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Research article

MICROBIAL QUALITY OF THE LAKE INSIDE THIRUVANANTHAPURAM ZOOLOGICAL GARDEN, KERALA, INDIA.

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ABSTRACT: A study of the microbial water quality of the lentic body inside Thiruvananthapuram Zoological Garden was carried out. The water in the lake is used for cleaning the cages of the zoo animals and for their bath. Total viable count, coli form count and *E.coli* count for the water found was exceeding the limits as prescribed by WHO and CPCB. Prevalence of indicator bacteria like *Escherichia coli* was observed during the study period. Isolation of *Staphylococcus sp* and *Pseudomonas sp* was done and are identified and confirmed biochemically. *Klebsiella oxytoca* and *Enterobacter cloacae* were identified by the sequencing of 16S rRNA and COI genes. The sequence and phylogenetic similarity search done with all entries in the DNA sequence database, GenBank using BLAST and identified. Bacterial results hint towards the pollution status of the water body. Bacterial count studies showed a higher number of bacterial colonies present in the water during the Monsoon Period. The animals under capture are more sensitive to diseases than in wild. A regular monitoring of the water is needed to check water borne diseases.

Key words: Water Quality, Zoological Garden, Thiruvananthapuram, *Escherichia coli*, BLAST

INTRODUCTION

The examination of bacteriological water quality based on technical standards is obligatory for use-related aspects such as for drinking water production, irrigation or recreation. Bacteria play an important role in global ecosystems which are being important factors in controlling the quality of water and are fate determinators of environmental pollution (Atlas & Bartha, 1993). Bacteriological assessment of water is an important tool to study the water quality and status of hazardous microorganisms.

The seriousness of pathogen contamination is reflected in the maximum contaminant level goals of zero for microbial pathogens, which were established through Safe Drinking Water Act (SDWA) regulations. These goals conform to the position of the World Health Organization (WHO,1993) which states: '….there is no tolerable lower limit for pathogens, and water intended for consumption, for preparing food and drink, or for personal hygiene, should thus contain no agents pathogenic for humans.' Water quality standards are based on the concentrations of bacterial indicator organisms and the designated use of the water body. Measuring microbial indicators is less expensive, easier and more common than measuring pathogens directly. However, indicator organisms used by monitoring programmes are limited in their ability to predict pathogen presence and health risks (Arnone and Joyce 2007). Zoos, conservation parks and wildlife-rehabilitation centres which house a wide range of captive wildlife are for aesthetic, educational and conservation purposes (Yvonne *et al* ,2009) Inadequate information of diseases is a major limiting factor for the captive zoo animals (Rajasekharaiah *et al* 1971). Central Zoo Authority (CZA) is the prime statutory body which regulates Zoological parks in India. The main objectives of CZA are to provide the urban population with a window to nature and to serve as green lungs for the polluting environment. Zoos shall extend their expertise and help to State Governments and local authorities to create nature parks extending over extensive areas near big cities. Every Zoo shall maintain a healthy, hygienic and natural environment in the zoo, so that the visitors get an adequate opportunity to experience a natural environment. A water body inside the zoo has its own importance, as the quality of water may affect the animals when it is provided to them. Captive animals maybe exposed to high-density and high-stress environments which can result in increased susceptibility to infection and disease. They are also exposed to species of parasites that they would not usually encounter in the wild (Theron & Cloete, 2002).

Zoo should be aware of the necessary water quality standards for all species in their care. Regular monitoring and recording of water quality should be performed and water quality should be maintained to appropriate levels. Considerably no study has yet been carried out in this lake inside Thiruvananthapuram Zoo regarding the bacterial water quality.

The present study enabled a comprehensive analysis of bacterial status including the pollution indicator bacteria of the lake.

MATERIALS AND METHODS

Study Area

The Thiruvananthapuram Museum and Zoo is one of the oldest of its kind in India located at the heart of the city, is at **N 08˚30' E 076˚57'**. Swathi Thirunal (1813-1847), illustrious king and music composer who ruled southern Kerala (Travancore) during 1830-1847 may be said to be the visionary behind establishment of the Thiruvananthapuram Museum and Zoo (Nair, A.S. 2003). The Zoo was established as an annexe to the Museum in 1857 in order to attract more visitors. The expansive lake inside the Thiruvananthapuram Zoo - complete with an island, lush vegetation and the consequent abundance of winged visitors - has never failed to arrest the attention of visitors. The lake is extended over 7693.93 sq.m, often named as the 'The lungs of the city'. A sketch of the lake showing the sampling sites is shown in Figure 1.

