Published in final edited form as:

Water Air Soil Pollut. 2011 November 1; 222(1-4): 305–314. doi:10.1007/s11270-011-0825-6.

Comparison of the Transport of Tetracycline-Resistant and Tetracycline-Susceptible *Escherichia coli* Isolated from Lake Michigan

Jacob J. Walczak,

Department of Geosciences, University of Wisconsin-Milwaukee, Milwaukee, WI, USA

Sonia L. Bardy,

Department of Biological Sciences, University of Wisconsin-Milwaukee, 3209 N Maryland Ave, Milwaukee, WI 53211, USA

Lucia Feriencikova, and

Department of Geosciences, University of Wisconsin-Milwaukee, Milwaukee, WI, USA

Shangping Xu

Department of Geosciences, University of Wisconsin-Milwaukee, Milwaukee, WI, USA

Shangping Xu: xus@uwm.edu

Abstract

It was recently reported that tetracycline could enhance the mobility of manure-derived *Escherichia coli* within saturated porous media (Walczak et al. (Water Research 45:1681–1690, 2011)). It was also shown, however, that *E. coli* from various sources could display marked variation in their mobility (Bolster et al. (Journal of Environmental Quality 35:1018–1025, 2009)). The focus of this research was to examine if the observed difference in the mobility of manure-derived tetracycline-resistant (tet^R) and tetracycline-susceptible (tet^S) *E. coli* strains was source-dependent. Specifically, *E. coli* were isolated from Lake Michigan, and the influence of tetracycline resistance on Lake Michigan-derived *E. coli* was investigated through column transport experiments. Additionally, a variety of cell morphology and surface properties were determined and related to the observed bacterial transport behavior. Our experimental results showed that, consistent with previous observations, the deposition rate coefficients of the tet^R *E. coli* strain was ~20–100% higher than those of the tet^S *E. coli* strain. The zeta potential of the tet^R *E. coli* cells was ~25 mV more negative than the tet^S *E. coli* cells. Because the surfaces of the *E. coli* cells and the quartz sands were negatively charged, the repulsive electrostatic double-layer interaction between the tet^R *E. coli* cells and the quartz sands was stronger, and the mobility of the tet^R *E. coli* cells in the sand packs was thus higher. The tet^R *E. coli* cells were also more hydrophilic than the tet^S *E. coli* cells. Results from migration to hydrocarbon phase (MATH) tests showed that about ~35% more tet^S *E. coli* cells partitioned to the hydrocarbon phase. As it was previously shown that cell hydrophobicity could enhance the attachment of bacterial cells to quartz sand, the difference in cell hydrophobicity could also have contributed to the observed higher mobility of the tet^R *E. coli* cells. The size of the tet^R and tet^S *E. coli* cells were similar, suggesting that the observed difference in their mobility was not size-related. Characterization of cell surface properties also showed that tet^R and tet^S *E. coli* cells differed slightly in cell-bound lipopolysaccharide contents and had distinct outer membrane protein profiles. Such difference could alter cell surface properties which in turn led to changes in cell mobility.

Keywords

Antibiotic-resistant bacteria; *Escherichia coli*; Bacteria transport; Groundwater contamination

1 Introduction

Due to the widespread use of antibiotics by humans and within the animal farm environment, a wide variety of antibiotic-resistant bacteria (ARB) have been detected in domestic wastewater (Andersen 1993; Luczkiewicz et al. 2010; Reinthaler et al. 2003; Salmore et al. 2006), discharge of wastewater treatment plant (Andersen 1993; Kim and Aga 2007; Lefkowitz and Duran 2009; Luczkiewicz et al. 2010; Rijal et al. 2009), as well as manure and agricultural discharge (Halbert et al. 2006; Hofacre et al. 2000; Hu et al. 2008; Parveen et al. 2006; Ray et al. 2006; Sato et al. 2004; Varga et al. 2008). The spread of the ARB in the environment is being increasingly recognized as a serious threat to public health (World Health Organization 2003).

In the USA, groundwater represents the primary source of drinking water for more than 40% of the total population and accounts for 87% and 29% of domestic and public water supply, respectively (Hutson et al. 2004; Kenny et al. 2009). A thorough understanding of the transport of ARB within the groundwater system is critical to the protection of groundwater from contamination of ARB. Additionally, because granular bed (e.g., silica sand, anthracite coal) is commonly used to remove particulate materials (e.g., bacteria) from drinking water (Cleasby and Logsdon 1999), insight into the transport of ARB in sand packs can also help assess and improve the removal of ARB in drinking water treatment processes.

