

Klebsiella pneumoniae: A Growing Concern in a Tertiary Care Hospital

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ABSTRACT

Background: Gram negative organisms are an important cause of community and nosocomial infections of which *Klebsiella pneumoniae* is a leading cause and has become quite notorious in many health care settings, being resistant to almost all the antimicrobials except a few. This can be attributed to its ability to produce extended Spectrum β - Lactamases (ESBLs).

Objective: The present study was carried out to determine the prevalence of Multidrug resistant and ESBL producing *Klebsiella pneumoniae* in various clinical samples and to detect their antibiotic susceptibility and resistance pattern as not much literature is available on it from our area.

Materials And Methods: A total of 3018 samples of urine, pus, sputum, blood and other body fluids received at the Department of Microbiology from both inpatients and outpatients were processed, out of which 122 isolates were identified as *Klebsiella pneumoniae* positive by standard microbiological techniques. Antibiotic susceptibility test was performed for different antibiotics using Kirby-Bauer disc diffusion method. Isolates were evaluated for ESBL production by Double Disc Synergy Test and Confirmatory Method: National Committee for Clinical Laboratory Standard (NCCLS) Phenotypic confirmatory combination disc diffusion test.

Results: Out of a total of 3018 samples received 670 were culture positive for different organisms. 122 isolates were identified as *Klebsiella pneumoniae* (18.20%), of which 55 (47%) were Multidrug resistant (MDR) and 49 (53%) were found to be ESBL producing by Confirmatory method. Our study showed good sensitivity of *Klebsiella pneumoniae* to Imipenem accounting for 96.87% in pus, 92.3% in sputum, 95.23% in urine, 91.6% in blood samples. In our centre isolates of

Klebsiella pneumoniae on an average were sensitive to Imipenem (93.93%), followed by Amikacin (81.8%), Gentamycin (71.96%), Piperacillin-Tazobactam (69.7%), Ciprofloxacin (39.4%), Cefotaxime (25%) and Cefpodoxime (18.23%). The sensitivity was almost negligible for Amoxicillin (2.2%) and Trimethoprim sulphamethoxazole (TMP-SMX) (2.3%) respectively.

Conclusion: The study shows that *Klebsiella pneumoniae* has emerged as an important cause of antimicrobial resistance due to ESBL Production and their resistance to third generation cephalosporin may be attributed to injudicious and inappropriate use of these antibiotics in our hospital setting. Therefore following antibiotic stewardship and good hospital infection control policy is mandatory to reduce drug resistance and help reduce patient morbidity, mortality as well as financial burden on health care settings.

Keywords: *Klebsiella Pneumoniae*, Antimicrobial Resistance, MDR, ESBLs.

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INTRODUCTION

Klebsiella pneumoniae is an opportunistic pathogen that causes a notable proportion of community and hospital acquired infections including urinary tract, pneumonia, septicaemia and soft tissue infections. It is naturally resistant to many antibiotics like ampicillin,¹ amoxicillin and also broad-spectrum cephalosporin because of production of extended spectrum β -lactamases (ESBLs).^{2,3} ESBLs are the enzymes that have the ability to hydrolyze and cause resistance to various types of newer β -lactam antibiotics, including the broad-spectrum (or third generation) cephalosporins (eg. cefotaxime, ceftriaxone,

ceftazidime) and monobactams (eg. aztreonam), but not the cephamycins (eg. cefoxitin and cefotetan) and carbapenems (eg. imipenem, meropenem and etrapenem). These enzymes are sensitive to β -lactamase inhibitors (sulbactam, clavulanic acid, and tazobactam). ESBL pose serious therapeutic challenge to clinicians due to limited therapeutic options mainly caused by *Klebsiella* species.⁴ *Klebsiella pneumoniae* has become a leading cause of morbidity & mortality in population. As a cause of nosocomial Gram negative bacteremia, *Klebsiella* is second only to *E. coli*. in some centres.⁵ The widespread use of antibiotics in

hospital has lead to emergence of Multi drug resistant organisms of low virulence like *Klebsiella* causing serious infections. Antimicrobial resistance in Gram negative bacilli is a serious concern in nosocomial infections. This can be attributed to production of Extended Spectrum Beta Lactamases (ESBLs) by *Klebsiella* that has been rising over the past few years & spreading world wise causing a serious threat for treatment of hospital acquired infections. These *Klebsiella pneumoniae* are also showing co-resistance to other antimicrobials like quinolones & aminoglycosides antibiotics. In India high prevalence of ESBL producing *Klebsiella pneumoniae* strains have been reported by various groups varying from 4%-83%.⁶ A study conducted in Aligarh tertiary care centre has reported 30.18% ESBL producing *Klebsiella pneumoniae* from different clinical samples.⁷