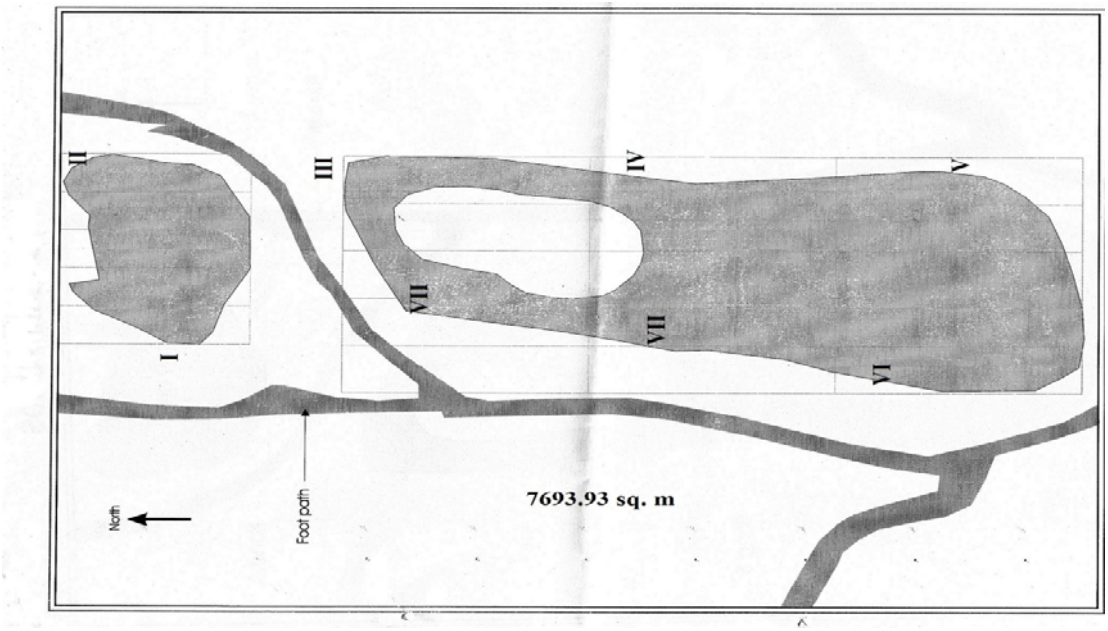

Figure.1: Sketch of the Thiruvananthapuram Zoo Lake

Identification of Bacteria

The water samples were collected for one year (April 2013 to March 2014) with the help of pre- sterilized screw capped bottles and streak plate method containing three different agar plates Nutrient Agar, MacConkey Agar and Eosin Methylene Blue (EMB) Agar media had been adopted for survey of bacteria. The cultural characteristics such as colour and surface of colonies were observed along with any other peculiar feature of culture growth. Morphology of these cultures was studied by gram staining technique. In order to identify bacteria, different methods which were used are Cultural characteristics, Morphology, Pigment production, Staining reaction and Biochemical reaction. Bacterial colonies were identified on the basis of different biochemical test *viz*, H_2S production, Citrate utilization test, Catalase test, Oxidase test, Indole Production test , Voges-Proskauer test, Methyl Red test, Lactose reduction test and Motility. Morphological and biochemical tests were done for the identification of particular bacteria as per methods given in Central Diagnostic Laboratory Manual, Bergey's Manual (9^{th} edn) and Cowan and Steel's Manual (3^{rd} edn).

Molecular Identification of the Isolate

DNA extraction and PCR Amplification

The genomic DNA was isolated from the given organism using NucleoSpin® Tissue Kit (Macherey-Nagel).

