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Multidrug Resistant Pathogenic *Escherichia coli* Status in Water Sources and Yamuna River in and Around Mathura, India

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Abstract: The present study was taken to understand the status of water resources in the holy city of Mathura, India. As it is a tourist place and pilgrims come from all around the world, there are more chances of spread of pathogens through them. The pathogens which are particularly excreted through urine and feces are most commonly excreted out. *E. coli* is one of them which causes many severe diseases particularly in neonatal calves or neonates of human. As the city has its limitation of accommodation and river Yamuna is also shrinking, the pressure of millions of pilgrims has enormous, drastic and stipulated effect on water resources particularly river Yamuna. The samples collected showed the presence of pathogenic *E. coli*. Out of total 100 samples 40 were found positive for *E. coli*. The 60 samples of different water resources showed the presence of *E. coli* in 26 samples while 14 were found positive out of 40 samples of Yamuna water. The total coliforms count ranged between 1.08 $\log_{10}$ CFU m L^{-1} to 6.34 $\log_{10}$ CFU m L^{-1} in drinking water sources and waste water, respectively. The high percentage of positive samples and coliforms count may be because of the method and place of sample collection. When these isolates were analyzed for antibiotic sensitivity pattern against some commonly used antibiotics. They showed a variable effectiveness against them. The number of resistant isolate is significant enough to make an alert at the earliest to protect the water resources and also to secure better and brighter future of human population.

Key words: Coliforms, antibiogram, water samples, Yamuna River, *Escherichia coli*

INTRODUCTION

The demands for safe water for human consumption and recreational activities have increased rapidly in recent years due to human exponential growth. The impact of this growth has affected the performance of waste water treatment facilities and changed the biological and chemical stability of watersheds throughout the nation. One of the most significant challenges facing the Nation is to meet the standards established by regulatory agencies. The quality of potable water depends on water source and its treatment, if any. Place of site related data on water source gives important information for improvement of environmental conditions (Arnold *et al.*, 2013). The drinking water may be contaminated with pathogens, toxic metals, chemicals like pesticides, herbicides etc (Azizullah *et al.*, 2011). Mostly occurrence of water borne disease outbreak is observed due to compromise with the safety of drinking water. It is mainly might be because of threat by pathogenic microorganisms

due to inadequate water treatment and compromise with distribution system (Azizullah *et al.*, 2011; Opisa *et al.*, 2012). Besides the conventional pathogens which are transmitted by water, several emerging water-borne pathogens have become increasingly important during the test decade or so. *E. coli* is most commonly water dependent bacteria responsible for various ailments in human (Levine, 1987; Dhama *et al.*, 2013). Now a day, emergence and dissemination of antimicrobial resistance in bacteria is well documented worldwide (Cohen, 2000; Verma *et al.*, 2007; Kumar *et al.*, 2011, 2012a) and considered as one of the biggest issue in public health globally (Romeu Alvarez *et al.*, 2012). *E. coli* is most commonly observed gastrointestinal flora and is supposed to be an important reservoir of transferable antibiotic resistance by accepting and transferring plasmids. These plasmids can easily be transferred to other species (Feng *et al.*, 2002). Although, *E. coli* is a commonsal harmless inhabitant of the intestinal tract but now it has been seen as a pathogenic species due to

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involvement in many disease conditions in human and animals and remarkable versatility (Hussain and Saikia, 2000). Hence, active and continous monitoring of the antimicrobial resistant pattern of *E. coli* isolates recovered from disease conditions is of public health importance. Therefore, this study investigated presence of *E. coli* in water sources in Mathura city and the occurrence of multidrug resistance bacteria not only to commonly used antibiotics, but also to the broad spectrum antibiotics.

MATERIALS AND METHODS

Sample collection: Samples of wastewater, surface, ground and drinking waters were collected from the entire region of the city of Mathura. Wastewater samples were collected from the stretch of holy river Yamuna transverse through the city and down steam. Surface water samples were collected from the river Yamuna upstream of Mathura. Ground and drinking water samples were collected from hand pumps and municipal water supplies, respectively. Samples were collected in sterilized screw-capped bottles making sure that there will be no additive, transported to the laboratory in cold and processed within 6 h of their collection.

