Original Article

DOI: 10.7860/JCDR/2021/48845.15036

Microbiology Section

A Study on Phenotypic Association between Biofilm and Drug Resistant *Klebsiella* Species Isolated from Urinary Tract Infections

ISHRATH RAZIA RIAZ[1], ANUSHA HINDUPUR[2], AKHIL RAJ THOTA[3], ARULMOZHI BALAKRISHNAN[4], ISWARYA ANBAZHAGAN[5], INDRA PRIYA PALANISAMY[6], PRABU DHANDAPANI[7]

ABSTRACT

Introduction: Urinary Tract Infections (UTI's) are considered to be one of the most prominent infections, worldwide. *Klebsiella* species are the commonest cause of community and hospital acquired UTIs. Biofilm production and hyper-mucous production are the important virulence factors among the pathogenic strains of *Klebsiella* species. Biofilm-associated and Hyper-Mucoviscosity (HMV) variants of *Kiebsiella* spp. is potent enough to easily colonise the urinary tract, develop the invasiveness of infection and mainly contribute in higher resistance to most classes of antibiotics. Understanding the virulence factors associated with Multi Drug Resistant (MDR) *Klebsiella* spp. would aid in estimating the resistance related mortality.

Aim: To identify and comparatively analyse the HMV, biofilm-producing capacity and drug resistance pattern of archived *Klebsiella* spp. isolates from UTI.

Materials and Methods: The present retrospective analysis was done from January to March 2020 on 207 archived *Klebsiella* spp. isolates collected between December 2017 to January 2019. The presumptive identification of *Klebsiella* spp. was done using standard microbiological techniques. Antimicrobial susceptibility test was performed as per Clinical and Laboratory Standards Institute (CLSI) guidelines. *Klebsiella* spp. were tested for biofilm production using microtiter plate method and HMV phenotype by string test. Descriptive statistics and Student's t-test were performed to analyse the data.

Results: A total of 207 *Klebsiella* isolates were used in this study and majority (94.6%) of the isolates were identified as *Klebsiella pneumoniae* subspecies pneumoniae. Out of 207 isolates, 14 isolates (6.8%) were Extensively Drug Resistant (XDR); 141 isolates (68.1%) were MDR; 43 isolates (20.8%) were resistant to one or two class of antibiotics. Biofilm detection assay showed 201 (97.1) out of 207 were strong biofilm producers and 6 (2.9%) were moderate biofilm producers. String test for HMV detection showed only 2 (1%) isolates were positive HMV producers. Among the biofilm producers, majority of the *Klebsiella* isolates were found to be MDR.

Conclusion: Resistance in *Klebsiella* spp. is an evolving problem. Majority of the drug resistant *Klebsiella* isolates used in present study were strong biofilm producers. This study emphasises on the sensible use of last resort drugs to cut down the evolution of resistant strains.

Keywords: Antibiotic resistance, Biofilm production, Hyper-mucoviscosity

INTRODUCTION

The UTI is one of the most common and frequent bacterial infection in both the community and hospital setting worldwide and it is an important health problem in all age groups. UTIs account for more than 7 million visits to doctor's place and complicate over 1 million hospital admissions annually. Community Acquired (CA)-UTIs affect more than 150 million people annually while the prevalence of healthcare associated-UTIs ranges between 1.4- 5.1%. Members of the Enterobacteriaceae family are well-known for the cause of UTIs, predominantly *Escherichia coli (E. coli)* followed by *Klebsiella* and *Proteus* [1,2].

Klebsiella is a widely recognised opportunistic pathogen often acting as agents of bacteremia's, 6-17% UTIs, respiratory tract infections, gastrointestinal tract infections and wound infections, particularly in intensive care unit patients and immunocompromised patients [3]. Success of *K. pneumoniae* as a pathogen associated with both CA infections and hospital acquired infection has been recognised by the World Health Organisation (WHO), due to the major factor that it can confer high-tolerance level of antibiotic resistance [4]. Carbapenems and colistin are the last resort drugs, while fosfomycin and tetracycline are used as alternative drug options and spread of resistance to these antibiotics further increases the concern [5,6]. *K. pneumoniae* isolates have been associated with hyper production of capsular polysaccharide i.e., HMV phenotype which further confers resistance to serum complement and phagocytosis by white blood cells [7]. *K. pneumoniae* is also known for its capability to form biofilms, that supports the bacterial attachment to biotic and abiotic surfaces, protecting antibiotics penetration and reducing its effects [8,9].

