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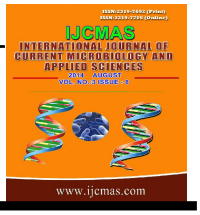

Original Research Article

Nosocomial Infection due to Multidrug Resistant (MDR) *Escherichia coli* and *Klebsiella pneumoniae* in Intensive Care Unit

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Keywords

Nosocomial Infection, MDR, *Escherichia coli*, *Klebsiella pneumoniae*

A B S T R A C T

To determine the Multidrug Resistance (MDR), Extended Spectrum β-lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae*. The study was conducted at a centralized microbiology laboratory, MGM Hospital Mumbai (from February 2012 to February 2013). ESBL production test was done by Double Disk Synergy Test and Confirmatory Method: National Committee for Clinical Laboratory Standard (NCCLS) Phenotypic confirmatory combination disc diffusion test. A total of 122 isolates *Klebsiella pneumoniae* 77 (63.11%) and *Escherichia coli* 45(36.88%) in which MDR 100(81.96%). *Klebsiella* spp 45.45% and *Escherichia coli* 50% was ESBL producer. The findings document evidence of the spread of Multidrug Resistance ESBL-producers nosocomial isolates.

Introduction

Nosocomial infection is defined as an infection which develops 48 hours after hospital admission or within 48 hours after being discharged (Costantini et al.; 1987) that was not incubating at the time of admission at hospital (Ferrer M et al.; 2008). Patients admitted to the ICU have been shown to be at particular risk of acquiring nosocomial infection with a prevalence rate as high as 30% (Caraven DE et al.; 1998). The risk of nosocomial infection in ICU is 5–10 times greater than those acquired in general medical and surgical wards (Vincet JL et al.; 1995).

The most common hospital infections are blood stream infection (BSI) (32%-53%) (Gaynes et al.; 1996, Sohn AH et al.; 2001) followed by pneumonia (12%-18%), ear, nose and throat infections (8% 21%), gastrointestinal infections and necrotizing enterocolitis (NEC) (5%-11%), and urinary tract infections (17%).(Yalah M.et al.;2006). Klebsiella account for 6 to 17% of all nosocomial urinary and shows an even higher incidence in specific group of patients at risk (Bennet CJ et al.; 1995). As a cause of nosocomial Gram– negative bacteremia, Klebsiella is second only to

Escherichia coli (Yinnon et al.; 1996). Antimicrobial resistance among Gram-negative bacilli represents a major problem in nosocomial infection (Pitout et al.; 1997) increasing with both morbidity and mortality greater when infection is caused by drug resistant organisms (Hosein et al.; 2002). Extended Spectrum beta lactamases (ESBL) pose serious therapeutic challenge to clinicians due to limited therapeutic options mainly caused by *Escherichia coli* and *Klebsiella species* (Philip. J et al.;2005, David L.Patrson et al.; 2005). The emergence of extended-spectrum β-lactamase (ESBL)- producing bacteria, particularly *Escherichia coli* and *Klebsiella pneumoniae*, is now a critical concern for the development of therapies against bacterial infection. Since the early 1980s, the number of nosocomial infections by ESBL producing, gram-negative bacteria has been increasing worldwide, and β-lactamase production has become a major causative factor for increasing resistance to antibiotics (Canton et al.; 2006, Pitout JDD et al.; 2008). The present study was planned to determine frequency of ESBL production among nosocomial isolates of *Klebsiella pneumoniae* and *Escherichia coli* from MGM Hospital, New Mumbai.

