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Microbiology Section

Risk Factor Analysis in Clinical Isolates of ESBL and MBL (Including NDM-1) Producing *Escherichia coli* and *Klebsiella* Species in a Tertiary Care Hospital

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ABSTRACT

Background: Extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) producing Gram negative organisms are emerging as a worldwide public health concern.

Aim: To elucidate risk factors for infection with ESBL and MBL (also NDM-1) producing *E. coli* and *Klebsiella* spp.

Materials and Methods: A prospective observational study was conducted from November 2010 to March 2012. ESBL production was detected using ESBL E-test, MBL by MBL E-test and NDM-1 by polymerase chain reaction (PCR). Risk factors analysed includes age, sex, clinical specimen, type of infection, duration of hospital stay prior to collection of sample, admitting ward, antimicrobial susceptibility, previous antibiotics used, co-morbid illnesses like diabetes mellitus, immunodeficiency, low birth weight, respiratory/neurological/cardiac/haematological/liver diseases, malignancy, urinary or central venous catheter, ventilatory support, surgical procedures and dialysis.

Statistical analysis: z-test or Fisher's exact test.

Results: *E. coli* – ESBL producing isolates *E. coli* revealed female preponderance, equal incidence of hospital and community acquired infections, mostly from surgical wards, isolated from urine, age group among females >20-30 years and among males >28 days-1 year. They showed high resistance to cephalosporins, monobactam, penicillin but low resistance to carbapenems and aminoglycosides. Co-morbid conditions observed were surgery, urinary catheterisation, haematological disease, ventilatory support, diabetes mellitus and neurological disease. MBL producing strains were mainly from females, surgical wards, (including both NDM-1 isolates), hospital acquired infections, isolated from body fluids (NDM-1 positive), female genital tract specimen and urine (one NDM-1 positive). NDM-1 positive isolates belonged to age groups >5-10 year and >0-28 days and underwent surgery and urinary catheterisation.

Klebsiella spp.- ESBL producing isolates showed female preponderance, hospital acquired infections, from surgical wards, high resistance levels to cephalosporins, fluoroquinolones, monobactam, but low levels to carbapenems, among males isolated from pus in age group >0-28 days and >28 days -1 year and among females from urine in >20-30 years, no significant difference when correlated with risk factors. MBL (NDM-1) producing isolates were mainly from females with age range 0 days to 70 years, mainly admitted to ICU/postoperative wards with urinary catheter in-situ, ventilatory support, surgery, diabetes mellitus, haematological and neurological disease.

Conclusion: Risk factors for infections due to ESBL and MBL producing Gram Negative Bacteria (GNB) should be clearly identified to reduce their spread and to optimise antibiotic use.

Keywords: Antimicrobial susceptibility, Antimicrobial therapy, E-test, Polymerase chain reaction

INTRODUCTION

Bacteria, especially gram negative bacteria, are showing increased resistance to the current antibiotics and drug development programmes seem insufficient to provide therapeutic cover in the near future. Repeated courses of antimicrobial therapy are common in acutely ill, febrile patients, who frequently have endotracheal tubes, urinary catheters and central venous catheters [1]. In combination with host factors, indwelling devices are routes for transmission and colonization of resistant infections [2]. Lengthy or unjustified use of antibiotics helps microbes to undergo mutations which help them to resist antibiotics and become new dominant strains [3].

The incidence of infection due to extended-spectrum β-lactamase (ESBL) producing organisms has increased sharply especially in *Escherichia coli* and *Klebsiella pneumoniae* and also by *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* The growing prevalence of ESBL producing organisms is responsible for increased use of carbapenems. But extensive and sometimes unnecessary use of carbapenems has facilitated the emergence of carbapenem resistant bacteria by production of carbapenem hydrolysing enzyme, metallo-β-lactamase (MBL). MBL producing *Pseudomonas* and *Acinetobacter* isolates are common cause of nosocomial infections but other MBL producing gram negative bacteria like *E. coli* and *Klebsiella* spp. are also emerging as a worldwide public health concern, especially in USA, Greece and Israel [4]. Data from India is currently insufficient.

This study was carried out to elucidate risk factors for infection with ESBL and MBL producing *E. coli* and *Klebsiella* spp.

MATERIALS AND METHODS

A prospective observational descriptive study was carried out in the Department of Microbiology, Lady Hardinge Medical College, New Delhi and associated hospitals from November 2010 to March 2012.

Three hundred and fifty strains each of *E. coli* and *Klebsiella* spp. isolated from various clinical samples of inpatients were collected consecutively. Repeat isolates with the same antibiogram from the same patient were excluded from the study.

Processing of Specimens: Collection, transport and processing of specimens were done as per standard protocol [5,6].

Antimicrobial Susceptibility Tests

1) Disk Diffusion Tests: All identified strains were tested for antimicrobial susceptibility by Kirby-Bauer method on Mueller-Hinton Agar (MHA) medium according to criteria recommended by Clinical and Laboratory Standards Institute (CLSI) [7].

