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Occurrence of Klebsiella pneumoniae and Salmonella spp. in environmental samples

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Abstract

The present study was undertaken for the isolation and identification of Klebsiella pneumoniae and Salmonella spp. from environmental samples. A total of 120 soil and 120 water samples were collected from ponds and rivers in Thrissur. The samples were subjected to conventional culture techniques and K. pneumoniae and Salmonella spp. isolates were detected in 57.50 and 14.58 per cent samples, respectively. There were no significant differences between the occurrence of K. pneumoniae and Salmonella spp. in different soil and water samples, (p≥0.05). The isolates were subjected to molecular confirmation targeting rpoB gene for K. pneumoniae and invA gene for Salmonella spp. Out of the 138 K. pneumoniae and 35 Salmonella spp. isolates analysed, 86.96 and 97.14 per cent isolates were found positive on PCR. The results of the study revealed that environment acts as an important reservoir of K. pneumoniae and Salmonella spp.

Keywords: *Klebsiella pneumoniae*, *Salmonella spp., environment, water*

Gram negative bacterial infections are highly significant public health concern worldwide. Among the Gram negative bacterial agents, *K. pneumoniae* and *Salmonella* spp. are important in terms of virulence and pathogenicity. These bacteria are often detected in the environment from which they enter the human body directly and through contamination of food materials. *Klebsiella pneumoniae* is an opportunistic pathogen and causes several types of infections in humans, including respiratory tract, urinary tract and bloodstream infections. These infections usually occur but are not limited to hospitalised or immunocompromised patients. The pneumonia caused by *Klebsiella* spp. causes mortality in as high as 50 per cent of the patients (He *et al*., 2016). In addition, they have an ability to adhere to the surfaces of urinary catheters and ventilators, forming biofilms causing catheter-associated urinary tract infections and ventilator-associated pneumonia, making it the common cause of death in patients admitted to intensive care units. The bacteria are main agents in the transmission and maintenance of antimicrobial resistance (AMR). The species

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Table 1. Primers used for PCR

Genes	Primer	Primer sequence	Size (bp)	Reference
*rpo*B	F R	5'CAACGGTGTGGTTACTGACG 3' 5'TCTACGAAGTGGCCGTTTTC 3'	108	Bobbadi *et al.* (2020)
*inv*A	F R	5'ATCTCCGTTGCACTCTTTGC 3' 5'ACCATCATCATCATCGTCCA 3'	244	Amini *et al*. (2010)

Table 2. Thermal cycling conditions for amplification

Steps	*rpo*B gene		*inv*A gene	
Initial denaturation	95°C; 5 min		94°C ;1 min	
Denaturation	95°C;1 min	35 cycles	94°C; 30 sec	35 cycles
Annealing	55°C; 1 min		60°C ; 30 sec	
Extension	72°C ;2 min		72°C; 2 min	
Final extension	72°C ;10 min		72°C; 10 min	
Hold	4°C; 10 min		4°C; 10 min	

Table 3. Occurrence of *K. pneumoniae* in water and soil samples

Sl. No.	Source	Samples Analysed	Positive Samples		*Chi*- square	p-value
			Number	Per cent		
1.	Pond Water	60	42	70.00	0.463^{ns}	0.496
2.	Pond Soil	60	37	61.67		
3.	River Water	60	33	55.00	0.866^{ns}	0.352
4.	River Soil	60	26	43.33		
Total		**240**	**138**	**57.50**		

p< 0.05 – significant, ns - Non significant

Table 4. Occurrence of *Salmonella* spp. in water and soil samples

Sl. No.	Sample	Samples Analysed	Positive Samples		*Chi*- square	p-value
			Number	Per cent		
1.	Pond Water	60	10	16.67	3.718^{ns}	0.054
2.	Pond Soil	60	6	10.00		
3.	River Water	60	10	16.67	3.765^{ns}	0.052
4.	River Soil	60	9	15.00		
Total		**240**	**35**	**14.58**		

p< 0.05 – significant, ns - Non significant

K. pneumoniae is again constituted by three different subspecies; *K. pneumoniae* subsp. *pneumoniae, K. pneumoniae* subsp*. ozaenae* and *K. pneumoniae* subsp*. Rhinoscleromatis* (Ko *et al.*, 2002).