PCR Amplification of the 16s rRNA gene was performed using the universal primers

Primers used

Target	Primer Name	Direction	Sequence (5' → 3')
16S rRNA	16S-RS-F	Forward	CAGGCCTAACACATGCAAGTC
	16S-RS-R	Reverse	GGGCGGWGTGTACAAGGC

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

Agarose Gel electrophoresis of PCR products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. 1 µl of 6X loading dye was mixed with 5 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was a 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad) (Figure 2).

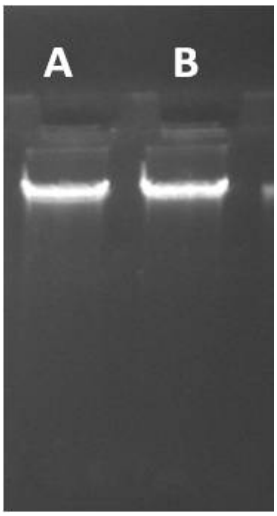

Figure 2: Image showing Gel Documentation of DNA

ExoSAP-IT Treatment

ExoSAP-IT (USB) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications.

Five micro litres of PCR product is mixed with 2 µl of ExoSAP-IT and incubated at 37^{o}C for 15 minutes followed by enzyme inactivation at 80^{o}C for 15 minutes.

Sequencing using BigDye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems , USA) following manufactures protocol.

The PCR mix consisted of the following components:

PCR Product (ExoSAP treated)	-	10-20 ng
Primer	-	3.2 pM (either Forward or Reverse)
Sequencing Mix	-	0.28 µl
Reaction buffer	-	1.86 µl
Sterile distilled water	-	make up to 10µl

The sequencing PCR temperature profile consisted of a 1st cycle at 96°C for 2 minutes followed by 30 cycles at 96°C for 30 sec, 50°C for 40 sec and 60°C for 4 minutes.

After post sequencing clean up air dried PCR product was sequenced in ABI 3730 DNA Analyzer (Applied Biosystems).

Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.6 (Drummond et al., 2012). Bioinformatics analysis Sequences were compared to the non-redundant NCBI database by using BLASTN, with the default settings used to find the most similar sequence and were sorted by the E score.

RESULTS AND DISCUSSIONS

Bacterial Indicators

During the study period, only 14 isolates were obtained for pure culture and by Gram's staining, one was Gram +ve Cocci and one was Gram –ve Cocci, 2 were Gram +ve Bacilli and 8 were Gram –ve Bacilli. Gram-positive non-motile oxidase –ve cocci was identified as *Staphylococcus* sp. The Gram-negative rods with IMVC test (Indole, Methyl Red, Vogues Proskauer, Citrate)++-- that gave a green metallic sheen colonies in EMB agar were identified as Escherichia coli, and Gram negative rods with greenish pigment production and discolouration of the BHA agar that gave catalase +ve oxidase +ve, Indole –ve results were identified was Pseudomonas sp.

Molecular Identification

After the DNA extraction of the organisms, the PCR amplification is carried and DNA was sequenced. The PCR amplification on agarose gel is given in fig 3.

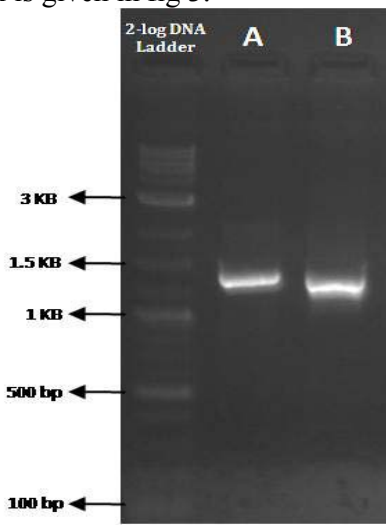

Figure 3: PCR Amplification on Agarose Gel.

The isolated bacteria were having 99% similarity with *Klebsiella oxytoca* (Sample A) (Shin et al 2012) and 99% similarity with *Enterobacter cloacae* (Sample B) (Ren et al 2010) for the second isolate.
A list of 14 isolates and their identification are given in Table 1 and the Total Viable Count and *E.coli* count for the three seasons are given in Table 2.

