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Baseline

Baseline screening for the presence of antimicrobial resistance in *E. coli* isolated from Kuwait's marine environment

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ABSTRACT

Here we present the findings of a study where 598 isolates of *Escherichia coli* (351 derived from seawater; 247 derived from the Venus clam, *Circenita callipyga*) were obtained from Kuwait's marine environment. Isolates were screened for their potential resistance to an array of 23 commonly deployed frontline antibiotics. Results demonstrate the resistant was widespread across all sites with high-levels of resistance (seawater: summer 89–64%; winter 90–57% and biota: summer 77%; winter 88%) observed to at least 1 of the 23 antibiotics tested. Resistance to Ampicillin was by far the most widely observed profile in seawater and biota across both summer and winter seasons, with 55.9 to 70.9% isolates displaying resistance to this antibiotic. This study demonstrates the potential of AMR screening to be used in Kuwait to detect issues related to water quality and the consequences it may pose for human health.

1. Baseline paper

Antimicrobial agents are widely used across health care and agricultural sectors to treat a range of disease conditions (Baquero et al., 2008; Martinez, 2009). However, concern has been raised over their indiscriminate, or poorly regulated use and the adverse impact this may have on the environment (Kümmerer, 2009; Taylor et al., 2011; Williams et al., 2016). Antimicrobial resistance (AMR) is known to span all classes of natural and synthetic antibiotics and the phenomenon is considered to be one of the foremost global health care problems (D'Costa et al., 2006; WHO, 2005; O'Neill, 2016). It has become increasingly clear that the diversity and abundance of antibiotic resistance in the environment has been underestimated, with potentially widespread ramifications (Williams et al., 2016). Recently, there has been an increasing amount of interest in the role that the marine environment plays in accentuating drug resistance (Taylor et al., 2011). It has a high and diverse bacterial loading and is a known sink for a multitude of clinical bacteria and contaminants. Therefore, marine ecosystems are not only an important reservoir for AMR, but they also drive its emergence (Taylor et al., 2011; Williams et al., 2016).

Recent studies suggest that the Gulf Cooperative Council (GCC) region is susceptible to the emergence of AMR genes and bacteria (Balkhy et al., 2016). However, little attention has been paid to the role that the marine environment may play in acting as a sink, or promoting the emergence of AMR. Where available, studies have identified the presence of AMR bacteria in fish and seawater collected from locations close to sewage discharges (Al-Bahry et al., 2009) and within the effluent itself (Al-Bahry et al., 2011). The presence of AMR bacteria has also been used as an indicator to monitor the exposure of green turtles (*Chelonia mydas*) to different marine pollutants (Al-Bahry et al., 2012).

The marine environment of Kuwait is known to be heavily impacted by large volumes of partially or untreated sewage discharge (Lyons et al., 2015; Saeed et al., 2015; Al-Sarawi et al., 2015). An assessment of the degree of sewage contamination in Kuwait's marine environment used both microbial water quality data and concentrations of faecal sterols in sediment to reveal regular breaching of regional water quality guidelines, with clear pollution hot spots within Kuwait Bay and along the Arabian (Persian) Gulf coast (Devlin et al., 2015; Lyons et al., 2015; Saeed et al., 2015). It is also known that wide array of chemical pollutants are associated with these effluents, some of which have antimicrobial modes of action (Smith et al., 2015; Saeed et al., 2017).

In Kuwait, research into AMR has been restricted to the clinical setting with high levels of resistance documented against a range of front line antibiotics (Jamal et al., 2013; Zhang et al., 2006). To date no studies have addressed the issue of AMR in marine systems, which represent a key data gap in Kuwait. Here data is presented from a baseline

