

Antibiotic sensitivity profile of *Escherichia coli* in water samples from water hand pumps in selected areas in Iligan City, Philippines

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Abstract. Increasing resistance of *Escherichia coli* to antimicrobial agents has been a growing problem as it compromises antibiotic efficacy in treating infections caused by this potentially pathogenic bacteria. Antibiotic-resistant strains of this bacterial species are readily acquired through water sources. This study was conducted to test antibiotic sensitivity of *E. coli* isolated from local alternative water supplies such as water hand pumps that provide water for domestic demands. *E. coli* was isolated from the water samples collected from 50 water hand pumps in selected areas in six barangays in Iligan City, Philippines. The bacterial samples were tested for their phenotypic sensitivity against four antibiotics: amoxicillin (25 µg), cefoxitin (30 µg), ciprofloxacin (5 µg), and gentamicin (10 µg). The *E. coli* isolates were most susceptible to ciprofloxacin with non-resistance percentage at 35%. It was also towards this antibiotic that most number of the isolates (29/100) had displayed intermediate resistance. Amoxicillin had shown the least efficacy against *E. coli* as exhibited by 96% non-susceptible isolates. Fifty-one percent of the tested bacteria demonstrated multiple drug resistance to at least three of the antibiotics. Results indicate the presence of antibiotic-resistant and multiple drug-resistant *E. coli* in the water supplies used by the local communities for domestic activities.

Key Words: antibiotic-resistant bacteria, coliform, drug resistance, susceptibility.

Introduction. *Escherichia coli* is part of the normal flora of human and animal fecal matter which may contaminate soil and water (Moş et al 2010). *E. coli* in water sources is accountable for disease outbreaks (CNN Library 2013; Thenmozhi 2010; Craun et al 2006) and mortality worldwide in recent years. Infection with this bacteria may be transmitted through accidental ingestion or deliberate consumption and direct contact with *E. coli*-contaminated water. Although most *E. coli* strains are harmless, some are pathogenic and can cause diseases (Li et al 2012) such as diarrhea, urinary tract infections, respiratory problems, and even life-threatening bloodstream illnesses (Centers for Disease Control and Prevention 2013) among others as new pathogenic strains of these bacteria have been emerging. *E. coli*'s escalating resistance over the past years (Iqbal et al 2002) is becoming a problem as it renders infections more difficult to treat, compromising efficiency of present therapeutic remedies (Patoli et al 2010). These drug-resistant *E. coli* strains are easily acquired via water sources (Collignon 2009) as water ecosystems are known reservoir for antibiotic-resistant bacteria and antibiotic resistance genes, which may transmit resistance to opportunistic pathogens (Xi et al 2009), such as *E. coli* bacteria.

In Iligan City, Philippines, water hand pumps are used as alternative water sources in areas where residents lack access to or have insufficient supply of water from the city's water distribution system. The water from the hand pumps is used for drinking, cooking, personal hygiene, household sanitation practices, and other domestic activities. Despite these varied and vital uses of water inside the homes, there is lack of information on the antibiotic profiles of isolated *E. coli* strains from this type of water supply. A local surveillance would just be important to determine the antibiotic sensitivity of potentially-pathogenic bacteria detected in the domestic water sources. This study was conducted to

examine the antibacterial agent susceptibility of *E. coli* isolated from these local alternative water sources supplying water for household needs.

Materials and Methods

Bacterial strains. *E. coli* was isolated from water samples collected from 50 water hand pumps in six selected areas in Iligan City, Philippines, namely: Barangays Hinaplanon, Sta. Filomena, Luinab, Upper Hinaplanon, San Roque, and Bagong Silang. Water samples were collected in January to April 2013. Bacterial identity was confirmed through cultural characterization, Gram staining and microscopy, and biochemical testing (Adejuwon et al 2011).

Antibiotic sensitivity testing. Antibiotic sensitivity testing was done on 100 *E. coli* isolates from the water samples, employing the disk diffusion technique (Ravi et al 2010), specifically Kirby-Bauer disk diffusion test to determine bacterial antibiotic susceptibility profiles. Bacterial suspensions of 24-hour old *E. coli* isolates were prepared based on 0.5 McFarland turbidity standard (1.5×10^8 bacterial cells/mL). Bacterial suspensions were swab-streaked and allowed to grow on Mueller Hinton Agar (MHA) plates where the standard antibiotic disks, specifically amoxicillin (25 μ g), cefoxitin (30 μ g), ciprofloxacin (5 μ g), and gentamicin (10 μ g) and a blank paper disk pipetted with 10- μ L sterile distilled water serving as the negative control were aseptically placed at an even distance from each other. These antibiotics were chosen to represent various antibiotic groups. Amoxicillin, one of the oldest and regularly used antibiotic, belongs to the penicillin group while cefoxitin is a second-generation cephalosporin. Both antibiotic groups subsequently belong to the beta-lactam drug family whereas ciprofloxacin and gentamicin are second-generation fluoroquinolone and aminoglycoside drugs, respectively. All these antibiotics are readily available and widely used against a broad spectrum of human diseases including those infections caused by *E. coli*. After incubation of the MHA plates, zones of inhibition were measured and *E. coli* isolates were categorized as resistant, intermediate resistant, or susceptible to a particular antibiotic based on the Clinical Laboratory Standard Institute (CLSI) standard zone diameter interpretation chart (Hudzicki 2013).

