

## **Rapid selection of antimicrobial resistant bacteria in complex water systems by chlorine and pipe materials**

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## **Abstract**

The presence of antimicrobial resistant bacteria and their genes in water supply system is an emerging issue. Resistant bacteria are difficult to treat and tend to horizontally transfer genes to other populations, including pathogens, thus complicating clinical treatments. However, the acute (short-term) response of bacteria to disinfection and exposure to various pipe materials remain unknown, and require investigation. Their responses to chlorination may mirror those of other microbial toxicants (e.g., subinhibitory antibiotics); as such, we test the hypothesis that pipe materials and the presence of chlorine could immediately stress bacteria and differentially select for antimicrobial resistant bacteria and biofilms.

Microcosm were created with polyvinylchloride, copper, and cement pipes, in which susceptible and resistant bacteria were competitively challenged to the presence of chlorination (0.5 mg/mL) over 5 days. Viable counts determined bacterial survival; while quantitative polymerase chain reaction was used to discern those with resistance genes. Physico-chemical parameters and chlorine levels monitored the environmental conditions to which the bacteria were exposed.

Results demonstrated that, in 56% of the cases, resistant bacteria became immediately enriched into biofilms due to chlorine exposure. In respects to pipe materials, greater proportion of resistant bacteria were found in biofilms on PVC and copper pipes, which represent common plumbing materials. The consequence is if bacteria contaminate drinking water systems, chemical conditions may immediately select for resistant strains, which could have unforeseen consequences in water safety.

## **Key words**

biofilm; chlorine; disinfectant; water supply, antibiotic resistant bacteria

# 1 **1. Introduction**

2 Antimicrobial resistance has emerged globally as an environmental problem, but it can cross  
3 into being a food/water issue (e.g., Rusu, et al. 2015; Daghrir & Drogui 2013). Water-  
4 treatment plants and distribution systems use filtration and disinfection to control  
5 microorganisms and maintain the potability of water (Morente et al. 2013). While generally  
6 effective, bacteria (including those resistant) invade water systems via line disruptions or  
7 compromised cisterns, which introduce additional bacterial hazards. One cannot fully  
8 eradicate antimicrobial-resistant bacteria at normal treatment dosages; rather, much higher  
9 dosages often become required. Unfortunately, higher concentrations produce odour and taste  
10 problems and are difficult to achieve and maintain in water. While disinfectants help  
11 attenuate bacteria, nonetheless, they can selectively cause robust bacteria to proliferate (e.g.,  
12 *Aeromonas*; McBain et al. 2004) and enrich antimicrobial-resistant bacteria.

13 The emergence, enrichment and transfer of resistance traits among bacteria depend on  
14 the microbial conditions in water distribution system. For example, biofilm-forming bacteria  
15 facilitate the attachment of microorganisms along the distribution system, and create  
16 community structures with greater complexity in terms of bacterial species than those in  
17 finished water (Chu et al. 2003), enhancing the capability to retain and protect pathogens.  
18 Furthermore, biofilm structures, with increased cell densities, facilitate the horizontal gene  
19 transfer of resistant genes. Moreover, bacterial physiology and metabolic rates vary along  
20 biofilm depth and reflect the gradients of nutrients versus chemical toxicants; the  
21 consequential reduced growth rates impacts biocide efficacy (Boe-Hansen et al. 2002).  
22 Protective layers of biofilm bacteria provide better tolerance to metal ions and other toxic  
23 substances, and improve survivability. Ultimately, biofilms in distribution systems become a  
24 generator and repository of resistance traits.

25 Conditions within water systems impact microorganisms and further contribute to the  
26 formation, composition, and proliferation of biofilms, e.g.: source water (Zeng et al. 2013),  
27 excretion of extracellular polymeric substances (Fish et al. 2015), physico-chemical  
28 conditions of water, hydraulic operations and flow rates (Douterelo et al. 2016), and  
29 composition of pipe materials (Nguyen et al. 2012).

