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Concomitant Antibiotic and Mercury Resistance Among Gastrointestinal Microflora of Feral Brook Trout, *Salvelinus fontinalis*

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Abstract

Twenty-nine bacterial isolates representing eight genera from the gastrointestinal tracts of feral brook trout *Salvelinus fontinalis* (Mitchell) demonstrated multiple maximal antibiotic resistances and concomitant broad-spectrum mercury (Hg) resistance. Equivalent viable plate counts on tryptic soy agar supplemented with either 0 or 25 μM HgCl_2 verified the ubiquity of mercury resistance in this microbial environment. Mercury levels in lake water samples measured 1.5 ng L^{-1} ; mercury concentrations in fish filets ranged from 81.8 to 1,080 ng g^{-1} and correlated with fish length. The presence of similar antibiotic and Hg resistance patterns in multiple genera of gastrointestinal microflora supports a growing body of research that multiple selective genes can be transferred horizontally in the presence of an unrelated individual selective pressure. We present data that bioaccumulation of non-point source Hg pollution could be a selective pressure to accumulate both antibiotic and Hg resistant bacteria.

Introduction

Mercury is pervasive in the environment, however, many bacterial species can adapt to its presence by invoking efficient Hg-detoxification systems [8, 24]. The most common bacterial Hg resistance mechanism is reduction of mercuric ion [Hg(II)] to the elemental form [Hg(0)] catalyzed by the enzyme mercuric reductase encoded by the gene *merA* [8]. Bacteria may also contain the gene *merB* that encodes organomercurial lyase, which catalyzes the detoxification of organomercuric compounds. The genes *merA* and *merB* are components of the *mer* operon, which is frequently associated with mobile genetic elements, including transposable elements and plasmids. Therefore, Hg resistance is readily transferable among bacterial species by horizontal gene transfer (HGT) mechanisms [8, 27, 41]. The *mer* operon is closely linked genetically with antibiotic resistance genes [27, 46], and antibiotic resistance determinants are frequently organized in gene cassette systems that contain genes conferring resistance to a wide variety of antibiotics [36].

Antibiotic resistance gene transfer is a commonly observed phenomenon in antibiotic-replete environments such as hospitals, aquaculture, and agriculture [4, 16]. However, antibiotic resistance determinants are also disseminated throughout bacterial communities in environments lacking antibiotics [4, 7]. One proposed mechanism for the persistence of antibiotic resistance in environments devoid of antibiotics is via co-resistance. Co-resistance occurs when the genes encoding resistance phenotypes are linked in close proximity on the same mobile genetic element [7]. This physical linkage allows for the co-selection of other genes located on the same mobile genetic element. Associations between heavy metal exposure and specific patterns of antibiotic resistance in bacteria have been reported [26, 27, 40].

Linkage between bacterial Hg resistance and antibiotic resistance has been documented in a wide range of habitats, including oral and fecal microbial flora of primates [46], oral microbial flora of patients with Hg amalgam dental fillings [43], fish gastrointestinal tracts [2], mine sediments [30], and freshwater microcosms [40]. Although all of the above studies on co-selection of metal and antibiotic resistance involve environments either contaminated with toxic metals or were experimental studies in which metal exposure was directly manipulated to test for co-selection in bacterial communities, we reported recently that co-selection of Hg and antibiotic resistance was observed in bacterial strains isolated in the natural environment of a sphagnum bog [45]. We found that this environment contained sufficient levels of Hg to exert selective pressure on indigenous sphagnum bog bacteria to produce the mercury and antibiotic resistance phenotype. Furthermore, bacterial strains were isolated from bog core samples dating back 2,000 years before present (ybp), i.e., significantly before the era of antimicrobial chemotherapy [45].

These bacterial isolates harboring mobile genetic elements that encode *mer* operons are often shown to be associated with antibiotic resistance gene cassettes [25]. For example, we have reported that the multiple drug- and mercury resistance phenotype was co-transferable via conjugation from *Aeromonas salmonicida* subsp. *salmonicida* AS03 to various gram-negative recipient bacteria, and that antibiotic resistance cassettes and the *mer* operon were closely linked physically on a conjugative IncA/C plasmid [27].