Prevalence of virulent and XDR *Klebsiella* is increasing gradually in our setting which is a significant global burden of public health. The increase in resistance of *Klebsiella* spp. is leading to doubtfulness of drug of choice. This emergence of drug resistance can lead to end of antibiotic era. Thus, the aim of the study was to understand, analyse, compare the phenotypic dynamics of antibiotic resistance and virulence in special reference to biofilm and HMV of *Klebsiella* spp. in Chennai, Tamil Nadu, India.

MATERIALS AND METHODS

The present retrospective study included 207archived *Klebsiella* spp. isolates which were collected from UTI patients and maintained at the Department of Microbiology, Dr. ALM PG IBMS, University of Madras, Tamil Nadu, India, were included in the study and were stored and maintained at -20ºC between December 2017 to January 2019. Retrospective analysis of these archived isolates was performed during Janurary 2020 to March 2020.

The isolates were revived in Macconkey agar and the colonies were subjected to gram staining and biochemical tests to confirm the *Klebsiella* spp.

Antimicrobial Susceptibility Testing

All the isolates were subsequently subjected to antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method [10]. Extended Spectrum ß-Lactamase (ESBL) resistance was detected using ceftazidime (30 µg), aztreonam (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg) discs for screening, followed by confirmatory test using ceftazidime (30 µg) and ceftazidime/clavulanic acid (10/5 µg) discs. The imipenem-EDTA Combined Disc Test (CDT) was performed to confirm carbapenem resistance among the study isolates, while AmpC lactamases resistance was detected using cefoxitin (30 µg) [11]. Resistance to fluoroquinolones was detected using discs of nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), ofloxacin (5 µg), and levofloxacin (5 µg). Gentamicin (10 µg) and amikacin (30 µg) discs were used to detect resistance to aminoglycosides [11]. The isolates were also tested against other antibiotics like ampicillin (10 µg), piperacillin (100 µg), co-trimoxazole (1.25/23.75 µg), meropenem (10 µg), cefepime (30 µg), azithromycin (15 µg), fosfomycin (200 µg), tetracycline (30 µg) and chloramphenicol (30 µg). *E.coli* (American Type Culture Collection (ATCC) 25922) and *Klebsiella* (ATCC 700603) were used as control strains. The inhibition zones were measured, and results were interpreted according to the CLSI guidelines [11].

Detection of HMV Phenotype using String Test

The HMV phenotype was determined using a modified string test. *Klebsiella* isolates was cultured on MacConkey agar and the 24 hours culture of *Klebsiella* obtained were touched with an inoculation loop and then the loop was lifted from the agar surface. Strains were defined as HMV if there was formation of a viscous string longer than 5 mm [7].

Biofilm Detection Assays

The biofilm producing capacity of the *Klebsiella* isolates was determined by Congo Red Agar (CRA) method and tissue culture plate method. The qualitative detection of biofilm production was performed by CRA method. Briefly, the *Klebsiella* isolates were inoculated in the CRA medium, composed of brain heart infusion broth (37 gm/L), sucrose (5 gm/L), agar number 1 (10 gm/L) and Congo red dye (0.8 gm/L). Plates were incubated at 37°C for 24 to 48 hours aerobically. Black colonies with a dry crystalline consistency indicated biofilm production [Table/Fig-1].

[Table/Fig-1]: Black crystalline biofilm production by standard control strain of *E. coli* in Congo Red Agar (CRA) medium.

Tissue culture plate method, which is a quantitative, gold-standard method for biofilm detection was performed for all the *Klebsiella* isolates. Briefly, 1 µL of overnight culture was inoculated into 100 µL of fresh Luria-Bertani (LB) broth in each well of a 96-well polystyrene plate. Negative control wells contained uninoculated sterile broth. After 5-24 hours of static incubation at 37°C, non-adherent bacteria were removed by gentle tapping and the wells were washed gently three times with 200 µL of Phosphate Buffer Saline (PBS) (pH 7.2). The bacteria were then stained with 200 µL of 0.1% crystal violet for one hour. The supernatant was discarded, and plates were washed with 200 µL of PBS to remove unattached cells. The biofilm-bound dye was then eluted with 200 µL of 70% acetic acid, and the optical density at 595 nm (OD_{595}) was determined. The suitable positive control was used. Each assay was performed in triplicate. Based on the adherence capabilities, the strains were classified as given in [Table/Fig-2] [9].