Following antimicrobial agents (Hi-Media) were used for antibiotic susceptibility testing: Amikacin (30 µg), Ampicillin (10 µg), Cotrimoxazole (1.25/23.75 µg), Ertapenem (10 µg), Aztreonam (30 µg), Gatifloxacin (5 µg), Cefazolin (30 µg), Imipenem (10 µg), Cefotaxime (30 µg), Norfloxacin (10 µg), Ceftazidime (30 µg), Tetracycline (30 µg), and Ciprofloxacin (5 µg).

2) Minimum Inhibitory Concentration (MIC) Determination: MIC of isolates which were positive for both ESBL and MBL was determined using microbroth dilution [8]. The isolates which showed multidrug resistance with this method were tested for NDM-1 production.

Detection of ESBL and MBL Production: The strains resistant to Ceftazidime and/or Cefotaxime were tested for ESBL production by ESBL E-test [9]. The strains which showed resistance to imipenem were tested for MBL production by MBL E-test [10].

Detection of NDM-1 Production: DNA was extracted from the strains by heat boil method and this DNA was subjected to single target PCR. Amplified products (250 bp) were analysed by electrophoresis in 2% agarose gels stained with ethidium bromide. *E. coli* NDM-1 positive controls were included. (Thermo Scientific Gene Ruler 1 kb DNA Ladder 250 to 10,000 bp).

Risk factor Analysis: The isolates positive for ESBL production and MBL production were analysed for various risk factors by means of a review of medical charts. Data obtained included age, sex, clinical specimen, hospital or community acquired infection, duration of hospital stay prior to collection of clinical sample, admitting ward, antimicrobial susceptibility, previous antibiotics used, presence of co-morbid illnesses like diabetes mellitus, immunodeficiency, low birth weight, respiratory/ neurological/ cardiac/ haematological/ liver diseases, malignancy, the presence of urinary or central venous catheter, ventilatory support, surgical procedures and dialysis. Nosocomial infections were defined according to Center for Disease Control and Prevention criteria.

STATISTICAL ANALYSIS

The association of variables was compared by the use of z-test or Fisher's exact test as appropriate.

RESULTS

E. coli: 192 two ESBL positive isolates were obtained from 186 patients. Analysis revealed female preponderance (65.1%) and the difference was statistically significant (p<0.001). Significantly higher isolates (p<0.0001) were obtained from males in >28 days -1 year age group and in females in >20-30 years age group [Table/Fig-1].

Irrespective of gender, significantly higher number of isolates (p<0.001) were obtained from urine in both males and females (34.8% and 43.1% respectively). In males the frequency of isolation was followed by pus (30.4%) and blood (14.5%) (p <0.01), in females by female genital tract specimens (26.0%) (p<0.0001) and pus (17.9%) [Table/Fig-2].

[Table/Fig-3] shows the distribution of number of ESBL producing *E. coli* isolates in relation to the duration of stay of the patient in the hospital prior to collection of clinical sample. Isolates showed equal percentage of hospital acquired and community acquired infection [Table/Fig-4]. However, on comparison of 50% of community acquired ESBL producing isolates individually with those isolated at different intervals from patients hospitalized for >48 hours, the observation was statistically significant (p<0.0001). Significantly higher number of isolates were received from surgical wards (55.8%) as compared to medicine (28.6%) and ICU/Post op (15.6%) wards [Table/Fig-5]. The ESBL producing isolates showed an overall high levels of resistance to cephalosporins, monobactam, penicillin, fluoroquinolones and tetracycline (>88%) but low levels to carbapenems and aminoglycosides (<25%) [Table/Fig-6] (p<0.0001).

Cephalosporins, carbapenems (p>0.05), penicillins, metronidazole and aminoglycosides were prescribed to patients infected with ESBL producing *E. coli* for 1-7 days prior to sending the sample. [Table/Fig-7].

Significantly higher number of patients of ESBL positive isolates (p<0.0001) had undergone surgery (61.83%), 58.6% had urinary catheter in-situ, 18.28% had a haematological disease, 8.06% required ventilatory support (p<0.0001), 11.83% had diabetes mellitus and 5.91% had a neurological disease (p<0.01) [Table/Fig-8].

Only 4 isolates were positive by the MBL E-test (all these were ESBL producers). Only 2 isolates showed NDM-1 production (one was strongly positive and one was weakly positive) [Table/Fig-9]. [Table/Fig-10] shows the characteristics of MBL and NDM-1 positive isolates.

Klebsiella spp.***:*** 169 isolates positive for ESBL production by E-test were received from 166 patients. Two isolates were of *K. oxytoca*.

