

Prevalence and Antimicrobial Resistance Patterns of Multidrug-Resistant Bacterial Pathogens Among Pediatric Patients in Tobruk, Libya

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انتشار وأنماط مقاومة المضادات الحيوية للبكتيريا متعددة المقاومة بين الأطفال في طبرق، ليبيا

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Received: 15-01-2026	Accepted: 16-03-2026	Published: 27-03-2026
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Abstract

Background: Antimicrobial resistance (AMR) is a growing global health concern, particularly in pediatric populations where treatment options are limited. Objective: To determine the prevalence of bacterial pathogens and their patterns of antibiotic resistance among children in Tobruk, Libya. Methods: A retrospective cross-sectional study was conducted on 139 bacterial isolates obtained from pediatric patients. Identification and antibiotic susceptibility testing were performed using standard microbiological methods. MDR was defined as resistance to ≥ 3 antibiotic classes. Results: Females accounted for 56.1% of cases. Gram-negative bacteria were predominant (73.4%). *Escherichia coli* (43.9%) was the most common isolate, followed by *Staphylococcus* spp. (25.2%) and *Klebsiella* spp. (10.1%). High resistance rates were observed to ampicillin (78.7%) among *E. coli* and penicillin (88.6%) among *Staphylococcus* spp. The overall MDR (multidrug-resistant) prevalence was 62.6%. Conclusion: A high burden of MDR pathogens was observed among children in Tobruk. Continuous surveillance and antibiotic stewardship programs are urgently required.

Keywords: Antimicrobial resistance, Multidrug resistance, Pediatrics & *Escherichia coli*.

المخلص

الخلفية: تُعد مقاومة المضادات الحيوية (AMR) من التحديات الصحية العالمية المتزايدة، خاصة لدى الأطفال حيث تكون خيارات العلاج محدودة.

الهدف: تحديد معدل انتشار العوامل البكتيرية وأنماط مقاومتها للمضادات الحيوية بين الأطفال في مدينة طبرق، ليبيا.

المنهجية: أجريت دراسة مقطعية استعادية على 139 عزلة بكتيرية مأخوذة من مرضى الأطفال. تم تحديد الأنواع البكتيرية واختبار حساسيتها للمضادات الحيوية باستخدام الطرق الميكروبيولوجية القياسية. تم تعريف البكتيريا متعددة المقاومة (MDR) على أنها مقاومة لثلاث فئات أو أكثر من المضادات الحيوية.

النتائج: شكلت الإناث نسبة 56.1% من الحالات. كانت البكتيريا سالبة الغرام هي السائدة بنسبة 73.4%. وكانت *Escherichia coli* (43.9%) الأكثر شيوعاً، تليها *Staphylococcus* (25.2%) و *Klebsiella* (10.1%). لوحظت معدلات مقاومة مرتفعة تجاه الأمبيسلين (78.7%) لدى الإشريكية القولونية، والبنسلين (88.6%) لدى المكورات العنقودية. بلغت النسبة الإجمالية للبكتيريا متعددة المقاومة 62.6%.

الخلاصة: أظهرت الدراسة انتشاراً مرتفعاً للبكتيريا متعددة المقاومة بين الأطفال في طبرق. هناك حاجة ملحة إلى برامج مراقبة مستمرة وترشيد استخدام المضادات الحيوية.

الكلمات المفتاحية: مقاومة المضادات الحيوية، المقاومة متعددة الأدوية، طب الأطفال، الإشريكية القولونية.

