Impact of wave action and rainfall on incidence and antibiotic resistance of total coliforms in Southern California beaches
Rebecca Hernandez, Ismael Acedo and Jesse G. Dillon

ABSTRACT

Coliforms are important bacterial contamination indicators in recreational waters. Little is known about the antibiotic resistance of coliforms from Southern California beaches. This study examined the numbers of coliforms as well as the incidence of antibiotic-resistant coliforms in beaches with restricted and non-restricted wave action by sampling from the shores of both types of beaches following dry and wet weather. Total coliforms were selected by membrane filtration onto mEndo agar and then enumerated. Randomly selected isolates from each location were screened for resistance to nine classes of antibiotics by disk diffusion, and the multiple antibiotic resistance (MAR) index was calculated. Numbers of total coliforms were significantly higher following rain compared to dry weather. Total coliform numbers were not significantly elevated at non-restricted wave action sites. Restricted wave action sites had a 78.5% increase in MAR index following wet weather compared to dry weather. Resistance to ampicillin was observed in almost 50% of isolates and was not significantly impacted by wave action or weather. Minimum inhibitory concentration testing revealed that many isolates were highly resistant to ampicillin. This study is the first to report on the antibiotic resistance of coliforms found in Southern California beaches and highlights the prevalence of ampicillin resistance.

Key words | ampicillin, beaches, minimum inhibitory concentration, multiple antibiotic resistance, total coliforms

HIGHLIGHTS

- Total coliforms increased after rain and were higher at protected beaches.
- MAR index was higher at protected beaches following rainfall.
- Only 3.1% of total coliforms were multiple antibiotic resistant.
- 45.3% of total coliforms were resistant to ampicillin, many up to 128 μg ml⁻¹.

INTRODUCTION

Antibiotic resistance is becoming a pressing public health issue, and we are in danger of entering a post-antibiotic era (World Health Organization 2014). While antibiotic resistance is very common in clinical settings due to the overuse and inappropriate prescription of antibiotics (Ventola 2015), antibiotic resistance primarily evolved in natural environments (Martínez 2008); however, the degree of antibiotic resistance in these settings is less well studied.
Since aquatic environments harbor a diverse array of bacteria and are often impacted by contaminants, these settings are important reservoirs for antibiotic resistance (Taylor et al. 2011). Antibiotic-resistant coliforms have been isolated from a variety of aquatic locations, including rivers (Chitanand et al. 2010; Middleton & Salierno 2013; Kumar et al. 2018; Sanderson et al. 2018) and seawater (Cooke 1976; Rabbia et al. 2016; Hernandez et al. 2019).

Conjugation, transformation, and transduction (all means of the horizontal transfer of genes, such as those encoding antibiotic resistance) occur in aquatic settings (Taylor et al. 2011). Antibiotic-resistant pathogens found in marine environments pose a poorly characterized public health danger (Salyers et al. 2004).

In recreational beaches such as those found in Southern California, fecal indicator bacteria (FIB), such as coliforms, are assayed by local water treatment agencies to test water quality (Boehm et al. 2002). Coliforms can be divided into two subgroups: total coliforms (found in both fecal matter and soil) and fecal coliforms (found in fecal matter; includes fewer species than the total coliform group) (Mishra et al. 2018). Most coliforms are non-pathogenic; however, their presence indicates fecal pollution and the potential for the presence of pathogens (Harmon et al. 2014). Antibiotic resistance poses a potential challenge in the treatment of environment acquired infections; however, antibiotic resistance is not screened for in agency assays.