Bacteriological results hint towards the pollution status of the water body. Bacterial count studies showed a higher number of bacterial colonies present in the water during the Post-Monsoon period. The faecal coli form bacteria *Escherichia coli* in water are a recommended indicator of faecal contamination for water. *E.coli* is present in the gastro- intestinal tracts of warm- blooded birds and animals. The lake is associated with a good number of avifaunal visitors and any contamination in this lake is due to avian excrements rather humans. And the presence and source of enteric bacteria may be from these birds. Birds' excrements are a potential source of biogenic elements, responsible for eutrophication of water bodies (Gwiazda 1996), as well as pathogenic microorganisms (Buck 1990, Fricker 1984, Kapperud, Rosef 1983, Kuhn et al. 2002, Palmgren et al. 1997, Seymour et al. 1994). As a result of the microbiological contamination of surface waters, water quality deteriorates and humans or animals in contact with such water risks higher incidence of illnesses (Hagedorn et al. 1999, Medema et al. 1997).
The permissible level of *E.coli* in recreational water is 400 CFU/ml and is Nil for drinking water (WHO).

Although not necessarily agents of diseases, faecal coli form bacteria may indicate the potential presence of disease carrying organism. In India, Patra *et al* (2009) showed a positive relationship between faecal indicators and pathogenic microorganisms. E.coli was observed in all the seasons and the prevalence was found rather high than as prescribed by WHO and CPCB. *Pseudomonas sp* acts as an indicator of organic pollution. Different bacterial genera which were identified are indicators of organic pollution as in the case of Thiruvananthapuram Zoo Lake. The organic pollution may be due to increasing organic matter like the presence of leaf litter from the Rain tree *Samanea saman*, (jacq) Merr located along shores of the lake

Table-1: Microbial Identification

Growth	NA	MA	MA	NA	MA	MA	NA	NA	EMB	NA	MA	MA	MA
Colour of the colony	C	D R	C L	Y	P	C	C	Y	G M	C	P	P	C L
Gram's staining and Morphology	-ve B	+ve B	+ve B	-ve B	-ve B	-ve B	+ve C	-ve B	-ve B	-ve C	-ve B	-ve B	-ve B
Motility	-	-	+	-	?	+	?	?	+	-	-	+	+
Catalase	-	+	+	-	+	+	+	+	+	-	+	+	+
Oxidase	+	+	+	-	-	+	+	+	-	+	-	-	-
Indole	-	?	+	-	-	-	-	+	+	+	-	+	-
MR	+	+	-	+	+	+	+	+	+	-	+	+	-
VP	+	-	+	+	?	?	-	+	+	+	-	-	-
Citrate	?	+	?	+	?	+	-	-	?	-	+	+	-
H_2S	-	?	?	+	-	+	+	+	+	+	-	?	-
Nitrate											-	-	-
Urease											+		
Sucrose													+
Lactose		+		+	+		-	-	+	-		+	?
Adanitol											+		-
Dulcitol											?		
Mannitol												+	+
Organism	UD	UD	UD	UD	UD	*Pseudomonas sp.*	UD	*Staphylococcus sp.*	*Escherichia coli*	UD	*Klebsiella oxytoca*	*Enterobacter cloaca*	UD

(NA- Nutrient Agar; MA- MacConkey Agar; EMB- Eosin Methylene Blue; B- Bacilli; C- Cocci; D R- Dark Red; ?- Not Known; C L- Colour Less; Y- Yellow; P- Pink; C- Cream; G M- Green Metallic sheen; M R- Methyl Red; V P- Vogues Proskauer; UD- Unidentified)

Table 2: Total coli form count and *E.coli* count

Study Period	*E.coli* Count	Total Viable Count
Pre- Monsoon	90 CFU/ml	854 CFU/ml
Monsoon	335 CFU/ml	2301 CFU/ml
Post- Monsoon	88 CFU/ml	3965 CFU/ml

(CFU – Coli form Unit)

Bacteria of the genus *Klebsiella* are opportunistic pathogens that can lead to severe diseases such as septicaemia, pneumonia, urinary tract infections, and soft tissue infections (Kovtunovych *et al* 2003).