Average OD value	Biofilm production
OD≤ODc*	Nil
ODc<OD≤2×ODc	Weak
2ODc<OD≤4×ODc	Moderate
4×ODc<OD	Strong

[Table/Fig-2]: Formula to interpretate biofilm production.
*ODc-OD cut-off-Three standard deviations above the mean OD of the negative control

STATISTICAL ANALYSIS

The data obtained by quantitative biofilm assay was analysed by Student's t-test using the online statistical software, GraphPad QuickCalcs. The results were expressed in the form of mean±SD (Standard Deviation). The p-values <0.05 were considered to be statistically significant.

RESULTS

Bacterial strains: Among the 207 *Klebsiella* isolates, 196 (94.6%) isolates were *Klebsiella pneumoniae* subspecies pneumoniae, 2 (1.0%) isolates were *Klebsiella oxytoca*, 2 (1.0%) isolates were *Klebsiella aerogenes* and 7 (3.4%) belonged to *Klebsiella pneumoniae* subspecies *ozaenae*.

Drug resistance: Out of 207 isolates included in the study, 14 isolates (6.8%) were XDR; 141 isolates (68.1%) were MDR; 43 isolates (20.8%) were resistant to only one or two classes of antibiotic, while 9 isolates (4.3%) were totally susceptible. ESBL production was observed in 159 (76.8%) of the isolates. Amp-C lactamases were detected in 141 (68.1%) of the isolates. By CDT, carbapenem resistance was detected in 74 (35.7%) of the isolates.

Resistance to cotrimoxazole, cefepime and azithromycin was observed in 147 (71%), 159 (76.8%) and 165 (79.7%) of the *Klebsiella* isolates respectively. Highest sensitivity was observed for chloramphenicol 112 (54.1%), followed by fosfomycin 108 (52.2%). The [Table/Fig-3] summarises the antibiotic profile of *Klebsiella* isolates used in this study.

Eighty-one different combinations of drug resistance patterns were observed among the 207 *Klebsiella* isolates included in the study (supplementary). Combination 19 (C19) was the most frequently observed combination with 14 (6.8%) isolates belonging to XDR category, followed by C60 and C67 with 13 (6.3%) and 11 (5.3%) of the isolates belonging to MDR category respectively. The details of other drug resistance combination patterns are shown in [Table/Fig-4].

String test: Out of 207 isolates, only 2 (1.0%) isolates showed the HMV phenotype [Table/Fig-5].

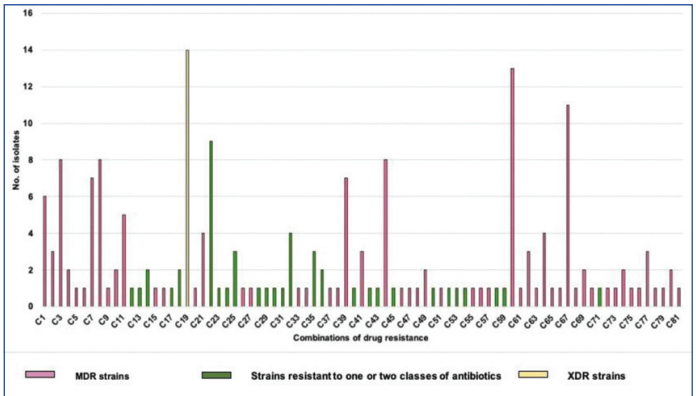
Biofilm detection: In biofilm detection by CRA method, none of the isolates showed black crystalline formation.

In titre plate method, based on the ODc cut-off=0.048, 201 (97.1%) of the *Klebsiella* isolates were found to be strong biofilm producers (mean OD=0.88±1.06) and 6 (2.9%) of the isolates showed moderate biofilm formation (mean OD=0.17±0.02) [Table/Fig-6].