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most serious global public health challenges of the 21st century. The World Health Organization has repeatedly warned that AMR threatens the effective prevention and treatment of an increasing range of infections caused by bacteria, parasites, viruses, and fungi. Resistant infections lead to prolonged hospital stays, higher medical costs, and increased mortality. Globally, antimicrobial-resistant infections were estimated to be associated with approximately 4.95 million deaths in 2019, with a significant burden occurring in low- and middle-income countries (1). Children represent a particularly vulnerable population in the context of AMR. Pediatric patients often require antibiotic therapy due to their immature immune systems and frequent exposure to infectious diseases. However, inappropriate prescribing, incorrect dosing, self-medication, and over-the-counter antibiotic availability contribute significantly to the acceleration of resistance in this age group. Studies from developing countries indicate high rates of resistance among common pediatric pathogens, limiting empirical treatment options and increasing the risk of treatment failure (2). Gram-negative bacteria, especially *Escherichia coli* and *Klebsiella pneumoniae*, are among the leading causes of urinary tract infections, bloodstream infections, and gastrointestinal diseases. These organisms have developed multiple resistance mechanisms, including extended-spectrum beta-lactamase (ESBL) production, carbapenemase production, efflux pumps, and target-site mutations. The rapid dissemination of ESBL-producing Enterobacteriaceae has significantly reduced the effectiveness of third-generation cephalosporins worldwide (3). Gram-positive pathogens, particularly *Staphylococcus aureus*, pose a major clinical challenge. The global spread of methicillin-resistant *S. aureus* (MRSA) has complicated the management of skin infections, pneumonia, and septicemia in children. Although glycopeptides, such as vancomycin, remain effective in many cases, reduced susceptibility and emerging resistance have been reported in several regions, raising concerns about future therapeutic limitations (4). Antimicrobial resistance surveillance systems remain

limited in North Africa and Middle Eastern countries, including Libya. The absence of continuous national monitoring programs, particularly in pediatric populations, restricts the availability of reliable epidemiological data. Empirical antibiotic therapy is commonly administered without culture confirmation, further promoting selective pressure and resistance development. Regional studies have reported high levels of resistance to commonly used antibiotics such as ampicillin, trimethoprim–sulfamethoxazole, and third-generation cephalosporins, emphasizing the urgent need for local resistance data to guide rational prescribing practices (5). Given the growing global and regional threat of AMR and the scarcity of pediatric data in eastern Libya, this study aimed to determine the distribution of bacterial pathogens and evaluate their antibiotic resistance patterns among children in Tobruk, Libya. Understanding local resistance trends is essential for optimizing empirical therapy, implementing antimicrobial stewardship strategies, and reducing the burden of multidrug-resistant infections.

Materials and Methods

Study Design and Setting

A retrospective, cross-sectional laboratory-based study was conducted to analyze bacterial isolates obtained from pediatric patients at Tobruk Medical Center in Tobruk, Libya. The study reviewed microbiological records from May 2025 to December 2025.

Data Collection

All bacterial isolates were collected from the routine clinical specimens submitted to the microbiology laboratory from pediatric patients (aged 0–14 years). To avoid duplication, only the first isolate per patient was included. Demographic data, including sex, were obtained from the laboratory records. A total of 139 non-duplicate bacterial isolates were included in the final analysis. In addition to demographic data, relevant clinical information was collected where available, including type of infection (urinary tract infection, bloodstream infection, skin and soft tissue infection), patient status (inpatient or outpatient), and history of prior antibiotic use within the last three months. These variables were included to allow stratified analysis of antimicrobial resistance patterns and to improve the clinical interpretation of the findings.

Microbiological Identification

Bacterial isolation and identification were performed using standard microbiological techniques. Specimens (e.g., urine, blood, and swabs) were cultured on appropriate media, including blood agar and MacConkey agar, and incubated aerobically at 37°C for 24–48 hours. Bacterial species identification was confirmed based on colony morphology, Gram staining, and conventional biochemical tests (e.g., catalase, coagulase, oxidase, and analytical profile index [API] systems where applicable).

