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Prevalence, Antibiotic Susceptibility Testing, Beta-Lactamase Production and *mcr-1* Gene Detection in Uropathogenic *Klebsiella Pneumoniae* Isolated from A Tertiary Care Hospital in Bhopal: A Prospective Study

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Abstract

Introduction: *Klebsiella pneumoniae* (*K pneumoniae*) is an inhabitant of the nasopharynx and gastrointestinal tract and it is capable of causing a variety of infections, including urinary tract infection (UTI), pneumonia, liver abscess and septicemia. UTI in humans can be hospital and community-acquired. UTI should initially be treated with Co-trimoxazole, Nitrofurantoin, 1st generation cephalosporins, and Ciprofloxacin. Still, in India, frequent usage of broad-spectrum antibiotics such as cephalosporins, carbapenems and colistin for getting immediate response has led to resistance to these drugs. *K pneumoniae* possess several different mechanisms of drug resistance for survival. ESBL, MBL and AmpC beta-lactamase production is one of the dominant mechanisms to inactivate the beta-lactam antibiotics. UTI caused by MDR *K pneumoniae* is often treated with carbapenems and colistin. Inappropriate doses and frequent usage of these antibiotics make bacteria resistant therefore it is important to know about the susceptibility of antibiotics against *K pneumoniae* before giving broad-spectrum antibiotics in the local community for the better management of UTI.
Methods: The present study is a prospective study. All clean-catch, mid-stream urine samples were collected in the sample collection centre from the patients suspecting UTI. Semi-quantitative culture method (SQCM) was used to isolate K pneumoniae. SQCM is a routinely used culture method as a diagnostic criterion for patients having a UTI. SQCM indicates the bacterial count present in the urine sample. Firstly, *K pneumoniae* was isolated and identified followed by Antimicrobial-susceptibility testing (AST). After the AST, double disc synergy test checked the production of ESBL, MBL and AmpC beta-lactamase. Lastly, colistin resistance in K pneumoniae was determined by the E-strip method and *K pneumoniae* strains positive by E-strip method were further screened for mcr-1 gene by PCR.
Results: A total of 11740 urine samples were received and processed. 2465 (21%) samples showed significant growth of uropathogens. Out of all pathogens, 332 (13%) were identified as *K pneumoniae*. Other 2133 (87%) pathogens were identified as Enterobacteriaceae members, *Pseudomonas aeruginosa*, *Burkholderia*, *Acinetobacter baumannii*, *Enterococcus*, and *Staphylococcus*. Of all the antibiotics we tested in our study, colistin (87%), carbapenems (78-79%) were the most and penicillin (00-43%) group was the least sensitive. ESBL, AmpC and MBL were 203 (61%), 126 (38%) and 83 (25%) respectively in *K pneumonia*. Colistin resistance was shown by 43 (13%) *K pneumonia* strains and out of these 43, only 08 (19%) strains were positive for mcr-1 gene.
Conclusion: In the present study, *K pneumonia* isolates were most sensitive to

colistin followed by carbapenems. Beta-lactamase enzymes production were demonstrated and the involvement of mcr-1 gene in colistin resistance was found. Implementing urine culture and sensitivity in our hospital is essential for the effective treatment of UTI as clinicians become aware of the antibiotic sensitivity pattern against *K pneumoniae*.

