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Short Communication

Potential public health significance of faecal contamination and multidrug-resistant *Escherichia coli* and *Salmonella* serotypes in a lake in India

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SUMMARY

Objective: To assess the prevalence of faecal coliform bacteria and multiple drug resistance among *Escherichia coli* and *Salmonella* serotypes from Vembanadu Lake.

Study design: Systematic microbiological testing.

Methods: Monthly collection of water samples were made from ten stations on the southern and northern parts of a salt water regulator constructed in Vembanadu Lake in order to prevent incursion of seawater during certain periods of the year. Density of faecal colifrom bacteria was estimated. *E. coli* and *Salmonella* were isolated and their different serotypes were identified. Antibiotic resistance analysis of *E. coli* and *Salmonella* serotypes was done and the MAR index of individual isolates was calculated.

Results: Density of faecal coliform bacteria ranged from mean MPN value 2900 -7100/100ml. Results showed multiple drug resistance pattern among the bacterial isolates. *E. coli* showed more than 50% resistance to amickacin, oxytetracycline, streptomycin, tetracycline and kanamycin while *Salmonella* showed high resistance to oxytetracycline, streptomycin, tetracycline and ampicillin. The MAR indexing of the isolates showed that they have originated from high risk source such as humans, poultry and dairy cows.

Conclusions: The high density of faecal coliform bacteria and prevalence of multi drug resistant *E. coli* and *Salmonella* serotypes in the lake may pose severe public health risk through related water borne and food borne outbreaks.

Faecal contamination is considered to be the main contributor of enteric pathogens to natural water resources. Infections originating from such sources, especially diarrhoea and typhoid fever, are highly endemic to India, and are also a major public health concern among children in other developing countries. The main pathogens accountable for these illnesses are diarrhoeagenic serotypes of *Escherichia coli* and *Salmonella enterica* serotypes typhi and paratyphi.[1,2] Infections are further aggravated by the emergence and dissemination of antimicrobial resistance among these pathogens, and reported

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outbreaks due to multidrug-resistant diarrhoeagenic *E. coli* and *Salmonella* serotypes from India further challenges the efficiency of antibiotic treatment in the country.[2,3]

Vembanadu Lake, the largest lake in India, lies 0.6–2.2 m below mean sea level along the south-west coast of India (9°35′N 76°25′E). Its maximum depth is 12 m. The lake is used by over 1.6 million people, directly or indirectly, for agriculture, fishing, transportation and recreation; therefore, the monitoring of microbiological quality and related risk assessment is of particular health significance. The present study analysed the prevalence of faecal coliform bacteria at 10 stations, and the incidence of multidrug resistance among *E. coli* ($n = 33$) and *Salmonella* ($n = 26$) serotypes isolated from Vembanadu Lake.

Each month, water samples were collected aseptically from a depth of 1 m from five stations on the southern side (enclosed region) and five stations on the northern side (open region) of the saltwater regulator in Vembanadu Lake. This regulator was constructed to prevent incursion of seawater during certain periods of the year. Samples were collected in sterile screw-capped bottles (500 ml) and transported to the laboratory in an ice box. Prevalence estimate of faecal coliform bacteria were made, and *E. coli* and *Salmonella* were isolated. The three-tube most probable number (MPN) method was used to estimate the prevalence of faecal coliform bacteria and for isolation of *E. coli*. Membrane filtration followed by the selective enrichment method was used for the isolation of *Salmonella*. The procedures for the isolation of *E. coli* and *Salmonella* have been described in detail elsewhere.[4] Serotyping of *E. coli* and *Salmonella* strains were carried out at the National *Salmonella* and *Escherichia* Centre, Kasauli, Himachal Pradesh, India.

Antimicrobial susceptibility testing was performed using the disc diffusion method as described previously.[5] In total, 10 antibiotics were used: ampicillin (10 μg); amikacin (30 μg); chloramphenicol (30 μg); ciprofloxacin (10 μg); gentamycin (10 μg); kanamycin (30 μg); nalidixic acid (30 μg); oxytetracyline (30 μg); streptomycin (30 μg) and tetracycline (30 μg). Multiple antibiotic resistance (MAR) indices for the individual isolates were calculated by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics to which the isolate was exposed, as described previously.[5] A MAR index >0.2 is considered to have originated from a high-risk source of contamination.

The density of faecal coliform bacteria ranged from a mean MPN value of 2900–7100 per 100 ml; this exceeds the limits set by the World Health Organization for recreational water (200 faecal coliforms per 100 ml). Fourteen different 'O' antigenic serotypes of *E. coli* were isolated: O75; O63; O92; O41; O132; O32; ROUGH; UT (untypable); O114; O20; O26; O9; O2 and O7. This is the first report on the isolation of certain *E. coli* serotypes (pathogenic O114, O26 and O7; non-pathogenic O75, O92, O41 and O132) from this lake. *Salmonella* serotypes including paratyphi A, B and C, and *S. newport* were also isolated. All *E. coli* serotypes tested (33 strains) were resistant to more than three antibiotics. MAR indices ranged between 0.4 and 0.8. The highest resistance was found for amikacin (96.96%), followed by oxytetracycline (93.93%), kanamycin (90.90%), streptomycin (84.84%), tetracycline (69.6%), nalidixic acid (45.45%), ampicillin (33.33%) and gentamycin (27.27%). The lowest resistance was found for ciprofloxacin (6.06%) and chloramphenicol (9.09%). More importantly, uropathogenic serotypes of *E. coli* (O2, O7) and enteropathogenic serotypes (O114, O20, O9) were resistant to more than four antibiotics, with MAR indices from 0.4 to 0.7.