30 Pipes comprise of various metals: galvanized iron, lead, copper, cast iron, steel  
31 (Nguyen et al. 2012); plastic materials: chlorinated polyvinyl chloride, polypropylene,  
32 polyethylene, polyvinylchloride and other materials: cementitious composites, such as concrete  
33 (Niquette et al. 2000); and elasto-materials. Pipe materials and biofilms differentially affect

34 each other; e.g., the presence of slime-forming bacteria facilitate metal tolerance, and biofilm  
35 bacteria produce organic acids, which contribute to the dissolution of metals and cement.  
36 Consequently, exposure to leached materials from pipes can enhance antimicrobial-  
37 resistance development, as frequently observed in contaminated environments in nature (e.g.,  
38 Rodgers et al. 2018; Abdu et al. 2017). Ultimately, disinfection strategies and surface  
39 exposures may effectively attenuate most bacteria in distribution networks (e.g., Dong et al.  
40 2018; Li et al. 2017), but could the stressors selectively contribute to resistance problem?

41 Here, we aimed to determine whether disinfectants and pipe material differentially  
42 affect bacteria with antimicrobial resistance. We employed experimental microcosms to  
43 examine how factors selectively influenced bacterial populations in a municipal water supply  
44 system, which included the formation of biofilms. These microcosms represented  
45 miniaturised analogues of actual distribution systems, and the bacteria were harvested from  
46 tap water; we hypothesized that different pipe material and disinfectant residuals impact  
47 microbial communities to select resistant bacteria and biofilm formation.

## 48 **2. Materials and methods**

### 49 **2.1 Microcosm set-up**

50 Experimental design involved laboratory microcosms with autoclaved phosphate  
51 buffer saline in Millipore water (10 mM, pH 7) having polyvinylchloride, copper and cement  
52 pipes (internal volume 102 cm<sup>3</sup>, surface area 240 cm<sup>2</sup>) versus presence/absence of free  
53 chlorine (0.5 mg/mL), as such six independent experiments were conducted. Peristaltic  
54 pumps (MP model M312) provided continuous recirculation (2 mL/min). Before each  
55 experiment, the microcosms, pipes and tubing were disinfected twice by 500 mg/L of sodium  
56 hypochlorite for 2 hours.

### 57 **2.2 Bacterial strains**

58 *Bacillus*, *Paenibacillus*, and *Micrococcus* were isolated from drinking water, except *Bacillus*  
59 *subtilis* R2 (the National Collection of Type Cultures, NCTC 10400, UK). The presence of  
60 antibiotic resistant genes (*sul1* and *sul2*), mobile genetic elements (*intI1*) and chlorine  
61 susceptibility were determined as described previously (Khan et al. 2016a,b). The paired  
62 microcosm assays (chlorinated and chlorine free) were run with two populations of each  
63 genus having different level of resistance; *sul1* (*Bacillus* and *Paenibacillus*), *sul2* and *intI1*  
64 (*Micrococcus*).

## 65 **2.3 Experimental operation**

66 Overnight culture in 50-mL Luria-Bertani broth was centrifuged 3x (3000 rpm, 10 min),  
67 washed with phosphate buffer saline, added (final concentration  $1 \times 10^5$  cell/mL) to  
68 microcosms, and recirculated for five days at 20 °C ( $\pm 2$ ), with 1-mL harvested from each  
69 pipe on days 0, 3 and 5 for viable counts (Nutrient Agar). Biofilm samples were taken on day  
70 5 from 4-cm<sup>2</sup> area using sterile swabs; and 50-mL water were filtered through 0.22  $\mu$ m  
71 membrane filter (Millipore, UK) on days 0 and 5. Filters and swabs were stored at -20 °C. An  
72 additional 30-50-mL were collected on days 3 and 5 to measure temperature, pH (Model S40  
73 SevenMulti™, Mettler Toledo™), dissolved oxygen (DO 200 meter, VWR), total organic  
74 carbon (Teledyne Tekmar Apollo 9000 Combustion TOC Analyzer), and free chlorine (N, N-  
75 diethyl-p-phenylenediamine colorimetric method, Hach).

