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Emergence of Drug Resistance among ESBL Producing Klebsiella Species Along With the Detection of Minimum Inhibitory Concentration of Fluoroquinolones: A Hospital Based Study from Dakshina Kannada, Karnataka

Jitendra Chandra Devrari[1], Sateesh K Malkappa[2], Vidya Pai[3]
[1]PhD Scholar, Department of Microbiology, Yenepoya Medical College, Yenepoya University, Mangalore
[2]Associate professor, Department of Microbiology, Yenepoya Medical College, Yenepoya University, Mangalore
[3]Professor and Head, Department of Microbiology, Yenepoya Medical College, Yenepoya University, Mangalore

Original Research Article

***Corresponding author**
Dr Sateesh K Malkappa

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Abstract: Extended-spectrum β-lactamase (ESBL) production is the major resistance mechanism to β-lactam antibiotics in *Enterobacteriaceae*. In addition, emergence of plasmid-mediated quinolone resistance (PMQR) in ESBL-producing isolates has become a global threat for treatment of these infections. The aim of the study was to analyze ESBL production among *Klebsiella* species by combined disc diffusion method and to detect fluoroquinolone resistance by Kirby Bauer disc diffusion method along with the MIC by E- strip method. A total of 200 isolates of *Klebsiella* species were collected from different non repeated clinical samples and Antibiotic susceptibility test with special reference to fluoroquinolone antibiotics was performed by disc diffusion and ESBL production was confirmed by combined disc diffusion method. Among 200 *Klebsiella* isolates, 121(60.5%) were ESBL producers. Out of these 121 ESBL isolates, Multidrug-resistance was observed in 80.9 % isolates. Fluoroquinolone resistance was detected among ESBL isolates of which; 76% were resistant to nalidixic acid and 76% to ciprofloxacin, 74.4% levofloxacin, 76% to sparfloxacin and 76% to moxifloxacin. For carbapenem antibiotics; 55.4 % and 54.5% ESBL isolates were susceptible to imipenem and meropenem respectively. Resistance to Piperacillin and Piperacillin/tazobactam were 90.1% and 72.7%, respectively. Bacterial resistant profile found to be quite high to ESBL production with quinolone resistance in the majority of the *Klebsiella* isolates, which are emerging because of prescription of improper antibiogram schedules.
Keywords: *Klebsiella species*, ESBL, Fluoroquinolone resistant, Minimum inhibitory concentration.

INTRODUCTION

Antibiotic-resistant *Enterobacteriaceae* have increased significantly from past several decades and it is emerging worldwide which is causing a real health issue that is expensive to treat. *Klebsiella pneumoniae* is one of the most common pathogen associated with drug resistance and can exhibit resistance to multiple antibiotics [1]. *Klebsiella pneumoniae* is an opportunistic pathogen causing several nosocomial infections such as urinary tract infections, pneumonia, septicemia, and soft tissue infections [2]. Extended spectrum β lactamase (ESBL) isolates were first detected in Western Europe in the mid-1980s. Since then, their incidence has been increasing steadily. ESBLs are able to hydrolyze 3 and 4 generation cephalosporins and monobactam. ESBL producing strains are inhibited by β-lactamase inhibitors (clavulanic acid, sulbactam and tazobactam) [3]. *β-l*actam antimicrobial agents represent the most common treatment for bacterial infections and continue to be the leading cause of resistance to *β*-lactam antibiotics among Gram-negative bacteria worldwide. The persistent exposure of bacterial strains to a multitude of *β*-lactams has induced dynamic and continuous production and mutation of *β*- lactamases in these bacteria, expanding their activity even against the newly developed *β*-lactam antibiotics. These enzymes are known as extended-spectrum *β*-lactamases (ESBL) [4]. ESBL producing strains often exhibit multidrug resistance, including resistance to aminoglycosides and fluoroquinolones, limiting the therapeutic options. The typical character of ESBL is their ability to hydrolyse oxyimino-cephalosporins and aztreonam while being inhibited by β-lactamase inhibitors [5]. Fluoroquinolones are broad-spectrum antibiotics that are used to treat several Gram negative and Gram-

positive bacterial infections. Since 1960, fluoroquinolones have become prevalent in the treatment of urinary, respiratory, gastrointestinal, urogenital, intra-abdominal, and skin infections. Quinolones are a group of synthetic antibacterial agents against gram negative bacteria that are widely used in routine clinical practice. Quinolones inhibit the function of bacterial DNA gyrase and topoisomerase IV. First and second generation fluoroquinolones selectively inhibit the topoisomerase II ligase domain or DNA gyrase activity whereas third and fourth generations fluoroquinolones are with more tendency for topoisomerase IV ligase [6]. Aim of this study to determine antimicrobial susceptibility testing among ESBL and Non ESBL *Klebsiella species* and Analysis the fluoroquinolone resistant and their MIC among ESBL producing *Klebsiella* isolates.

