**Advances in Bioresearch**
Adv. Biores., Vol 10 (5) September 2019: 60-65

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.10.5.6065

Advances in Bioresearch

## ORIGINAL ARTICLE

# Coliform contamination as an indicator for potability of different drinking water sources in and around the Nainital City, Western Himalaya, Uttarakhand

**Devendra Singh Bisht [1]*, Neha Giri [1], Anchal Lodhi [2] and Deepak Kumar Arya [1]***
1. Department of Zoology, D S.B. Campus, Kumaun University, Nainital, Uttarakhand, India
2. Department of Biotechnology, Bhimtal Campus, Kumaun University, Nainital, Uttarakhand, India
*Corresponding Authors: bisht25devendra@gmail.com, dee.in@rediffmail.com

**ABSTRACT**
*Based on the presence or absence of indicator bacteria i.e., Escherichia coli, drinking water quality supply of Nainital city was investigated from January to December in 2018. The potability assessments of 28 water samples, collected aseptically in sterilized container from treated and untreated water sources, were carried out by determining total plate count and total coliform bacteria count. During the present study, treated water samples were found most suitable for drinking when compared with the samples of untreated as per defined standards of potability by World Health Organization (WHO). The antimicrobial resistance among confirmed E. coli isolates from water samples was also determined by disk diffusion method. All isolates of E. coli were found resistant against Cefotaxime and Penicillin while antibiotics such as Gentamycin and Norfloxacin showed highest susceptibility for isolates investigated in the present study. It is recommended that the current situation of untreated water sources is degrading for drinking purpose and thus, requires an immediate action towards improvement of their quality for the welfare of the residing people. Safe and pure drinking water should also be provided by authorities and municipal corporations to entire population of Nainital City (Uttarakhand, India).*
**Keywords:** *Antimicrobial resistance, Coliform, E. coli, Potability, Treated and untreated water.*

Received 01.05.2019 Revised 18.07.2019 Accepted 09.08.2019

**How to cite this article:**
D S Bisht , N Giri, A Lodhi and D K Arya.Coliform contamination as an indicator for potability of different drinking water sources in and around the Nainital City, Western Himalaya, Uttarakhand . Adv. Biores., Vol 10 [5] September 2019 60-65.

## INTRODUCTION

Water is significant and fundamental element for our life support system. Water quality is a critical factor affecting human health and welfare[1]. Approximately 3.1% of deaths (1.7 million) and 3.7% of disability adjusted-life-years (54.2 million) worldwide are attributable to unsafe water. Global recent estimates suggested that 768 million people did not use an improved source for drinking water and 2.5 billion people lacked access to an improved sanitation facility [24]. In India, it has been estimated that annually 37.7 million people get affected by water associated diseases [26]. Of these, more than 50% are caused due to microbial intestinal infections [6]. The most common and widespread health risk associated with drinking water is contamination, whether directly or indirectly by human or animal excreta. Water gets contaminated by enteric pathogens such as coliforms group of bacteria, thus are considered as the most reliable indicators of fecal contamination [18] .These indicators are used to assess the potential public health risk of drinking water and their absence or presences are the key elements of most drinking water quality guidelines.

In the recent decades, drinking water pollution by fecal coliform bacteria harboring resistance genes has become a water quality issue of national scope and importance. *E. coli* can be considered as an important vehicle for the spread of resistance genes due to its plenty in such environments with a high risk of transfer from environment to human pathogens [14, 15]. Globally, especially in developing countries due to the development of anti-microbial resistivity in clinical as well as environmental isolates of *E. coli*,

concerns have been there regarding the potability of water [5, 9, 19]. In spite of high prevalence of such cases in India, very few studies have been conducted on the occurrence of antibiotic resistance in *E.coli* mainly from fresh water sources of state Uttarakhand [3, 8, 20]. Furthermore, there is extreme paucity of information on the presence of *E.coli* and their association with antimicrobial resistance in drinking water sources from Himalayan region of Kumaun. Therefore, the present investigation was aimed primarily to determine the *E.coli* as indicator bacteria of water contamination and secondarily to assess their association with antibiotic resistance patterns from different drinking water sources in the urban city of Nainital, Uttarakhand (India).