Many factors, including wave action and rain, impact bacterial levels in marine environments and may also affect the level of antibiotic resistance. The dilution of bacteria in water is partly controlled by wave action (Boehm 2003). Low wave action sites, such as those where water circulation is reduced by a breakwater or harbor, are often associated with elevated bacterial levels as well as other pollutants (Zanoni et al. 1978; McLellan et al. 2007; Li et al. 2014). Higher numbers of genetic markers for antibiotic resistance were found in polluted water compared to unpolluted water (Carnelli et al. 2017). Another study (Lin et al. 2004) reported a significantly higher proportion of enteric bacteria (including coliforms) isolated from a polluted river location as resistant to specific antibiotics compared to enteric bacteria isolated from a non-polluted river. Rain affects bacterial water quality and leads to higher levels of fecal pollution (World Health Organization 2003) due to stormwater runoff and fecal matter washing into the water (Reeves et al. 2004). Recent studies (Zhang et al. 2016; Sanderson et al. 2018) have shown that rain is associated with higher incidences of antibiotic-resistant bacteria.

Despite the risks to public health associated with fecal contamination at public beaches, especially after rainfall events when contamination of public beaches is most common, and the large amount of resources dedicated to routine water quality testing, to our knowledge, there have been no studies investigating antibiotic-resistant coliforms in seawater in Southern California. Many studies have addressed antibiotic resistance in fecal coliforms or Escherichia coli at other sites (Middleton & Salierno 2013; Dhiman et al. 2016; Rabbia et al. 2016; Carnelli et al. 2017; Kumar et al. 2018; Sanderson et al. 2018; Hernandez et al. 2019), but few have focused on total coliforms (Cooke 1976; Chitanand et al. 2010; Azzam et al. 2019). By examining total coliforms, we were able to more broadly investigate the incidence of coliform antibiotic resistance. Studies addressing this will help identify when and where antibiotic-resistant coliforms are most prevalent and determine the degree of multidrug resistance at local beaches. The objectives of this study were to compare the impact of rainfall and exposure to wave action on the abundance and antibiotic resistance profiles of total coliforms isolated from Southern California beaches.

**METHODS**

**Sample collection**

Seawater samples were collected by hand in sterile 500 ml Nalgene bottles in ankle-deep water from three restricted wave action sites: Cabrillo Beach (inside the San Pedro breakwater), Mother’s Beach in Long Beach (inside Alamitos Bay), and Mother’s Beach in Newport Beach (inside Newport Harbor), as well as from three non-restricted wave action sites: Cabrillo Beach (outside the San Pedro breakwater; no significant river input), Seal Beach (open coastline; 170 m away from the San Gabriel River mouth), and Newport Beach (open coastline; 7.9 km away from the Santa Ana River; Supplemental Figure S1). Samples were kept on ice during transport back to the laboratory and processed...
Cultivation and enumeration of total coliforms

Water samples were vacuum filtered in aliquots of 0.5, 5, 20, 50, and 75 ml onto a sterile membrane filter with a 47 mm diameter and 0.45 μm pore size (Eaton et al. 1999) following swirling to ensure even distribution of bacteria. To account for high bacterial concentrations, wet weather samples diluted to 10⁻¹ and 10⁻²; 0.1 and 1 ml were added to the sterile membrane filter from each dilution. Following filtration, the filter unit was rinsed with 100 ml sterile phosphate buffer solution, and the filter was removed using alcohol-flamed forceps, rolled across 7 ml mEndo agar (Oxoid) in 50 mm Petri plates, and then incubated at 35 °C for 20–22 h (Eaton et al. 1999). E. coli was used as a positive control for total coliforms, Pseudomonas aeruginosa was used as a non-coliform control, and a blank plate that only received sterile phosphate buffer solution was included as a sterility control. Total coliforms were enumerated using standard methods (Eaton et al. 1999).

Antibiotic resistance testing

Twenty coliform colonies (where possible) were randomly selected from each site and streaked three times to ensure purity. The Clinical and Laboratory Standards Institute (CLSI) disk diffusion guidelines (Clinical and Laboratory Standards Institute 2012) were used to test isolates for antibiotic resistance on Mueller-Hinton agar plates.

For each isolate, a direct colony suspension was prepared using a McFarland standard, as described above, diluted 1:10 (~10⁴ CFU ml⁻¹) in Mueller-Hinton Broth and two μl of the 1:10 dilution were pipetted onto Mueller-Hinton Agar plates containing 0, 8, 16, 32, 64, and 128 μg ml⁻¹ (Andrews 2001; Clinical and Laboratory Standards Institute 2016). Triplicate plates for each treatment were incubated at 35 °C for 24 h and observed for growth. The lowest concentration at which no growth was observed was recorded as the minimum inhibitory concentration (MIC).