Genus Salmonella is composed of two separate species; *S. bongori* and *S. enterica* and encompasses over 2500 known serotypes, all of which are considered to have the potential to cause diseases in humans. *Salmonella* spp. shows different types of disease manifestations in human *viz*., enteric fever, gastroenteritis, bacteraemia and other extra intestinal complications and chronic carrier state. Salmonella is responsible for 93.8 million cases of gastroenteritis worldwide annually with 1,55,000 deaths (Grimont and Weill, 2007). Considering the gravity of the infections caused by these bacteria, the present study was carried out to study the occurrence of *K. pneumoniae* and *Salmonella* spp. in environmental samples.

Materials and methods

A total of 120 water samples were collected from Thrissur district, which included

pond water samples (60) and river water samples (60). From the respective pond sides and river banks, 60 soil samples each were also collected. The samples were collected during a span of 10 months, from September 2019 to July 2020. Sampling was performed across the three river basins in Thrissur district *viz*., Chalakkudy, Karuvannur and Kechery.

Approximately 250 mL water sample and 100g soil sample each were collected from each sampling site in sterile containers. The samples were then transported and processed in the Quality Control Laboratory, Department of Veterinary Pubic Health, College of Veterinary and Animal Sciences, Mannuthy. All the samples were subjected to isolation and identification of *K. pneumoniae* (Kumar *et al*., 2013) and *Salmonella* spp. (APHA, 2012) by conventional culture techniques with some modifications.

For isolation of *K. pneumoniae*, samples were enriched on nutrient broth (NB). Five grams of soil sample was transferred to 45 mL NB, which was incubated at 37°C for 24 h. In case of water samples, five millilitres of each sample was transferred to 45 mL NB. This was followed by selective plating onto MacConkey agar (MCA) and incubation at 37°C for 24 h. Characteristic large, pink mucoid colonies were selected and further confirmed by biochemical tests (Fig. 1). For isolation of *Salmonella* spp., the samples were enriched on tetrathionate (TT) broth, followed by selective plating on Xylose Lysine Deoxycholate (XLD) agar and incubated at 37°C for 24 h. Characteristic red colonies with black centres were selected and later confirmed by biochemical tests (Fig. 2). The significance of occurrence of *K. pneumoniae* and *Salmonella* spp. among different sources was analysed using chi-square test with the help of IBM – SPSS version 24.0.

The isolates were further subjected to molecular confirmation by polymerase chain reaction (PCR) targeting *rpo*B gene for *K. pneumoniae* (Bobbadi *et al.*, 2020) and *inv*A gene for *Salmonella* spp. (Amini *et al*., 2010). The primer sequences and cyclical conditions of the genes are mentioned in Table 1 and Table 2.

Results and discussion

Out of the 240 samples analysed, 138 (57.50 per cent) were found positive for *K. pneumoniae* by conventional culture techniques (Fig. 1). Of this, 54.35 per cent were from water samples and the rest 45.65 per cent were isolated from soil samples. On statistical analysis, there was no significant difference ($p \geq 0.05$) between the occurrence of *K. pneumoniae* in different water and soil samples (Table 3). Similar results were obtained in studies conducted by Podschun *et al.* (2001) in Germany and Tafoukt *et al*. (2017) in Algeria.

In case of *Salmonella* spp., 35 out of the 240 samples analysed (14.58 per cent) were found positive (Fig.2). Of this, water samples and soil samples constituted 57.14 per cent and 42.86 per cent, respectively. There was no significant difference ($p \geq 0.05$) between the occurrences of *Salmonella* spp. in different water and soil samples (Table 4). In a study