Total count, isolation and identification of *E. coli*: About 0.5 mL of water samples were inoculated onto MacConkey lactose agar (Difco) for coliforms counts. Plates with counts between 30-300 colonies were selected for the determination of colony forming unit per mL (CFU m L^{-1}). The procedure adopted for the isolation of *E. coli* from sample was as described by Edwards and Ewing (1972). All the water samples were streaked on MacConkey's Lactose Agar (MLA) after incubation at 37°C for 24-48 h. Pink colonies suspected of *E. coli* from each MLA plate were picked up and restreaked on Eosin Methylene Blue (EMB) for observing the metallic sheen. Bacterial isolates suggestive of *E. coli* on MLA and EMB were studied on the basis of their cultural, morphological and motility characteristics. Then biochemical studies of *E. coli* isolates were carried out as per the method of Ceuickahnk *et al.* (1975).

Assessment of pathogenicity: All the isolates were assessed for their pathogenicity by Congo red binding and production of hemolysins. For this the colonies confirmed biochemical, cultural and morphological basis were grown in were grown at 37°C for 24 h on Tryptic soy agar supplemented with 0.02% Congo red (Sigma) and 0.15% bile salt (Difco) and 5% sheep blood agar, respectively. Positive colonies for Congo

red binding appeared red in color whereas haemolytic isolates produced a clear zone of haemolysis in surrounding to colonies.

Antibiogram: *In vitro* antibiotic susceptibility testing of all the isolates of *E. coli* recovered during study was performed by standard procedure (Bauer *et al.*, 1966). Various antibiotic discs viz., Streptomycin (10 µg), Chloramphenicol (30 µg), Amikacin (30 µg), Ampicillin (10 µg), Cephalexin (30 µg), Tetracycline (30 µg), Gentamicin (10 µg), ciprofloxacin (5 µg) and Norfloxacin (10 µg) were applied. Twelve hours old nutrient broth cultures of *E. coli* isolates were used and spreaded on the nutrient agar plate by sterile cotton swab and allowed to dry. The antibiotic discs were placed on culture plates with the help of sterile fine pointed for up at a distance of approximately 2.5 cm and incubated at 37°C overnight. The susceptibility of *E. coli* to the antibiotic agents was determined by observing the presence of zone of inhibition around the discs. The diameters of the zone of inhibition around discs were measured in mm using a millimeter scale. The zone size around each antibiotic disc was categorized into sensitive, intermediate or resistant according to NCCLS (2002).

RESULTS AND DISCUSSION

A total of 100 samples were collected from different water sources. Out of these, 60 were from drinking water resources and 40 from holy river, Yamuna. Each sample was screened for total coliforms counts; isolation and identification of *E. coli*. The total coliforms count ranged between 1.08 $\log_{10}$ CFU m L^{-1} to 6.34 $\log_{10}$ CFU m L^{-1} in drinking water and waste water, respectively. The high percentage of positive samples and coliforms count may be because of the method and place of sample collection. The identification was carried out on the basis of cultural characters on MacConkey's Agar medium and Eosin methylene blue Agar medium. Bright pink colored colonies due to lactose fermentation were taken as suggestive of *E. coli* and colonies exhibiting metallic seen on EMB agar were further confirmed with Gram's staining and biochemical tests in addition to motility test using hanging drop method. Each isolate was then subjected to various biochemical tests such as indole test, Methyl Red (MR), vogesproskauer (VP) and nitrate reduction test in addition to the fermentation of glucose, sucrose, inositol, maltose, mannitol and lactose. The isolates found Indole, Methyl Red, and Nitrate positive and V.P. negative were confirmed as *E. coli*. They also showed the fermentation of sugar typical of *E. coli* characteristics. All the isolates had Congo red binding however only nine *E. coli* isolates caused hemolysis in sheep blood agar plates.

Table 1: Drug sensitivity pattern of *E. coli* isolates

Drug sensitivity	Streptomycin (10 µg)	Chloramphenicol (30 µg)	Amikacin (30 µg)	Ampicillin (10 µg)	Cephalaxin (30 µg)	Tetracycline (30 µg)	Gentamicin (10 µg)	Ciprofloxacin (5 µg)	Norfloxacin (10 µg)
Resistant	24	10	26	22	28	32	18	14	16
Resistance of %	60	25	65	55	70	80	45	35	40
Sensitive	16	30	14	18	12	8	22	26	24
Total	40	40	40	40	40	40	40	40	40