Materials and Methods

The study was conducted at the Department of Microbiology, M.G.M. Medical College and Hospital. Navi Mumbai. The duration of the study was 1 year (from February 2012 to February 2013). Clinical specimens obtained from patients with nosocomial infection yielded 122 isolates (*77 Klebsiella pneumoniae and* 45 *Escherichia coli*). Clinical samples including urine, blood, pus, sputum, ET-secretion, were collected from variety of patients from intensive care unit. The isolated bacterial species were identified by morphology, cultural characteristics and bio-chemical reactions according to the standard techniques (Cruickshank R et al.; 1089). After identifying the isolate, their antibiotic sensitivity test was done on Muller Hilton Agar using the Kirby Bauer method. The plates were read out after overnight incubation and by measuring the zone of inhibition around the antibiotics as per CLSI standards. Isolates were tested against amikacin, ampicillin, ampicillin/sulbactam, piperacillin, piperacillin-tazobactam, cephotaxime, ceftazidime, cefoxitin, norfloxacin, nitrofurantoin, gentamicin, ciprofloxacin, levofloxacin and imipenem.(Hi Media, India) (CLSI 2010). Isolates were labelled as MDR if they were resistant to at least two classes of first line agents including ampicillin, trimethoprim-sulfamethoxazole, floroquinolones (ciprofloxacin and ofloxacin), gentamicin and cephalosporins (cephotaxime, ceftriaxone and ceftazidime. The degree of sensitivity is determined by measuring the zones of inhibition of growth around the disc. *Escherichia coli* strain ATCC 25922 and *Klebsiella pneumoniae* ATTC 62003 were used as control strains **(**Reenaset et al.; 2011)

Detection of ESBL

Double Disk Synergy Test: (AMC) Amoxyclav disc was kept in the centre and both the cephalosporin discs that were Ceftazidime (CAZ) and Ceftriaxone (CTR) kept at a distance of 25 mm on either side of Amoxyclav (AMC). Extension of edge of inhibition zone of cephalosporin towards AMC indicated potential ESBL Producer (Jerlin et al.; 1998, Suryavanshi et al.;2011).

Confirmatory Method: National Committee for Clinical Laboratory Standard (NCCLS) Phenotypic confirmatory combination disc diffusion test. A disc of ceftazidime (30μg) alone and ceftazidime + clavulanic acid (30μg/10) were placed at a distance of 25 mm centre to centre, on a

MHA plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37˚C. An increase in inhibition zone diameter of ≥5mm for a combination disc versus ceftazidime disc alone confirmed ESBL production (Cormican et al.; 2011).

Results and Discussion

Table no 1. A total of 482 clinical samples 122 isolates *Klebsiella pneumoniae* 77 (63.11%) and *Escherichia coli* 45(36.88%).Out of 122 isolates 100 were multi drug resistance (MDR) in which males 48 and females 52. Similar study by Renuka Rampur et al. has reported the 199(51.8%) males and 185(48.2%) females (Renuka et al.; 2013).Table 2. Shows *Escherichia coli* were highly sensitive to imipenem (95.34%), nitrofurantoin (71.54%) and *Klebsiella pneumoniae* imipenem (97.46%), nitrofurantoin (61.78%).

In one study on nosocomial UTI in Nepal, the most commonly isolated pathogens were *Escherichia coli* and *Klebsiella pneumoniae*, for which higher susceptibilities were seen for amikacin (87.2%), ciprofloxacin (74.8%), ceftazidime (71.5%), and gentamicin (70.4%), however, the following were less susceptible for nitrofurantoin (35%), cephalexin (49.7%), and ampicillin (50.5%) (Das et al.; 2006). In the present study, Table 3. ESBL production out of 67 MDR 35 was found among the clinical isolates of *Klebsiella pneomoniae.* This is similar to another study, where ESBL production was observed in 40% of *Klebsiella pneumoniae* isolated (Babypadmini et al.; 2004). As regards the distribution of ESBL-producing *Klebsiella pneumoniae* in the various clinical specimens, the highest rate was recorded in ET-secretion (40%), followed by sputum (25.71%), blood (14.28%), pus (11.42%), and urine (8.57%). This is in contrast to the work done by Anwar et al., where the highest rate was found in blood (50%), followed by urine (43.2%), wound swabs (40.3%) and other samples (37.5%) (Anwar et al.; 2007). In the present study, Table 4. ESBL production out of 45 MDR 19 was found among the clinical isolates of *Escherichia coli.* This is similar to another study, where ESBL production was observed in 15.7% of *Klebsiella* species isolated (Husan et al.; 2009).