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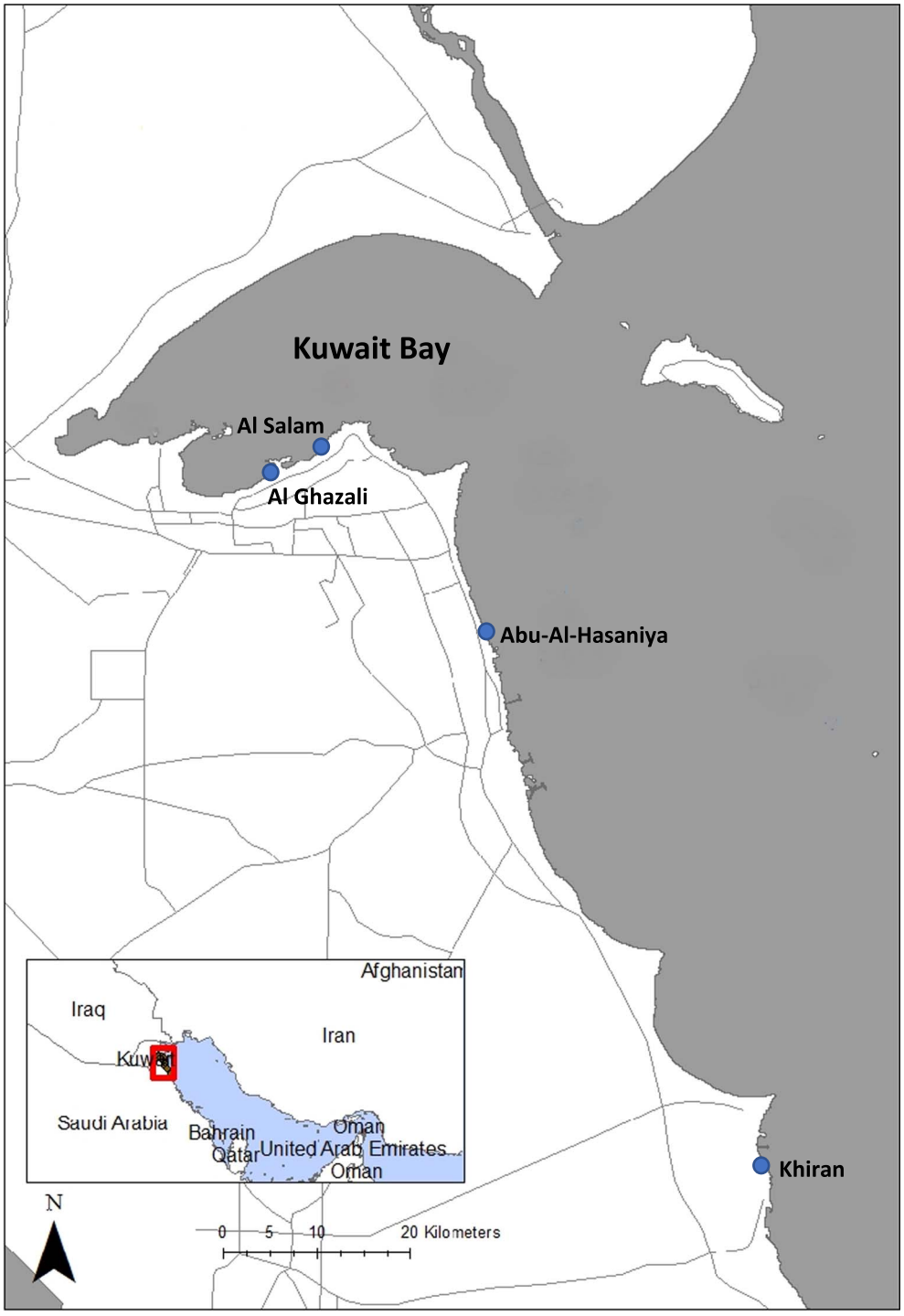

Fig. 1. Map of sampling locations around the coastline of Kuwait.

survey that is the first to obtain information on the prevalence of AMR within bacterial isolates collected from Kuwait's marine environment. In total 598 isolates of *Escherichia coli* were isolated from seawater and biota (Venus clam, *Circenita callipyga*) and screened for resistance against a panel of 23 antibiotics.

Seawater samples for AMR screening were collected during both winter (December 2014–February 2015) and summer (July – August 2015) seasons from sites along the Kuwait coastline. Bivalves samples were collected across summer (July–August 2015) and winter (December 2015–February 2016) seasons (Fig. 1). Physical-chemical parameters (pH, water temperature and salinity) were recorded at each sampling location using a portable multi-parameter water quality instrument (Hanna instruments model no. HI 9828, USA). Salinity (mean 36‰ ± 2.0) and pH (mean 8.2 ± 0.2) values were similar across sampling periods, whereas water temperatures varied between summer (34 °C ± 2.0) and winter (17.2 °C ± 2.0) seasons. Sites were selected to provide representative locations around known sewage outlets within Kuwait Bay (Al-Salam, Al-Ghazali) and along the Arabian Gulf Coast (Abu-Al-Hasaniya). At each site, 6 seawater samples (1 l) were collected close to known effluent discharge points. For comparison, seawater samples were collected at Khiran as a reference site. Samples of Venus clam were only available from Al-Salam. Multiple seawater samples at each location were taken into 1 l sterile polyethylene bottles and stored on ice, before transporting to the laboratory.