Of the 201 strong biofilm producers, 13 (6.5%) were XDR strains (mean OD=0.43±0.18), 138 (68.6%) were MDR strains, 41 (20.4%)

Antibiotics	Concentration (µg)	Interpretation		
		Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Cephalosporins				
Cefoxitin- 2nd Gen	30	141 (68.1)	9 (4.3)	57 (27.5)
Cefotaxime- 3rd Gen	30	175 (84.5)	13 (6.3)	19 (9.2)
Ceftriaxone- 3rd Gen	30	164 (79.2)	9 (4.3)	34 (16.4)
Ceftazidime- 3th Gen	30	159 (76.8)	9 (4.3)	39 (18.8)
Cefepime- 4th Gen	30	159 (76.8)	5 (2.4)	43 (20.8)
Carbapenems				
Meropenem	10	108 (52.2)	22 (10.6)	77 (37.2)
Imipenem	10	74 (35.7)	44 (21.3)	89 (43.0)
Fluoroquinolones				
Nalidixic acid- 1st Gen	30	155 (74.9)	4 (1.9)	48 (23.2)
Ciprofloxacin- 2nd Gen	5	157 (75.8)	15 (7.2)	35 (16.9)
Norfloxacin- 2nd Gen	10	153 (73.9)	5 (2.4)	49 (23.8)
Ofloxacin- 2nd Gen	5	146 (70.5)	11 (5.3)	50 (24.2)
Levofloxacin- 2nd Gen	5	149 (72.0)	7 (3.4)	51 (24.7)
Aminoglycosides				
Gentamycin	10	102 (49.3)	26 (12.6)	79 (38.2)
Amikacin	30	111 (53.6)	19 (9.2)	77 (37.2)
Penicillin's				
Ampicillin	10	198 (95.7)	4 (1.9)	5 (2.4)
Piperacillin	100	141 (68.1)	29 (14.0)	37 (17.9)
Other antibiotics				
Ceftazidime/ Clavulanic acid	10.0/5.0	163 (78.74)	0	44 (21.25)
Aztreonam	30	149 (71.98)	12 (5.79)	46 (22.22)
Azithromycin	15	165 (79.71)	2 (0.96)	40 (19.32)
Tetracycline	30	127 (61.35)	5 (2.41)	75 (36.23)
Chloramphenicol	30	86 (41.54)	9 (4.34)	112 (54.10)
Trimethoprim Sulfamethoxazole (Co-trimoxazole)	1.25/23.75	147 (71.01)	3 (1.44)	57 (27.53)
Fosfomycin	200	85 (41.06)	14 (6.76)	108 (52.17)

[Table/Fig-3]: Antibiogram of *Klebsiella* isolates included in this study.



[Table/Fig-4]: Different combinations of drug resistance observed among the study isolates.

isolates were resistant to either one or two classes of antibiotics only (mean OD=0.44±0.20) and 9 (4.5%) of the *Klebsiella* isolates were susceptible to all the antibiotics (mean OD=0.43±0.19). Among the six moderate biofilm producing strains, 1 (16.7%) isolate was found to be XDR (mean OD=0.19±0) and 3 (50%) strains belonged to MDR category (mean OD=0.16±0.02) 2 (33.3%) isolates were resistant to either one or two classes of antibiotics only.

On analysing the OD values of XDR (mean OD=0.41±0.18) and MDR (mean OD=0.44±0.2) *Klebsiella* isolates, no significant variation was observed (p=0.5529). Similarly, there was a very little

[Table/Fig-5]: Viscous string denoting Hyper-Mucoviscosity (HMV) of the *Klebsiella* strain.

OD value	Biofilm production level	Biofilm producers n (%)
ODc cut-off - 0.048		
>0.048 to ≤0.096	Weak	0/207
>0.096 to ≤0.192	Moderate	6/207, (2.9%)
>0.192	Strong	201/207, (97.1%)

[Table/Fig-6]: Biofilm production of the *Klebsiella* strain by titre plate method.

variation between the OD values of XDR and susceptible (mean OD=0.43±0.19) strains (p=0.8015).

DISCUSSION

In the present study, the biofilm forming ability of the *Klebsiella* isolates with respect to antibiotic resistance was analysed, owing to the rapid emergence of MDR and XDR *Klebsiella* strains. In this study, majority of the *Klebsiella* isolates obtained from urine samples were identified as *K. pnuemoniae*. The result of this study was in line with other studies that have documented *K. pnuemoniae* as the predominant cause of UTI:3.8% of *K. pnuemoniae* isolates vs 0.7% of other *Klebsiella spp.* in Nepal [12]; 8.78% vs 5% in Pakistan [13] and 65.5% vs 34.5% in Ghana [14]. Mohammed MA et al., reported that *K.pneumonia* (16.3%) was the only *Klebsiella spp.* identified among 160 culture positive urine samples [15]. *K. oxytoca* as a cause of bacterial UTI is being frequently reported from other studies [14-17]. In the present study, 1% of the *Klebsiella* isolates were identified as *K.oxytoca*. In studies from Iraq and Zimbabwe, *K.oxytoca* was identified in 11% and 7% of the *Klebsiella* strains obtained from urine samples respectively [16,17]. In another study, *K.oxytoca* accounted for 38% of the *Klebsiella*-associated UTI [14]. In a study by Mohammed MA et al., *K.pneumoniae* subsp. *pneumoniae* (16.3%) was the most frequently detected *Klebsiella* species in urine samples, which was similar to present study. Also, in this study, *K.oxytoca* was identified in 2.5% of 160 bacterial species isolated from urine samples [15].