Statistical analyses

All statistical assessments were run in Minitab (v. 18). Total coliforms 100 ml⁻¹ data, MAR indices, and the proportion of isolates resistant to each antibiotic were first evaluated for equal variances and normality using residuals versus fits plots and normal probability plots. Colony-forming units 100 ml⁻¹ data were log₁₀ transformed. MAR indices and the proportion of isolates resistant to each antibiotic data did not need to be transformed. Split-plot analysis of variances (ANOVAs) were run to compare variance among mean total coliform CFU 100 ml⁻¹ as well as MAR indices across weather type (wet versus dry) and wave action (restricted versus non-restricted). The whole plot factor was wave action (restricted or non-restricted wave action), and the split unit factor was weather (a given site...
included both wet and dry samples). To more closely inspect the effects of rain among non-restricted wave action sites, a two-way ANOVA was performed to compare mean total coliform CFU 100 ml\(^{-1}\) across weather type and sites (Cabrillo Beach outside the breakwater, Seal Beach, and Newport Beach). Two-sample \(t\) tests were used to compare the mean proportion of isolates resistant to each antibiotic following wet and dry weather as well as the mean proportion of isolates resistant to each antibiotic isolated from restricted and non-restricted wave action sites.

MIC data were scaled from 1 to 6 with 8 \(\mu\)g ml\(^{-1}\) – 1, 16 \(\mu\)g ml\(^{-1}\) – 2, 32 \(\mu\)g ml\(^{-1}\) – 3, 64 \(\mu\)g ml\(^{-1}\) – 4, 128 \(\mu\)g ml\(^{-1}\) – 5, and >128 \(\mu\)g ml\(^{-1}\) – 6. Scaled MICs were assessed for equal variances and normality via a residuals versus fits plot and normal probability plot. A Mann–Whitney U test was used to compare scaled MICs for isolates obtained following wet and dry weather and from restricted and non-restricted wave action sites.

**RESULTS**

**Total coliform enumeration**

Total coliform counts were significantly higher following wet weather compared to dry weather (\(F = 18.49, p = < 0.001\)). However, despite an apparent increase in mean numbers in restricted sites (Figure 1), there was no significant difference between sites with restricted and non-restricted wave action (\(F = 1.85, p = 0.245\)), nor a significant interaction term (\(F = 0.71, p = 0.404\)). However, when specific sites and time points were examined, some interesting observations relating to the impacts of wet weather were found (Figure 1). Seal Beach (a non-restricted wave action site) had higher numbers of total coliforms compared to other non-restricted wave action sites following wet weather (Figure 1; 126.3% average difference from Cabrillo Beach outside the breakwater; 200.0% average difference from Newport Beach). The two-way ANOVA comparing mean total coliforms across weather type and sites, among non-restricted wave action sites only, revealed that there was a significant interaction between weather and site (\(F = 6.55, p = 0.005\)). Wet weather at Cabrillo Beach outside the breakwater was associated with a 34.0% increase in total coliforms, and wet weather at Newport Beach was associated with a 27.3% decrease in total coliforms (Figure 1). At Seal Beach, wet weather resulted in a 109.9% increase in total coliforms compared to dry weather, showing that out of the three non-restricted wave action sites, Seal Beach is most impacted by rainfall. Notably, the highest numbers of total coliforms following wet weather were seen during the first wet sampling date at all sites (data not shown). Newport Beach consistently had the lowest number of total coliforms following both weather types (Figure 1).
MAR indices

Neither wave action nor weather had a significant effect on MAR index ($F = 0.35, p = 0.587$; $F = 2.26, p = 0.139$, respectively). However, there was a significant interaction between wave and weather on MAR index ($F = 4.25, p = 0.044$). Wet weather was associated with a 78.5% higher MAR index compared to dry weather at restricted wave action sites, whereas at non-restricted wave action sites, wet weather MAR index was 8.5% lower than dry weather MAR index (Figure 2). Following dry weather, Cabrillo Beach outside the breakwater had the highest overall MAR index (average MAR index = 0.099276), while Mother’s Beach in Newport Beach had the highest overall MAR index following wet weather (average MAR index = 0.1257).