Table 2: Antibiotic sensitivity pattern of isolates of drinking water resources

Drug sensitivity	Streptomycin (10 µg)	Chloramphenicol (30 µg)	Amikacin (30 µg)	Ampicillin (10 µg)	Cephalaxin (30 µg)	Tetracycline (30 µg)	Gentamicin (10 µg)	Ciprofloxacin (5 µg)	Norfloxacin (10 µg)
Resistant	16	4	16	16	20	24	12	8	12
Sensitive	10	22	10	10	6	2	14	18	14
Total	26	26	26	26	26	26	26	26	26

Table 3: Antibiotic sensitivity pattern of isolates of Yamuna water

Drug sensitivity	Streptomycin (10 µg)	Chloramphenicol (30 µg)	Amikacin (30 µg)	Ampicillin (10 µg)	Cephalaxin (30 µg)	Tetracycline (30 µg)	Gentamicin (10 µg)	Ciprofloxacin (5 µg)	Norfloxacin (10 µg)
Resistant	8	6	10	6	8	8	6	6	4
Sensitive	6	8	4	8	6	6	8	8	10
Total	14	14	14	14	14	14	14	14	14

Antibiotic sensitivity testing of each isolate was carried out by using disc diffusion method against nine commonly used antibiotic discs (Hi media, Mumbai) (Table 1). The drug sensitivity pattern of isolates revealed the resistance against two or more antibiotics in both type of samples (Table 2 and 3). Isolates recovered from drinking water showed multiple resistances against all the antibiotics. Resistance was highest against tetracycline (80%) followed by streptomycin (60%). Sensitivity was highest with fluroquinolones.

A pattern of multiple drug resistance was observed (Fig. 1). No isolate was sensitive to all the antimicrobial agents used for the study (Fig. 2). The highest rates of resistance were against Tetracycline (80%), followed by Cephalaxin (70%), Amikacin (65%) and Streptomycin (60%) (Fig. 1).

In the last few decades, the frequency and spectrum of antimicrobial-resistant infections have increased tremendously. Certain infections that are essentially untreatable have begun to occur as epidemics both in the developing world and even in institutional settings in the United States and other developed regions (FAO, OIE, WHO, 2003). This antimicrobial resistance is resulting in increased morbidity, mortality, and health-care costs (Cohen, 1992). In these outbreaks *E. coli* is major pathogen and food and water borne outbreaks of *E. coli* have been documented from a number of countries (Bartlett, 1996; Ogden *et al.*, 2001). *E. coli* is a member of fecal coliforms that contaminate the drinking water from human and animal fecal waste. In developing countries including India, drinking water supply lines and waste water drainage system are laid side by side, thus increasing the chances of water contamination (Malik *et al.*, 1996).
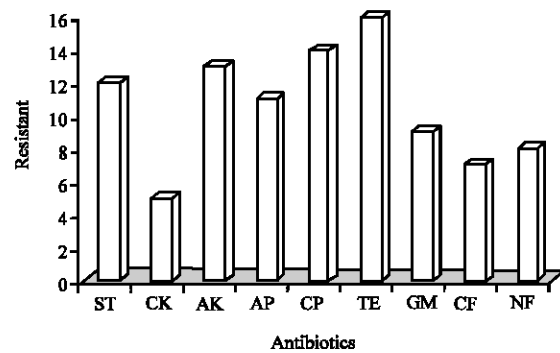

Fig. 1: The percentage of antibiotic resistance of *E. coli* isolates to nine antibiotics, ST: Streptomycin, CK: Chloramphenicol, AK: Amikacin, AP: Ampicillin, CP: Cephalaxin, TE: Tetracycline, GM: Gentamicin, CF: Ciprofloxacin, NK: Norfloxacin
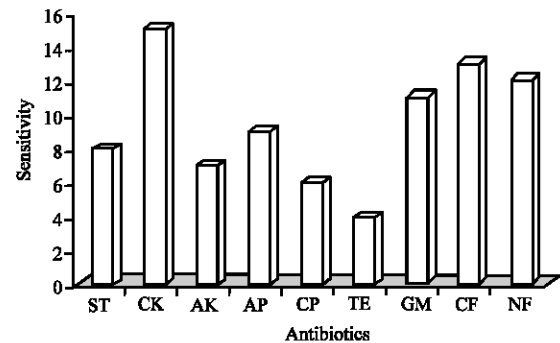

