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# Biotyping and Antibiotic Susceptibility Pattern of Klebsiella Isolates Recovered From Tertiary Care Hospital

KEYWORDS: Klebsiella, bityping, antibiotic susceptibility

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**ABSTRACT** *Klebsiella species are implicated in causing community acquired as well as hospital acquired infections. In clinical bacteriological laboratories these bacteria are identified only up to genus level and to some extent up to species level. This degree of sub division is of little assistance in hospital epidemiological investigations. The present study was conducted to know the biotypes prevalent in Klebsiella genus by numerical coding system. Out of 200 isolates of Klebsiella , two biotypes were recovered.Klebsiellaoxytoca strains were more resistant to commonly used antibiotics compared to Klebsiellapneumoniae. Klebsiellapneumoniae strains were more sensitive to Imipenem (89%) and Amikacin (75%). Klebsiellaoxytoca strains were also more susceptible toImipenem (80%) and Amikacin (70%) compared to other drugs.*

**Introduction**

Klebsiellapneumoniae is one of the causative agents in community acquired as well as hospital acquired pneumonias. K.pneumoniae and K.oxytoca are implicated in causing hospital acquired infections like urinary tract infections, pneumonias,septicemia and wound infections.[1]

Klebsiella accounts for 6 to 17% of all nosocomial urinary tract infections and shows an even higher incidence in specific groups of patients at risk i.e. patients with neuropathic bladders or with diabetes mellitus. Klebsiella species are second only to E.coli to cause nosocomial Gram negative bacteremias. In clinical bacteriological laboratories these bacteria are identified only to genus level and to some extent species level. This degree of sub division is of little assistance in hospital epidemiological investigations.[2]

To identify the source of infection in hospital out breaks, different typing methods like biotyping, serotyping, phage typing and klebocin typing will be helpful. As clinical isolates of Klebsiella shows considerable differences in biochemical reactions, biochemical charecterisation by biotyping was taken for study which can be useful as a tool in detection of hospital outbreaks.

The genus Klebsiella was classified into five species namely K.pneumoniae, K.oxytoca, K.terrigena,K.ornithinolytica and K.planticola, according to the Bergey's manual of systemic bacteriology.Klebsiellapneumoniae is having three subspecies K.pneumoniae, K.ozaenae and K.rhinoscleromatis.[3]

But according to the recent studies (Drancourt) depending upon 16Sr RNA and rpoB gene, the genus Klebsiella was classified into three species namely,K.pneumoniae, K.oxytoca and K.granulomatis. K.pneumoniaecontain three subspecies Klebsiellapneumoniae, Klebsiellaozaenae and Klebsiellarhinoscleromatis. Species likeK.Terrigena,K. planticola,K.ornithinolytica were kept in separate genus Raoultella.[1]

**Material and Methods**

A total of 4276 samples were collected from patients attending outpatient departments and patients admitted in hospital as in-patients. Samples include urine,sputum,blood,wound swab,pus,endotracheal tube,pleural fluid and tip of intravenous catheter. All the samples were processed in the microbiology laboratory based on conventional methods. Samples were inoculated on MacConkey agar and blood agar. Plates were incubated at 37 c overnight in incubator. Colonies of Klebsiella species were suspected by their large size and mucoid nature on MacConkey agar and blood agar. The colonies were identified by their morphology, Gram stain,negative staining,motility,oxidase test,catalase,nitrate reduction test and reaction in triple sugar iron agar,indole,MR,VP,citrate, urease,lysine,ornithine decarboxylase, arginine dihydrolase and fermentation of sugars like glucose,lactose,sucrose,xylose,maltose,mannitol and dulcitol.

Gram negative short,stout capsulated,non motile, fermenter (lactose fermenting and non lactose fermenting), able to reduce nitrates in to nitrates,catalase test positive and oxidase test negative were biotyped.

Species and biotype identification was done by numerical biotyping by a set of tests

**Coding of biochemical tests**

The biochemical tests were divided into three groups A,B &C, which are detailed in the following tables. The various combinations of reactions in each group of tests were allocated code numbers. The numerical biotype of a strain was expressed by combining the code numbers for each group of tests. Thus a strain with the reactions (− + + −) in group A,(+ + − −) in group B, and (+ + − + )in group C was given a numerical biotype of 1/1/1.[4]

**Table no:1**
**Coding system for numerical biotyping of Klebsiella group A tests**

| INDOLE | VP | CITRATE | MR | CODE |
|---|---|---|---|---|
| − | + | + | − | 1 |
| − | − | + | + | 2 |
| − | + | − | + | 3 |
| − | − | − | + | 4 |

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| | | | | |
|---|---|---|---|---|
| + | + | + | – | 5 |
| + | – | + | + | 6 |
| + | + | – | – | 7 |
| + | – | – | + | 8 |
| + | + | + | + | 9 |
| – | + | + | + | 10 |

