SHORT COMMUNICATION

Antimicrobial Resistance Pattern of Klebsiella spp. Isolated from Animal Handlers

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ABSTRACT

Klebsiella spp. are ubiquitous members of Enterobacteriaceae that inhabit the gastrointestinal tract of humans and animals. Opportunistic pathogens like Klebsiella pneumoniae and K.oxytoca are capable of causing nosocomial infections of surgical wounds, urinary tract, biliary tract, lower respiratory tract etc. and can also harbour genes coding for antimicrobial resistance (AMR). For the present study, 480 hand and nasal swabs were collected from 120 animal handlers in an attempt to isolate Klebsiella spp. Out of 118 presumptive colonies of Klebsiella spp. obtained by conventional culture method, 67 were confirmed as the same by biochemical tests and polymerase chain reaction that targeted gyrA gene. AMR pattern of those confirmed isolates done by disc diffusion method revealed maximum resistance to penicillin (100%) followed by enrofloxacin (94.73%) and maximum susceptibility to gentamicin (84.21%) followed by tetracycline (68.42%).

Keywords: Klebsiella spp., Animal handlers, Polymerase Chain Reaction (PCR), Antimicrobial Resistance (AMR)

The genus Klebsiella of gram negative, capsulated, non motile bacilli includes 6 species and 3 subspecies. 4 species related to humans include Klebsiella pneumoniae, K.oxytoca, K.granulomatis and K.varicola (Euzéby, 1997). Klebsiella pneumoniae is a leading cause of hospital-acquired (HA) infections and neonatal sepsis globally (Falade et al., 2011). Widely considered an opportunistic pathogen, K. pneumoniae can be carried asymptomatically in the intestinal tract, skin, nose, and throat of healthy individuals (Podschun et al., 1998; Brisse et al., 2001) but can also cause a range of infections in hospitalized patients, most commonly pneumonia, wound, soft tissue, or urinary tract infections. Failure to follow appropriate hand hygiene practices can aid in the spread of multiresistant organisms and has been counted as a significant contributor to infectious diseases outbreaks by the World Health Organisation. K. pneumoniae has been found capable to resist many antibiotics especially third generation cephalosporins like cefotaxime, ceftriaxone and ceftazidime (Yeh et al., 2007). K. pneumoniae mostly contains extended spectrum beta-lactamase genes (SHV, TEM and CTX-M) that are encoded by plasmid. These genes have shown resistance to many types of antibiotics (more than three classes), making the bacteria multidrug resistant (Ahmed et al., 2014). Keeping the above points in mind the present study was designed to isolate Klebsiella spp. from the hand and nasal swabs of animal handlers, to confirm them by biochemical tests and molecular method and to determine their current antibiotic resistance profile.

A total of 480 hand and nasal swabs were collected from 120 individuals handling 6 different species of animals, namely, cattle, sheep and goat, pig, poultry, pet dogs and laboratory animals, with informed consent. The samples were taken from palm of left and right hands, left and right nostrils of each individual animal handler separately using sterile cotton swabs dipped in PBS. Collected samples were kept along with ice packs and immediately transported to Department of Veterinary Public Health and
Epidemiology, Madras Veterinary College and were stored at 4°C till processing.

Isolation attempts for *Klebsiella* spp. was done by streaking the samples on HiCrome™ Klebsiella Selective Agar Base with added Klebsiella selective supplement, incubated at 37°C for 24 hours. After the period of incubation, colonies with purple or magenta colour were taken and stored as glycerol stock of *Klebsiella* species.

Biochemical characterization of the obtained isolates were done by *klebsiella* specific tests (indole, methyl red tests: negative, voges-proskauer, citrate, urease: positive, Triple sugar Iron Agar test: yellow/yellow). DNA extraction was done by boiling and snap chilling method. PCR confirmation of obtained isolates were performed targeting the ‘gyrA’ gene (Brisse et al., 2001) with \( \text{gyrA-A} \) 5’-CGCGTACTATACGCCATGAACGTA-3’ and \( \text{gyrA-C} \) 5’-ACCGTTGATCACTTCGGTCAGG-3’ as forward and reverse primers respectively, yielding a product size of 441 bp. Amplification reactions were carried out in a final volume of 25 µl containing reaction mixture and DNA, subjected to initial denaturation at 94°C for 4 minutes, 30 cycles of denaturation at 94°C for 45 seconds, annealing at 57°C for 45 seconds and elongation step at 72°C for 50 seconds followed by final extension at 72°C for 10 minutes.