## 76 **2.4 DNA extraction and quantitative polymerase chain reaction**

77 DNA were extracted using the ISOLATE II Genomic DNA kit (Bioline, UK) per  
78 manufacturer's instructions. Quantitative polymerase chain reaction (qPCR) were used to  
79 enumerate *Bacillus* (DeClerck, et al. 2004), *Paenibacillus* (Pettersson, et al. 1999), and  
80 *Micrococcus* species (Walcott and Gitaitis, 2000). qPCR involves the amplification (copying)  
81 of specific DNA sequences in a manner with timing of fluorescent detection to count the  
82 number of genes in a sample. Strains were discerned with primers associated with their  
83 antibiotic resistance genes (*sul1*, *sul2*; Pei et al. 2006) or mobile genetic element (*int11*; Luo,  
84 et al. 2010); 16S-rRNA genes were quantified as a surrogate measure of 'total bacteria'.  
85 Serially diluted DNA standards ( $10^8$ - $10^2$  genes/mL) and a control without DNA template  
86 were run with each qPCR assay.

## 87 **2.5 Data Analyses**

88 From qPCR data, we calculated %resistance [resistance genes]/[total genus] and bacterial  
89 abundances on pipe surfaces and water. Fold increases in total bacteria and %resistance were  
90 calculated by comparing abundances between days 0 and 5. Abundances were log-  
91 transformed for presentation and statistical (e.g., normality) purposes. Data were analysed by  
92 Excel (Microsoft Office 2010) and MiniTab version 17.

## 93 **3. Results and discussion**

### 94 **3.1 Abundances of bacteria in biofilm**

95 Microbial populations responded almost immediately by attaching to surfaces, with a greater  
96 percentage of those having resistant traits—suggesting that conditions in drinking water can  
97 select for bacterial contaminants with antimicrobial resistance. Surface swabs on day 5 for  
98 biofilm formation indicated that in 2/3 of the cases bacteria adhered themselves onto  
99 surfaces (Figure 1). Bacterial viability declines under stressful conditions, which resulted in  
100 difference in plate (viability) and genetic counts; however, the likely cause for declining gene  
101 counts in the water is the surface-attachment of cells, which would escape detection in the  
102 water. One exception was *Bacillus*, where only 35% of total bacteria and 65% of the resistant  
103 strain were found in biofilms in the polyvinylchloride microcosms. Further, only the resistant  
104 strains of *Micrococcus* and *Paenibacillus* were found on copper surfaces. Percentages of total  
105 and resistant populations were equivalent for other treatments.

106 Here, up to  $10^{6.5}$  cfu/cm<sup>2</sup> were found on surfaces in 5 days; more than reported previously  
107 (i.e.,  $10^5$  cfu/cm<sup>2</sup>; Frias et al. 2001). Bacteria showed different biofilm potentials  
108 *Paenibacillus* > *Bacillus* > *Micrococcus* (Figure 1) depending on pipe materials. Reported  
109 trend is as follow: polyvinylchloride < copper < steel < iron < cement < plastic (e.g.  
110 polyethylene) < elastomeric surfaces (Yu et al. 2010), with some variation in adhesion  
111 potentials (Niquette et al. 2000). Different in this study, polyvinylchloride and copper pipes  
112 allowed more bacterial attachment with chlorine. Biofilm formation was faster on plastic  
113 pipes than copper pipes, due to copper toxicity (Yu et al. 2010); however in this experiment,  
114 phosphate levels may have confounded effects of copper toxicity.

### 115 **3.2 Water-borne resistant and total populations**

116 Each pair of bacteria was added (totalling  $2 \times 10^5$  cfu/mL, log 5.3) into the microcosms;  
117 heterotrophic plate counts determined viabilities (Table 1). In unchlorinated systems, viable-  
118 cell counts declined by various orders of magnitude, except *Micrococcus*, which showed  
119 greater viability between days 3-5 in polyvinylchloride and copper pipes. Chlorination  
120 compromised viabilities by 1-2 orders greater reduction than untreated systems (Table 1). No  
121 viable *Micrococcus* were recovered from any chlorinated microcosm on day 3; although  
122 *Paenibacillus* and *Micrococcus* showed some recovery by day 5 in copper and  
123 polyvinylchloride pipes, respectively.