Female preponderance (54.8%) was seen. Amongst males, 21.3% each were in the age group of >0-28 days and >28 days-1 year followed by 12% from the age group of >20-30 years. Among the females, 33% isolates were from the age group >20-30 years followed by 14.3% from the age group >30-40 years [Table/Fig-1].

In males, 32.1% ESBL producing *Klebsiella* spp. isolates were obtained from pus followed by 26.9% from respiratory secretions and17.9% from urine. In females, the frequency of isolation was maximum for urine (42.9%) followed by pus (24.2%) [Table/Fig-2]. The difference in frequency of isolation between males and females

Age	*E. coli*			*Klebsiella* spp.		
	Male	Female	Total	Male	Female	Total
>0d - 28d	5 (7.7)	2 (1.7)	7 (3.8)	16 (21.3)	7 (7.7)	23 (13.9)
>28d - 1y	21 (32.3)	6 (5.0)	27 (14.5)	16 (21.3)	2 (2.2)	18 (10.8)
>1y - 5y	8 (12.3)	11 (9.1)	19 (10.2)	5 (6.7)	8 (8.8)	13 (7.8)
>5y - 10y	6 (9.2)	3 (2.5)	9 (4.8)	2 (2.7)	9 (9.9)	11 (6.6)
>10y - 20y	1 (1.5)	4 (3.3)	5 (2.7)	6 (8.0)	8 (8.8)	14 (8.4)
>20y - 30y	3 (4.6)	53 (43.8)	56 (30.1)	9 (12.0)	30 (33.0)	40 (23.5)
>30y - 40y	5 (7.7)	12 (9.9)	17 (9.1)	5 (6.7)	13 (14.3)	19 (10.8)
>40y - 50y	5 (7.7)	11 (9.1)	16 (18.6)	6 (8.0)	2 (2.2)	9 (4.8)
>50y - 60y	7 (10.8)	12 (9.9)	19 (10.2)	5 (6.7)	6 (6.6)	11 (6.6)
>60y - 70y	3 (4.6)	6 (5.0)	9 (4.8)	4 (5.3)	4 (4.4)	8 (4.8)
>70y - 80y	1 (1.5)	1 (0.8)	2 (1.1)	1 (1.3)	1 (1.1)	2 (1.2)
>80y - 90y	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.1)	1 (0.6)
Total	65 (34.9)	125 (65.1)	186	75 (45.2)	91 (54.8)	166

[Table/Fig-1]: Correlation of isolates with age and sex of patients

Specimen	*E. coli*			*Klebsiella* spp.		
	Male	Female	Total	Male	Female	Total
Blood	10 (14.5)	5 (4.1)	15 (7.8)	13 (16.7)	8 (8.8)	21 (12.4)
Body Fluids	5 (7.2)	4 (3.3)	9 (4.7)	3 (3.8)	0 (0.0)	3 (1.8)
Female Genital Tract Specimens	0 (0)	32 (26.0)	32 (16.7)	0 (0.0)	12 (13.2)	12 (7.1)
Oral Secretions	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.6)	1 (1.1)	3 (1.8)
Pus	21 (30.4)	2 (17.9)	43 (22.4)	25 (32.1)	22 (24.2)	47 (27.8)
Respiratory Secretions	8 (11.6)	6 (4.9)	14 (7.3)	21 (26.9)	9 (9.9)	30 (17.8)
Stool	1 (1.4)	1 (0.8)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
Urine	24 (34.8)	53 (43.1)	77 (40.1)	14 (17.9)	39 (42.9)	53 (31.4)
Total	69 (35.9)	123 (64.1)	192	78 (46.2)	91 (53.8)	169

[Table/Fig-2]: Correlation of isolates with type of specimen and sex of patients

Duration of Stay	*E. coli*	*Klebsiella* spp.
0 - 48 hrs	96 (50.0)	74 (43.8)
>48 hrs - 7 days	53 (27.6)	58 (34.3)
>7 days - 14 days	28 (14.6)	26 (15.4)
>14 days - 21 days	9 (4.7)	7 (4.1)
>21 days - 28 days	1 (0.5)	4 (2.4)
>28 days	5 (2.6)	0 (0.0)
Total	192	169

[Table/Fig-3]: Correlation of isolates with duration of stay in hospital

Type of Infection	*E. coli*	*Klebsiella* spp.
Hospital acquired	96 (50.0)	95 (56.2)
Community acquired	96 (50.0)	74 (43.8)
Total	192	169

[Table/Fig-4]: Correlation of isolates with type of infection

Ward	*E. coli*	*Klebsiella* spp.
ICU/ Post op Ward	30 (15.6)	33 (19.5)
Medicine Ward	55 (28.6)	48 (28.4)
Surgery Ward	107 (55.8)	88 (52.1)
Total	192	169

[Table/Fig-5]: Correlation of isolates with admitting wards

was statistically significant (p<0.01) except for pus, blood and body fluids (p>0.05).

