Int.J.Curr.Microbiol.App.Sci (2016) *5*(9): 549-554

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 9 (2016) pp. 549-554**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.509.061

Change in Antibiotic Sensitivity Pattern of *Klebsiella pneumoniae*: A Two and Half Year Study

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Keywords

Klebsiella pneumoniae, Antibiotic Susceptibility pattern, Imipenem.

Article Info

Accepted:
20 August 2016
Available Online:
10 September 2016

A B S T R A C T

In the era of drug resistant microorganisms, we are left with very few choices of antibiotics. *Klebsiella pneumoniae* is among the most common gram negative bacteria encountered by physicians worldwide which were often resistant to many antibiotics. This study was aimed to evaluate change in Antibiotic Susceptibility pattern of *Klebsiella pneumoniae* isolated from various clinical samples over a period of Two and a Half year which can guide future treatment option for this organism. Total of 416 Klebsiella pneumonia isolated from various clinical samples and their antibiotic sensitivity were studied. Antibiotic Susceptibility pattern showed change in sensitivity pattern for most of the antibiotics over a period of time. Imipenem showed much reduction in sensitivity 94.25% in February 2014 – January 2015, 70.76 % in February 2015 – January 2016 and 36.17 % in February 2016 – July 2016. Increase in susceptibility towards Gentamicin (from 16.66 % to 29.78 %) and Amikacin (29.31 % to 40.42 %) was observed.

Introduction

In the era of drug resistant microorganisms, we are left with very few choices of antibiotics. If we do not use available antibiotics judiciously, it can lead to increased resistance among microorganisms over a period of time. Antibiotics provide the main basis for the therapy of microbial infections. Since the discovery of these antibiotics and their use as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains of several groups of microorganisms (Firdaus Jahan *et al.*, 2011; Harbottle *et al.*, 2006).

Klebsiella pneumoniae is a Gram negative bacilli, belonging to Tribe II – Klebsielleae of the *Enterobacteriaceae* family. *Klebsiella pneumoniae* is among the most common gram negative bacteria encountered by physicians worldwide (Lin *et al.*, 2010). *Klebsiella* spp. is often resistant to many antibiotics, including cephalosporins and

aminoglycosides (Vinetz, 2007). These bacteria have become important pathogens in nosocomial infections (Nordamann *et al.,* 2009), which have been well documented in United States (Graybill *et al.,* 1973) and India (Mathur *et al.,* 1991).

The Review by Munoz-Price *et al.,*2013 emphasizes the importance of *Klebsiella pneumoniae* strains that produce *K pneumoniae* carbapenemases (KPC-KP) that have taught the medical community to fear rapid in-hospital transmission and excess mortality caused by carbapenem resistance (Nordmann *et al.,* 2009).

The importance of *K. pneumoniae* in the ever increasing number of gram negative aerobic bacillary nosocomial infections in the United States (Graybill *et al.,* 1973) and India (Mathur *et al.,* 1991) has been well documented. Epidemic and endemic nosocomial infections caused by *K. pneumonia* species are leading causes of morbidity and mortality (Cryz *et al.,* 1985). Recently, World Health Organization also warned the community that multidrug resistant bacteria are emerging worldwide which is a big challenge to healthcare. If we don't take immediate action then only handful antibiotics will be left to cure diseases (Young Soo, 2011).

This study is aimed to evaluate change in Antibiotic Susceptibility pattern of *Klebsiella pneumoniae* isolated from various clinical samples over a period of Two and a Half year which can guide future treatment option for this organism.

Materials and Methods

This study was conducted at SBKS Medical Institute and Research Centre, Vadodara, Gujarat. Over a period of two and half year (February 2014 to July 2016) total of 416 *Klebsiella pneumoniae* isolated from various clinical samples and their antibiotic sensitivity were studied.

Isolation and Identification of *Klebsiella pneumoniae*

Clinical samples received in sterile container were inoculated on to Blood agar and Mac Conkey agar plates and incubated overnight at 37^0C. *Klebsiella pneumoniae isolates* were identified by their morphology and biochemical characteristics. Morphology of *Klebsiella* identified were large gray dome shaped colonies on Blood agar and lactose fermenting mucoid colonies on Mac Conkey agar. The biochemical characteristics identified were negative Indole test, positive Voges-Proskauer test, positive Citrate utilization test, positive Urease test, acid and gas production from glucose, lactose, sucrose and mannitol sugar fermentation tests (Collee *et al*., 14th Ed) (Betty A. Forbes *et al.,* 12th Ed) (Koneman *et al.,* 6th Ed).