124 Both the susceptible and resistant strains were simultaneously challenged in the  
125 microcosms and monitored by quantitative PCR to differentiate strains. Their presence in  
126 water declined across all treatments (Table 2), except for *Paenibacillus* and *Micrococcus* spp.  
127 in the polyvinylchloride and cement treatments. In 44.4% of the cases, the resistant strains

128 also declined, but at a lesser rate—resulting in mixed populations skewed towards a greater  
129 proportion of resistant bacteria.

130 Viability counts (Table 1) and gene detection (non-viable) are not analogous. Genetic  
131 (resistance and 16S-rRNA) traits indicate the presence of bacteria in the system; whereas  
132 plate counts indicate whether bacteria are capable of growing. Although not growing, their  
133 presence in the water (even with chlorine) could increase the prevalence of resistant genes if  
134 conditions change.

135 Low concentrations of disinfectants exert selection pressure on resistant populations,  
136 but other driving force i.e. pipe materials showed differential selection patterns in  
137 *Micrococcus* species (Table 1). Further, pipe materials impacted bacterial concentrations;  
138 e.g., polyvinylchloride pipes had greatest abundances of bacteria; and copper, the least, which  
139 may be attributed to its antimicrobial effects. Depending on the bacteria, resistant populations  
140 also proliferated in circulating water e.g., *Micrococcus* and *Paenibacillus* (Table 2).

### 141 **3.2 Water chemistry**

142 Water conditions remained similar: 3.3 mg/L dissolved oxygen ( $\pm 0.7$ , 95% confidence  
143 intervals);  $87.4 \pm 58.4$  mg/L total organic carbon; and initial chlorine 0.50 mg/L, but declined  
144 to 0.15 ( $\pm 0.06$ ) and 0.06 ( $\pm 0.03$ ) on days 3 and 5, respectively. Initial pHs were 7.6 ( $\pm 0.1$ );  
145 however, elevated pHs were observed in copper ( $9.4 \pm 1.8$ ) and cement microcosms ( $9.0 \pm$   
146 1.3). Neither pH nor dissolved oxygen were impacted by inoculations or chlorination.

147 The dissolved oxygen and total organic carbon were within World Health Organization  
148 guidelines for drinking water. Dissolved oxygen influences corrosion in water pipes and  
149 bacterial activity, while the organic carbon increases chlorine demand and microbial growth.  
150 Here, any residual organic carbon likely reduced the free chlorine concentration as observed.

### 151 **3.4 Relationship between physicochemical properties, abundances of genes, and** 152 **bacteria**

153 Comparison of population abundances and viabilities to the physico-chemical conditions  
154 (Table 3) indicated that dissolved oxygen increased bacterial abundances, and might have an  
155 inverse impact on relative abundance of resistant strains (though significant in *Paenibacillus*  
156 assays only). Occasional correlations occurred between organic carbon and bacterial  
157 abundances. Further, elevated pH reduced total bacteria, which were observed by inverse  
158 correlations in absolute abundances, and positive correlations in relative numbers—suggesting  
159 that presence of resistant strains were less impacted. There were no significant correlations

160 between viable counts and resistant gene abundances, nor chlorine and resistant genes; while  
161 interesting, this does not surprise as gene presence does not represent viability, as discussed  
162 previously.

#### 163 **4. Conclusion**

164 This study demonstrated the factors affecting the fate of antimicrobial resistance in water-  
165 distribution systems; pipe material, residual disinfectant, and water age shift community  
166 dynamics. Resistant bacteria developed biofilm on polyvinylchloride and copper pipes  
167 (66.7%) more than cement pipes (16.7%). Biofilm-forming bacteria behave differently than  
168 planktonic cells, and due to physiological adaptations, they exhibit greater resistance to  
169 disinfectants as 55.6% cases showed more resistant population in biofilm in the presence of  
170 chlorine. In term of total population more bacteria were in biofilm  $10^{6.5}$  cfu/mL. Further,  
171 increased population densities and selective pressures by sub-inhibitory concentrations of  
172 disinfectants and pipes create conditions, underwhich microorganisms acquire new traits and  
173 become more resistant.

