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Antibiotic Resistance Patterns of Pathogenic Bacteria in Children Under 5 Years Old with Suspected Septicemia in Kerkuk, Iraq

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ABSTRACT

Objective: *Septicemia, a dangerous blood disease, is a life-threatening condition that can affect infants and young children, especially in less developed countries.* ***Methods:*** *This study involves a bacteriological investigation to correlate the pattern of resistance of pathogenic bacteria isolates against antibiotic resistance patterns in septicemia cases of children under 5 years old admitted to Kerkuk Gynecology and Children Hospital in Iraq for the period extended from July 2024 to Jan. 2025. A total of 120 children (72 Female and 48 Male) aged 1–60 months old, compared with 40 similar age healthy children. The isolated pathogenic bacteria were also subject to biochemical tests and antibiotic susceptibility profiling.* ***Results:*** *Babies and infants ages ranged between 1-18 months were the most susceptible ages in comparison with older children. The most common bacteria isolated from blood samples were Staphylococcus aureus (31.6%) and Escherichia coli (25.5%). Septicemia isolates exhibited significant resistance to antibiotics, i.e., Azithromycin (AZM) and Cephalexin (CEV), while Meropenem (MRP) and Vancomycin (VAN) were effective against most isolates.* ***Novelty:*** *The specific cell culture is deemed mandatory as a quick check-up for antibiotic resistance prior to administration for babies. The applications of the results can help enhance the handling of sepsis in infants and younger children, especially in poorer regions of Kirkuk.*

INTRODUCTION

Sepsis is a life-threatening health condition that occurs when the body's immune system has an extreme response to an infection, causing organ dysfunction [1]. The body's reaction causes damage to its own tissues and organs, and can lead to shock, multiple organ failure, and sometimes death, if not recognized early and treated promptly. Sepsis can affect anyone, particularly older people, very young, pregnant meanwhile other health problems are at higher risk. Common signs of sepsis are many; they include fever, fast heartbeat, rapid breathing, confusion, and body pain, which can lead to septic shock and consequent death. It is usually caused by bacteria, but other microorganism agents are also possible. The presence of bacteria in the blood and the consequent inflammatory reaction represent the hallmarks of septicemia that further develops causing sepsis as a dangerous illness; more precisely, blood poisoning indicates infectious bacteria poisoning the blood by itself or by their toxins [2]. Hence, any positive blood culture with systemic bacterial infection consider as septicemia. Mortality and hospitalization are common in children affected by septic shock around the world. Meanwhile, the reports of the World Health Organization (WHO) refer to about 85% of deaths in newborn results from infections, including tetanus, pneumonia, and sepsis that leads to death in about 40% of affected infants, mostly in developing countries [3]. The

infants and young children with septicemia are at significant risk of dying, especially in underdeveloped nations with inadequate medical infrastructure [4]. Symptoms in children >5 years are many e.g. the skin looks bluish, or pale; the child feels very sleepy, difficult to rouse or wake; with fast breathing; feeling cold; with a rashly and fade skin and suffers convulsion. Many of these children get potentially fatal sepsis when they have a fever [5]. One of the most significant complications that frequently arises from infections in septicemia is linked to risk factors, i.e., age, gender, nutritional condition, medical history, and test results [6]. On an international scale, annually, about 3 million newborns and 1.2 million children have a dangerous medical condition caused by the impact of harmful bacteria in the blood. The resistant pathogens are a cause of sepsis, leading to death in 30% of neonatal cases. Meanwhile, every year, more than 75,000 infants and children in the USA develop severe sepsis, where almost 7,000 (9.3%) die. This number is more than the deaths from cancer in children. Although it is a major cause of global morbidity and mortality, sepsis still lacks targeted therapy. The bacteria *Escherichia coli* and *Staphylococcus aureus* are the most common pathogenic bacteria found in septicemia cases, with resistance profiles comparable to those of frequently administered antibiotics [7]. The immune system of children is still developing, making them more susceptible to septicemia and more vulnerable than older children. The risk is further increased by immunosuppression, hospitalization, infections, and pre-existing conditions [5]. Identification of bacteria and their resistance is the gold standard for a conclusive diagnosis of septicemia in blood culture [8].