In this study, majority of the *Klebsiella* isolates were sensitive to chloramphenicol, followed by fosfomycin and imipenem. However, contrasting results have been documented in other studies. While *Klebsiella* strains showed maximum sensitivity to aztreonam, levofloxacin and piperacillin in a study from Turkey, another study from Russia found that all the urinary *K. pneumoniae* strains were sensitive to meropenem, amikacin and ciprofloxacin [18,19]. In line with present study findings, a study from North India found that the *Klebsiella* isolates were sensitive to fosfomycin and carbapenem group [20]. In South India, it was reported that the urinary *K. pneumoniae* isolates were sensitive to amikacin, ceftazidime and ceftriaxone [21].

High resistance was shown by the *Klebsiella* isolates used in this study to the commonly prescribed antibiotics like ampicillin, cotrimoxazole

and ciprofloxacin. Similar results have been documented in other studies [21-23]. This could be due to the fact that these antibiotics have been used as first line drugs for UTI for many years, which has resulted in greater resistance in the community.

ESBL producers are more often implicated in UTIs, with prolonged duration of hospital stay, increased cost of hospitalisation and high mortality rate associated with ESBL-producing *Klebsiella* spp [24]. In present study, ESBL production was seen in 76.8% of the *Klebsiella* isolates which was very high when compared to other studies [21,25,26].

Carbapenems are the first choice of treatment for infections caused by *Klebsiella* strains producing ESBL and Amp-C enzymes [27-29]. The emergence of carbapenem-resistant *K. pneumoniae* has become a global threat. The results of this study showed that 35.7% of the *Klebsiella* isolates were carbapenem resistant, which was inconsistent with a previous study from South India [21].

String test revealed that only two isolates belonged to the HMV phenotype. This was in contrast to the results obtained by Vuotto C et al., wherein none of the *Klebsiella* spp isolates were classified as HMV, despite producing moist mucoid colonies [30].

It has been reported that the CRA method has very poor sensitivity in detecting biofilm when compared with the tissue culture plate method [8]. Similarly, in the present study none of the isolates were found to produce exopolysaccharide by CRA, while all the 207 isolates were found to be biofilm producers by tissue culture plate method. CRA method was not recommended for biofilm detection by Knobloch JK et al., in their study [31].

Biofilm producing organisms are considered to be intrinsically more resistant to antimicrobial agents. In the present study, 97.1% of the *Klebsiella* spp isolates were strong biofilm producers. However, no significant association was found between antibiotic resistance and biofilm forming ability, contradicting the results of previous reports [30-32]. In support of present study findings, Nirwati H et al., observed no association between *K. pnuemoniae* MDR strains and biofilm production based on statistical analysis [33].

This study emphasises on the judicious use of last line drugs and provides adequate knowledge about the current trends in susceptibility patterns of urinary *Klebsiella* spp. isolates and also confers in-depth phenotypic correlation between drug resisting and biofilm producing capacity among urinary *Klebsiella* spp. isolates.

Limitation(s)

The present study focused on the phenotypic correlation in association in depth without any molecular analysis and a detailed molecular level research has to be conducted further to understand insights of the biofilm-associated antimicrobial resistance.

CONCLUSION(S)

In this study, majority of the *Klebsiella* strains were resistant to most classes of the drugs and resistance profile was found to be high with ESBL production. In this study, there was high incidence of MDR *Klebsiella* spp and majority of MDR *Klebsiella* isolates were strong biofilm producers. Hence, biofilm may be one of the important factors for the drug resistance among the *Klebsiella* species causing UTI.

