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Multi-Drug-Resistant *Enterobacteriaceae* Causing Urinary Tract Infections At Al- Marj Teaching Hospital

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ABSTRACT

Urinary tract infections (UTIs) are one of the most common infections in hospitals. Studies on the causative agents of UTIs in Libya and their susceptibility to antimicrobials are few and were mainly carried out in the two main cities in Libya, Tripoli and Benghazi. The study aims to identify the Gram-negative bacteria that cause UTIs at Al-Marj Teaching Hospital as well as determine the antibiotic sensitivity (AST) of the isolated bacteria. Between March and September 2018, 163 urine samples were collected from the inpatients at the AL-Marj teaching hospital. The samples were inoculated onto CLED agar and incubated at 37^oC for 24 hours. The phoenix system was used for the identification of bacteria and AST. Of 163 samples, significant growth was seen in 50 samples (30.6%). Of them, 27 were Gram-positive and 23 were Gram-negative, which comprised *Escherichia coli* 16 (32 %), *Klebsiella pneumoniae* 6 (12%), and *Citrobacter freundii* 1 (2.0%). The percentage of extended spectrum beta lactamase (ESBL) producers in *E. coli* and *K. pneumoniae* was observed to be 68.7% and 50%, respectively. While carbapenamase producers were observed to be 6.2% for *E. coli* and 16.6% for *K. pneumoniae*. The highest resistance rates were against Ampicillin (86.9%), Cephalothin (82.6%), Cefuroxime (73.9%), Cefepime (69.5%), Ampicillin-Clavulanate, and Ceftriaxone (65.2%), respectively. In conclusion, *E. coli* and *K. pneumoniae* were the most common microorganisms causing UTIs. The increased prevalence of multi-drug-resistant organisms limits the available treatment options for infections caused by these organisms. Therefore, the identification and reporting of ESBL-producing organisms is crucial for clinical decision-making.

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1. Introduction

Urinary tract infection (UTI) is the most common infectious disease in clinical practice [1]. It can affect various parts of the urinary tract. Although both males and females are exposed to the infection, women are more affected due to their morphology and reproductive physiology [2]. It ranges from the presence of bacteria in urine without symptoms (asymptomatic bacteriuria), to a wide variety of symptoms ranging from mild bacteraemia, sepsis, or even death [3]. This problem is spread across all age groups. However, different factors, including race, genetic factors, age, gender, sexual activity, nocturnal enuresis, and circumcision in boys, may influence the appearance of UTIs [4].

The major causative organisms responsible for most UTI cases are bacteria, mainly Gram-negative species [5]. Therefore, the pathogens traditionally associated with UTI are known to change many of their features, particularly due to their antimicrobial resistance (AMR) patterns[6].

The increase of AMR in bacterial pathogens is of worldwide concern; uropathogens are showing higher rates of resistance, according to the SENTRY Antimicrobial Surveillance program. Particularly, the rising prevalence of AmpC-lactamases and extended spectrum beta lactamase (ESBL) producing microbes raises concerns about multidrug resistance [7]. Since the initiation of antimicrobial therapy in UTI is empirical, a huge need for AMR exists at local, national and international levels. But this has to be balanced with the inappropriate usage of broad-spectrum antibiotics leading to the emergence of resistant strains [8] ; therefore the knowledge of the AMR patterns of common uropathogens is required [9].

This study conducted at Al-Marj teaching hospital in Libya, aimed to investigate the bacterial identification and antimicrobial susceptibility patterns of urinary pathogens using Phoenix BD.

2. Methodology

2.1. Study area and data collection

A total of 163 urine samples were collected from inpatients AL-Marj teaching hospital, between March 2018 and September 2018. The samples comprised of 115 midstream urine, 14 suprapubic aspiration and 33 catheter specimens and one by bag of urine collection. These samples were directly taken to the laboratory for standard microbiological analysis.