The expanding antibiotic resistome threatens both public health and agricultural yields [12, 16]. Once present in a biological system, resistance determinants seldom remain restricted to a single organism. HGT permits efficient exchange of mobile genetic elements between unrelated species, genera, and even domains [9, 14]. Commensal bacterial populations are notorious reservoirs of antibiotic resistance determinants due to their long-term biological stability [20, 21, 23]. The gastrointestinal tract microenvironment, a common habitat for

commensal flora, is an ideal environment for the spread of resistance genes via HGT given its temporal stability, abundant species diversity and high bacterial density [39, 42]. Consequently, antibiotic resistance determinants may be perpetuated and disseminated in this gene reservoir.

Mercury contamination in northeastern North America is inordinately high. Biological mercury hotspots have been identified in this continental region, and they have received particular attention due to both human and ecological effects of this toxic metal [1, 13]. Among these identified hotspots are the western lakes region of the upper Androscoggin and Kennebec Rivers, Maine, USA, where water columns contain total mercury levels of ~ 1.5 ng L⁻¹ [13]. Oligotrophic, pristine lake systems such as these are frequently associated with increased methylmercury accumulation [11]. Hence, the possibility exists that antibiotic resistant bacteria are being propagated in these environments in the presence of indirect selection forces like non-point source mercury pollution.

Piscivorous adult brook trout are top trophic food chain predators in oligotrophic Maine waters. To correlate tissue mercury levels and mercury-resistant bacteria in the brook trout gastrointestinal tract, feral brook trout were collected. Our results suggest that Hg exposure may indirectly enhance antibiotic resistance and horizontal spread through the gut commensal bacterial community.

Materials and Methods

Subjects

Nine brook trout were trap-netted by Maine State Fisheries biologists from Kennebago Lake, Maine, USA (45°07'N, 71°11'W) and euthanized using MS 222 [6]. Trap-net sampling was done in three periods over 2 years in August 2004 (FBT1), September 2005 (FBT3), and October 2005 (FBT4). Fish length was measured. FBT1 samples represented pooled tissues from three individual fish.

Microbial Sample Collection

Within 2 h of euthanasia, the gut from each trout was extracted aseptically. Ingesta and gut epithelial scrapings were collected and weighed. Gastrointestinal samples from three fish collected in August 2004 (FBT1) were pooled. Serial dilutions of ingesta material in sterile phosphate buffer (Hardy Diagnostics, Santa Maria, CA, USA) were inoculated on tryptic soy agar (TSA) plating media (Difco, Sparks, MD, USA) or TSA amended with 25 μ M HgCl₂. All isolations were performed from TSA plating media following incubation at 22 °C for 24–48 h. In experiments comparing mercury-susceptible to mercury-resistant culturable populations, viable plate counts from suspensions of gut and ingesta in phosphate buffer were plated in triplicate.

Mercury Analysis

Seven 1 g filet samples were analyzed for total mercury content (Frontier Geosciences, Seattle, WA, USA). Samples were homogenized and digested in a 70:30 mixture of nitric and sulfuric acids and mercury concentrations were determined using cold vapor atomic fluorescence spectrometry.

Bacterial Isolate Identification

Following genomic DNA extraction (E.Z.N.A., Omega Bio-tek, Inc., Doraville, GA, USA), 29 randomly selected isolates were identified based on 16S rRNA gene [28] sequence similarities (BLASTn against GenBank at <http://www.ncbi.nlm.nih.gov/BLAST>) [5]. Forward and reverse sequencing of a 174–200 bp hypervariable region of the 16S rRNA

gene was accomplished using the Big Dye Terminator Reaction Kit (Applied Biosystems, Foster City, CA, USA) and an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). API 20E strips (BioMérieux, Marcy-L'Etoile, France) were used to classify an isolate in the event of multiple equally scored BLASTn results and were run in parallel with quality control strain *Escherichia coli* ATCC 25922 as recommended by the manufacturer.

Antibiotic and Mercury Minimum Inhibitory Concentration Assays

The antibiotic minimal inhibitory concentration (MIC) values for each isolate were determined on Sensititre® dried susceptibility panels MJ (gram-negative) and GPN2F (gram-positive) (Trek Diagnostic Systems, Westlake, OH, USA). Quality control strains *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* ATCC 29212 were inoculated in parallel to the isolates as suggested by the manufacturer. Other than incubating MIC panels for 4 days at 22 °C, the guidelines set by the National Committee for Clinical Laboratory Standards were followed [29]. A complete table of all of the antibiotics tested from MJ and GPN2F panels is provided in the supplementary tables.