Results and Discussion. Figure 1 shows that the *E. coli* isolates were most susceptible to ciprofloxacin as they had the lowest resistance percentage (36%) indicating that this antibiotic is the most efficient among the four antimicrobial agents used in this study. Ciprofloxacin also had the highest number (29/100) of isolates exhibiting intermediate resistance, which implied possible non-susceptibility by this bacteria at forthcoming time to this antibacterial agent.

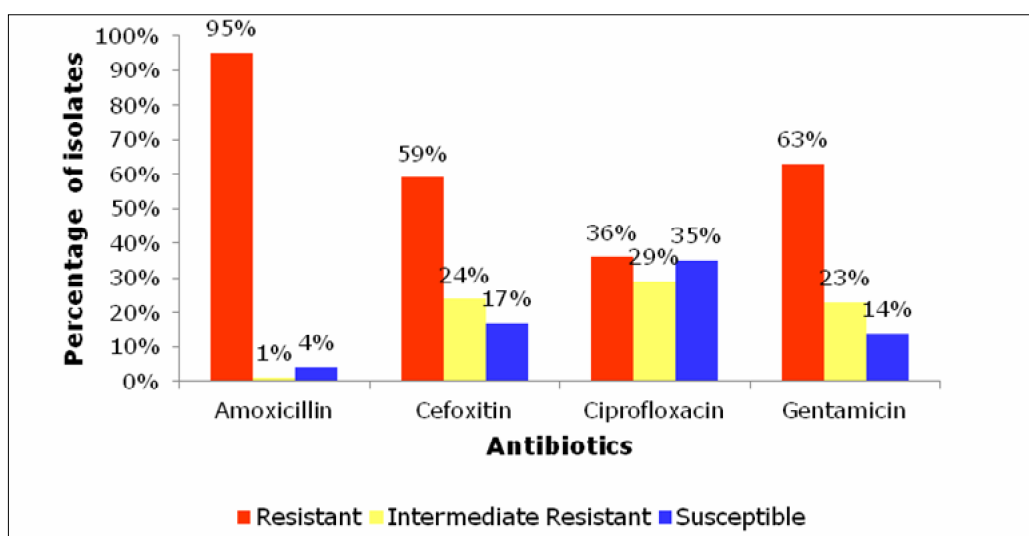


Figure 1. Percentage of *E. coli* isolates showing sensitivity towards each of the four antibiotics.

Eapen et al (2005) and Middleton & Ambrose (2005) reported that ciprofloxacin is the most effective antibiotic versus Gram-negative microorganisms isolated from stool and urine samples and against *E. coli* (100%) detected from fecal matter of Canada geese (*Branta canadensis*). One hundred percent sensitivity to this antibiotic was also observed in *E. coli* from different water sources in Kashmir, India (Rather et al 2013) and in Aleltu neighborhood of Kelala district in South Wollo Administrative Zone, Northeast Ethiopia (Bahiru et al 2013).

The amoxicillin antibiotic showed no efficacy in inhibiting most of the isolates' growth as was demonstrated by the lack of zone of inhibition in 96% of the tested *E. coli*. This high percentage of amoxicillin non-susceptibility was similar to the findings of a local research study conducted by Nuñez et al (2012), which showed that all 26 (100%) *E. coli* isolates from fecal samples of children aged under five years old with diarrhea were resistant to the antibiotic. In a study by Nazir et al (2005), however, 50% of *E. coli* from different water samples remained susceptible to this antibiotic. High resistance of *E. coli* isolated from aquatic environment to amoxicillin was also reported in the study by Bahiru et al (2013) wherein non-susceptibility to the bactericidal agent was exhibited by 100% of the isolates, much higher than what was previously reported in Ethiopia.

This low efficiency of amoxicillin in eradicating *E. coli* growth could be attributed to its widespread and recurring use both in hospital and community settings against a wide array of diseases. Amoxicillin is one of the oldest but still most commonly prescribed antibiotics worldwide. Even without physician's prescription, over-the-counter services have led to the increase in the use (Wood 1999) abuse and misuse of medicines (Cooper 2013). Marlière et al (2000) reported that amoxicillin is the most regularly used antibiotic in the Brazilian households. Surveillance data have shown that *E. coli* resistance is considerably highest for those antibiotics that are used for the longest time among humans and animals (Tadesse et al 2012). With no strict regulations for over-the-counter drugs and high level of inappropriate and irrational use of amoxicillin (Elsiddi 2010), high bacterial resistance rate against this antibacterial agent would be likely. Amoxicillin is a semi-synthetic derivative of the misused antibiotic penicillin (Derderian 2007).