Therefore we need to keep an eye on changing pattern of sensitivity to antimicrobial agents so that we decrease the morbidity, mortality & financial burdens in our health care settings. The present study was carried out to determine prevalence of *Klebsiella* infection in our hospital, RIMS, Ranchi, their antibiogram & presence of resistant strains in various samples.

MATERIALS AND METHODS

The present study was carried out at the Department of Microbiology, RIMS, Ranchi. from May 2016 to Feb 2017 . Various clinical samples obtained from both inpatients and those attending OPD were collected during this period. Clinical samples included urine, pus, sputum and blood. Samples were obtained from both inpatients and outpatients, of all age group and sex. *Klebsiella pneumoniae* ATCC 62003 and E.coli ATCC 25922 were used as control strains.

Characterization of Bacterial Isolates

Pus, urine, sputum, blood samples were aseptically inoculated on to Blood and Mac Conkey agar plates and incubated overnight at 37° C. *Klebsiella* isolates were identified by their colony morphology, and biochemical reactions according to the standard techniques⁸. Morphology of *Klebsiella* identified were large dome shaped colonies on blood agar and lactose fermenting mucoid colonies on Mac Conkey agar. In Gram staining, gram negative, short, plump straight rods were seen. The biochemical characters

identified were positive citrate utilization test, positive urease test, acid and abundant gas production from glucose, lactose, sucrose, maltose and mannitol sugar fermentation tests⁹. After identification of isolates, antibiotic sensitivity was performed by Kirby Bauer disc diffusion method on Mueller Hinton Agar plates. Isolates were tested against Amoxycillin (30µg), Amikacin(30µg), piperacillin- tazobactam (100/10µg), cefotaxime (30µg), ciprofloxacin(5µg), norfloxacin (10µg), levofloxacin (5µg), erythromycin (15µg), gentamicin (10µg), ampicillin(30µg), netilmycin (30µg), imipenem (10µg), cefpodoxime (10µg), nitrofurantoin (300µg), trimethoprim- sulphamethoxazole(10µg) (HiMedia, Mumbai India). The plates were read after overnight incubation and by measuring the zone of inhibition around the antibiotics interpreted as Resistant, Intermediate and Sensitive as per CLSI standards¹² (CLSI 2012). Isolates were labelled as MDR if they were resistant to at least two classes of first line agents including Amoxycillin, Ampicillin, fluoroquinolones like ciprofloxacin, norfloxacin, levofloxacin, gentamicin and cephalosporins like cefotaxime (30µg) and cefpodoxime (10µg) with zone sizes ≤ 27mm & ≤ 22mm respectively were used as a screening method for ESBL production.

Detection of ESBL

Double Disc Synergy Test: (AMC). Amoxyclav disc was kept in the centre & both the cephalosporin discs that were Ceftazidime & Cefotaxime kept at a distance of 25mm on either side of Amoxyclav. If there was an extension of the zone of inhibition towards Amoxyclav, it was an indication for the potential ESBL Producer.^{10,11}

Confirmatory Method

National Committee for Clinical Laboratory Standards (NCCLS) Phenotypic confirmatory combination disc diffusion test. A disc of ceftazidime (30µg) and cefotaxime (30µg) alone & ceftazidime + clavulanic acid (30µg/10µg) and cefotaxime+ clavulanic acid (30/10µg) 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 and cefotaxime disc alone confirmed ESBL production.⁷

Table 1: Distribution of MDR & ESBL Producer among *Klebsiella pneumoniae*

Sample	No. of samples (%)	MDR(%)	ESBL(%)
Blood	15(12.29)	9(16.36)	7(14.28)
Pus	33(27.04)	22(40)	21(38.18)
Urine	61(50)	17(30.9)	15(30.61)
Sputum	13(10.65)	7(12.72)	6(12.24)
TOTAL	122	55	49

