FREQUENCY OF ESBL PRODUCTION AND CARBAPENEM RESISTANCE IN URINARY ISOLATES FROM A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: To determine the frequency of ESBL production, Carbapenem resistance and antimicrobial susceptibility in *Escherichia coli* and *Klebsiella pneumoniae* urinary isolates from a tertiary care hospital.

Materials and Methods: This cross-sectional study was performed from July to December, 2017 in the Department of Microbiology, Armed Forces Institute of Pathology in collaboration with CMH Rawalpindi, AFBMTC and AFIU Intensive Care Units. All urine specimens collected from indoor and outdoor patients yielding growth of *E.coli* and *K. pneumoniae* were processed as per standard protocols. ESBL production was detected by phenotypic confirmatory disc diffusion test as per CLSI guide lines. The antimicrobial susceptibility testing was performed by modified Kirby Baur disc diffusion method and the results were interpreted as per CLSI guidelines. Carbapenem resistance was reconfirmed by breakpoint MICs on VITEK 2 systems-version 08.01.

Results: Among 622 samples, 499 yielded *E.coli* (80.22%) and 123(19.77%) *K.pneumoniae.* Out of 499 *E.coli*, 320 (64%) were ESBL producers and 30(6%) were Carbapenem resistant. Out of 123 *K.pneumoniae* isolates, 73 (59%) were ESBL producers and 28 (23%) were carbapenem resistant. Fosfomycin and Nitrofurantoin were found sensitive in most of isolates with only 3% and 9% resistance respectively.

Conclusion: This study has highlighted the high frequency of ESBL production in uropathogens and significant Carbapenem resistance in *K.pneumoniae* in our setup. Detailed analysis of antibiogram will not only be useful in formulating the antibiotic policy but also strong antimicrobial stewardship program.

Key Words: Carbapenem resistance, ESBLs, *Escherichia coli, Klebsella pneumoniae.*

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INTRODUCTION

Urinary tract infections (UTIs) are second most common infections in the body and accounts for an estimated 150 million cases annually with approximate expenditure of 6 billion dollars globally. Enterobacteriaceae including *Escherichia coli (E.coli)* and *Klebsiella pneumoniae* (*K.pneumoniae)* are considered as most common infectious agents causing UTIs. Wide spread use of antimicrobials including β lactams have resulted in increased antibiotic resistance and appearance of Multi drug resistant organisms (MDROs). ESBLs are enzymes that confer resistance to third and fourth generation cephalospoprins and monobactams; and are widely present in clinical isolates throughout the world [1, 2].

Carbapenems including meropenem and imipenem are considered as last line therapy for treating infections caused by members of Enterobacteriaceae [3, 4]. Widespread use of these drugs has also resulted in emergence of resistance. It is predicted that antibiotic resistance causes 10 million annual deaths throughout the globe and has become a vital threat for mankind [5]. In the United States, approximately 10% of nosocomial infections; are caused by *K. pneumoniae* [6]. More over Carbapenem-Resistant *K.pneumoniae* (CRKP) is a serious cause of health care-associated infections and antibiotic-resistance as listed by WHO and CDC [7]. Infections caused by these agents pose difficulty in effective treatment; thereby, leads to increased mortality rates [8]. Considering these facts, this study endeavour to determine antimicrobial susceptibility and frequency of ESBL production and Carbapenem resistance among *Escherichia coli* and *K.pneumoniae* uropathogens, to help and guide clinicians in selecting appropriate empirical treatment, to decrease not only morbidity but also mortality especially in hospitalized patients.

MATERIALS AND METHOD

This was a cross sectional observational study and was carried at the department of Microbiology AFIP Rawalpindi; in collaboration with CMH Rawalpindi, AFBMTC and AFIU from June 2017 to December 2017. All urine specimens collected from indoor and outdoor patients yielding

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growth of *E.coli* and *K. pneumoniae* processed as per standard protocols were included in study.

