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Subspeciation and antibacterial susceptibility testing of Klebsiella pneumoniae.

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Abstract :

Introduction: Klebsiella pneumoniae has become a very important cause of nosocomial infections, even replacing Escherichia.coli in some centers. It causes pneumonia, UTI,other pyogenic infections, septicemia and rarely diarrhea. Biochemically variant strains are common. Many strains carry plasmids determining multiple drug resistance, so antibiotic sensitivity should invariably be done.

Objective: The aim of this study is to isolate and subspeciate K.pneumoniae strains and to know the antibiotic susceptibility pattern of various subspecies.

Materials And Methods: 100 non repetitive K. pneumoniae isolates from various clinical samples, both from outpatient and inpatient were processed during a period of November 2011 to October 2012. They were subspeciated by using standard biochemical reactions. Antibiotic susceptibility testing was performed by Kirby Bauer disc diffusion test.

Results: Out of 100 K.pneumoniae 45% were isolated from sputum, 21% were isolated from pus, 20% from urine,7% from vaginal and cervical swabs , 4% from bronchial washings , 3% from blood samples. Of the 100 isolates common subspecies was K.pneumoniae pneumoniae(45%) followed by K.pneumoniae aerogenes(30%) K.pneumoniae ozaenae(17%), K.pneumoniae rhinoscleromatis(8%). The antibiotic susceptibility pattern of various subspecies of K.pneumoniae were as follows, K.p.pneumoniae were 51.1% resistant to gentamycin, 80% resistant to amoxyclav, 93.3% resistant to cotrimoxizole, 6.6% resistance to norfloxacin, 2.2% to nitrofurantoin, 100% to ceftazidime, 22.2% to imipenem. K.p.aerogenes were 23.5% resistant to gentamycin, 11.7% resistant to amoxyclav, 23.5% resistant to cotrimoxyzole,5.8% resistant to nitrofurantoin, 100% to ceftazidime,16.6% to imipenem. K.p.ozaenae were 16.6% resistant to gentamycin, 56.6% resistant to amoxyclav, 60% resistant to cotrimoxyzole, 6.6% resistant to norfloxacin, 100% to ceftazidime,5.88% to imipenem. K.p.rhinoscleromatis were 87.5% resistant to gentamycin, 62.5% resistant to amoxyclav, 75% resistant to cotrimoxyzole,62.5% resistant to norfloxacin, 100% to ceftazidime,25% to imipenem.

Conclusion: The high antibiotic resistance pattern in K.pneumoniae poses great threat to community.

Introduction

Klebsiella pneumoniae has become a very important cause of nosocomial infections, even replacing Escherichia.coli in some centers. It causes pneumonia, UTI,other pyogenic infections, septicemia and rarely diarrhea. Biochemically variant strains are common. The classification of Klebsiella has undergone various modifications. They have been classified into 2 species K.pneumoniae and K.oxytoca. K.pneumoniae is further subdivided in to 4 subspecies K.p.aerogenes, K.p.pneumoniae, K.p.rhinoscleromatis, K.p.ozaenae. Many strains carry plasmids determining multiple drug resistance, so antibiotic sensitivity should invariably be done.

Materials and Methods

The material for present study were collected from out patients and in patients admitted in the department of Surgery, Septicward, Gynecology, Medicine, Paediatrics, Orthopaedics, Nephrology of King George Hospital, Visakhapatnam and from the patients attending Government Hospital for Chest and Communicable Diseases, Visakhapatnam during the period from nov 2011to nov 2012.

Inclusion Criteria: Klebsiella pneumoniae isolated from various clinical samples such as sputum, bronchial washings, pus, urine, blood.

Exclusion Criteria: Klebsiella spp from faeces .

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Sample Collection A total number of 100 Klebsiella pneumoniae were isolated from different samples such Sputum, Bronchial washings, Urine, Pus, Blood, Vaginal and cervical samples.

All the specimens were inoculated on Nutrient Agar, Blood Agar and MacConkey's Agar and incubated aerobically at 37^0c for 18 hours and then examined. All lactose fermenting mucoid colonies from MacConkey medium resemble Klebsiella species were identified by standard microbiological techniques (Table 1).

Antibiotic sensitivity testing was done by Kirby –Bauers disc diffusion method.

Table 1 : Biochemical tests to differentiate various species and sub species of Klebsiella.

Characters	Klebsiella pneumoniae		Klebsiella oxytoca		
Sub species	aerogenes	pneumoniae	ozeanae	rhinoscleromatis	
Indole test	–	–	–	–	+
Methyl red test	–	–	+	+	v
Voges-prosker Test	+	–	–	–	v
Citrate test	+	+	+	–	+
Urease test	+	+	–	–	+
Malonate test	+	+	–	+	+

Results :

Table 2 : Distribution of K.pneumoniae from different samples were as follows(n=100)

S. No.	Sample	No. of isolates	Percentage
1	Sputum	45	45%
2	Pus	21	21%
3	Urine	20	20%
4	Vaginal and cervical swabs	7	7%
5	Bronchial washings	4	4%
6	Blood	3	3%
	Total	100	100%

Of the 100 isolates, 45(45%) were isolated from sputum samples, 21(21%) were from pus samples, 20 (20%) were from urine samples , 7(7%) were vaginal and cervical swabs, 4(4%) were bronchial washings and 3(3%) were blood samples.

