the placenta. It plays a pivotal role in the exchange of oxygen, nutrients, and waste between the mother and fetus during pregnancy. This study aimed to identify bacterial isolates from newborns with umbilical cord infections in Baghdad Governorate. One hundred swabs were collected from newborns with umbilical cord infections who visited the pediatric consultants at Al-Kadimiya Teaching Hospital and Al-Karkh Hospital. The swabs ranged in age from 3 days to 28 days and were of both sexes. The study was conducted from October 1, 2024, to February 1, 2025. Phenotypic and biochemical results revealed that 100 samples were isolates, of which 29.16 were identified as *Staphylococcus aureus*, 29.16 were identified as *Escherichia coli*, 29.16 were identified as *Pseudomonas*, 8.33 were identified as *Enterococcus*, and 4.16 were identified as *Bacteroides*. Molecular screening was performed using PCR. Biofilm production results also showed a

significant decrease in the number associated with biofilm formation, indicating that the bacterial species is directly proportional to the isolate type and sample collection time.

Keywords: Bacterial isolates, newborns, umbilical cord, PCR

Introduction:

The umbilical cord is a vital physiological structure that connects the fetus to the placenta. It plays a pivotal role in the exchange of oxygen, nutrients, and waste products between the mother and fetus during pregnancy. The umbilical cord consists of a single vein that transports oxygenated blood from the placenta to the fetus, and two arteries that transport deoxygenated blood and waste products from the fetus to the placenta. These vessels are surrounded by a protective gelatinous tissue known as Wharton's jelly, which provides support and protection from compression (Alatyyat et al., 2020).

In Nigeria, studies have shown that umbilical cord infections account for between 10% and 19% of neonatal hospital admissions, with an estimated mortality rate of 30% to 49% (Abdel

Razek et al., 2023; Abdulrazzaq and Ali, 2025). Nigeria has a neonatal mortality rate of 37 deaths per 1,000 live births, and the neonatal mortality rate varies by place of residence, region, maternal education level, and household income. Mortality rates in urban areas remain consistently lower than those in rural areas, with infant mortality rates 43% higher in rural areas (86 deaths per 1,000 live births) than in urban areas (60 deaths per 1,000 live births) (Cui et al., 2017). Most cases of umbilical cord infection are attributed to skin or environmental bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp.*, and *Pseudomonas aeruginosa*. These infections can progress to serious systemic complications such as sepsis, especially in premature or immunocompromised infants (Du et al., 2020). *P. aeruginosa* causes disease in healthy individuals and often infects immunocompromised persons.

P. aeruginosa is a Gram-negative rod-shaped bacterium, measuring between 0.5 and 0.8 μm . These bacteria occur singly or in short chains, are characterized by one or several polar flagella, and are non-spore-forming. They are found in soil, plants, and mammalian tissues (Al-Azzawi and Abdullah, 2019). These bacteria can survive on water and surfaces. *Pseudomonas aeruginosa* is a Gram-negative, rod-shaped, aerobic bacterium that can be isolated from various environments and medical devices using its specific binding factors, such as flagella, papillae, and thin biofilms. Aerobic *P. aeruginosa* is abundant in natural and artificial environments, including lakes, hospitals, and household drains (Al-Mhesin et al., 2024). It is positive for catalase and oxidase tests. These bacteria resemble members of the Enterobacteriaceae family, but they require oxygen to survive because they live by oxidative means, deriving energy from carbohydrates rather than fermenting. They are also known as nonfermenters because they do not ferment the sugar glucose (Abdullah and Dahham, 2023).