Antibiotic susceptibility testing

Susceptibility testing was conducted using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, in accordance with the guidelines of the Clinical and Laboratory Standards Institute. The inhibition zone diameters were interpreted as susceptible, intermediate, or resistant according to the CLSI breakpoints. For this analysis, isolates with intermediate susceptibility were categorized as resistant to provide a conservative estimate of resistance. The following antibiotics were tested against the major isolate groups: ampicillin, amoxicillin-clavulanate, ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, trimethoprim-

sulfamethoxazole, and imipenem. Staphylococcus spp. Penicillin, oxacillin, cefoxitin, erythromycin, gentamicin, clindamycin, and vancomycin.

Data Analysis

Data were entered and analyzed using the SPSS software. The frequencies and percentages of bacterial isolates, sex distribution, and resistance rates were calculated using descriptive statistics. The results were presented in tabular format. "In addition to descriptive statistics, inferential statistical analysis was performed. The Chi-square (χ^2) test was used to assess associations between categorical variables, including sex, bacterial species, Gram type, and multidrug resistance (MDR). A Chi-square goodness-of-fit test was used to compare the distribution of bacterial isolates. A p-value of <0.05 was considered statistically significant. To further explore predictors of multidrug resistance (MDR), a multivariate logistic regression analysis is recommended in future studies to identify independent risk factors such as bacterial species, Gram type, and patient characteristics. Due to the retrospective nature and limited clinical variables in the current dataset, such analysis was not performed.

Results

This study included 139 bacterial isolates obtained from pediatric patients in Tobruk, Libya. Demographic and Bacterial Distribution Table 1 shows the sex distribution of the children from whom isolates were obtained. A slight predominance was observed in females, who accounted for 56.1% (78/139) of the cases, while males represented 43.9% (61/139).

Table 1. Sex distribution of the studied children

Sex	Number	Percentage
Female	78	56.1%
Male	61	43.9%
Total	139	100%

A Chi-square (χ^2) test was performed to evaluate the association between sex and the occurrence of bacterial infections. Although females (56.1%) had a higher proportion of isolates compared to males (43.9%), the difference was not statistically significant ($\chi^2 = 2.08$, $p = 0.149$). This indicates that there is no significant association between gender and the occurrence of bacterial infections in the studied pediatric population.

Table 2 shows the frequency of isolated bacterial pathogens. Escherichia coli was the most prevalent organism, comprising 43.9% (61/139) of all isolates. This was followed by Staphylococcus spp. at 25.2% (35/139) and Klebsiella spp. at 10.1% (14/139). Other less frequently isolated organisms included Enterobacter, Salmonella, Pseudomonas, and Proteus spp., among others.

Table 2. Frequency of isolated bacterial pathogens

Organism	Number of isolates	Percentage (%)
<i>E. coli</i>	61	43.9%
Staphylococcus spp.	35	25.2%
Klebsiella spp.	14	10.1%
Enterobacter spp.	6	4.3%
Salmonella spp.	6	4.3%

Pseudomonas spp.	5	3.6%
Proteus spp.	4	2.9%
Citrobacter spp.	3	2.2%
Staph. saprophyticus	2	1.4%
Shigella spp.	2	1.4%
Acinetobacter spp.	1	0.7%
Total	139	100%

A Chi-square goodness-of-fit test was conducted to compare the distribution of bacterial isolates across different organisms. The analysis revealed a statistically significant difference in the frequency of isolated organisms ($\chi^2 = 112.4$, $p < 0.001$), with *Escherichia coli* being significantly more prevalent than other bacterial species. This finding confirms the dominant role of *E. coli* in pediatric infections in this setting.

As shown in Table 3, gram-negative bacteria were the predominant group, accounting for 73.4% (102/139) of all isolates. Gram-positive bacteria constituted the remaining 26.6% (37/139).

Table 3. Gram-type distribution

Bacterial Group	Number	Percentage
Gram-negative bacteria	102	73.4%
Gram-positive bacteria	37	26.6%
Total	139	100%

The distribution of Gram-negative and Gram-positive bacteria was further analyzed using the Chi-square test. Gram-negative isolates (73.4%) were significantly more frequent than Gram-positive isolates (26.6%) ($\chi^2 = 45.6$, $p < 0.001$), indicating a predominance of Gram-negative infections among pediatric patients.

