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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 inhabits the gastrointestinal tract of humans and animals. Opportunistic pathogens like *Klebsiella pneumonia* 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.variicola* (Euzeby, 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-proskeur, 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 '*gyr*A' gene (Brisse *et al.,* 2001) with *gyrA-A* 5'-CGCGTACTATACGCCATGAACGTA-3'and *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 enlongation 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).

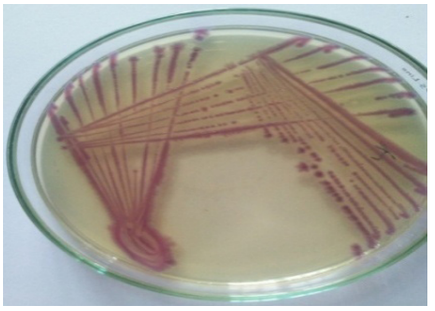

Fig. 1: Purple coloured colonies on selective agar

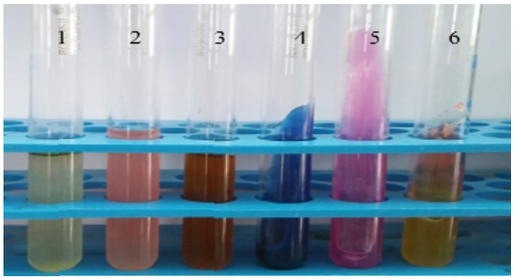

Fig. 2: Biochemical characterization

1. Indole negative, 2. Methyl red negative, 3. Voges Proskeur positive, 4. Citrate positive, 5. Urease positive, 6. TSI – Y/Y

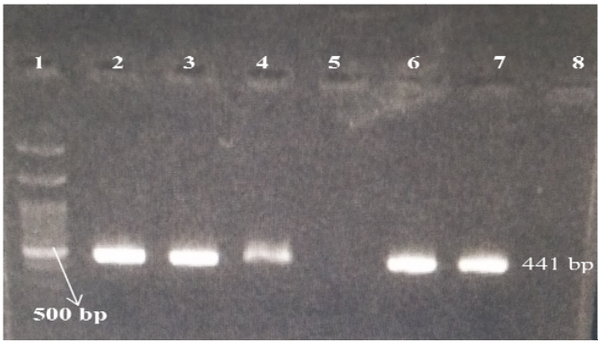

Fig. 3: Agarose gel showing amplification products of gyrA of *klebsiella* spp. (441 bp); Lane 1: 100bp DNA ladder, Lane 2: Positive control, Lane 3, 4, 6, 7: Amplified product of gyrA gene, Lane 5: Negative sample, Lane 8: Negative control

Tabular representation of molecular characterization is given below (Table 1). Maximum harvest of *Klebsiella* spp. sampling site wise was from right hand of pig handlers. The Antibiotic sensitivity test results is summarised below (Table 2).

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

Table 1: Molecular characterization of Klebsiella isolates with PCR

Category of animal handlers	No: of isolates by culture	Percentage Positive (%)	PCR Positive	Percentage Positive (%)
Cattle	14	11.9	7	50
Sheep & Goat	33	27.9	22	66.6
Pig	37	31.3	28	75.7
Poultry	10	8.5	2	20
Pet dog	14	11.9	4	28.57
Laboratory animals	10	8.5	4	40
Total	**118**		**67**	**56.77**

Table 2: Antibiotic sensitivity test results for Klebsiella isolates

Drugs	Number of resistant isolates	Total number of isolates screened
Penicillin	19 (100%)	19
Ceftriaxone	13 (68.4%)	19
Tetracycline	4 (21.05%)	19
Enrofloxacin	18 (94.7%)	19
Gentamicin	—	19

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 rseistance 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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REFERENCES

Aher, T., Roy, A. and Kumar, P. 2012. Molecular detection of virulence genes associated with pathogenicity of *Klebsiella* spp. isolated from the respiratory tract of apparently healthy as well as sick goats. *Isr. J. Vet. Med*., **67**(4): 249-252.

Ahmed, O.I., Soha, A.E., Tamer, M.A. and Iman, Z.A. 2014. Detection of bla SHV and bla CTX-M genes in ESBL producing *Klebsiella pneumoniae* isolated from Egyptian patients with suspected nosocomial infections. *Egypt. J. Med. Hum. Genet*., **14**: 277-283.

Akter, J., Masudul Azad, A.M. and Al Forkhan, M. 2013. Study on prevalence and antibiotic resistance pattern of Klebsiella isolated from clinical samples in south east region of Bangladesh. *Am. J. Drug Discov. Dev*., **4**: 73-79.

Aljanaby, A.A.J. and Alhasani, A.H.A. 2016. Virulence factors and antibiotic susceptibility patterns of multidrug resistance *Klebsiella pneumoniae* isolated from different clinical infections. *Afr. J. Microbiol. Res*., **10**(22) : 829-843.

Asha, A., Karnaker, V. K. and Rekha Rai. 2017. Characterization and Antibiogram of Klebsiella isolated from clinical samples. *Int. J. Curr. Microbiol. App. Sci*., **6**(7): 386-396.

Brisse, S. and Verhoef, J. 2001. Phylogenetic diversity of *Klebsiella pneumonia* and *Klebsiella oxytoca* clinical isolates revealed by randomly amplified polymorphic DNA, gyrA and parC genes sequencing and automated ribotyping. *Int. J. Syst. Evol. Microbiol*., **51**: 915-924.

Chakraborty, S., Mohsina, K., Sarker, P.K., Alam, M.D., Karim, M. and Sayem, S.M. 2016. Prevalence, antibiotic susceptibility profiles and ESBL production in *Klebsiella pneumoniae* and *Klebsiella oxytoca* among hospitalized patients. *Period biol*., **118**(1): 53-58.

Euzeby, J.P. 1997. List of Bacterial Names with Standing in Nomenclature: a Folder Available on the Internet. *Int. J. Syst. Bacteriol*., **47**(2): 590–592.

Falade, A.G. and Ayede, A.I. 2011. Epidemiology, aetiology and management of childhood acute community-acquired pneumonia in developing countries—a review. *Afr. J. Med Sci.,* **40**(4): 293–308.

Namratha, K.G., Sreeshma, P., Subbannayya, K., Dinesh, P.V. and Champa, H. 2015. Characterization and Antibiogram of *Klebsiella* spp. Isolated from Clinical Specimen in a Rural Teaching Hospital. *Sch. J. App. Med. Sci*., **3**(2E): 878-83.

Pitout, D.D., Hossain, A. and Nancy D.H. 2004. Phenotypic and Molecular Detection of CTX-M- β-Lactamases Produced by *Escherichia coli* and *Klebsiella* spp. *J. Clin. Microbiol*., **42**(12): 5715–5721.

Podschun, R. and Ullmann, U. 1998. *Klebsiella* spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin. Microbiol. Rev*., **11(**4): 589–603.

WHO Guidelines on Hand Hygiene in Healthcare, 2009. World Health Organization, Geneva, Switzerland.

Yeh, K.M., Kurup. A., Siu, L.K., Koh. Y.L., Fung, C.P., Lin, J.C., Chen, T.L., Chang, F.Y. and Koh, T.H. 2007. Capsular serotype K1 or K2, rather than magA and rmpA, is a major virulence determinant for *Klebsiella pneumoniae* liver abscess in Singapore and Taiwan. *J. Clin. Microbiol*., **45**(2): 466-471.