Antibiotic resistance

As previously mentioned, some sites (especially Newport Beach) regularly had lower numbers of total coliforms, preventing us from consistently analyzing 20 isolates from each site every time. This resulted in a total of 1,035 isolates tested against antibiotics. Resistance was observed against all nine antibiotics tested; however, ampicillin resistance was by far the most common (Table 1). Overall, 469 isolates (out of 1,035 total isolates; 45.3%) were resistant to ampicillin. An intermediate number of isolates were resistant to nitrofurantoin, tetracycline, cefotaxime, and sulfamethoxazole-trimethoprim, while resistance to meropenem, chloramphenicol, amikacin, and ciprofloxacin was very rare (Table 1). There were no significant differences in the proportion of bacteria resistant to any antibiotic following wet versus dry weather. There also were not any significant differences in the proportion of resistant isolates from restricted versus non-restricted wave action sites to any antibiotics. MAR (defined as resistance to three or more antibiotic classes) was uncommon with only 32 of 1,035 isolates (3.1%) in this category. Sixty-nine were resistant to two antibiotics, while the highest number (414) were resistant to only a single antibiotic tested here.

Ampicillin MIC

Because of the obvious importance of ampicillin resistance in our coastal total coliform isolates, we determined the degree of resistance via the MIC method for isolates showing ampicillin resistance. Generally, very high levels of ampicillin resistance were observed at all sites with >50% of isolates resistant to at least 32 μg ml$^{-1}$ (Figure 3). While some variation in MIC was observed, no clear patterns in ampicillin resistance of isolates was seen based on wet versus dry weather ($W = 14,648; p = 0.721$) or restricted versus non-restricted comparisons ($W = 14,297; p = 0.757$). Overall, 27.9% of isolates had an ampicillin MIC of >128 μg ml$^{-1}$.

Figure 2 | MAR indices at all sites (classified as non-restricted or restricted wave action) following dry and wet weather. Error bars show 95% confidence intervals based on individual standard deviations. N = 5 for each weather type at each site. CO, Cabrillo Beach outside breakwater; NB, Newport Beach; SB, Seal Beach; CI, Cabrillo Beach inside breakwater; LB, Mother’s Beach in Long Beach; and MB, Mother’s Beach in Newport Beach.
DISCUSSION

Consistent with prior studies, total coliforms were significantly elevated following rain compared to dry weather (Boehm et al. 2002; Reeves et al. 2004; McLellan et al. 2007; Griffith et al. 2010; Harmon et al. 2014). During rainfall events, rain washes pollutants (including fecal matter) into aquatic environments (Boehm et al. 2002; Reeves et al. 2004), resulting in increased bacterial levels. Counts (CFU ml⁻¹) obtained in the current study differed from past studies (both higher and lower), even those conducted in Southern California. This is not unexpected, as FIB have been shown to vary over time and are impacted by multiple factors (Boehm et al. 2002).

While mean total coliforms were elevated at restricted wave action sites, they were not significantly higher compared to non-restricted wave action sites. This was surprising, given that past studies have found higher levels of bacteria in restricted wave action sites (Zanoni et al. 1978; Kim et al. 2007; McLellan et al. 2007). This discrepancy could be due to different methodologies targeting different bacterial types in those studies and that they were performed in freshwater habitats instead of the ocean. We specifically enumerated total coliforms, while McLellan et al. (2007) only enumerated *E. coli*, and Kim et al. (2007) enumerated heterotrophic bacteria.