The isolates' Hg MIC values on both inorganic HgCl₂ and organic phenylmercuric acetate (PMA) were determined by published methods using TSA plating media solidified with Noble agar (Difco Laboratories, Sparks, MD, USA) [44]. Bacterial suspensions (4.0 µl, 2.0 McFarland standard) of fresh (24–48 h) solid plating media cultures in sterile, demineralized water were inoculated on TSA Hg MIC plating media and incubated for 4 days at 22 °C. Two control strains, mercury-susceptible *Bacillus subtilis* 168 (Bacillus Genetics Stock Center) and mercury-resistant *E. coli* SK1592, were inoculated alongside the isolates [15].

Amplification of *merA* in Bacterial Isolates

Genotypic confirmation of inorganic mercury resistance was accomplished with PCR amplification of *merA*. Amplification of an 288-bp *merA* fragment was also done using primers A1s.F and A5-n.R described by [32].

Nucleotide Sequence Accession Numbers

Sequences for all isolates have been deposited in the GenBank database and have been assigned the following nucleotide accession numbers: partial 16S rRNA sequences DQ530318–DQ530346; partial *merA* sequences DQ530313–DQ530317.

Statistical Analysis

All data were tested for normality with the Shapiro–Wilk *W* test. Parametric CFU data were then analyzed statistically using a paired two-tailed *t* test ($P = 0.22$). Mercury levels in fish filets were compared to known water concentrations and the published average concentration of fish filets in the Northeastern North America. The degree of association between antibiotic and mercury resistance was measured using correlation.

Results

Mercury concentrations in trout filets ranged from 81.8 to 1,080 ng g⁻¹ with a median of 235 ng g⁻¹ (Table 1). Data were not normally distributed (Shapiro–Wilks test $W = 0.7878$; $P = 0.0310$). Mean mercury concentrations in brook trout filets of northeastern North America are ~200 ng g⁻¹ [19]. Wilcoxon Signed Ranks test comparing the sample median to Kamman et al. [19], revealed no statistical difference. The filet mercury levels had an exponential correlation with the length of the fish as suggested by al-Hashimi and al-Zoerba [3] (Fig. 1).

Viable plate counts on TSA ranged from 4.30×10^7 to 1.31×10^9 CFU g⁻¹ with a mean of 5.2×10^8 CFU g⁻¹ (Table 1). Data were normally distributed (Shapiro–Wilks test $W = 0.9271$; $P = 0.5268$). Viable plate counts on TSA amended with 25 μ M HgCl₂ ranged from 4.08×10^7 to 8.92×10^8 CFU g⁻¹ with a mean of 3.79×10^8 CFU g⁻¹. Data were normally distributed (Shapiro–Wilks test $W = 0.9037$; $P = 0.3540$). There was no significant difference between the two growth conditions by paired two-tailed t test ($P = 0.22$). Comparison of the total number of culturable bacteria for each fish versus the number of mercury-resistant culturable bacteria by paired t test demonstrated statistical difference ($P = 0.016$) in only one fish (09.05B); the other five fish showed no significant difference in the number of bacteria grown in the two plating conditions.

Twenty-nine mercury-resistant culturable bacteria were randomly chosen and characterized from nine trap-netted *Salvelinus fontinalis* gastrointestinal samples over a 2-year period. The isolates represented seven gram-negative genera and one gram-positive genus (Table 2). Many of these genera, including *Aeromonas*, *Carnobacterium*, *Pseudomonas*, and *Shewanella*, are documented commensal bacteria of salmonid gut [10, 17, 33]. The six *Carnobacterium* spp. isolates have identical partial 16S rRNA gene sequences suggesting that these isolates are of the same strain (Table 2c), although they were isolated from different feral brook trout at different times. Three of the *Aeromonas* spp. (group 2: FBT3-5, FBT4-3, and FBT4-5) were identical based on the partial 16S rRNA gene sequence obtained; these isolates also came from fish collected at two different samplings. Two other pairs, *Hafnia* FBT4-2 and FBT4-11, and *Serratia* FBT3-1 and FBT3-16, also have the same 16S rRNA gene sequence but came from the same sampling and therefore may just be duplicate isolates. The other isolates have unique partial 16S rRNA gene sequences that suggest these represent different bacterial isolates.

