International Journal of Research Studies in Biosciences (IJRSB)
Volume 3, Issue 7, July 2015, PP 72-79
ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)
www.arcjournals.org

Bacteriological Assessment of Water Samples of Indore City: A Study of Prevalence of Drug Resistant Bacteria

Nandini Phanse[1*], Vartika Mishra[1], Shivangi Agrawal[1]

[1]Department of Microbiology, PMB Gujarati Science College, Indore (M.P.)
[*]*nandiniphanse@rediffmail.com*

Pragya Rathore[2]

[2]Department of Biotechnology, SIMS, Indore

Abstract: *The issue of quality water supply is assuming paramount importance in Indore city in view of rapid urbanization. A major problem of the city of Indore regarding water reservoirs is the disposal of industrial and domestic waste in the Khan River flowing in the city thus creating hazards to public health. Water samples collected from area along the Khan River were analysed to assess their potability. In the present study it has been found that polluted water of Khan River has seeped into the ground water and has polluted bore wells present in colonies along the river belt. Furthermore, in some residential areas mixing of sewage with underground pipelines of municipal water supplies has also been found. Out of the 64 water samples analysed, 36 were found to be non-potable with the occurrence of drug resistant bacteria in 86% of the non-potable samples. 65% of the household municipal water supply was potable while only 22% samples of ground water from bore wells were potable.*

Keywords: *Ground water, municipal water supply, non-potable water, coliforms, drug resistant bacteria*

1. INTRODUCTION

Maintenance of the microbiological quality of water has been used as an important means of preventing waterborne disease (WHO Report, 2008).The major sources of drinking water include the municipal water supply (treated water), ground water from bore wells, surface water of rivers, lakes etc. These sources of water can become contaminated at the original water source, during treatment, or during distribution to the home; due to discharges from domestic wastes, industries, mining activities, and flow from irrigation which add chemicals like pesticides, fertilisers etc.[1]. Wastewater discharges in fresh waters are the major source of feacal microorganisms, including pathogens [2, 3, 4].

Ground water is an important source of drinking water. There is a common misconception among the people that ground water is generally safe for human consumption. This is not true particularly in the high density residential areas where sewage disposal practices are not proper and sewage continuously seeps in the ground thus contaminating ground water bodies. The problem of contamination of ground water quality is more acute in areas that are densely populated [5]. This affects the quality and purity of ground water thus directly affecting human health.

Pollution of water by microbial contaminants of faecal origin through sewage not only leads to spread of water borne infections [6] but also involves the spread of multiple drug resistant organisms in the environment. As reported in *The Lancet Infectious Diseases*, bacteria carrying a gene that confers resistance to a major class of antibiotics have shown up in samples of drinking water and sewage seepage from New Delhi [7].The environmental spread of drug resistant bacteria is a matter of concern as it can lead to transmission of antibiotic resistance to pathogenic bacteria. Treatment of infections with antibiotic resistant bacteria is extremely difficult [8]. In this context detection of the presence of coliforms as indicators of faecal pollution and the occurrence of transmissible antibiotic resistance among coliforms in drinking water is essential.

The issue of quality water supply is assuming paramount importance in Indore city in view of rapid urbanisation. A major problem of the city of Indore regarding water reservoirs is the disposal of industrial and domestic waste in the Khan river flowing in the city thus creating hazards to public health [9]. A recent news article published in the newspaper, Dainik Bhaskar (DB Star, 22.07.14) reporter Avinash Ravat has stated that the polluted water of Khan River has seeped into the ground water and has polluted it [10].

The present work is an attempt to examine the water quality of various resources in Indore city and gain information about the quality of water in terms of its potability and prevalence of drug resistant bacteria transmitted through it.

2. MATERIALS & METHODS

2.1. Study Area and Collection of Samples

Different locations of Indore city present along the bank of river Khan, were selected for collection of water samples (Table 01).

Most of the samples collected for analysis were ground water sources. Along with these, treated water sources from tap water and municipal water supply tanks (municipal water supply) were also analysed for a comparative study. A total of 64 water samples were collected from bore wells, tap water (municipal supply water) and tanks storing municipal water, from each site.

About 100 ml samples were collected in non reactive borosilicate glass or plastic bottles sterilized by autoclaving at 121°C, 30 min.