Few studies, however, have examined the transport of ARB in saturated porous media. Rahe et al. (1978) reported that wastewater-derived *Escherichia coli* that were resistant to novobiocin, tetracycline, and nalidixic acid could move over a distance of >15 m within 1 h in saturated Oregon hillslope soils. Rysz and Alvarez (2006) reported that tet^R *Burkholderia cepacia* displayed high mobility in columns packed with quartz sands, but the influence of tetracycline resistance on the transport of *B. cepacia* was not specifically examined. We recently examined the transport of manure-derived tet^R and tet^E *E. coli* in saturated porous media and found that the tet^R *E. coli* strains exhibited significantly higher mobility (Walczak et al. 2011). Measurement of bacterial cell surface properties showed that the tet^R *E. coli* strains had more negative zeta potential values than the tet^S *E. coli* strains. The difference in cell surface properties was related to the absence and presence of several proteins on the outer membrane of the bacterial cells, some of which were likely related to bacterial tetracycline resistance (Walczak et al. 2011).

The tet^R and tet^S *E. coli* strains used in the previous study were obtained from the same milking cow in order to minimize the variations in cell properties that can be caused by different growth condition and nutrition status of different cows (Walczak et al. 2011). It is known, however, that *E. coli* from different sources can have marked variations in the transport behavior in saturated porous media (Bolster et al. 2010; Bolster et al. 2009; Foppen et al. 2010; Lutterodt et al. 2009). In this research, we used *E. coli* strains isolated from Lake Michigan to further investigate the effects of tetracycline resistance on the transport of *E. coli* in saturated porous media. We also examined the relationship between *E. coli* mobility and cell properties to identify the key factors that led to the different transport behavior of the tet^R and tet^S *E. coli* strains.

2 Materials and Methods

2.1 Isolation and Antimicrobial Susceptibility Test of *E. coli*

The *E. coli* strains used in this research were isolated from water samples collected from Lake Michigan (Bradford Beach, Milwaukee, WI, USA). The sampling site is about 1 mile south of Milwaukee's Linnwood drinking water treatment plant, which, on average treats ~100 million gallons of water each day and supplies drinking water to the city of Milwaukee and its suburban areas. About 4 miles to the south of the sampling site is the Jones Island wastewater treatment plant (WWTP). The treatment capacity of the WWTP is ~100 million gallons per day, and the treated wastewater is discharged into Lake Michigan along the shoreline.

The water samples were collected along the shoreline of Lake Michigan using sterile medium glass bottles early in the morning when the beach was empty. The samples were immediately put on ice and processed within 30 min upon return to the laboratory. The protocols established by US EPA was used to isolate *E. coli* (US EPA 2000; Walczak et al. 2011). Briefly, the collected water samples were filtered through sterile Polyvinylidene Fluoride 0.45 μm membranes (Millipore); the membranes were then placed onto modified mTEC agar plates (Becton Dickinson), which were incubated at 35°C for 2 h and then 44.5°C for 22 h. Red or magenta colonies on the mTEC agar plates were tentatively identified as *E. coli*, which were then confirmed with Enterotube II (Becton Dickinson) and MacConkey II agar plates containing 4-methylumbelliferyl-D-glucuronide (Becton Dickinson).

The *E. coli* isolates were subject to antibiotic susceptibility tests using Mueller-Hinton agar plates (Clinical and Laboratory Standards Institute 2006). Seven representative antibiotics were selected for the test, and for each antibiotic, two concentrations were used (Table 1). The isolates were assayed to determine their antibiotic resistance patterns. To study the effects of tetracycline resistance on mobility, two *E. coli* strains with different tetracycline resistance patterns were selected (Table 1). Polymerase chain reaction (PCR) assays were then performed to detect the 20 *tet*^R genes (Hu et al. 2008). The primers used for the PCR assays and the protocols can be found elsewhere (Hu et al. 2008; Walczak et al. 2011). The *tetB* gene was detected in the plasmid of the *tet*^R strain and all tetracycline resistance genes, including *tetB*, were not detected in the *tet*^S strain (Fig. 1). This PCR result was consistent with the manure-derived strains used in the previous research (Walczak et al. 2011). The two selected *E. coli* strains were preserved in 20% glycerol under -80°C.

2.2 Column Transport Experiments

The setup of the column transport experiments was similar to that employed in Walczak et al. (2011). Briefly, vertically oriented, duplicate glass columns (Kontes) measuring 2.5 cm in diameter and 15 cm in length were packed with quartz sands (US Silica), which were cleaned alternately using hot, concentrated HNO₃ (to remove surface metal oxide/hydroxide coatings) and diluted NaOH solution (to remove colloid-sized particles on the surface of sand). The size of the sand was within 0.707–0.841 mm. The flow (downward) was regulated using peristaltic pumps (Cole-Parmer) at a constant specific discharge of 0.31 cm/min. The porosity of the sand was 0.356 as determined using the bulk density method (Weight 2008). Prior to the injection of cell suspensions, the packed columns were equilibrated with >40 pore volumes of appropriate electrolyte solution (i.e., 1–100 mM KCl). The electrolyte solutions were selected to cover to ionic strength range of natural groundwater (Freeze and Cherry 1979).