The pathogenic bacteria thus isolated were mostly belonging to the Enterobacteriaceae. The presence of Enterobacteriaceae members in the tested water samples indicates the faecal pollution. A good number of resident breeding Cormorant populations can be found at anytime here in and around Thiruvananthapuram Zoo lake. Moreover, Wiśniewska et al (2007) has shown a positive relationship between Cormorant population and Enterobacteriaceae bacteria from Długie Wigierskie Lake, which are situated in Wigry National Park in Poland.

The study reveals that the concentrators of organic materials from a wide range of sources can support bacterial populations, a portion of which are capable of responding positively to the Total Coli form and Faecal Coli form tests.

Naturally, water contains a large number and variety of micro organisms which does not necessarily, make such water not portable (Sandhya *et al*., 2014).

CONCLUSION

The presence of pathogenic bacteria such as *E.coli, Enterobacter cloacae, Klebsiella oxytoca, Pseudomonas sp* and *Staphylococcus sp* indicates that the water is unfit for either utility or drinking purposes. Similar findings were done by Shittu *et al*., (2008) and Sandhya *et al* (2014). The incidence of health risks when this water gets into contact with animals under capture is high. The animals under capture are more susceptible to diseases than the one in wild. Thus the study strongly recommends proper management and preservation of the quality of the Lake inside the Thiruvananthapuram Zoo and the use of safe and purified form when it is given to the animals for cage cleaning and their bath.

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Abbreviations: WHO- World Health Organization; CPCB- Central Pollution Control Board; COI genes- Cytochrome c oxidase subunit I; BLAST- Basic Local Alignment Search Tool.

REFERENCES

Achuthsankar, S. Nair (2003). Dept. of Culture, Govt of Kerala, India, Centre for Development of Imaging Technology & Vailoppally Sanskriti Bhavan A Brief History of the Trivandrum Museum, Zoo and Gardens, http://www.swathithirunal.in/rlinsti/musiume.htm

Atlas, R.M and Bartha, R. (1993). Microbial ecology: Fundamentals and Application (3rd Edition) (Redwood City, Benjamin) pp 576.

Barrow, G. I and Feltham, R.K.A. (2004). Cowan and Steel's Manual for the Identification of Medical Bacteria Cambridge University Press pp 352.

Buck, J.D. (1990). Isolation of Candida albicans and halophilic Vibrio spp. from aquatic birds in Connecticut and Florida. Appl. Environ. Microbiol. 56: 826-828.

Central Pollution Control Board www.cpcb.nic.in

Drummond, A.J, Ashton, B., Buxton, S, Cheung, M, Cooper, A, Duran, C, Heled, J, Kearse, M, Markowitz, S, Moir, R, Stones-Havas, S, Sturrock, S, Swidan, F, Thierer, T, Wilson, A. (2012). Geneious v5.6, Available from www.geneious.com

Fricker, C.R. (1984). A note on salmonella excretion in the black-headed gull (Larus ridibundus) feeding at sewage treatment works. J. Appl. Bacteriol., 56: 499-502.

General Policy about Zoos. Available at www.cza.nic.in/general.html

Gennadiy, Kovtunovych. Tetyana, Lytvynenko. Valentyna, Negrutska. , Olena, Lar., Sylvain, Brisse and Natalia, Kozyrovska. (2003). Identification of Klebsiella oxytoca using a specific PCR assay targeting the polygalacturonase pehX gene Research in Microbiology 154 :587–592.

Gwiazda, R. (1996). Contribution of water birds to nutrient loading to the ecosystem of mesotrophic reservoir. Ekol. Pol., 44: 289-297.

Hagedorn, C, Robinson, S.L, Filtz, J.R, Grubbs, S.M, Angier, T.A. Reneau, Jr. R.B. (1999). Determining sources of fecal pollution in a rural Virginia watershed with antibiotic resistance patterns in fecal streptococci. Appl. Environ. Microbiol, 65: 5522-5531.

Hanna, Wiśniewska, Stanisław, Niewolak, Ewa, Korzeniewska and Zofia, Filipkowska. (2007). Enterobacteriaceae Family Bacteria In A Mesotrophic Lake (Lake Długie Wigierskie) In The Presence Of Black Cormorants (Phalocrocorax Carbo) Pol. J. Natur. Sc., Vol 22(3):486-499,

John, G, Holt and David, Heodricks, Bergey. (1993). Bergey's Manual of Determinative Bacteriology. Wolter Kluwer Publishers pp 816.