Samples were assessed within 24 hours for heterotrophic plate count (CFU/ml), presence of coliforms (Total Coliforms and Fecal Coliforms) by using standard protocol of American Public Health Association [11]. All samples were analysed for heterotrophic plate count on R2A agar medium, total coliform (TC) count by multiple fermentation tube method [11]. Confirmed phase was performed using brilliant green lactose bile broth and MacConkey agar medium was used for completed phase.

Suspected colonies were then identified by microscopically and by standard biochemical tests.

For detection of thermo tolerant fecal coliforms, elevated temperature test was performed using EC broth. Faecal coliform bacteria are differentiated in the laboratory by their ability to ferment lactose, with production of acid and gas at 44.5 °C within 24 h.

Presence or absence of *Salmonella* in the water samples was checked by enrichment in selenite broth and isolation and confirmation on Wilson -Blair medium.

2.2. Antibiotic Resistance Profile

Antibiotic sensitivity testing was done using standardized KirbyBauer Disc Diffusion Test [12].The isolated bacterial colonies which were identified by standard biochemical characters were tested against eight commonly used antibiotics, i.e. Ciprofloxacin(5µg), Carbenicillin (100µg), Choramphenicol(30µg), Cefoperazone(75µg), Tetracycline(30µg), Amikacin(30µg), Gentamicin(10µg), Ampicillin(10µg),Ceftriaxone (30µg) and Norfloxacin (10µg). Bacteriological culture media and antibiotic discs of Hi-Media Pvt. Ltd. Mumbai, were used.

3. RESULTS & DISCUSSION

The bacteriological analysis of our studies, depicted in Table 01, shows coliform contamination in 78 % of ground water samples and 35 % of household municipal water supply while all water samples analysed from municipal water tank were potable. Among all the sites selected for the study, 56% samples showed TC and FC and heterotrophic bacterial counts above permissible levels, as per WHO [13]. According to WHO guidelines, potable water samples should have a zero count of TC and FC. Also, the heterotrophic bacterial counts in water which is used for drinking purpose should not be above 100cfu/ml. In such circumstances, the present study revealed that most of the ground water samples did not meet the parameters of the bacteriological quality.

In areas lying close to the Khan river, the highly polluted water of the of Khan River has seeped into the ground water and has polluted it. Water of bore wells present in colonies along the river belt has thus been severely polluted [10]. The Khan River passes through the city, and flows from Devguradia to Kabit Khedi through heart of city. In this journey about 25 km of Khan River passes through the city areas. (Fig.01). Due to urbanization of the city the river has become a waste water disposal site. The results of the present work have also revealed the household municipal water supply in most colonies has also been severely polluted and is heavily contaminated with coliforms. In contrast to this, the water analysed from the municipal water tanks which supplies water in respective areas, was not contaminated and was safe for drinking purpose. The drinking water supply in most parts of the city is through supply of water from river Narmada (municipal water supply).In the high density residential areas where sewage disposal practices are not proper, mixing of sewage with ground water and underground pipelines of municipal water supplies occurs. This is a shocking reality about 2007, unhygienic conditions in Indore. Similar reports were given by Anand et al., 2006; Krishnan et al., in their study on water analysis [14,1].

Table01. *Bacteriological analysis of water samples from different locations of Indore city*