The *E. coli* cells used for the experiments were cultured in Luria-Bertani broth at 37°C and then harvested using centrifuge (4,000×g, 4°C, 10 min). The pellets were rinsed four times

using appropriate KCl solution to remove the growth medium. The cell suspension was diluted to $\sim 4 \times 10^7$ cells mL^{-1} for the column transport study. The pH of both the background electrolyte solution and cell suspension was ~ 5.7 . The injection of cell suspension lasted for ~ 3 pore volumes, following which the columns were flushed using KCl solutions of the same ionic strength. The column effluents were monitored using a UV/Vis spectrophotometer (Shimadzu, UV1700) equipped with quartz flow-through cells for bacterial concentrations (wavelength, 220 nm).

We calculated the clean-bed deposition rate coefficient (K_d) to describe the deposition kinetics of *E. coli* cells in saturated sands (Kretzschmar et al. 1999):

$$K_d = -\frac{v}{\theta L} \ln \left(\frac{C}{C_0} \right) \quad (1)$$

Where v is approach velocity (centimeters per minute), L is the length of the packed bed (centimeters), θ is the porosity of the porous medium (cubic centimeters per cubic centimeter), C is concentration of bacteria cell in the effluent under “clean-bed” conditions (cell per milliliter), and C_0 is the bacteria concentration in the influent (cell milliliter). The values of $\frac{C}{C_0}$ were determined by calculating the average normalized breakthrough concentrations (i.e., $\frac{C}{C_0}$) measured between 1.8 and 2 pore volumes (Walczak et al. 2011; Walker et al. 2005).

2.3 Cell Characterization

Bacterial transport in saturated porous media is governed by the energy interactions between the bacterial cells and the surface of the solid matrix, which are influenced by a variety of cell properties such as size, zeta potential, and hydrophobicity (Elimelech et al. 1998). The cell surface properties were usually related to factors such as cell-bound proteins and lipopolysaccharides (LPS; Foppen and Schijven 2006; Lutterodt et al. 2009). In this research, cell properties were characterized and related to bacterial transport behavior.

The zeta potential of the freshly harvested bacterial cells suspended in appropriate KCl solutions was measured using a Brookhaven ZetaPALS analyzer. The hydrophobicity of the cells was determined through microbial adhesion to hydrocarbon test (Pembrey et al. 1999). Briefly, 1 mL of *n*-dodecane and 4 mL of cell suspension were mixed and vortexed for 2 min. The vials were allowed to stand still for 15 min, and then cell concentrations in the aqueous phase were determined at a wavelength of 546 nm. Cell hydrophobicity was calculated as the fraction of bacterial cells that partitioned into the hydrocarbon phase. To quantify EDTA-extractable cell-bound proteins, fresh cell suspensions ($\sim 3 \times 10^8$ cell/ml) were prepared and mixed with 2.5% EDTA solution on a 3:2 (v/v) basis (final EDTA concentration 1%; Zhang et al. 1999). Following a 30-min incubation period at 4°C, the mixture was centrifuged at $10,400 \times g$ (4°C) for 50 min to remove cell debris. The supernatant was then decanted and filtered through 0.45- μm filters. Protein contents in the filtrates were quantified using the Coomassie brilliant blue method developed by Bradford (1976). Human serum albumin was used as a standard to calibrate the Bradford method. To extract cell-bound LPS, 5 mL cell suspension was placed into 50 mL centrifuge tubes and subject to sonic disruption (50 W, 20 s; Liu et al. 2007). Following a centrifuge step ($10,000 \times g$, 40 min, 4°C), the supernatant was filtered using 0.2- μm cellulose acetate filters. The contents of LPS in the filtrates were determined using the phenol-sulfuric acid method (DuBois et al. 1956). Standard solutions of xanthan gum were used to calibrate this method.

In addition to the total amount of EDTA-extractable, cell-bound proteins, the outer membrane proteins (OMPs) of the *E. coli* cells were extracted and profiled using sodium dodecyl-sulfate polyacrylamide gel (SDS-PAGE; Ben Abdallah et al. 2009; Gatewood et al. 1994; Xu et al. 2006). For this purpose, *E. coli* cells were harvested (4,000×g, 10 min, 4°C) and rinsed twice with sterile 0.15 M KCl. Cell suspensions (5 mL) were disrupted by intermittent sonic oscillation (50 W, eight cycles of 15 s of sonication, VirTis Virsonic). The resulting suspension was centrifuged at 5,000×g for 40 min to remove cellular debris, and the supernatant was transferred and centrifuged at 100,000×g for 40 min at 4°C. The pellets were treated using 2% sodium lauryl sarcosinate (Sigma) at room temperature for 1 h and then centrifuged again at 100,000×g (40 min, 4°C). The pelleted OMPs thus produced were suspended in 0.25 mL sterile 0.15 M NaCl solution and resolved on 10% sodium dodecyl-sulfate polyacrylamide gel (Laemmli). The gel was fixed in 4% perchloric acid and strained using 0.1% Coomassie G250 in hot 4% perchloric acid (Faguy et al. 1996).

A Nikon Eclipse 50i microscope, which was equipped with a Photometric Coolsnap ES digital camera and MetaMorph software, was used to determine the size of the *E. coli* cells suspended in various electrolyte solutions. The length and width of a minimum of 35 cells were determined using the ImageJ software, and the equivalent radii of the cells were then calculated.

