MediPharm

International Journal of MediPharm Research

ISSN: 2395-423X www.medipharmsai.com

Vol.01, No.01, pp 14-18, 2015

Efficacy of antibiotics against *Klebsiella* spp isolated from various clinical sources

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Abstract : The pathogenicity of *Klebsiella* is a major problem in most hospitals because of their resistance to multiple antibiotics and potential transfer of plasmids to other organisms. Isolation and enumeration of different bacterial species were made from 250 clinical samples collected from various sites of different patients in Chennai of India during 2013. The samples were subjected to different processes in order to isolate the causative agents of different diseases. The isolated organisms were *Klebsiella*, *E. coli*, *Staphylococcus*, and *Salmonella*, among these *Klebsiella* was the dominant one comprised 63% of total positive samples. Out of 75 clinical samples, 95 were positive and the rest (155) were sterile samples. Antibiotic sensitivity as well as determination of minimum inhibitory concentration (MIC) of antibiotic-Cefotaxime on *Klebsiella* species was done by tube dilution technique. The organisms resistant to all were categorized in 1st line of antibiotics, those are intermediate sensitive were categorized in 2nd line of antibiotics and finally the remaining were kept for sensitive with 3rd line of antibiotics. It was found the antibiotics like Cefotaxime, Amykacin, Netilmycin, Cetpirane, Cifriaxone, Ampisulbactum, Azithromycin and Gentamycin were useful for most *Klebsiella* infection which arrested bacterial cell wall synthesis, in turn inhibited the bacterial growth.

Key Words: *Klebsiella*, Clinical samples, MIC, 1st, 2nd & 3rdline of antibiotics.

Introduction

The genera *Klebsiella* cause a wide range of infections in man and known as one of the principal microbe responsible for various hospital infections, have become dangerous mainly for people with impaired immunological responses[1,2]. The emergence of this microbe's resistant to multiple antimicrobials has made the treatment of these infections very difficult. Many antibiotics at levels below their minimal inhibitory concentration (MIC) can affect bacteria in ways other than the expected bactericidal action *Klebsiella* are ubiquitous in nature. In humans, they may colonize the skin, pharynx or gastrointestinal tract. They may also colonize sterile wounds and urine. Carriage rates vary with different studies. They are opportunistic pathogens found in the environment and in mammalian mucosal surfaces[3,4]. The principal pathogenic reservoirs of infection are the gastrointestinal tract of patients and the hands of hospital personnel. The acquisition of various species of *Klebsiella* has become a major problem in most hospitals because of their resistance to multiple antibiotics and potential transfer of plasmids to other organisms[5,6,7]. *K. pneumonie* infections are common in hospitals where they cause pneumonia and urinary tract infections in catheterized patients. *K. pneumoniae* is second to *E. coli* as a urinary tract pathogen. *Klebsiella* infections are encountered far more often now than in

the past. This is probably due to the bacterium's antibiotic resistance properties. *Klebsiella* species may contain resistance plasmids (R-Plasmids), which confer resistance to antibiotics such as ampicillin and carbenicillin[8]. Extensive use of broad spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and subsequently, the development of multi drug-resistant strains that produce extended – spectrum-B-lactamase (ESβl)[9]. These strains are highly virulent, show capsular type K 55, and have an extraordinary ability to spread. Treatment of *Klebsiella* infections depends on the organ system involved. In general, the initial therapy of patients with possible bactericemia is empirical. The choice of a specific antimicrobial agent depends on local susceptibility patterns. The present study was carried out to isolate *Klebsiella* sp. from different samples and was subjected to record its sensitivity pattern by serological tests from capsular antigen separation technique and antibiogram.

Materials and Methods

The study was conducted at Microbiology Laboratory, Department of Biomedical Engineering, Sathyabama University, Chennai during 2003. Different samples were collected from Urine, Blood and Miscellaneous samples like sputum, pus, cerebrospinal fluid (CSF), Pleural fluid, Bronchial aspirate, Nasal swab, vaginal swab, Throat swab, Ear swab, Semen, Umbilical swab, etc., One or more than one samples were collected from different patients of different age groups. The samples from different patients was collected through a syringe with or without needle and transferred to a sterile container. If the collected sample is very thick, then it was scooped off or coaxed into a sterile container using a swab. Swabs must be well loaded, to saturation if possible. Simultaneously a duplicate swab was also taken for each sample for the microscopic view and for other culture. The work has gone through direct Gram's staining, plate culture and to get the confirmatory conclusion through biochemical methods. The culture was streaked over the various media like Nutrient Agar Media, Mac Conkey Agar Media, Blood Agar, Urochrome Agar and Selective Media. The plates were kept inside the incubator for 24 hours and the morphological identification of various cultures was studied. The Gram's strain method was done firstly to get the confirmation whether the same was containing any organism or sterile. Various organisms like *Escherichia coli, Staphylococcus aureus, Klebsiella, Pseudomonas* and *Salmonella* were isolated by different methods (Cappuccino and Sherman 2004) employed. The most frequently found organisms were *Klebsiella* and *Staphylococcus aureus.*

