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Antibiotic Resistance Pattern of Bacterial Isolates Obtained from Two Ponds of Patna (Bihar), India

Pallavi

Assistant Professor, Department of Botany, Ganga Devi Mahila Mahavidyalaya, Patna
(Constituent College Under Patliputra University), Kankarbagh, Patna-800020, Bihar, India

Abstract: ***Pathogenic coliform bacteria present in water bodies remain harmful and became much harmful in case of development of multidrug resistance within them. The antibiotic resistance pattern of pathogenic bacteria was evaluated during present research work. Out of the total isolates (83) of E. coli obtained from the water sample of Gardanibagh Kacchi Talab, 32.53% showed multidrug resistance against all tested antibiotics. 18.52% of K. preumoniae, 34.62% of P. aeruginosa and 66.67% of Salmonella sp. obtained from the water sample of this pond showed multidrug resistance against all tested antibiotics. As well as, out of the total isolates (40) of E. coli obtained from water sample of Adalatganj pond, 60% showed multidrug resistance against all four tested antibiotic drug during present research work. 19.44% of K. peumoniae and 48.15% of P. aeruginosa obtained from water samples of this pond exhibited multidrug resistance. Cent per cent Salmonella sp. obtained from this pond exhibited multidrug resistance. These pathogenic bacteria remain as causative agent for several human diseases such as dysentery, diarrhoea, cholera, typhoid etc. Thus, the result of present research work will remain helpful in direction of the protection of human health of the adjoing areas of these ponds.***

Keywords: Antibiotic, Resistance, Gardanibagh Kacchi Talab, Adalatganj Pond.

1. Introduction

Microbes are essential for recycling biomass and maintain the chemistry of any aquatic ecosystem. Microorganisms can be applied to device solutions for environmental problems created by humans. Thus microbiology has much global relevance and is of vital importance to the future of humankind. The chemical and microbial contamination of water is a global health concern. Proper monitoring and management of water distribution system has recently generated substantial interest among researchers (Rajabir *et al*., 2020). Water-borne diseases are a major concern in economically developing countries such as India, where water supply and sanitation service do not keep pace with increasing population growth, urbanization and industrialization. Inadequate water availability, poor quality of the water supply, poorly maintained water and sewage pipelines, improper disposal of human, animal and household wastes, and lack of awareness among people about good sanitation are the main factors that are responsible for water pollution (Rajabir *et al*., 2020).

Pathogenic *E. coli* remain responsible for bloody diarrhea in humans. It is also a common pathogen of human urinary tract infections (Swerdlow *et al*., 1992). Skorczewski *et al*. (2013) observed that fecal coliform bacteria obtained from a pond of Poland showed resistance to Ampicillin and sensitivity to Gentamycin. They also observed high value of multiple antibiotic resistance for these fecal coliform bacteria. The level of antibiotic resistance in fecal bacteria can be used to determine the impact of anthropogenic activities on aquatic ecosystem. The fecal coliform bacteria develops antibiotic resistance in polluted water bodies with hospital and domestic wastes.

Thus, it is clear that assessment of water ecosystems remain as current task for Microbial Biotechnologists with regard to presence of antibiotic resistant fecal coliforms in water bodies. The rising trend of the use of antibiotics leads to evolution of resistance in fecal coliform bacterial strains. The increasing prevalence of antibiotic resistant bacterial strains poses serious threat to human health. Karaca *et al*. (2023) analysed the antibiotic susceptibility level of *E. coli* isolated from the water samples obtained from Orontes river (Turkey). They also observed highest level of resistance to ampicillin as 40%. During their research study *E. coli* isolates exhibited susceptibility against twelve types of antibiotics at different level. Thus present research work related with evaluation of antibiotic resistance pattern of fecal coliform bacterial isolates obtained from two lentic water bodies of Patna town.

2. Materials and Method

During this research work antibiotic sensitivity of obtained bacterial isolates from Adalatganj Pond and Gardanibagh Kacchi Talab (Patna) were examined to assess the level of pollution with different types of antibiotics in these lentic ecosystems. Antibiotic resistance is one of the characteristics of fecal coliform bacteria to determine the anthropogenic impact on these water bodies. These water bodies of Patna town are characterized by different level of anthropogenic activities. Clean and sterilized plastic bottles of one litre capacity with screw cap were used for sampling of surface water from two ponds of Patna town. Sampling was conducted in the month of July, 2024. Collected samples containing bottles were carried to the laboratory and preserved at 4^{o}C in a refrigerator for further bacteriological analysis. Disposable gloves were worn during sampling of water.

Identification of bacteria was conducted by Gram staining technique, counting of colony forming units, IMVIC test, oxidation-fermentation test and oxidase test. Antibiotic sensitivity tests for bacterial isolates were conducted with four types of antibiotic drugs such as Amplillin, Erythromycin, Gentamycin and Tetracycline by Kirby-Bauer Disc diffusion method.