These bacteria can grow and survive even in minimally nutritious conditions, allowing them to survive for long periods on surfaces and medical instruments, contributing to hospital-acquired infections. They contribute to the production of two important pigments used in laboratory tests: pyocyanin, which gives a blue-green color to pus in cases of wounds and burns, and pyoverdine, which tends to be yellow-green and fluoresces under ultraviolet light. These bacteria are highly resistant to disinfectants, increasing their role in hospital-acquired infections. Studies have shown that it can grow in media containing hexachlorophene and soap solutions, as well as in disinfectants and detergents (Sudhakar et al., 2015). It also grows on MacConkey agar, and its colonies appear pale due to its inability to ferment lactose (non-lactose fermentation), which has an odor reminiscent of fermented wine. When grown on blood agar, it forms dark colonies surrounded by a transparent halo, indicating its ability to lyse blood, and to produce beta-hemolytic lysis due to the secretion of the enzyme hemolysin. It can also grow at temperatures as high as 42°C (Ahmed and Ibrahim, 2022). *P. aeruginosa* possesses several factors that make it capable of causing infection. Among these factors are enzymes such as T3SS Ex, ExoT, ExoU, and ExoY, as well as exotoxin A, phospholipase C, and proteases, which cause tissue destruction and help the bacteria resist immune responses. In addition, the ability of these

bacteria to stimulate secreted virulence factors such as pyocyanin and alkaline protease enhances their survival.

This type of bacteria enters these patients through burns and wounds. These bacteria are considered opportunistic pathogens and have the potential to cause hospital-acquired infections. When these bacteria infect a host, they begin an adhesion phase using flagella and pili, as well as the enzyme exoenzyme S, which damages cells and leads to tissue destruction (Said et al., 2024). Inflammation is also triggered by virulence factors, including exotoxin A, a protein that harms phagocytes by forming a complex with elongation factor 2 (EF2), which inhibits protein production. As a result, tissue necrosis occurs and wound healing is delayed, potentially leading to death in burns. The effect is similar to diphtheria toxin (Samad et al., 2019). *P. aeruginosa* is a prevalent pathogen responsible for healthcare-associated infections in hospitalized patients (Standring, 2021), posing significant therapeutic challenges due to its inherent resistance mechanisms and rapid acquisition of resistance genes (Yin et al., 2025). According to 2019 data from CHINET surveillance in China, resistance rates to imipenem and meropenem among *P. aeruginosa* isolates were reported at 27.5% and 23.5%, respectively (Tuon et al., 2022).

High resistance rates contribute to outbreaks of hospital-acquired infections and severe infections at multiple sites, including the respiratory and urinary tracts. urinary tracts, skin, and soft tissues, which are associated with negative clinical outcomes such as increased resource consumption, higher healthcare costs, and increased morbidity and mortality (Abbood and Hateet, 2025). Therefore, the aim of study was to estimate of the evolutionary origin and virulence factors of bacterial species causing umbilical cord infections in newborns.

Materials and Methods:

Bacterial Isolates:

One hundred swabs were collected from newborns with umbilical cord infections who visited the pediatric consultation rooms at Al-Kadhimiya Teaching Hospital and Al-Karkh Hospital. Their ages ranged from 3 days to 28 days and were of both sexes. The samples were collected between October 1, 2024, and February 1, 2025.

Identification of Bacterial:

Bacterial isolates were identified based on morphological characteristics, including the shape, color, and texture of colonies growing on selective media, namely MacConkey agar and EMB agar. The isolates were then subjected to microscopic examination. Smears were prepared from these colonies after re-purification on Blood agar and MacConkey agar, stained with Gram stain, and examined under oil-based microscope at 100x magnification.

Biofilm formation test:

Two methods were used to detect biofilm formation, as follows:

A: Congo red agar method. Isolates to be tested for their ability to form biofilms were inoculated onto Congo red medium, and the plates were incubated at 37°C for 24 hours. All isolates were then examined for color change after 24-48 hours of incubation. Biofilm-forming isolates were detected by the appearance of a black color, while non-film-forming isolates were red (Chegini et al., 2020).

B: Phenotypic detection of biofilm using the tube method. Test tubes containing 10 ml of brain-heart infusion broth medium with 2% glucose were inoculated with a 24-hour-old bacterial culture. They were then incubated at 37°C for 48 hours. The contents of the tubes were then poured out, washed with phosphate buffer saline, dried upside down, and stained with crystal violet. They were left to stand for half an hour. The stain was then removed and the tubes left to dry. The result of the test was the formation of a violet ring on the tube walls.

Statistical analysis:

All results of the current study were statistically analyzed to determine significant differences. The Statistical Package for Social Sciences (SPSS), version 27, was used, along with the chi-square test. Significant differences were determined at a probability level of 0.05.