2.2. Isolation and identification of *Enterobacteriaceae*

After the collection of the samples (approximately 10 ml) in sterile containers, the samples were examined within 2 hrs, as described by Pezzlo et al [10]. A standard bacteriological loopful (0.01ml) of urine was directly inoculated and spread over the surface of sterile agar on a CLED agar plate, then incubated aerobically at 37°C for 24 hours [11].

On the second day, if there is pure and significant bacterial growth, a Gram stain is applied to know the Gram reaction of the isolates, and then the isolates were sub-cultured on blood agar in preparation for their identification by the Phoenix system. On the third day and upwards, the BD Phoenix system for identification (ID) and antimicrobial susceptibility testing (AST) was used according to the manufacturer’s recommendations. A colony of the culture on the blood agar was taken by swab and mixed with the ID solution to match the turbidity of a 0.5 McFarland standard by using a Crystal Spec nephelometer. Twenty-five microliters of the mixture were transferred to an AST bottle, and one drop of ID indicator was added to the AST tube and mixed gently. The panel was then loaded into the instrument within 30 min of inoculation for identification and antibiotic susceptibility [12,13].

2.3. Statistical analysis:

Data were analyzed using SPSS (Statistical Package for Social Sciences (statistics for windows, version 23.0. Armonk, NY: IBM Corp). Descriptive data was compared using Chi-Square and its alternative Fisher exact test were used, and graphs and tables were created to demonstrate results.

3- Results

Out of the 163 urine specimens examined, 103 (63.6%) were collected from females, while 60 (36.4%) were collected from males. The specimens were collected from individuals spanning various age groups, ranging from a few days old to ninety years old with mean age of 40 years, as indicated in table 1.

Out of 163 urine samples, 50 samples were had significant bacterial growth, of them 35 (70%) were from female individuals and 15 (30%) were from male individuals.

The distribution of positive samples based on age and gender reveals that the age group associated with sexual activity in females (21-40 years) exhibited the highest prevalence of UTIs, as depicted in table 2.

The overall prevalence of the uropathogens was 50 (30.9%), as shown in figure 1.

Table 1. The distribution of samples among different age groups

Age (years)	NO. of samples	Percentage %
< 20	50	30.7 %
21-40	53	32.5 %
41-60	29	17.8 %
61-90	31	19.0 %
Total	163	100 %

Table 2. Association between gender and presence of significant growth among different ages

Fisher test ,

Age	Gender		Total	*P value*
	Male	**Female**		
< 20	8	4	12	0.0036*
21- 40	1	13	14	
41- 60	3	7	10	
61-90	4	10	14	
Total	15	34	50	

Using Exact *

Statistically significant of females sexually active age.

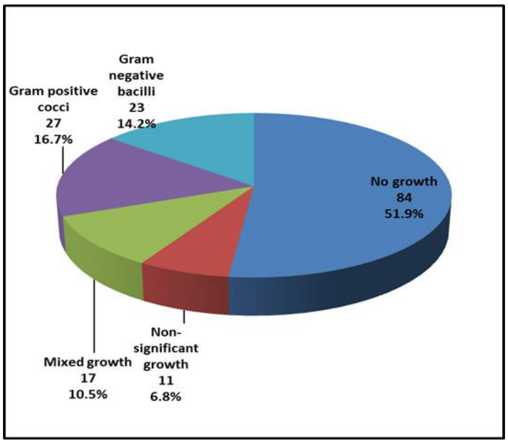

Figure 1. Percentages of bacterial growth

Table 3. Frequency and types of Enterobacteraece isolated from urine culture

Organism	NO. of samples	Percentage %
E. coli	16	32 %
K. pneumonia	6	12 %
C. freundii	1	2 %
Total	23	46 %

The majority of the pathogenic microorganisms isolated in this study belonged to the *Enterobacteriaceae* family. *E. coli* was identified as the most common causative agent of UTIs 16 (32 %), followed by *K. pneumoniae* 6 (12 %), and *C. freundii* 1 (2.0%) as shown in table 3.

The antimicrobial resistance and sensitivity profile of the isolated organisms showed that all the bacterial isolates were resistant against commonly used antibiotics. The highest resistant rate was Ampicillin 88.6%, Cephalothin 82.6%, Cefepime 69% and Ampicillin-Clavulanate and ceftriaxone 65%.