There was a significant interaction between weather and site on total coliforms 100 ml⁻¹ among the three non-restricted wave action sites with Seal Beach showing higher numbers of total coliforms following wet weather. This could be because Seal Beach is adjacent to the mouth of the San Gabriel River, which receives input from 689 square miles of Los Angeles County; the lower watershed has especially impaired water quality (California State Water Resources Control Board 2019), whereas the other sites are not near river inputs.

As expected, the highest numbers of total coliforms were observed following the first rainfall event of the season. The so-called ‘first flush’, after prolonged dry weather, can bring high levels of accumulated debris/bacteria into the ocean from upper watershed regions, as well as from land sources (Ackerman & Weisberg 2005). Another study in Southern California (Griffith et al. 2010) reported that FIB are highest in the ocean after early season storms compared to late season storms.

One of the key questions we asked in this study was what is the incidence of MAR in coliforms at Southern California beaches. To our knowledge, this is the first study to address this question in North American coastal beaches. Our values were even less than the 8% of *E. coli* isolates from Antarctic seawater impacted by treated wastewater
Figure 3 | Number of isolates at each location (in dry and wet weather) growing on plates containing ampicillin concentrations ranging from 0 to 128 μg ml⁻¹.
effluent reported as multiple antibiotic resistant (Hernandez et al. 2019).

Sites with a MAR index greater than 0.2 are considered potentially hazardous to human health (Krumpelman 1985). It is reassuring that only one sample in the current study (Wet Sample 3 from the restricted wave action Mother’s Beach in Newport Beach) had a MAR index above 0.2. Consistent with the low incidence of MAR, the MAR indices found in this study were low compared to other studies. The MAR indices of total coliforms from contaminated Indian rivers up to 0.43 were reported by Chitanand et al. (2010). E. coli from water draining into the Nile River had MAR indices ranging from 0.4 to 0.51 (Azzam et al. 2019). In the United States, Dhiman et al. (2016) found fecal coliforms isolated along a river in Washington, DC had a MAR index of 0.07, while those collected from untreated sewage overflow had a MAR index of 0.36. Thus, our values are more similar to less impacted US sites.

Despite our lower overall MAR values, rainfall events did result in elevated MAR indices at restricted wave action sites. Previous studies have reported increased antibiotic resistance in rivers and lakes after rain (Zhang et al. 2016; Sanderson et al. 2018). Other studies have shown that polluted water contains many antibiotic-resistant bacteria (Lin et al. 2004; Chitanand et al. 2010; Carnelli et al. 2017). Rain results in increased levels of pollutants, especially in restricted wave action sites. As coliforms have a short life-span (hours to days) in seawater (Fujioka et al. 1988; Statham & McMeekin 1994), it is likely that antibiotic-resistant coliforms from more polluted sources washed into restricted wave action sites during wet weather via runoff.

Although MAR was rare in this study, ampicillin resistance was found in almost half of all isolates, including both dry and wet, and restricted and non-restricted isolates, indicating that ampicillin resistance is widespread among Southern California coliforms. The prevalence of ampicillin resistance has been found in other habitats as well. Multiple studies conducted on the antibiotic resistance of E. coli from Antarctic seawater have reported that resistance to ampicillin was most common (Rabia et al. 2016; Hernandez et al. 2019). Additionally, most E. coli isolated in the Washington DC area river study were resistant to ampicillin (Dhiman et al. 2016).

Interestingly, the level of ampicillin resistance determined by MIC was very high in all locations in our study with the majority of isolates resistant to at least 32 μg ml⁻¹. MIC values were not significantly elevated following wet weather; however, wet weather isolates generally had higher mean ampicillin MICs compared to dry isolates. This suggests that total coliforms may be resistant to ampicillin at higher levels following wet weather; however, it is likely that this could not be detected statistically due to a lack of power in our design. Past studies have also reported high ampicillin MIC values. Ash Mauck & Morgan (2002) reported that ampicillin MIC of Gram-negative bacteria from multiple rivers in the United States was greater than 256 μg ml⁻¹ for 98% of isolates. MICs of fecal coliforms from Washington, DC rivers were also high, some values reaching up to 1,000 μg ml⁻¹ (Dhiman et al. 2016).