Ethical Approval:

This study was approved by the local Publication Ethics Committee at Al-Qadisiyah University, Iraq, according to the numbered letter.

Results and Discussion:

The morphological and biochemical results of the 100 samples revealed that 29.16 of these isolates were identified as *Staphylococcus aureus*, 29.16 as *Escherichia coli*, 29.16 as *Pseudomonas*, 8.33 as *Enterococcus*, and 4.16 as *Bacteroides*. Molecular characterization was performed using PCR, with primer amplification results showing that all bacterial isolates were identified, as shown in Table (1). The data showed a probability value of <0.0001 at a significance level of 0.01. According to the results, of the 100 samples collected, 9 isolates (9%) were found to be *Pseudomonas aeruginosa*, consistent with the findings of (Abdullah and Dahham, 2023; Sabah and Kadhim, 2025). This is lower than the findings of (Chegini et al., 2020), which ranged from 15.4% to 19.4%.

This discrepancy may be attributed to differences in sample size, study population, length of hospital stay, patient exposure to high-risk medical devices, and prescription of antibiotics without antibiotic susceptibility testing (Chen et al., 2020; Schwartzman et al., 2024). Inconsistencies in the prevalence of *Pseudomonas aeruginosa* between studies may also be due to variations in clinical samples, hospitals, populations examined, geographic regions, and healthcare practices (Fatima et al., 2012). Bacterial culture results (Figure 1) showed 48% positive growth and 52% no growth. This is consistent with (Hashemi et al., 2017; Mohammed et al., 2017), who isolated a range of bacteria from wounds, and 10% each from the ear, urethra, tonsils, and umbilical cord.

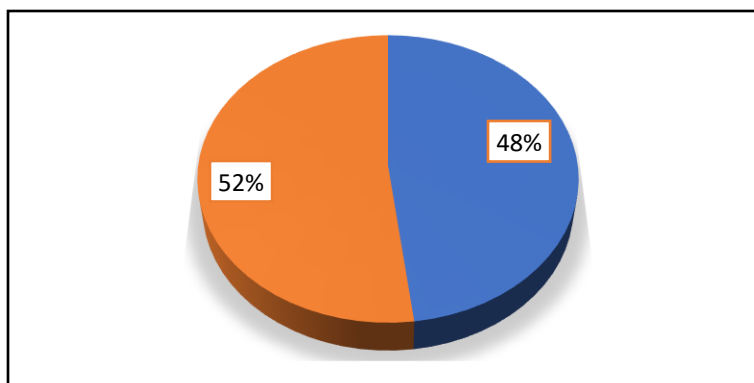


Figure (1) Results of bacterial culture on different media (red is Positive for growth; blue is not growth)

Table (1) Types of bacterial isolation from the umbilical cord of children (total number of samples: 100)

Bacterial Type	Number	Percentage
<i>Staphylococcus aureus</i>	14	29.16
<i>Escherichia coli</i>	14	29.16
<i>Pseudomonas bacteria</i>	14	29.16
<i>Enterococcus bacteria</i>	4	8.33
<i>Bacteroides bacteria</i>	2	4.16
Total Count	48	100
Chi-square Value	25.70	
Calculated Probability Value	<0.0001*	

* Significant differences were found at the 1% significance level.

The results of biofilm production showed a clear decrease in the numbers associated with biofilm formation, indicating that the bacterial species is directly proportional to the increase in bacterial species. Significant differences ($P < 0.05$) were observed among the other isolates, with *Staphylococcus aureus* accounting for 3 isolates (42.85%), *Escherichia coli* accounting for 6 isolates (85.71%), *Pseudomonas aeruginosa* accounting for 3 isolates (42.85%), *Enterococcus bacteria* accounting for 2 isolates (100%), and *Bacteroides bacteria* accounting for 1 isolate (100%). The values were calculated based on (Jimoh et al., 2022; Kareem et al., 2024). Transcriptomic analysis provides valuable insights into the mechanisms by which biofilms exhibit increased antibiotic susceptibility. Overall, it can be an effective strategy to enhance biofilm antibiotic susceptibility and provide insights into the mechanisms of antimicrobial activity. Differences in antimicrobial efficacy may be attributed to their specific mechanisms of action.

