Isolation, biotyping and antibiotic sensitivity pattern of *Klebsiella pneumoniae* from pus and uterine discharge samples of bovine

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ABSTRACT

It is essential to conduct microbiological testing to both identify as well as to adopt control strategies of infection. Gram negative organisms are involved in a variety of infections. *Klebsiella pneumoniae* has become one of the more common cause of nosocomial infection. One of the more important aspect of *Klebsiella* associated infections is the emergence of multi-drug resistant strains. The present study is conducted to isolate *Klebsiella*; biotype the organism (upto species level); and to see the antibiotic sensitivity pattern as the organism is of global concern. A total of 17 pus and 27 uterine discharge samples are included in the present study. Of the total number of 44 samples processed 28 (63.6 per cent) samples are found to be culture positive for *Klebsiella pneumoniae*. Moreover, the organism is found to be sensitive to antibiotics: enrofloxacin, oxytetracycline and tazobactum; and resistant to: ampicillin and combination of ampicillin and cloxacillin. So it can be said that the present study reveals the incidence of infections due to *Klebsiella pneumoniae* along with the tendency towards resistance to β-lactamase antibiotics. This is a major problem to community health and challenging to animal health care. Revealing the morphological and biochemical properties along with the resistance pattern of *Klebsiella pneumoniae* in north-eastern part of India will help to guide uses of antibiotics appropriately and judiciously. This will also help in formulation of antibiotic policies.

Keywords: Antimicrobial, drug resistance, infection, nosocomial, *Klebsiella pneumoniae*

Abbreviations: ESBL - Extended Spectrum Beta Lactamase

1. INTRODUCTION

In 1883 Friedlander isolated a capsulated bacillus from the lungs of patients who had died of pneumonia (Murray, 2005). This was named after him as Friedlander's bacillus. Later on this organism was given the generic name of *Klebsiella*, which is ubiquitously present and reported worldwide. *Klebsiella* is a gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the Enterobacteriaceae family (Koneman, 2006). It is the second most popular member of the aerobic bacterial flora of the human intestine. It is the most common causative agent of nosocomial and community acquired infections. It has even replaced *Escherichia coli* in some centers as a nosocomial pathogen. It causes pneumonia, urinary tract infection, other pyogenic infections, septicemia and rarely diarrhea (Kapil, 2013). Biochemically typical strains of *Klebsiella pneumoniae* are resistant to a wider range of antibiotics than are most *Escherichia coli* strains. They are nearly always naturally resistant to ampicillin (Murray, 2005). Resistance of *Klebsiella* to previously sensitive antibiotics is also increasing in the recent years due to overuse and misuse of antimicrobial agents and or natural causes. Of particular concern is the Extended Spectrum Beta Lactamase (ESBL) producing *Klebsiella pneumoniae* that have been steadily increasing over the past years and rapidly spreading worldwide that cause a serious threat for healthcare associated infections. Increasingly the ESBL *Klebsiella pneumoniae* are also showing co-resistance to other antimicrobial agents like quinolones and aminoglycoside antibiotics. Both morbidity and mortality is increased when infection is caused by these drug resistant organisms. Antibiotic sensitivity pattern may change from time to time and place to place. Therefore updated knowledge of the drug resistance pattern in a particular region is useful in clinical practice (Ahmad et al., 2009; Sarathbabu et al., 2012). This work gives an account of isolation of *Klebsiella* from clinical pus and uterus discharge samples, their antibiogram and presence of resistant strains in various samples.

2. MATERIALS AND METHODS

Collection of samples

This study was carried from March 2015 to June 2016 at the Department of Microbiology, College of Veterinary Sciences and Animal Husbandry. A total of 17 pus and 27 uterine discharge samples were collected during this period with universal safety precautions and were transported to the laboratory without delay. The pus samples were either aspirated by disposable syringes or collected onto sterile cotton tipped swabs. Samples were obtained from all age groups of dairy cows.