At this point, we do not have a clear understanding of why ampicillin resistance is so much more common than other classes of antibiotics we tested, although ampicillin is a commonly used broad-spectrum penicillin class drug that has been in use since the 1940s. These antibiotics work by binding to penicillin-binding proteins, which inhibits cross-linking of peptidoglycan layers in the bacterial cell wall and results in cell death (Kaushik et al. 2014). Since penicillin class antibiotics are unstable in aquatic environments, aquatic total coliforms are likely not developing ampicillin resistance due to aquatic exposure to ampicillin. Resistant bacteria are likely being washed into aquatic sources from other environments that could have higher levels of ampicillin.

The most common resistance mechanism to ampicillin is the production of beta-lactamase, a bacterial enzyme that hydrolyzes the beta-lactam ring of these antibiotics, rendering them ineffective (Livermore 1995). Another possible resistance mechanism is the use of efflux pumps. These pumps are commonly utilized by Pseudomonas species; however, they have also been reported in the coliform bacteria E. coli and Klebsiella pneumoniae (Cag et al. 2016). The specific mechanism of the resistance of our beach isolates should be examined in future studies.

CONCLUSIONS

As shown in previous studies, total coliform values were highest following rainfall. This study was the first to examine the antibiotic resistance of total coliforms in Southern
California beaches. MAR was surprisingly uncommon in this study, which is a positive finding for public health. However, resistance to ampicillin was found in almost half of all isolates. This is concerning, given that ampicillin is used to treat many bacterial infections. Additional studies identifying the specific resistance mechanisms (e.g. beta-lactamase, efflux pumps, and R-factors) as well as determining the susceptibility of total coliform isolates to ampicillin combination drugs are needed. Overall, our study highlights the importance of avoiding recreational water activities following wet weather and points to the need for future work investigating antibiotic resistance in coliforms from beaches. Additional investigations into the incidence of antibiotic resistance in non-coliform bacterial groups including native marine strains are also warranted.

ACKNOWLEDGEMENTS

We want to acknowledge Dr Erika Holland and Dr Bruno Pernet for providing helpful suggestions and feedback on the experimental design and writing of this manuscript and to Dr Bengt Allen for advice with statistical analyses. Funding for this project was provided by the CSU Council on Ocean Affairs, Science and Technology award CSUCOAST-HERREB-CSULB-AY1718 (to R.H.) and the National Institute of General Medical Sciences of the National Institutes of Health under award numbers UL1GM118979, TL4GM118980, and RL5GM118978 (to I.A./J.D.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Ackerman, D. & Weisberg, S. B. 2003 Relationship between rainfall and beach bacterial concentrations on Santa Monica Bay beaches. *Journal of Water and Health* 1, 85–89.

Andrews, J. M. 2001 Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy* 48, 5–16.

Ash, R. J., Mauck, B. & Morgan, M. 2002 Antibiotic resistance of Gram-negative bacteria in rivers, United States. *Emerging Infectious Diseases* 8, 713–716.

Azzam, M. I., Ezzat, S. M., Othman, B. A. & El-Dougdoug, K. A. 2019 Antibiotics resistance phenomenon and virulence ability in bacteria from water environment. *Water Science* 31, 109–121.

Boehm, A. B. 2003 Model of microbial transport and inactivation in the surf zone and application to field measurements of total coliform in North Orange County, California. *Environmental Science & Technology* 37, 5511–5517.

Boehm, A. B., Grant, S. B., Kim, J. H., Mowbray, S. L., McGee, C. D., Clark, C. D., Foley, D. M. & Wellman, D. E. 2002 Decadal and shorter period variability of surf zone water quality at Huntington Beach, California. *Environmental Science & Technology* 36, 3885–3892.

Cag, Y., Caskurlu, H., Fan, Y., Cao, B. & Vahaboglu, H. 2016 Resistance mechanisms. *Annals of Translational Medicine* 4, 326.

