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Relationship between antibiotic- and disinfectant-resistance profiles in bacteria harvested from tap water

Sadia Khan [a, b], Tara K. Beattie [a], Charles W. Knapp [a, *]

[a] Department of Civil and Environmental Engineering, James Weir Building, 75 Montrose Street, Glasgow G1 1XJ, UK
[b] Department of Environmental Engineering, NED University of Engineering and Technology, University Road, Karachi 75270, Pakistan

HIGHLIGHTS

- Twenty two genera were isolated from chlorinated drinking-water with a range of susceptibilities to chlorine and antibiotics.
- Chlorine-resistant bacteria had higher MICs for tetracycline, sulfamethoxazole and amoxicillin.
- In the presence of free chlorine, antibiotic-sensitive bacteria survival was less than antibiotic-resistant bacteria.

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Abbreviations:
ARB
Antibiotic-resistant bacteria
ARG
Antibiotic resistance genes
PBS
Phosphate buffer saline
DPD
N,N-diethyl-p-phenylenediamine
PCR
Polymerase chain reaction
DNA
Deoxyribonucleic acid

ABSTRACT

Chlorination is commonly used to control levels of bacteria in drinking water; however, viable bacteria may remain due to chlorine resistance. What is concerning is that surviving bacteria, due to co-selection factors, may also have increased resistance to common antibiotics. This would pose a public health risk as it could link resistant bacteria in the natural environment to human population. Here, we investigated the relationship between chlorine- and antibiotic-resistances by harvesting 148 surviving bacteria from chlorinated drinking-water systems and compared their susceptibilities against chlorine disinfectants and antibiotics. Twenty-two genera were isolated, including members of *Paenibacillus*, *Burkholderia*, *Escherichia*, *Sphingomonas* and *Dermacoccus* species. Weak (but significant) correlations were found between chlorine-tolerance and minimum inhibitory concentrations against the antibiotics tetracycline, sulfamethoxazole and amoxicillin, but not against ciprofloxacin; this suggest that chlorine-tolerant bacteria are more likely to also be antibiotic resistant. Further, antibiotic-resistant bacteria survived longer than antibiotic-sensitive organisms when exposed to free chlorine in a contact-time assay; however, there were little differences in susceptibility when exposed to monochloramine. Irrespective of antibiotic-resistance, spore-forming bacteria had higher tolerance against disinfection compounds. The presence of chlorine-resistant bacteria surviving in drinking-water systems may carry additional risk of antibiotic resistance.

1. Introduction

Antibiotic-resistant bacteria (ARB) and their genes (ARG) are considered emerging environmental contaminants with a widespread distribution (Pruden et al., 2006; Diehl and Lapara, 2010; Dodd, 2012; Chen et al., 2015) with natural and anthropogenic

* Corresponding author. Present addresses: Department of Civil and Environmental Engineering, Level 5, James Weir Building, 75 Montrose Street, Glasgow G1 1XJ, UK.
E-mail address: charles.knapp@strath.ac.uk (C.W. Knapp).

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activities contributing to its development and dispersion in the environment (Allen et al., 2010; Gaze et al., 2011; Wellington et al., 2013) and water bodies (Pruden et al., 2012; Su et al., 2012). As the demand for safe drinking-water increases around the world (Brettar and Hofle, 2008), these compromised natural-water resources could more increasingly become considered as sources of either drinking-water or contamination to the system.

Drinking-water treatment plants use a number of treatment methods to improve water quality: e.g., flocculation, sedimentation, filtration, and disinfection. Among the processes, chemical disinfection contributes greatly to the control of microorganisms from treatment plant to point of use (Berry et al., 2006). However, it is known that chemical disinfection has limitations in its immediate and prolonged effectiveness, and multiple factors reduce the effectiveness of disinfectants against bacterial populations (Scully et al., 1999; Cherchi and Gu, 2011; Jaglic et al., 2012; Bessa et al., 2014) including the presence of organic matter having amino nitrogen compounds (Scully and Hartman, 1996), bacterial growth phase (Cherchi and Gu, 2011) and the presence of extracellular polymeric matrix (Bridier et al., 2011; Wong et al., 2010).

It has increasingly been discovered that resistance traits horizontally transfer in microbial communities due to either cross-resistance (e.g., efflux mechanisms capable of detoxifying multiple stressors) or co-resistance (e.g., closely linked genetic traits on a mobile genetic element) factors. For example, Templeton et al. (2009) found greater frequency of chlorine tolerance among antibiotic-resistant *Escherichia coli* as compared to antibiotic-sensitive *E. coli* grown in the presence of chlorine (Templeton et al., 2009). Genetic factors, such as class 1 and class 2 integrons that transfer multiple resistance genes could be responsible for such traits (Gillings et al., 2009; Ozgumus et al., 2009; Koczura et al., 2012; Mokracka et al., 2012; Su et al., 2012; Hsu et al., 2014; Chen et al., 2015).