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#### 177 **References**

- 178 Abdu N, Abdullahi AA, Abdulkadir A (2017) Heavy metals and soil microbes. *Environ Chem Lett*  
179 15(1): 65-84. doi:10.1007/s10311-016-0587-x
- 180 Boe-Hansen R, Albrechtsen H-J, Arvin E, Jorgensen C (2002) Bulk water phase and biofilm growth  
181 in drinking water at low nutrient conditions. *Water Res* 36: 4477-4486.
- 182 Chu C, Lu C, Lee C, Tsai C (2003) Effects of chlorine level on the growth of biofilm in water pipes. *J*  
183 *Environ Sci Health Part A*, 38(7): 1377-1388. doi:10.1081/ese-120021132
- 184 Daghrir R, Drogui P (2013) Tetracycline antibiotics in the environment: a review. *Environ Chem Lett*.  
185 11(3): 209-227. doi.org/10.1007/s10311-013-0404-8
- 186 DeClerck E, Van Mol K, Jannes G, Rossau R, De Vos P (2004) Design of a 5' exonuclease-based  
187 real-time PCR assay for simultaneous detection of *Bacillus licheniformis*, members of the '*B.*  
188 *cereus* group' and *B. fumarioli* in gelatine. *Lett Appl Microbiol* 39 (1):109-115.  
189 doi:10.1111/j.1472-765X.2004.01550.x
- 190 Dong F, Li C, Lin Q, Duan H (2018) Effect of pipe materials on disinfection by-products and  
191 bacterial communities during sulfamethazine chlorination in a pilot-scale water distribution  
192 system. *Environ Chem Lett*. doi:10.1007/s10311-018-00823-3



193 Douterelo I, Jackson M, Solomon C, Boxall J (2016) Microbial analysis of in situ biofilm formation in  
194 drinking water distribution systems: implications for monitoring and control of drinking water  
195 quality. *Appl Microbiol Biotechnol* 100(7): 3301-3311. doi:10.1007/s00253-015-7155-3

196 Fish KE, Collins R, Green NH, Sharpe RL, Douterelo I, Osborn AM, Boxall JB (2015)  
197 Characterisation of the physical composition and microbial community structure of biofilms  
198 within a model full-scale drinking water distribution system. *PLoSOne* 10(2): e0115824.  
199 doi:10.1371/journal.pone.0115824

200 Frias J, Ribas F, Lucena F (2001) Effects of different nutrients on bacterial growth in a pilot  
201 distribution system. *Antonie van Leeuwenhoek* 80: 129-138.  
202 <https://doi.org/10.1023/A:1012229503589>

203 Khan S, Beattie TK, Knapp CW (2016a) Relationship between antibiotic- and disinfectant-resistance  
204 profiles in bacteria harvested from tap water. *Chemosphere* 152: 132-141.  
205 doi:10.1016/j.chemosphere.2016.02.086

206 Khan S, Knapp CW, Beattie TK (2016b) Antibiotic resistant bacteria found in municipal drinking  
207 water. *Environ Process* 3: 541-552. doi:10.1007/s40710-016-0149-z

208 Li C, Feilong D, Feng L, Zhao J, Zhang T, Cizmas L, Sharma VK (2017) Bacterial community  
209 structure and microorganism inactivation following water treatment with ferrate(VI) or  
210 chlorine. *Environ Chem Lett* 15(3): 525-530

211 Luo Y, Mao D, Rysz M, Zhou Q, Zhang H, Xu L, Alvarez PJJ (2010) Trends in antibiotic resistance  
212 genes occurrence in the Haihe River, China. *Environ Sci Technol* 44 (19):7220-7225.  
213 doi:DOI: 10.1021/es100233w

