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Contamination of drinking water from improved sources with antimicrobial resistant *Escherichia coli*

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Key words: *Escherichia coli*, Drinking water, Antibiotic resistance, Improved water sources.

Abstract

In this study, the microbiological quality of household tap water samples from the piped distribution system of Iligan City was assessed for the presence of total coliforms and *Escherichia coli*. Based on microbial risk assessment specific for drinking water, 60 out of the 108 samples exceeded the value for gastrointestinal risk and of which 54 (90%) exceeded both international and Philippine drinking water guidelines of less than one colony-forming unit (CFU) of *E. coli* per 100 milliliters of water sample. Fifty-four isolates were cultured and each isolate was tested for susceptibility against four antibiotics namely ampicillin, chloramphenicol, gentamicin, and tetracycline. Thirty-three (61%) of the isolates have shown susceptibility to all four antibiotics. The isolates have also exhibited low resistance rates to these antimicrobials as none was non-susceptible to chloramphenicol, only five (9.2%) were gentamicin-resistant, and six (11.1%) were resistant towards each of ampicillin and tetracycline. The findings of this preliminary study increases awareness on the presence of antibiotic-resistant *E. coli* strains in the drinking water system of Iligan City. Although the definite impact of these resistant strains to human health is not yet fully established, it is important to conduct constant monitoring as ignorance to potential risks may result to unnecessary waterborne disease outbreaks.

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Introduction

Water is at the core of sustainable development and is critical for socio-economic development, healthy ecosystems, and for human survival itself (United Nations-Water, 2014). Thus, everyone is entitled to "sufficient, safe, acceptable, physically accessible, and affordable water for personal and domestic uses" as provisioned by the United Nations through the Human Right to Water and Sanitation (United Nations Committee on Economic, Social and Cultural Rights, 2002). The Joint Monitoring Programme for Water Supply and Sanitation (JMP) of World Health Organization (WHO) and United Nations Children's Fund (UNICEF) has established a standard set of drinking-water and sanitation categories that are used for monitoring purposes. An "improved" drinking-water source, by the nature of its construction and when properly used, adequately protects the source from outside contamination, particularly fecal matter ([a]World Health Organization/United Nations Children's Fund, 2013). Improved source types include piped water into dwelling, yard, or plot, standpipe, borehole, protected dug well or spring, and rainwater. Unimproved source types are those that do not protect water from outside contamination such as unprotected wells, unprotected springs, surface waters, and tanker trucks (Bain *et al.*, 2014).

WHO and UNICEF reported in 2012 that the world had met the Millennium Development Goal (MDG) Target 7c last 2010 which reduced by half the proportion of people without sustainable access to safe drinking water and basic sanitation. The declaration was based on the assessments done by categorizing water sources according to the JMP water ladder ([a]World Health Organization/United Nations Children's Fund, 2013) but fails to account for microbial water quality resulting to overestimation of the number of people using safe water (Onda *et al.*, 2012). This was supported by the study of Bain *et.al.* (2014), which monitored water quality data in five countries and yielded substantially reduced estimates of the proportion of the population with access to safe water.

Escherichia coli remains the universal standard for microbiological parameters (Dunn *et al.*, 2014) and current World Health Organization (WHO) guidelines still recommend detection of *E. coli* as indicator of the effectiveness of disinfection processes, and as index organism for the potential presence of fecal contamination and waterborne pathogens (World Health Organization, 2014). The use of *E. coli* as indicator organism is also recommended by the 2007 Philippine National Standards for Drinking Water (PNSDW) which ensured the strict compliance of water quality monitoring in order to provide access to safe water supply for the promotion and protection of public health (Department of Health, 2007).