## MATERIAL AND METHODS

### Study area

City Nainital is located at 29°21' to 29°24' N latitudes and 79°25' to 79°29' E longitudes and has an elevation of 1938 m above mean sea level surrounded by mountains on three sides and is an internationally recognized tourism hot spot within the Kumaun Himalayan Region of state Uttarakhand. The climate of this region is wet monsoon and temperate. The whole year can be divided into five seasons i.e. spring, summer, monsoon, autumn and winter. The region is famous for long cold and snowy winter and short summer. The average annual temperature is 13°C. The average annual rainfall around the catchment area is about 1,636 mm. Vegetation is covered with mixed oak-conifer forests in and around the city. Nainital also act as a base for other tourist places in the vicinity of the city and as per Census conducted in 2011, it provides permanent shelter to approximately 41,377 people. The major sources of water supply in the city include natural lake, springs, water tanks, municipal water and others. The combination of processes like pre-treatment, flocculation, sedimentation, rapid sand filtration and chlorination are used for water treatment distributed in the town on the daily basis through municipal body and corporations. However, a small section of people is directly dependent on the untreated water sources for their daily water demands.

### Collection of water samples

Water samples were collected in sterile plastic bottles (Genaxy Scientific Private Limited) containing 4-5 drops of sodium thiosulphate to neutralize any residual chlorine (required only in the case of treated water samples). Samples were placed in $4^0$C cooling boxes for transport to the laboratory and processed within 6 hours of collection.

### Microbial examination of water samples

### Total viable count

The total viable count of bacteria were determined by serial dilution and plating on Nutrient agar (Hi-Media, Mumbai). 1 ml of the water from $10^0$ and $10^1$ dilutions were added to petridishes (Genaxy Scientific Private Limited) and 20 ml of nutrient agar was poured over and mixed well for each water sample. Duplicate plates for each dilution were used. After the plates were set, one set of plates were incubated at $35^0$C for 24 hours and the other set at 20°C for 48 hours. All colonies on selected plates were counted after the incubation [1].

### Most probable number technique

Detection of total coliforms was determined by MPN test. This test was carried out in three stages namely presumptive test, confirmative test and complete test according to the WHO recommendations

### Presumptive test

A sets of three tubes inoculated with 10 ml of Lactose broth (Hi-Media, Mumbai) of different strengths (Single and double) with samples of 10, 1, 0.1 ml. The inoculated tubes for coliform were incubated at $35^0$C for 48 hours. All The tubes were observed after 48 hours for any vigorous effervescence or bubble formation when tubes were shaken gently. Absence of air bubble in any test tube confirmed the absence of coliform group and do not need further confirmation or completed test. Tubes showing air bubbles were tested further for final confirmation of coliforms [1].

### Confirmed test

The positive presumptive tubes were further used to accomplish confirmative test. 1 ml of sample from positive presumptive tubes were transferred to separated media tubes of Brilliant Green Lactose Bile (BGLB) broth (Hi-Media, Mumbai) which were incubated at $35^0$C for 48 hours and gas positive tubes were recorded. The results were expressed as MPN per 100 ml of the sample.

### Completed test

In the completed test, the positive BGLB samples were streaked in Eosin methylene blue (EMB) agar (Hi-Media, Mumbai) plates and incubated at $37^0$C for 24 hours [17]. Isolated colonies were picked from confirmatory test plates on respective media were identified on the basis of their morphological (Gram-negative, motile, or nonmotile, nonsporing. rod-shaped, aerobic or facultative anaerobic that ferment

lactose with gas formation within 48 hours at $37^0$C) and their biochemical properties following Bergey's Manual of determination Bacteriology 1994. This confirmed the presence of *E. coli* in different water samples [7].

**Antimicrobial susceptibility tests**

Sensitivity pattern of all the confirmed *E. coli* isolates was carried out by Kirby-Bauer disc diffusion method [4] using Mueller Hinton agar as per the recommendations of the Clinical and Laboratory Standards Indtitute (CLSI). Isolates of *E. coli* were then evaluated for their antimicrobial susceptibility patterns against fourteen clinically significant antibiotics viz.; Amikacin 30 µg, Ampicilin 10 µg, Cefixime 5 µg, Cefotaxime 30 µg, Cefuroxime 30 µg, Chloramphenicol 30 µg, Erythromycin 15 µg, Gentamycin 10 µg, Kanamycin 30 µg, Nalidixic acid 30 µg, Norfloxacin 10 µg, Penicillin 10 µg, Streptomycin 10 µg, Tetracycline 30 µg. Antimicrobial sensitivity was determined by measuring the zone of inhibition. The isolates were categorized into 3 groups i.e resistant, intermediate and susceptible on the basis of measuring zone diameter measured in millimetre (mm) by CLSI standards. All the dehydrated culture media and antibiotic discs were obtained from Hi Media Laboratories Private Limited, Mumbai, India.