While all isolates were found to have less resistance to Amikacin, Ertapenem, Imipenem, Nitrofurantoin, and Piperacillin-Tazobactam with 4.3% each, as shown in figure 2.

There are some cross-resistance and cross-sensitivity that are nearly complete for Ciprofloxacin and Levofloxacin.

E. coli were higher resistance which constitute 33 (67.34%) of all isolated bacteria against the most type of antibiotics (58.85 %) and the highest resistance was seen among Cephalothin (87.5%), Ampicillin (81.3%), Cefuroxime (80.0%), Ceftriaxone (68.8%), Aztreonam (62.5%), in the other hand *K. pneumoniae* has highest resistance to ampicillin (83.3%), ampicillin-clavulanate (83.3%), Aztreonam (66.7%), as shown in figure 3 and 4. Different resistant strains of Enterobacteraece were produced ESBL and carpenamase where highest resistance found in strains of *E. coli* produced ESBL and carpenamase are 11 (68.75%) and 1 (6.2%) while in *K. pneumonia* 3 (50%) and 1 (16.6) respectively as shown in table 4.

4- **Discussion**

The emergence and prevalence of multi-drug-resistant *Enterobacteriaceae* (MDR-EB) causing UTIs carriage a significant challenge to healthcare systems worldwide. The study analyzed 163 urine specimens, with 50 samples showing significant bacterial growth. The prevalence of UTIs was higher in females (70%) compared to males (30%). The age group associated with sexual activity in females (21-40 years) exhibited the highest prevalence of UTIs. These findings are consistent with previous studies that have reported higher rates of UTIs among women and sexually active individuals [14,15].

The majority of the isolated uropathogens belonged to the *Enterobacteriaceae* family, with *E. coli* being the most common causative agent of UTIs (32%), followed by *K. pneumoniae* (12%) and *C. freundii* (2.0%). These results align with local study in Teaching Hospital in Zawiya city [16] and also in Messalata Central Hospital, Libya [17]. As well as with global trends where *E. coli* is frequently implicated as the primary pathogen causing UTIs [18].

Antimicrobial susceptibility testing revealed alarmingly high rates of resistance among the isolated organisms against commonly used antibiotics. The highest resistance rates were observed for Ampicillin, Cephalothin, cefepime, Ceftraxone and Ampicillin-Clavulanate. These findings highlight the urgent need for antibiotic stewardship programs and surveillance of antimicrobial resistance in healthcare settings [19].

Interestingly, the isolated organisms showed sensitivity to Daptomycin, indicating its potential as alternative treatment options for MDR-EB infections. These findings are consistent with previous studies that have reported the effectiveness of this antibiotics against MDR-EB strains [20,21].

Cross-resistance and cross-sensitivity were observed among certain antibiotics. For example, Ciprofloxacin and Levofloxacin. Additionally, Cefotaxime and Ceftriaxone displayed nearly complete cross-resistance and cross-sensitivity. These findings highlight the importance of selecting appropriate antibiotics based on their individual susceptibility profiles [22].

Further analysis of the resistance profiles showed that *E. coli* exhibited the highest resistance rates among all isolated bacteria, particularly against Cephalothin, Ampicillin, Cefuroxime, Ceftriaxone, and Aztreonam. *K. pneumoniae* displayed the highest resistance rates against Ampicillin, Ampicillin-clavulanate, and Aztreonam. The findings are consistent with a study conducted in Tripoli [3]. These results are also consistent with global trends, as both *E. coli* and *K. pneumoniae* are well-known MDR-EB pathogens associated with UTIs and other healthcare-associated infections [23].

Furthermore, the study identified strains of *E. coli* and *K. pneumoniae* that produced extended-spectrum β-lactamases (ESBL) and carbapenemases. The prevalence of ESBL-producing *E. coli* was 68.75%, while carbapenemase production was identified in 6.2% of *E. coli* strains. Among *K. pneumoniae* isolates, 50% produced ESBL, and 16.6% produced carbapenemases. These findings indicate the presence of highly resistant strains capable of hydrolyzing a broad range of β-lactam antibiotics, further complicating treatment options [24].