Antimicrobial-resistant *E. coli* have been detected in a variety of food sources including vegetables, meat, and poultry (Sáenz *et al.*, 2001; Phongpaichit *et al.*, 2007; Van *et al.*, 2007; Phongpaichit *et al.*, 2008; Jouini *et al.*, 2009; Thorsteinsdottir *et al.*, 2010; Wu *et al.*, 2010; Tadesse *et al.*, 2012) as well as in drinking water (Xi *et al.*, 2009; Coleman *et al.*, 2012; Amin, 2014). Recent researches have highlighted soil and water environments as recipients, reservoirs, and sources of antibiotic resistance genes (ARGs) of clinical concern ([a]Martinez, 2009; Wright, 2010). Information about antibiotic resistant *E. coli* from water is certainly lacking in the Philippines. Although antibiotic resistance is clearly a global challenge (Pruden *et al.*, 2013), information even at the community level is an absolute necessity.

This pilot study is conducted to assess the microbiological quality of the improved drinking water system of Iligan City and to determine the prevalence of antibiotic-resistant *E. coli.* The information collected will be critical in the development of more robust risk assessments for drinking water quality which may contribute in minimizing antibiotic resistance.

Materials and methods

For this cross-sectional study, 108 water samples were collected at consumer points from various households in Iligan City that satisfied the following inclusion criteria: supply comes from an improved water sources which are centrally treated, direct consumption of tap water without further treatment, and willingness of household members to participate.

Total Fecal Coliform (TFC)

Water samples were analysed in a microbiological services laboratory where users of private sources submit samples for detection of coliforms and *E. coli*. The Most Probable Number (MPN) of total coliforms bacteria were determined by multiple tube fermentation technique. Total coliform were calculated from MPN tables as per 100 mL (World Health Organization, 1997).

Isolation and Detection of E. coli

The samples were tested for presumptive *E. coli* isolates via defined substrate method (Edberg *et. al*., 1991). Isolates were then selected and subcultured in a differential coliform medium to obtain pure cultures. A series of biochemical tests for confirmative identification (Zahera *et al*., 2011) were performed.

Antimicrobial Susceptibility Testing

Screening for antibiotic resistance was performed using the Kirby-Bauer disc diffusion method (Bauer *et al*., 1966) and as described by Akinyemi *et al.* (2005), Oyetao *et al.* (2007), Duru and Mbata (2010), and Manji *et al.* (2012) and. *E. coli* isolates were tested for their resistance to four most commonly used antimicrobials on discs containing ampicillin (AMP10µg), chloramphenicol (C30µg), gentamicin (CN10µg), and tetracycline (TE30µg). The concentrations of the antimicrobial discs were selected based on the internationally recognized standards and guidelines on antimicrobial routine testing and reporting on Enterobacteriaceae provided by the Clinical and Laboratory Standards Institute (CLSI) (Odwar *et al*., 2014). Each test was performed in triplicate for each *E. coli* isolate and antimicrobial. Inoculated agar plates were incubated at 37°C for 18-24 hours. The susceptibility zones were also measured and interpreted according to criteria set by the CLSI (2012).

Results and discussion

Detection and Enumeration of Fecal Coliforms in Potable Water Samples

The microbiological quality of 108 water samples collected from consumer points accessed through the piped distribution system of Iligan City was assessed. All the 108 water samples in this study were from improved water sources and based on the JMP guidelines are thus, considered to be safe. However, at the time the JMP water quality ladder was created, there were no simple, affordable ways to regularly and routinely measure the microbial quality of water to quantify its safety within the survey programs being used (Baum *et al.,* 2014).

Despite coming from improved water sources, sixty of the 108 samples were found to be contaminated with fecal coliform with MPN values ranging from 2.3 to >1100 per 100 mL which can be considered as unsafe for human consumption. This value is lower than several studies on contaminated drinking water (Shar *et al*., 2007; Ram *et al*., 2008; Freeman *et al*., 2012; Nabeela *et al*., 2014; Schriewer *et al*., 2015) however, a value less than one (1) fecal coliform per 100 mL sample is the internationally accepted value for a microbiologically safe drinking water ([b]World Health Organization/United Nation Children's Fund, 2013; World Health Organization, 2014).

