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Multi-Drug Resistance in Indian Rivers: Review

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Abstract

Now a day, riverine system is becoming under pressure due to antibodies interference which highly affect the fauna and flora biodiversity, and economic activities. These antibodies have significantly increased the antibodies resistant microbes present in the aquatic environment which act as reservoirs including rivers, sewage, ocean water and drinking water. Bacterial resistance is the superb example of biological evolution. Further, eutrophication of rivers and lakes facilitates the growth and survival of enteric pathogens. There is no regulation in India of the use of antibiotics. Therefore, this study is focused on increased surveillance of surface waters and development of prevention strategies for protection of public health.

Keywords: *Antibodies, Pathogens, Eutrophication, Bacterial resistance.*

Introduction

The Pressure on Riverine water resources is becoming serious problem across the Asia, which is witnessing rapid growth which is attributed to the human population growth, urbanization and economic activities. In several developing countries together with Asian country that has as several as fourteen rivers and a number of other cities aboard them, an outsized population depends on untreated water from rivers, lakes, wells and alternative surface water resources for drinking, bathing, laundry, recreation and alternative domestic functions (Obi et al 2004; Qadri et al. 2005).

The discovery of antibiotics in the 20th Century marked a watershed in the treatment of infections. The ability to treat the intense infections of the pre-antibiotic era stirred advances in medical fields and enlarged the scope of medical aid. Antimicrobial resistance, a world problem, is especially pressing in developing countries wherever the communicable disease burden is high and cost constrains the replacement of older antibiotics with newer, more expensive ones. The increased use of antibiotics has resulted in the in a significant increase in the numbers of antibiotic resistant bacteria present in aquatic environments. The phenomenon of bacterial drug resistance was first documented in 1951. Management of common and lethal bacterial infections has been critically compromised by the appearance and rapid spread of antibiotic-resistant bacteria. The microorganism sickness burden in Asian nation is among the best within the world (WHO 2011); consequently, antibiotics can play a vital role in limiting morbidity and mortality within the country. As a marker of sickness burden, respiratory illness causes an calculable 410,000 deaths in Asian nation annually (Mathew, J.L. 2009), and it is the number-one killer of children (Levine, O.S., Cherian, T. 2007). Bacterial resistance is the superb example of biological evolution. It is also the consequence of a wide variety of biological, but mostly of non-biological factors: marketing, economics, legislation, and education. In 2000, the world Health Organization warned that "the world might be plunged back to the 'pre-antibiotic era' once individuals usually died from diseases that in trendy time are simply treated with antibiotics. 'Drug choice pressure' is that the single most significant consider the evolution of drug resistance in microorganism. The explanations for drug pressure multi-factorial and involve each human and animal use. Though drug resistance is primarily a medical drawback, the factors that influence the unfold of resistance ar ecological, medical specialty, cultural, social, and economic.

"Drug Resistance refers to a situation in which the drugs that usually destroy the bacteria no longer do so." Antibiotic resistance has been detected in various aquatic environments which emerged as reservoirs including rivers, sewage, ocean water and drinking water (Hermansson et al., 1987; Ash et al., 2002; Reinthaler et al., 2003; Schwartz et al., 2003). Increased introduction of antimicrobial agents into the environment via medical therapy, municipal sewage and wastes (Bruneau et al. 2004; Qadri et al. 2005; Hamelin et al. 2006), agriculture and animal husbandry has resulted in selective pressures

on bacterial populations (Col and O'Conner, 1987) as well as the indiscriminate use of antibiotics have spread drug-resistant microbes to all parts of the world. Further, eutrophication of rivers and lakes facilitates the growth and survival of enteric pathogens (Olapade et al. 2006). Internationally several rivers have become reservoirs of antibiotic resistant microbes (Pathak et al., 1993; Ash et al., 2002; Ram et al., 2003).

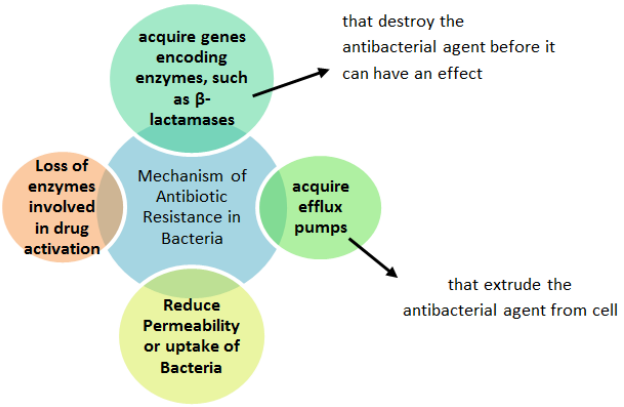
There is no regulation in India of the use of antibiotics. A second cause is counterfeit medication. Antibiotic use in agriculture is additionally making some drug resistant bacterium, which might be transmitted to humans. The resistance rates will highest to those antibiotics in use the longest.