The high prevalence of MDR-EB strains causing UTIs at Al-Marj teaching hospital underscores the urgent need for infection control measures, antimicrobial stewardship programs, and improved surveillance to combat the spread of drug-resistant pathogens. Implementing strategies such as regular monitoring of resistance patterns, optimizing antibiotic prescribing practices, and promoting infection prevention measures can help mitigate the impact of MDR-EB infections [25] (WHO, 2019).

5- Conclusions

In conclusion, this study provides valuable insights into the prevalence, bacterial identification, and antimicrobial resistance patterns of MDR-EB causing UTIs at Al-Marj teaching hospital in Libya. The results highlight the alarming rates of antibiotic resistance among uropathogens and emphasize the need for evidence-based strategies to guide appropriate antibiotic use and prevent the further dissemination of drug-resistant strains.

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Conflict of Interest

No conflict of interest was declared by the authors.

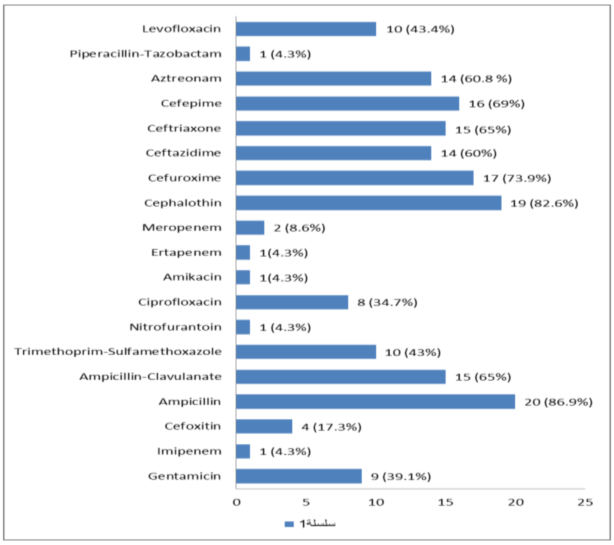

Figure 2: Antimicrobial resistance and sensitivity profile of the isolated *Enterobacteraece*