3 Results and Discussion

3.1 Transport of tet^R and tet^S *E. coli* Strains in Sand Packs

The measured breakthrough concentrations of the tet^R *E. coli* cells were higher than the corresponding breakthrough concentrations of the tet^S *E. coli* cells, and, in general, the breakthrough concentrations decreased when the ionic strength increased from 1 to 100 mM, particularly within the range of 1 to 30 mM (Fig. 2). The calculated deposition rate coefficients (K_d) increased from 0.015 min⁻¹ (1 mM KCl) to 0.052 min⁻¹ (100 mM KCl) for tet^R and from 0.035 min⁻¹ (1 mM KCl) to 0.061 min⁻¹ (100 mM KCl) for tet^S (Fig. 3). Across the range of 1 to 100 mM KCl, the K_d values of tet^R were all significantly lower than the K_d values of tet^S, suggesting that tet^R *E. coli* cells had higher mobility than the tet^S *E. coli* cells. The values of K_d increased with ionic strength for both tet^R and tet^S *E. coli* strains, suggesting higher ionic strength facilitated the removal of *E. coli* cells from the moving aqueous phase.

3.2 Comparison of *E. coli* Cell Properties

Published results showed that transport of bacterial cells in porous media depends on a range of factors such as the Lifshitz-van der Waals force, electrostatic double-layer (EDL) interactions and hydrophobicity interactions (Ong et al. 1999; Walker et al. 2004). These factors in turn are affected by cell size and various cell surface properties (Lindqvist and Bengtsson 1991; Ong et al. 1999).

Measurement of the size of *E. coli* cells suspended in 1–100 mM KCl indicated that cell size did not vary with ionic strength, and the sizes of the tet^R and tet^S *E. coli* cells were similar. The observed difference in the mobility of tet^R and tet^S *E. coli* cells was thus not size-related (Fig. 4a).

The zeta potential values of both the solid matrix and the bacterial cells strongly influence the EDL interactions between them. Under the experimental conditions, the surface of the sands and the bacterial cells displayed negative zeta potential values (Fig. 4b), suggesting repulsive EDL interactions. The zeta potentials of the tet^R *E. coli* strain were more negative than those of the tet^S strain (Fig. 4b). As a result, the repulsive interaction between the surface of quartz sands and the tet^R *E. coli* cells were stronger and the deposition rates

should thus be lower. In general, the zeta potential values of the quartz sands, as well as the tet^R and tet^S *E. coli* strains became less negative with increasing ionic strength. This trend indicated that the repulsive forces between the *E. coli* cells (both tet^R and tet^S strains) and quartz sands decreased with increasing ionic strength. As a result, the mobility of the *E. coli* cells became lower when ionic strength increased. This is consistent with the observed relationship between the deposition rate coefficients and ionic strength (Fig. 3).

The hydrophobicity of the *E. coli* strains selected for this experiment was found to be dependent on ionic strength, and the tet^S *E. coli* cells were generally more hydrophobic than the tet^R *E. coli* cells (Fig. 4c). It was previously suggested that cell hydrophobicity could enhance the attachment of bacterial cells to clean quartz sands (Bolster et al. 2006; Mccaulou et al. 1994; Walker et al. 2005). Walker et al. (2005), for instance, reported that the more hydrophobic stationary-phase *E. coli* D21g (34% of cells partitioned into the hydrocarbon phase) displayed slightly lower mobility than the less hydrophobic exponential-phase cells (16% of cells partitioned into the hydrocarbon phase). It was possible that the variation in the hydrophobicity of the tet^S and tet^R cells contributed to the observed difference in their mobility.

LPS and OMPs represent the major components of the outer membrane of Gram-negative bacteria such as *E. coli*. They maintain the integrity of the outer membrane, protect the membrane from various attacks, and define cell surface properties (Wang and Quinn 2010). For the *E. coli* strains used in this research, cell-bound LPS contents varied between 22 and 35 $\mu\text{g}/10^8$ cells for the tet^R *E. coli* cells and between 20 and 24 $\mu\text{g}/10^8$ cells for the tet^S *E. coli* cells, respectively (Fig. 4d). These values were comparable to previous observations for manure-derived *E. coli* strains (Haznedaroglu et al. 2008; Walczak et al. 2011). Within the range of 1–30 mM of KCl, the tet^R *E. coli* cells had higher LPS contents than the tet^S ones. Because LPS could occupy as much as 75% of the surface of *E. coli* cells and the polysaccharides component of *E. coli* LPS is primarily hydrophilic (Caroff and Karibian 2003; Wang and Quinn 2010), it was likely that the observed more negative zeta potential and lower hydrophobicity of the tet^R *E. coli* cells were related to the greater abundance of LPS. For instance, it was previously reported that the zeta potential of *E. coli* K12 D21g, which produces LPS, was more negative than that of *E. coli* K12 21f2g, which was a mutant of D21 with a truncated LPS molecule (Walker et al. 2004). Accordingly, *E. coli* K12 D21 exhibited higher mobility than the mutant D21 f2g in saturated sand packs.

