

FREQUENCY AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF GRAM-NEGATIVE BACILLI ISOLATED FROM URINE SPECIMENS AT A TERTIARY CARE SETTING

Aamir Hussain¹, Ihsan Ullah², Humera Adeeb³, Mubarak Zeb⁴

ABSTRACT:

OBJECTIVES:

To find out the frequency and pattern of conventional antibiotic susceptibility of gram-negative bacilli cultured from urine specimens of patients at a tertiary care setting.

METHODOLOGY:

This study was conducted at the Microbiology Department of Combined Military Hospital Multan from June 2016 to May 2017. The data in this retrospective descriptive study was collected from urine culture records of the Microbiology Department, CMH Multan. Only those urine specimens who revealed positive gram-negative bacilli cultures were included in the study. Drug susceptibility patterns of these isolates were recorded against routinely used antibiotics (e.g. Nitrofurantoin, Imipenem, Sulbactam-cefoperazone, Gentamicin and Ciprofloxacin) and evaluated accordingly.

RESULTS:

A total of 1703 urine specimens were submitted for culture and antibiotics susceptibility testing during the period of study. A total of 128 specimens showed growth of gram-negative rods. Imipenem (95% sensitivity), Sulbactam- Cefoperazone (88% sensitivity) and Nitrofurantoin (87% sensitivity) were highly effective antibiotics against the cultured gram-negative bacilli in the study.

CONCLUSION:

This study showed that *E. coli* is the commonest cause of urinary tract infection (UTIs), followed by *Klebsiella* and *Enterobacter* species among gram-negative bacilli in our set up. In-vitro efficacy of Imipenem, Sulbactam-Cefoperazone and Nitrofurantoin was found to be the highest against these gram-negative bacilli as compared to other antimicrobials. On the contrary, in-vitro efficacy of ciprofloxacin and gentamycin was found to be extremely low.

KEYWORDS: Antimicrobial Susceptibility, *Escherichia Coli*, Gram-Negative Bacilli, Urine Culture, Urinary Tract Infection

How to cite this article:

Hussain A, Ullah I, Adeeb H, Zeb M. Frequency and Antimicrobial Susceptibility Pattern of Gram-Negative Bacilli Isolated from Urine Specimens at A Tertiary Care Setting. J Gandhara Med Dent Sci. 2022;9(1): 15-19
<https://doi.org/10.37762/jgm.9-1.126>

Correspondence:

²Ihsan Ullah, Associate Professor, Khyber Medical University, Peshawar.

+92-313-9558883

✉: drihsan.ibms@kmu.edu.pk

¹Lieutenant Colonel, Combined military Hospital, Multan

³Trainee Community Medicine, Khyber Medical College, Peshawar

⁴Senior Medical Specialist, District Health System, Peshawar

INTRODUCTION:

Urinary tract infections (UTIs) are one of the most common bacterial infectious diseases encountered in clinical practice. UTIs include microbial infection of the urinary tract including urinary bladder and urethra. Females are more frequently affected by UTI due to their short urethra length. An estimated 150 million cases of UTIs are reported worldwide annually with the highest incidence in the developing world^{1,2}. These infections account for a significant morbidity and high medical costs, on part of patients as well as institutions/hospitals, especially in elderly males and females of all age groups³. Clinically, patients suffering from UTIs may present with a wide variety of symptoms. Most of the UTIs are asymptomatic or may start with some form of mild symptoms and then gradually increase in severity ending with cystitis leading to severe septicemia⁴. Urinary tract infections are most commonly caused by gram-negative bacteria, but gram-positive bacteria may also cause it in certain conditions. Few cases of UTIs are also caused by certain medically important fungi, which are commonly detected by their non-responsive nature to antibiotics. Gram-negative bacteria are the most common bacterial pathogens in such infections where *Escherichia coli* being the major etiological agent among gram-negative bacteria, followed by *Klebsiella pneumoniae*^{5,6}. *Escherichia coli*, an important pathogen alone accounts for approximately 80-90% of UTI. Around 30-50% of community acquired and nosocomial urinary tract infections are caused by *E.coli*, which shows the importance of this pathogen⁷. Patients presenting with symptoms of urinary tract infections are usually prescribed empirical antibiotic treatment in our set up. Culture and sensitivity testing is usually not prescribed as it takes a few days to show the causative organism and its antibacterial susceptibility results. The empirical antibiotic prescription leads to development of resistance in these pathogens^{8,9}. Recent data suggest an increasing trend of antibiotic resistance among