Bacterial resistance to antimicrobial agents, that is increasing worldwide, is often caused by the acquisition of latest genes instead of by mutation (Recchia, G.D. et al., 1995; Hall, R.M. et al., 1995). An efficient means of acquiring new genes is by mobile genetic elements such as resistance (R)-plasmids and Transposons. Recently, a novel class of naturally occurring mobile genetic elements, Integron, have been described as vehicles for the acquisition of antimicrobial resistance genes (Hall, R.M. et al., 1995). Horizontal and vertical transfer can occur readily, as shown by the widespread acquisition of these gene cassettes among the Enterobacteriaceae and *Pseudomonas* spp.

Antibiotic Resistance in Bacteria

Microbial populations develop resistance to antimicrobials through several mechanisms. Some species of bacteria are innately resistant to ≥ 1 class of antimicrobial agents. Of greater concern are cases of acquired resistance, where initially greater populations of bacteria become resistant to an antimicrobial agent and proliferate and spread under the selective pressure of use of that agent. The rate at which an individual gene mutates to express an antimicrobial resistance phenotype is a complex phenomenon in which environment, cell physiology, bacterial genetics, and population dynamics all play roles (Martinez and Baquero, 2000). In addition, for full resistance to occur, mutations must develop within multiple genes because of genetic redundancy in the antimicrobial targets. A primary example of this is the fluoroquinolone target gene gyrA, gyrB, parA, and parC that are all targets for the fluoroquinolone antimicrobial, and all must have mutations for full resistance phenotypes to develop (Martinez and Baquero, 2000). Severalmechanisms of antimicrobial resistance are promptly unfold to a range of microorganism genera.

Bacteria may acquire several genes for a metabolic pathway which ultimately produce altered bacterial cell walls that no longer contain the binding site of the antimicrobial agent, or bacteria may acquire mutations that limit access of antimicrobial agents to the intracellular target site via down regulation of porin genes. Two conditions are required for antibiotic resistance to develop in microorganism. First, the organism should acquire contact with the antibiotic.



Then, resistance against the agent should develop, beside a mechanism to transfer the resistance to female offspring organisms or on to different members of constant species. every antibiotic operates at a selected website among the microorganism cell. For example (Levy, S.B. 1998; O'Grady, F. et al., 1997):

- Some target the cell walls (e.g., bacitracin, cephalosporins and penicillins)
- Some target cell membranes (e.g., ionophores and polymyxins)
- Some target cell components responsible for the synthesis of

a) Proteins (e.g., aminoglycosides, chloramphenicol and tetracycline)
b) RNA (e.g., rifamycins)
c) DNA (e.g., nalidixic acid and quinolones)
d) particular biochemical pathways such as folate synthesis (e.g., methotrexate and sulfonamides)

Thus, when resistant organisms arise, their resistance is specific to particular antibiotics.

Transfer of antimicrobial resistance genes in aquatic environment:

It was earlier stated that antibiotic resistance genes could be originated in the antibiotic-producer organisms and afterwards transferred to pathogenic bacteria (Benveniste, R. and Davies, J. 1973) for example: tetracycline resistance genes *otrA* and *otrB* that have been found in the tetracycline-producing organism *Streptomyces rimosus* and in pathogenic microbacteria (Pang, Y. et al 1994). Acquisition and transfer of antibiotic resistance and virulence factor genes by the bacteria via these methods:

1) Horizontal gene transfer

About 50 years have passed since the discovery that bacteria are able to exchange genetic information. Antibiotic resistance can be developed through the acquisition of antibiotic resistance genes by horizontal or lateral gene transfer (Davis, J. E. 1997), which has a highly relevant role in the emergence and further spread of antibiotic resistance genes among pathogenic bacteria.