The antimicrobial susceptibility testing was performed by modified Kirby baur disc diffusion method as per CLSI guidelines. The tested antimicrobials with potency as per CLSI guidelines were Ampicillin (in *E.coli* only), Amoxicillin-clavulanate, Cefepime, Ceftriaxone, Ceftazidime, Imipenem, Meropenem, Ciprofloxacin, Tazobactam-pipracillin (TZP), Co-triamoxazole, Gentamicin, Nitrofurantoin and Fosfomycin. ESBL production was detected by phenotypic confirmatory disc diffusion test as per CLSI guide lines. Isolates with zone sizes of < 23mm for imipenem and meropenem or both were reconfirmed by Vitek 2 systems-version 08.01 break point MICs. Isolates resistant to either imipenem or meropenem alone or both were considered as Carbapenem resistant. Quality control testing was done by *E.coli* ATCC(25922) and *K.pneumoniae* ATCC (700603). Data was analysed by SPSS 21. Descriptive statistics including frequencies and percentages were analyzed for susceptibility of isolates to different antimicrobials.

RESULTS

In our study, total 622 clinical isolates of *E.coli* and *K. pneumoniae* were yielded from urine cultures. Among these 499 (80.22%) were *E.coli* and 123 (19.77%) were *K. pneumoniae*. Out of 499 *E.coli*, 320 (64%) were found ESBL producers and 30 (6%) were Carbapenem resistant. Meropenem was resistant in 30 (6%) and imipenem in 17(3.4%) isolates. Thirteen isolates that were resistant to meropenem were found intermediate to imipenem. Susceptibility to other antimicrobials (% resistance) is shown in Figure-1.

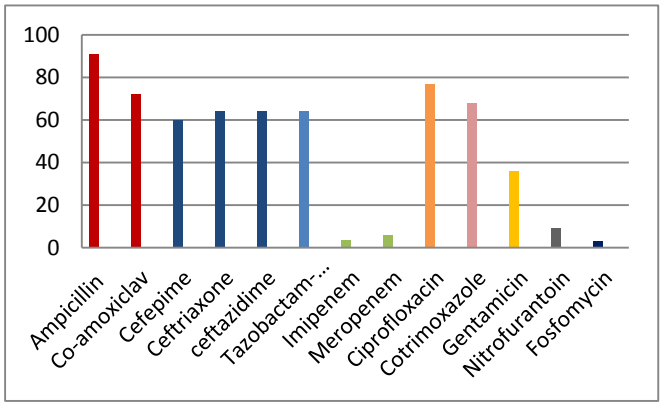

Figure-1: Susceptibility pattern (% resistance) of *E.coli* urinary isolates to different antimicrobials.

Out of 123 *K.pneumoniae* isolates, 73 (59%) were ESBL producers and 28 (23%) were carbapenem resistant. Among Carbapenem resistant isolates, all 28 of the isolates were found resistant to Meropenem and 15 were resistant to Imipenem. Out of 13 showed total isolates resistant to meropenem, having intermediate susceptibility to imipenem. Susceptibility to other anti-microbials (% resistance) is shown in Figure-2.

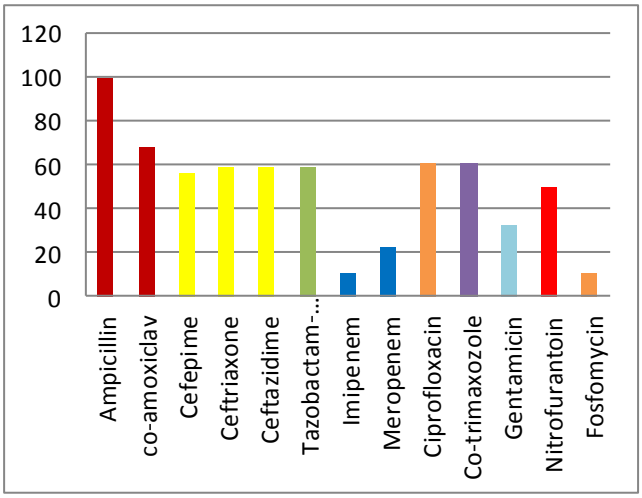

Figure-2. Susceptibility pattern (% resistance) of *K.pneumoniae* urinary isolates to different antimicrobials.

Overall susceptibility (% Resistance) of urinary isolates yielded in our study is shown in Table-1.

Table-1: Overall susceptibility pattern (Resistance percentage) of *E.coli* and *K.pneumoniae* Urinary Isolates (n= 622).