Antimicrobial susceptibility testing

All isolates were subjected to antimicrobial susceptibility testing by modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Hi-Media) and interpreted as per CLSI guidelines (CLSI, 2014). Culture inoculum of the isolate with a turbidity equivalent to 0.5 McFarland standard ($1.5x10^8$ CFU/ml) was prepared and lawn cultured on the Mueller-Hinton agar. Antibiotic discs were applied to the Mueller Hinton agar surface with the help of sterile forceps. The antibiotics chosen for the study were Ciprofloxacin (5 µg), Cefoxitin (30 µg), Gentamicin (10µg), Amikacin (30µg), Imipenem (10µg), Amoxycillin/ Clavulanic acid (20/10 µg), Cotrimoxazole (1.25/23.75µg), Cefuroxime (30 µg), Cefepime (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Cefixime (5 µg). The plates were then incubated at 37°C for 24 hours. Antimicrobial activity was indicated

by an inhibition zone. The diameter of the inhibition zones was measured in millimeter using a calibrated scale. An organism was interpreted as susceptible, intermediate or resistant according to diameter of the inhibition zone.

Results and Discussion

Study was conducted at Clinical Microbiology Laboratory situated in Dhiraj Hospital, SBKS Medical Institute & Research Centre, Piparia, Vadodara.

Total of 416 Klebsiella spp isolated over the period of two and a half year (February 2014 to July 2016) were subjected for Antibiotic Susceptibility testing. Out of 416 isolates maximum were from pus sample (36.78%) followed by Urine sample (33.89 %), Sputum (12.02 %), Blood (9.62 %) and Endotracheal (7.69 %) samples.

Klebsiella pneumoniae isolated from February 2014 – January 2015 was 174 (41.83 %), from February 2015 – January 2016 was 195 (46.87 %) and from February 2016 – July 2016 was 47 (11.30 %).

Antibiotic Susceptibility pattern shows change in sensitivity pattern for most of the antibiotics. Compared to February 2014 – January 2015 there is a visible change in sensitivity pattern in February 2015 – January 2016 and in February 2016 – July 2016. Imipenem showed much reduction in sensitivity 94.25% in February 2014 – January 2015, 70.76 % in February 2015 – January 2016 and 36.17 % in February 2016 – July 2016.

Surprisingly *Klebsiella pneumoniae* shows increase in susceptibility towards Gentamicin and Amikacin. It was 16.66 % in February 2014 – January 2015, 27.17 % in February 2015 – January 2016 and 29.78 % in February 2016 – July 2016 for Gentamicin. It was 29.31% in February 2014 – January 2015, 35.38% in February 2015 – January 2016 and 40.42% in February 2016 – July 2016 for Amikacin.

Klebsiella spp is now considered as one of the important cause of mortality in infectious Diseases due to high level of resistance towards most of the antibiotics available.

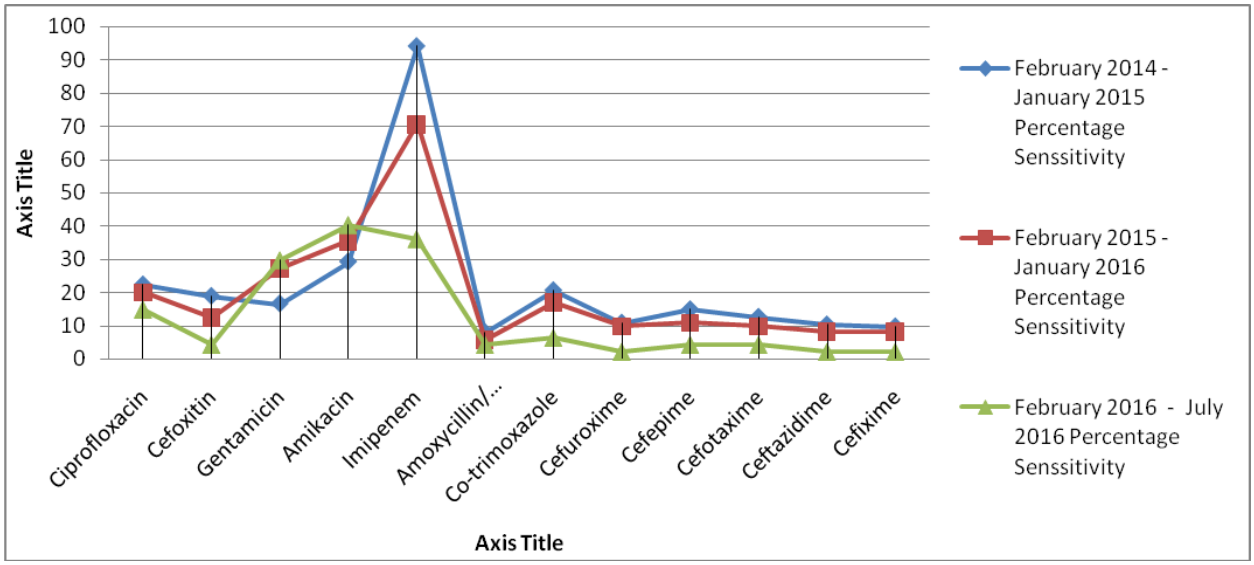
Table.1 Sample wise distribution of *Klebsiella pneumoniae*

