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Original article

Prevalence of Common Bacterial Species in Clinical Specimens Referred to Medical Laboratories in Tarhuna Region and Their Most Appropriate Available Antibiotics

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Abstract
Antimicrobial resistance (AMR) represents a growing public health threat globally, with Libya experiencing particularly high resistance rates due to fragile health systems and limited surveillance. Understanding local resistance patterns is essential for guiding empiric antibiotic therapy and antimicrobial stewardship. A retrospective analysis was conducted on bacterial isolates from clinical specimens referred to medical laboratories in the Tarhuna region. Bacterial identification and antimicrobial susceptibility testing were performed using standard microbiological procedures. Resistance rates were calculated for Escherichia coli, Klebsiella species, and Staphylococcus species against commonly used antibiotics. E. coli isolates demonstrated low resistance profiles, with Cefotaxime resistance at 14.3% and Trimethoprim resistance at 10%, while complete susceptibility (0% resistance) was observed for Erythromycin, Gentamicin, Ciprofloxacin, Tetracycline, and Ceftriaxone. Klebsiella isolates showed variable resistance: Nalidixic acid (25%), Augmentin (25%), Ampicillin/Cloxacillin (33.3%), and Tetracycline (7.7%), with full susceptibility to Cefotaxime, Gentamicin, Ciprofloxacin, Ceftriaxone, and Streptomycin. Staphylococcus species exhibited concerning resistance patterns, particularly to Ampicillin (92.9%), Nalidixic acid (92.9%), and Fusidic acid (84%), while Trimethoprim, Cefuroxime, and Streptomycin showed 0% resistance. This study reveals markedly lower resistance rates in Tarhuna compared to national Libyan averages, suggesting geographic heterogeneity in AMR patterns within Libya. The findings highlight the critical importance of local susceptibility data for guiding antibiotic selection and the need for expanded surveillance across all Libyan regions.
Keywords: Antimicrobial Resistance, Escherichia Coli, Klebsiella, Staphylococcus, Libya.

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most pressing public health challenges of the 21st century, threatening to reverse decades of medical progress in the treatment of infectious diseases (1). The World Health Organization has declared AMR among the top ten global public health threats facing humanity, with low- and middle-income countries bearing a disproportionate burden of resistance-related morbidity and mortality (2). In 2019, an estimated 4.95 million deaths were associated with bacterial AMR globally, with the highest burden observed in sub-Saharan Africa (3). Libya, a North African country experiencing prolonged conflict and health system fragility, faces particular challenges in combating antimicrobial resistance. The country's health infrastructure has suffered from years of instability, leading to disrupted surveillance systems, limited laboratory capacity, and weakened regulatory frameworks for antibiotic dispensing (4). Recent systematic reviews have documented alarmingly high resistance rates among clinical isolates from Libyan patients, with pooled resistance exceeding 75% in some pathogen-antibiotic combinations (5). A meta-analysis of Libyan studies from 1970 to 2024 estimated 2,183 deaths attributable to AMR in 2024 alone, representing 9.7% of total mortality (5).

The most common bacterial pathogens isolated from clinical specimens in Libya include *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, though their relative prevalence varies by region and specimen type (6). In Tobruk, studies have reported *Staphylococcus aureus* (33.6%) as the predominant isolate, followed by *E. coli* (31.5%) and *Klebsiella* spp. (26.2%), a distribution that deviates from the global norm where *E. coli* typically dominates (6). Bloodstream infection studies from Libyan hospitals confirm the predominance of Gram-negative pathogens, with *E. coli* (32.0%) and *K. pneumoniae* (25.9%) accounting for the majority of isolates (7).

Antimicrobial susceptibility testing practices in Libyan laboratories have raised concerns regarding adherence to international standards. A recent evaluation in Gharyan revealed widespread deviations from EUCAST guidelines, including the use of non-recommended culture media (nutrient agar instead of Mueller-Hinton agar), excessive numbers of antibiotic disks per plate, and specimen overloading, all of which compromise result accuracy and clinical utility (8). Such methodological inconsistencies may contribute to the wide variations in reported resistance rates across different Libyan regions and underscore the need for standardized approaches to susceptibility testing (9). The emergence of multidrug-resistant organisms in Libyan healthcare settings has been associated with poor clinical outcomes. Patients infected with multidrug-resistant Gram-negative bacteria have demonstrated 30-day mortality rates of 32.1%, compared to 18.8% for those with susceptible infections (10). Independent predictors of mortality include inappropriate

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empiric therapy, intensive care unit admission, and high comorbidity burden, highlighting the critical importance of accurate, locally-relevant susceptibility data to guide initial antibiotic selection (10).

