

# Multi Drug Resistant *Klebsiella* Isolates in Burn Patients: A Comparative Study

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## ABSTRACT

**Introduction:** Infections are the most common complications in the burn patients admitted to the hospitals leading to high morbidity and mortality. *Klebsiella* is one of the most frequently isolated bacteria from burn wounds.

**Materials and Methods:** We studied antimicrobial susceptibility patterns of *Klebsiella* isolates from burn patients. In this cross-sectional study wound swabs from 1294 patients hospitalized in burnward were collected for bacteriological examination. Antibiotic sensitivity testing of *Klebsiella* isolates was done by modified Stokes disc diffusion method.

**Results:** Out of 883 isolates from 1294 patients 195 were found to be *Klebsiella* spp. Based on the biochemical properties 153 isolates were *Klebsiella pneumoniae*, 37 were *Klebsiella oxytoca* and 5 were others species. In our study we found that 54% of the *Klebsiella* isolates were multidrug resistant as they were resistant to at least one antibiotic of three or more different groups of antibiotics.

**Conclusion:** Rate of isolation of *Klebsiella* as well as its resistance for commonly used antibiotics is increasing over the time.

**Keywords:** Antibiotic susceptibility, Prevalence, Wound swabs

## INTRODUCTION

Infections are the most common complications in the burn patients admitted to the hospitals leading to high morbidity and mortality [1]. Burn patients are more prone to nosocomial infections as compared to patients hospitalized for other reasons. This is often attributed to immunocompromized state of burn patients due to disrupted skin barrier, altered physiology, longer stay in hospital and invasive interventions. Prompt antibiotic treatment can be life saving in burn patients. Increasing resistance is the major hurdle to achieve this goal. For an empirical therapy one requires a prior knowledge of prevalence of common bacteria and their antibiotic resistance pattern in that area.

*Klebsiella* is one of the most frequently isolated bacteria from burn wounds [2]. *Klebsiella* is the most common member of family *Enterobacteriaceae*, known to produce plasmid mediated extended spectrum beta lactamases (ESBLs) which are horizontally transferable [3]. In recent years, multidrug resistant *Klebsiella* is reported more frequently as a major hurdle in the treatment of infections [4].

We studied antimicrobial susceptibility patterns of *Klebsiella* isolates from burn patients. Along with the commonly used antibiotics, we also included Carbapenems and Polymyxins for antibiotic susceptibility testing.

## MATERIALS AND METHODS

A cross-sectional study was conducted at Department of Microbiology, Maulana Azad Medical College, New Delhi in 2012-13. Wound swabs from 1294 patients hospitalized in burnward were collected for bacteriological examination. Swabs were inoculated on blood agar, Mac Conkey agar and brain heart infusion broth. Plates and the broth were incubated at 37°C for overnight. Culture plates were checked for the bacterial growth next day.

All bacterial isolates were examined for colony characteristics, Gram staining, motility and biochemical tests. Biochemical tests employed were oxidase, catalase, nitrate, urea hydrolysis, citrate utilization, sugar fermentation, Indole production test and H<sub>2</sub>S production on TSI agar.

## Antibiotic sensitivity

Antibiotic sensitivity testing of *Klebsiella* isolates was done by modified Stokes disc diffusion method. A suspension of 0.5 McFarland standards was prepared from the colonies of isolated organism and was inoculated along with control strains on Mueller Hinton agar plates by sterile swabs. Antibiotic discs were applied on agar and kept for overnight incubation. The antibiotics that were included for testing were cephalexin (30mcg), ceftriaxone (30mcg), cefotaxime (30mcg), amoxicillin (20mcg), ciprofloxacin (5mcg), gentamicin (10mcg), amikacin (30mcg), imipenem (10mcg), meropenem (10mcg), piperacillin tazobactam (100/10), netilmicin (10mcg), polymyxin B (300unit) and colistin (10mcg).

## STATISTICAL ANALYSIS

The data collected was analysed with the aid of the Statistical Package for Social Sciences (Version 10) computer software. A p-value < 0.05 was taken as significant.

## RESULT

Out of 883 isolates from 1294 patients 195 were found to be *Klebsiella* spp. Based on the biochemical properties 153 isolates were *Klebsiella pneumoniae*, 37 were *Klebsiella oxytoca* and 5 were others species.