Sample No.	Site of sample collection	Type of water supply	Heterotrophic plate count (CFU/ml)	Total Coliform count/100 ml (MPN)	* Fecal Coliforms
S 1	Gangour Ghat	Municipal water supply (household tap water)	$45x 10^2$	> 240	+
S 2	Gangour Ghat	Bore well	360	21	+
S 3	Chatri Baug	Bore well	570	38	+
S 4	Vainkatesh temple area	Bore well	240	15	-
S 5	Chatri Baug, Jairampur colony	Municipal water supply tank	53	-	-
S 6	Harsiddhi	Bore well	$76x 10^2$	> 240	+
S 7	Moti Tabela	Bore well	$41 x 10^2$	240	-
S 8	Juni Indore	Bore well	$54 x 10^2$	> 240	+
S 9	Juni Indore	Municipal water supply (household tap water)	630	15	+
S 10	Chawni	Bore well	320	15	-
S 11	Parsi Mohalla	Bore well	$65 x 10^2$	38	-
S 12	Nai Anaj Mandi	Bore well	$89 x 10^2$	> 240	-
S 13	Gumashta Nagar	Bore well	$49 x 10^2$	240	+
S 14	Scheme No. 71	Municipal water supply tank	58	-	-
S 15	Scheme No. 71	Bore well	360	5	-
S 16	Subhash Nagar	Municipal water supply (household tap water)	79	-	-
S 17	Pardesipura	Bore well	$97 x 10^2$	240	+
S 18	Nehru Nagar	Bore well	$71 x 10^2$	240	+
S 19	Manik Baug Road	Municipal water supply (household tap water)	62	-	-
S 20	Manik Baug Road	Bore well	$61 x 10^2$	> 241	-
S 21	Lokmanya nagar	Municipal water supply (household tap water)	49	-	-
S 22	Kesar Baugh Road	Bore well	$87 x 10^2$	> 240	+
S 23	Lokmanya nagar	Municipal water supply (household tap water)	47	-	-
S 24	Lalaramnagar	Bore well	79	-	-
S 25	Mahesh nagar	Bore well	650	21	-
S 26	Radha nagar	Bore well	$74 x 10^2$	38	+
S 27	Navlakha	Bore well	$52 x 10^2$	96	+
S 28	South Tukoganj	Bore well	96	-	-
S 29	South Tukoganj	Municipal water supply (household tap water)	316	5	+
S 30	Yeshwant Club area	Municipal water supply tank	69	-	-
S 31	Killa Maidan Road	Municipal water supply (household tap water)	83	-	-
S 32	Banganga	Bore well	$49 x 10^2$	38	+
S 33	Banganga	Municipal water supply (household tap water)	$21 x 10^2$	15	-
S 34	Tilak Nagar	Bore well	86	-	-
S 35	Tilak Nagar	Municipal water supply (household tap water)	72	-	-
S 36	Suhkaliya	Municipal water supply tank	93	-	-
S 37	Geeta Bhavan area	Municipal water supply (household tap water)	58	-	-
S 38	Geeta Bhavan area	Bore well	69	-	-
S 39	Annapurna Road	Municipal water supply (household tap water)	85	-	-
S 40	Annapurna Road	Bore well	91	-	-
S 41	Usha Nagar	Bore well	77	-	-
S 42	Usha Nagar	Municipal water supply (household tap water)	63	-	-
S 43	Vaishali nagar	Bore well	$85 x 10^2$	12	+
S 44	Sudama Nagar	Municipal water supply (household tap water)	63	-	-
S 45	Sudama Nagar	Bore well	$72 x 10^2$	38	-
S 46	Prikanko Colony	Bore well	$54 x 10^2$	240	+
S 47	Prikanko Colony	Municipal water supply	49	-	-

		(household tap water)			
S 48	Rajendra Nagar	Bore well	71 x 10^2	>240	+
S 49	Rajendra Nagar	Municipal water supply (household tap water)	53x 10^2	98	-
S 50	Chandrabhaga area	Bore well	103 x 10^2	>240	+
S 51	Chandrabhaga area	Municipal water supply (household tap water)	98 x 10^2	240	-
S 52	Muktidham area (Near Sarvate bus stand)	Bore well	114 x 10^2	>240	+
S 53	Muktidham area (Nea Sarvate bus stand)	Municipal water supply (household tap water)	98 x 10^2	96	+
S 54	Rambaug	Bore well	37 x 10^2	38	+
S 55	Rambaug	Municipal water supply (household tap water)	72	-	-
S 56	Vijaynagar	Bore well	84	-	-
S 57	Vijaynagar	Municipal water supply (household tap water)	61	-	-
S 58	Vijaynagar	Municipal water supply tank	85	-	-
S 59	Sukhaliya	Municipal water supply (household tap water)	39 x 10^2	96	-
S 60	Sukhaliya	Bore well	95 x 10^2	>240	+
S 61	Veena Nagar	Bore well	98 x 10^2	240	-
S 62	Veena Nagar	Municipal water supply (household tap water)	52	-	-
S 63	Shreenagar	Bore well	77	-	-
S 64	Shreenagar	Municipal water supply (household tap water)	54	-	-

* + = Acid & gas production by coliforms in EC medium at 44.5 °C within 24 h - = Absent