California State Water Resources Control Board 2019 San Gabriel River Watershed. California Water Boards. https://www.waterboards.ca.gov/rwqcb4/water_issues/programs/regional_program/Water_Quality_and_Watersheds/san_gabriel_river_watershed/summary.shtml (accessed 20 January 2019).

Carnelli, A., Mauri, F. & Demarta, A. 2017 Characterization of genetic determinants involved in antibiotic resistance in *Aeromonas* spp. and fecal coliforms isolated from different aquatic environments. *Research in Microbiology* 168 (5), 461–571.

Chitanand, M. P., Kadam, T. A., Gyananath, G., Totewad, N. D. & Balhal, D. K. 2010 Multiple antibiotic resistance indexing of coliforms to identify high risk contamination sites in aquatic environment. *Indian Journal of Microbiology* 50, 216–220.

Clinical and Laboratory Standards Institute 2012 Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard, 11th edn. CLSI Document M02-A11, Wayne, PA.

Clinical and Laboratory Standards Institute 2016 Performance Standards for Antimicrobial Susceptibility Testing. CLSI Supplement M100S, Wayne, PA.

Cooke, M. D. 1976 Antibiotic resistance among coliform and fecal coliform bacteria isolated from sewage, seawater, and marine shellfish. *Antimicrobial Agents and Chemotherapy* 9, 879–884.

Dhiman, G., Burns, E. N. & Morris, D. W. 2016 Using multiple antibiotic resistance profiles of coliforms as a tool to investigate combined sewage overflow contamination. *Journal of Environmental Health* 79, 36–39.

Eaton, A., Clesceri, L., Greenberg, A. & Franson, M. 1999 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, American
Water Works Association, Water Environment Federation, Washington, DC.

Fujioka, R. S., Hashimoto, H. H., Siwak, E. B. & Young, R. H. 1981 Effect of sunlight on survival of indicator bacteria in seawater. *Applied and Environmental Microbiology* **41**, 690–696.

Griffith, J. F., Schiff, K. C., Lyon, G. S. & Fuhrman, J. A. 2010 Microbiological water quality at non-human influenced reference beaches in southern California during wet weather. *Marine Pollution Bulletin* **60**, 500–508.

Harmon, S. M., West, R. T. & Yates, J. R. 2014 Identifying fecal pollution sources using 3M Petrifilm count plates and antibiotic resistance analysis in the Horse Creek Watershed in Aiken County, SC (USA). *Environmental Monitoring and Assessment* **186**, 8215–8227.

Hernandez, F., Calisto-Ulloa, N., Gomez-Fuentes, C., Gomez, M., Ferrer, J., Gonzalez-Rocha, G., Bello-Toledo, H., Botero-Coy, A. M., Boix, C., Ibanez, M. & Montory, M. 2019 Occurrence of antibiotics and bacterial resistance in wastewater and sea water from the Antarctic. *Journal of Hazardous Materials* **363**, 447–456.

Hinton, M., Hedges, A. J. & Linton, A. H. 1985 The ecology of *Escherichia coli* in market calves fed a milk-substitute diet. *Journal of Applied Bacteriology* **58**, 27–35.

Kaushik, D., Mohan, M., Borade, D. M. & Swami, O. C. 2014 Ampicillin: rise fall and resurgence. *Journal of Clinical and Diagnostic Research* **8** (5), ME01–ME03.

Kim, Y. O., Yang, E. J., Kang, J. H., Shin, K., Chang, M. & Myung, C. S. 2007 Effects of an artificial breakwater on the distributions of planktonic microbial communities. *Ocean Science Journal* **42**, 9–17.

Krumperman, P. H. 1985 Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Applied and Environmental Microbiology* **46**, 165–170.

Kumar, S., Tripathi, V. R., Vikram, S., Kumar, B. & Garg, S. K. 2018 Characterization of MAR and heavy metal-tolerant *E. coli* o157:H7 in water sources: a suggestion for behavioral intervention. *Environment, Development and Stability* **20**, 2447–2461.