214 McBain AJ, Ledder RG, Moore LE, Catrenich CE, Gilbert P (2004) Effects of quaternary-  
215 ammonium-based formulations on bacterial community dynamics and antimicrobial  
216 susceptibility. *Appl Environ Microbiol* 70(6), 3449-3456. doi:10.1128/AEM. 70.6.3449-  
217 3456.2004

218 Morente OE, Fernandez-Fuentes MA, Grande Burgos MJ, Abriouel H, Perez Pulido R, Galvez A  
219 (2013) Biocide tolerance in bacteria. *Int J Food Microbiol* 162(1): 13-25.  
220 doi:10.1016/j.ijfoodmicro.2012.12.028

221 Nguyen C, Elfland C, Edwards M (2012) Impact of advanced water conservation features and new  
222 copper pipe on rapid chloramine decay and microbial regrowth. *Water Res* 46(3): 611-621.  
223 doi:10.1016/j.watres.2011.11.006

224 Niquette P, Servails P, Savoir R (2000) Impacts of pipe materials on densities of fixed bacterial  
225 biomass in a drinking water distribution system. *Water Res* 34(6): 1952-1956. DOI:  
226 10.1016/S0043-1354(99)00307-3

227 Pei R, Kim S-C, Carlson KH, Pruden A (2006) Effect of river landscape on the sediment  
228 concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Water*  
229 *Research* 40 (12):2427-2435. doi:10.1016/j.watres.2006.04.017

230 Pettersson B, Rippere KE, Yousten AA, Priest FG (1999) Transfer of *Bacillus lentimorbus* and  
231 *Bacillus popilliae* to the genus *Paenibacillus* with emended descriptions of *Paenibacillus*  
232 *lentimorbus* comb. nov. and *Paenibacillus popilliae* comb. nov. Int J Sys Bacteriol 49 (531-  
233 540)

234 Rodgers K, McLellan I, Peshkur T, Williams R, Tonner R, Hursthouse AS, Knapp CW, Henriquez FL  
235 (2018) Can the legacy of industrial pollution influence antimicrobial resistance in estuarine  
236 sediments? Environ Chem Lett. doi.org/10.1007/s10311-018-0791-y