Antibiotics	Percent resistance
Ampicillin	92.7%
Co-amoxiclav	71.38%
Cefepime	59.32%
Ceftriaxone	63.18%
Ceftazidime	63.18%
Tazobactum piperacillin	59.96%
Imipenem	4.8%
Meropenem	9.32%
Ciprofloxacin	81.67%
Co-trimoxazole	66.88%
Gentamicin	37.29%
Nitrofurantoin	17.20%
Fosfomycin	4.98%

DISCUSSION

Major problems with broad spectrum antibiotics therapy resulting in treatment failure commonly occur while treating ESBLs and carbapenemases producing pathogens [9]. In ICU patients overuse or misuse of antibiotics not only increases financial burden but also leads to development of antimicrobial resistance as these patients are more prone to infection and colonization by various pathogens [10].

The resistance to third generation cephalosporins in *Enterobacteiacae* has been increased recently and has been reported by many hospitals worldwide [11,12,13,14]. In our study 64% *E.coli* and 59% *k.pnemoniae* isolates were found ESBLs producers. Like, there has been various research studies conducted in different parts of world and a recent study by Obdsu *et al*. (2018) have shown frequency of ESBLs in *E.coli* ranging from 38.3% to 85.9% in *E.coli* and 45.1% to 93.1% in *K.pneumoniae* [15-20]. The high resistance of *E.coli* to extended spectrum β lactams is probably due to the excessive use, over reliance on third generation cephalosporins and lack of regulated hospital antibiotic policy in our country, as was also reported in other study [21].

In our study, ESBL production was found high in *E.coli* strains, as compared to *K.pneumoniae* strains. The same finding was also reported in many studies [22, 23, 24, 25]. Worldwide as well as geographically, the occurrence of ESBLs among clinical isolates not only vary greatly but are also changing rapidly over the time [23].

In the early 2000, Carbapenem resistant *K.pneumoniae* was a grave threat to public health due to its massive out breaks (26 and 27). At present, it is endemic in most parts of the world. In our study 23% of *K.pneumoniae* and 6% of *E.coli* were found carbapenem resistant. A study conducted by Budek *et al*. in (2014) showed 22% carbapenem resistance each in E.*coli* and *K. pneumoniae*[28]. Another study conducted by Park, liu and furuya (2016) depicted decreasing trend of Carbapenem resistant *K. pneumoniae* in hospital acquired infections from 2006 to 2014. [29].

All the carbapenemase-producing organisms were found highly resistant to most other antibiotic classes, including aminoglycosides and fluoroquinolones in addition to to β-lactam drugs in our study. The co-resistance to non-β-lactam antibiotics might be due to simultaneous presence of other drug resistance mechanisms in addition to ESBL genes as was reported in other study [30]. High prevalence of multidrug resistance and extended spectrum β lactamases in uropathogen *E.coli* was also reported by Ranjini *et al*. (2015) [31]

In our study, nitrofurantoin showed very low resistance in uropathogens. Study conducted by Shukumaran and Kumar, (2017) had also found that nitrofurantoin remained to be least resistant among uropathogens in outdoor and indoor patients.

In our study, fosfomycin resistance was 3% in *E.coli* and 11% in *K. pneumonia*, which is quite comparable to a study by Ito**,** *et al*. (2017) showing 94.9% of the isolates susceptible to fosfomycin [32]. This observation revealed that fosfomycin can be used reliably as empirical treatment option in urinary tract infections.

The limitation of inability to detect cephalosporin and carbapenem resistance at molecular level is beyond the scope of our study.

CONCLUSION

This study has highlighted the high frequency of ESBL production in uropathogens and significant Carbapenem resistance in *K.pneumoniae* in our setup. Detailed analysis of antibiogram will not only be useful in formulating the hospital antibiotic policy but also strong antimicrobial stewardship program.

The significant rate of carbapenemase producing Gram-negative uropathogens showed by this study is extremely worrisome. It is recommended that there is a dire need of a strong antimicrobial stewardship program to be practiced by concerned health care providers with more emphasis on infection control measures to prevent the spread of these multidrug resistant bugs.

AUTHORS CONTRIBUTION

Riffat Bushra: Principal author, paper writing
Wajid Hussain: Data collection, supervision of the project
Gohar Zaman: Approval and proof reading
Umer Khursheed: Literature review
Muhammad Kaleem: Statistical Analysis
Tahir Khadim: Approval and supervision of the overall project.

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