Li, Y., Liu, F. & Wu, J. 2014 Study on pollution characteristics in west breakwater in Haizhou Bay. *Marine Sciences* **38**, 84–89.

Lin, J., Biyela, P. T. & Puckree, T. 2004 Antibiotic resistance profiles of environmental isolates from Mhlathuze River KwaZulu-Natal. *Water SA* **30**, 23–28.

Livermore, D. M. 1995 Beta lactamases in laboratory and clinical resistance. *Clinical Microbiology Reviews* **8**, 557–584.

Martínez, J. L. 2008 Antibiotics and antibiotic resistance genes in natural environments. *Science* **321**, 365–367.

McLellan, S. L., Hollis, E. J., Degas, M. M., Van Dyke, M., Harris, J. & Scopel, C. O. 2007 Distribution and fate of *Escherichia coli* in Lake Michigan following contamination with urban stormwater and combined sewer overflows. *Journal of Great Lakes Research* **33**, 566–580.

Middleton, J. H. & Salierro, J. D. 2013 Antibiotic resistance in triclosan tolerant fecal coliforms isolated from surface waters near wastewater treatment plant outflows (Morris County, NJ, USA). *Ecotoxicology and Environmental Safety* **88**, 79–88.

Mishra, M., Arukha, A. P., Patel, A. K., Behera, N., Mohanta, T. K. & Yadav, D. 2018 Multi-drug resistant coliform: water sanitary standards and health hazards. *Frontiers in Pharmacology* **9**, 311.

Rabbia, V., Bello-Toledo, H., Jiménez, S., Quezada, M., Domínguez, M., Vergara, L., Gómez-Fuentes, C., Calisto-Ulloa, N., González-Acuña, D., López, J. & González-Rocha, G. 2016 Antibiotic resistance in *Escherichia coli* strains isolated from Antarctic bird feces, water from inside a wastewater treatment plant, and seawater samples collected in the Antarctic Treaty area. *Polar Science* **10**, 123–131.

Reeves, R. L., Grant, S. B., Mrse, R. D., Copil-Oancea, C. M., Sanders, B. F. & Boehm, A. B. 2004 Scaling and management of fecal indicator bacteria in runoff from a coastal urban watershed in southern California. *Environmental Science & Technology* **38**, 2637–2648.

Salyers, A. A., Gupta, A. & Wang, Y. 2004 Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends in Microbiology* **12**, 412–416.

Sanderson, C. E., Fox, J. T., Dougherty, E. R., Cameron, A. D. S. & Alexander, K. A. 2018 The changing face of antibiotic resistance: a dynamic reflection of antibiotic resistance across landscapes. *Frontiers in Microbiology* **9**, 1894.

Statham, J. A. & McMeekin, T. A. 1994 Survival of faecal bacteria in Antarctic coastal waters. *Antarctic Science* **6**, 333–338.

Taylor, N. G., Verner-Jeffreys, D. W. & Baker-Austin, C. 2011 Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance? *Trends in Ecology and Evolution* **26**, 278–284.

Ventola, C. L. 2015 The antibiotic resistance crisis. *Pharmacy and Therapeutics* **40**, 277–283.

WHO 2005 Guidelines for Safe Recreational Water Environments, Report, World Health Organization, Geneva, Switzerland.

WHO 2014 Antimicrobial Resistance: Global Report on Surveillance, Report, World Health Organization, Geneva, Switzerland.

Zanoni, A. E., Katz, W. J., Carter, H. H. & Whaley, R. C. 1978 An *in situ* demonstration of the disappearance of coliforms in Lake Michigan. *Journal of the Water Pollution Control Federation* **50**, 321–330.

Zhang, S., Pang, S., Wang, P., Wang, C., Han, N., Liu, B., Han, B., Li, Y. & Anim-Larbi, K. 2016 Antibiotic concentration and antibiotic-resistant bacteria in two shallow urban lakes after stormwater event. *Environmental Science and Pollution Research* **23**, 9984–9992.

First received 27 April 2020; accepted in revised form 10 July 2020. Available online 3 August 2020.