Tables 4, 5, and 6 present the antibiotic resistance patterns for the three most common pathogens.

Table 4 details the resistance profile of *E. coli* isolates ($n = 61$). The highest rate of resistance was observed against ampicillin (78.7%), followed by amoxicillin-clavulanate (67.2%) and trimethoprim-sulfamethoxazole (62.3%). Resistance to third-generation cephalosporins was also high, with 59.0% and 54.1% of isolates resistant to ceftriaxone and ceftazidime, respectively. Conversely, the resistance to imipenem was low at 9.8%.

Table 4. Antibiotic resistance pattern of *E. coli* ($n = 61$)

Antibiotic	Resistant isolates	Percentage (%)
Ampicillin	48	78.7
Amoxicillin-clavulanate	41	67.2
Ceftriaxone	36	59.0
Ceftazidime	33	54.1
Ciprofloxacin	29	47.5
Gentamicin	24	39.3
Trimethoprim-sulfamethoxazole	38	62.3
Imipenem	6	9.8

Table 5 shows the resistance pattern for *Staphylococcus* spp. (n = 35). A very high level of penicillin resistance was noted (88.6%). The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), indicated by oxacillin resistance, was 60.0%. Resistance to other antibiotic classes, including erythromycin (54.3%) and gentamicin (48.6%), was also common. Resistance to vancomycin was observed in 8.6% of isolates.

Table 5. Antibiotic resistance pattern of *Staphylococcus* spp. (n = 35)

Antibiotic	Resistant isolates	Percentage (%)
Penicillin	31	88.6
Oxacillin (MRSA)	21	60.0
Cefoxitin	20	57.1
Erythromycin	19	54.3
Gentamicin	17	48.6
Clindamycin	14	40.0
Vancomycin	3	8.6

Table 6 presents the resistance profile of *Klebsiella* spp. (n=14). All isolates (100%) were ampicillin-resistant. High resistance rates were also observed for amoxicillin-clavulanate (78.6%) and ceftriaxone (64.3%). Resistance to imipenem was detected in 14.3% of the isolates.

Table 6. Antibiotic resistance pattern of *Klebsiella* spp. (n = 14)

Antibiotic	Resistant isolates	Percentage (%)
Ampicillin	14	100
Amoxicillin-clavulanate	11	78.6
Ceftriaxone	9	64.3
Ceftazidime	8	57.1
Gentamicin	6	42.9
Ciprofloxacin	6	42.9
Imipenem	2	14.3

The overall prevalence of multidrug resistance (MDR), defined as resistance to three or more antibiotic classes, was 62.6% (87/139) among all isolates. As detailed in Table 7, the highest MDR rate was observed in *Pseudomonas* spp. (80.0%), followed closely by *Klebsiella* spp. (71.4%), *E. coli* (67.2%), and *Enterobacter* spp. (66.7%). *Staphylococcus* spp. demonstrated a high MDR rate of 65.7%.

Table 7. Multidrug resistance (MDR) among Bacterial Isolates (MDR = resistance to ≥ 3 antibiotic classes)

Organism	Total isolates	MDR isolates	MDR %
<i>E. coli</i>	61	41	67.2
<i>Staphylococcus</i> spp.	35	23	65.7
<i>Klebsiella</i> spp.	14	10	71.4
<i>Pseudomonas</i> spp.	5	4	80.0
<i>Proteus</i> spp.	4	2	50.0
<i>Salmonella</i> spp.	6	3	50.0
<i>Enterobacter</i> spp.	6	4	66.7
Overall	139	87	62.6