As per WHO guidelines (World Health Organization, 2011), the contaminated samples were further categorized: 28 samples are of intermediate risk (MPN value of 1–10/100 mL), 13 are at high risk (MPN is >10–100/100 mL) and the remaining 19 contaminated samples are at very high risk (MPN is >100/100 mL). The presence of coliform bacteria in the water suggest a possible risk of exposure to potentially pathogenic microorganisms (Allevi *et al*., 2013); it can predict the possibility, but not the

certainty, of the presence of pathogenic microbes that can cause hazardous diseases (Nabeela *et al*., 2014).

Antibiotic Susceptibility Testing of Escherichia coli Isolates

Fifty four (90%) out of the 60 samples positive for coliform yielded *E. coli* isolates. The presence of *E. coli* in the water sample is indicative of fecal contamination in the water supply system (Talukdar *et al*., 2013). It becomes a serious threat when these *E. coli* strains exhibit resistance to multiple antibiotics.

The 54 *E. coli* isolates that were subjected to Kirby Bauer Disc Diffusion Method for its antibiotic phenotypes were first examined for the differences of the sizes of the clearing zones between the four different types of antibiotics. Chloramphenicol exhibited the largest diffusion zones ranging from 18 millimeters (mm) to 39 mm (<x>= 25.61, s=4.11) (Table 1) indicating greater susceptibility of the isolates to the antibiotic. The antibiotic gentamicin had the lowest mean zone of inhibition at 18.89 mm with clearing zones ranging from 0-30 mm.

Table 1. Range of the sizes of zones of inhibition exhibited by the *Escherichia coli* against the four antibiotics and number of *E. coli* isolates showing sensitivity towards each of the antibiotics.

ANTIBIOTIC	SIZES OF ZONES OF INHIBITION (mm)	NUMBER OF ISOLATES PER PHENOTYPE		
		Susceptible	Intermediate	Resistant
Gentamicin	0 - 30	47	2	5
Tetracycline	8 - 35	48	-	6
Ampicillin	0 - 45	43	5	6
Chloramphenicol	18 - 39	54	-	-

Based on the current guidelines of CLSI (2015), the average zone of diameter of each isolate will determine what phenotype it will fall under: susceptible, intermediate, or resistant. The "susceptible" category implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used. The "intermediate" category includes isolates which response rates may be lower than for susceptible isolates implying clinical efficacy in body sites where the drugs are physiologically concentrated or when a higher than normal dosage of a drug is used. Finally, the "resistant" category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules (Clinical and Laboratory Standards Institute, 2014).

In the present study, most of the isolates were still susceptible to all antibiotics. In fact, thirty-three isolates (61.1%) were still susceptible to all four test antibiotics. This is in contrast to previous studies where there were relatively high frequencies of antibiotic resistant *E. coli* isolated from drinking water: 93% of all *E. coli* isolates of the study of Walia *et al.* (2004) exhibited resistance to two or more antibiotics; 73% of *E. coli* isolates from drinking water samples from Bangladesh were found to be resistant to at least one of the 10 antibiotics used in the study done by Talukdar *et al*. in 2013.

Antibiotic resistant *E. coli* from drinking water sources is a source of concern since the presence of *E. coli* in the environment poses both immediate concerns due to the widespread cases of disease outbreaks associated with water sources contaminated with pathogenic variants of *E. coli* (Janezic *et al*., 2013). Furthermore, the antibiotic resistant *E. coli* represents a significant reservoir of genetic determinants of antimicrobial resistance and its long-term persistence may facilitate the spreading of antibiotic resistance to other microorganisms in the environment (Talukdar *et al*., 2013).

Resistance of the *E. coli* isolates against chloramphenicol was not observed and is in conformity with the results obtained from drinking water isolates of Kathmandu (Subba *et al.*, 2013). Two previous studies were able to isolate chloramphenicol-resistant *E. coli*, although it was not observed in high abundance – 15.3% of isolates from Mathura, India (Anita *et al.*, 2014) and 8% of total isolates from a study in Bangladesh (Talukdar *et al.*, 2013).