Inorganic mercury (HgCl₂) and organic mercury (PMA) MIC assays performed on the 29 bacterial isolates demonstrated inorganic mercury MIC of 100–250 μ M and an organic mercury MIC of 8 to >16 μ M (Table 2). These results classify all of the bacterial isolates as having “broad-spectrum” mercury resistance and putatively contain both *merA* and *merB* genes [8].

The antibiotic MIC values for each isolate were determined on Sensititre® dried susceptibility panels MJ (gram-negative) and GPN2F (gram-positive) (Trek Diagnostic Systems, Westlake, OH, USA). *Pseudomonas* FBT4-23 demonstrated the greatest antibiotic resistance with maximal resistance to 14 of the 22 tested antibiotics (Table 2b), whereas *Shewanella* FBT3-6 represented the most susceptible strain with maximal resistance to only one antibiotic (Table 2b). All isolates exhibited at least low-level resistance to multiple antibiotics, particularly against the penicillin and cephem families. Most gram-negative isolates also demonstrated considerable resistances to β -lactam/ β -lactamase inhibitor combinations, as well as tetracycline, and sulfisoxazole. *Carnobacterium* isolates all exhibited maximal resistance to ceftriaxone, clindamycin, and gentamicin, and, unlike the majority of the gram-negative isolates, did not demonstrate tetracycline resistance (Table 2c). All of the *Carnobacterium* isolates appear to be identical based on the partial 16S rRNA gene sequencing, suggesting that these might be independent isolates of the same bacterial strain. However, there is some difference in the resistance profile of the bacteria, which may indicate subtle differences between individual isolates.

merA, the gene that encodes mercuric reductase, conveys Hg(II)-resistance via reduction of Hg(II) to volatile Hg(0). Genotypic confirmation of mercury resistance was accomplished with PCR amplification of a segment of the *merA* gene. A short 288 bp segment was identified in five *Serratia* isolates, while the remaining isolates did not amplify a product using these *merA* primers. Though it is likely the remaining isolates also carry *merA* based

on phenotypic observation of mercury resistance, the high diversity of *merA* confounds efforts to design a primer set to universally amplify the gene [32].

Serratia merA fragments were sequenced using both amplification primers, and BLASTn searches of these sequences produced significant alignments with known *merA*. The partial *merA* sequences obtained from four isolates showed similarity to reported plasmid-borne *mer* operons and each showed ~90 % nucleic acid identity to each other (data not shown). The partial *merA* sequence from FBT1-21 shows ~90 % nucleic acid identity to plasmid pNAL21202 from *Nitrosomonas* sp. (Genbank accession CP002554). Sequences obtained from FBT1-21 and FBT1-23 show ~84 % nucleotide identity to plasmid pMATVIM-7 isolated from *P. aeruginosa* (Genbank accession AM778842). Sequences obtained for FBT3-8 and FBT3-16 appear related to a multiple-metal resistance plasmid pMOL30 identified in *Cupriavidis metallidurans* CH34. All four of the sequences that match plasmid-borne *mer* operons are ~84–91 % identical to *Comomonas testosteroni* plasmid pI2 (Genbank accession JF274989).

Isolate FBT3-1 shows a partial *merA* sequence similarity to chromosomal *mer* operons identified in *Achromobacter* sp. AO22 (Genbank accession EU969790) and *P. aeruginosa* PA7 (Genbank CP000744). This isolate also showed similar sequence identity to two *merA* sequences identified from floodplain soil (*Variovorax* sp. Is-D287 and *Cupriavidus* sp. IS-D310, Genbank accession EF455072 and EF455069, respectively).

Discussion

The feral brook trout collected show a bioaccumulation of mercury within muscle tissue in an exponential relationship relative to overall fish length (Fig. 1). This is similar to the results observed by al-Hashimi and al-Zoerba [3]. The apparent bioaccumulation of mercury in the fish tissue (range of 81.8–1,080 ng g⁻¹) from the surrounding water (1.5 ng L⁻¹) is in agreement with the concentration factors of 5,000–100,000 reported by others [18, 38].