3. Result and Discussion

All together 247 bacterial colonies of fecal origin were identified after culture of samples obtained from two ponds of Patna town. Out of 247 colonies, 123 were of *E. coli* 63 of *Klebsiella pneumoniae,*53 of *Pseudomonas aeruginosa* and 8 of *Salmonella* sp. 83 colonies of *E. coli,* 27 colinies of *K. pneumoniae,* 26 colonies of *P. aeruginosa* and 06 colonies of *Salmonella* sp. were identified in water samples of Gardanibagh Kacchi Talab. 40 colonies of *E. coli*, 36 colonies of *K. pneumoniae,* 27 colonies of *P. aeruginosa* and only two colonies of *Salmonella* sp. were identified in water sample obtained from Adalatganj Pond (Table-1).

The zone of inhibition affected by Ampicillin upto 11 mm, Gentamycin upto 12 mm, Erythromycin upto 13 mm and Tetracycline upto 14 mm indicates about resistant nature of respective bacteria. The zone of inhibition above these data indicated about susceptible or intermediate nature of bacterial isolates with respect of tested antibiotics (Table-2).

Table 1: Bacterial colonies observed in water samples of the Ponds of Patna (on culture medium CFU/ml)

Sl. No.	Bacterial isolates	No. of bacterial Isolates obtained		
		Gardanibagh Kacchi Talab	Adalatganj Pond	Total
1.	*Escherichia coli*	83	40	123
2.	*Klebsiella pneumoniae*	27	36	63
3.	*Pseudomonas aeruginosa*	26	27	53
4.	*Salmonella* sp.	06	02	08
	Total	142	105	247

Table 2: Standard Zone of Inhibition pattern (in mm) of Bacteria against different antibiotics drugs

S. No.	Antibiotics	Disc conc.	Diameter of Zone of Inhibition		
			Resistant	Susceptible	Intermediate
1.	Ampicillin	10µg	≤11	≥14	12-13
2.	Erythromycin	15µg	≤13	≥18	14-17
3.	Gentamycin	10µg	≤12	≥15	13-14
4.	Tetracycline	30µg	≤14	≥19	15-18

Note: As per Kirby-Bauer Antibiotic Susceptibility Test Manual.

Table 3: Antibiotic Resistance Pattern of Bacterial isolates obtained from Gardanibagh Kacchi Talab

Sl. No.	Bacterial Isolates	Antibiotic Resistant Isolates							
		Ampicillin		Erythromycin		Gentamycin		Tetracycline	
		No.	%	No.	%	No.	%	No.	%
1.	*Escherichia coli*	83	100	69	83.13	59	71.08	58	69.88
2.	*Klebsiella pneumoniae*	25	92.59	26	96.30	05	18.52	27	100
3.	*Pseudomonas aeruginosa*	23	88.41	21	80.77	14	83.85	25	96.15
4.	*Salmonella* sp.	05	83.83	04	66.67	05	83.33	06	100

Table 4: Antibiotic Resistance Pattern of Bacterial isolates obtained from Adalatganj Pond

Sl. No.	Bacterial Isolates	Antibiotic Resistant Isolates							
		Ampicillin		Erythromycin		Gentamycin		Tetracycline	
		No.	%	No.	%	No.	%	No.	%
1.	*Escherichia coli*	39	97.50	35	87.50	30	75.00	38	95.00
2.	*Klebsiella pneumoniae*	36	100	30	83.33	36	100	33	91.67
3.	*Pseudomonas aeruginosa*	24	88.89	22	81.48	13	48.15	24	88.89
4.	*Salmonella* sp.	02	100	02	100	01	50.00	02	100

It became evident from the data presented in Table-3 that out of 83 isolates of *E. coli* obtained from Gardanibagh Kacchi Talab, all showed resistance against Ampicillin and 69, 59 and 58 isolates showed resistance against Erythromycin, Gentamycin and Tetracycline respectively. Out of the 27 isolates of *K. pneumoniae* obtained from water sample of this pond, all showed resistance against Tetracycline as well as 26, 25, and 05 isolates exhibited resistance against Erythromycin, Ampicillin and Gentamycin respectively. Total 06 isolates of Salmonella sp. were isolated from this pond, among them all exhibited resistance against Tetracycline, 05 against Amplicillin and Gentamycin as well as 04 against Erythromycin.

Data mentioned in Table-4 indicates that out of the 40 isolates of *E. coli* obtained from water samples of Adalatganj Pond, 39,38,35 and 30 exhibited resistance against Ampicillin, Tetracycline, Erythromycin and Gentramycin respectively. Altogether 36 isolates of *K. pneumoniae* were obtained from the water sample of this pond, out of which 33 and 30 remained resistant against Tetracycline and Erythromycin respectively. But all strains of *K. pneumonia* remained resistance against Ampicillin and Gentamycin. Altogether 27 isolates of *P. aeruginosa* were obtained from the water sample of this pond, out of which 24, 22 and 13 remained resistant against Ampicillin & Tetracycline, Erythromycin and Gentamycin respectively. Out of the 02 isolates of *Salmonella* sp. obtained from the water sample of this pond, one remained resistant against Gentamycine. Both isolates of *Salmonella* sp. obtained from water of this pond showed resistance against Ampicillin, Erythromycin and Tetracycline. Thus it became evident that most of the bacterial isolated obtained from different ponds exhibites multi-drug

resistance against more than two antibiotics tested during this study.