Determination of (MIC) of antibiotic–Cefotaxime on *Klebsiella* species by tube dilution technique:

Minimum inhibitory concentration (MIC) of the antibiotics is defined as the highest dilution of the antibiotic showing no visible turbidity. To measure the bactericidal concentration, it is necessary to subculture the tubes showing no visible growth on the agar plates. The highest dilution yielding no growth is the bactericidal concentration. A stock solution of the antibiotic was prepared and followed the serial dilutions by dissolving 100mg of cefotaxime to 1ml of sterile distilled water in a test tube. Serial dilution was continued until the final dilution containing 6.25 µg/ml of the antibiotic obtained. One drop of young culture was added into each tube starting form 100 µg/ml of the antibiotic to 6.25 µg/ml of the antibiotic. One test tube was kept as control containing only broth and culture. The tubes are kept for incubation at 37^{o}c for 5 hours and subculture into agar plates which are kept overnight incubation at 37^{o}c. The colonies were observed. The highest dilutions which did not show growth of the organism was taken as the Minimum Inhibitory Concentration (MIC) of the antibiotic on *Klebsiella* sp. Antibiotic sensitivity Test was also carried out by Kriby Bauer's method[10] on Muller Hinton agar plates.

Results

Out of the total samples collected, nearly 40 were blood samples, 70 were urine samples and 140 were miscellaneous samples. The miscellaneous samples carried 80 samples which were collected from nasal swab, Throat Swab and Bronchial aspirate, 30 samples from the sputum pus cerebrospinal spiral fluid, pleural fluid, semen and 30 samples were from vaginal swab, ear swab and umbilical swab. Among the blood, urine and miscellaneous samples *Klebsiella* spp were found maximum than compared to the other organism (Fig 1). Out of the samples, 155 samples were confirmed as sterile and 95 were found positive. Fig 2 shows that among these 95 positive samples, *Klebsiella* were (63%) and remaining (37%) *Escherichia coli, Staphylococci, Streptococci,Candida* etc. Table 1 shows that the highest percentage, 70 % *Klebsiella* were found in miscellaneous culture followed by urine and the least amount of blood sample contains only 10%. *Klebsiella* showed some mucoid and non-mucoid colonies in different types of media. In Nutrient Agar media, the

maximum mucoid colony was shown by miscellaneous sample (36), followed by urine (10) and Blood (4), where as the non-mucoid colony was same for blood and urine. Similarly, the Mac-Conkey agar media showed the same mucoid colony like Nutrient Agar media but there was no mucoid colony was found from the selective media. The biochemical test was carried out in two batches. Each batch was containing 30 *Klebsiella* positive samples. Out of them, 3 Blood samples, 6 urine samples, 21 miscellaneous samples. 30 samples of each batch showed negative response towards Iodole Test and the Methyl Red Test in urine showed 4 positive and 2 negative cases and Blood sample showed 2 positive and one negative result while 15 miscellaneous samples were positive and 6 showed negative response.

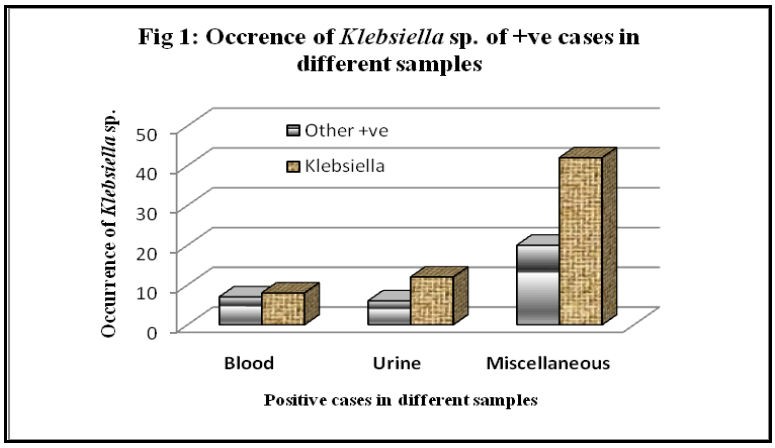
After several dilutions and standardization of the antibiotics, the Minimum Inhibitory Test was done. From that standardization, it was found that there was no growth from 50 µg /ml which was taken as the cut of value or the MIC of the antibiotic Cefotaxime for the genus *Klebsiella*. All *Klebsiella* samples were examined for antibiotic sensitivity using Kirby Bauer disc diffusion technique and *Klebsiella* was found highly sensitive to Amykacin, Cefotaxime, Gentamycin, Ceftazidine, Azithromycin and Augmentin. They showed moderate sensitivity to Ampisulbactum, Ceftriaxone, Cefpirane, Netilmycin and resistant to Ampicilin, Cephalaxin, Cefadroxyl, Tobramycin, Pefloxacin, Levofloxacin and Ciprofloxacin etc. (Table 2)