Simultaneous bacterial resistance to both ionic and organic mercury species, a phenotype known as broad-spectrum mercury resistance suggests the isolates have been exposed to some form of mercury [8]. High concentrations of mercury in the fish gastrointestinal tract are likely due to the diet of brook trout, including smaller fish and crayfish in which methylmercury accumulation has been documented [11, 19, 35]. Furthermore, viable plate counts on 0 and 25 µM HgCl₂ TSA demonstrated no significant difference in culturable bacteria between the two growth conditions by paired *t* test in five of six fish samples (Table 1). These data suggest that the presence of mercury resistance among the culturable gut bacteria is nearly, if not totally, ubiquitous.

Efforts to determine the mercury resistance mechanism resulted in the identification of five *Serratia* isolates with *merA*. Four *Serratia* spp. isolates showed partial *merA* sequences similar to plasmid-borne *merA* from *P. aeruginosa*, *C. testosteroni*, and *C. metallidurans* that all share an organization of the *mer* operon of *merRTPADE* [22]. Isolate FBT3-1 shows a *merA* sequence with similarity to putative chromosomal *merRTPADE* operons of *Achromobacter* sp. and *P. aeruginosa* PA7 [31, 34, 37]. The inability to amplify *merA* from the remaining isolates may be the result of divergent *merA* sequences that have evolved [8, 32] or could represent a novel mechanism of resistance.

Numerous strains demonstrated organomercurial resistance, including the five *Serratia* isolates from which *merA* (inorganic mercury resistance) was amplified and sequenced. While *merB* was not assayed in our experiments, it is interesting to note that the closest matches based on *merA* sequences identified are to plasmids or chromosomal *mer* operons of the *merRTPADE* conformation, with no indication of the presence of *merB* in these

operons. This does not address the presence of *merB* within the *Serratia* isolates, but suggests that if present, there would be a different transposon arrangement that would include *merB*.

Antibiotic resistance patterns within the isolates do not show one common antibiotic resistance phenotype among all of the isolates. The one *Pseudomonas* sp. isolate (FBT4-23) shows maximal resistance to 14 of 22 antibiotics tested while the two *Shewanella* isolates (FBT3-6 and 4-19) show overall few maximal antibiotic resistances (one and three, respectively). Overall, the gram-negative isolates showed resistance to different cell wall synthesis inhibitor drugs tested, including maximum resistance to ampicillin (17), ampicillin/sulbactam (15), cefazolin (16), and cephalothin (19) (Table 2a). Only the *Hafnia* sp. isolates showed resistance to protein synthesis inhibitors tetracycline and nitrofurantoin (Table 2a, Supplemental Table 1a). No gram-negative bacteria showed resistance to DNA synthesis inhibitors. All of the *Carnobacterium* sp. isolates, which based on partial 16S rRNA sequencing appear to be identical, demonstrated maximal resistance to the cell wall synthesis inhibitors oxacillin +2 % NaCl and ceftriaxone and the protein synthesis inhibitors clindamycin and gentamycin (Table 2c). These *Carnobacterium* spp. isolates may be duplicates of one isolate, although there are two from each fish harvest and subtle differences in the antibiotic resistance profile (Table 2c; Supplemental Table 1c), suggesting that there are a minimum of three individual *Carnobacterium* spp. evaluated with this research.

The advent of widespread antibiotic resistance threatens to further complicate medical treatment and diminish agricultural revenues in the future. Though curbing antibiotic usage will slow the dissemination of these resistance determinants, the presence of antibiotic resistance in the absence of the selective pressure of antibiotics suggests other agents are able to indirectly select for its proliferation. This phenomenon in trout gut microflora is possibly the result of mercury contamination given the established correlation between antibiotic resistance and Hg resistance, and Maine's elevated levels of atmospheric mercury deposition. It appears, based on the *merA* sequencing of the five isolates and the overall antibiotic resistance patterns, that HGT would not be occurring from one donor, but may be more restricted within the individual fish gut microflora.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Exponential correlation between fish length (cm) and total file Hg concentration (ng g⁻¹). An exponential curve fit was applied to the data and the regression value is shown

Table 1

Viable plate counts, fish filet mercury concentration and length of fish sampled over three periods and 2 years