Despite growing recognition of the AMR crisis in Libya, significant gaps remain in our understanding of resistance patterns at the sub-national level. Most published studies have focused on major coastal cities such as Tripoli, Benghazi, and Tobruk, leaving inland regions like Tarhuna underrepresented in the national AMR landscape (11). This geographic heterogeneity in resistance patterns is well-documented globally and necessitates local surveillance to inform region-specific treatment guidelines (4). The present study aims to address this knowledge gap by characterizing the bacterial species isolated from clinical specimens referred to medical laboratories in the Tarhuna region and determining their antimicrobial susceptibility profiles. By identifying the most common pathogens and the most effective available antibiotics, this research seeks to provide evidence-based guidance for empiric therapy in this region and contribute to the growing body of Libyan AMR data.

Methods

Study Design and Setting

This retrospective laboratory-based study analyzed bacterial isolates obtained from clinical specimens submitted to medical diagnostic laboratories in the Tarhuna region, Libya. Tarhuna is a city located in northwestern Libya, approximately 90 kilometers southeast of Tripoli.

Bacterial Isolates

The study included clinical isolates of Escherichia coli, Klebsiella species, and Staphylococcus species recovered from various clinical specimens (including urine, wound swabs, blood, and other body fluids) submitted for routine diagnostic testing. Isolates were included regardless of patient age, gender, or clinical diagnosis.

Bacterial Identification

Bacterial identification was performed using standard microbiological techniques, including colony morphology assessment on selective and non-selective media, Gram staining, and conventional biochemical testing (12).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the disk diffusion method on Mueller-Hinton agar according to standard procedures (13). Although recent evaluations have identified deviations from standardized protocols in some Libyan laboratories (8), this study adhered to Clinical and Laboratory Standards Institute (CLSI) guidelines for test performance and interpretation (13). For *E. coli* isolates, susceptibility was tested against: Cefotaxime, Trimethoprim, Erythromycin, Gentamicin, Ciprofloxacin, Tetracycline, and Ceftriaxone. For *Klebsiella* isolates, susceptibility was tested against: Nalidixic acid, Augmentin (amoxicillin-clavulanate), Ampicillin/Cloxacillin, Tetracycline, Cefotaxime, Gentamicin, Ciprofloxacin, Ceftriaxone, and Streptomycin. For *Staphylococcus* isolates, susceptibility was tested against a comprehensive panel including: Ampicillin, Nalidixic acid, Fusidic acid, Ampicillin/Cloxacillin, Clindamycin, Erythromycin, Amoxicillin, Vancomycin, Azithromycin, Gentamicin, Tetracycline, Norfloxacin, Ceftriaxone, Trimethoprim, Cefuroxime, Streptomycin, Doxycycline, Ciprofloxacin, and Nitrofurantoin. Zone diameters were measured and interpreted as susceptible, intermediate, or resistant according to CLSI breakpoints (13). For this analysis, isolates categorized as intermediate were considered non-susceptible and included in the resistance calculations.

Data Analysis

Resistance rates were calculated as the percentage of isolates demonstrating non-susceptibility (resistant or intermediate) to each antibiotic tested. For each bacterial species, antibiotics were categorized based on observed resistance levels:

High resistance (≥50%): Agents with limited clinical utility
Moderate resistance (10-49%): Agents requiring caution and susceptibility confirmation
Low resistance (<10%): Agents with excellent activity, representing optimal therapeutic options

The most appropriate antibiotics were defined as those demonstrating 0% resistance among tested isolates, indicating complete susceptibility of all tested strains.

Ethical Considerations

This study utilized anonymized laboratory data obtained from routine diagnostic testing. No patient identifiers were collected, and no interventions were performed on patients. The study was conducted in accordance with ethical principles for research using existing data and received approval from the relevant institutional review bodies.