Three fundamental mechanisms have been identified which mediate horizontal gene transfer (HGT) between bacteria. These are designated; (1) conjugation (Lederberg and Tatum 1946) which is relatively common cell contact dependent para-sexual process, whereby specific plasmids or transposons transfer from donor to recipient cells. It is therefore an important means by which antibiotic resistance is spread between bacteria. An example of this is the floR gene encoding florofenicol resistance in *E.coli* found in cattle (Cloeckaert et al., 2000). Conjugative plasmids have been identified in a wide range of Gram-negative and Gram-positive bacteria. An important feature of some of these conjugative plasmids is their potential to mobilise even chromosomally located genes to recipient cells (Haas and Holloway 1976). (2) transformation (Avery et al. 1944) which is defined as the uptake, integration and stable inheritance of cell-free DNA by bacterial and archeal cells. The frequency with which bacteria acquire DNA from the environment depends on several factors including cell wall structure and bacterial species. Bacterial cells have to be "competent" to acquire extraneous DNA by transformation. Bacteria such as *Campylobacter* are thought to be naturally competent. There are differences in the process among gram-positive and gram-negative bacteria. The process involves the specific recognition sequences in order for the new DNA to be taken up by the bacteria (Elkins et al., 1991). (3) transduction (Zinder and Lederberg 1952), in which the introduction of genetic material into a bacterium take place by a bacterial virus called bacteriophage, or transduction. This concept has been used in molecular cloning by introducing vector DNA; including antimicrobial resistance, through bacteriophage λ for some time (Sambrook et al., 1989). There are two kinds of transduction mediated by two different classes of bacteriophages. In specialised transduction, only certain genes adjacent to the phage genome, which is integrated into specific sites of the bacterial chromosome, can be transferred upon excision and subsequent phage propagation. In contrast, in generalised transduction virtually any gene of a bacterial host can be transduced after accidental packaging of host DNA into a phage head. Since phages often display a narrow host range of infection, transduction is not considered to contribute essentially to gene exchange among distantly related bacteria.

2) R- Plasmid mediated Transfer of Antibiotic Resistance

The Evolution of multi drug resistant plasmids in pathogens is a comparatively recent phenomenon which came into existence after the introduction of antibiotics after 1940s. Horizontal transfer of plasmid encoded genes is the primary reason for the dissemination of resistance genes in the environment (Spratt, B.G. 1994). Plasmid transfer between bacteria occurs in a variety of natural habitats, e.g., wastewater (Mach, P.A. et al 1982), sewage (Fontaine, H.D. et al 1976 and Grabow, W.O.K. et al 1973), river water (Bale, M.J. et al 1988 and Grabow, W.O.K. et al 1975), lake water (O'Morchoe, S.B. et al 1988), sediments (Stewart, K.R. et al 1980), and soil (Smit, E. Et al 1995 and Van Elgas, J.D. 1984). A single plasmid can carry a number of genes coding for multiple drug resistance (Day, M. et al 1998). Bacterial resistance to antibiotics is located in plasmids of 1-30 megadaltons molecular weight [Kobori, H. et al., 1984]. Genes assembled in plasmids protect bacterial populations against antibiotics. It is the R plasmid that plays a substantial role in bacterial resistance to antibiotics [Klech, W. J. et al., 1978, Silva, A. L. et al., 1995]. The R plasmid can be transferred between various strains of bacteria through conjugation and transformation processes [Herwig, R.P. et al., 1997]. There are four classic mechanisms of resistance specified by plasmids: inactivation, impermeability, bypasses and altered target site; all occur in aquatic environments [Davies, J. et al., 1978]. Resistance can also be associated with the production of enzymes that modify and inactivate antibiotics [Koch, A. L. 1981]. According to Hermansson et al. [Hermansson, M. et al., 1987] some strains of bacteria resistant to antibiotics do not contain any plasmids. In such a case bacterial resistance to antibiotics depends on the mobile genetic elements, called transposons [Herwig, R.P. et al., 1997].

3) Transposons

While plasmids act as vectors of resistance genes, the genes themselves are most often located on discrete movable DNA elements called Transposons (Tenover, F.C. 1991). The important process in the gene pick up is done by transposons carrying multiple antibiotic resistance genes. Transposons are unique in that they have the ability of excising themselves from one genetic locus and moving to another, whether it is within the same bacteria or bacteria in other taxa (Ochman et al., 2000). Transposons can be transferred through all of the methods mentioned above namely, conjugation, transformation, and transduction. Transposons play a significant role in antimicrobial resistance development because they often contain gene sequences mediating antimicrobial resistance called integron gene sequences.

