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ANTIMICROBIAL RESISTANCE AMONG *E. COLI* & *K. PNUMONIAE* ISOLATED FROM HINDON RIVER

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ABSTRACT

Hindon River originates from the upper Shivalik in lower Himalayan range of District Saharanpur Uttar Pradesh (India). The river passes through six districts, including Noida and Ghaziabad, in Uttar Pradesh before it meets the Yamuna ahead of Dankaur (Uttar Pradesh). Two of its tributaries – Kali River and Krishna River - in Ghaziabad are also equally polluted like the main river. Paper mills, sugar manufacturing plants, alcohol distillation units and slaughterhouses located at places like Saharanpur, Muzaffarnagar and Baghpat are blamed for the pollution. The Hindon Air Force Base of the Indian Air Force also lies on its bank in the Ghaziabad district on the outskirts of Delhi. The aim of this study was to assess the level of different Parameters (Isolation of *E.coli* & *K.pneumoniae,* Biochemical test & Antimicrobial susceptibility test) in Hindon River at Indrapuram, Ghaziabad (Delhi). A total of 4 stations, covering the upstream and downstream sites of Hindon, were selected for this study.

KEYWORDS: *E. coli*, *K. pneumonia*, Hindon River.

INTRODUCTION

Hindon River, a tributary of Yamuna River, is a river in India that originates in the Saharanpur District, from Upper Shivalik in Lower Himalayan Range.[1][2] The river is entirely rainfed and has a catchment area of 7,083 square kilometers.[4][5] The main tributary of Hindon River is Kali River; it originates from the Doon valley and travels 150 kilometers passing through Saharanpur, Muzaffarnagar, Meerut and Baghpat districts of Uttar Pradesh.[3] The Kali River is too polluted river, the industrial waste from the Saharanpur adds into it and then it add to Hindon and waste water from the industrial belt from Saharanpur to Noida

release into the Hindon further treatment or not.[9][10][13] Effluent discharged from sugar and paper mills, slaughter houses and chemical industries in Uttar Pradesh(India) are degrade the water quality in the Hindon, so much so that the water is not fit for bathing.[6][7][8] The Central Pollution Control Board (CPCB) submitted an affidavit in the National Green Tribunal showing that the water in the Hindon River does not meet the prescribed standard of primary water quality criteria for bathing.[16][17][18] Government take the appropriate steps for set up the STP(Sewage Treatment plant) on the Hindon river for the treatment of waste water and give the instructions to industries for set up Effluent treatment plant for the treatment of effluent before release into the river.[14] But the reality is that there is not so good working for the waste water treatment on Hindon River, further most of the STP plants are not in good working condition and industries not set up well efficient working ETP plant.[15] Villagers say that the situation was different a decade ago.

METHODOLOGY

We have selected a Hindon river tributary from Indrapuram (Ghaziabad) over a length of about 4 km adjacent to which a flyover is being constructed it is placed at Latitude 28.641485, Longitude: 77.371385 and many of the industries which are installed near Indrapuram released their effluent in it. Four Sampling sites were selected to obtain the sample of water over a time period of Three months and tests such as Isolation of *E.coli* & *K. pneumonia*, Biochemical test, Antimicrobial susceptibility Test & Phenotypic Detection Test will be carried. Total 21 samples were taken and tested in laboratory to give the results.

All the chemicals and reagents used were of analytical grade and were procured from Hi Media (India). All glass wares and other containers were thoroughly cleaned and finally rinsed with double glass distilled water several times prior to use.

Soon after collection, samples were screened for the presence of bacterial isolates using Luria agar and Luria Broth. Lactose fermenting Gram negative bacterial colonies were initially assessed based on their characteristic growth on MacConkey agar and Eosin Methylene Blue (EMB) agar followed by the IMViC standard biochemical tests (Clinical and Laboratory Standards Institute, 2010).

Antibiotic Susceptibility Test & Phenotypic Detection Test

After identification, screening for ESBL production was performed Ceftazidime(30mcg), Cefpodoxim(10mcg), cefotaxime(30mcg), Ampicillin(10mcg), ciprofloxacin(5mcg),

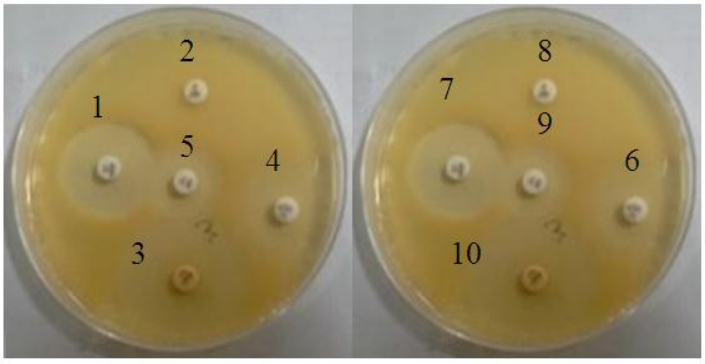
Amikacin(30mcg), Metronidazole(4mcg), Meropenem(10mcg), Azithromycin(15mcg) and ceftriaxone(30mcg)by the Kirby Bauer disc diffusion method using Mueller Hinton Agar (MHA) plates. Phenotypic detection Test was then performed by placing discs containing cephalosporin alone (Cefotaxime(30mcg), Ceftazidime(30mcg), Cefepime(30mcg), Cefepirome(30mcg), alone and combination with clavulanic acid (10mcg).