[Table/Fig-3] shows the distribution of number of ESBL producing *Klebsiella* spp. isolates in relation to the duration of stay of the patient in the hospital prior to collection of clinical sample. Both the *K. oxytoca* strains were isolated from patients with stay <48 hours. Therefore, majority of the infections were hospital acquired [Table/Fig-4].

Upon correlating with the admitting wards, significantly higher numbers were received from surgical wards (52.1%) as compared to medicine wards (28.4%) and ICU and postoperative wards (19.5%) (p<0.0001) [Table/Fig-5]. Both the *K. oxytoca* isolates were received from surgical wards.

Irrespective of type of specimen, the ESBL producing isolates showed high levels of resistance to cephalosporins, fluoroquinolones, monobactam, tetracycline and penicillin but low levels to carbapenems [Table/Fig-11].

In patients infected with ESBL producers, penicillins, aminoglycosides and cephalosporins were prescribed upto 7 days prior to sending the sample for investigation [Table/Fig-7] while relatively fewer patients had received antibiotic beyond 7 days and the difference was statistically significant (p<0.0001). No significant difference was observed between ESBL producing and non-producing *Klebsiella* isolates when correlated with co-morbidities and other risk factors [Table/Fig-8].

Twenty one isolates were positive by the MBL E-test (17 were also ESBL producers). Eight isolates were strongly positive for NDM-1 production while 1 was weakly positive [Table/Fig-9]. [Table/Fig-10] shows the characteristics of MBL and NDM-1 positive isolates.

DISCUSSION

Our study detected high incidence of ESBL production amongst *E. coli* and *Klebsiella* spp. although incidence of MBL production was not very high.

E. coli

The present study revealed female preponderance (65.1%), with significantly higher isolates from males in >28 days - 1 year age group and in females > 20 - 30 years. Rodriguez-Bano et al., also found female preponderance (57%) but the median age was 70 years (age range, 15 to 92 years) [11]. However, the study done by Lautenbach et al., showed male preponderance in the median age of 54 years (17-80 years) [12].

Similar to our study, Lautenbach et al., found specimens from urinary tract (51.5%), wound (15.2%), CVP lines (12.1%), respiratory (9.1%) and abdomen (3%) as sites of infection [12]. Rodriguez-Bano et al., isolated ESBL producing *E. coli* from urine (92%) and blood (12%) [11].

In contrast to our study, Lautenbach et al., observed that ESBL positive patients were more likely to have nosocomial infection (97%) and longer hospitalization prior to infection (median 11 days) and did not find any significant difference when hospital locations of case and control patients were compared [12].

Overall high levels of resistance to cephalosporins, monobactam, penicillin, fluoroquinolones and tetracycline (>88%) was seen but low levels to carbapenems and aminoglycosides (<25%) (p< 0.0001). This finding was in conformity with Shobha et al., who detected resistance of ESBL producing *E. coli* as 0% for imipenem, 11.5% for amikacin, 70% for gentamicin, 58% for trimethoprim-sulfamethoxazole, 93.4% for ciprofloxacin, 90% for norfloxacin and 93.4% for nalidixic acid [13]. Similarly Chaudhuri et al., detected very low resistance levels to imipenem (3%) and ertapenem (5%), amikacin (15%) and piperacillin-tazobactam (24%) [14]. Rodriguez-Bano et al., detected low resistant levels to imipenem (0%), amikacin (4%), gentamicin (26%) and high resistance levels to ciprofloxacin (78%), cotrimoxazole (71%) and amoxicillin-clavulanate (52%) [11]. Similarly Jitsurong et al., demonstrated high resistance levels to