Fig. 2: Sensitivity percentage of *E. coli* isolates to antibiotics. ST: Streptomycin, CK: Chloramphenicol, AK: Amikacin, AP: Ampicillin, CP: Cephalaxin, TE: Tetracycline, GM: Gentamicin, CF: Ciprofloxacin, Nk: Norfloxacin

Various studies have shown the resistance of E. coli in river water (Walia *et al.*, 2004; Patoli *et al.*, 2010; Romeu Alvarez *et al.*, 2012). The presence of resistance against fluoroquinolones is also supported by Hooper (2001) as the emergence of resistance to the beta-lactams and the fluoroquinolone antibiotics which has become more serious in recent decades is mainly due to the broad use of fluoroquinolones (Romeu Alvarez *et al.*, 2012). This problem is compounded by the continued emergence of antibiotic resistance to a growing number of antibiotics; i.e., carbenicillin, tetracycline, streptomycin (Walia *et al.*, 2004) norfloxacin, amoxycillin, trimethoprim, nitrofurantoin (Goettsch *et al.*, 2000) and nalidixic acid, gentamicin, cefuroxime (Shehabi *et al.*, 2004) etc. Injudicious use of antibiotics is the main factor resulting in this emergence, selection and dissemination of drug-resistant pathogens in human as well as veterinary medicine (Smith *et al.*, 2005; Verma *et al.*, 2007; Sukumaran *et al.*, 2012; Kumar *et al.*, 2012b, 2013). Therefore, there is a need for continuous surveillance of antimicrobial resistance trends worldwide particularly among organisms resident in the gastrointestinal tract of farm-animals. These organisms are also implicated in infectious diseases in human as these might be the major source of water contamination. Implementing antibiotic use strategies at all levels will decrease the risk and the clinical threat posed by antimicrobial resistance due to use and misuse of antibiotics.

CONCLUSION

The places like Mathura are very sensitive in respect to public health concern. The gathering of thousands of people all over the world throughout year put enormous pressure on river Yamuna and local authorities. Disposure of human and animal wastes directly into river without proper treatment is further aggregating the scenario. Presence of pathogenic *E. coli* is mainly due to the sewage inclusion in river and also due the contamination of drinking water sources with human waste. Moreover, multi drug resistant pattern of pathogenic *E. coli* are also of major concern and can lead to hazard at any time and posing one of the most significant challenges to meet the standards established by regulatory agencies.

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REFERENCES

Arnold, M., J.A. VanDerslice, B. Taylor, S. Benson and S. Allen *et al.*, 2013. Drinking water quality and source reliability in rural Ashanti region, Ghana. J. Water Health, 11: 161-172.

Azizullah, A., M.N. Khattak, P. Richter and D.P. Hader, 2011. Water pollution in Pakistan and its impact on public health: A review. Environ. Int., 37: 479-497.

Bartlett, C.L.R., 1996. An overview of emerging foodborne and waterborne diseases. Eastern Mediterranean Health J., 2: 51-60.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Ceuickahnk, R., J.P. Duguid, B.P. Marimann and R.H.A. Swain, 1975. Medical Microbiology. 12th Edn., Churchill Livingston, Edinburgh.

Cohen, M.L., 1992. Epidemiology of drug resistance: Implications for a Post-antimicrobial Era. Science, 257: 1050-1055.

Cohen, M.L., 2000. Changing patterns of infectious disease. Nature, 406: 762-767.

Dhama, K., S. Rajagunalan, S. Chakraborty, A.K. Verma and A. Kumar *et al.*, 2013. Food-Borne Pathogens of Animal Origin-Diagnosis, Prevention, Control and Their Zoonotic Significance: A Review. Pak. J. Biol. Sci., 16: 1076-1085.

Edwards, P.R. and W.H. Ewing, 1972. Identification of Enterobacteriaceae. 3rd Edn., Burgess Publishing Co., Minneapolis.

FAO, OIE, WHO, 2003. Joint FAO/OIE/WHO expert workshop on non-human antimicrobial usage and antimicrobial resistance: Scientific assessment. December 1-5, 2003, Geneva.

Feng, P., S.D. Weagant, M.A. Grant and W. Burkhardt, 2002. Enumeration of *Escherichia coli* and the Coliform Bacteria. In: Bacteriological Analytical Manual, FDA (Eds.). U.S. Food and Drug Administration, Silver Spring, MD., USA.