Low prevalence values for resistant isolates were observed for the remaining three antibiotics, tetracycline (11.1%) ampicillin (11.1%) and gentamicin (9.2%). However, despite its low frequency, the most prevalent resistant phenotype were observed for tetracycline and ampicillin, closely followed by resistance to gentamicin.

There were only six (11.1%) tetracycline-resistant isolates in the study which is the lowest so far compared to previous published studies of antibiotic phenotypes of *E. coli* isolates from drinking water as it ranged from 16% in Jordan (Shehabi *et al.*, 2006) to 45% in a study in Bangladesh (Talukdar *et al.*, 2013), 92.3% of the total isolates in a study in India (Anita *et al.*, 2014) and 93.5% of *E. coli* isolates studied in Kathmandu (Subba *et al.*, 2013). Tetracyline resistance among *E. coli* isolates have already been reported by various investigators although sample sources were primarily food animals and derived meats (Teshager *et al.*, 2000; Saenz *et al.*, 2001; Schroeder *et al.*, 2003; Johnson *et al.*, 2005).

Theoretically there should be low prevalence of ampicillin resistance as this antibiotic is inactivated by chromosomal beta-lactamases produced by many enterobacterial strains including *E. coli* (Goñi-Urriza *et al.*, 2000). This could be the reason of low ampicillin resistant rates in this study (11.1%). However, it is in contrast to similar studies where the lowest frequency of ampicillin resistance was at 26% (Shehabi *et al.*, 2006) and the highest at 61.5% (Anita *et al.*, 2014).

Gentamicin resistance among the *E. coli* isolates in this study was only at 9.2% which is at the lower end of frequency spectrum of previous studies. The isolates in the study done in Bangladesh were mostly still susceptible to this antibiotic with only 1% being resistant to gentamicin (Talukdar *et al.*, 2013). *E. coli* isolates from Jordan and India exhibited gentamicin resistance at values of 17% and 46.2% respectively (Shehabi, 2006; Anita *et al.*, 2014). Only seven (12.9%) isolates exhibited an intermediate type of antibiotic phenotype: two for gentamicin and five for ampicillin. Despite its low values, this is indicative of possible lowered susceptibilities to these two antibiotics.

Conclusion

The widespread overuse and misuse of antibiotics has resulted to a growing global health concern of antibiotic resistance. There is a definite paucity of information on how the selective pressure of clinical antibiotic usage can affect environmental communities in aquatic ecosystems and which bacterial groups might be responsible for dissemination of antibiotic resistance genes (ARGs) into the environment (Huerta *et al.*, 2013). The isolation of antibiotic resistant *E. coli* in drinking water as reported in this study could only be explained as either due to the entrance of antibiotic-resistant bacteria in some part of the water distribution system or to other unknown mechanisms of resistance emergence and/or selection, within the system (Figueira *et al.*, 2012).

Although there is very limited literature about antibiotic resistance in drinking water, the isolation of resistant *E. coli* strains from the piped distributions systems of Iligan City raises concern for both public health issues as well as the probable impact to the natural microbiota of this aquatic environment. Despite the fact that the specific impacts of the antibiotic resistant bacteria present in drinking water

may have on human health are still unknown (Vaz-Moreira *et al.*, 2014), it does not discount the probability of having implications for the health of the urban population. Furthermore, the presence of antibiotic resistant *E. coli* is postulated to increase the chance of lateral gene transfer events (Lopez-Cerero *et al.*, 2011) which might lead to a creation of a natural reservoirs of resistance phenotypes ([b]Martinez, 2009).

Further studies can provide more detailed analysis on the antibiograms of *E. coli* isolates from drinking water which will give a better understanding on the characteristics of the isolates making it possible to manage public health concerns. Additionally it will promote the creation of effective intervention strategies that would limit the spread of resistant strains and will contain the threat of resistance from ballooning.

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Conflicts of Interest

The authors confirm that this article content has no conflicts of interest.

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