Ceftazidime (30 µg) and Cefotaxime (30 µg) alone and in combination with clavulanic acid (10 µg) 30 mm apart on MHA plates. A ≥5 mm increase in zone diameter around the disc with antibiotic plus clavulanic acid relative to the discs with antibiotics alone was considered positive for ESBL production (Clinical and Laboratory Standards Institute, 2012).

RESULTS

In total, 14*E. coli*and 7 *Klebsiella Pneumoniae* were isolated non selective conditions (according antibiotic resistance). All the selected wild-type *E.coli* & *K. pneumonia* strains were further confirmed by biochemical test and the results of all these tests were similar for all the strains. The presence of the acquired resistances in the total population was tested for their susceptibility to 10 antibiotics.

Almost all ESBL-producing isolates (80%) were multiresistant (resistant to three or more antibiotic groups). ESBL positive *E.coli* & *K.pneumoniae* isolates showed resistance to Ceftazidime(30mcg) (82%), Cefpodoxim(10mcg) (56%), Cefotaxime(30mcg) (72%), Ampicillin(10mcg) (100%), Ciprofloxacin(5mcg) (100%), Amikacin(30mcg) (11٪), Metronidazole(4mcg) (70٪), Meropenem(10mcg) (30٪), Azithromycin(15mcg) (59%) and Ceftriaxone(30mcg) (71%). High levels of resistance to 7 to 8 antimicrobial agents were observed in the presence of multidrug-resistant ESBL & PMQR-producing *E.coli* & *K.pneumoniae.*



A **B**

Figure 1: Mueller-Hinton agar plate showing Antibiotic susceptibility testing of *E.coli* & *K. pneumonia* strains by disk diffusion test.

Table 1: Scoring of antibiotic resistance of (n = 21).

S.No.	Antibiotics	No. (%) of resistant strains	No. (%) of susceptible strains
1	Ampicillin	100 %	0 %
2	Cefotaxime	72%	28 %
3	Ceftriaxone	71 %	29 %
4	Ciprofloxacin	100 %	0 %
5	Cefpodoxim	56 %	44 %
6	Ceftazidime	82 %	18 %
7	Metronidazole	70 %	30 %
8	Meropenem	30 %	70 %
9	Azithromycin	59 %	41 %
10	Amikacin	11 %	89 %

Phenotypically conformation of ESBL&PMQR

Almost all 73% were showed ESBL Positive *E.coli* and *Klebsiella Pneumoniae* isolates showed resistance to cephalosporin alone (cefotaxime(30mcg) alone and with Clavulanic acid(10mcg) 73%, ceftazidime(30mcg) alone and with clavulanic acid (10mcg) 74%, Cefepime(30mcg) alone and with Clavulanic acid (10mcg) (73%), and cefepirome(30mcg) alone and combination with clavulanic acid (10mcg) 73%.

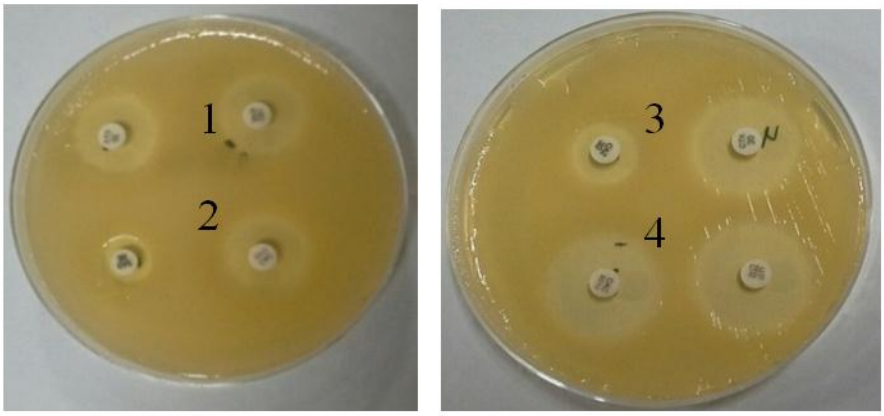


Figure 2: Mueller-Hinton agar plate showing ESBL&PMQR production of *E. coli*&*K. Pneumonia* strains detected by Phenotypic disk confirmatory test.

Table 2: Scoring of antibiotic resistance of (n = 21).

S.No.	Method	Antibiotic Disk	No (%)of Resistant ESBL producing strains
1	PDCT	Cefotaxime alone and with Clavulanic acid	73 (%)
2	PDCT	Ceftazidime alone and with Clavulanic acid	(74%)
3	PDCT	Cefepime alone and with Clavulanic acid	(73%)
4	PDCT	Cefepirome alone and with Clavulanic acid	(74%)

CONCLUSION

On the basis of above parameters we can easily say that the Hindon river is highly polluted, due to the industrial effluents released into it. The limit of above parameters in the river is so high than the permissible limit. This water is neither able to use in washing, nor be in irrigation. The levels of different parameters which are so common parameters to represent the characteristics of water and waste water like Antibiotic Resistance bacteria of *E.coli* & *K. pneumonia.* Out of the four sites (From where the sample is collected), on the site, where the high amount of industrial effluent released into the river, the level of parameter has high than the other site. So, on the basis of above data we can easily say that the Hindon River is most polluted river of Uttar Pradesh (India), which also degrade the ground water. The ground water of most of the villages, towns and cities, which locate on the bank of this river, become highly polluted with yellow colour appearance. Many of the persons in those villages suffered from Diarrhea & Pneumonia after the use of this polluted water from the long time. So, there are so drastic conditions for the villagers and those who used to live near its bank.

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