Our measurements suggested no difference in the cell-bound protein contents for the tet^R and tet^S *E. coli* cells (Fig. 5a). SDS-PAGE analysis of OMPs, however, showed that the tet^R and tet^S *E. coli* strains had distinct OMP profiles (Fig. 5b). Specifically, there were three proteins enriched in the outer membrane of the tet^R strain (indicated by arrows in Fig. 5b). These proteins had approximate molecular masses of 22, 50, and 75 kDa. As previously suggested, the protein encoded by the *tetB* gene that was detected in the tet^R strain (Fig. 1) is not exposed to the outside of the bacterial cells, it was thus unlikely that the presence of *TetB* could substantially impact cell mobility (Walczak et al. 2011). The protein of 50 kDa, however, was likely the TolC protein, the outer membrane components of the AcrAB-TolC efflux pump that was reported to be related to tetracycline resistance (de Cristobal et al. 2006; Xu et al. 2006). Because proteins like TolC are located in the outer membrane, these proteins could lead to variations in cell surface properties, which in turn could alter cell transport behavior in saturated porous media (Walczak et al. 2011).

3.3 Comparison of tet^R and tet^S *E. coli* Strains Isolated from Different Sources

We previously reported that tetracycline resistance could enhance the mobility of manure-derived *E. coli* (Walczak et al. 2011). In this research, we further examine the effects of tetracycline resistance on the transport behavior of *E. coli* in saturated porous media using

strains isolated from Lake Michigan. All the tet^R strains used in both studies had similar antibiotic resistance patterns and possessed the *tetB* gene. Similarly, all the tet^S strains shared similar resistance patterns, and no tetracycline resistance gene was detected. Our results confirmed that tet^R *E. coli* strains had higher mobility than the tet^S strains in saturated porous media. This trend was also valid when the transport behavior of *E. coli* strains from different sources was compared, i.e., the tet^R strain obtained from Lake Michigan displayed higher mobility than tet^S strains retrieved from dairy manure; and the tet^R strains isolated from dairy manure also exhibited higher mobility than the tet^S strain obtained from Lake Michigan. In the meantime, we also observed increased variability when the transport of tet^R (or tet^S) *E. coli* strains from different sources was compared. As explained below, such variations could be related to differences observed in the surface properties of the *E. coli* cells of different sources.

All the *E. coli* strains used in both studies had comparable sizes, indicating that the observed difference in their mobility was not size-related. For both manure- and Lake Michigan-derived *E. coli* strains, we also observed that the tet^R *E. coli* strains had more negative zeta potentials than the tet^S *E. coli* strains (Walczak et al. 2011). Because the surface of the quartz sand was also negatively charged, the more negative zeta potentials of the tet^R *E. coli* cells led to stronger repulsive EDL interactions between the cells and the surface of the sand and lowered the possibility of cell attachment to the sand surface. The mobility of the tet^R *E. coli* strains was thus higher than that of the tet^S *E. coli* strains.

The surface properties of *E. coli* strains isolated from different sources (i.e., dairy manure and Lake Michigan) also displayed variations. For the *E. coli* strains isolated from manure, the tet^R strains were more hydrophobic than the tet^S strains, as suggested by the results of MATH tests, while the opposite trend was observed for the *E. coli* strains isolated from Lake Michigan (Walczak et al. 2011). It was thus unlikely that cell hydrophobicity played a major role in determining the transport behavior of tet^R and tet^S *E. coli* strains. Hydrophobicity, however, was reported to affect the transport bacterial cells (Bolster et al. 2006; Mccaulou et al. 1994). The differences in cell hydrophobicity therefore could have contributed to the variations in the transport of tet^R (or tet^S) *E. coli* strains of different sources. Additionally, while no difference was observed in LPS contents for the manure-derived *E. coli* strains, the measured LPS contents of the tet^R strain isolated from Lake Michigan seemed to be higher than those of the tet^S strains when ionic strength ranged from 1 to 30 mM. Although cell-bound LPS could affect bacterial transport through altering cell surface properties, the different patterns observed for *E. coli* strains isolated from different sources suggest that cell-bound LPS contents did not play major roles in determining the transport of tet^R and tet^S *E. coli* cells.

We found that tet^R and tet^S *E. coli* strains (either from dairy manure or from Lake Michigan) had substantial differences in OMP profiles. For the *E. coli* strains used in this research, there were three OMPs that were enriched in tet^R *E. coli* cells (size, 22, 50, and 75 kDa). In Walczak et al. (2011), there were four OMPs (size, 38, 42, 46, and 54 kDa) enriched in the tet^R *E. coli* cells and three OMPs (size, 33, 48, and 56 kDa) enriched in the tet^S *E. coli* cells. The difference of OMPs between tet^R and tet^S *E. coli* strains might have altered cell surface properties and subsequently affected cell mobility. In the meantime, the variations of OMPs within tet^R (or tet^S) *E. coli* strains might have led to variations in the mobility of tet^R (or tet^S) *E. coli* strains from different sources. Lutterodt et al. (2009) recently showed that an outer membrane protein (Ag43) of *E. coli* could enhance the attachment of *E. coli* cells to the surface of quartz sands. Our results further highlighted the potential influence of OMPs on the transport of *E. coli* cells and additional studies will be required to identify the proteins and quantify their influences on cell surface properties and bacterial transport in the natural environment.