Justification:

Despite recent legislative initiatives to enhance children's health and promote educational chances, in the protection of infectious illnesses, mortality remains high in Kirkuk, Iraq [9]. Hence, our inspiration has led us to investigate septicemia in children under five years old to: (1) correlate the incidence of septicemia infection to the pathogenic microorganisms and the alterations in blood parameters in septicemia of children under five in the province of Kirkuk. (2). Identify the patterns of antibiotic susceptibility of pathogenic bacteria isolated from septicemia cases.

RESEARCH METHOD

A cross-sectional study was conducted from October 30, 2024, to January 2025, including 120 children >5 years old presented in the Gynecology and Children's Hospital, Children's Hospital within Kerkuk city, Iraq, compared with another 40 Control healthy children aged >5 years old. A solution of 0.1% chlorhexidine was applied for infants under 8 weeks of age. Blood samples (2.5 mL) were collected in pediatric BHI bottles for the detection of bacterial sepsis and further bacteriological studies, including culture and sensitivity. Sterilization of culture media and solutions was performed using an autoclave at 121°C for 15 minutes, while glassware was dry-sterilized at 200°C for 2 hours [10].

A. Catalase Reagents:

Catalase activity was tested by adding 1 mL of 30% hydrogen peroxide to 9 mL of sterilized distilled water [11]. Meanwhile, Bromothymol blue (4%) was prepared in distilled water to assess bacterial motility [12]. Kovac's reagent was used to detect indole production from tryptophan oxidation [10]. Methyl Red reagent identified glucose metabolism, while Voges-Proskauer reagents-α-naphthol and 40% KOH- were used to detect neutral end-products from glucose fermentation.

B. Preparation of Culture Media:

(1). MacConkey Agar (MA): Isolates and identifies lactose-fermenting and non-lactose-fermenting bacteria. (2). Brain Heart Infusion Broth (BHI): Preserves bacterial isolates for long-term storage with 15% glycerol; (3). Mannitol Salt Agar (MSA): Selective for *Staphylococcus* species due to high NaCl (7.5-10%); (4). Kligler Iron Agar (KIA): Differentiates bacteria based on lactose/dextrose fermentation and hydrogen sulfide (H_2S) production; (5). Muller Hinton Agar (MHA): Used for antimicrobial susceptibility testing; (6). Peptone Water: Detects indole production; (7). Methyl Red and Voges-Proskauer Media: Determines bacterial ability to ferment glucose into stable acid or neutral end products; and (8). Simmon's Citrate Agar: Tests bacteria for utilization of sodium citrate as the sole carbon source and inorganic ammonium salts as the sole nitrogen source.

C. Prepared Culture Media:

Blood Agar (BA) and Chocolate Agar (CHA) were prepared after autoclaving and cooled to 45°C and 80°C, respectively, then supplemented with 5-10% blood for bacterial isolation [13].

D. Inoculation and Culture Procedure:

Blood samples were inoculated into BHI broth and incubated at 37°C for 5-7 days. Cultures were monitored daily using Gram staining and turbidity. Positive cultures were sub-cultured on selective media, including MSA, MA, and BA [14].

E. Gram Staining and Biochemical Identification:

Isolated colonies were Gram-stained to determine Gram reaction and morphology. Biochemical tests performed included Catalase, Coagulase, Oxidase, TSI, Urease, Indole, and Citrate utilization to identify bacterial species.

F. Antibiotic Sensitivity Testing:

The Kirby-Bauer disc diffusion method was used on Muller Hinton Agar. Bacterial colonies (24 hours old) were suspended in 0.9% saline and swabbed onto MHA plates. Antibiotic discs were applied within 15 minutes, incubated at 37°C for 24 hours, and inhibition zones measured to classify bacteria as sensitive, intermediate, or resistant according to Clinical and Laboratory Standards Institute (CLSI) [15].