The results of the current study showed the ability of bacterial isolates to produce biofilm after growing them on Congo Red agar medium for 24-48 hours at a temperature of 37°C. The results of the detection test using this method showed the production of biofilm in the isolates in the form of black colonies with black edges surrounding the colony, while the isolates that did not form biofilm were described as pink or red in color (Figure 2). Biofilm production is one of the virulence factors associated with pathogenicity.

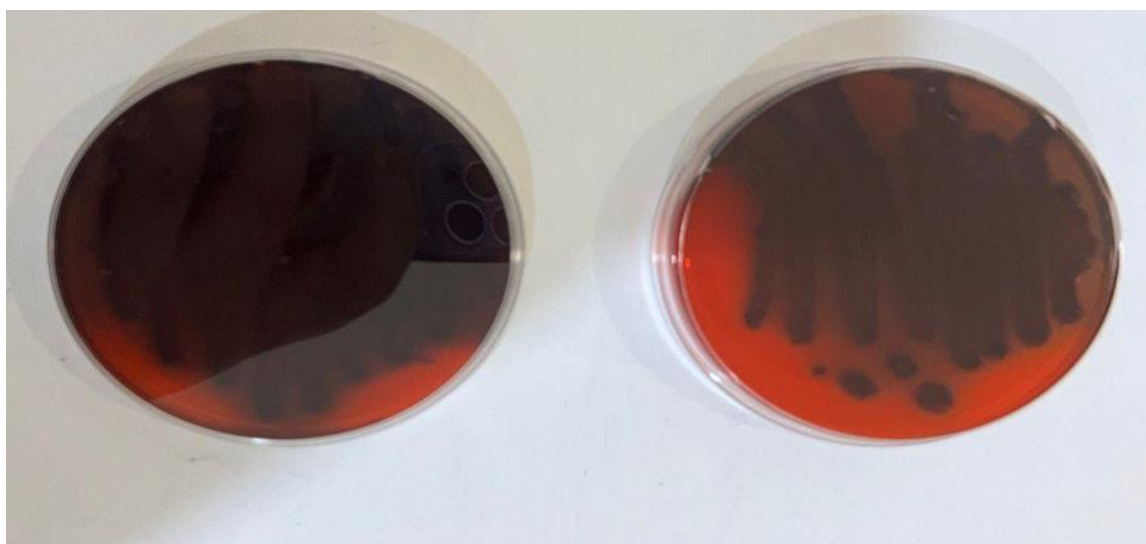


Figure (2) Biofilm-producing isolated bacteria on solid Congo red medium for 24-48 hours at 37°C.

The results of the current study showed that *S. aureus* and *P. aeruginosa* produced biofilms in 3 out of 7 isolates, representing 42.85% of the total, indicating that they are weakly adherent biofilm producers (Table 2). *E. coli* produced biofilms at a rate of 85.71%, meaning they are moderately adherent biofilm producers. Enterococcus bacteria, Bacteroides, produced biofilms at a rate of 100%, and are classified as strongly adherent biofilm-producing bacteria.

Table (2) Biofilm-producing ability of the bacteria under study using the Congo red staining method

Bacterial Species	Number of isolates tested	Positive biofilm production result	Percentages
<i>Staphylococcus aureus</i>	7	3	42.85
<i>Escherichia coli</i>	7	6	85.71
<i>Pseudomonas aeruginosa</i>	7	3	42.85
Enterococcus bacteria	2	2	100
<i>Bacteroides spp.</i>	1	1	100
Chi-square value	5.71		
Calculated probability value	0.222*		

* There are no significant differences at the 5% significance level.

Conclusions:

The study concludes that Phenotypic and biochemical results revealed that 100 samples were isolates, of which 29.16 were identified as *Staphylococcus aureus*, 29.16 were identified as *Escherichia coli*, 29.16 were identified as *Pseudomonas*, 8.33 were identified as *Enterococcus*, and 4.16 were identified as *Bacteroides*. Molecular screening was performed using PCR. Biofilm

production results also showed a significant decrease in the number associated with biofilm formation, indicating that the bacterial species is directly proportional to the isolate type and sample collection time.

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Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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