**Multiple antibiotic resistance (MAR) Index of isolates**

The MAR is used for analyzing the prevalence of *E. coli* isolated from drinking water sources. Those isolates which are found to be resistant to three or more than three groups of antibiotics were taken as MAR strains. MAR was determined by the number of antibiotics to which isolate was resistant divided by the total number of antibiotics was subjected for sensitivity test [11]. Isolates with an MAR index of higher than 0.4 is associated with human fecal source and less than 0.4 are said to non human fecal source of contamination occurs [13].

**RESULTS AND DISCUSSION**

A total of 28 drinking water samples including treated water (n=15) and untreated water sources (n=13) from January to December, 2018 were collected aseptically in sterilized container from different localities in the study area. The viable count of untreated water was higher than the treated water sources. In untreated water sources Raati-ghat (944 CFU/100 ml) was highest among all the analyzed samples, while the lowest was from Jiwaji cottage (476 CFU/100 ml) whereas for treated water sources bacterial counts was highest for Hari-nagar area (371 CFU/100 ml) and lowest was at Naina-Devi Temple (202 CFU/100 ml) (Table 1). The bacterial content of drinking water leaving piped water sources should contain only very low level of microorganisms and low level of these organisms indicated the treatment and disinfection process was effective in removing most pathogens [16].

Water sample which were collected from treated water sources have not shown any presence of coliform bacteria whereas the untreated water sources shown 0–3 coliforms in 100 ml of water. These values are however quite similar compared with results obtained from similar studies conducted recently [12]. Interestingly, in all the untreated samples, three water sources, i.e., Sataya-narayan temple, Naina-peak and Raati-ghat, exhibited green metallic sheen on EMB indicating the presence of fecal coliform bacteria i.e., *E. coli* rendering the water sample as non potable [25]. In this study total isolates of fecal coliform were identified as *E. coli* confirmed by IMViC tests (Table 2). These results should be considered indication of fecal pollution in drinking water sources. The presence of coliforms might be exposure of the springs to the external environment, most likely from animal's fecal matter such as monkeys, birds, langurs and other wild life animals [21].

The sensitivity test revealed that all the isolates were totally resistant to Cefotaxime and Penicillin (100%). Most of the isolates were found highly resistance to Cefixime and Amikacin (66.66%) and moderately resistant to Streptomycin (33.33%). On the other hand, all isolates were found sensitive to Gentamycin (100%) and Norfloxacin (100%). Highly sensitive to Chloramphenicol (83.33%), Tetracycline (66.66%), and moderately sensitive to Ampicilin (50%) followed by Cefuroxime (50%), Kanamycin (50%), and Streptomycin (33.33%) (Fig.1). Several studies also reported that isolates of *E. coli* have developed resistant pattern for different clinically important antibiotics from different drinking water sources [3]. Presence of high resistance to Cefotaxime and Penicillin can be justified by the fact that there have been used extensively for various bacterial diseases treatment [23].