REFERENCES

[1] Kalal BS, Nagaraj S. Urinary tract infections: A retrospective, descriptive study of causative organisms and antimicrobial pattern of samples received for culture, from a tertiary care setting. Germs. 2016;6(4):132-38.
[2] Tabak YP, Sung AH, Ye G, Vankeepuram L, Gupta V, McCann E. Attributable clinical and economic burden of carbapenem-non-susceptible gram-negative infections in patients hospitalised with complicated urinary tract infections. J Hosp Infect. 2019;102(1):37-44.
[3] Forbes BA, Sahm DF, Weissfeld AS. Study Guide for Bailey and Scott's Diagnostic Microbiology-E-Book. Elsevier Health Sciences; 2016 Jun 30.
[4] Mendelson M, Matsoso MP. The World Health Organization global action plan for antimicrobial resistance. SAMJ: South African Medical Journal. 2015;105(5):325.
[5] Sherry N, Howden B. Emerging gram-negative resistance to last-line antimicrobial agents fosfomycin, colistin and ceftazidime-avibactam–epidemiology, laboratory detection and treatment implications. Expert Rev Anti Infect Ther. 2018;16(4):289-306.
[6] Sahoo RK, Das A, Sahoo S, Gaur M, Rao EV, Subudhi E. The first report of colistin–carbapenem resistance in *Klebsiella pneumoniae* ST70 isolated from the pediatric unit in India. Braz J Microbiol. 2020;51(1):01-03.
[7] Mohammed ES, Flayyih MT. Detection of *rmpA* and *magA* genes and hypermucoviscosity phenotype in *Klebsiella pneumoniae* isolated from water samples in compare with clinical isolates. Curr Res Microbiol Biotechnol. 2018;6(1):1424-30.
[8] Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz J Infect Dis. 2011;15(4):305-11.
[9] Mathlouthi A, Pennacchietti E, Biase D. Effect of temperature, pH and plasmids on in vitro biofilm formation in escherichia coli. Acta Naturae. 2018;10(4):129-32.
[10] Bauer AT. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol. 1966;45:149-58.
[11] Performance Standards for Antimicrobial Susceptibility Testing; Twenty-eighth Informational Supplement. Wayne, Pennsylvania, USA: CLSI; 2018.
[12] Shakya P, Shrestha D, Maharjan E, Sharma VK, Paudyal R. ESBL production among *E. coli* and *Klebsiella* spp. causing urinary tract infection: A hospital-based study. Open Microbiol J. 2017;11:23-30.
[13] Dar SF, Atif MM, Arshad MH, Hayat MF, Dar TF. Urinary tract pathogens and their patterns of resistance to commonly used antibiotics. Rawal Med J. 2020;45(1):22-26.
[14] Quansah E, Amoah Barnie P, Omane Acheampong D, Obiri-Yeboah D, Odarkor Mills R, Asmah E, et al. Geographical distribution of β-Lactam resistance among *Klebsiella* spp. from selected health facilities in Ghana. Trop Med Infect Dis. 2019;4(3):117-30.
[15] Mohammed MA, Alnour TM, Shakurfo OM, Aburass MM. Prevalence and antimicrobial resistance pattern of bacterial strains isolated from patients with urinary tract infection in Messalata Central Hospital, Libya. Asian Pac J Trop Dis. 2016;9(8):771-76.
[16] Mbanga J, Dube S, Munyanduki H. Prevalence and drug resistance in bacteria of the urinary tract infections in Bulawayo province, Zimbabwe. East Afr J Public Health. 2010;7(3):229-32.
[17] AL-Khikani FH, Abadi RM, Ayit AS. Emerging carbapenemase *Klebsiella oxytoca* with multidrug resistance implicated in urinary tract infection. Biomedical Biotechnol Res J. 2020;4(2):148-51.
[18] Demir M, Kazanasmaz H. Uropathogens and antibiotic resistance in the community and hospital-induced urinary tract infected children. J Glob Antimicrob Resist. 2020;20:68-73.
[19] Khaertynov KS, Anokhin VA, Rizvanov AA, Davidyuk YN, Semyenova DR, Lubin SA, et al. Virulence factors and antibiotic resistance of *Klebsiella pneumoniae* strains isolated from neonates with sepsis. Front Med. 2018;5:225-33.
[20] Chooramani G, Jain B, Chauhan PS. Prevalence and antimicrobial sensitivity pattern of bacteria causing urinary tract infection; study of a tertiary care hospital in North India. Clin Epidemiol Glob Health. 2020;8(3):890-93.
[21] Dillirani V, Suresh R. A study on prevalence and antimicrobial resistance pattern of urinary *Klebsiella pneumoniae* in a tertiary care centre in South India. Int J Curr Microbiol App Sci. 2018;7(4):969-73.
[22] Basavaraj CM, Jyothi P. Antimicrobial resistance of *Klebsiella pneumoniae* strains from patients with urinary tract infections in SBMPMC hospital Bijapur, India. Int J Pharm Bio Sci. 2014;5:376-82.
[23] Pai V, Nair B. Etiology and sensitivity of uropathogens in outpatients and inpatients with urinary tract infection: Implications on empiric therapy. Ann Trop Med Public Health. 2012;5(3):181-84.
[24] Schwaber MJ, Navon-Venezia S, Kaye KS, Ben-Ami R, Schwartz D, Carmeli Y. Clinical and economic impact of bacteremia with extended-spectrum-β-lactamase-producing enterobacteriaceae. Antimicrob Agents Chemotherapy. 2006;50(4):1257-62.
[25] Romero ED, Padilla TP, Hernández AH, Grande RP, Vázquez MF, García IG, et al. Prevalence of clinical isolates of *Escherichia coli* and *Klebsiella* spp. producing multiple extended-spectrum β-lactamases. Diagn Microbiol Infect Dis. 2007;59(4):433-37.
[26] El Bouamri MC, Arsalane L, El Kamouni Y, Zouhair S. Antimicrobial susceptibility of urinary *Klebsiella pneumoniae* and the emergence of carbapenem-resistant strains: A retrospective study from a university hospital in Morocco, North Africa. Afr Journal Urol. 2015;21(1):36-40.
[27] Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: A clinical update. Clin Microbiol Rev. 2005;18(4):657-86.
[28] Pitout JD, Laupland KB. Extended-spectrum β-lactamase-producing enterobacteriaceae: An emerging public-health concern. Lancet Infect Dis. 2008;8(3):159-66.
[29] Jacoby GA. AmpC β-lactamases. Clin Microbiol Rev. 2009;22:161-82.
[30] Vuotto C, Longo F, Pascolini C, Donelli G, Balice MP, Libori MF, et al. Biofilm formation and antibiotic resistance in *Klebsiella pneumoniae* urinary strains. J Appl Microbiol. 2017;123(4):1003-18.
[31] Knobloch JK, Horstkotte MA, Rohde H, Mack D. Evaluation of different detection methods of biofilm formation in *Staphylococcus aureus*. Med Microbiol Immunol. 2002;191(2):101-06.