RESULTS AND DISCUSSION

The blood cultures from <5 years old pediatric patients with clinically identified symptoms of sepsis yielded a range of eight bacterial isolates [Table-1]. The majority of pathogens identified were Gram-positive (G+) bacteria, which accounted for a higher

proportion of cases than Gram-negative (G-) isolates. The most frequently isolated pathogens were *Staphylococcus aureus* (31.6%) and *Escherichia coli* (25.5%). Other isolates included *Pseudomonas aeruginosa, Enterococcus faecalis, Streptococcus viridans,* and *Streptococcus pyogenes,* as in other similar studies [16], [17].

Table 1. The percentages of the main eight types of pathogenic bacteria isolated from the blood of septic children under 5 years old.

Gram-Positive Bacteria	%	**Gram-Negative Bacteria**	%
Staphylococcus aureus	31.6%	*Escherichia coli*	25.5%
Streptococcus epidermidis	8.2%	*Klebsiella pneumoniae*	11.2%
Streptococcus viridans	6.1%	*Pseudomonas aeruginosa*	7.1%
Streptococcus pyogenes	5.1%		
Enterococcus faecalis	4.1%		

The presence of both G+ and G- bacteria highlights the polymicrobial nature of sepsis. The predominance of G- bacteria is consistent with previous studies, suggesting virulence factors like endotoxins contribute to pathogenicity and resistance [18], [19]. Both *E. coli and K. pneumoniae* are particularly known for their ability to cause nosocomial infections, a factor which may contribute to their high prevalence in cases of pediatric sepsis [20]. In contrast, G+ pathogens, particularly *S. aureus*, remain a major concern due to their ability to form biofilms and evade immune responses. The detection of *Pseudomonas aeruginosa and Enterococcus faecalis* further indicates the potential involvement of opportunistic pathogens, which can exacerbate the severity of the disease in immunocompromised children [21].

In order to accurately identify the bacterial species present in septic patients, a series of biochemical tests was also performed. These tests allowed differentiation based on metabolic and enzymatic activity and provided reliable confirmation of the isolates *Escherichia coli*, which was confirmed by lactose fermentation, acid, gas production on MacConkey agar, positive indole, methyl red tests, negative citrate and urease tests. The bacteria *Klebsiella pneumoniae* showed mucoid colonies, positive Voges-Proskauer, citrate utilization, and negative indole, while the bacteria *Staphylococcus aureus* were catalase- and coagulase-positive with β-hemolysis. *Pseudomonas aeruginosa* was oxidase-positive with blue-green pyocyanin pigment and a characteristic odor. The bacteria *Enterococcus faecalis* hydrolyzed bile esculin and grew in 6.5% NaCl, distinguishing it from other streptococci. These tests enabled accurate identification and guided antimicrobial therapy selection. These biochemical markers played a crucial role in the accurate classification of bacterial isolates, enabling the selection of appropriate antimicrobial therapy. The results underline the importance of laboratory identification techniques in the effective management of sepsis cases [22] (Table-A and 2B).

Table 2A. Biochemical characterization of the isolates, Biochemical Identification of Gram-Positive Bacteria.

Bacterium	Catalase	Coagulase	Mannitol Fermentation	Bile Esculin	Hemolysis	Other Tests
Staphylococcus aureus	+	+	-	-	B	-
Streptococcus epidermidis	+	-	-	-	Γ	Novobiocin Susceptible
Streptococcus viridans	-	-	-	-	A	Optochin Resistant
Streptococcus pyogenes	-	-	-	-	B	Bacitracin Sensitive
Enterococcus faecalis	-	-	-	+	Γ	PYR (+)

Table 2B. Biochemical Identification of Gram-Negative Bacteria.