uropathogens isolated from urine culture¹⁰. The antibiotic susceptibility pattern of uropathogens, especially gram-negative rods also vary according to different healthcare settings and geographical locations^{11,12}. Not much work has been done in this regard in the southern Punjab region of Pakistan. In the present study, we report the prevalence of gram negative bacilli among pathogens causing urinary tract infections along with their antimicrobial susceptibility profile, isolated from both indoor and outdoor patients of this tertiary care hospital of southern Punjab region of Pakistan.

METHODOLOGY:

Mid-stream urine samples were collected from all patients in sterile wide mouthed containers under aseptic conditions. Urine specimens were then inoculated on cysteine lactose electrolyte deficient (CLED) agar using a semi-quantitative strip method (Abtek Biologicals Ltd, Liverpool) and applying 0.2 µl per strip. The inoculated plates were then aerobically incubated at 37°C for 18-24 hours. Gram-negative rods (more than 20 colonies were considered significant) were provisionally identified by colony morphology and Gram's staining. Biochemical reactions were used for the final identification of bacterial species. For antimicrobial susceptibility testing, Mueller-Hinton agar (Oxoid, UK) was carefully inoculated with the test organism (0.5 McFarland standards), and a semi-confluent growth was obtained. Then appropriate antibiotic discs were applied on this MH Agar. Following overnight incubation in air at 35°C ±2, zone diameters were measured and interpreted as per CLSI guidelines¹³. The antibiotic discs and their concentration used were Nitrofurantoin (300µg), Imipenem (10µg), Sulbactam-cefoperazone (30/10) µg, Gentamicin (30µg), and Ciprofloxacin (5µg). Bacterial isolates were then classified as resistant, intermediate sensitivity or totally sensitive to the above antibiotics based on CLSI guidelines.

RESULTS:

Out of 1703 urine specimens submitted for culture and drug susceptibility, 128 (7.51%) yielded significant growth of gram-negative bacilli and *E.coli* was the most frequent one. *E.coli* was found in more than half of all the isolated gram-negative bacilli. The frequencies of all gram-negative bacilli cultured from these samples are shown in the Figure 1.

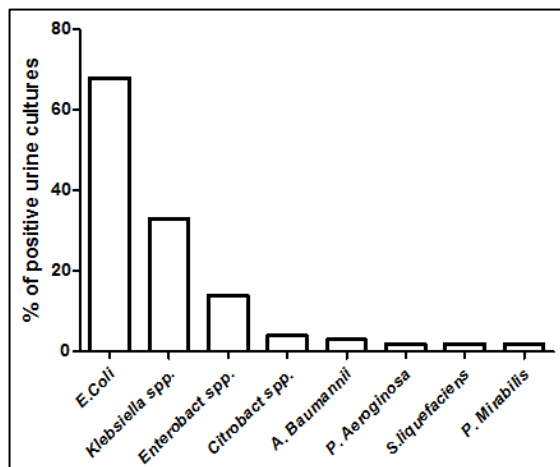


Figure1: Frequency of Gram Negative Bacilli Cultured from Urine Specimens (n=128)

Table 1: Antibiotic Susceptibility Profile of Gram-Negative Bacilli (n=128)

Gram Negative Bacilli	Imepenam (Sensitive) %	Nitrofurantoin (Sensitive) %	Ciprofloxacin (Sensitive) %	Sulbactam-Cefoperazone (Sensitive) %	Gentamycin (Sensitive) %
E. Coli	68	64	38	63	42
Klebsiella Spp.	31	33	20	29	21
Enterobacter Spp.	11	11	2	10	5
Citrobacter Spp.	3	4	2	2	1
Acinetobacter Baumannii	3	1	-	2	1
Pseudomonas Aeruginosa	1	1	-	1	-
Serratia Liquefaciens	2	-	-	1	-
Proteus Mirabilis	2	-	1	2	1
Total	121 (95%)	111 (87%)	63 (49%)	112 (88%)	70 (55%)