Table 2: Antibiotic sensitivity pattern of *Klebsiella pneumoniae*

Antibiotics	Pus		Sputum		Urine		Blood	
	S%	R%	S%	R%	S%	R%	S%	R%
Cefotaxime	15.62	84.37	30.76	69.23	34.92	65.08	41.66	58.33
Gentamycin	71.87	28.13	61.53	38.37	76.19	23.81	79.16	20.83
Ciprofloxacin	56.25	48.75	37.5	62.5	46.03	53.97	54.16	45.83
PiperacillinTazobactam	65.62	34.38	69.23	30.77	69.84	30.16	75	25
Amikacin	81.25	18.75	76.92	23.08	84.12	15.88	79.16	20.84
Imipenem	96.87	3.13	92.30	7.7	95.23	4.77	91.6	8.4

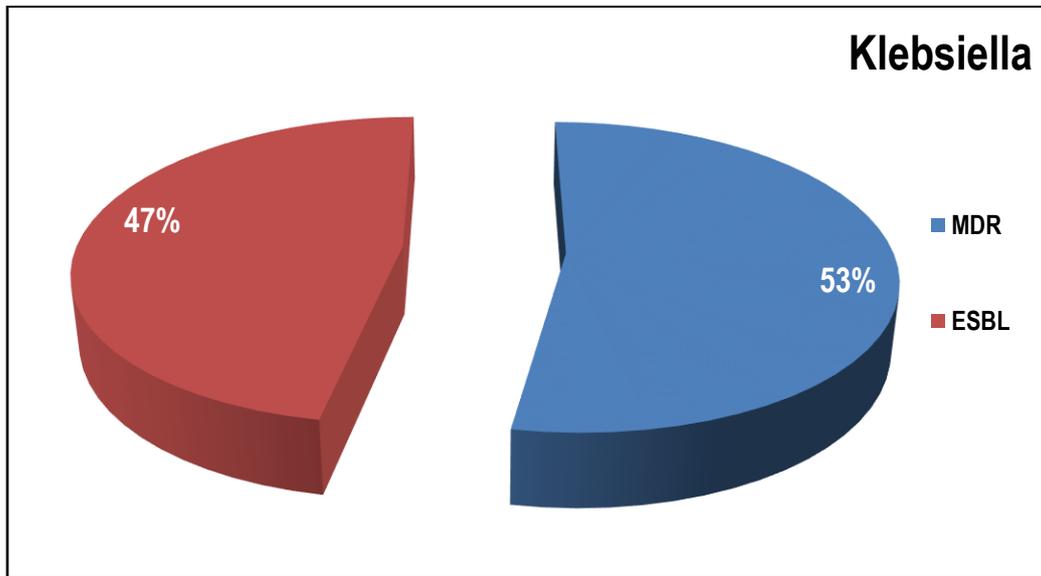


Chart 1: Distribution of ESBL producer among MDR in *Klebsiella*

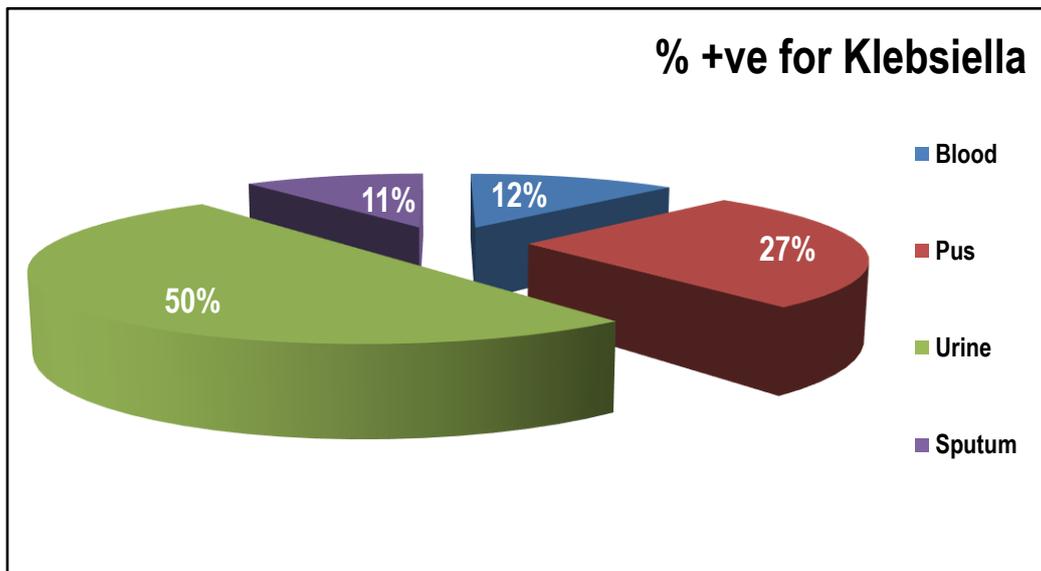


Chart 2: % of samples +ve for *Klebsiella*