	Oxidase	Indole	Urease	TSI	Citrate	MR/VP	Lactose Fermen' n	Other Tests
Escherichia coli	-	+	-	A/A gas	-	+/+	+	-
Klebsiella pneumoniae	-	-	+	A/A gas	+	-/+	+	Mucoid colonies
Pseudomonas aeruginosa	+	-	-	K/K No gas	+	-/-	-	Growth at 42°C

Antibiotic susceptibility profiles: Antimicrobial resistance revealed multidrug resistance, particularly among G- isolates. The bacteria *Escherichia coli* showed high resistance to ampicillin and cefotaxime, suggesting possible ESBL production, but susceptibility to imipenem, meropenem, amikacin, and gentamicin [23]. The *Klebsiella pneumoniae* exhibited resistance to third-generation cephalosporins and macrolides (azithromycin, ceftazidime), but susceptibility to colistin [24]. The *Staphylococcus aureus* isolates included MRSA (resistant to oxacillin, cephalosporins, and macrolides) and MSSA (susceptible to β-lactams) [25]. The *Pseudomonas aeruginosa* was resistant to ceftazidime and piperacillin but susceptible to colistin and carbapenems. *Enterococcus faecalis* showed moderate resistance to ampicillin and macrolides but was sensitive to vancomycin. Statistical analysis revealed a significant correlation between bacterial species and susceptibility profiles ($p<0.05$) [26]. The increasing prevalence of multidrug-

resistant isolates in pediatric septicemia poses a major challenge. High resistance to penicillins and cephalosporins underscores the need for antimicrobial stewardship [27]. The susceptibility of G bacteria to carbapenems remains important, but carbapenem-resistant strains are a concern. For G+ pathogens like *S. aureus*, MRSA complicates treatment, requiring alternatives like linezolid or vancomycin. Vancomycin remains effective against *Enterococcus faecalis*. Routine susceptibility testing and infection control measures are critical (Table-3) [28].

Table 3. Various antibiotics are used against isolated bacteria involved in septicemia. (P): penicillin; Azithromycin (AZM); Cephalexin (CEV); levofloxacin (LEV); Doxycycline (DOX); Amikacin AK; Meropenem (MRP); Augmentin (AUG); and vancomycin (VAN). The number of positive samples tested for each bacterium is shown. (NS): means insignificant; (*): significant ($p<0.05$) and (**): High significant ($p \leq 0.001$-0.0003).

Bacteria	P		AZM		CEV		LEV		DOX		AK		MRP		AUG		V A N	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Escherichia coli	11	2	4	9	1	12	2	11	12	1	3	10	1	12	9	4	11	2
Enterococcus faecalis	2	0	0	2	2	0	2	0	2	0	2	0	1	1	1	1	0	2
Klebsiella pneuomonia	3	2	5	0	2	3	1	4	1	4	4	1	2	3	4	1	3	2
Pseudomonas aeruginosa	6	0	6	0	4	2	5	1	4	2	3	3	4	2	5	1	6	0
S. epidermidis	3	2	4	1	4	1	4	1	2	3	1	4	3	2	1	4	4	1
Staphylococcus aureus	14	2	0	16	13	3	1	15	8	8	4	12	11	5	6	10	8	8
Streptococcus viridans	1	0	0	1	1	0	1	0	1	0	1	0	0	1	1	0	1	0
Streptococcus pyogenes	2	1	1	1	3	0	2	1	2	1	3	0	1	2	3	0	2	1
Total (51 each case)	42	9	20	31	30	21	18	33	32	19	21	30	23	28	30	21	35	16
Probability tests and significancy	NS $p \leq 0.271$		** $p<0.0003$		** $p<0.006$		** $p<0.008$		* $p<0.042$		** $p<0.011$		* $p<0.039$		* $P<0.051$		* $P<0.044$	

Limitations of the Study: A few inevitable limitations might have been encountered in the present study, e.g. duration of 7 months was an inadequate time factor, nor did it represent all the seasons in Kirkuk city, thereby affecting the generalization of the results. Moreover, some studied cases might have involved early treatment by the general practitioners (GPs) with antibiotics prior to hematological analysis, which might have compromised the results to a certain extent. A longer study period is recommended to achieve more significant results.