The *Salmonella* serotypes tested (26 strains) were also resistant to more than three antibiotics. The highest resistance was found for oxytetracycline (88.46%), followed by tetracycline (76.92%), streptomycin (73.07%), ampicillin (65.38%), amikacin (34.61%), kanamycin (42.3%) and nalidixic acid (23.07%). The lowest resistance was found for gentamycin (3.84%). However, all tested *Salmonella* strains were completely sensitive to chloramphenicol and ciprofloxacin, with MAR indices between 0.3 and 0.7.

The presence of pathogenic serotypes of *E. coli* and *Salmonella* in the lake is a serious health concern for the local population. Sporadic outbreaks of diarrhoea due to the prevailing clonal diversity among different categories of diarrhoeagenic *E. coli* and enteric fever due to *S. enterica* serovars paratyphi A, B and C and *S. newport* have been reported in India with an annual incidence of 3 million cases.[2,6] As *S. enterica* serovar paratyphi A has emerged as an important cause of enteric fever in India,[7] and humans are the only reservoir of this serovar, the source of *S. enterica* serovar paratyphi A in Vembanadu Lake could be faecal discharge by infected persons/carriers, indicating its high range of contamination. The problem could become more serious when these organisms acquire resistance against multiple drugs, because infection caused by such organisms will be difficult to treat. Relatively high levels (95%) of multidrug-resistant *E. coli* including pathogenic serotypes have been reported from aquatic environments.[5] It has also been demonstrated that within *S. enterica* subsp. *enterica*, there has been a serovar-dependent increase in acquired resistance to several antibiotics such as ampicillin, chloramphenicol and tetracycline, along with an increase in multidrug-resistant strains.[8]

The MAR indices (≥0.2) of *E. coli* and *Salmonella* isolates indicate that they have originated from high-risk sources of contamination, such as humans, poultry and dairy cows. This shows that the lake is experiencing severe pollution from different sources. The results show that the water quality is poor in terms of faecal coliform density and multidrug-resistant pathogenic *E. coli* and *Salmonella* serotypes, and may pose severe health risks to the population who use the water for different purposes. Government authorities need to take effective measures (e.g. proper sanitation facilities and wastewater treatment) to reduce pollution of the lake to avoid serious consequences for healthcare management within local communities.

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Competing interests

None declared.

REFERENCES

1. Kahali S, Sarkar B, Chakraborty S, Macaden R, Deokule JS, Ballal M, Nandy RK, Bhattacharya SK, Takeda Y, Ramamurthy T. Molecular epidemiology of diarrheagenic *Escherichia coli* associated with sporadic cases and outbreaks of diarrhea between 2000 and 2001 in India. *Eur J Epidemiol* 2004; **19**:473–9.
2. Threlfall EJ. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food and water-borne infections. *FEMS Microbiol Rev* 2002;**26**:141–8.
3. Rao PS, Rajashekar V, Varghese GK, Shivananda G. Emergence of multidrug resistant *Salmonella typhi* in rural Southern India. *Am 1 Trop Med Hyg* 1993;**48**:108–11.
4. Abhirosh C, Hatha AAM, Sherin V. Increased prevalence of indicator and pathogenic bacteria in Vembanadu Lake: a function of salt water regulator, along south west coast of India. *J Water Health* 2008;**6**:539–46.
5. Abhirosh C, Hatha AAM, Sherin V, Sheeja KM. Prevalence of multiple drug resistant *Escherichia coli* serotypes in a tropical estuary, India. *Microb Environ* 2008;**23**:153–8.
6. Misra RN, Bawa KS, Magu SK, Bhandari S, Nagendra A, Menon PK. Outbreak of multi-drug resistant *Salmonella Typhi* enteric fever in Mumbai Garrison. *Med J Arm for India* 2005;**61**: 48–50.
7. Gupta V, Kaur J, Chander J. An increase in enteric fever cases due to *Salmonella Paratyphi* A in and around Chandigarh. *Ind J Med Res* 2009;**129**:95–8.
8. Gallardo F, Ruiz J, Marco F, Towner KJ, Vila J. Increase in incidence of resistance to ampicillin, chloramphenicol and trimethoprim in clinical isolates of *Salmonella serotype Typhimurium* with investigation of molecular epidemiology and mechanisms of resistance. *J Med Microbiol* 1999;**48**:367–74.