Cent per cent of the total isolates (83) of *E. coli* obtained from water sample of Gardanibagh Kacchi Talab showed resistance against Ampicillin as well as 83.13%, 71.08%, and 69.88% remained resistant against Erythromycin, Gentamycin and Tetracycline respectively. Cent per cent of the total isolates (27) of *K. pneumoniae* obtained from the water sample of this pond remained resistant against Tetracycline as well as 96.30%, 92.59% and 18.52% of total isolates showed resistance against Erythromycin, Ampicillin and Gentamycin respectively. Out of the total (26) isolates of *P. aeruginosa* obtained from water sample of this pond 96.15%, 88.46%, 80.77% and 53.85% exhibited resistance against Tetracycline, Ampicillin, Erythromycin and Gentamycin respectively. Cent per cent of the total isolates (06) of *Salmonella* sp. obtained from the water of this pond remained resistant against Tetracucline and 83.66% remained resistant against Ampicillin and Gentamycin. Only 66.67% of *Salmonella* sp. obtained from this pond remained resistant against Erythromycin (Table-3).

Out of the total (40) isolates of *E. coli* obtained from water sample of Adalatganj Pond, 97.50%, 95%, 87.50% and 75% showed resistance against Ampicillin, Tetracycline, Erythromycin and Gentamycin respectively. Whereas, out of the total (36) isolated of *K. pneumoniae* obtained from this pond, cent per cent exhibited resistance against Ampicillin and Gentamycin. But 91.67% and 83.33% of the total isolates of this bacteria tested during present research work exhibited resistance against Tetracycline and Erythromycin respectively. Out of the total (27) isolates of *P. aeruginosa* obtained from water of this pond, 88.89%, 81.48% and 48.15% exhibited resistance against Ampicillin & Tetracycline, Erythromycin and Gentamycin respectively. Cent percent *Salmonella* sp. obtained from the water sample of this pond remained resistant against Ampicillin, Erythromycin and Tetrocycline but only 50 of them remained resistant against Genamycin (Table-4).

The fecal indicator bacteria *E. coli* was observed in all of the water samples collected from two ponds examined during present research work. It became evident that water of these ponds remain inundated with coliform bacteria. In addition to *E. coli*, other bacteria of fecal origin such as *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella* sp. were observed in all water samples collected from two ponds of Patna town. These bacteria pose a serious health concern to the local human community, because these bacteria may facilitate spread of diseases.

Reinthaler *et al*. (2023) observed *E. coli* isolates obtained from waste water resistant to Ampicillin. Kim *et al*. (2007) observed 23% resistance of *E. coli* obtained from waste water against tetracycline. Fluoroquinolone resistant *E. coli* was reported by Lautenbach *et al*. (2009). The resistance of these Gram negative bacteria may be due to chromosomal mutations or plasmid-mediated. Luczkiewicz *et al*. (2011) observed *E. coli* resistant to Ampicillin, Tetracycline and Ciprafloxacin. The resistance pattern of *E. coli* against different antimicrobial agents is considered as increasing environmental and health problems in different parts of India and Abroad. Resistance dissemination takes place generally through horizontal gene transfer.

Resistant *E. coli* strains bears multi-resistance plasmids. All the isolated bacterial strains during present research work showed multidrug resistance. These bacterial strains exhibited resistance against three or more antibiotics tested during present research work. Wolf ensberger *et al*. (2019) also isolated multidrug resistant Gram negative bacteria from lentic water bodies situated in urban area. Ho *et al*. (2021) also observed multi-drug resistant bacteria from a river estuary receiving urban waste. During their study, it was observed that these bacteria exhibited resistance against Erythromycin, Tetracycline at high degree and against carbapenem at low degree. The results of present study corresponds with results obtained by them.

4. Conclusion

Occurrence of highly resistant fecal bacteria in water bodies of Patna town indicates that ingestion of antibiotics provide selective pressure on these bacteria. In Patna town, antibiotics tested during present research work are readily made available over the counters of medical stores without prescription. This situation leads to indiscriminate use or misuse of antibiotics, which remain responsible for evolution of resistance in bacteria of fecal origin. Thus, co-transfer of resistance among different strains of bacteria takes place predominantly. Genes responsible for multi-antibiotic resistance remain located on plasmids of bacterial cell. It became evident from the results of present research work that a considerable number of fecal coliforms examined remain high and multi-antibiotic resistant strains. Periodic examination of hazardous coliform bacteria in water bodies of Patna town is essential for protection of public health.

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