MATERIAL AND METHODS

This prospective study was conducted in the Department of Microbiology at Yenepoya Medical College, Mangalore. A total 200 non duplicate *Klebsiella* isolates were obtained from different clinical samples in Microbiology laboratory of Yenepoya Medical College and Hospital after obtaining ethical clearance from the institutional ethics committe. The isolates were identified based on standard biochemical tests.

Antimicrobial Susceptibility Testing

AST were performed by Kirby Bauer disc diffusion method according to CLSI guidelines [7]. Antimicrobial discs (Hi-media laboratories Pvt. Limited, Mumbai) were used which include antimicrobials for screening of ESBL *Klebsiella* species: amikacin (10μg), amoxyclav (30μg), gentamicin (10 μg), cefotaxime (30μg), ceftazidime (30μg), imipenem (30μg), nalidixic acid (30μg), ciprofloxacin (5μg), levofloxacin (5μg), sparfloxacin (5μg), moxifloxacin (5μg), cotrimoxazole (1.25/23.75μg), tetracycline (30μg), nitrofurantoin (300μg). The results were interpreted as per the CLSI 2016 guidelines. *K. pneumoniae* ATCC 700603, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 strains were used as control throughout the study.

Screening of ESBL producing strains for *Klebsiella species*

Clinical and Laboratory Standards Institute has developed screening tests for identifying the ESBL-producing *Klebsiellla* species. According to CLSI guidelines, strains showing zone of inhibition of ≤22mm for ceftazidime, ≤27mm for cefotaxime, and ≤25mm for ceftriaxone were selected for conformational tests of ESBL.

Phenotypic Confirmatory Disc Diffusion Test (PCDDT) for ESBL

ESBL production was confirmed among potential ESBL producing isolates by phenotypic tests. Lawn culture of the organism was made and 3*rd*-generation cephalosporins ceftazidime (30 μg) disc and ceftazidime clavulanic acid (30/10μg) disc was placed with 25mm apart. An increase of ≥5mm in zone of inhibition for ceftazidime-clavulinic acid compared to ceftazidime was confirmed as ESBL producers. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used for quality control for ESBL tests.

MIC of Fluoroquinolone drugs by E strip

Minimum inhibitory concentration (MIC) of nalidixic acid, ciprofloxacin, levofloxacin, sparfloxacin, moxifloxacin was determined by the E-test method. The discs were obtained from Hi-media laboratories Pvt. Limited, Mumbai. Interpretative criteria used were as per the E-test manufacturer's guidelines and CLSI 2016. *E. coli* ATCC 25922 was used as quality control.

RESULTS

A total of 200 *Klebsiella species* isolates were obtained from different clinical samples in Microbiology laboratory of Yenepoya Medical College Hospital as shown in Table 1. Among 200 *Klebsiella* isolates 121 (60.5%) were ESBL producing while 79 (39.5%) were non ESBL producing. However multidrug-resistance (MDR) was observed in 98 (80.9%) of ESBL producers. Among 121 ESBL producers, 115 were *Klebsiella pneumoniae* and 6 were *K. oxytoca* whereas among Non ESBL producers, 73 were *Klebsiella pneumoniae* and 6 were *K. oxytoca*. Maximum number of ESBL producing *Klebsiella* were isolated from Pus (40.5%), followed by urine (23.1%), Sputum (19%), blood culture (9.1%), Ventilator aspirate (4.2%), body fluid (2.5%) and Endotracheal aspirate (0.8%) and Broncheoalveolar Lavage (0.8%) as shown in table 1. In this *Klebsiella* infection study 50-60 age group was more affected followed by 40-50 and 60-70 age groups and Male female ratio was 6:3 as shown table 2.