Antibiotic	Blood (n=15)	Body Fluids (n=9)	Female Genital Tract Specimen (n=32)	Pus (n=43)	Respiratory Secretions (n=14)	Stool (n=2)	Urine (n=77)	Total Isolates (n=192)
Amikacin	7 (46.7)	2 (22.2)	10 (31.3)	9 (20.9)	3 (21.4)	0 (0)	18 (23.4)	49 (13.3)
Ampicillin	14 (93.3)	9 (100.0)	25 (78.1)	37 (86.0)	13 (92.9)	2 (100.0)	70 (90.9)	170 (88.5)
Aztreonam	15 (100.0)	8 (88.9)	31 (96.9)	41 (95.3)	13 (92.9)	2 (100.0)	76 (98.7)	186 (96.9)
Cefazolin	15 (100.0)	9 (100.0)	32 (100.0)	43 (100.0)	14 (100.0)	2 (100.0)	76 (98.7)	191 (99.5)
Cefotaxime	15 (100.0)	9 (100.0)	32 (100.0)	43 (100.0)	14 (100.0)	2 (100.0)	77 (100.0)	192 (100.0)
Ceftazidime	15 (100.0)	9 (100.0)	32 (100.0)	43 (100.0)	14 (100.0)	2 (100.0)	77 (100.0)	192 (100.0)
Ciprofloxacin	12 (80.0)	8 (88.9)	28 (87.5)	40 (93.0)	11 (78.6)	2 (100.0)	70 (90.9)	171 (89.1)
Cotrimoxazole	14 (93.3)	6 (66.7)	25 (78.1)	32 (74.4)	11 (78.6)	2 (100.0)	57 (74.0)	147 (76.6)
Ertapenem	4 (26.7)	2 (22.2)	2 (6.3)	4 (9.3)	1 (7.1)	1 (50.0)	13 (16.9)	27 (14.1)
Gatifloxacin	12 (80.0)	8 (88.9)	24 (75.0)	37 (86.0)	10 (71.4)	2 (100.0)	58 (75.3)	151 (78.7)
Imipenem	1 (6.7)	1 (11.1)	0 (0)	2 (4.7)	0 (0)	0 (0)	1 (1.3)	6 (3.1)
Norfloxacin	13 (86.7)	8 (88.9)	27 (84.4)	39 (90.7)	12 (85.7)	2 (100.0)	70 (90.9)	171 (89.1)
Tetracycline	13 (86.7)	7 (77.8)	24 (75.0)	36 (83.7)	10 (71.4)	2 (100.0)	72 (93.5)	164 (85.4)

[Table/Fig-6]: Antimicrobial resistance of *E. coli* strains isolated from different specimen

Antibiotic	*E. coli*		*Klebsiella* spp.	
	1-7 Days	>7-14 Days	1-7 Days	>7-14 Days
Amoxicillin-Clavulanic acid	77 (40.1)	5 (2.6)	77 (45.6)	1 (0.6)
Ampicillin	32 (16.7)	0 (0)	13 (7.7)	0 (0)
Piperacillin-Tazobactam	18 (9.4)	3 (1.6)	58 (34.3)	3 (1.8)
Metronidazole	92 (47.9)	14 (7.3)	46 (27.2)	2 (1.2)
Ciprofloxacin	31 (16.1)	9 (4.7)	9 (5.3)	2 (1.2)
Ofloxacin	4 (2.1)	1 (0.5)	5 (3.0)	0 (0)
Levofloxacin	20 (10.4)	0 (0)	19 (11.2)	1 (0.6)
Norfloxacin	12 (6.3)	0 (0)	8 (4.7)	0 (0)
Amikacin	56 (29.2)	7 (3.6)	114 (67.5)	4 (2.4)
Gentamicin	35 (18.2)	7 (3.6)	11 (6.5)	2 (1.2)
Clindamycin	1 (0.5)	0 (0)	0 (0)	2 (1.2)
Cefotaxime	20 (10.4)	0 (0)	25 (14.8)	0 (0)
Ceftriaxone	45 (23.4)	6 (3.1)	34 (20.1)	4 (2.4)
Ceftazidime	2 (1.0)	1 (0.5)	3 (1.8)	0 (0)
Cefixime-Sulbactam	6 (3.1)	0 (0)	1 (0.6)	0 (0)
Cefepime	3 (1.6)	1 (.5)	2 (1.2)	0 (0)
Aztreonam	0 (0)	1 (0.5)	0 (0)	0 (0)
Imipenem	11 (5.7)	2 (1.0)	6 (3.6)	1 (0.6)
Meropenem	8 (4.2)	1 (.5)	17 (10.1)	2 (1.2)
Ertapenem	1 (0.5)	0 (0)	0 (0)	0 (0)
Azithromycin	3 (1.6)	1 (0.5)	3 (1.8)	0 (0)
Tobramycin	0 (0)	0 (0)	1 (0.6)	0 (0)
Colistin	0 (0)	0 (0)	1 (0.6)	1 (0.6)

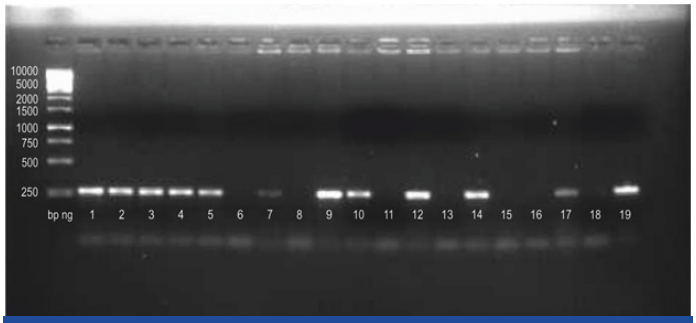
[Table/Fig-7]: Antibiotics taken prior to sending sample