Fish	TSA plates with HgCl ₂		Total Hg (ng g ⁻¹ muscle)	Length (cm)
	0 μ M (CFU g ⁻¹)	25 μ M (CFU g ⁻¹)		
08.04A	ND	ND	1,010 ^a	ND
09.05A	9.14E + 08	8.92E + 08	110	17.0
09.05B	1.31E + 09	5.75E + 08	137	20.9
09.05C	1.54E + 08	1.09E + 08	108	18.2
09.05D	4.90E + 08	4.82E + 08	81.8	16.3
10.05A	7.24E + 08	5.57E + 08	1,080	39.5
10.05B	4.30E + 07	4.08E + 07	235	26.0

^aPooled muscle from three individual fish

Table 2Hg MIC (μM) and Antibiotic MIC ($\mu\text{g mL}^{-1}$) of Feral Brook Trout Bacterial Isolates

Species	HgCl ₂ (μM)	PMA (μM)	Maximum resistances ^a
A. Enterobacteriaceae			
<i>Citrobacter</i> spp.			
FBT4-6	100	>16	amp, aug, faz, cep, fox
FBT4-10	100	>16	cep, fis
FBT4-12	100	>16	cep, fis
<i>Ewingella</i> sp.			
FBT1-15	100	>16	amp, aug, faz, cep, fur
<i>Hafnia</i> spp.			
FBT3-17	250	>16	amp, a/s, aug, faz, cep, tet, fis
FBT4-2	250	>16	cep, tet, cot, fis
FBT4-11	250	>16	amp, a/s, aug, faz, cep, fur, tet, cot, fis
FBT4-22	250	>16	amp, a/s, aug, faz, cep, tet, fis
<i>Serratia</i> spp.			
FBT1-8	100	>16	amp, faz, cep, fox, fur, tet, nit
FBT1-21	250	>16	amp, a/s, aug, faz, cep, axo, fur
FBT1-23	100	>16	amp, a/s, aug, faz, cep, axo, fur, fis
FBT3-1	250	>16	amp, mez, a/s, faz, cep, axo, fur
FBT3-8	250	>16	amp, mez, pip, a/s, faz, cep, axo, fur
FBT3-16	100	>16	amp, pip, a/s, aug, faz, cep, axo, fur
FBT4-19	100	>16	amp, a/s, aug, faz, cep, axo, fur, fis
B. Other gram-negative bacterial isolates			
<i>Aeromonas</i> spp.			
FBT3-2	100	16	amp, a/s
FBT3-5	250	>16	amp, a/s, tim, faz, cep, fis
FBT4-3	250	8	amp, a/s, faz, cep, fis
FBT4-5	100	>16	amp, a/s
FBT4-21	100	>16	amp, a/s, aug, faz, cep, fis
<i>Pseudomonas</i> sp.			
FBT4-23	100	>16	amp, mez, a/s, tim, aug, faz, cep, fox, taz, axo, fur, nit, cot, fis
<i>Shewanella</i> spp.			
FBT3-6	100	16	fis
FBT4-9	100	>16	faz, cep, fis
C. Gram-positive bacterial isolates			
<i>Carnobacterium</i> spp.			
FBT1-19	100	8	amp, pen, oxa, axo, cli, gen
FBT1-22	100	16	oxa, axo, cli, gen
FBT3-9	100	16	oxa, axo, cli, gen
FBT3-14	100	8	oxa, axo, cli, gen
FBT4-1	100	>16	amp, pen, oxa, axo, cli, gen, cip

Species	HgCl ₂ (μM)	PMA (μM)	Maximum resistances ^a
FBT4-18	100	8	oxa, axo, cli, gen

^a *a/s* ampicillin/sulbactam, *amp* ampicillin, *aug* amoxicillin/clavulanic acid, *axo* ceftriaxone, *cep* cephalothin, *cip* ciprofloxacin, *cli* clindamycin, *cot* trimethoprim/sulfamethoxazole, *faz* cefazolin, *fis* sulfisoxazole, *fox* ceftiofur, *fur* cefuroxime, *gen* gentamicin, *mez* mezlocillin, *nit* nitrofurantoin, *oxa* oxacillin +2 % NaCl, *pen* penicillin, *pip* piperacillin, *taz* ceftazidime, *tet* tetracycline, *tim* ticarcillin/clavulanic acid

For concentrations used, refer to Supplementary Tables 1 and 2