4) Integron

It was first identified by Stokes and Hall (1989). Integrons are thought to play a significant role in the rapid dissemination of antimicrobial resistance among bacteria (Ochman et al., 2000). Integron is the key structural constituent of a Transposon (Day, M. et al 1998). Integron is a mobile DNA element with a specific structure consisting of two conserved segments (5'CS and 3'CS) flanking a central region - "gene cassette" which acts as a site-specific recombination system capable of capturing or excising novel genetic elements. While most known cassette-associated genes located distal to the 5'CS region

encode resistance to antimicrobial drugs, some cassettes may include one or more open reading frames whose product(s) and corresponding function(s) remain to be defined (Hall, R.M. et al., 1995). In the 3'CS downstream of the gene cassette are two genes, one of which encodes resistance to quaternary ammonia compounds (qacEΔ1), while the other is the sulphonamide resistance determinant (sul1). Genes encoding functions like resistance to wide variety of antibiotic can be inserted in this region (Hall and Collis, 1995; Rowe-Magnus and Mazel, 1999; Ochman et al., 2000). These circular gene cassettes are exchanged between bacteria and are linearized by the integrase enzyme before being inserted at the integration site Integron gene sequences contribute to the spread of antimicrobial resistance alleles by lateral gene transfer of gene cassettes in a variety of enteric bacteria, including *Campylobacter spp*., *E.coli*, and Salmonella *enterica* serotype Typhimurium (Ochman, H. et al., 2000; Lucey, B. et al., 2000; Briggs, C.E. et al., 1999). Four different classes of Integrons, namely class 1, class 2, class 3, and class 4, have been described to date, each of which has several distinctive traits (Hall and Collis, 1995; Mazel et al., 1998). The four classes of Integrons differ primarily by the sequence of their integrase gene.

Gene Transfer between distinctly related Bacteria

There is a great diversity among bacteria, and they do not share all of the same biochemical and physiological pathways. Therefore, not all antibiotics are active against all bacteria and bacterial species can have intrinsic resistance to one or more antibiotics. Intrinsic resistance refers to resistant microorganisms without any chromosomal mutation or acquisition of plasmid carrying resistance factors. Inherent features of the bacterial cell prevent antimicrobial action, and these properties are typically characteristics of species. The extent of antibiotic use is a measure of the selection pressure exerted on bacteria (Schwartz et al. 1993). Bacteria have evolved diverse mechanisms to transmit resistance traits to other members of their own species and to other species. Genetic traits for antibiotic resistance are coded for in 2 places in bacteria:

- The chromosomes

Mutations can cause chromosomal genes that usually code for antibiotic sensitivity to start coding for resistance; such mutations occur at the rate of one per million to one per billion cells.

- The extrachromosomal elements

The extrachromosomal elements (plasmids and transposons) are smaller pieces of circular DNA, each equivalent in size to about 1% of a chromosome. Plasmids can be either non-conjugative or conjugative; the latter can move from one bacterium to another.

Genetic exchange is another mechanism by which antibiotic-resistant plasmids can move between bacteria. Some bacteria are considered “promiscuous,” because once they have acquired antibiotic-resistant plasmids, intra- and inter-species transfer of resistance occurs irrespective of the environment (i.e., whether or not antibiotics are present) (O’Grady, F. et al., 1997; Chadwick, D.J. et al., 1997; Marshall, B. et al., 1990).

Scenario of drug resistance in Indian Rivers

In many developing countries, a large population depends on untreated water from rivers, lakes, wells and other surface water resources for drinking, bathing, laundry, recreation and other domestic purposes (Obi et al. 2004; Qadri et al. 2005). The Potential sources of contamination in these countries are overburdened as a result of rapid urbanization and population growth. Besides domestic and wild animal defecation, malfunctioning of septic trenches, storm water drainage, municipal wastes and industrial effluents are also contributed to pollution to the rivers (Ahmed et al. 2005, Bruneau et al. 2004; Edge and Hill 2005; Qadri et al. 2005; Hamelin et al. 2006). All these contamination adversely affected the physico-chemical and biological quality of water. Bacterial contamination of rivers, particularly contamination with faecal derived bacteria, has long been a water quality issue due to the potential for disease transmission. Because of this and the potential for antibiotic resistance, there is a new level of risk associated with these bacteria. The prevalence of background level of antimicrobial-resistance is influenced by a variety of biotic and abiotic factors including geographical area and demography. Further, eutrophication of rivers and lakes facilitates the growth and survival of enteric pathogens (Olapade et al. 2006). Bacteria slowly but steadily synthesise and secrete a number of antibiotic substances into water, namely phenazines, pyrrolnitrin, bacteriocins, glycolipids and bromopyrrolic compounds [Dakhama A. et al., 1993; Lemos, M. L. 1991]. All of these antibiotic substances inhibit bacterial respiration and biosynthesis of cellular structures [BARON, S. S. et al., 1989; Jensen, L.M. 1984]. A large number of bacteria and actinomycetales occurring in aquatic ecosystems are capable of synthesising compounds of antibiotic nature [Klein, T.M. et al., 1986; Lemos, M. L. et al., 1985]. These inhibitory substances are 2,000-15,000 dalton large molecules and their concentration in water could be 1 μg • cm -3 (Lemos, M. L. et al., 1985).