**Table no:2**
**Coding system for numerical biotyping of Klebsiella group B tests**

| Lactose | Sucrose | Growth at $5^{O}c$ | $10^{O}c$ | $41^{O}c$ | Code |
|---|---|---|---|---|---|
| + | + | – | – | + | 1 |
| – | – | – | – | – | 2 |
| + | + | – | + | + | 3 |
| + | – | + | + | + | 4 |
| + | – | + | + | – | 5 |

**Table no:3**
**Coding system for numerical biotyping of Klebsiella C tests**

| Dulcitol | Lysine | Ornithine | Urease | Code |
|---|---|---|---|---|
| + | + | – | + | 1 |
| – | + | – | + | 2 |
| + | + | – | – | 3 |
| – | + | – | – | 4 |
| – | – | – | + | 5 |
| – | – | – | – | 6 |
| – | + | + | + | 7 |

**Table no:4**
**Common species of Klebsiella and their numerical codes**

| Klebsiella species | Numerical codes |
|---|---|
| K.pneumoniae | 1/1/1 |
| K.ozaenae | 4/2/3 |
| K.rhinoscleromatis | 4/2/6 |
| K.oxytoca | 5/3/1 |
| K.ornitholytica | 9/4/7 |
| K.planticola | 10/4/2 |
| K.terrigena | 3/5/4 |

Antibiotic susceptibility of all isolates was determined by Kirby – Bauer disc diffusion method on Muller Hinton agar by following CLSI guide lines.

**Results**

Out of 4276 samples processed, 200 isolates of Klebsiella species were recovered. 2 biotypes were distinguished. More number of Klebsiella isolates were recovered from urine samples ie 116 followed by sputum 36,pus 18,blood cultures and wound swab( 10 each),endotracheal aspiration (6),pleural fluid (2),Tip of venous catheter (2).(Table 5) Among the Klebsiella species recovered, K.pneumoniae was the predominant one (90%) compared to K.oxytoca (10%). More number of K.pneumoniaestrains(48%) were recovered from urinary tract infections, followed by pneumonia (18%). All K.oxytoca strains were recovered from urinary tract infections.(Table 6). More number of Klebsiella isolates were recovered between age groups of 20-40 years. Among them K.pneumoniae isolates were recovered more (53%) between the age group of 20-40 years and more number of K.oxytoca were isolated between the age group of 20-30 years.(Table 7)

Coming to the distribution of biotypes, numerical biotype 1/1/1 was the predominant (90%) followed by biotype 5/3/1(10%). Biotype 1/1/1 corresponds to K.pneumoniae and biotype 5/3/1 correspond toK.oxytoca.(Table 8).

Antibiotic susceptibility pattern of Biotype 1/1/1, K.pneumoniae showed that 89% strains were sensitive to Imipenem followed by 75% to Amikacin,56% to Gentamicin,46% to Piperacillin +Tazobactam,43% to cotrimoxazole,13% toceftazidime,35% to ciprofloxacin and 9% to Amoxycillin +clavulanic acid. Susceptibility ofuropathogenicK.pneumoniae to Norfloxacin and Nitrofurantoin was 70% and 34%.But susceptibility of K.pneumoniae to Norfloxacin varied among out patients and inpatients ie 90% and 50% respectively.

Biotype 5/3/1 (K.oxytoca) was highly sensitive to Imipenem(80%) followed byAmikacin(70%),Gentamicin(50%)Piperacillin+Tazobactam(40%),40% to Cotrimoxazole,10% to Ceftazidime,30% to Ciprofloxacin and 0% to Amoxicillin and clavulanic acid. Susceptibility to Norfloxacin and Nitrofurantoin was 30% and 50% respectively. (Table 10)

87% of K.pneumoniae and 90% of K.oxytoca were Extended spectrum β lactamase (ESBL) producers.

**Discussion**

In the present study among 200 strains of Klebsiella two biotypes were identified.90% were 1/1/1(K.pneumoniae) and 10% were 5/3/1 (K.oxytoca). These biochemical pattern was similar to some studies.[5]R.P.Rennieetal conducted studies in 1974 and 1978 on typing of Klebsiella.[4,6] In their studies they reported a higher number of biotypes ie 29 types and 24 numerical biotypesin the year 1974 and 1978 respectively. Identification of less number of biotypes in the present study compared to R.P.Rennieetal may be due to less strains subjected to typing i.e.200 strains compared to 640 strains in their study.[4]

In the present study out of 200 strains of Klebsiella90% were indole negative and 10% were indole positive. But R.P.Rennie et al reported 16% and35% indole positive strains in their studies done in 1974 and 1978.