Colony characterization and biotyping of bacterial isolates

Pus and uterus discharge samples were aseptically inoculated on to nutrient agar plates incubated overnight at 37 degree celsius. *Klebsiella* isolates were identified by their morphology and biochemical characteristics. Morphology of *Klebsiella* identified were...
large dome shaped colonies on brain heart infusion agar and lactose fermenting mucoid colonies on Nutrient agar. In gram staining, gram negative, short, plump, straight rods were seen. Negative staining for demonstration of capsule was also performed. The biochemical characters identified were positive catalase test, Voges-Proskauer (VP) test, positive citrate utilization test, positive urease test, acid and abundant gas production from glucose, lactose, sucrose, maltose and mannitol sugar fermentation tests (Koneman, 2006; Gerald collee, 2012).

**Antimicrobial susceptibility testing**

Was done for all the isolates on Mueller- Hinton agar plates by Kirby-Bauer disc diffusion method. The organism was found to be sensitive to antibiotics: enrofloxacin, oxytetracycline and tazobactum; and resistant to: ampicillin and combination of ampicillin and cloxacillin.

### 3. RESULTS

A total of 44 pus and uterine discharge samples were processed during our study period. The number and percentage (%) of culture positive samples have been shown in table 1 and figure 1. Antibiotic sensitivity pattern has been shown in table 2.

<p>| Table 1 |
| Number of culture positive for <em>Klebsiella pneumoniae</em> |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Total No of samples</th>
<th>Number of culture positive samples</th>
<th>% of culture positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus</td>
<td>17</td>
<td>10</td>
<td>58.8</td>
</tr>
<tr>
<td>Uterine discharge</td>
<td>27</td>
<td>18</td>
<td>66.7</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>28</td>
<td>63.6</td>
</tr>
</tbody>
</table>

<p>| Table 2 |
| Antibiotic sensitivity test for <em>Klebsiella pneumoniae</em> |</p>
<table>
<thead>
<tr>
<th>Samples</th>
<th>Enrofloxacin</th>
<th>Oxytetracycline</th>
<th>Tazobactum</th>
<th>Ampicillin</th>
<th>Ampicillin/ Cloxacillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Resistant</td>
<td>Resistant</td>
</tr>
<tr>
<td>Uterine discharge</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Resistant</td>
<td>Resistant</td>
</tr>
</tbody>
</table>
4. DISCUSSION

In animal husbandry numerous infections are caused by *Klebsiella* species. The strains that are responsible for causing infection are biochemically typical members of *Klebsiella pneumoniae* (Murray, 2005). The indiscriminate use of broad spectrum antibiotics have led to development of antibiotic drug resistance as is shown in this particular case study against ampicillin as well as ampicillin and cloxacillin combination. Serious nosocomial infections are caused by such multi-drug resistant strains (Sikarwar and Batra, 2011). This must be taken into account seriously. However in our study sensitivity to enrofloxacin, oxytetracycline and tazobactum are found to be high which is in good agreement with Manikandan and Amsath, 2013.

5. CONCLUSION

Revealing the morphological and biochemical properties along with the resistance pattern of *Klebsiella pneumoniae* in north-eastern part of India will help to guide uses of antibiotics appropriately and judiciously. This will also help in formulation of antibiotic policies.

**SUMMARY OF RESEARCH**

1. *Klebsiella pneumoniae* is ubiquitously present and reported worldwide. It is a gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the Enterobacteriaceae family.
2. Due to indiscriminate uses of antibiotics there is development of antibacterial drug resistance as is shown in case of nosocomial infections caused by *Klebsiella pneumoniae* viz., resistance to ampicillin and combination of ampicillin and cloxacillin.
3. That is why it is necessary to reveal the morphological and biochemical patterns of the organism so that there can be judicious uses of antibiotics in near future against this organism.

**FUTURE ISSUES**

In near future the indiscriminate uses of antibiotics will be minimized/ restricted once antibiotic sensitivity testing is done on regular basis. This will prevent the development of drug resistance strains of the organism *Klebsiella pneumonia* in nature.
DISCLOSURE STATEMENT
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