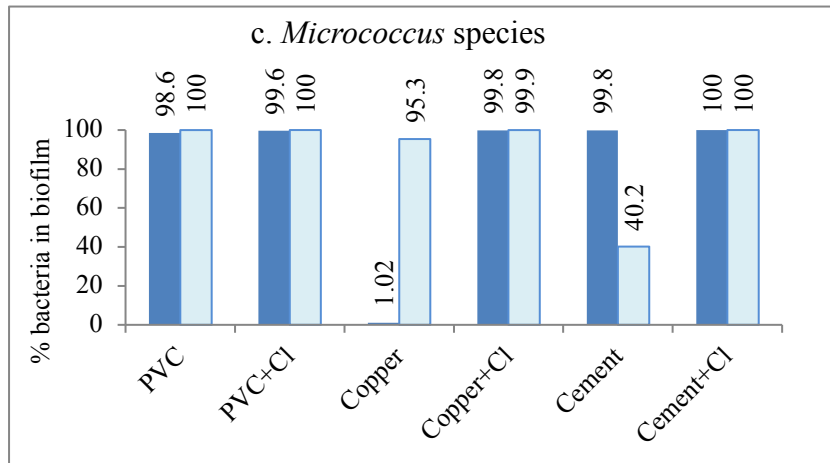
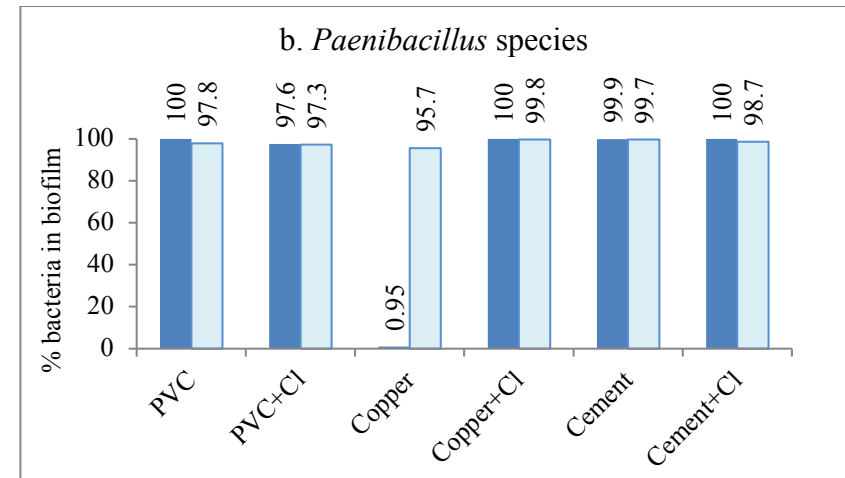
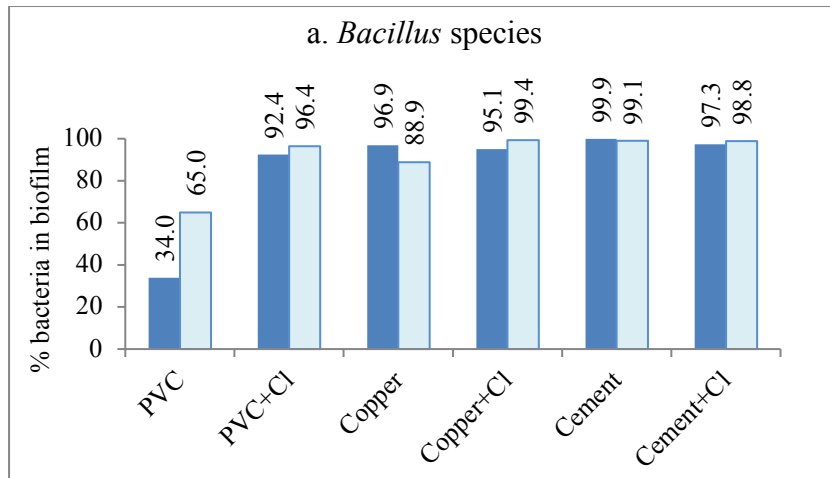
237 Rusu A, Hancu G, Uivarosi V (2015) Fluoroquinolone pollution of food, water and soil, and  
238 bacterial resistance. Environ Chem Lett 13: 21. doi.org/10.1007/s10311-014-0481-3

239 Walcott RR, Gitaitis RD (2000) Detection of *Acidovorax avenae* subsp. citrulli in watermelon seed  
240 using immunomagnetic separation and the polymerase chain reaction. Plant Disease 84  
241 (4):470-474. http://dx.doi.org/10.1094/PDIS.2000.84.4.470

242 Yu J, Kim D, Lee T (2010) Microbial diversity in biofilms on water distribution pipes of different  
243 materials. Water Sci Technol 61(1): 163-171. doi:10.2166/wst.2010.813

244 Zeng DN, Fan ZY, Chi L, Wang X, Qu WD, Quan ZX (2013) Analysis of the bacterial communities  
245 associated with different drinking water treatment processes. World J Microbiol Biotechnol  
246 29(9): 1573-1584. doi:10.1007/s11274-013-1321-5

247



**Figure 1.** Percentage of bacteria attached to surfaces after 5 days. Chlorination selection was found in resistant bacteria (*Bacillus* R1 and *Paenibacillus* R), which were higher in chlorinated pipes than non-chlorinated in all cases. Cl = chlorinated, PVC = polyvinylchloride. : ■ Total bacteria ▨ Resistant bacteria

**Table 1.** Heterotrophic Plate Count (log cfu/mL) in the different pipe material in the absence and presence of 0.5 mg/L free chlorine; each species was added on Day 0 at a concentration of 10<sup>5</sup> cfu/mL (log total bacteria = 5.3). cfu colony forming units, ND not detected