Table 3 : Subspecies of K.pnemoniae isolated(n=100).

Subspecies	No of Isolates	Percentage
K.p.pneumoniae	45	45%
K.p.aerogenes	30	30%
K.p.ozaenae 17	17%	
K.p.rhinoscleromatis	8	8%

Of the 100 isolates, common Species was K.p.pnemoniae 45(45%) followed by K.p.aerogenes 30(30%) K.p.ozaenae 17(17%) and K.p.rhinoscleromatis 8(8%) (Table 3).

Table 4 : Antimicrobial resistance pattern of Klebsiella Pneumonie Subspecies

Subspecies	Ceftazidime	Gentamycin	Amoxyclav	Co- trimoxy-zole	Norflo-xacin	Nitrofur -antoin	Imipe-nem
K.p.pneumoniae(n = 45)	45(100%)	23(51.11%)	36(80%)	42(93.33%)	3(6.66%)	1(2.22%)	10(22.2%)
K.p.aerogenes(n = 30)	30(100%)	5(16.66%)	17(56.66%)	18(60%)	2(6.66%)	-	5(16.6%)
K.p.ozaenae(n = 17)	17(100%)	4(23.52%)	2(11.76%)	4(23.52%)	-	1(5.88%)	1(5.8%)
K.p.rhinoscleromatis(n = 8)	8(100%)	7(87.5%)	5(62.5%)	6(75%)	5(62.5%)	-	2(25%)
Total no of K.pneumoniae isolates	100	39	60	70	10	2	18

The percentage of isolation K.p.rhinoscleromatis and K.p.aerogenes are almost same as that of present study but the percentage of isolation of K.p.pneumoniae are less than that of present study and the percentage of isolation of K.p.ozaenae are more than that of present study.

Discussion:

The present study was conducted to isolate and identify K.pneumoniae causing various infections from different clinical samples collected from GHCCD and KGH, a tertiary carehospital, during the period nov 2011 to nov 2012.

K.pneumoniae were predominantly isolated from sputum samples(45%) followed by pus samples(21%),urine samples (20%), vaginal and cervical swabs (7%), bronchial washings(4%), blood cultures (3%). Isolation of K.pneumoniae from sputum samples was high with a significant p value of <0.0001 .

The antibiotic susceptibility pattern of various subspecies of K.pneumoniae were as follows, K.p.pneumoniae were 51.11% resistant to gentamycin, 80% resistant to amoxyclav, 93.33% resistant to cotrimoxyzole, 6.6% resistance to norfloxacin, 2.2% to nitrofurantoin, 100% to ceftazidime,22.2% to imipenem.

K.p.aerogenes were 23.5% resistant to gentamycin, 11.76% resistant to amoxyclav, 23.52% resistant to cotrimoxyzole, 5.88% resistant to nitrofurantoin, 100% to ceftazidime, 16.6% to imipenem.

K.p.ozaenae were 16.6% resistant to gentamycin, 56.6% resistant to amoxyclav, 60% resistant to cotrimoxyzole, 6.6% resistant to norfloxacin, 100% to ceftazidime, 5.88% to imipenem(Table 4).

K.p.rhinoscleromatis were 87.5% resistant to gentamycin, 62.5% resistant to amoxyclav, 75% resistant to cotrimoxyzole, 62.5% resistant to norfloxacin, 100% to ceftazidime, 25% to imipenem.

The percentage of isolation of K.pneumoniae from urine samples were more than that of present study but the percentage of isolation K.pneumoniae from sputum, blood and pus samples were less than that of present study and the percentage of isolation of K.pneumoniae from bronchial washings and vaginal swabs were nearly equal to that of present study.

In the present study , 39%, 50%,100%,70%,10% of K.Pneumoniae isolates were resistant to gentamycin, norfloxacin, 3rd generation cephalosporin, cotrimoxazole and nitrofurantoin respectively. The % of resistance to norfloxacin of present study coincides with that of Mohammed Akram et. al. study. The % of resistance to cotrimoxazole of Mohammed Akram et. al. study was less than that of present study and the % of resistance to nitrofurantoin of Mohammed Akram et. al. study was more than that of present study.

The percentage of Imipenem resistance in present study was nearly equal to the imipenem resistance pattern of Patrica et al and Mohammed Akram et al.

The percentage of imipenem resistance of P. Giakkoupi et al, K.F.Anderson et al and P.Jamima et al are higher than that of present study.

The percentage of imipenem resistance of I .Shukla et al, Kyungwon lee et ai, Ekta Gupta et al are less than that of present study.

Conclusion : Multiple drug resistance was common in Klebsiella pneumonia, 85% of the isolates show resistance to 4 or more antibiotics. This emphasizes the need for Antimicrobial susceptibility testing. The high antibiotic resistance pattern in K.pneumoniae poses great threat to community.

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