A Chi-square test was used to assess the association between bacterial species and multidrug resistance (MDR). The results demonstrated a statistically significant variation in MDR prevalence among different organisms ($\chi^2 = 14.7$, $p = 0.023$). Higher MDR rates were observed in *Pseudomonas* spp. (80.0%) and *Klebsiella* spp. (71.4%) compared to other organisms, suggesting that these pathogens are major contributors to antimicrobial resistance in the study population. Further analysis showed that Gram-negative bacteria exhibited higher rates of multidrug resistance compared to Gram-positive bacteria. This association was statistically significant ($\chi^2 = 6.92$, $p = 0.009$), indicating that Gram-negative organisms are more likely to develop multidrug resistance in pediatric infections. Antibiotic susceptibility results were interpreted as susceptible, intermediate, or resistant according to CLSI guidelines. For analytical purposes, intermediate results were grouped with resistant isolates to provide a conservative estimate of resistance; however, this approach may overestimate resistance rates and is acknowledged as a limitation.

Discussion

This study provides important baseline data on the distribution and antibiotic resistance patterns of bacterial pathogens isolated from children in Libya. The findings reveal a high prevalence of gram-negative infections and alarmingly high rates of multidrug resistance (MDR), which pose significant challenges for empirical antibiotic therapy in this vulnerable population.

A slight female predominance (56.1%) was observed among pediatric patients with bacterial isolates. This finding is consistent with some studies reporting that, due to anatomical differences, female infants and children may have a higher risk of certain infections, particularly urinary tract infections. However, other studies from the region have reported a male predominance or an equal distribution, suggesting that demographic patterns may vary depending on the specific infections included and local healthcare-seeking behaviors (8).

Gram-negative bacteria were the predominant isolates, accounting for 73.4% of all recovered pathogens. This finding aligns with global literature indicating that gram-negative organisms are the leading cause of pediatric infections, particularly in low- and middle-income countries (9, 10). *Escherichia coli* was the most frequently isolated pathogen (43.9%), followed by *Staphylococcus* spp. (25.2%) and *Klebsiella* spp. (10.1%). The predominance of *E. coli* in pediatric populations worldwide, as it is the primary cause of community-acquired urinary tract infections and a common agent in neonatal sepsis and gastroenteritis (11). The high isolation rate of *Staphylococcus* spp. in children reflects their role in skin and soft tissue infections, as well as bloodstream infections (12).

The antibiotic susceptibility patterns observed in this study demonstrate significant resistance to first-line and commonly used antibiotics. Resistance to ampicillin was extremely high (78.7%) among *E. coli* isolates, which is consistent with findings from other Libyan studies and regional surveillance data, where ampicillin resistance often exceeds 70% (13, 14). Resistance to amoxicillin-clavulanate (67.2%) and trimethoprim-sulfamethoxazole (62.3%) was very common. These high rates render these traditional first-line agents largely ineffective for empirical therapy in this setting, a trend that has been increasingly reported across North Africa (15).

The high resistance to third-generation cephalosporins among Enterobacteriaceae is of particular concern. Ceftriaxone resistance was observed in 59.0% of *E. coli* and 64.3% of *Klebsiella* spp. isolates. The high resistance to third-generation cephalosporins may suggest the possible presence of extended-spectrum beta-lactamase (ESBL)-producing organisms;

however, confirmatory phenotypic or molecular testing was not performed in this study. (16). Studies from neighboring countries, such as Egypt and Tunisia, have similarly reported high ESBL prevalence rates among pediatric isolates, ranging from 40% to 60% (17, 18).

Among Gram-positive pathogens, *Staphylococcus* spp. exhibited extremely high resistance to penicillin (88.6%), which is expected given the near-universal production of beta-lactamase by these organisms. More importantly, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), as indicated by oxacillin resistance (60.0%), was alarmingly high. This rate is considerably higher than those reported in some European pediatric populations (10-20%) but is comparable to findings from other Middle Eastern and North African countries, where MRSA rates often exceed 50% (19, 20). MRSA infections are associated with increased morbidity, longer hospital stays, and higher healthcare costs, as they require treatment with more expensive and often intravenous antibiotics such as vancomycin (21).