Table-1: Sample wise distribution of *Klebsiella* isolates

Sample	ESBL (n=121)	Non ESBL (n=79)	Total
Pus	49 (40.5%)	34 (43%)	81
Urine	28 (23.1%)	12 (15.2%)	40
Sputum	23 (19%)	20 (25.3%)	43
Blood Culture	11 (9.1%)	06 (7.6%)	12
Ventilator aspirate	05 (4.2%)	00	05
Body fluid	03 (2.5%)	04 (5.1%)	07
High vaginal swab	00	03 (3.8%)	03
Endotracheal aspirate	01 (0.8%)	00	01
Broncheoalveolar Lavage	01 (0.8%)	00	01
Total	**121**	**79**	**200**

Table-2: Age and Gender wise distribution of Culture positive isolates

Age (In Years)	ESBL (n=121)		Non ESBL (n=79)		Total
	Male	Female	Male	Female	
0-10	06	01	01	00	08
11-20	01	02	03	00	06
21-30	08	05	03	05	21
31-40	07	04	12	05	28
41-50	12	10	10	05	37
51-60	20	10	13	09	52
60-70	19	08	07	03	37
Above 70	08	00	02	01	11
Total	**81**	**40**	**51**	**28**	**200**

Highest percentage of ESBL producing *Klebsiella spp*. is obtained from medicine followed by surgery, orthopedics and urology ward as shown in table 3. Antibiotic susceptibility pattern of ESBL and Non ESBL isolates is shown in table 3. Among the antibiotic sensitivity pattern of the ESBL isolates revealed that 55.4%, 54.5% of the isolates were sensitive to imipenem and meropenem respectively, 37.2% were sensitive to amikacin, 35.5 % were sensitive to gentamicin. High resistance was seen for Piperacillin (90.1%), amoxyclav (87.6%), nalidixic acid (76%), ciprofloxacin (75.2%), levofloxacin (74.4%), sparfloxacin (76%), moxifloxacin (76%), co-trimoxazole (69.4%), tetracycline (71.1%) and nitrofurantoin (84%) as shown in Table 4. Among the antibiotic sensitivity pattern of the Non ESBL isolates revealed that Amikacin (78.5%), Meropenem (77.2%), tetracycline (78.5%), cotrimoxazole (78.5%) were highest sensitive followed by nalidixic acid (72.1%), ciprofloxacin (75.9%), levofloxacin (77.2%), sparfloxacin (77.2%), moxifloxacin (77.2%), Piperacillin/Tazobactam (72.2%) and gentamycin (73.4%) as shown in Table 4.

Table-3: Distribution of ESBL producing according to ward in hospital

Wards	ESBL	Non ESBL	Total
Medicine	49	36	85
Surgery	37	23	60
Orthopedic	11	06	17
Urology	09	04	13
Nephrology	07	05	12
OBG	01	03	04
Oncology	02	01	03
Pediatric	01	01	02
Medical ICU	02	00	02
Surgical ICU	02	00	02
Total	**121**	**79**	**200**

MIC of fluoroquinolone resistant (FQR) among ESBL producing *Klebsiella* isolates

92 fluoroquinolone resistant ESBL producing *Klebsiella* isolates- MIC of 88 FQR were resistant to all FQ drugs with at maximum MIC value such as nalidixic

acid >256µg/ml, ciprofloxacin >32µg/ml, levofloxacin>32µg/ml, sparfloxacin >32µg/ml and moxifloxacin>32µg/ml, whereas 4 (4.3%) isolates had MIC of levofloxacin below 12 µg/ml i.e. 2 isolates had MIC at 12 µg/ml, one had at 8 µg/ml and one had at 2 µg/ml.

Table-4: Antimicrobial Susceptible pattern of ESBL and Non ESBL producing Klebsiella species