Introduction

Many infectious diseases can occur in humans during a lifetime and Urinary-tract infection (UTI) is one of them. UTI refers to an infection of any part of the urinary system from the urethra to the kidney [1, 2]. Women are more susceptible to UTI than men and nearly 50-60% of all women suffer from an episode of UTI at some point in their life [3, 4]. UTI can be caused by bacteria, fungi, virus and parasites and bacteria are most commonly responsible for causing UTI. The most common bacteria include members of *Enterobacteriaceae, Pseudomonas, Acinetobacter, Enterococcus and Staphylococcus*. Out of all, one of the most common bacteria causing UTI is gram-negative-coccobacillus, facultative-anaerobe, lactose-fermenter, capsulated *K pneumoniae* [4, 5]. Most of the time UTI is treated with broad-spectrum antibiotics like cephalosporins, carbapenems and colistin without performing urine culture and sensitivity in India. This inappropriate and frequent usage of such antibiotics has led to the emergence of antibiotic resistance among uropathogenic bacteria including *K pneumoniae*. *K pneumoniae* shows several mechanisms like beta-lactamases production, biofilm production, altered drug target, efflux pumps and decrease in porins to exhibit antibiotic resistance. Resistance through ESBL, MBL and AmpC beta-lactamase production is one of the common mechanisms. *K pneumoniae* is the most common multi-drug resistant (MDR) bacteria causing UTI. In recent years, colistin has been commonly used to treat UTI with MDR *K pneumoniae*. Frequent usage of colistin is increasing without having antibiotic susceptibility testing of *K pneumoniae* towards it which leads to resistance against colistin, which is a serious issue as colistin is considered a "last resort" antibiotic to combat UTI with MDR *K pneumoniae*. Expression of plasmid-located, mobile-colistin-resistant (*mcr* 1-10) genes are responsible for colistin-resistant in *K pneumoniae*. The emergence and spread of colistin resistance among *K pneumoniae* strains due to the transfer of mcr genes is alarming [5, 6]. The availability of sensitivity patterns of antibiotics help clinicians to choose the appropriate drug to cure UTI. Therefore, the aim of the study were:

1) Isolation of *K pneumoniae* with its antibiotic susceptibility
2) ESBL, MBL and AmpC beta-lactamase detection in *K pneumoniae*
3) mrc-1 gene detection in colistin-resistant *K pneumoniae*

Materials & Methods

The present study is a prospective study. This study occurred in the microbiology laboratory of LN Medical College Bhopal (MP) from January 2021 to January 2023 with approval from the Institutional Ethical (IE) Committee (LNCTU/PhD/Sept-19/RDC/2020/241). All urine samples were received from January 2021 to January 2023 were processed for isolation of *K pneumoniae*. Antimicrobial-susceptibility testing (AST) was performed. Double disc synergy phenotypic methods were used to detect the production of beta-lactamases (ESBL, MBL & AmpC). mcr-1 gene was detected in colistin-resistant *K pneumoniae*.

Study criteria

- Inclusion criteria: All *K pneumoniae* isolates from the urine samples were included.
- Exclusion criteria: Clinical isolates apart from *K pneumoniae* and the presence of multiple pathogenic micro-organisms were excluded. Urine samples received from patients suffering from recurrent UTI were also excluded from the present study.

Study procedure

All clean-catch, mid-stream urine (CCMSU) samples (11740) from in and outpatient departments received in the laboratory were processed as per the standard microbiological procedure for the isolation and identification of *K pneumoniae* [7]. AST for all *K pneumoniae* isolates were done on Mueller-Hinton agar (HiMedia) by disc diffusion (Kirby-Bauer) technique as per the Clinical and Laboratory Standards-Institute guidelines [8].

Extended-spectrum-β-lactamase (ESBL) detection [9, 10]:

- Screening test: Cefotaxime (30µg) and Ceftazidime (30µg) drugs were used for detecting resistant strains for ESBL-production.

- Confirmatory test: The isolates were further tested by the combined disc method. Cefotaxime (30µg), Cefotaxime-clavulanate (30/10µg) and Ceftazidime (30µg), Ceftazidime-clavulanate (30/10µg) were used for confirmation of ESBL producing strains. The zone differences of more than 5mm between Cefotaxime, Cefotaxime-clavulanate and Ceftazidime, Ceftazidime-clavulanate were considered positive.

Metallo-β-lactamase (MBL) detection [9, 10]:
- Screening test: Screening was done with an imipenem disc (10µg) and strains resistant to it were considered for MBL production.
- Confirmatory test: The screened isolates were confirmed by Imipenem (10µg) alone and with Imipenem+EDTA. >7mm zone difference between Imipenem and Imipenem+EDTA was considered positive.

AmpC β-lactamase detection [9, 10]:
- Screening test: Screening was done with Cefoxitin (30 µg) and strains resistant to it were considered for AmpC β-lactamase production.
- Confirmatory test: Cefoxitin alone and with a combination of cloxacillin were used for the confirmation. ≥4mm zone differences between these two were considered positive.

Colistin-resistant detection [11]:
Colistin Ezy MIC™ Strip (MICs 0.016 - 256 mcg/ml) by HiMedia was used for the detection of Colistin resistance and results were interpreted as per manufacture guidelines.

mcr-1 gene detection [12]:
Phenotypic confirmed colistin resistant *K pneumoniae* isolates's DNA was extracted by QIAGEN DNA extraction kit and extracted DNA was screened for presence of mcr-1 gene using PCR method.