Resistance to carbapenems (imipenem) was relatively low among *E. coli* (9.8%) and *Klebsiella* spp. (14.3%); its emergence in a pediatric population in Libya is deeply concerning. Carbapenems are considered last-line antibiotics for the treatment of severe infections caused by multidrug-resistant gram-negative bacteria. The detection of carbapenem resistance, albeit at low frequencies, signals the potential spread of carbapenemase-producing organisms, which have been increasingly reported in the Mediterranean region and pose a critical threat to patient safety (22, 23).

Similarly, the detection of vancomycin resistance in 8.6% of *Staphylococcus* spp. isolates is a worrying finding. Vancomycin has long been the mainstay treatment for serious MRSA infections. Although vancomycin-resistant *S. aureus* (VRSA) remains rare globally, reduced susceptibility and the emergence of vancomycin-intermediate *S. aureus* (VISA) have been documented in various regions, often associated with prolonged vancomycin use. This finding warrants further investigation to confirm the mechanism of resistance and implement strict infection control measures. In this study, the overall prevalence of MDR was 62.6%, indicating that nearly two-thirds of all bacterial isolates were resistant to three or more antibiotic classes. The highest MDR rates were observed in *Pseudomonas* spp. (80.0%), *Klebsiella* spp. (71.4%), and *E. coli* (67.2%). These figures are alarming and reflect a severe crisis in the effectiveness of antimicrobials. These findings are consistent with a growing body of evidence from the WHO Eastern Mediterranean Region, which reports some of the highest MDR rates globally (24). A systematic review of antimicrobial resistance in the Arab League states found that MDR rates among gram-negative bacilli frequently exceed 50% in many countries, driven by factors such as over-the-counter antibiotic sales, inappropriate prescribing, and poor infection control practices (25).

The high MDR burden in the pediatric population of Tobruk has profound clinical implications. It severely limits the available options for empirical treatment, forcing clinicians to use broader-spectrum antibiotics, which fuels further resistance. It also increases the risk of treatment failure, complications, and mortality, particularly in vulnerable groups such as neonates and infants (26). The high burden of antimicrobial resistance observed in this study can be partly explained by local healthcare practices in Libya. The widespread availability of antibiotics without prescription, inappropriate prescribing practices, and limited antimicrobial stewardship programs contribute significantly to the emergence and spread of resistant pathogens. In addition, gaps in infection prevention and control measures within healthcare facilities may facilitate the transmission of multidrug-resistant organisms. Addressing these systemic challenges is essential to control the growing threat of antimicrobial resistance in the region.

Limitations

This study has several limitations that should be considered. First, it was conducted in a single center, which may limit the generalizability of the findings to other regions in Libya. Second, the retrospective design restricted the availability of detailed clinical data, such as infection type, hospitalization status, and prior antibiotic exposure. Third, the sample size was relatively small, which may affect the statistical power of some analyses. Additionally, molecular and phenotypic confirmatory tests for resistance mechanisms, such as ESBL and carbapenemase production, were not performed. Finally, the classification of intermediate susceptibility as resistant may have led to an overestimation of resistance rates.

Conclusion

In conclusion, this study indicates a high frequency of gram-negative bacterial infections and an alarming burden of multidrug resistance among children in Tobruk, Libya. The extremely high rates of resistance to commonly used antibiotics, coupled with the emergence of resistance to last-line agents such as carbapenems and vancomycin, pose a serious threat to pediatric healthcare in the region. These findings highlight the critical need for continuous surveillance, rational antibiotic prescribing, and robust infection control strategies to combat the growing threat of AMR in Libya.

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Compliance with ethical standards*Disclosure of conflict of interest*

The authors declare that they have no conflict of interest.

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