Sample	Number of *Klebsiella* Isolated	Percentage
Pus	153	36.78
Urine	141	33.89
Sputum	50	12.02
Blood	40	9.62
Endo Tracheal Secretion	32	7.69
Total	416	100

Table.2 Antibiotic sensitivity pattern of *Klebsiella pneumoniae* over period of two and a half year

Antibiotic	February 2014 - January 2015		February 2015 - January 2016		February 2016 - July 2016	
	No of *Klebsiella* Isolated	Percentage Sensitivity	No of *Klebsiella* Isolated	Percentage Sensitivity	No of *Klebsiella* Isolated	Percentage Sensitivity
Ciprofloxacin	174	22.41	195	20	47	14.89
Cefoxitin		18.96		12.3		4.25
Gentamicin		16.66		27.17		29.78
Amikacin		29.31		35.38		40.42
Imipenem		94.25		70.76		36.17
Amoxycillin/ Clavulanic acid		8.04		5.6		4.25
Co-trimoxazole		20.68		16.92		6.38
Cefuroxime		10.91		9.74		2.12
Cefepime		14.94		10.76		4.25
Cefotaxime		12.64		9.74		4.25
Ceftazidime		10.34		8.2		2.12
Cefixime		9.77		8.2		2.12

Graph.1 Shows change in Antibiotic sensitivity pattern of *Klebsiella pneumoniae* over period of two and a half year



The present study showed decreased sensitivity toward cotrimoxazole (20.68 % in February 2014 - January 2015, 16.92 % in February 2015 - January 2016 and 6.38 % in February 2016 - July 2016) which is comparable with the study done in Pakistan (0 % sensitivity). (Ullah *et al.*, 2009) Such low level of sensitivity is also observed in a study done in Gwalior India (24 %). (Sikarwar *et al.*, 2011).

In our study, amoxicillin-clavulanate sensitivity of *Klebsiella* spp was 8.04 % in February 2014 - January 2015, 5.60 % in February 2015 - January 2016 and 4.25 % in February 2016 - July 2016.While it was 26.95% (2012) and 18.75% (2014) in a study by Sharma *et al.*, 2016.

Such type of sensitivity of 50% and 23.91 % was documented from studies done in Bhopal (Kumar *et al.*, 2013), and in North West Pakistan (Ullah *et al.*, 2009) respectively.

Ciprofloxacin Sensitivity was 22.41 % in February 2014 - January 2015, 20 % in February 2015 - January 2016 and 14.89 % in February 2016 - July 2016. Fluoroquinolones sensitivity in other studies are between 40% and 47% (Ullah *et al.*, 2009) (Sarathbabu *et al.*, 2012). Similarly, sensitivity to fluoroquinolones 22.23 % (2012) and 31.12 % (2014) observed in a study (Sharma *et al.*, 2016). High level of Sensitivity was seen to imipenem 88.06 % (2012) and 86.25 % (2014) in a study by Sharma N *et al.*, 2016. In a study done in Tamil Nadu, India.

sensitivity to imipenem was 86.1 % (Manikandan *et al.*, 2013). Our study showed decrease in sensitivity towards imipenem. Sensitivity to imipenem in our study was 94.25 % in February 2014 - January 2015, 70.76 % in February 2015 - January 2016 and 36.17 % in February 2016 - July 2016.

Study does not include patient data like age, gender and other history shows limitation of this study while large sample size over a long period as strength of this study.

In conclusion, our study shows decrease in sensitivity for Imipenem over the period. It can be due to over usage of imipenem in last few years as it was the most effective antibiotic available for *Klebsiella pneumoniae*.

Our study also shows increase in sensitivity towards gentamicin and amikacin which may be due to less usage of these antibiotics.

This study shows change in antibiotic sensitivity pattern of *Klebsiella pneumoniae*. We are left with very few choice of antibiotics for this type of organisms. Meticulous planning and judicious use of available antibiotics is the need of present time.

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How to cite this article:

Krunal Shah, S. Suguna Hemachander, S.J. Lakhani and Radhika Khara. 2016. Change in Antibiotic Sensitivity Pattern of *Klebsiella pneumoniae*: A Two and Half Year Study. *Int.J.Curr.Microbiol.App.Sci.* 5(9): 549-554. doi: http://dx.doi.org/10.20546/ijcmas.2016.509.061