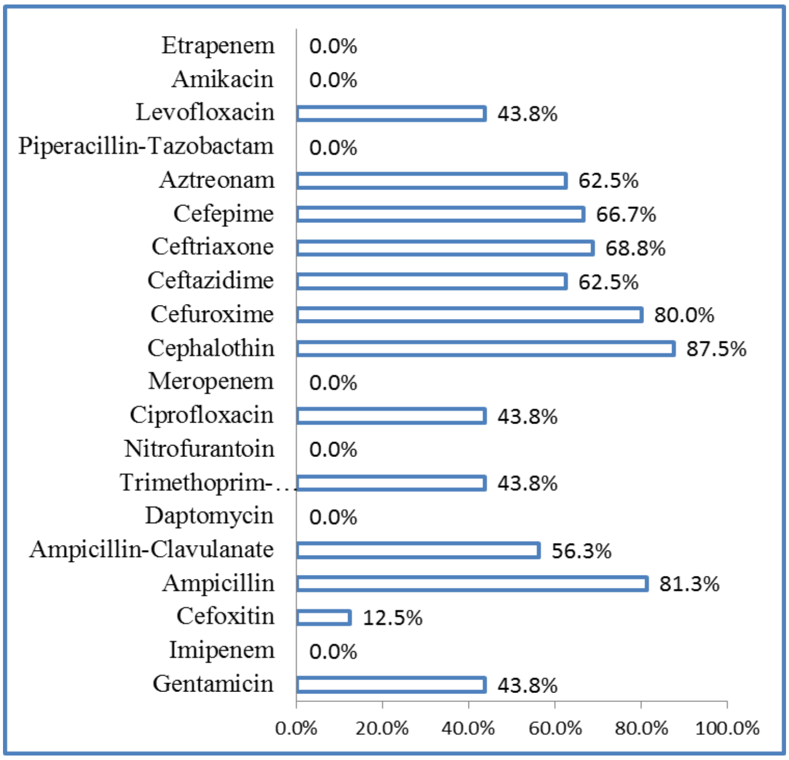

Figure 3: Antibiotics resistance profile for *E. coli*

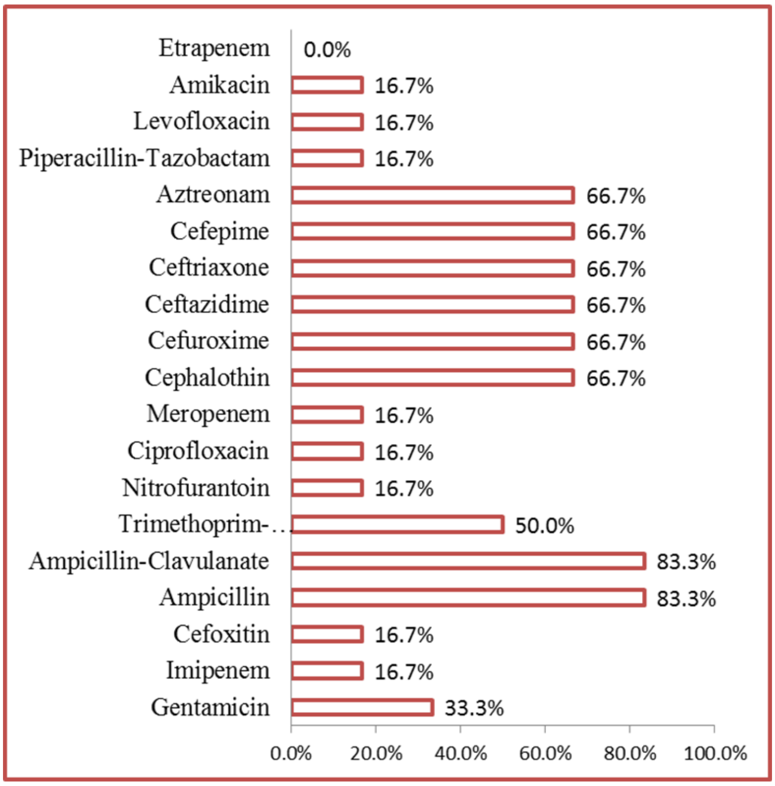

Figure 4: Antibiotics resistance profile for *K. pneumoniae*

Table 4. Resistant strains *Enterobacteraece* produced ESBL

Organism	n	%	Total
E. coli (ESBL)	11	68.75 %	16
*K. pneumoniae (*ESBL)	3	50%	6
E. coli (Carbapenamase producer)	1	6.2%	16
K. pneumoniae (Carbapenamase producer)	1	16.6%	6