Associated disease/ Risk factor	*E. coli*		*Klebsiella* spp.	
	ESBL positive	ESBL negative	ESBL positive	ESBL negative
DM	22 (11.83)	4 (2.63)	14 (8.3)	14 (7.7)
HIV Positive	4 (2.15)	1 (0.66)	0 (0)	1 (0.6)
Low Birth Weight	3 (1.61)	0 (0)	0 (0)	6 (3.3)
Respiratory Disease	4 (2.15)	2 (1.32)	6 (3.6)	7 (3.9)
Neurological Disease	11 (5.91)	0 (0)	2 (1.2)	5 (2.8)
Cardiac Disease	10 (5.38)	0 (0)	2 (1.2)	2 (1.1)
Chronic Liver Disease	2 (1.08)	0 (0)	0 (0)	4 (2.2)
Haematological Disease	34 (18.28)	2 (1.32)	9 (5.3)	17 (9.4)
Malignancy	13 (6.99)	0 (0)	7 (4.1)	12 (6.6)
Ventilator	15 (8.06)	1 (0.66)	21 (12.4)	26 (14.4)
Surgery	115 (61.83)	6 (3.95)	44 (26.0)	52 (28.7)
Dialysis	3 (1.61)	0 (0)	2 (1.2)	5 (2.8)
Central venous catheter	3 (1.61)	0 (0)	0 (0)	4 (2.2)
Urinary catheter	109 (58.60)	8 (5.26)	52 (30.8)	71 (39.2)

[Table/Fig-8]: Analysis of Co-morbidities and other risk factors with ESBL producing and non-ESBL producing isolates

ampicillin (100%), ceftazidime and cefotaxime (66.7% each) and cotrimoxazole (100%) and low resistance levels to amikacin and imipenem (0%) [15].

With respect to co-morbidities and other variables in present study, Lautenbach et al., also observed that ESBL positive patients were more likely to have urinary catheter (55%) or central venous catheter (58%) in place, less likely to have malignant disease (12%) and more likely to have renal insufficiency (36%) and diabetes mellitus



[Table/Fig-9]: Gel picture of NDM-1 PCR results
bp ng is a molecular weight marker. Last lane (19) is NDM-1 plasmid (positive control). Lane nos. 1,2,3,4,5,9,10.12 and 14 are strongly positive, 7 and 17 are weakly positive, 6,8,11,13,15,16 and 18 are negative. (Lane nos. 1, 6 and 7 represent *E. coli.* rest are Klebsiella)

(36%), had significant greater prior cumulative antibiotic exposure including extended-spectrum cephalosporins, fluoroquinolones, aminoglycosides, cotrimoxazole, vancomycin and metronidazole [12]. Rodriguez-Bano et al., observed that previous antimicrobial agent use (67%) especially use of fluoroquinolones (41%), diabetes mellitus (41%), neoplasia (12%), respiratory disease (10%), chronic liver disease (2%), presence of urinary catheter (22%), surgery (8%), old age and male gender were frequently associated with ESBL-producing *E. coli* infection [11].

In the present study, amongst the MBL producing strains, one each was isolated from body fluid (NDM-1 positive) and female genital tract specimen and two from urine (one was NDM-1 positive). All were females, 3 were admitted in surgical wards (including both NDM-1 isolates) and 1 in medicine ward, all had hospital acquired infections. NDM-1 positive isolates patients belonged to age groups >5-10 year and >0-28 days and underwent surgery and required urinary catheter. This was in contrast to the finding of Deshpande et al., who isolated 9 MBL (NDM-1) positive *E. coli* from respiratory secretion (1), blood (2) and urine (6) and 7 samples were from wards while 2 from ICU [16], and Chakraborty et al., who observed that MBL positive isolates were mainly from male patients in the old age group (61-80 years) and 54.16% patients suffered from diabetes mellitus [17]. However, Kumarasamy et al., isolated NDM-1 producing *E. coli* primarily from community acquired urinary tract infections, pneumonia and blood stream infections. The age range was 4-66 years with a mean of 36 years and a female to male ratio of about two to one [18].

Klebsiella spp.

The present study showed female preponderance (54.8%) with significantly higher isolates from males in the age group of >0 day - 1 year and in females in >20-30 years of age group. Marra et al., did not find any significant age or gender difference between ESBL producing and non-producing *K. pneumoniae* [19]. Lautenbach et al., observed ESBL producers to be significantly younger and more frequently males [12].

Similar to our study, Lautenbach et al., found specimen for urinary tract (51.5%), wound (15.2%), CVP lines (12.1%), respiratory (9.1%) and abdominal (3%) as sites of infection and observed that ESBL positive patients were more likely to have nosocomial infection (97%) and longer hospitalization prior to infection (median 11 days) and did not find any significant difference when hospital locations of case and control patients were compared [12].

Both Marra et al., and Mathur et al., found the proportion of ESBL positive isolates to be highest from ICU in contrast to our study [19,20].