CONCLUSION

Fundamental Finding : The results provide important insights into the pathogenic bacterial etiology of pediatric septicemia and associated antibiotic resistance patterns and highlight the predominance of G+ bacteria in septicemic children >5 years of age and points to the increasing trend of multidrug resistance. **Implication :** These patterns require immediate diagnostic action and rational use of antibiotics to improve patient outcomes and reduce the burden of pediatric sepsis. The high prevalence of multidrug-resistant G- bacteria emphasizes the need for prudent antibiotic use and continuous surveillance of resistance development. Early detection of pathogens through biochemical identification and susceptibility testing remains essential to guide empirical therapy and improve clinical outcomes. As antimicrobial resistance continues to rise, it is imperative that healthcare professionals apply evidence-based treatment strategies and infection control measures to effectively combat septicemia. **Limitation :** The results are constrained by the growing complexity of antimicrobial resistance patterns, which limits the effectiveness of empirical treatment approaches in pediatric septicemia cases. **Future Research :** Future research should focus on the molecular mechanisms of resistance and explore new therapeutic options to address the growing challenge of drug-resistant infections in pediatric patients. Further molecular studies are recommended to explore resistance mechanisms and track epidemiologic changes in bacterial profiles over time.

REFERENCES

[1] M. Singer *et al.*, "The third international consensus definitions for sepsis and septic shock (Sepsis-3)," *JAMA*, vol. 315, no. 8, pp. 801–810, 2016, doi: 10.1001/jama.2016.0287.

[2] R. S. Watson *et al.*, "The epidemiology of severe sepsis in children in the United States," *Am. J. Respir. Crit. Care Med.*, vol. 167, no. 5, pp. 695–701, 2003.

[3] World Health Organization, "Sepsis: A global priority," 2024. [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/sepsis

[4] UNICEF, *Child Mortality Report 2021*, 2021. [Online]. Available: https://data.unicef.org/resources/child-mortality-report-2021/

[5] R. K. Saleh, A. M. Ahmed, and R. H. Jaffar, "Antibiotic resistance profiles of pathogenic bacteria in septicemia," *Iraqi J. Med. Sci.*, vol. 18, no. 3, pp. 175–183, 2020.

[6] S. R. Ali, H. Khan, and A. Mahmood, "Impact of conflict on healthcare systems in post-war regions," *J. Global Health*, vol. 10, no. 2, pp. 235–244, 2018.

[7] L. Yang *et al.*, "Impact of the 2017 American Academy of Pediatrics guideline on hypertension prevalence compared with the Fourth Report in an international cohort," *Hypertension*, vol. 74, no. 6, pp. 1343–1348, 2019, doi: 10.1161/HYPERTENSIONAHA.119.13807.

[8] C. Fleischmann-Struzek and K. Rudd, "Challenges of assessing the burden of sepsis," *Med. Klin. Intensivmed. Notfmed.*, vol. 118, suppl. 2, pp. 68–74, 2023, doi: 10.1007/s00063-023-01088-7.

[9] World Health Organization, *Monitoring Health for the SDGs: Sustainable Development Goals*, 2017. [Online]. Available: https://www.who.int/publications/i/item/9789241565486

[10] C. R. Mahon, D. C. Lehman, and G. Manuselis, *Textbook of Diagnostic Microbiology*, 5th ed. China: Elsevier, 2015.

[11] H. J. Benson, *Microbiology Applications: Laboratory Manual in General Microbiology*, 8th ed. New York, NY, USA: McGraw-Hill, 2002, pp. 185–188.

[12] J. F. McFaddin, *Biochemical Tests for Identification of Medical Bacteria*, 3rd ed. Philadelphia, PA, USA: Lippincott Williams & Wilkins, 2000.