Antibiotics (µg)	ESBL *Klebsiella species* (n=121)			Non ESBL *Klebsiella* (n=79)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Amikacin(10)	45 (37.2%)	02 (1.6%)	74 (61.2%)	62 (78.5%)	-	17 (21.5%)
Gentamicin (10)	43 (35.5%)	03 (2.5%)	75 (62%)	58 (73.4%)	-	21 (26.6%)
Amoxyclav (30)	13 (10.7%)	02 (1.6%)	106 (87.6%)	40 (50.6%)	-	39 (49.4%)
Piperacillin (100)	12 (9.9%)	-	109 (90.1%)	53 (67%)	-	26 (33%)
Piperacillin/Tazobactam (100/10)	32 (26.4%)	01 (0.8%)	88 (72.7%)	57 (72.2%)	-	22 (27.8%)
Imipenem (10)	67 (55.4%)	-	54 (44.6%)	61 (77.2%)	-	18 (22.8%)
Meropenem (10)	66 (54.5%)	-	55 (45.5%)	62 (78.5%)	-	17 (21.5%)
Ceftazidime (30)	-	-	121 (100%)	79 (100%)	-	00
Cefotaxime (30)	-	-	121 (100%)	49 (62%)	-	30 (38%)
Cefepime (30)	07 (5.8%)	-	114 (94.2%)	54 (68.4%)	-	25 (31.6%)
Nalidixic acid (30)	22 (18.2%)	07 (5.8%)	92 (76%)	57 (72.1%)	01 (1.3%)	21 (26.6%)
Ciprofloxacin (5)	22 (18.2%)	07 (5.8%)	92 (76%)	60 (75.9%)	01 (1.3%)	18 (22.8%)
Levofloxacin (5)	29 (24%)	02 (1.6%)	90 (74.4%)	61 (77.2%)	-	18 (22.8%)
Sparfloxacin (5)	28 (23.2%)	01 (0.8%)	92 (76%)	61 (77.2%)	-	18 (22.8%)
Moxifloxacin (5)	28 (23.2%)	01 (0.8%)	92 (76%)	61 (77.2%)	-	18 (22.8%)
Co-trimoxazole (25)	37 (30.6%)	-	84 (69.4%)	62 (78.5%)	-	17 (21.5%)
Tetracycline (10)	35 (28.9%)	-	86 (71.1%)	62 (78.5%)	-	17 (21.5%)
Nitrofurantoin (300) (only for urine Sample)	03 (12%)	01 (4%)	21 (84%)	08 (72.7%)	01 (9.1%)	02(18.2%)

STATISTICAL ANALYSIS

Descriptive statistics was used for analysis. Proportions were used to study the resistance pattern of *Klebsiella* and variables were expressed as percentages. All the data were expressed as table's diagrams.

DISCUSSION

Infection caused by *Klebsiella species* is the second most common cause of hospital acquired infection in India and other countries. In recent years, a significant increase in ESBL producing *Klebsiella species* was reported worldwide. In the present study, the prevalence of ESBL producing *Klebsiella* is 60.5 %. Another study by Arijit Bora *et al*. also had results in accordance with this study which showed 67.2 % ESBL producing *Klebsiella* infection [8]. In the present study, the highest percentage of ESBL producing Klebsiella was obtained from pus (40.5%) followed by urine (23.1%), sputum (19%) and blood culture (9.1%) samples.

Present study showed that the ESBL producing *Klebsiella spp*. is also resistant to all fluoroquinolones used in this study i.e. upto 74.4% to 76% which is higher than a study done by Namratha W Nandihal *et al.* which had only 38.5% resistance for quinolones [9]**.** Carbapenems are often considered to be the last line of effective treatment available for infections caused by MDR *Enterobacteriaceae*. In the present study 55.4% and 54.5% isolates were sensitive to Imipenem and

Meropenem respectively, whereas another study done by Vemula Sarojamma *et al.* found that the 84% isolates were sensitive to Imipenem [4]. Most of these ESBL producers were multidrug resistant with a high level of resistance to more than three groups of antibiotics. In this study, we found that 80.9 % *Klebsiella spp*. were MDR and simultaneously resistant to fluoroquinolones i.e. 74.4% to 76 % similarly a study done by Neetu Sharma *et al*. also showed that 67 % were MDR *Klebsiella* [10]. In a study done in Iran by Fereshteh Raei *et al.* 2014 showed resistance to nalidixic acid, ciprofloxacin and levofloxacin i.e. 61.9%, 65.2% and 52.1% respectively which is nearly similar to our study i.e. nalidixic, ciprofloxacin and levofloxacin were 76%, 76%, and 74.4% [11]. However another study done by Devjyoti Majumdar *et al*., Lesley R Varughese *et al.*, Nivedita Dasgupta N *et al*. showed higher resistance for Ciprofloxacin as compared to our study which was 92.3%, 100% and 84.5% respectively [12−14].