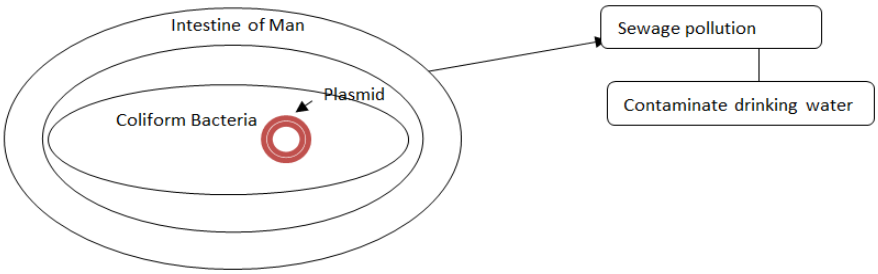
Concentrations of antibiotics in surface waters, i.e. in rivers and lakes, are in the low microgram per litre range for most compounds (Kummerer, K. 2004).

The rivers Ganga, Gomati, Narmada, Yamuna, Krishna, and Godavari etc. are major source of water supply to a great number of populations and receive untreated domestic waste water and waste from agriculture, health sector, and practices of holy-dip and crematory processes. The rivers exhibit high faecal coliform density and the highest bacterial resistance.

Antibiotic studied in Indian rivers

S. No.	Pathogens	Rivers	Antibiotics	References
1.	*E.coli*	Ganga	37% Ch, 20% A and Ac, 13% Pc, 49% Ch, 52% AK, 97% Ne	Ram, S., Vajpayee, P., Shanker, S.2007
2.	*STEC*	””	80% Ac, 29.4% Ch, 23.5% Pc, 11.8% T, 5.9% Cf	””
3.	*ETEC*	””	66.7% Ac, 33.3% Ch, 26.7% T	””
4.	*E.coli*	””	88% AK, 44% C, 44% T, 45% Na, 27%Tr	Mandal, D.M. et al 2011
5.	*Enterococci*	””	High frequency of MAR	Lata, P. et al. 2001
6.	*E.coli*	Gomati	90% OT, 57.8% T, 77.8% Nr, 7.8% Ct, 65.6% AK, 21.1% C, 48.9% G, 3.3% Co, 37.8% Pc, 15.6% K	Ram, S. et al 2008
7.	Ciprofloxcin resistant *E. coli*	””	Resistant to multiple antimicrobial agents	Alam et al. Karlowsky, et al. 2006
9.	*E. coli*	””	57.8% T	Singh et al 2004,
10.	*Vibrio cholerea, Aeromonas spp. Bacillus* spp. and *Micrococcus* spp.	””	Resistant to multiple antimicrobial agents	Pathak, et al. 1993
11.	*EHEC*	””	45.5% Resistant to multiple antimicrobial agents	Ram, S., et al., 2008
12.	*Bacillus spp.* *Actinobacter* *Klebsiella* *Microbacterium* *Lactobacillus* *Clostridium* *Rhizobium* *Yersinia* *Carnybacterium* *Serratia* *Proteus* *Enterobacter*	Krishna Godavari sediments	A, S C, A, S A S A, C C A, C A, C A, C C A, C	Ruban, P., Gunaseelan, C., 2011
13.	*Klebsiella*	Narmada	72.5% Ac, 62.5% A, 44% Ct, G	Sharma, A. et al., 2007
14.	*Klebsiella*	Yamuna	40% G, 33.3% A, 33.3% Cefuroxime, 80% Cefriaxone, 20% Co, 20% Cf, 13.3% Cefriaxone, 66.6% C, Cf	Singh, N. et al., 2011

A- ampicillin, Ac- amoxycillin, AK- amikacin, Cf- ciprofloxacin, Ch- cephalothin, Co- cotrimoxazole, N- neomycin, Na- nalidixic acid, Nx- norfloxacin, Pc- piperacillin, S- streptomycin, T- tetracycline, C- Chloramphenicol, OT- Oxytetracycline, G- Gentamicin, K- Kanamycin, Ct- Ceftazidime, Ne- Neomycin, Tr- Trimethoprim, Nr- Norfloxacin, Ct- Cephotaxime.