Goettsch, W., W. Van Pelt, N. Nagelkerke, M.G.R. Hendrix and A.G.M. Buiting *et al.*, 2000. Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in the Netherlands. J. Antimicrob. Chemother., 46: 223-228.

Hooper, D.C., 2001. Emerging mechanisms of fluoroquinolone resistance. Emerg. Infect. Dis., 7: 337-341.

Hussain, I. and G.K. Saikia, 2000. Isolation and characterization of bacteria from diarrhoeic bovine calves. Indian J. Comp. Microbiol. Immunol. Infect. Dis., 21: 125-127.

Kumar A., A.K. Verma, Parul and V.P. Singh, 2011. Prevalence and antibiotic profile of bacterial isolates in chicken meat in Western U.P. J. Vet. Pub. Health, 9: 111-114.

Kumar, A., A.K. Verma, N.K. Gangwar and A. Rahal, 2012a. Isolation, characterization and antibiogram of mycoplasma bovis in sheep pneumonia. Asian J. Anim. Vet. Adv., 7: 149-157.

Kumar, R., A.K. Verma, A. Kumar, M. Srivastava and H.P. Lal, 2012b. Prevalence and antibiogram of campylobacter infections in dogs of Mathura, India. Asian J. Anim. Vet. Adv., 7: 434-440.

Kumar, A., A.K. Verma, S. Malik, M.K. Gupta, A. Sharma and A. Rahal, 2013. Occurrence of extended spectrum β-lactamases producing alpha hemolytic *Escherichia coli* in Neonatal diarrhea. Pak. J. Biol. Sci., (In Press)

Levine, M.M., 1987. *Escherichia coli* that cause diarrhea: Enterotoxigenic, enterophatogenic, enteroinvasive, enterohemorrhagic and enteroadherent. J. Infect. Dis., 155: 377-389.

Malik, I.A. and W. uz-Zaman Tariq, 1996. Hepatitis E in Pakistan. Eastern Mediterranean Health J., 2: 121-128.

NCCLS, 2002. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals: Approved Standard. 2nd Edn., National Committee for Clinical Laboratory Standards, Wayne, PA., USA., ISBN: 1-56238-461-9.

Ogden, L.D., D.R. Fenlon, A.J. Vinten and D. Lewis, 2001. The fate of *Escherichia coli* O157 in soil and its potential to contaminate drinking water. Int. J. Food Microbiol., 66: 111-117.

Opisa, S., M.R. Odiere, W.G. Jura, D.M. Karanja and P.N. Mwinzi, 2012. Faecal contamination of public water sources in informal settlements of Kisumu City, western Kenya. Water Sci. Technol., 66: 2674-2681.

Patoli, A.A., B.B. Patoli and V. Mehraj, 2010. High prevalence of multi-drug resistant *Escherichia coli* in drinking water samples from Hyderabad. Gomal J. Med. Sci., 8: 23-26.

Romeu Alvarez, B., P. Salazar Jimenez, D. Lugo Moya, N.M. Rojas Hernandez and C.A. Eslava Campos, 2012. Antimicrobial susceptibility of *Escherichia coli* isolates from river water ecosystems. Revista Cubana Medicina Tropical, 64: 132-141.

Shehabi, A.A., A.M. Mahafzah and K.Z. Al-Khalili, 2004. Antimicrobial resistance and plasmid profiles of urinary *Escherichia coli* isolates from Jordanian patients. Eastern Mediterranean Health J., 10: 322-328.

Smith, D.L., J. Dushoff and J.G. Jr. Morris, 2005. Agricultural antibiotics and human health. PLoS Med., Vol. 2 10.1371/journal.pmed.0020232

Sukumaran, D.P., S. Durairaj and M.H. Abdulla, 2012. Antibiotic resistance of *Escherichia coli* serotypes from Cochin estuary. Interdiscip. Perspect. Infect. Dis., Vol. 2012 10.1155/2012/124879

Verma, A.K., D.K. Sinha and B.R. Singh, 2007. Salmonella in apparently healthy dogs. J. Vet. Public Health, 5: 37-39.

Walia, S.K., A. Kaiser, M. Parkash and G.R. Chaudhry, 2004. Self-transmissible antibiotic resistance to ampicillin, streptomycin and tetracyclin found in *Escherichia coli* isolates from contaminated drinking water. J. Environ. Sci. Health A, 39: 651-662.