DISCUSSION:

Increasing antibiotic resistance is a serious health concern in the present daytime. These resistant bacteria not only increase the cost of treatment for both patients as well as hospitals/institutions but are also a leading cause of treatment failures. The findings of this study have shown that more than half (>53%) of all the culture positive gram-negative isolates were identified as E.coli. Literature search about isolation of E.coli as uropathogens has shown that it is different for different regions of the world. It has shown to vary from 25% (which was shown in a Nigerian study) up to 81%, which was reported in a study from Nepal^{14,15}. Frequency of isolation of E.coli as uropathogens in our study is almost close to the frequency reported from other local and regional studies. A study from Khyber Teaching Hospital Peshawar, conducted in 2002, showed that 57% of all their isolates from culture of urine specimens were E.coli¹⁶. Similarly another study from Ayub Medical College Abbottabad showed that 77% of all urine cultures revealed E.coli¹⁷. Two different

studies conducted at the Armed Forces Institute of Pathology Rawalpindi, have shown frequency of isolation of E.coli as 52% and 63%¹⁸. Another study from India has reported 62% of their uropathogens as E.coli¹⁹. Contrary to these findings, two different studies conducted in India and Nepal in 2009 reported the isolation rate of E.coli to be 36% and 25% of the total culture positive urine samples from two centers respectively^{20,21}. Klebsiella and Enterobacter species were the next most commonly isolated gram-negative bacteria. These findings were consistent with the reports from other studies as well^{22,23}. The results our study has revealed that imipenem, Sulbactam-cefoperazone and Nitrofurantoin are the most effective drugs against gram negative pathogens causing urinary tract infections, as 95% isolates were susceptible to imipenem while more than 85% of the isolates were sensitive to Sulbactam-cefoperazone and nitrofurantoin. Similar susceptibility profile for imipenem has been reported from a study conducted in India, where 95% of urinary isolates were susceptible to carbapenems. Nitrofurantoin

has also shown a similar susceptibility pattern in two previous studies conducted in the Armed Forces Institute of Pathology in 2004 and 2010. According to these two studies 88% and 87% of E.coli were susceptible to nitrofurantoin respectively^{18,24}. Sensitivity pattern of organisms against Sulbactam-Cefoperazone was 88%. Similar sensitivity profile was also reported from a study conducted in India, where 93% of the E.coli isolates were found to be sensitive to this drug¹⁹. On the contrary, in vitro susceptibility results of urinary gram-negative pathogens against Gentamycin and ciprofloxacin were quite low, as almost 50% of all the isolates were sensitive to both these antibiotics. As regards the susceptibility results for ciprofloxacin, our isolates were less resistant compared to another study conducted in Peshawar in 2005/2006 where only 28% of E.coli isolated were susceptible to this compound²⁵. As not much clinical details were provided with the urine specimens submitted for culture and antibiotic susceptibility testing, so clinical correlation could not be done for the in vitro susceptibility test results. However this important laboratory data shall be quite helpful in formulating empirical treatment options for urinary tract infections, in this part of the world. Other laboratories /diagnostic centers should also publish their data regarding urinary pathogens which will help clinicians in recommending empirical treatment for urinary tract infections.

CONCLUSION:

E.coli is the most common gram-negative bacillus causing urinary tract infections (UTIs) in our set up. Although these gram negative urinary pathogens have developed resistance against certain routinely used antibiotics like Ciprofloxacin but still show more than 80% sensitivity to antibiotics like Nitrofurantoin and Carbapenems (imipenem). Carbapenems should be considered as a drug of choice in these infections because these pathogens are 95% susceptible to it.

CONFLICT OF INTEREST: None

FUNDING SOURCES: None

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CONTRIBUTORS

1. **Aamir Hussain** - Concept & Design; Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript
2. **Ihsan Ullah** - Concept & Design; Data Analysis/Interpretation; Drafting Manuscript; Critical Revision; Supervision; Final Approval
3. **Humera Adeeb** - Data Analysis/Interpretation; Drafting Manuscript; Critical Revision; Final Approval
4. **Mubarak Zeb** - Drafting Manuscript; Critical Revision; Final Approval



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