Statistical analysis
The p-value was calculated and a p-value of <0.05 was considered statistically significant.

Results
A total of 11740 urine samples were collected from outdoor and indoor patients and processed in the microbiology laboratory. Out of all these samples, 2465 (21%) samples showed significant growth of uropathogens. Of all pathogens, 332 (13%) were identified as *K pneumoniae* with a significant value ($p < 0.05$). Of 11740 urine samples, maximum were obtained from outdoor (7866; 67%) than indoor (3874; 33%) departments. *K pneumoniae* were isolated more from indoor samples (209; 63%) comparative to outdoor samples (123; 37%) as mentioned in table 01. Most of the samples belonged to female gender (7161; 61%) than male (4579; 39%). Majority of *K pneumoniae* were isolated from female patients (219; 66%) than male (113; 34%) as shown in table 02. Maximum samples were received from Obstetrics-Gynecology department and maximum *K pneumoniae* strains were recovered from surgery department as described in table 03. *K pneumoniae* showed maximum sensitivity towards polymyxin (87%) followed by carbapenems (78-79%), aminoglycoside (76-78%), nitrofurantoin (67%), chloramphenicol (62%), cephalosporins (32-49%), tetracycline (41%), fluoroquinolones (33-36%) and least sensitivity was shown by penicillins as concluded in table 04. Occurrence of ESBL, AmpC and MBL were 61% (203), 38% (126) and 25% (83) respectively in *K pneumoniae* as described in table 05. Out of 43 colistin-resistant *K pneumoniae* isolates, only 8 (19%) strains were positive for mcr-1 gene as shown in table 06.

Table 01: Location-Wise Distribution of Urine Samples and *K pneumoniae*

Location	**Samples (11740)**	***K pneumoniae* (332)**
Outdoor	7866 (67%)	123 (37%)
Indoor	3874 (33%)	209 (63%)

Table 2: Gender-Wise Distribution of Urine Samples and *K pneumoniae*

Gender	**Samples (11740)**	***K pneumoniae* (332)**
Female	7161 (61%)	219 (66%)
Male	4579 (39%)	113 (34%)

Table 3: Distribution of *K pneumoniae* Isolates Among Different Departments

S. No.	Departments	Samples	*K pneumoniae*
1.	OBG	3756 (32%)	90 (27%)
2.	Surgery	3287 (28%)	113 (34%)
3.	Medicine	2583 (22%)	63 (19%)
4.	Orthopedics	1057 (9%)	43 (13%)
5.	Pediatrics	822 (7%)	20 (6%)
6.	Others	235 (2%)	03 (1%)
Total		**11740 (100%)**	**332 (100%)**

Table 4: AST Pattern on Uropathogenic *K pneumoniae*

S. No.	Group	Antibiotic	Sensitive (%)	Resistant (%)
1	**Penicillin**	Ampicillin	00	100
2		Amoxycillin-Clavulanic acid	31	69
3		Piperacillin/Tazobactum	43	57
4	**Cephalosporin**	Cefazolin	32	68
5		Cefuroxime	29	71
6		Ceftriaxone	28	72
7		Cefotaxime	28	72
8		Ceftazidime	28	72
9		Cefepime	49	51
10	**Carbapenems**	Imipenem	78	22
11		Meropenem	79	21
12	**Combination**	Cotrimoxazole	28	72
13	**Chloramphenicol**	Chloramphenicol	62	38
14	**Tetracycline**	Tetracycline	41	59
15	**Aminoglycoside**	Amikacin	78	22
16		Gentamycin	76	24
17	**Fluoroquinolones**	Ciprofloxacin	36	64
18		Levofloxacin	33	67
19	**Nitrofuran**	Nitrofurantoin	67	33
20	**Polymyxin**	Colistin	87	13