Bacteria	Number	Percentage (%)
<i>Acinetobacter</i>	70	7.92
<i>Citrobacter</i>	9	1.01
<i>E. coli</i>	125	14.15
<i>Enterobacter</i>	8	0.9
<i>Klebsiella</i>	195	22.08
<i>Morganella</i>	1	0.1
<i>Proteus</i>	71	8.04
<i>Providentia</i>	15	1.6
<i>Pseudomonas</i>	248	28.08
<i>Staphylococcus</i>	141	15.96
Total	883	99.84

[Table/Fig-1]: Isolation of different organism in burn wound infection

*Klebsiella* was the second most common bacteria isolated. On comparison with previous studies from India and abroad we found this unusually high rate of isolation.

On antibiotics susceptibility testing *Klebsiella* strains from clinical cases were found highly susceptible to imipenem, meropenem, colistin and polymyxin B. At the same time over 50 to 60% strains were found resistant to amoxicillin, gentamicin, ceftriaxone and cefotaxime. Most of the isolates were resistant to cephalixin. 55% of the isolates were resistant of third generation cephalosporins [Table/Fig-2].

<i>Klebsiella isolates (195)</i>				
	Sensitive	Intermediate Sensitive	Resistant	Resistant (%)
Cephalexin	6	1	188	96
Ceftriaxone	83	4	108	55
Cefotaxime	82	5	108	55
Amoxicillin	73	1	121	62
Ciprofloxacin	72	16	97	48
Gentamicin	77	1	117	60
Amikacin	28	4	163	84
Imipenem	147	12	36	18
Meropenem	152	19	19	10
Piperacillin/tazobactam	121	22	52	26
Netilmicin	120	4	71	36
Polymyxin B	180	1	14	7
Colistin	178	1	16	8

[Table/Fig-2]: Percentage of resistant *Klebsiella isolates* for different antibiotics

Multi drug resistant <i>Klebsiella isolates (106)</i>				
	Sensitive	Intermediate Sensitive	Resistant	Resistant (%)
Cephalexin	1	0	105	99
Ceftriaxone	75	3	28	26
Cefotaxime	75	3	28	26
Amoxicillin	8	12	86	81
Ciprofloxacin	0	0	106	100
Gentamicin	0	0	106	100
Amikacin	69	11	26	24
Imipenem	73	16	17	16
Meropenem	90	2	14	13
Piperacillin/tazobactam	2	0	104	98
Netilmicin	94	2	10	9
Polymyxin B	63	1	42	39
Colistin	89	1	16	15

[Table/Fig-3]: Multidrug resistant *Klebsiella*

## DISCUSSION

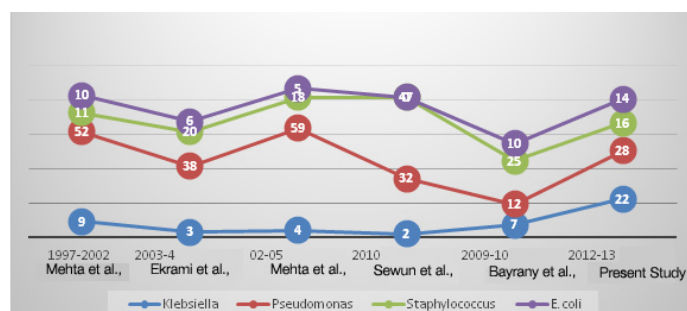
In our study we found that 54% of the *Klebsiella isolates* were multidrug resistant as they were resistant to at least one antibiotic of three or more different groups of antibiotics. Sensitivity pattern of multi drug resistant isolates is shown in [Table/Fig-3].

In the present study we have shown that Gram negative bacteria are the most common bacteria causing burn wound infection. Gram-negative isolates were the five times more in number than Gram-positive pathogens this is consistent with other studies [5-7]. However, some workers have found Gram positive organisms as predominant isolates from burn wounds [8,9].

*Klebsiella* species was the second most frequent pathogen isolated after *Pseudomonas* (28%), accounting for 22% of the total and 26% of all the Gram negative isolates. This is consistent with another study from India which reported *Pseudomonas aeruginosa* (36%)

	Present study (2012-13)	Mehta et al., [2] 2007 (1997-02)	Mehta et al., [2] 2007 (2002-05)	Sewunet et al., [13] 2013	Ekrami A et al., [14] 2007	Bayram Y et al., [15] 2013 (2009-2011)
<i>Pseudomonas</i>	28	59	52	32	38	12
<i>Staphylococcus</i>	16	18	11	47	20	25
<i>Klebsiella</i>	22	4	9	2	3	7
<i>E. coli</i>	14	5	10	---	6	10
<i>Enterobacter</i>	1	4	2	---	2	9
<i>Proteus</i>	8	3	2	17	5	---
<i>Providentia</i>	2	---	---	2	---	---
<i>Acinetobacter</i>	8	7	14	---	10	24
<i>Citrobacter</i>	1	---	---	---	2	---
<i>Morganella</i>	0.1	---	---	---	---	---