Acknowledgments

SX was supported by University of Wisconsin-Milwaukee and University of Wisconsin Ground-water Research Program (WR10R007). SLB is the recipient of an NIH grant (5R00GM083147-04). We thank Dr. Douglas A. Steeber, Dr. Heather A. Owen, Thomas Schuck, and Steven E. Hardcastle for their assistance.

References

- Andersen SR. Effects of waste-water treatment on the species composition and antibiotic-resistance of Coliform bacteria. *Current Microbiology*. 1993; 26:97–103.
- Ben Abdallah F, Kallel H, Bakhrouf A. Enzymatic, outer membrane proteins and plasmid alterations of starved *Vibrio parahaemolyticus* and *Vibrio alginolyticus* cells in seawater. *Archives of Microbiology*. 2009; 191:493–500. [PubMed: 19373459]
- Bolster CH, Walker SL, Cook KL. Comparison of *Escherichia coli* and *Campylobacter jejuni* transport in saturated porous media. *Journal of Environmental Quality*. 2006; 35:1018–1025. [PubMed: 16738386]
- Bolster CH, Haznedaroglu BZ, Walker SL. Diversity in cell properties and transport behavior among 12 different environmental *Escherichia coli* isolates. *Journal of Environmental Quality*. 2009; 38:465–472. [PubMed: 19202016]
- Bolster CH, Cook KL, Marcus IM, Haznedaroglu BZ, Walker SL. Correlating transport behavior with cell properties for eight porcine *Escherichia coli* isolates. *Environmental Science & Technology*. 2010; 44:5008–5014. [PubMed: 20540542]
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 1976; 72:248–254. [PubMed: 942051]
- Caroff M, Karibian D. Structure of bacterial lipopolysaccharides. *Carbohydrate Research*. 2003; 338:2431–2447. [PubMed: 14670707]
- Cleasby, JL.; Logsdon, GS. Granular bed and percoat filtration. In: Letterman, RD., editor. *Water quality and treatment: A handbook of community water supplies*. 5. New York: McGraw-Hill, Inc; 1999. p. 8.1-8.99.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests; approved standard. 9. Wayne PA: 2006.
- de Cristobal RE, Vincent PA, Salomon RA. Multidrug resistance pump AcrAB-TolC is required for high-level, Tet(A)-mediated tetracycline resistance in *Escherichia coli*. *The Journal of Antimicrobial Chemotherapy*. 2006; 58:31–36. [PubMed: 16687460]
- DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*. 1956; 28:350–356.
- Elimelech, M.; Gregory, J.; Jia, X.; Williams, RA. Particle deposition and aggregation measurement, modelling, and simulation. Oxford: Butterworth-Heinemann; 1998.
- Faguy DM, Bayley DP, Kostyukova AS, Thomas NA, Jarrell KF. Isolation and characterization of flagella and flagellin proteins from the thermoacidophilic archaea *Thermoplasma volcanium* and *Sulfolobus shibatae*. *Journal of Bacteriology*. 1996; 178:902–905. [PubMed: 8550530]
- Foppen JWA, Schijven JF. Evaluation of data from the literature on the transport and survival of *Escherichia coli* and thermotolerant coliforms in aquifers under saturated conditions. *Water Research*. 2006; 40:401–426. [PubMed: 16434075]
- Foppen JW, Lutterodt G, Roling WFM, Uhlenbrook S. Towards understanding inter-strain attachment variations of *Escherichia coli* during transport in saturated quartz sand. *Water Research*. 2010; 44:1202–1212. [PubMed: 19765793]
- Freeze, RA.; Cherry, JA. *Groundwater*. Engle-wood Cliffs: Prentice-Hall; 1979.
- Gatewood DM, Fenwick BW, Chengappa MM. Growth-condition dependent expression of *Pasteurella-Haemolytica* a1 outer-membrane proteins, capsule, and Leukotoxin. *Veterinary Microbiology*. 1994; 41:221–233. [PubMed: 7975148]
- Halbert LW, Kaneene JB, Ruegg PL, Warnick LD, Wells SJ, Mansfield LS, et al. Evaluation of antimicrobial susceptibility patterns in *Campylobacter* spp isolated from dairy cattle and farms