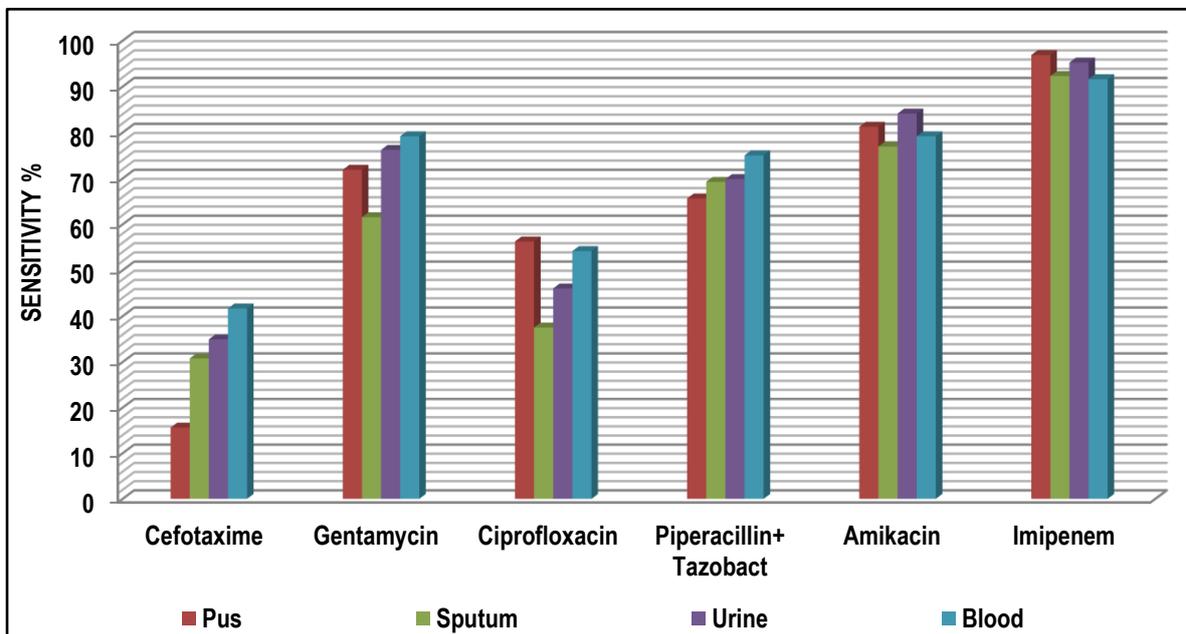
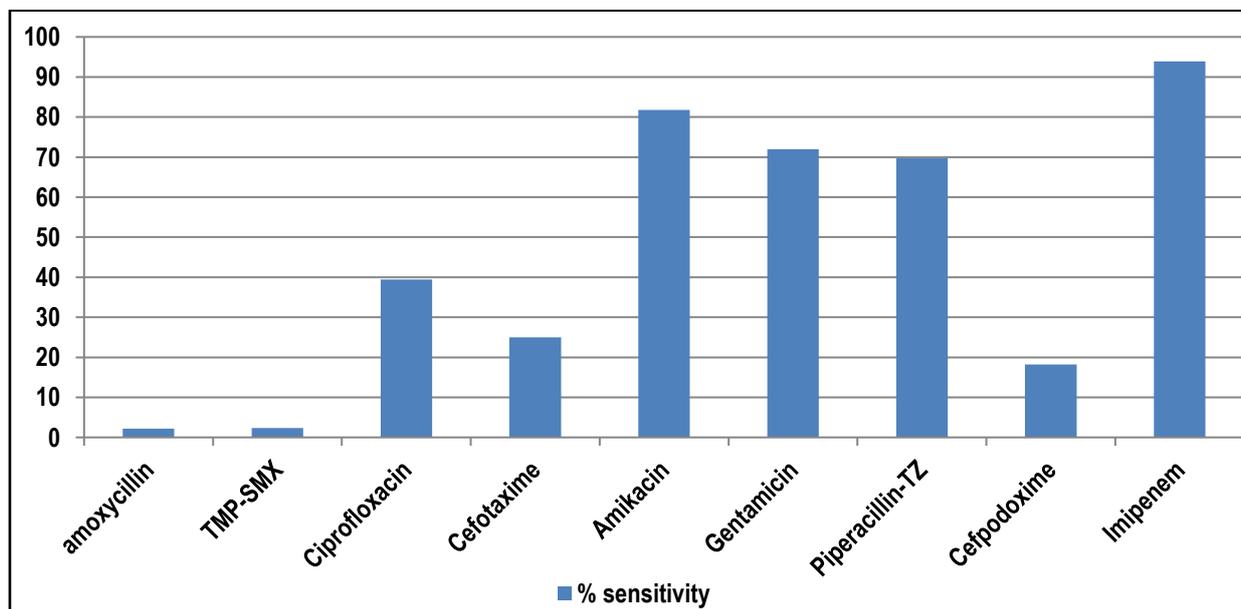
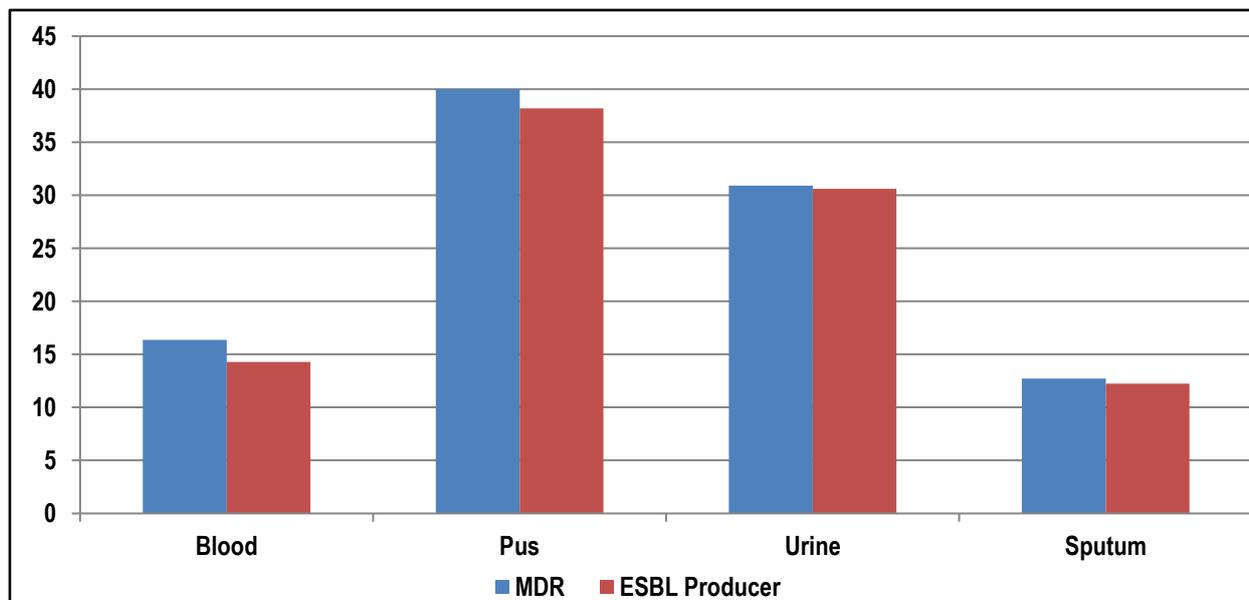


Chart 3: Antibiotic sensitivity pattern of *Klebsiella* to different drugs

Chart 4: Average sensitivity of *Klebsiella* to different antibioticsChart 5: Percentage MDR and ESBL Producing *Klebsiella pneumoniae*