[Table/Fig-4]: Comparison of isolates in various studies

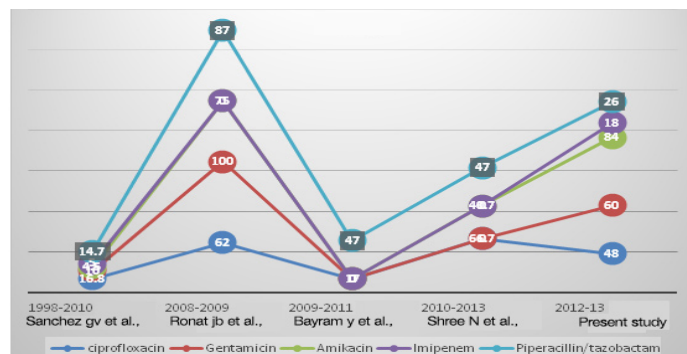


[Table/Fig-5]: Isolation rate of common bacteria in different studies

as the most frequently isolated pathogen [10]. Many studies have reported *Klebsiella* as the most common organism [11,12]. We have shown that percentage isolation of *Klebsiella* (22%) was significantly higher than previous studies [Table/Fig-4,5], and we gave special attention to its antibiotic susceptibility pattern.

Antibiotics	<i>Klebsiella isolates resistant to antibiotics (%)</i>				Our study
	Sanchez GV et al., [16]	JB Ronat et al., [17]	Bayram Yet et al., [15]	Shree N et al., [19]	
Ciprofloxacin	16.8	62	17	66.7	48
Gentamicin	10	100	0	--	60
Amikacin	4.5	75	0	40.7	84
Imipenem	5	0	0	0	18
Piperacillin/tazobactam	14.7	87	47	47	26

[Table/Fig-6]: Comparison of resistant *Klebsiella isolates* for common antibiotics



[Table/Fig-7]: *Klebsiella* resistance pattern from 1998 to 2013

There is great variability in antibiotics resistant percentage in various studies. Comparison of the common antibiotics is shown in [Table/Fig-6]. In this study we found a high rate of resistance for imipenem (18%). Bayraam et al., and Ronat et al., have found no isolate resistant for imipenem however Sanchez et al., has shown only 5% resistance for imipenem [15-17].

Fifty four per cent of the *Klebsiella* isolates were multi drug resistant. They were resistant to at least one antibiotic of three or more antibiotics group of different mechanism of action. All of the multidrug resistant isolates were resistant to ciprofloxacin and Gentamicin. Only two isolates of *Klebsiella* species were sensitive for piperacillin/tazobactam which is a commonly used antibiotic combination used for the Gram negative bacteria.

If we see the resistance pattern of *Klebsiella* spp over the years, its resistance has not come down. In our study 16 % resistance for Imipenem is very high comparing to the other studies in which very less resistance (0 to 5%) for imipenem was observed. This might have happen due to the inclusion of only burn patients in our study. For gentamicin people have reported resistance from 0-100%. But for other antibiotics overall trends show that there is no decrease in their resistance pattern.

Most MDR isolates were resistant to cephalosporins which are commonly used for *Klebsiella* infection as monotherapy and in combination with other. Although plasmid mediated resistance to broad spectrum antibiotics is a common phenomenon among the members of family *Enterobacteriaceae* but it is more commonly seen with *Klebsiella* spp. These bacteria inactivates broad spectrum antibiotics by producing extended spectrum beta lactamase enzymes. Although in our study MDR *Klebsiella* showed lowest resistance to meropenem, imipenem, netilmicin, and colistin, but there were isolates which were resistant to these antibiotics. Being plasmid mediated this resistance can be transferred horizontally from one bacterium to another. Which is an alarming state and needs to be addressed by prompt identification, isolation and treatment of the infection cases. Successful decrease in mortality and morbidity has been reported in burn patients by prompt isolation and treatment [18].

## CONCLUSION

We have shown that *Klebsiella* stays as an important isolate from burn wound infections and its rate of isolation has increased in last ten years. Also, it appears that the resistance to the commonly used antibiotics is increasing over the time. There is a need for similar studies from the other health care facilities.

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