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Results

Escherichia coli Isolates

A total of 14 E. coli isolates recovered from clinical specimens in Tarhuna laboratories were analyzed for antimicrobial susceptibility. The resistance profile of E. coli to commonly used antibiotics is presented in Table 1.

Table 1: Antimicrobial Resistance Rates of E. coli Isolates

Antibiotic	Resistance Rate (%)
Cefotaxime	14.3
Trimethoprim	10.0
Erythromycin	0
Gentamicin	0
Ciprofloxacin	0
Tetracycline	0
Ceftriaxone	0

The highest resistance among E. coli isolates was observed against Cefotaxime (14.3%), followed by Trimethoprim (10.0%). Notably, complete susceptibility (0% resistance) was demonstrated against five antibiotics: Erythromycin, Gentamicin, Ciprofloxacin, Tetracycline, and Ceftriaxone. These agents therefore represent the most appropriate antibiotic options for E. coli infections in the Tarhuna region.

Klebsiella Isolates

A total of 20 Klebsiella species isolates exhibited a more varied resistance profile compared to E. coli, as shown in Table 2. The highest resistance rate among Klebsiella isolates was observed against Ampicillin/Cloxacillin (33.3%), followed by Nalidixic acid (25.0%) and Augmentin (25.0%). Tetracycline demonstrated moderate activity with 7.7% resistance. Five antibiotics showed complete activity against all tested Klebsiella isolates: Cefotaxime, Gentamicin, Ciprofloxacin, Ceftriaxone, and Streptomycin. These agents constitute the optimal therapeutic options for Klebsiella infections in this region

Table 2: Antimicrobial Resistance Rates of Klebsiella Isolates

Antibiotic	Resistance Rate (%)
Ampicillin/Cloxacillin	33.3
Nalidixic acid	25.0
Augmentin	25.0
Tetracycline	7.7
Cefotaxime	0
Gentamicin	0
Ciprofloxacin	0
Ceftriaxone	0
Streptomycin	0

Staphylococcus Isolates

A total of 41 Staphylococcus species isolates demonstrated the most concerning resistance patterns, with several antibiotics showing high resistance rates exceeding 50%. Table 3 presents the complete resistance profile. Nine antibiotics demonstrated high resistance rates exceeding 50%, with the highest observed for Ampicillin (92.9%) and Nalidixic acid (92.9%). Fusidic acid (84.0%), Ampicillin/Cloxacillin (80.0%), and Clindamycin (72.7%) also showed concerningly high resistance. Notably, Vancomycin—often considered an antibiotic of last resort for Gram-positive infections—exhibited 55.6% resistance among Staphylococcus isolates. Four antibiotics fell into the moderate resistance category, ranging from Gentamicin (29.4%) to Ceftriaxone (22.6%). The low resistance category included six antibiotics, with three agents demonstrating complete activity (0% resistance): Trimethoprim, Cefuroxime, and Streptomycin. Doxycycline (7.7%), Ciprofloxacin (12.1%), and Nitrofurantoin (12.5%) also maintained good activity against the majority of isolates.

Table 3: Antimicrobial Resistance Rates of Staphylococcus Isolates

Resistance Category	Antibiotic	Resistance Rate (%)
High Resistance (≥50%)	Ampicillin	92.9
	Nalidixic acid	92.9
	Fusidic acid	84.0
	Ampicillin/Cloxacillin	80.0
	Clindamycin	72.7

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	Erythromycin	66.7
	Amoxicillin	60.0
	Vancomycin	55.6
	Azithromycin	53.8
Moderate Resistance (10-49%)	Gentamicin	29.4
	Tetracycline	27.8
	Norfloxacin	25.9
	Ceftriaxone	22.6
Low Resistance (<10%)	Doxycycline	7.7
	Ciprofloxacin	12.1
	Nitrofurantoin	12.5
	Trimethoprim	0
	Cefuroxime	0
	Streptomycin	0

Summary of Optimal Antibiotic Options

Based on the resistance patterns observed, the most appropriate antibiotic options for each bacterial species in the Tarhuna region are summarized in Table 4. Doxycycline, Ciprofloxacin, and Nitrofurantoin demonstrated low but non-zero resistance (7.7-12.5%) and remain valuable options with susceptibility confirmation. Ciprofloxacin and Gentamicin emerged as broadly effective agents across both Gram-negative species, while Ceftriaxone showed excellent activity against both E. coli and Klebsiella. For staphylococcal infections, Trimethoprim, Cefuroxime, and Streptomycin represent the most reliable empiric options based on the complete susceptibility of tested isolates.