Fig1. *City of Indore*

Another dimension of the present study was to check the prevalence of multiple drug resistance in the organisms isolated from water samples. The results of the susceptibility of the bacteria isolated from the analysed samples, showed that contaminants were resistant to commonly used antibiotics (Table 2). Fig. 02 shows the percentage of *E.coli* , *Enterobacter*, *Kleibsella*, *Citrobacter*, *Pseudomonas* isolates resistant to antibiotics. The bacterial strains isolated from water samples included E.coli, *Enterobacter aerogenes*, *Kleibsella pneumoniae*, *Citrobacter fruendii*, *Pseudomonas species and Salmonella typhi*. The presence of these organisms is not only of public health importance as coliforms are indicators of fecal contamination and because of their potential as pathogens but also because of the fact that they are resistant to antibiotics. Antibiotic resistant coliform bacteria are common in the intestine of man and as a result of sewage pollution they may become widely disseminated into the environment and transfer antibiotic resistance to other sensitive coliforms or enteric pathogens [15, 16, 17]. Multi-drug resistant (MDR) strains of *E. coli* in drinking water due to mixing of sewage lines with drinking water supplies have been reported [18,19].More recently, disease-causing bacteria carrying the new genetic resistance to antibiotics, NDM-1, have been discovered in New Delhi's drinking water supply [7]. The presence of MDR bacteria in environmental samples has important implications for people who are reliant on public water and sanitation facilities.

Table2. *Antibiotic resistance pattern of bacterial isolates obtained from non-potable water samples*

S.No.	Sample No.	Bacterial Isolates	Antibiotic Resistance
1	S 1	*E. coli* *Enterobacter aerogenes*	Ampicillin, Gentamycin Ampicillin
2	S 2	*E. coli* *Pseudomonas aeruginosa*	Ampicillin, Gentamycin Gentamycin
3	S 3	*Enterobacter aerogenes* *Pseudomonas aeruginosa* *Citrobacter fruendii*	Ciproloxacin, Norfloxin, Ampicillin Amikacin, Gentamycin -
4	S 4	*Enterobacter aerogenes* *Citrobacter fruendii*	Norfloxin, Ampicillin Norfloxacin, Gentamyci
5	S 6	*E. coli* *Enterobacter aerogenes*	Tetracycline, Cholramphenicol, Ampicillin Ampicillin, Chloramphenicol
6	S 7	*Enterobacter aerogenes* *Pseudomonas aeruginosa*	- Amikacin
7	S 8	*E. coli* *Pseudomonas aeruginosa* *Salmonella typhi*	Carbenicillin, Norfloxcin, Ampicillin Amikacin, Gentamycin -
8	S 9	*E. coli* *Enterobacter aerogenes*	- -
9	S 10	*Enterobacter aerogenes* *Pseudomonas aeruginosa*	Ampicillin, Norfloxacin -
10	S 11	*Enterobacter aerogenes* *Pseudomonas aeruginosa*	- Ampicillin, Gentamycin
11	S 12	*E. coli* *Enterobacter aerogenes* *Salmonella typhi*	Carbenicillin, Norfloxcin, Ampicillin - -
12	S 13	*E. coli* *Enterobacter aerogenes* *Pseudomonas fluorescens*	- - -
13	S 15	*E. coli* *Klebsiella pneumoniae*	Carbenicillin, Ampicillin Carbenicillin, Ampicillin, Cefoperazone
14	S 17	*E. coli* *Enterobacter aerogenes* *Pseudomonas fluorescens*	- Carbenicillin, Amikacin Tetracycline, Ampicillin
15	S 18	*E. coli* *Enterobacter aerogenes* *Salmonella typhi*	Ampicillin, Amikacin - Ampicillin
16	S 20	*E. coli* *Enterobacter aerogenes*	Carbenicillin, Norfloxcin, Ampicillin -
17	S 22	*E. coli* *Enterobacter aerogenes*	Ampicillin, Ciprofloxacin Carbenicillin, Norfloxcin, Ampicillin
18	S 25	*Enterobacter aerogenes* *Pseudomonas aeruginosa*	Tetracycline, Ampicillin , Carbenicillin Gentamycin, Amikacin
19	S 26	*E. coli* *Pseudomonas aeruginosa* *Salmonella typhi*	Amikacin, Ampicillin - -
20	S 27	*E. coli* *Pseudomonas aeruginosa* *Klebsiella pnuemoniae*	Tetracycline, Ampicillin Norfloxacin, Amikacin -
21	S 29	*E. coli* *Enterobacter aerogenes* *Citrobacter fruendii*	Ampicillin, Amikacin - -