Treatment	<i>Bacillus</i>		<i>Paenibacillus</i>		<i>Micrococcus</i>	
	Day-3	Day-5	Day-3	Day-5	Day-3	Day-5
Polyvinyl chloride	2.9	2.0	3.2	1.5	1.6	2.8
Polyvinyl chloride+Chlorine	ND	ND	1.2	0.5	ND	1.9
Copper	2.8	1.2	3.3	2.0	0.9	2.8
Copper+Chlorine	0.3	ND	1.2	1.7	ND	ND
Cement	2.9	2.6	3.5	2.4	3.5	3.0
Cement+Chlorine	ND	ND	1.4	0.7	ND	ND

**Table 2.** Changes in total and resistant bacterial abundances after 5 days in microcosms as determined by polymerase chain reaction. + increase, - decrease, 0 unchanged, ND = not detected, Cl = chlorine, PVC = polyvinylchloride.

Assay	Organism Type	PVC	PVC + Cl	Copper	Copper + Cl	Cement	Cement + Cl
<i>Bacillus</i>	Total <i>Bacillus</i>	-	-	-	-	-	-
	% Resistant	-	-	+	-	-	-
<i>Paenibacillus</i>	Total <i>Paenibacillus</i>	ND	+	-	-	-	ND
	% Resistant	+	+	0	-	-	0
<i>Micrococcus</i>	Total <i>Micrococcus</i>	-	-	-	-	ND	ND
	% Resistant	+	-	+	+	+	-

**Table 3.** Pearson correlation analysis between physicochemical properties and bacterial abundances. Significant limit was  $p < 0.10$ . DO = dissolved oxygen, TOC = total organic carbon, Cl = chlorine, HPC = heterotrophic plate count (log transformed).

Assay	Genes	Pearson correlation	Water					Biofilm				
			DO	TOC	Cl	pH	HPC	DO	TOC	Cl	pH	HPC
<i>Bacillus</i>	<i>Bacillus</i> R1/ total bacteria	<i>r</i> = value	-0.17	0.03	-0.60	0.54	0.28	0.43	-0.50	-0.59	<b>0.95</b>	-0.23
		<i>p</i> value	0.69	0.95	0.12	0.17	0.51	0.39	0.31	0.91	<b>0.00</b>	0.66
	Total bacteria	<i>r</i> = value	<b>0.63</b>	-0.23	0.32	-0.55	0.20	-0.39	-0.00	0.29	-0.67	0.38
		<i>p</i> value	<b>0.10</b>	0.59	0.44	0.15	0.63	0.45	0.99	0.58	0.14	0.46
<i>Paenibacillus</i>	<i>Paenibacillus</i> R/ total bacteria	<i>r</i> = value	<b>-0.89</b>	<b>0.78</b>	0.01	<b>0.82</b>	-0.10	-0.10	0.15	0.34	0.43	0.33
		<i>p</i> value	<b>0.02</b>	<b>0.07</b>	0.95	<b>0.05</b>	0.33	0.87	0.81	0.57	0.47	0.59
	Total bacteria	<i>r</i> = value	<b>0.88</b>	<b>-0.78</b>	-0.04	-0.81	0.06	-0.09	-0.12	-0.51	-0.35	-0.19
		<i>p</i> value	<b>0.02</b>	<b>0.07</b>	0.94	0.05	0.92	0.89	0.84	0.38	0.57	0.76
<i>Micrococcus</i>	<i>Micrococcus</i> S/ total bacteria	<i>r</i> = value	-0.31	0.33	-0.17	<b>0.85</b>	-0.23	0.63	0.31	0.01	-0.32	-0.42
		<i>p</i> value	0.50	0.43	0.72	<b>0.02</b>	0.62	0.18	0.55	0.99	0.54	0.71
	Total bacteria	<i>r</i> = value	0.46	-0.30	0.20	<b>-0.88</b>	0.12	0.05	<b>-0.82</b>	0.72	-0.47	-0.53
		<i>p</i> value	0.29	0.51	0.67	<b>0.01</b>	0.80	0.93	<b>0.04</b>	0.11	0.35	0.28