[13] P. M. Tille, *Bailey & Scott's Diagnostic Microbiology*, 13th ed. St. Louis, MO, USA: Mosby Elsevier, 2014.
[14] A. E. Brown, *Benson's Microbiological Applications: Laboratory Manual in General Microbiology*, 10th ed. New York, NY, USA: McGraw-Hill, 2007.
[15] Clinical and Laboratory Standards Institute, *Performance Standards for Antimicrobial Susceptibility Testing*, 26th ed. Wayne, PA, USA, 2016.
[16] G. G. Zhanel *et al.*, "Antimicrobial susceptibility of 22,746 pathogens from Canadian hospitals: Results of the CANWARD study," *J. Antimicrob. Chemother.*, vol. 68, suppl. 1, pp. i7–i22, 2013, doi: 10.1093/jac/dkt022.
[17] S. Ali and M. Assafi, "Prevalence and antibiogram of *Pseudomonas aeruginosa* and *Staphylococcus aureus* clinical isolates from burns and wounds in Duhok City, Iraq," *J. Infect. Dev. Ctries.*, vol. 18, no. 1, pp. 82–92, 2024, doi: 10.3855/jidc.18193.
[18] R. W. Al-Hassan, "Correlation of pathogenic bacteria with blood parameters in septicemia cases among under-five-year-old patients," M.Sc. thesis, Fac. Med., Near Middle East Univ., Nicosia, Northern Cyprus, 2025.
[19] K. Roszak *et al.*, "Endotoxin's impact on organism: From immune activation to tolerance and beyond," *J. Clin. Med.*, vol. 14, no. 18, Art. no. 6478, 2025, doi: 10.3390/jcm14186478.
[20] M. Abuolfotoh *et al.*, "Prevalence of nosocomial infections caused by multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* encoded by different genes," *Microbes Infect. Dis.*, vol. 6, no. 1, pp. 278–287, 2025, doi: 10.21608/mid.2024.309925.2195.
[21] M. V. Kuznetsova *et al.*, "Nosocomial *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*: Sensitivity to chlorhexidine-based biocides and prevalence of efflux pump genes," *Int. J. Mol. Sci.*, vol. 26, no. 1, Art. no. 355, 2025, doi: 10.3390/ijms26010355.
[22] S. Akter and M. Uddin, "Biochemical tests for bacterial identification," *ResearchGate*, 2023. [Online].
Available:
https://www.researchgate.net/publication/391346437_Biochemical_Tests_for_Bacterial_Identification
[23] N. Mousa *et al.*, "Antibiotic resistance profiles and risk factors of multidrug-resistant *Escherichia coli* in a large tertiary care hospital in a low- and middle-income country," *Sci. Rep.*, vol. 15, no. 1, Art. no. 26667, 2025, doi: 10.1038/s41598-025-12384-x.
[24] S. Kothari *et al.*, "Third-generation cephalosporin resistance in *Klebsiella pneumoniae* isolates: An emerging threat," *Int. J. Basic Clin. Pharmacol.*, vol. 2, no. 1, pp. 56–60, 2017.
[25] A. Alghamdi *et al.*, "Antimicrobial resistance in methicillin-resistant *Staphylococcus aureus*," *Saudi J. Biol. Sci.*, vol. 30, no. 4, Art. no. 103604, 2023, doi: 10.1016/j.sjbs.2023.103604.
[26] J. Denissen *et al.*, "Prevalence of ESKAPE pathogens in the environment: Antibiotic resistance status, community-acquired infection, and risk to human health," *Int. J. Hyg. Environ. Health*, vol. 244, Art. no. 114006, 2022, doi: 10.1016/j.ijheh.2022.114006.
[27] S. K. Ahmed *et al.*, "Antimicrobial resistance: Impacts, challenges, and future prospects," *J. Med. Surg. Public Health*, vol. 2, Art. no. 100081, 2024, doi: 10.1016/j.glmedi.2024.100081.
[28] I. Gajic *et al.*, "A comprehensive overview of antibacterial agents for combating multidrug-resistant bacteria: Current landscape, development, future opportunities, and challenges," *Antibiotics*, vol. 14, no. 3, Art. no. 221, 2025, doi: 10.3390/antibiotics14030221.

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