**Table 1: Potability in various treated and untreated water sources in the Nainital city of Kumaun Himalaya.**

| S.no | Sample Site | Total plate count (CFU/100m) | Total coliforms (MPN/100ml) | Production of green sheen on EMB agar media | Potability |
|---|---|---|---|---|---|
| **Untreated Water (Natural springs)** | | | | | |
| 1 | Naina-peak | 676 | 1 | Positive | Non-potable |
| 2 | Sataya-narayan Temple | 912 | 2 | Positive | Non-potable |
| 3 | Sipayi dhara | 502 | 1 | Negative | Potable |
| 4 | Chuna dhara | 646 | 1 | Negative | Potable |
| 5 | Veer bhatti | 510 | 2 | Negative | Potable |
| 6 | Tur tur dhara | 543 | 1 | Negative | Potable |
| 7 | Jiwaji cottage | 476 | 0 | Negative | Potable |
| 8 | Gufa Mahadev upper | 551 | 1 | Negative | Potable |
| 9 | Gufa Mahadev Lower | 577 | 1 | Negative | Potable |
| 10 | Hanumangari road | 744 | 0 | Negative | Potable |
| 11 | Near Main Pump House | 480 | 1 | Negative | Potable |
| 12 | Raati-ghat | 944 | 3 | Positive | Non-potable |
| 13 | Near Krishnapur | 510 | 1 | Negative | Potable |
| **Treated Water (Piped-water sources)** | | | | | |
| 1 | Tallital bazaar | 270 | 0 | Negative | Potable |
| 2 | Near, Post-Office Tallital | 237 | 0 | Negative | Potable |
| 3 | Rickshaw-stand Tallital | 233 | 0 | Negative | Potable |
| 4 | Thandi Road | 270 | 0 | Negative | Potable |
| 5 | Naina devi Temple | 202 | 0 | Negative | Potable |
| 6 | Masjid Chauraha | 237 | 0 | Negative | Potable |
| 7 | Bada bazaar Mallital | 270 | 0 | Negative | Potable |
| 8 | Hari-nagar Area | 371 | 0 | Negative | Potable |
| 9 | Cantonment area | 237 | 0 | Negative | Potable |
| 10 | Snow view | 271 | 0 | Negative | Potable |
| 11 | Saat Number Area | 351 | 0 | Negative | Potable |
| 12 | Sukhataal Area | 303 | 0 | Negative | Potable |
| 13 | Ayaar Patha | 270 | 0 | Negative | Potable |
| 14 | Police line | 202 | 0 | Negative | Potable |
| 15 | Birla chungi | 321 | 0 | Negative | Potable |

**Table 2: Morphological and biochemical characteristics of isolates of Non-potable water sources in the Nainital city of Kumaun Himalaya.**

| Isolates | Morphological characteristics | Methyl Red | Indole | Voges-Proskauer | Citrate | Screened bacteria as *E. coli* |
|---|---|---|---|---|---|---|
| **Sataya-narayan temple** | POSITIVE | POSITIVE | POSITIVE | NEGATIVE | NEGATIVE | *E. coli* present confirmed |
| **Naina- peak** | POSITIVE | POSITIVE | POSITIVE | NEGATIVE | NEGATIVE | *E. coli* present confirmed |
| **Raati-ghat** | POSITIVE | POSITIVE | POSITIVE | NEGATIVE | NEGATIVE | *E. coli* present confirmed |

**Table 3: Multiple antibiotic resistance index of *E. coli* isolates from non potable water in the Nainital city of Kumaun Himalaya.**

| Number of isolates | MAR value |
|---|---|
| 1 | 0.3 |
| 2 | 0.4 |
| 3 | 0.3 |
| 4 | 0.3 |
| 5 | 0.3 |
| 6 | 0.3 |