High levels of resistance to cephalosporins, fluoroquinolones, monobactam, tetracycline and penicillin but low levels to carbapenems was seen in this study which was in conformity with Shobha et al., who detected antibiotic resistance of ESBL-

No	Specimen	Admitting Ward	Age Group	Sex	Co-morbidity	Type of Infection	ESBL	NDM-1
E. coli								
1	Urine	Surgery	>5y - 10y	F	Urinary catheter	HA	Positive	Strongly positive
2	Body fluid	Surgery	>0d - 28d	F	Surgery	HA	Positive	Weakly positive
3	Urine	Medicine	>10y - 20y	F		HA	Positive	Negative
4	Female genital tract specimen	Surgery	>20y - 30y	F	Surgery	HA	Positive	Not tested
Klebsiella spp.								
1	Blood	Surgery	>50 - 60y	F		HA	Positive	Strongly positive
2	Blood	Surgery	>60y - 70y	F	Surgery, Ventilator, Urinary catheter	HA	Positive	Strongly positive
3	Pus	Surgery	>30y - 40y	F	Surgery, Urinary catheter, Haematological disease	HA	Positive	Strongly positive
4	Respiratory secretion	ICU/Post-op	>28d - 1y	M	Ventilator, Urinary catheter	HA	Positive	Strongly positive
5	Respiratory secretion	ICU/Post-op	>1y - 5y	M	Surgery, Ventilator, Urinary catheter	HA	Positive	Strongly positive
6	Respiratory secretion	ICU/Post-op	>20y - 30y	F	Ventilator, Urinary catheter, Diabetes mellitus	HA	Positive	Strongly positive
7	Respiratory secretion	ICU/Post-op	>40y - 50y	F	Ventilator, Urinary catheter, Haematological disease	HA	Positive	Strongly positive
8	Respiratory secretion	ICU/Post-op	>20y - 30y	F	Ventilator, Urinary catheter	HA	Positive	Strongly positive
9	Blood	ICU/Post-op	>0d - 28d	M	Ventilator, Urinary catheter	CA	Positive	Weakly positive
10	Pus	Surgery	>20y - 30y	F	Surgery, Urinary catheter	CA	Positive	Negative
11	Pus	Surgery	>20y - 30y	M	Surgery, Urinary catheter, Neurological disease	HA	Positive	Negative
12	Respiratory secretion	ICU/Post-op	>0d - 28d	M		HA	Positive	Negative
13	Respiratory secretion	ICU/Post-op	>0d - 28d	M	Ventilator, Urinary catheter	HA	Positive	Negative
14	Urine	ICU/Post-op	>20y - 30y	F	Surgery, Urinary catheter	HA	Positive	Negative
15	Urine	Surgery	>10y - 20y	F	Urinary catheter	HA	Positive	Negative
16	Female genital tract specimen	Surgery	>30y - 40y	F		CA	Negative	Not tested
17	Pus	Medicine	>50y-60y	M	Diabetes Mellitus	CA	Negative	Not tested
18	Pus	Surgery	>20y - 30y	F	Surgery, Urinary catheter	HA	Positive	Not tested
19	Respiratory secretion	ICU/Post-op	>28d - 1y	F	Ventilator, Urinary catheter	HA	Positive	Not tested
20	Urine	ICU/Post-op	>60y-70y	M	Ventilator, Urinary catheter	HA	Negative	Not tested
21	Urine	ICU/Post-op	>30y - 40y	F		HA	Negative	Not tested

[Table/Fig-10]: Characteristics of MBL producing and NDM-1 positive isolates

producing *Klebsiella* spp. as 0% for imipenem, 14% for amikacin, 69% for gentamicin, 71% for trimethoprim-sulfamethoxazole, 90% for ciprofloxacin, 94% for norfloxacin and 94% for nalidixic acid [13]. Similarly Chaudhuri et al., detected low levels of resistance to imipenem (6%) and ertapenem (20%), amikacin (26%) and piperacillin-tazobactam (41%) [14]. Jitsurong et al., demonstrated high levels of resistance to ampicillin (100%), ceftazidime (100%), cefotaxime (56.3%) and cotrimoxazole (81.3%) and low levels of resistance to amikacin (16.8%) and imipenem (0%) [15].

In the present study, penicillins, aminoglycosides and cephalosporins were prescribed upto 7 days in patients infected with ESBL producing *Klebsiella* prior to sending the sample for investigation ($p<0.0001$). No significant difference was observed between ESBL producing and non-producing *Klebsiella* isolates when correlated with co-morbidities and other risk factors. However, the need for ventilatory support and central venous catheters was more in ESBL producing *Klebsiella* strains as reported by Marra et al., [19]. Lautenbach et al., observed that ESBL positive patients had urinary catheter (55%) or central venous catheter (58%) in place, were less likely to have malignant disease (12%) and more likely to have renal insufficiency (36%) and diabetes mellitus (36%), had significant greater prior cumulative antibiotic exposure including to extended-spectrum cephalosporins, fluoroquinolones, aminoglycosides, cotrimoxazole, vancomycin and metronidazole [12].