Fig. 1. *Klebsiella pneumoniae* on MCA

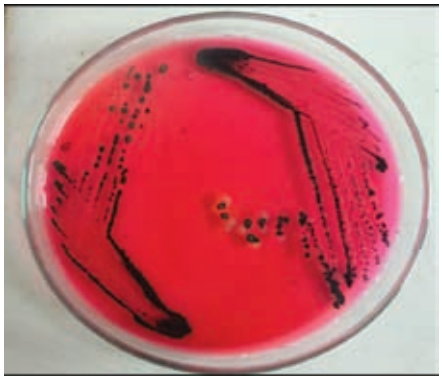

Fig. 2. *Salmonella* spp. on XLD Agar

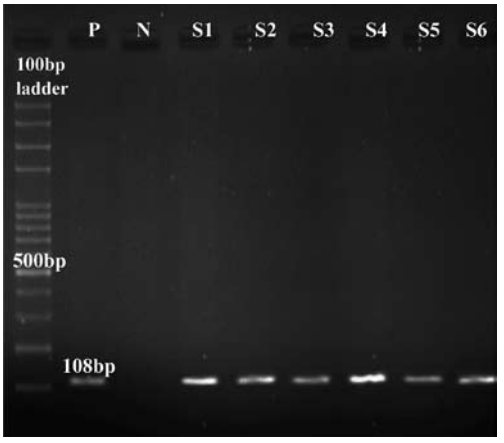

Fig. 3. Identification of *rpo*B gene. P- Positive Control N- Negative Control S1, S2, S3, S4, S5, S6- Samples

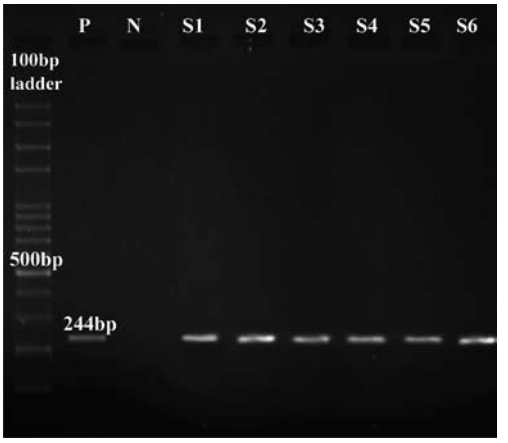

Fig. 4. Amplification of *inv*A gene. P- Positive Control. N- Negative Control. S1, S2, S3, S4, S5, S6- Samples

conducted by Keelara *et al*. (2013) in USA, 11.7 per cent of environmental samples were found to be positive for *Salmonella* spp., which accorded with the present study.

The isolates were then subjected to PCR for molecular confirmation. To confirm *K. pneumoniae* isolates, the house keeping gene *rpo*B was used. He *et al*. (2016) reported this gene to be a suitable PCR target for species level differentiation of *K. pneumoniae.* Of the 138 *K. pneumoniae* isolates identified by culture techniques, 120 isolates (86.96 per cent) were found positive (Fig. 3) by PCR. In a study conducted by Bobbadi *et al.* (2020) in Andhra Pradesh, 90 per cent of *K. pneumoniae* isolates were confirmed by PCR targeting *rpo*B gene.

To confirm *Salmonella* spp. isolates, conserved virulence gene *inv*A was used. Of the 35 isolates identified as *Salmonella* spp. by conventional culture techniques, 34 (97.14 per cent) generated amplicons specific for *inv*A (Fig. 4). Chaudhary *et al.* (2015) from Ahmedabad analysed different environmental samples for the presence of *inv*A and concluded that it was the major gene coding for the virulence of *Salmonella* spp.

The results of the present study show that environment could be a potent source of *K. pneumoniae* and *Salmonella* spp. The pathogens gain entry to the human body directly, causing infections in susceptible patients. In addition, pond and river water are routinely used for the irrigation of agricultural fields, paving way for the contamination of fresh produce by the bacteria. There is also high chance of acquisition of resistant infections as more often than not, these bacteria may contain resistance genes, making the infections untreatable with many of the routine antibiotics.

Conclusion

The present study demonstrated that antibiotic resistant *K. pneumoniae* and *Salmonella* spp. are present in the environment in abundance. A coordinated One Health approach is required at local and national level taking into account the role of environment in antibiotic resistance transmission, so that appropriate control measures can be put in place to limit the emergence and spread of AMR. This entails an integrated approach of surveillance at human, animal and environmental systems which should be coupled with awareness and education of public and professionals to contain global resistance load.

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Conflict of interest

The authors declare that they have no conflict of interest.

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