n= number of organisms

References

1. Sekmenli T, Kara F. Association of vesicoureteral reflux and renal scarring in urinary tract infections. Pediatr Int. 2018;116(4):542-547.
2. Ashur AB, El Magrahi H, Elkammoshi A, Alsharif H. Prevalence and antibiotics susceptibility pattern of urine bacterial isolates from Tripoli Medical Center (TMC), Tripoli, Libya. Iberoam J Med. 2021;3(3):221-226.
3. Salem MA, et al. Bacterial Profile of Urinary Tract Infection and Antimicrobial Susceptibility Pattern Among Patients Attending at Bushra. J Microbiol Infect Dis. 2018;7(4):2671-2675. doi: 10.17554/j.issn.2224-3992.2018.07.788
4. Feld LG. Urinary Tract Infections and Vesicoureteral Reflux in Infants and Children. Pediatr Infect Dis J. 2013;31(11):451. doi: 10.1542/pir.31-11-451
5. Birnie K, et al. Comparison of microbiological diagnosis of urinary tract infection in young children by routine health service laboratories and a research laboratory: Diagnostic cohort study. PLoS One. 2017;12(2):e0171113.
6. Bagchi I, Jaitly NK, Thombare VR. Microbiological evaluation of catheter-associated urinary tract infection in a tertiary care hospital. People J Sci Res. 2015;8:23-9.
7. Baloch BK, et al. Antibiotic Antibiogram in Patients with Complicated Urinary Tract Infections in Nephrology Unit of South Waziristan. Cureus. 2022;14(10).
8. O’Grady MC, et al. Empirical treatment of urinary tract infections: how rational are our guidelines? J Antimicrob Chemother. 2019;74(1):214-217.
9. Prais D. Bacterial susceptibility to oral antibiotics in community-acquired urinary tract infection. Arch Dis Child. 2003;88(3):215-218.
10. Pezzlo M. Laboratory diagnosis of urinary tract infections: guidelines, challenges, and innovations. Clin Microbiol Newsl. 2014;36(12):87-93.
11. Hilt EE, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. J Clin Microbiol. 2014;52(3):871-876. doi: 10.1128/JCM.02876-13
12. Hara CMO. Evaluation of the Phoenix 100 ID/AST System and NID Panel for Identification of Enterobacteriaceae, Vibrionaceae, and Commonly Isolated Nonenteric Gram-Negative Bacilli. J Clin Microbiol. 2006;44(3):928-933. doi: 10.1128/JCM.44.3.928
13. Carroll KC, et al. Evaluation of the BD Phoenix automated microbiology system for identification and antimicrobial susceptibility testing of staphylococci and enterococci. J Clin Microbiol. 2006;44(6):2072-2077.
14. Khawcharoenporn T, Vasoo S, Singh K. Urinary tract infections due to multidrug-resistant Enterobacteriaceae: prevalence and risk factors in a Chicago emergency department. Emerg Med Int. 2013;2013:258517.
15. Mazzariol A, Bazaj A, Cornaglia G. Multi-drug-resistant Gram-negative bacteria causing urinary tract infections: a review. J Chemother. 2017;29(sup1):2-9.
16. Abujnah AA, et al. Multidrug resistance and extended-spectrum β-lactamases genes among Escherichia coli from patients with urinary tract infections in Northwestern Libya. Libyan J Med. 2015;10(1).
17. Mohammed MA, et al. Prevalence and antimicrobial resistance pattern of bacterial strains isolated from patients with urinary tract infection in Messalata Central Hospital, Libya. Asian Pac J Trop Med. 2016;9(8):771-776.
18. Vachvanichsanong P, McNeil EB, Dissaneewate P. Extended-spectrum beta-lactamase Escherichia coli and Klebsiella pneumoniae

urinary tract infections. Epidemiol Infect. 2021;149:e12.

19. Llor, C., Bjerrum, L., & Strandberg, E. L. Microbiology of urinary tract infections in primary care patients: a cross-sectional study in the community in Spain and Sweden. BMC Family Practice. 2020; 21(1), 1-9.
20. Gales, A. C., Seifert, H., Gur, D., Castanheira, M., & Jones, R. N. Antimicrobial susceptibility of Gram-negative bacteria isolated from patients hospitalized in intensive care units in United States and European hospitals. 2019; 2009–2011). Diagnostic Microbiology and Infectious Disease, 73(4), 328-334.
21. Kuti, J. L., Patel, A. A., Coleman, C. I., & Nicolau, D. P. Antimicrobial activity of daptomycin against gram-negative pathogens: Results from SURVEILLANCE studies. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 2020; 40 (9), 839-847.
22. Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., & Monnet, D. L. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection. 2012;18 (3), 268-281.
23. Pitout, J. D., Nordmann, P., & Poirel, L. Carbapenemase-producing Klebsiella pneumoniae, a key pathogen set for global nosocomial dominance. Antimicrobial Agents and Chemotherapy. 2018; 62(1), e01019-17.
24. Paterson, D. L., & Bonomo, R. A. Extended-spectrum β-lactamases: a clinical update. Clinical Microbiology Reviews. 2018; 18(4), 657-686.
25. World Health Organization. (2019). Antimicrobial resistance: Global report on surveillance. WHO