Antibiotic Resistance in treated and untreated drinking water and effluents

Several workers have drawn attention to the incidence of antibiotic resistance among coliforms in treated and untreated drinking water (Grabow, W.O.K. et al 1975, Armstrong, J.L. et al 1981). The process of contamination of drinking water is given above.

In the South East Sikkim, Sikkim, North Tripura, Tripura and Leh, Jammu and Kashmir, the total of 231 thermotolerant coliforms were isolated from rural drinking water. Of these, 220 isolates were resistant to ampicillin, chloramphemicol, streptomycin and tetracycline. Apart from increased resistance to chloramphemicol and streptomycin, the thermotolerant coliforms exhibited a lower level of antibiotic resistance than the coliforms. Thermotolerant coliforms exhibited single, double and multiple antibiotic resistances (Gaur, A. et al 1992). Antibiotic resistance among coliform bacteria

in drinking water, as reported by various workers, has ranged from 70 to 96% (LeClerc, H. et al 1978, Ramteke, P.W. et al 1990, Grabow, W.O.K. et al 1974).

Goni-Urizza et al., 2000, found a correlation between resistant bacteria in rivers and urban water input. Antimicrobial resistance was also found in marine bacteria and bacteria living in estuaries (Cohen E. et al., 1986; Barkay, T. et al., 1995). Gentamicin resistance genes were found in *Acinetobacter, Pseudomonas,* Enterobacteriaceae, and in phylogenetically distant bacteria such as members of alpha and beta proteobacteria in coastal water polluted with sewage water (Heuer, H. et al., 2002).

Why is Resistance a Concern?

There are number of reasons why bacterial resistance should be a concern. Prolonged therapy with antimicrobial agents may also lead to the development of low level resistance that compromises therapy. The consequences of resistance affect not only the ability to treat the infection, but also the cost and duration of treatment (Ismaeel, 1993). During recent decades, antibiotics have been widely used as therapy for bacterial infections in humans and animals, and as growth promoters in agriculture and aquaculture, increasing proportion of antibiotic resistant bacteria in various environments.

The riverine environment in India is conducive to microbial growth due to warm humid conditions with cyclic periods of wet and dry weather and eutrophication. Hence, the close contact of the human population with surface water will enrich the environmental gene pool of bacterial isolates that may serve as reservoirs of virulence genes and lead to emergence of new pathogenic variants.

Future aspects

Presence of antibiotic resistance bacteria in the aquatic environment may be an indication that the area is contaminated with antibiotics. Such an area may foster adaptation and selection leading to antibiotic resistant organisms. The growing quantity of antibiotics/antimicrobial drugs used will probably increase antibiotic resistivity in pathogenic bacteria of the river system. The issue of antibiotic resistance has received considerable attention due to the problem of the emergence and rapid expansion of antibiotic-resistant pathogenic bacteria. Many scientists now show that bacteria in polluted rivers become resistant to a range of antibiotics. International experts fear that this may contribute to the development of untreatable infectious diseases worldwide.

Conclusion

Whether antibiotics are given as treatments to humans or animals or as additives in animal feeds, their misuse is at the heart of the antibiotic-resistance problem. Emergence of antibiotic resistance in pathogenic bacteria of the river water is directly associated with indiscriminate and excess use of antibiotics for treatment of infectious disease, which is a serious public health issue because it limits the therapeutic options. It is necessary that as for as possible water sources must be protected from contamination by human and animal waste, which can contain a variety of pathogens, and the parasites that will expose the community to the risk of outbreaks and other infectious disease. Therefore, now the aquatic environment acts not only as a reservoir of clinical resistance genes, but also as a medium for the spread and evolution of resistance genes and their vectors.

Detection of multi-antimicrobial-agent resistant bacteria in rivers is alarming. Other than pilgrims, the human population may be at great risk of contracting infections due to use of the river water daily for bathing, washing laundry, and cooking. Therefore, increased surveillance of surface waters and development of prevention strategies for protection of public health is necessary.

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