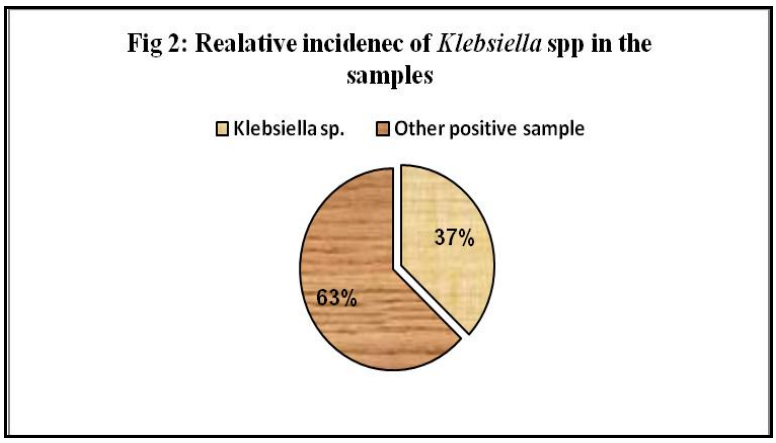
Table 1: Percentage occurrence of *Klebsiella* spp in different samples.

Samples	Total No. of patient	Positive sample	Negative sample	No. of *Klebsiella* spp	Percentage (%) of *Klebsiella* sp.
Blood	40	13	27	6	10
Urine	70	20	50	12	20
Miscellaneous	140	62	78	42	70
Total	250	95	155	60	100

Table 2: Protective capacity of antibiotics against *Klebsiella* spp

Sl. No.	Name of the antibiotics	Abbreviation	Antibiotic Sensitivity		
			Sensitive	Intermediate	Resistance
1	Ampicillin	Amp	-	-	+
2	Amoxycillin	Ax	-	-	+
3	Augmentin	Ag	+	-	-
4	Ampi/Sulfactum	A/S	-	+	-
5	Amykasin	Amk	+	-	-
6	Azithromyin	Az	+	-	-
7	Ciprofloxacin	Cif	-	-	+
8	Ceftazidime	For	+	-	-
9	Cefpirave	Cfr	-	+	-
10	Ceftriaxone	Cft	-	+	-
11	Cefotaxime	Omt	+	-	-
12	Cefazolin	Cef	-	-	+
13	Cefoperazone	Cp	-	-	+
14	Cefazoxin	Cz	-	-	+
15	Magnex	Mgx	+	-	-
16	Cephalaxin	Ce	-	-	+
17	Cefactor	Cr	-	-	+
18	Cefadroxyl	Cd	-	-	+
19	Gentamycin	G	+	-	-
20	Levofloxacin	Lev	-	-	+
21	Ofloxacin	Of	-	-	+
22	Pefloxacin	Pf	-	-	+
23	Netilmycin	N	-	+	-
24	Tobramycin	Tb	-	-	+
25	Timentin	Tn	+	-	-





Discussion

Hospital acquired infections can be divided into initial phase of bacterial colonization followed by tissue invasion. Miscellaneous colonization of *Klebsiella* has been reported maximum[11], *Klebsiella* was found to be a major infectant to the normal person as well as to the patients. The present analysis showed a clear data about different bacterial flora, which the concentration of *Klebsiella* was more in comparison to all the samples collected[12]. The miscellaneous samples showed abundant presence of *Klebsiella* (66%) followed by urine (21%) and blood samples (14%). Individual samples of blood showed 32.5% of Gram negative bacteria out of 40 samples, which in turn showed 46.15% *Klebsiella* positive. The remaining 53.85% were mixed bacterial flora (Table 1)

From urine samples collected from patients, 28.57% showed positive for the presence of bacterial flora, from which *Klebsiella* was 60 % and 40 % by others. Similarly, the miscellaneous samples collected from various patients showed 44.28% positive response for the presence of bacterial flora. *Klebsiella* covered 67.74% from the positive miscellaneous samples, which was found to be the maximum percentage compared to urine and blood (Fig 1). From the above data, it is clearly observed that maximum *Klebsiella* is present in the respiratory tract, which causes the outcome infection among hospitalized patients[13,14] (Fig 2). The epidemiological infection in hospital studied by Montgomirie[15], who found that urine sample was dominant with various *Klebsiella* species, especially *K. aerogenes*, which was recovered maximum from the urological ward[16].The biochemical test indicated the specific identification and confirmed test of the *Klebsiella* sp., like *Klebsiella aerogenes, Klebsiella edwarsii, Klebsiella pneumonia, K. rhinoscleromatis* and *Klebsiella ozaene* [17].

Rennie and Duncan[17] studied about the serological typing of *Klebsiella*. Gel diffusion method was carried out by Ouchterlory's technique in small flat Petri dishes (2.5 cm in diameter) and on microscopic slides as well[18]. Towbin et al [19] also opined their research work about the electrophoretic transfer of protein. The cell surface hydrophobicity and serum sensitivity of *Klebsiella* after treatment with minimum inhibitory concentration (MIC) of cefotaxime were studied. The present research studies analyzed that the MIC rate was minimum up to 50µg/ml confirmed with the previous work by Hostacka and Majtan[20] and Shibl [21] (Table 2).

The work on disc diffusion method adopted by Kadurugamuwa et al [22] and Tateda et al [23] have been agreed with the present report of our study.

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