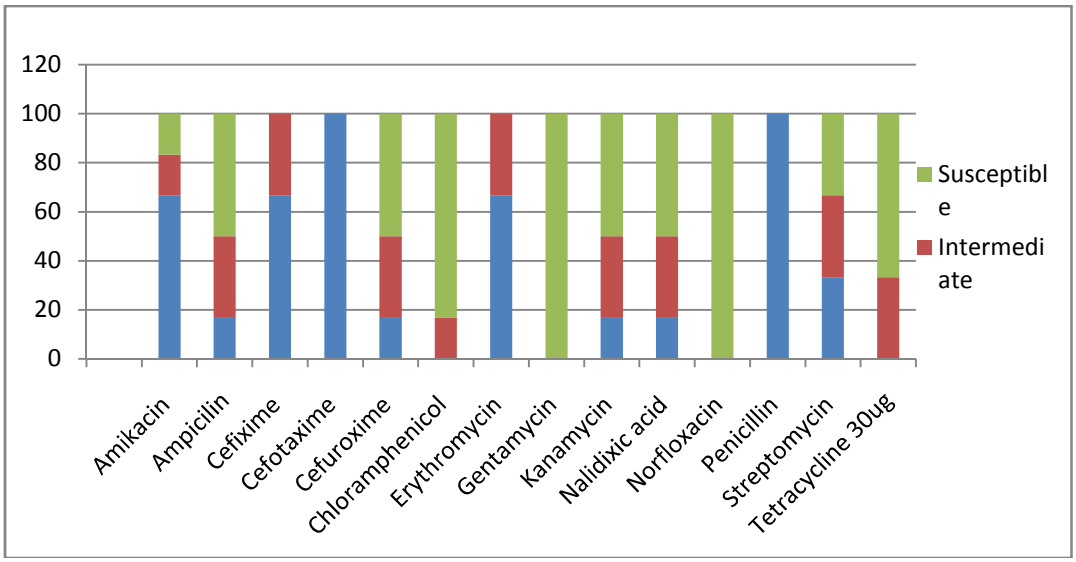


**Figure 1**: Percentage of Antibiotic susceptibility pattern of *E.coli* isolates from Non potable water-sources.

Multiple antimicrobial resistances (MAR) indexing is a method which accurately determines the source of fecal contamination by correlating with antibiotic resistance profile of the isolates [22]. In the current study, MAR index for the isolates was generated and shown in Table 3. All the isolates of *E. coli* were having MAR value greater than 0.2 showing the multi-drug resistance and also is an indicator with a high risk of contamination (e.g. animal farms, increased human population) [10]. MAR bacteria themselves are menace to human as well animal's population. Similar observation made by Chatterjee *et al.,* [8] who reported high risk MAR index of *E. coli* from water samples of different hilly locations of Uttarakhand region. The results of this study suggested that the bacterial strains are becoming resistant intrinsically in vivo and fecal dissemination during rainfall or surface runoff into the water sources might have lead to the occurrence of these bacteria into the water system.

## CONCLUSION

The study showed that the presence of these indicator organisms in water samples indicates the extent of water contamination in drinking water sources of the city. Occurrence of antimicrobial resistant bacteria emphasizing on high awareness in this regard. By applying these tests, we can check the quality of safe drinking water. The contaminated water may be made good for use through filtration, sedimentation, disinfection by chlorination and some physical methods like osmosis, distillation and U.V light. Although the water board of the city certifies water quality regularly, independent monitoring of drinking water needs to be done on a regular basis with a system of feedback and corrective measures.

## ACKNOWLEDGEMENT

This study was partially supported by UGC- BSR Research Start-Up-Grant {No. F. 30-74/2014 (BSR) Date-22.01.2014}.We are thankful to the Head, Department of Zoology, D.S.B. Campus, Nainital for providing necessary facilities for the present research work. Thanks are also to the Water Board of Nainital city for the help and support during this investigation.