The rising trend of MDR is seen over the successive years, which is an alarming situation. In this present study, we found that resistance to all tested fluoroquinolone antibiotics and multidrug resistance was found to be significantly higher in ESBL producing *Klebsiella species* as compared to the non-ESBL isolates. However, among the quinolones, the highest rate of resistance was observed for nalidixic, ciprofloxacin, sparfloxacin, moxifloxacin and levofloxacin and there was a significant association between resistances to the ESBL producing isolates.

CONCLUSION

Bacterial resistance profile found to be quite high to ESBL production with quinolone resistance in *Klebsiella* isolates, which are emerging because of prescription of improper antibiogram schedules. So it may be concluded that quinolone resistance with ESBL production is a serious public health problem and requires continuous surveillance, monitoring and revision of the antibiotic use policies and appropriate selection of antibiotics by clinicians.

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REFERENCES

1. Yahya Mohsen SM, Hamzah HA, Imad Al-Deen MM, Baharudin R. Antimicrobial Susceptibility of Klebsiella pneumoniae and Escherichia coli with Extended-Spectrum β-lactamase associated Genes in Hospital Tengku Ampuan Afzan, Kuantan, and Pahang. Malays J Med Sci. 2016; 23(2): 14-20.
2. Peymani A, Farivar TN, Nikooei L, Najafipour R, Javadi A, Pahlevan AA. Emergence of plasmid-mediated quinolone-resistant determinants in Klebsiella pneumoniae isolates from Tehran and Qazvin provinces, Iran. J prev med hyg. 2015; 56: 61-65.
3. Sharma M, Pathak S and Srivastava P. Prevalence and antibiogram of Extended Spectrum β-Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing Escherichia coli and *Klebsiella spp.* Journal of Clinical and Diagnostic Research. 2013; 7(10): 2173-2177.
4. Sarojamma V and Ramakrishna V. Prevalence of ESBL-Producing *Klebsiella pneumoniae* Isolates in Tertiary Care Hospital. ISRN Microbiology. 2011: 1-5.
5. Krishnamurthy V, Vijaykumar GS, Kumar SM, Prashanth HV, Prakash R and Nagaraj ER. Phenotypic and Genotypic Methods for Detection of Extended Spectrum β Lactamase Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Ventilator Associated Pneumonia. Journal of Clinical and Diagnostic Research. 2013; 7(9): 1975-1978.
6. Fabrega A, Madurga S, Giralt E and Vila J. Mechanism of action of and resistance to quinolones. Microbial Biotechnology. 2009; 2(1): 40–61.
7. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: 26th Informational Supplement (M100-S26). Wayne, PA: CLSI, 2016.
8. Bora A, Hazarika NK, Shukla SK, Prasad KN, Sarma JB and Ahmed G. Prevalence of *bla*TEM, *bla*SHV and *bla*CTX-M genes in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from Northeast India. Indian Journal of Pathology and Microbiology. 2014; 57(2): 249-254.
9. Nandihal NW. Profile of Urinary Tract Infection and Quinolone Resistance among *Escherichia coli* and *Klebsiella* species isolates. Int. J. Curr. Microbiol. App. Sci. 2015; 4(7): 749-756.
10. Sharma N, Gupta AK, Walia G and Bakhshi R. A retrospective study of the changing trends of antimicrobial resistance of *Klebsiella pneumoniae* isolated from urine samples over last 3 years (2012-2014). Journal of Natural Science, Biology and Medicine. 2016; 7(1): 39-42.
11. Raei F, Eftekhar F and Feizabadi MM. Prevalence of Quinolone Resistance Among Extended-Spectrum β -Lactamase Producing Uropathogenic *Klebsiella pneumoniae.* Jundishapur J Microbiol. 2014;7(6):10887-5.
12. Majumdar D, Sharan H and Singh DN. Fluoroquinolone Resistant *Escherichia Coli* and *Klebsiella Spp.* in Community-Acquired Urinary Tract Infections in Rural Kanpur, India. Journal of

Clinical and Diagnostic Research. 2012; 6(6): 978-981.

13. Varughese LR and Beniwal V. High quinolone resistance pattern among enteric pathogens isolated from patients with urinary tract infection. Indian Journal of Biotechnology. 2015; 14: 167-171.
14. Dasgupta N, Dhar D, Kharkongor N, Chakravarty A and Bhattacharjee A. Molecular detection of *aac(6')-Ib-cr* among clinical Enterobacterial isolates conferring quinolone resistance:- A study from North east India. Journal of Microbiology and Infectious Diseases. 2016; 6 (3): 97-102.