RESULTS AND DISCUSSION

A total of 3018 clinical samples of urine, pus, sputum and blood were processed during our study period among which 670 were found to be culture positive for different organisms. Out of them 122(17.91%) samples were cultures positive for *Klebsiella pneumoniae*. In the present study culture positivity for *Klebsiella* in various samples (Chart-2) shows urine was 48.8% which is different from study done by R. Sarathbabu et al (2012), followed by pus 26.4%, which is somewhat similar to Valarmathi et al. (2013), blood 12% and sputum 10.4%, different from that reported by Ravichitra et al (2014).¹⁴⁻¹⁶ The antibiotic sensitivity is very high for Imipenem being more than 90% and was quite good for Aminoglycosides in our study. The fluoroquinolones were of moderate sensitivity but resistance to third generation cephalosporins was very high, on an average (Chart-4). Amoxicillin was not sensitive at all showing natural resistance of *Klebsiella* to it in our center (Table-2, Chart-3). The present study showed very good sensitivity to Imipenem accounting for 96.87% sensitive in pus, 95.23% in urine, 92.3% in sputum, 91.6% in

blood samples. The urinary isolates of *Klebsiella* showed highest sensitivity to Imipenem (95.23%) which is different from reports by Manikandan in Tamil Nadu, India, where the sensitivity to Imipenem was 86.1%.²⁸, followed by Amikacin (84.12%), Gentamycin (76.19%) and Piperacillin-Tazobactam (69.84%) somewhat in accordance to study done by Das et al. (2006).¹⁷ The fluoroquinolones and nitrofurantoin were of moderate sensitivity (46.03%) and (41.4%) respectively. For Nitrofurantoin, resistance was 58.54% which is similar (53%) to a study done by Sarathbabu et al.(2012)¹⁴ in Andhra, Pradesh, India. *Klebsiella* showed negligible sensitivity to Amoxicillin and TMP-SMX similar to that reported by Sharma et al (2016)¹⁸ and Ullah (2009) where *Klebsiella* was absolutely resistant to TMP-SMX.⁸ Also study done by Sikarwar et al(2011)¹⁹ reported high level of resistance. Although fluoroquinolones are considered as the most effective drugs for urinary pathogens our study showed that the sensitivity was moderate (46.03%) somewhat in accordance to study done by Ullah et al (2009) and Sarathbabu et al (2012) between 53%-60%.^{9,10} Resistance of Amikacin and gentamycin in our study was

15.88% and 23.8% in accordance with a study done by Nasehi et al(2010)²⁸ where resistance was of the range of 16% for amikacin and 19.4% for gentamycin.

Isolates were labeled as MDR if resistant to at least two classes of first line agents including amoxicillin, fluoroquinolones (ciprofloxacin, levofloxacin), cephalosporins (cefotaxime, ceftriaxone, ceftazidime, cefpodoxime), gentamicin. In study done in our tertiary care centre most of the isolates of *Klebsiella* have been MDR. Out of 122 culture positive isolates 55(53%) were MDR and around 49(47%) were ESBL producers (Chart -1). As regards, the distribution of ESBL Producers in our study is similar (40.16%) to a study done by Babypadmini et al (2004)²¹ and Chaudhary et al (2014)²⁶ where ESBL Production was observed in 40% of *Klebsiella pneumoniae* isolated. This study is quite different from that done by Ravichitra et al (2014) where ESBL production was only 15.8% of *Klebsiella* isolates. Also studies done by Faezabadi et al (2006)²⁴ and Ramazanzadeh et al(2009)²⁵ showed 44.5% and 34.8% of ESBL producers. Study by Yoshiakeda et al showed 45.45% of ESBL producers whereas our study showed ESBL production by *Klebsiella pneumoniae* to be 40.4% in various clinical specimen (Table1,Chart-2), the highest rate being recorded in pus samples (38.18%), followed by urine (30.61%), blood (14.28%) and sputum (12.24%) which is in contrast to work done by Anwar et al (2007)²⁰ where highest rate was found in blood (50%) followed by urine (43.2%) and other samples (37.5%). Our study showed 30.61% urine samples producing ESBL similar to that reported by Shamweel et al (2000).²³ Some other studies have reported ESBL producing *Klebsiella* to be as high as 80% by Shanthi et al (2010).²² Present study provides information regarding the prevalence of *Klebsiella pneumoniae* and also their resistance to most of the available antibiotics in our health care setting. These multidrug resistant organisms have become a potent threat to the clinicians causing treatment failures and are major challenges which can only be handled by good infection control policies of the hospital and above all antibiotic stewardship.

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