Wastewater treatment studies (Diehl and Lapara, 2010; Burch et al., 2013) have reported decreases in total bacteria, but an increased ratio of resistant bacteria (Galvin et al., 2010; Guo et al., 2014; Al-Jassim et al., 2015) following treatment; a similar trend may occur in drinking-water systems (Bergeron et al., 2015). There have been reports of drinking-water treatment plants (DWTP) (Armstrong et al., 1981, 1982; Xi et al., 2009; Farkas et al., 2013; Pruden et al., 2006) and water distribution systems (DWDS) (Laroche et al., 2010; Talukdar et al., 2013; Xi et al., 2009) influencing the emergence and spread of antibiotic-resistance. For example, relative abundance of sulfonamide resistance genes increased from 3.5% to 33% in DWTP (Chao et al., 2013) and a broader range of ARGs were found (Fahrenfeld et al., 2013). Stressful environments such as extreme pH, high salinity, nutrient deprivation (Bessa et al., 2014), oxidation (Scully et al., 1999), or chlorine exposure (Ridgway and Olson, 1982) promote populations with greater resistance. Sub-inhibitory concentrations not only select resistant populations, but could invoke a stress response which may include genetic exchange.

Bacteria opportunistically colonise water distribution systems (Wang et al., 2013) and water meters (Hong et al., 2010). Additionally, localised disruptions in the distribution mains (e.g., in building cisterns and plumbing) also introduce bacterial populations, which may include agents of waterborne disease, increased health risks and maintenance costs to the system (Falkinham et al., 2015).

This study compares the susceptibilities of bacteria harvested from drinking-water taps to chlorine disinfectants and four antibiotics: tetracycline (TET), sulfamethoxazole (SMX), ciprofloxacin (CIP) and amoxicillin (AMX). We hypothesized that bacteria isolated from water taps would have enhanced disinfectant- and antibiotic-resistance profiles. Further, we determine whether disruptions to service lines provide a source of contamination and increase the risk of ARB and ARG.

2. Methods

2.1. Sampling and bacteria isolation

In the UK, most drinking-water is sourced from surface water (Scottish-Water, 2012a,b) and does not deviate from many conventional water-treatment works: screening, coagulation, flocculation, sedimentation or clarification, filtration (rapid gravity, slow sand, or membrane), and pH adjustment. Both chlorination and chloramination are used for disinfection in Scotland, UK to provide good quality water for human use. Monochloramine is used in the distribution system as it has a longer residence time than chlorine and produces fewer by-products.

To compare tolerances between disinfection and antibiotics, bacteria were harvested from 52 water samples, collected from flushed (5 min) taps in Glasgow, Scotland, UK. Samples were collected in pre-sterile screw capped bottles and brought to the laboratory for processing within two hours to minimise changes in the samples. Thirty-eight samples were collected from buildings that had tank cisterns for drinking-water storage, with tank capacities ranged from 16,000 to 27,000 L; the remaining 14 samples were from closed systems.

A vacuum-filtration method, with 0.22 μm pore-size cellulose-nitrate gridded membrane filters (Millipore, UK) was used to harvest cells from 100 mL of each water sample; the filter was placed on a Standard Plate Count Agar plate APHA (Oxoid, UK) and incubated for 48 h at 35 ± 2 °C for the development of colonies. The plastic lid was retained to minimise aerosol contamination; sterilised distilled water was used as controls. Isolated bacterial strains were preserved by using a bacterial bead preservation kit (Cryo vials TS/71-MX, Technical Service Consultants Ltd. UK) and stored at −80 °C throughout the study period. For each set of experiments, one bead was taken out from the cryovials, grown in LB broth overnight, and streaked on a Nutrient Agar (Oxoid, UK) plate to obtain isolated colonies.

2.2. Identification of bacteria isolates

Representative colonies were selected for phylogenetic characterisation by sequencing the V4 region of each 16S-rRNA gene. The DNA of bacterial isolates was extracted by a thermal freeze thaw method (Knapp et al., 2012), alternating between −80 °C and 70 °C in 100 μL PBS (phosphate buffer solution; pH 7.4). PCR reaction was performed with a Bio-Rad iQ5 Real-Time PCR Detection System. Forward and reverse primers (Sigma–Aldrich, Life Sciences, UK) were V4-16S-515F (5′-TGTGCCAGCMGCCGCGGTAA) and V4-16S-806R (5′-GGCTACHVGGGTWTCTAAT) (Caporaso et al., 2011). Each PCR reaction contained 10 μL of Universal Supermix (Bio-Rad, UK), 500 ηM of each primer, 0.1 μL SYBR green, 6 μL of nuclease free water and 3 μL of DNA template. A PCR run consisted of initial denaturation at 95 °C for 3 min followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 30 s and then a 10 min final extension at 72 °C. PCR product length was verified on 2% agarose gel (Bio-Rad, UK) with ethidium bromide (Sigma–Aldrich, UK) and a 50-bp DNA ladder.

A QIAquick PCR Purification Kit (Qiagen, UK) was used to purify PCR products. DNA concentrations were determined by the EPOCH™ Microplate spectrophotometric system (BioTek, UK). Five μL of purified DNA was mixed with the same volume of 5 μM forward primer solution in total volume of 10 μL. Sequencing for the identification of bacteria was performed by LightRun Sequencing