Table 4: Optimal Antibiotic Options by Bacterial Species

Bacterial Species	Optimal Antibiotics (0% Resistance)
E. coli	Ciprofloxacin, Gentamicin, Ceftriaxone, Erythromycin, Tetracycline
Klebsiella	Ciprofloxacin, Gentamicin, Ceftriaxone, Cefotaxime, Streptomycin
Staphylococcus	Trimethoprim, Cefuroxime, Streptomycin, Doxycycline, *Ciprofloxacin*, Nitrofurantoin*

Discussion

This study provides the first detailed characterization of antimicrobial resistance patterns among common bacterial pathogens in the Tarhuna region of Libya, revealing several important findings with implications for clinical practice and public health policy. The most striking observation is the markedly lower resistance rates compared to national Libyan averages reported in recent meta-analyses (5). The resistance rates observed for *E. coli* in Tarhuna (Cefotaxime 14.3%, Trimethoprim 10%) are substantially lower than those reported from other Libyan regions. A recent study from Gharyan documented overall *E. coli* resistance of 83%, with particularly high rates against commonly used antibiotics (11). Similarly, the meta-analysis by Bleiblo and colleagues reported widespread resistance to third-generation cephalosporins and fluoroquinolones among Enterobacterales isolates nationally (5). The complete susceptibility of *E. coli* to Ciprofloxacin, Gentamicin, and Ceftriaxone in Tarhuna stands in stark contrast to these national trends and suggests significant geographic heterogeneity in resistance patterns within Libya (4).

Klebsiella isolates in this study demonstrated moderate resistance to older antibiotics (Ampicillin/Cloxacillin 33.3%, Nalidixic acid 25%) but complete susceptibility to newer agents, including Cefotaxime, Ceftriaxone, and Ciprofloxacin. This pattern differs from the national landscape, where third-generation cephalosporin and fluoroquinolone resistance among *Klebsiella* is increasingly reported (14). The 7.7% resistance to Tetracycline among *Klebsiella* isolates is consistent with findings from other Libyan studies where tetracyclines have maintained reasonable activity against Enterobacterales (6). The resistance profile of *Staphylococcus* isolates in Tarhuna reveals both concerning trends and reassuring findings. The extremely high resistance to Ampicillin (92.9%), Nalidixic acid (92.9%), and Fusidic acid (84%) aligns with the well-documented ubiquity of penicillin resistance in staphylococci globally (12). However, the 55.6% resistance to Vancomycin is particularly alarming, as vancomycin has traditionally been reserved as a last-line agent for serious Gram-positive infections (10). This finding exceeds previously reported vancomycin resistance rates in Libya and warrants urgent confirmation through molecular studies to distinguish true resistance from technical artifacts (8).

Conversely, the complete susceptibility of *Staphylococcus* isolates to Trimethoprim, Cefuroxime, and Streptomycin provides valuable therapeutic options. Trimethoprim, in particular, represents an affordable, orally available option for outpatient management of staphylococcal infections (15). The 7.7% resistance to Doxycycline and 12.1% to Ciprofloxacin also position these agents as reasonable alternatives with susceptibility testing (16).

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The substantial differences between resistance patterns in Tarhuna and those reported from other Libyan regions warrant careful consideration of potential explanatory factors. Several hypotheses may account for this geographic heterogeneity. First, antibiotic prescribing practices and consumption patterns likely vary considerably across Libyan regions. Studies from Gharyan have documented widespread inappropriate antibiotic use, with 70% of pharmacists dispensing antibiotics without prescriptions and 40% providing incorrect dosages (17). Such practices drive resistance through selective pressure, and regional differences in the intensity of antibiotic misuse could explain variable resistance rates. Tarhuna, as a smaller inland city, may have different antibiotic access patterns compared to major coastal urban centers (4). Second, population mobility and healthcare-seeking behaviors differ between regions. Tarhuna's proximity to Tripoli (85 km) means that patients with complicated infections may seek care at hospitals in the capital, potentially skewing the case mix in local laboratories toward less complex, community-acquired infections with lower resistance probabilities (11).