Table 5: ESBL, AmpC and MBL Production in Uropathogenic *K pneumoniae*

***K pneumoniae* (332)**	**ESBL**	**AmpC**	**MBL**
	203 (61%)	126 (38%)	83 (25%)

Table 6: Presence of mcr-1 gene in Colistin-Resistant *K pneumoniae* isolates

***K pneumoniae* (43)**	***mcr-1* gene present**	***mcr-1* gene absent**
	8 (19%)	35 (81%)

Discussion

In the present study prevalence of uropathogenic *K pneumoniae* was 13%. Mehrishi *et al*. [13] documented 9.78% prevalence in Himachal Pradesh which was a little less to our finding. On the other hand, researchers from Gujarat, India showed 26.79% prevalence which was on higher side of our study [14]. Ding Y *et al*. [15] studied occurrence of *K pneumoniae* from 2011-19 and they mentioned that occurrence was between 6.7-9.6% which was concordant to present results. AST pattern of the uropathogenic bacteria varies from country to country and hospital to hospital as it depends on how frequently antibiotics are being prescribed without considering culture and sensitivity methods hence, we conducted this research in our hospital to provide proper antibiotic sensitivity pattern to our clinicians for the effective treatment of UTI. Out of all used antibiotics to cure UTI, colistin and carbapenem were most sensitive, followed by aminoglycoside and nitrofurantoin against *K pneumoniae* in current research. Mehrishi *et al*. [13], Rakesh *et al*. [16] and Deshpande *et al*. [17] also concluded that the highest sensitivity were shown by carbapenem and aminoglycoside. We observed less sensitivity from penicillins followed by cotrimoxazole and fluoroquinolones whereas Mehrishi *et al*. [13] reported less sensitivity towards penicillins followed by 2nd

generation cephalosporins and fluoroquinolones, Rakesh *et al*. [16] documented penicillins followed by 2nd generation cephalosporins and Aminoglycoside, Deshpande *et al*. [17] mentioned fluoroquinolones and Nitrofuran. In our study, K pneumoniae have produced 61% (203) ESBL, 38% (126) AmpC and 25% (83) MBL. In contrast to the present study, other authors have noticed lower ESBL prevalence in *K pneumoniae* from 33-48% [13, 14, 15]. On other side, Mirza S *et al*. [18] observed a very high prevalence (86%) in Pune. Kolhapure RM *et al*. [19] and Sinha P *et al*. [20] documented only 10% and 24% occurrence of AmpC beta-lactamase respectively which were less to our outcome. Several authors [15, 21] have reported MBL prevalence from 3-40% in *K pneumoniae*, which was similar to our study. 13% *K pneumoniae* strains from our hospital were resistant to colistin. Bhaskar BH *et al*. [22] from Manipal hospital demonstrated 28% resistance to colistin which was at higher side whereas Sodhi K *et al*. [23] reported 9% which was lower to our present research. The presence of mcr-1 gene in *K pneumoniae* is the main reason for colistin resistance and 19% (08) of colistin resistance strains were positive for *mcr*-1 gene in our research. Other 81% (35) strains are colistin resistant due to the presence of one of these other mcr genes (mcr-2, 3, 4, 5, 6, 7, 8, 9, 10). Scientists from Egypt have documented 84% presence of mcr-1 gene in *K pneumoniae* which was quite high [24] whereas MH Gharaibeh *et al*. [25] reported only 1.1% which was much lower than our findings. The strengths of the present study were it revealed the prevalence of *K pneumoniae* as a uropathogen with its antibiotics sensitivity, beta-lactamses production were checked and involvement of mcr-1 gene was checked in coilistin resistant *K pneumoniae*. The research is incomplete without mentioning the limitations of the study therefore we want to state that we did not add other common uropathogens for their prevalence and sensitivity pattern. We did not detect the presence of other genes in colisitn-resistant strains and genes associated with MBL, ESBL and AmpC β-lactamase production because of time and resource restrictions.

Conclusions

The prevalence of uropathogenic *K pneumoniae* was found to be 13%. Hence, it is important to know its antibiogram locally. Since carbapenems have demonstrated good sensitivity, they can be used as the empirical treatment of choice for multi-drug resistant gram-negative uropathogens. Emergence of antibiotic-resistance in *K pneumoniae* causing UTI has increased therefore careful selection of antibiotics after the AST and conservative use of reserve antibiotic (colistin) are very crucial to stop the emergence of resistance. Our research has shown the involvement of mcr-1 gene in colistin-resistant *K pneumoniae* isolated in our hospital. In conclusion, it is of crucial importance that microbiology laboratories perform AST for these antimicrobials and guide the clinicians on the usage of culture and sensitivity in the appropriate choice of antibiotic therapy for the better management of UTI in patients attending our hospital.

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