22	S 32	*E. coli* *Enterobacter aerogenes* *Pseudomonas aeruginosa*	Ampicillin, Amikacin - Norfloxacin, Amikacin, Gentamycin
23	S 33	*Enterobacter aerogenes* *Pseudomonas aeruginosa*	Carbenicillin, Ampicillin Gentamycin
24	S 43	*E. coli* *Enterobacter aerogenes*	Amikacin, Ampicillin, Chloramphenicol Amikacin, Gentamycin
25	S 45	*E. coli* *Enterobacter*	Ampicillin, Gentamycin -
26	S 46	*E. coli* *Enterobacter aerogenes*	Ampicillin, Cefoperazone, Norfloxacin Ampicillin, Cefoperazone, Norfloxacin Tetracycline, Carbenicillin,
27	S 48	*Citrobacter fruendii* *Pseudomonas fluorescens* *Klebsiella pneumoniae*	Ampicillin , Amikacin Gentamycin, Amikacin Ampicillin , Amikacin
28	S 49	*Citrobacter fruendii* *Pseudomonas aeruginosa*	Ampicillin Ampicillin
29	S 50	*E. coli* *Enterobacter aerogenes*	Amikacin, Ampicillin Ampicillin
30	S 51	*E. coli* *Citrobacter fruendii*	Ampicillin, Gentamycin Ampicillin
31	S 52	*Enterobacter aerogenes* *Pseudomonas aeruginosa*	Ampicillin, Amikacin Gentamycin, Amikacin,Ciprofloxacin
32	S 53	*Enterobacter aerogenes*	Ampicillin, Cefoperazone
33	S 54	*E. coli* *Klebsiella pneumoniae*	- -
34	S 59	*Enterobacter aerogenes*	Ampicillin, Amikacin
35	S 60	*E. coli* *Enterobacter aerogenes*	Ampicillin Amikacin Ampicillin
36	S 61	*Enterobacter aerogenes*	-

- Sensitive to all antibiotics, under study

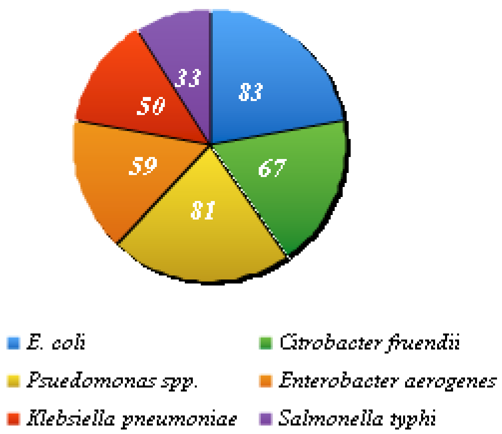

Figure02. *Percentage of antibiotic resistant bacterial isolates*

4. Conclusion

This study showed that the discharged industrial and domestic effluent in the Khan River flowing through the city of Indore has not only severely polluted it but the contaminated water has seeped into the ground water thus affecting its potability too. Furthermore, due to the lack of proper sewerage system, mixing of sewage with underground pipelines of municipal water supplies also occurs. This study also showed the occurrence of antibiotic resistant bacteria in the analysed water samples. The continued emergence of resistance to antibiotics in faecal contaminants found in drinking water is a major problem. Occurrence and prevalence of these resistant strains in environment is a cause of concern as they can act as a vehicle to disseminate antibiotic resistance to other bacteria.

Acknowledgements

The financial assistance provided by UGC, CRO for performing the present work is deeply acknowledged. I also express my thanks to the management and principal of PMB Gujarati Science College, Indore, providing instrumental and technical support.

REFERENCES

[1]. Krishnan R., RadhaK., Dharmaraj and Ranjitha Kumari., A comparative study on physicochemical and bacterial analysis of drinking, borewell and sewage water in 3 different places of Sivakashi, J.Environ. Biol. 28, 105-108(2007).

[2]. WHO (World Health Organization), Guidelines for Drinking-water Quality, Incorporating 1st and 2nd Addenda. Volume 1, Recommendations, 3rd ed.; WHO: Geneva, Switzerland (2008).

[3]. Fenwick A., Waterborne Diseases—could they be Consigned to History? *Science. 313*, 1077–1081(2006).