Third, laboratory practices and quality assurance may vary regionally. A recent evaluation in Gharyan revealed widespread deviations from standardized susceptibility testing protocols, including the use of inappropriate culture media and excessive antibiotic disks per plate (8). If such practices are less prevalent in Tarhuna laboratories, the resistance data may more accurately reflect true susceptibility patterns. Conversely, methodological differences could also produce artificially low resistance rates if testing conditions favor organism growth and larger inhibition zones (9). Fourth, temporal trends in resistance may contribute to observed differences. Resistance is dynamic and generally increases over time with continued antibiotic pressure. The national meta-analysis included studies spanning five decades, and regional differences may partly reflect different time periods of data collection (5).

The resistance patterns identified in this study have direct implications for empiric antibiotic selection in the Tarhuna region. For suspected Gram-negative infections, Ciprofloxacin, Gentamicin, and Ceftriaxone emerge as excellent empiric options with predicted susceptibility exceeding 90% for both *E. coli* and *Klebsiella*. This finding is particularly valuable for urinary tract infections, where *E. coli* predominates (6), and for intra-abdominal infections where Gram-negative pathogens are common (12). For staphylococcal infections, the optimal empiric choices are Trimethoprim, Cefuroxime, and Streptomycin. The high rate of Vancomycin resistance (55.6%) argues against its routine use for empiric coverage of staphylococcal infections in this region, a finding that contradicts international guidelines recommending vancomycin for serious MRSA infections (10). This resistance pattern requires urgent validation, as it would fundamentally alter treatment algorithms if confirmed. The availability of multiple oral options (Trimethoprim, Doxycycline, Ciprofloxacin) with good activity against staphylococci facilitates outpatient management of skin and soft tissue infections, reducing the need for hospitalization and parenteral therapy (16). However, the 12.1% Ciprofloxacin resistance among staphylococci suggests that susceptibility testing should guide fluoroquinolone use for serious infections.

From a public health perspective, this study underscores the critical importance of local antimicrobial resistance surveillance. National and regional resistance data, while valuable for policy development, cannot substitute for locally-generated susceptibility information to guide individual patient care (4). The marked geographic heterogeneity observed between Tarhuna and other Libyan regions reinforces the WHO's call for decentralized surveillance systems that capture sub-national resistance patterns (2). The findings also highlight opportunities for antimicrobial stewardship interventions. The complete susceptibility of *E. coli* and *Klebsiella* to multiple antibiotic classes suggests that resistance has not yet become entrenched in this region, providing a window of opportunity for preserving antibiotic effectiveness through rational use (1). Educational interventions targeting prescribers, dispensers, and the public could help maintain the current favorable resistance profile (17). The concerning vancomycin resistance among staphylococci, if confirmed, would signal the emergence of a public health crisis requiring immediate action, including infection control measures to prevent transmission of resistant strains and enhanced surveillance to monitor spread (10).

Conclusion

This study provides the first comprehensive analysis of antimicrobial resistance patterns among common bacterial pathogens in the Tarhuna region of Libya. The findings reveal a uniquely favorable resistance profile compared to national averages, with E. coli and Klebsiella isolates demonstrating complete susceptibility to multiple antibiotic classes and Staphylococcus isolates showing several highly effective options. However, the concerning vancomycin resistance observed among staphylococci warrants urgent investigation and confirmation. These results underscore the critical importance of local susceptibility data for guiding empiric antibiotic therapy and highlight the marked geographic heterogeneity in resistance patterns within Libya. Strengthening laboratory capacity, standardizing susceptibility testing methods, and establishing sustainable surveillance systems are essential priorities for preserving antibiotic effectiveness and improving patient outcomes across all Libyan regions.

Conflict of interest. Nil

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