## REFERENCES


1. Adekunle L.V. Sridhar M, Ajayi AA, Oluwade PA, Olawuyi JF, (2004) An Assessment of the Health and Social Economic Implications of Sachet Water in Ibadan Nigeria: A Public Health Challenge. *African J. Bioremedia Res;* **7**:5-8
2. American Public Health Association, (1998). Standard Methods for the Examination of Water and Wastewater, 20th edition; American public Health Association: Washington, DC, U.SA
3. Baghel V. S., Singh J and Gopal K, (2003). Antibiotic resistance among enteric bacteria isolated from runoff of the Gangotri glacier, Western Himalaya. *Indian J. Environ Biology*. **24**: 349-356
4. Bauer AW., Kirby W M M., Sherris J C. and Turck M, (1966). Antibiotic susceptibility testing by standard single disc method, *American J. Clinical Pathology*. 45: 493-496
5. Burke V, Richter D, Greskowiak J, Mehrtens A, Schulz L and Massmann G, (2016). Occurrence of antibiotics in surface and groundwater of a drinking water catchment area in Germany. *Water Environ Research* **88**: 652-659
6. Cabral, J. P, (2010**).** Water Mirobiology. Bacteria Pathogens and Water. *Int. J. Environ. Res. Pub. Health***. 7**: 3657-3703
7. Cappucino G.J., Natalie S, (2006). Media & Reagents. Microbiology; A laboratory manual. **6**: 491-502
8. Chatterjee R, Sinha S, Aggarwal S, Dimri AG, Singh D, Goyal P, (2012). Studies on susceptibility and resistance patterns of various E. coli isolated from different water samples against clinically significant antibiotics, *Int. J.Bioassays* 01:156–161
9. Gautam R, Chapagain ML, Acharya A, Rayamajhi N, Shrestha S, Ansari S and Upadhaya G, (2013). Antimicrobial susceptibility patterns of Escherichia coli from various clinical sources, *J. Chitwan Medi Colleg*.3: 14–17
10. Kaneene B.J., Miller R., Sayah R., Jihnson Y.J., Gilliland D and Gardiner J.C, (2007). Considerations when using discrimination function analysis of antimicrobial resistance profiles to identify sources of fecal contamination of surface water in Michigan. *Appl. Environ. Microbio*.73: 2878-2890.
11. Krumperman P.H, (1983)**.** Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of faecal contamination of foods. *Appl. Environ. Microbio. 46:165-170*
12. Maindoli S. D., Singh P., Nutan., Pant M., Chanyal P.C and Gupta P.K, (2018). Assessment of water quality of some fresh water mountain springs in Naini lake basin of Kumaun Himalaya.. *Int. J. Advan. Res and Develop*, **3**(2):707-712.
13. Mohanta T., and Goel S, (2014). Prevalence of antibiotic-resistant bacteria in three different aquatic environments over three seasons. *Environ. Moniter. Assessment.* 186: 5089–5100. doi: 10.1007/s10661-014-3762-1
14. Ozgumus OB, Sevim EC, Alpay-Karaoglu S, Sandalli C and Sevim A, (2007). Molecular characterization of antibiotic resistant Escherichia coli strains isolated from tap and spring waters in a coastal region in Turkey. *J. Microbio.* 45: 379-387
15. Patoli AA, Patoli BB and Mehraj V, (2010). High prevalence of multi-drug resistant *Escherichia coli* in drinking water samples from Hyderabad. *Gomal J. Medi. Sci*. 8: 23-26
16. Raju N. S., Roopavathi C., Ramachandra Kini K and Niranjana S.R, (2011). Assessment of coliform contamination in drinking water from source to point of use in Mysore city of Karnataka, India.35th WEDC Conference, Loughborough, U K
17. Rathaur V. K., Pathania M., Jayara A and Yadav N, (2014). Lineal study of acute diarrhea caused by bacterial enteropathogens. *J. Clinic and Diagnos Res* 8(5) 1-5
18. Scott T. M., Salina P., Rosen K. M., Tamplin J.B., Farran M.L., Koo S.R., Sood A., Singh K.D., Pandey P and Sharma S, (2008). Assessment of Bacterial indiators and physiochemical parameters to investigate pollution status of Gangetic river system of Uttarakhand (India). *Ecol. Indiators* 8: 709-717
19. Sharma BC and Rai B, (2012). Incidence of multi-drug resistance in Escherichia coli strains isolated from three lakes of tourist attraction (Mirik lake, Jorepokhari lake and Nakhapani lake) of darjeeling hills, India, *Indian J. Fundamen and Appli Life Sci*. **2**: 108–114.
20. Sood A., Singh K.D., Pandey P and Sharma S, (2008). Assessment of bacterial indicators and physicochemical parameters to investigate pollution status of Gangetic river system of Uttarakhand (India). *Ecol. Indicat.* **8**: 709-717
21. Stephen E and Ijah UJJ, (2006). Microbiology and Physico-chemistry of River Kaduna, Nigeria receiving water discharged from Hydroelectric Power Dam. *J. Environ Sci*; 10(1):17-26
22. Tambekar D.H., Wankahde S.J., Yadav S.D and Tambekar S.D, (2008). Correlation of antibiotics resistance profiling of E. coli and source of feacal pollution in water. *Poll. Res*., 27: 507-510.
23. Vantarakis A., Veineri D., Komniou G and Papapetropoulou M, (2006). Differentiation of fecal Escherichia coli from human and animals by multiple antibiotic resistance analysis. *Lett. Appl. Microbiol*. 42: 71-77
24. WHO and UNICEF (2013). Progress on sanitation and drinking water.
25. World Health Organization (2011). Microbial fact sheets, In : Guidelines for drinking-water rd quality. (1) 3 ed. Recommendations. World Health Organization: 221-295. World Health Organization, Geneva
26. WHO. (2008). Facts and figures: Water, sanitation and hygiene links to health.