[32] Subramanian P, Shanmugam N, Sivaraman U, Kumar S, Selvaraj S. Antiobiotic resistance pattern of biofilm-forming uropathogens isolated from catheterised patients in Pondicherry, India. Australas Med J. 2012;5(7):344-48.

[33] Nirwati H, Sinanjung K, Fahrunissa F, Wijaya F, Napitupulu S, Hati VP, et al. Biofilm formation and antibiotic resistance of *Klebsiella pneumoniae* isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. BMC Proc. 2019;13(11):01-08.

PARTICULARS OF CONTRIBUTORS:
1. Research Scholar, Department of Microbiology, Dr ALM PG IBMS, University of Madras, Chennai, Tamil Nadu, India.
2. Senior Research Fellow, Department of Laboratory Division, National Institute of Epidemiology, Indian Council of Medical Research, Chennai, Tamil Nadu, India.
3. Postgraduate Student, Department of Microbiology, Dr ALM PG IBMS, University of Madras, Chennai, Tamil Nadu, India.
4. Postgraduate Student, Department of Microbiology, Dr ALM PG IBMS, University of Madras, Chennai, Tamil Nadu, India.
5. Postgraduate Student, Department of Microbiology, Dr ALM PG IBMS, University of Madras, Chennai, Tamil Nadu, India.
6. Postgraduate Student, Department of Microbiology, Dr ALM PG IBMS, University of Madras, Chennai, Tamil Nadu, India.
7. Assistant Professor, Department of Microbiology, Dr ALM PG IBMS, University of Madras, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Prabu Dhandapani,
Dr ALM PG IBMS, University of Madras, Taramani, Chennai, Tamil Nadu, India.
E-mail: bruibms@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Feb 10, 2021
- Manual Googling: May 06, 2021
- iThenticate Software: May 29, 2021 (14%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? No
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Feb 07, 2021**
Date of Peer Review: **Apr 06, 2021**
Date of Acceptance: **May 11, 2021**
Date of Publishing: **Jun 01, 2021**