[4]. GeorgeI., Crop P. and ServaisP. Use of β-D-Galactosidase and β-D-Glucuronidase Activities for Quantitative Detection of Total and Faecal Coliforms in Wastewater, Can. J. Microbiol. 47, 670–675(2001).

[5]. Prakash K. and Somsekar R., Groundwater quality assessment on Anekal Taluk, Banglore urban district, Indian J. Environ.Biol. 27, 633-637(2006).

[6]. AsboltN.J., Microbial contamination of drinking water and disease outcomes in developing regions, *Toxicology.* 229-238 (2004).

[7]. Walsh T., Weeks J., Livermore D. and Toleman M., Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. The Lancet Infectious Diseases. doi:10.1016/S1473-3099(11), 70059-7 (2011).

[8]. El-Astal Z., Bacterial pathogens and their antimicrobial susceptibility in Gaza Strip, Palestine, Pakistani J. Medical Science. 20, 365-70(2004).

[9]. Rama RaoS.V., Singh V.P. and MallL.P., Pollution studies of River Khan (Indore), India-Biological assessment of pollution, Water Research. 12 (8), 555–559(1978).

[10]. Rawat A., Article published in Hindi newspaper, Dainik Bhaskar, DB Star, Indore (22.07.14).

[11]. APHA, Standard methods for examination of water and waste water, 21stEdn. Washington D.C. (2005).

[12]. Bauer A., Kirby W., Sherris J. and TurchM., Antibiotic susceptibility testing by standardized single disc method, American J. Clinical Pathology. 6, 493–496(1966).

[13]. WHO, WHO-Guidlines for drinking water quality. Volume 1 (1984).

[14]. AnandC., Akolkar P. and Chakrabarti R., Bacteriological water quality status of river Yamuna in

[15]. Delhi, J. Environ. Biol. 27, 97-107(2006).

[16]. Adesoji A.T. and OgunjobiA.A., Occurrence of Multidrug-Resistant Bacteria in Selected Water Distribution Systems in Oyo State, Nigeria, Global Veterinaria. 11 (2), 214-224 (2013).

[17]. Osterblad M, Hakanen A, Manninen R, LeistevuoT, Peltonen R, Meurman O, et al., A between-Species Comparison of Antimicrobial Resistance in Enterobacteria in Fecal Flora, Antimicrobial Agents and Chemotherapy. 44, 1479-84(2000).

[18]. Gaur A., Ramteke P., Pathak S.and Bhattacherjee W., Transferable antibiotic resistance among thermotolerant coliforms from rural drinking water in India, Epidemiology and Infection. 109(1), 113–120(1992).

[19]. AtifA., Patoli B. and Mehraj B., High prevalence of multi-drug resistant *Escherichiacoli* in drinking water samples from Hyderabad, Gomal J. Medical Sciences. 8 (1), 23-26 (2010).

[20]. Walia S.K., Kaiser A., Parkash M. and Chaudhry, G.R., Self-transmissible Antibiotic Resistance to Ampicillin Streptomycin, and Tetracyclin found in *Escherichiacoli* Isolates from contaminated Drinking water, J. of Environmental Science and Health. 39, 651-62(2004).

AUTHORS' BIOGRAPHY

Dr. Nandini Phanse, M.Sc. Ph.D. Microbiology, with 23 years of enriched experience in the field of education. Presently, functioning as Head of Microbiology department of PMB Gujarati Science College, Indore, (M.P.). A top notch academician, with a passion of teaching & research, she has published 20 research papers in national and international journals and 04 books. She has submitted 04 gene sequences of industrially important microorganisms to NCBI GeneBank & also deposited a bacterial culture to Microbial Type Culture Collection (MTCC) and Gene Bank, Chandigarh. She has two UGC funded research projects to her credit.

Dr. Pragya Rathore, M.Sc. Ph.D. Microbiology, is an Associate Professor & Head, Sanghvi Institute of Management & Science, Indore. She has actively involved in research and teaching and has an experience of 18 years with excellent track record. She has published 30 research papers in national and international journals and has published 03 books with international publishers. She has submitted 04 gene sequences of industrially important microorganisms to NCBI GeneBank & also deposited a bacterial culture to Microbial Type Culture Collection (MTCC) and Gene Bank, Chandigarh. She has undertaken a research project sponsored by UGC.