- managed organically and conventionally in the midwestern and northeastern United States. *Journal of the American Veterinary Medical Association*. 2006; 228:1074–1081. [PubMed: 16579787]
- Haznedaroglu BZ, Bolster CH, Walker SL. The role of starvation on *Escherichia coli* adhesion and transport in saturated porous media. *Water Research*. 2008; 42:1547–1554. [PubMed: 18037467]
- Hofacre CL, de Cotret AR, Maurer JJ, Garritty A, Thayer SG. Presence of fluoroquinolone-resistant coliforms in poultry litter. *Avian Diseases*. 2000; 44:963–967. [PubMed: 11195655]
- Hu JY, Shi JC, Chang H, Li D, Yang M, Kamagata YC. Phenotyping and genotyping of antihiotic-resistant *Escherichia coli* isolated from a natural river basin. *Environmental Science & Technology*. 2008; 42:3415–3420. [PubMed: 18522127]
- Hutson, SS.; Barber, NL.; Kenny, JF.; Linsey, KS.; Lumia, DS.; Maupin, MA. Estimated use of water in the United States in 2000, US Geological Survey Circular 1268. Reston: US Geological Survey; 2004. p. 46
- Kenny, JF.; Barber, NL.; Hutson, SS.; Linsey, KS.; Lovelace, JK.; Maupin, MA. Estimated use of water in the United States in 2005. 2009.
- Kim S, Aga DS. Potential ecological and human health impacts of antibiotics and antibiotic-resistant bacteria from wastewater treatment plants. *Journal of Toxicology and Environmental Health Part B: Critical Reviews*. 2007; 10:559–573.
- Kretzschmar R, Borkovec M, Grolimund D, Elimelech M. Mobile subsurface colloids and their role in contaminant transport. *Advances in Agronomy*. 1999; 66:121–193.
- Lefkowitz JR, Duran M. Changes in antibiotic resistance patterns of *Escherichia coli* during domestic wastewater treatment. *Water Environment Research*. 2009; 81:878–885. [PubMed: 19860144]
- Lindqvist R, Bengtsson G. Dispersal dynamics of groundwater bacteria. *Microbial Ecology*. 1991; 21:49–72.
- Liu Y, Yang CH, Li J. Influence of extracellular polymeric substances on *Pseudomonas aeruginosa* transport and deposition profiles in porous media. *Environmental Science & Technology*. 2007; 41:198–205. [PubMed: 17265948]
- Luczkiewicz, A.; Jankowska, K.; Fudala-Ksiazek, S.; Olanczuk-Neyman, K. Antimicrobial resistance of fecal indicators in municipal wastewater treatment plant. *Water research*; 2010. (in press)
- Lutterodt G, Basnet M, Foppen JWA, Uhlenbrook S. The effect of surface characteristics on the transport of multiple *Escherichia coli* isolates in large scale columns of quartz sand. *Water Research*. 2009; 43:595–604. [PubMed: 19042002]
- Mccaulou DR, Bales RC, Mccarthy JF. Use of short-pulse experiments to study bacteria transport through porous-media. *Journal of Contaminant Hydrology*. 1994; 15:1–14.
- Ong YL, Razatos A, Georgiou G, Sharma MM. Adhesion forces between *E-coli* bacteria and biomaterial surfaces. *Langmuir*. 1999; 15:2719–2725.
- Parveen S, Lukasik J, Scott TM, Tamplin ML, Portier KM, Sheperd S, et al. Geographical variation in antibiotic resistance profiles of *Escherichia coli* isolated from swine, poultry, beef and dairy cattle farm water retention ponds in Florida. *Journal of Applied Microbiology*. 2006; 100:50–57. [PubMed: 16405684]
- Pembrey RS, Marshall KC, Schneider RP. Cell surface analysis techniques: what do cell preparation protocols do to cell surface properties? *Applied and Environmental Microbiology*. 1999; 65:2877–2894. [PubMed: 10388679]
- Rahe TM, Hagedorn C, McCoy EL, Kling GF. Transport of antibiotic-resistant *Escherichia coli* through Western Oregon Hillslope soils under conditions of saturated flow. *Journal of Environmental Quality*. 1978; 7:487–494.
- Ray KA, Warnick LD, Mitchell RM, Kaneene JB, Ruegg PL, Wells SJ, et al. Antimicrobial susceptibility of *Salmonella* from organic and conventional dairy farms. *Journal of Dairy Science*. 2006; 89:2038–2050. [PubMed: 16702267]
- Reinthal FF, Posch J, Feierl G, Wust G, Haas D, Ruckebauer G, et al. Antibiotic resistance of *E-coli* in sewage and sludge. *Water Research*. 2003; 37:1685–1690. [PubMed: 12697213]
- Rijal GK, Zmuda JT, Gore R, Abedin Z, Granato T, Kollias L, et al. Antibiotic resistant bacteria in wastewater processed by the metropolitan water reclamation district of greater Chicago system. *Water Science and Technology*. 2009; 59:2297–2304. [PubMed: 19542634]