Table.1 Prevalence of MDR *Klebsiella pneumonie* and *Escherichia coli*

Organism	Total no of isolates	Male Patients	Female Patients	MDR	
				Males	Females
Klebsiella Pneumoniae	77(63.11)	42(75)	35(53.03)	39(81.25)	28(53.84)
Escherichia coli	45(36.88)	14(25)	31(46.96)	9(18.75)	24(46.15)
Total	122(100)	56(100)	66(100)	48(100)	52(100)

Table.2 Antimicrobial susceptibility of *Escherichia coli* and *Klebsiella pneumoniae*

Organism	A/S (%)	AK (%)	CIP (%)	CX (%)	CTX (%)	CAZ (%)	PIT (%)	GEN (%)	NIT (%)	IPM (%)
E. coli	35.32	27.23	45.76	43.24	45.02	50.43	62.12	60.34	71.54	95.34
Klebsiella pneumoniae	42.23	34.78	52.55	64.45	51.34	52.67	72.69	70.98	61.78	97.46

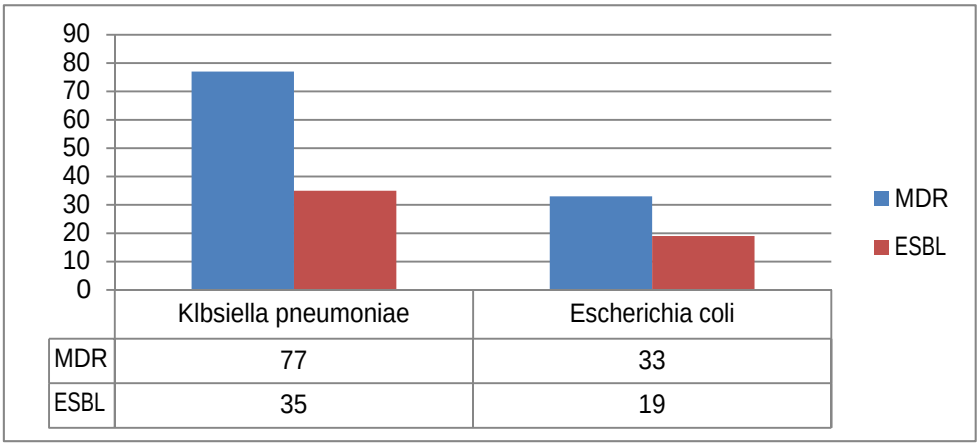
Table.3 Distribution of MDR and ESBL Producer among *Escherichia coli*

Specimen	Specimen No (%)	E. coli (%)	MDR (%)	ESBL (%)
Blood	142(29.46)	4(8.88)	3(9.09)	2(10.52)
Pus	72(14.93)	5(11.11)	2(6.06)	1(5.26)
Urine	125(25.93)	27(60)	22(66.66)	13(68.42)
ET-secretion	98(20.33)	5(11.11)	3(9.09)	2(10.52)
Sputum	45(9.33)	4(8.88)	3(9.09)	1(5.26)
Total	482	45	33	19

Table.4 Distribution of MDR and ESBL Producer among *Klebsiella pneumoniae*

Specimen	Specimen No (%)	*Klebsiella pneumoniae* (%)	MDR (%)	ESBL (%)
Blood	142(29.46)	9(11.68)	8(11.94)	5(14.28)
Pus	72(14.93)	9(11.68)	8(11.94)	4(11.41)
Urine	125(25.93)	7(9.09)	6(8.95)	3(8.57)
ET-secretion	98(20.33)	29(37.66)	25(37.31)	14(40)
Sputum	45(9.33)	23(29.87)	20(29.85)	9(25.71)
Total	482	77	67	35

Figure.1 Distribution of ESBL producer among MDR



As regards the distribution of ESBL-producing *Escherichia coli* in the various clinical specimens, the highest rate was recorded in urine (40%), followed by ET-secretion, blood (10.52%), and pus, sputum (5.26%). Similar study by Anwar et al. shows highest rate was recorded in blood 50% followed by urine 43.22% and pus 40.27% (Anwar et al.; 2007).

Table 5. *Shows Klebsiella pneumoniae* 45.45% were ESBL producer whereas *Escherichia coli* out of 50% were ESBL producer but study by Yoshiaki ikeda et al. *Escherichia coli* 21.4% were ESBL producer (Yoshiaikeda 2012). other study reported Klebsiella spp was much high 80% ESBL producer (Sahanti 2010).

The high prevalence of ESBL in *Escherichia coli* and *Klebsiella pneumoniae* is associated with a multitude of infections in hospitalized patients. Emphasis must be placed on the rational and judicious use of all antimicrobial agents. Accurate detection of ESBL producers, their treatment strategies and infection control policies are of paramount importance in curtailing this growing epidemic.

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