In the present study biotypes identified were correspond to two species i.e.Klebsiellapneumoniae (1/1/1) and K.oxytoca (5/3/1). This isin correlation with Agarwal et al who reported two species i.e.K.aerogenes (84%) and K.oxytoca (16%).[7] This was also in correlation with other studies. But R.J.Fallon in their study reported isolation of five Klebsiella species i.e.K.aerogenes(44%),K.ozaena (41%),K.edwardsiivaredwardsii(9%), K.edwardsiivaratlantae (3%) and K.pneumoniae (2%).[8]

In the present study out of 200 strains of Klebsiella isolated ,more number of strains recovered from urinary tract infections (58%) followed by other infections. This was in correlation with the study of Aggarwal et al ,who reported more number of Klebsiella strains were isolated from urinary tract infections i.e.42%.[7]

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Among K.pneumoniae isolates 11% showed resistance to Imipenem followed by 25% toAmikacin. Resistance to Amikacin was less in some other studies.[9,10]Gupta et al., from Delhi 17 reported 6.9% of meropenem resistance and 4.3% of imipenem resistance in Klebsiella[11] and a study from Kanpur reported no carbapenem resistance among K.pneumoniae tested[12]. In one study from south India 43.6% of resistance to meropenem, 32% to imipenem, 20.3% to ertapenem was observed.[13]In the present study 87%,65%,30%,66%of strains of K.pneumoniae were resistant to Ceftazidime, Ciprofloxacin,Norfloxacin and Nitrofurantoin respectively.C.Kamatchi et al from Chennai reported 69% of resistance to Ceftazidime,41% of resistance to Ciprofloxacin,39% of resistance to Norfloxacin.

Biotype 5/3/1 (K.oxytoca) was more resistant to antobiotics compared to K.pneumoniae. Imipenem resistance was 20% and resistance to Amikacin was 30%, which is high compared to K.pneumoniae. Samewas observed with other drugs like Ceftazidime 90%,Cprofloxacin 70%,Norfloxacin 70% and Nitrofurantoin 50%,Piperacillin +Tazobactum 60%.

ESBL production was high in our study i.e. 87% in K.pneumoniae and 90% in K.oxytoca.This was observed in some studies.[14]

**Table 5**
**Sample wise distribution of Klebsiella isolates n=200**

| Type of the sample | **Klebsiella isolates** |
|---|---|
| Urine | 116(58%) |
| Sputum | 36(18%) |
| Pus | 18(9%) |
| Blood cultures | 10(5%) |
| Wound swab | 10(5%) |
| Endotracheal aspiration | 6(3%) |
| Pleural fluid | 2(1%) |
| Tip of intravenous catheter | 2(1%) |

**Table 6**
**Klebsiella species isolated from different infections n=200**

| Infections | Total | K.pneumoniae | K.oxytoca |
|---|---|---|---|
| Urinary tract infections | 116 | 96 (48%) | 20(10%) |
| Pneumonias | 36 | 36(18%) | - |
| Pyogenic infections | 18 | 18 (9%) | - |
| Septicemias | 10 | 10(5%) | - |
| Wound infections | 10 | 10 (5%) | |
| Endotracheal aspirations | 6 | 6 (3%) | - |
| Pleural fluid | 2 | 2(1%) | - |
| Intravenous catheter | 2 | 2(1%) | - |
| Total | 200 | 180 (90%) | 20(10%) |

**Table 7**
**Age wise distribution of Klebsiella species n =200**

| Age | Number of Klebsiella isolates | K.pneumoniae | K.oxytoca |
|---|---|---|---|
| 0-10 | 6 | 4(2%) | 2(1%) |
| 11-20 | 24 | 22(11%) | 2(1%) |
| 21-30 | 76 | 66(33%) | 10(5%) |
| 31-40 | 44 | 40(20%) | 4(2%) |
| 41-50 | 20 | 20(10%) | - |
| 51-60 | 14 | 14(7%) | - |
| 61-70 | 10 | 8(4%) | 2(1%) |
| 71-80 | 6 | 6(3%) | - |

**Table 8**

| Species | Typical code |
|---|---|
| K.pneumoniae | 1/1/1 |
| K.oxytoca | 5/3/1 |

**Table 9**
**Distribution of Klebsiella isolates by numerical biotype**

| Biotype | No. of strains (%) |
|---|---|
| Indole negative 1/1/1 | 90 (90%) |
| Indole positive 5/3/1 | 10 (10%) |

**Table 10**
**Antibitic susceptibility pattern of biotypes recovered**

| Antibiotics | Biotype 1/1/1 (K.pneumoniae) % sensitive | Biotype 5/3/1 (K.oxytoca) % sensitive |
|---|---|---|
| Amoxicillin +clavulanic acid | 9% | 0% |
| Gentamicin | 56% | 50% |
| Amikacin | 75% | 70% |
| Ciprofloxacin | 35% | 30% |
| Cotromoxazole | 43% | 40% |
| Ceftazidime | 13% | 10% |
| Piperacillin +tazobactam | 46% | 40% |
| Imipenem | 89% | 80% |
| Norfloxacin | 70% | 30% |
| Nitrofurantoin | 34% | 50% |

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