- Rysz M, Alvarez PJJ. Transport of antibiotic-resistant bacteria and resistance-carrying plasmids through porous media. *Water Science and Technology*. 2006; 54:363–370. [PubMed: 17302340]
- Salmore AK, Hollis EJ, McLellan SL. Delineation of a chemical and biological signature for stormwater pollution in an urban river. *J Wat Health*. 2006; 4:247–262.
- Sato K, Bartlett PC, Kaneene JB, Downes FP. Comparison of prevalence and antimicrobial susceptibilities of *Campylobacter* spp. isolates from organic and conventional dairy herds in Wisconsin. *Applied and Environmental Microbiology*. 2004; 70:1442–1447. [PubMed: 15006764]
- US EPA. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. 2000. p. 52 <http://www.epa.gov/nerlcwww/RecManv.pdf>
- Varga C, Rajic A, McFall ME, Reid-Smith RJ, Deckert AE, Pearl DL, et al. Comparison of antimicrobial resistance in generic *Escherichia coli* and *Salmonella* spp. cultured from identical fecal samples in finishing swine. *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire*. 2008; 72:181–187. [PubMed: 18505208]
- Walczak JJ, Bardy SL, Feriencikova L, Xu S. Influence of tetracycline resistance on the transport of manure-derived *Escherichia coli* in saturated porous media. *Water Research*. 2011; 45:1681–1690. [PubMed: 21211813]
- Walker SL, Redman JA, Elimelech M. Role of cell surface lipopolysaccharides in *Escherichia coli* K12 adhesion and transport. *Langmuir*. 2004; 20:7736–7746. [PubMed: 15323526]
- Walker SL, Redman JA, Elimelech M. Influence of growth phase on bacterial deposition: Interaction mechanisms in packed-bed column and radial stagnation point flow systems. *Environmental Science & Technology*. 2005; 39:6405–6411. [PubMed: 16190193]
- Wang X, Quinn PJ. Lipopolysaccharide: Biosynthetic pathway and structure modification. *Progress in Lipid Research*. 2010; 49:97–107. [PubMed: 19815028]
- Weight, WD. *Hydrogeology field manual*. 2. New York: McGraw-Hill; 2008.
- World Health Organization. Geneva: 2003. 1st Joint FAO/OIE/WHO expert workshop on non-human antimicrobial usage and antimicrobial resistance: Scientific assessment. <http://www.who.int/foodsafety/publications/micro/en/amr.pdf>
- Xu CX, Lin XM, Ren HX, Zhang YL, Wang SY, Peng XX. Analysis of outer membrane proteome of *Escherichia coli* related to resistance to ampicillin and tetracycline. *Proteomics*. 2006; 6:462–473. [PubMed: 16372265]
- Zhang XQ, Bishop PL, Kinkle BK. Comparison of extraction methods for quantifying extracellular polymers in biofilms. *Water Science and Technology*. 1999; 39:211–218.

Fig. 1. PCR detection of *tetB* in the *E. coli* isolates. *Lane 1:* tet^S, *lane 2:* tet^R, *lane M:* 100 bp markers (New England Biolabs). The size of the *tetB* amplicon was 206 bp

Fig. 2. Breakthrough concentrations of the tet^R (a) and tet^S (b) *E. coli* strains in saturated porous media under ionic strength conditions of 1, 3, 10, 30, and 100 mM KCl. For clarity purpose, only one in five data points were shown

Fig. 3.

Deposition rate coefficients (K_d , per minute) for the two *E. coli* strains under ionic strength conditions of 1, 3, 10, 30, and 100 mM KCl. The values of K_d were calculated using Eq. 1. *Error bars* present standard deviation of duplicate experiments. Some error bars are smaller than the size of symbols

Fig. 4.

a Size (equivalent radius) of the bacterial cells suspended in 1, 3, 10, 30, and 100 mM of

KCl. The equivalent size was calculated as $\sqrt{\frac{L_C \times W_C}{\pi}}$, where L_C and W_C represent the length and width of the cell, respectively. *Error bars* represent the standard deviation of a minimum of 35 measurements. **b** Zeta potential of the quartz sand and *E. coli* cells. *Error bars* present the standard deviation of triplicate measurements; each measurement contained a minimum of five runs. **c** Results of MATH test that were expressed as the fraction of bacterial cells that partitioned into the hydrocarbon phase. *Error bars* present the standard deviation of triplicate measurements. **d** Comparison of the LPS contents of the tet^R and tet^S *E. coli* cells suspended in 1, 3, 10, 30, and 100 mM KCl. The *error bars* represent standard deviation of triplicate extraction attempts

Fig. 5.
a Protein contents of the *tet^R* and *tet^S* *E. coli* cells suspended in 1, 3, 10, 30, and 100 mM KCl. The *error bars* represent standard deviation of triplicate extraction attempts. **b** Outer membrane protein profiles of *tet^S* (lane 1) and *tet^R* (lane 2) strains. Molecular masses are shown on the *left*. The three proteins that are enriched in the *tet^R* strain are indicated with *arrows*

Table 1

Antibiotic resistance patterns of the tet^R and tet^S *E. coli* strains used for the transport experiments

<i>E. coli</i> isolate	Tetracycline		Ampicillin		Cephalothin		Gentamicin		Ciprofloxacin		Erythromycin		Sulfamethoxazole	
	4 ^a	16	8	32	8	32	4	16	1	4	3	15	38	152
tet ^R	+	+	+	-	+	+	-	-	-	-	+	+	-	-
tet ^S	-	-	+	-	+	+	-	-	-	-	+	+	-	-

^aThe concentrations of the antibiotics are expressed in milligrams per liter