The antimicrobial resistance pattern of a maximum of four isolates per category of animal handlers (total of 19 isolates) was determined by Kirby- Bauer disc diffusion method against a group of five antibiotics- Penicillin, ceftriaxone, enrofloxacin, tetracycline and gentamicin as per CLSI guidelines.

Out of 118 presumptive colonies of *Klebsiella* spp. (Fig. 1) which was obtained on selective agar, 67 were confirmed by biochemical tests (Fig. 2) and PCR (Fig. 3).

Most of the bacterial pathogens causing infections in humans come from animals to humans or from humans to animals, either through direct contact or through food products or contaminated water. The present study yielded 67 isolates (13.9%) (67/480) of *Klebsiella* spp. from the hand and nasal swabs of animal handlers. In a similar fashion, other workers had isolated Klebsiella spp from various clinical specimens and showed an isolation rate of 5.11% by Namratha et al. (2015) and 24% by Chakraborthy et al. (2016).

Results of our study indicated the prevalence rate of *Klebsiella* spp. in nasal swabs to be 43.3% (29/67) which
is way too high in contrast to the prevalence of 5.6% from 102 nasal swabs and 96 tissue samples of apparently healthy and sick goats collected from established farms and a local meat market by Aher and co-workers. Variation in the results between these two research findings would be attributed due to various reasons like nature of sampling, source of sampling, processing methodology, location of sample collection etc.

Antibiotic resistance pattern for 19 Klebsiella isolates analyzed by disc diffusion method revealed maximum resistance to penicillin (100%) followed by enrofloxacin (94.7%) and maximum susceptibility to gentamicin (84.21%) followed by tetracycline (68.42%). Aljanaby et al. (2016) obtained 13.168% of K. pneumoniae from a total of 464 samples from patients with urinary tract infections, respiratory tract infections, burns infections and blood infections. Antibiotic susceptibility test results revealed 100% resistance against amoxicillin, 87.5% against ceftriaxone, 31.25% against gentamicin, 34.37% against tetracycline and 43.75% against ciprofloxacin which are similar to results obtained in our present study where in, maximum resistance was observed against penicillin and ceftriaxone, least resistance against tetracycline and gentamicin. 92% susceptibility against ciprofloxacin by Klebsiella isolates was found out by Akter et al. (2013) which is in contrast to our study results that showed 94.73% resistance against enrofloxacin belonging to same class of antibiotics. Pitout et al. (2004) in their study determined that ESBL producing K. Pneumonia strains were 100% resistant to cefpodoxime and piperacillin, 43% resistant to cefotaxime and 14% resistant to ceftazidime which is in favour of our study that showed more resistance against Beta lactam group of antibiotics.

Another study by Asha et al. (2017) on Klebsiella isolates observed maximum resistance (68.5% - 69.5%) to the third generation cephalosporins which is almost similar to our present study results that showed 68.42% resistance to ceftriaxone.

Our study showed the prevalence of Klebsiella spp. from different categories of animal handlers. Resistance of these isolates to the commonly used antibiotics indicate that AMR strains are being circulated, which needs to be combated to encounter treatment failure scenarios and to safeguard human health.

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I am thankful to my guide, teachers and colleagues for their kind support and help during the entire period of my study.

### Table 1: Molecular characterization of Klebsiella isolates with PCR

<table>
<thead>
<tr>
<th>Category of animal handlers</th>
<th>No: of isolates by culture</th>
<th>Percentage Positive (%)</th>
<th>PCR Positive</th>
<th>Percentage Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>14</td>
<td>11.9</td>
<td>7</td>
<td>50</td>
</tr>
<tr>
<td>Sheep &amp; Goat</td>
<td>33</td>
<td>27.9</td>
<td>22</td>
<td>66.6</td>
</tr>
<tr>
<td>Pig</td>
<td>37</td>
<td>31.3</td>
<td>28</td>
<td>75.7</td>
</tr>
<tr>
<td>Poultry</td>
<td>10</td>
<td>8.5</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Pet dog</td>
<td>14</td>
<td>11.9</td>
<td>4</td>
<td>28.57</td>
</tr>
<tr>
<td>Laboratory animals</td>
<td>10</td>
<td>8.5</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>118</strong></td>
<td><strong>67</strong></td>
<td><strong>56.77</strong></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Antibiotic sensitivity test results for Klebsiella isolates

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Number of resistant isolates</th>
<th>Total number of isolates screened</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>19 (100%)</td>
<td>19</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>13 (68.4%)</td>
<td>19</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>4 (21.05%)</td>
<td>19</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>18 (94.7%)</td>
<td>19</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>—</td>
<td>19</td>
</tr>
</tbody>
</table>
REFERENCES