The 21 MBL producing isolates were mainly from females (61.9%) ($p<0.001$) isolated from respiratory secretions (8), pus (5), urine (4), blood (3) and female genital tract specimens (1). The 9 NDM-1 positive isolates were mainly from females, obtained from pus (1), blood (3) and respiratory secretions (5) with age range from 0 days to 70 years. This was similar to the finding of Deshpande et al., who isolated 10 MBL (NDM-1) positive *K. pneumoniae* obtained from blood, pus and swab (1 each), urine (3) and respiratory secretions (4) [16] and Chakraborty et al., who observed that MBL positive isolates were mainly from male patients in the old age group (61-80 years) and 54.16% patients suffered from diabetes mellitus [17]. Kumarasamy et al., detected 21.3% of isolates as MBL producing *K. pneumoniae* from community acquired urinary tract infections, pneumonia and blood-stream infections. The age range was 4-66 years (mean 36 years) and female to male ratio of about two to one [16].

In the present study, patients with MBL positive (including NDM-1 positive) isolates were mainly admitted to ICU/postoperative wards similar to Deshpande et al., [16]. We observed that 76.2% of MBL positive patients had urinary catheter in-situ, 47.6% required ventilatory support, 33.3% underwent surgery, 9.5% had diabetes mellitus, 9.5% haematological diseases and 4.8% neurological disease. The respective percentages of NDM-1 positive isolates was 88.9%, 77.8%, 33.3%, 11.1%, 22.2% and 0%. Cagnacci et al., observed that all patients infected with MBL producing *K. pneumoniae* had an underlying disease, had undergone surgery (55.6%), had malignancy (22.2%) and all had nosocomial infections [21].

Antibiotic	Blood (n=15)	Body Fluids (n=9)	Female Genital Tract Specimen (n=32)	Pus (n=43)	Respiratory Secretions (n=14)	Stool (n=2)	Urine (n=77)	Total Isolates (n=192)
Amikacin	10 (47.6)	1 (33.3)	2 (16.7)	13 (27.7)	12 (40.0)	2 (66.7)	13 (24.5)	53 (31.4)
Ampicillin	21 (100.0)	2 (66.7)	11 (91.7)	45 (95.7)	30 (100.0)	3 (100.0)	52 (98.1)	164 (97.0)
Aztreonam	21 (100.0)	3 (100.0)	11 (91.7)	47 (100.0)	28 (93.3)	3 (100.0)	53 (100.0)	166 (98.2)
Cefazolin	21 (100.0)	3 (100.0)	11 (91.7)	47 (100.0)	30 (100.0)	3 (100.0)	53 (100.0)	168 (99.4)
Cefotaxime	21 (100.0)	3 (100.0)	11 (91.7)	47 (100.0)	30 (100.0)	3 (100.0)	53 (100.0)	168 (99.4)
Ceftazidime	21 (100.0)	3 (100.0)	11 (91.7)	47 (100.0)	30 (100.0)	3 (100.0)	53 (100.0)	168 (99.4)
Ciprofloxacin	12 (57.1)	1 (33.3)	9 (75.0)	36 (76.6)	20 (66.7)	2 (66.7)	37 (69.8)	117 (69.2)
Cotrimoxazole	17 (81.0)	2 (66.7)	10 (83.3)	46 (97.9)	24 (80.0)	2 (66.7)	48 (90.6)	149 (88.2)
Ertapenem	4 (19.0)	0 (0)	1 (8.3)	10 (21.3)	6 (20.0)	1 (33.3)	7 (13.2)	29 (17.2)
Gatifloxacin	8 (38.1)	1 (33.3)	8 (66.7)	31 (66.0)	16 (53.3)	3 (100.0)	31 (58.5)	98 (58.0)
Imipenem	2 (9.5)	0 (0)	1 (8.3)	6 (12.8)	2 (6.7)	0 (0)	4 (7.5)	15 (8.9)
Norfloxacin	13 (61.9)	1 (33.3)	8 (66.7)	33 (70.2)	19 (63.3)	3 (100.0)	36 (67.9)	113 (66.9)
Tetracycline	19 (90.5)	2 (66.7)	11 (91.7)	38 (80.9)	23 (76.7)	2 (66.7)	42 (79.2)	137 (81.1)

[Table/Fig-11]: Antimicrobial resistance of Klebsiella spp. strains isolated from different specimens

CONCLUSION

ESBL and MBL production is now a significant problem in hospitalised patients globally and have a significant impact on several important clinical outcomes. The present study identified prior antibiotic use, surgery, haematological disease, diabetes mellitus, neurological disease, respiratory disease, urinary catheterisation and mechanical ventilation as important risk factors associated with ESBL and MBL producing organisms. Risk factors for infections due to these should be clearly identified so that effective strategies are developed to reduce spread of these infections and to optimise antibiotic use.

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