The second most effective antibiotic was cefoxitin having maximum efficacy to 59% of the tested microorganisms. Gentamicin was the third most effective with 63% non-resistant isolates. None of the isolates showed sensitivity towards distilled water. Detection of these antibiotic resistant strains of *E. coli* may challenge the safety of using the domestic water sources not only at present but in the future as well. *E. coli* isolates collected in a London teaching hospital from the years 1995 to 2000 were analyzed for their antibiotic sensitivity in a study by Shannon & French (2004) and it was revealed that resistance of the isolated bacteria towards amoxicillin, cefuroxime (a second-generation cephalosporin like cefoxitin), gentamicin, and ciprofloxacin was increasing annually. Compounding the problem is the widespread misuse of various antibiotics in the Philippines, which if not regulated may cause the emergence of more potent antimicrobial-resistant strains of the bacteria.

Overall, 96% of the *E. coli* isolates have shown resistance towards at least one of the antibiotics used. Multidrug resistance in this study is defined as non-susceptibility to more than two (Bahiru et al 2013; Bean et al 2008; Iqbal et al 2002) of the four antibiotics. Only 4% of the *E. coli* isolates have shown susceptibility to all four antibiotics (Table 1). Eighteen *E. coli* strains were non-susceptible to only one antibiotic, all were resistant towards amoxicillin. Multidrug resistance (Figure 2) was exhibited by 51 of the 100 isolates: 23 are nonsusceptible to three antibiotics and 28 (55%) have shown resistance to all four. The occurrence of these multidrug-resistant *E. coli* strains in the water supplies may cause transmission of resistance genes to other bacteria present in the water sources (Patoli et al 2010; Xi et al 2009).

Table 1

Percentage of *E. coli* isolates showing resistance to multiple antibiotics

<i>Resistance to antibiotics</i>	<i>Percentage of isolates</i>
Resistance to none of the antibiotics	4%
Resistance to 1 antibiotic	18%
Resistance to 2 antibiotics	27%
Resistance to 3 antibiotics*	23%
Resistance to 4 antibiotics*	28%

*considered multiple drug resistance.

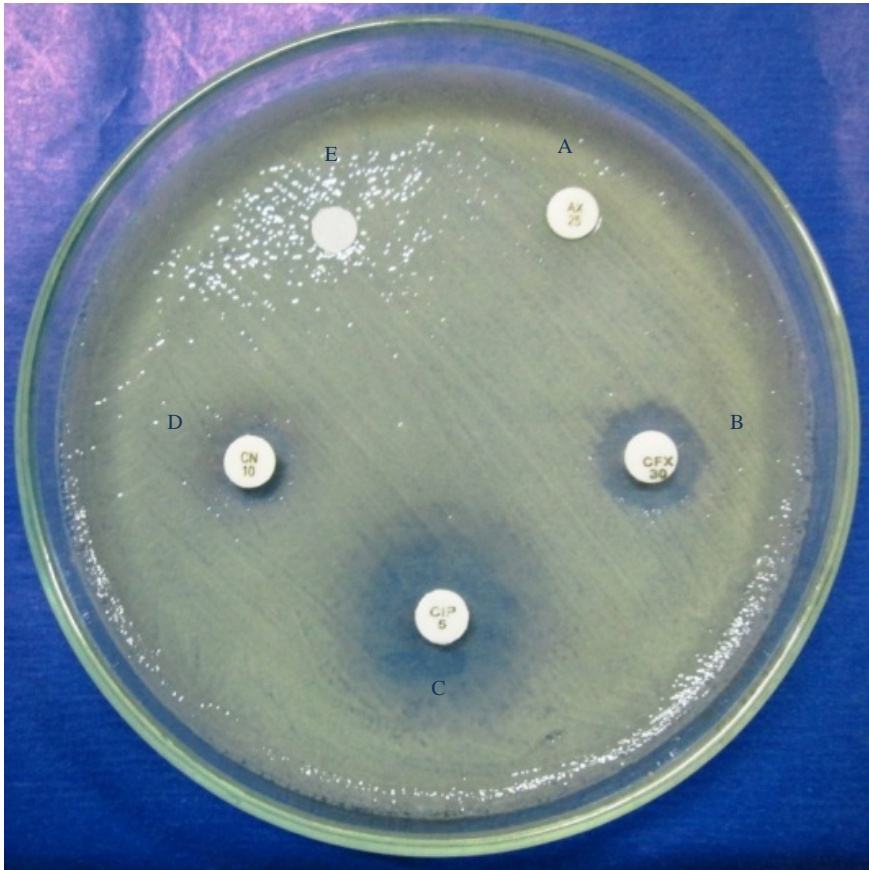


Figure 2. Representative *E. coli* isolate displaying multidrug resistance (A-Amoxicillin, B-Cefoxitin, C-Ciprofloxacin, D-Gentamicin, E-distilled water).

Conclusions. Antibiotic-resistant *E. coli* and multiple drug non-susceptible strains are present in the water samples from water hand pumps in selected areas in Iligan City, Philippines. Detection of these strains in water sources signals the need for further local and national surveillance on the presence of these bacteria in the domestic water supplies. This is a cause of concern for the communities and health agencies, particularly in the utilization of water from hand pumps.

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