Kapperud, G, Rosef. O. (1983). Avian wildlife reservoir of Campylobacter fetus subsp. jejuni, Yersinia spp., and Salmonella spp. in Norway. Appl. Environ. Microbiol. 45: 375-380.

Kuhn, R.C, Rock, C.M. and Oshima, K.H. (2002). Occurrence of Cryptosporidium and Giardia in wild ducks along the Rio Grande River valley in southern New Mexico. Appl. Environ. Microbiol., 68: 161-165.

Medema, G.J, Van Asperen, I.A and Havelaar, A.H. (1997). Assessment of the exposure of swimmers to microbiological contaminants in fresh waters. Wat. Sci. Tech., 35: 157-163.

Palmgren, H., Sellin, M., Bergstrom, S., Olsen, B. (1997). Enteropathogenic bacteria in migrating birds arriving in Sweden. Scand. J. Infect. Dis., 29: 565-568.

Patra, A.K. Acharya, B.C and Mohapatra, A (2009). Occurrence and distribution of bacterial indicators and pathogens in coastal waters of Orissa, Indian J. Mar. Sc., 38(4), , 474-480.

Rajasekhariah, G. R, Hedge, K.S, Gowda, R.N.S., Rahman, S.A, & Rao, H.S., (1971). A study of some parasites from panther cubs (Felix pardes) with the description of Eimeria anakalensis, Mysore. Journal of Agricultural Science. 5: 404-409.

Rajesh, Rathore. (2013). Manual of Bacterial Disease Investigation 6th ed. Central Disease Diagnostic Laboratory , Centre for Animal Disease Research and Diagnosis , Indian Veterinary Research Institute, Izatnagar, Bareilly, UP

Russell, D. Arnone and Joyce, Perdek, Walling. (2007). Water borne pathogens in urban watersheds Journal of Water and Health. 05(1): 149-162

Sandhya, Deepika, D, .Jyothi N S M and Laxmi Swomya K (2014). Physico-Chemical and microbial analysis of water bodies in Andhra University, Visakhapatnam, India Int J Curr Sci 12: E 72-78.

Sang, Heum, Shin, Sewhan, Kim, Jae, Young, Kim, Soojin, Lee, Younsoon, Um, Min-Kyu, Oh, Young-Rok, Kim, Jinwan, Lee and Kap-Seok, Yang. (2012). Complete Genome Sequence of Klebsiella oxytoca KCTC 1686, used in the production of 2, 3- butanediol. J. Bacteriol. 194(9), 2371-2372.

Seymour, C, Lewis, R.G, Kim, M, Gagnon, D.F, Fox, J.G, Dewhirst, F.E, Paster, B.J. (1994). Isolation of Helicobacter strains from wild bird and swine feces. Appl. Environ. Microbiol. 60: 1025-1028.

Shittu.O B, Olaitan,.J O, Amusa.T S (2008). Physico-chemical and bacteriological analyses of water used for drinking and swimming purposes in Abeokuta, Nigeria. African Journal of Biomedical Research 11:285-290.

Theron, J, Cloete, T.E, (2002). Emerging waterborne infections: contributing factors, agents and detection tools. Crit. Rev. Microbiol. 28, 1–26.

WHO (World Health Organization) (1993). Waterborne Infections, Guidelines for Drinking Water Quality, 2nd edn, Geneva, www.studiengang-wasser.de/files/VorlHygieneTeil5.pdf.

Yan, Ren, Yi, Ren, Zhemin, Zhou, Xi, Guo, Yayue, Li, Lu, Feng and Lei, Wang. (2010). Enterobacer cloacae subsp.cloacae ATCC 13047 Chromosomes, complete genome. J. Bacteriology. 192 (9) 2463-2464

Yvone, A.L. Lim, Monica M. Lai, Mohammed A.K. Mahdy, H.R. Mat Naim , Huw V. Smith, (2009). Molecular detection of Giardia contamination in water bodies in a zoo. Environmental Research: 109 857-859.

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