A new dimension in rapid water quality assessment

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1 Introduction

Microbial pollution of bathing waters from various sources, such as sewage discharge and agricultural runoff, poses a substantial risk to public health. Therefore, monitoring bathing water quality is vital to mitigate the health risks associated with the recreational use of bathing waters. Conventional testing for bacterial contamination relies on culture-based methods to enumerate faecal indicator bacteria (FIB), which often takes over 48 hours and water quality is thus retrospective with potential impacts on human health.

To address this issue, Molendotech have developed a novel rapid water quality kit (Bacterisk) that provides near real-time assessment of bacterial contamination of water which can be conducted by non-specialist staff *in. situ* (Figure 1).

Figure 1. A Bacterisk kit being used *in situ*.

Research by Molendotech has shown the applicability of using endotoxin as a marker of faecal contamination of seawater (1,2). This study was undertaken to evaluate the performance of the Bacterisk assay as a rapid risk assessment tool to assess the quality of coastal bathing waters in the southwest of England and further highlight the disadvantages of traditional culture-based techniques.

2 Methods

Water sampling
A total of 119 coastal water samples were collected across the southwest of England. Briefly, 1000 mL samples were taken using sterile bottles 30 cm and then transported in the dark and tested within 4 hours or stored in a fridge (2-8 °C) and tested no later than 24 hours after collection.

Bacterial culture identification
Appropriate volumes of each water sample (1, 10, and 100 mL) were aseptically filtered through a 0.45 µm membrane (Whatman, UK). Membranes were placed on membrane TBX agar (Oxoid, UK) and incubated at 30°C for 4 hours, then at 37°C for 14 hours for the detection of presumptive *E. coli* or on Slanetz and Bartley medium (Oxoid, UK) and incubated at 36°C for 44 hours for the detection of presumptive intestinal enterococci.

Bacterisk Assay
The Bacterisk assay (Molendotech Ltd., Brixham, UK; www.molendotech.com) was performed following the manufacturer's instructions, taking only 15 minutes.

Sequencing analysis
Bacterial gene sequencing was performed by Eurofins using the INVIEW Microbiome Profiling package with amplification and Illumina MiSeq sequencing of the hypervariable regions in the 16S rRNA gene (16S V1-V3, 16S V3-V4 or 16S V3-V5).

3 Results

A total of 119 coastal water samples were analysed in parallel by the Bacterisk assay to calculate Endotoxin Risk Units (ERU) and by membrane filtration to enumerate the levels of *E. coli* and intestinal Enterococci (CFU/100 mL). Figure 2 shows the water quality of the water samples determined by Bacterisk and compared with culture of *E. coli* and enterococci by membrane filtration method. An ROC analysis (2) of the data from Figure 2A gave an area under the curve of 0.932 (p=0.0001) and sensitivity of 74% and specificity of 94% .

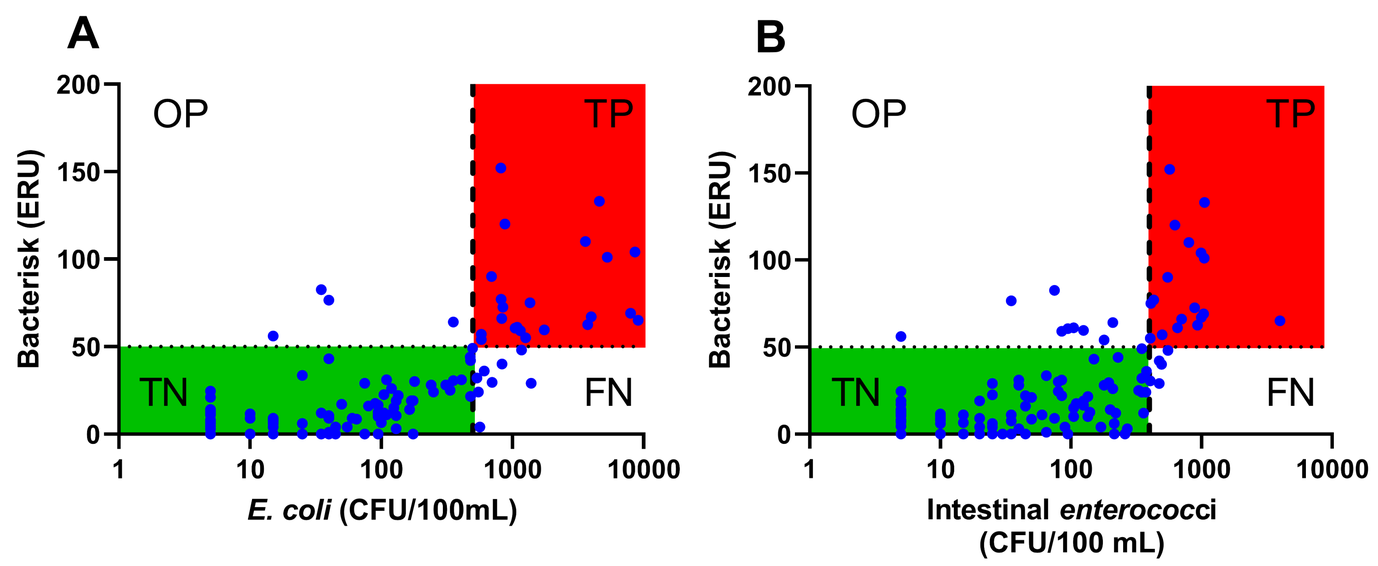

Figure 2. Scatter plot displaying the Bacterisk Endotoxin Risk Units against (A) *E. coli* and (B) Enterococci enumerated using the membrane filtration method on TBX agar for Coastal water samples. The chart is split into quadrants based on the ERU threshold (Y axis 50) and (A) E. coli cut-off (X axis 500CFU/100mL), (B) Enterococci cut-off (X axis 200CFU/100mL). n=119. TN: true negative, TP: true positive, FN: false negative, OP: off-target positive

Due to Bacterisk determining the levels of a Gram-negative molecule (endotoxin) as a marker of water contamination, it not only provides good correlation with *E. coli* but will respond to other Gram-negative bacteria including non-FIB. These may be pathogenic and of concern for human health. These have been included in our data as 'other' or 'off-target' positives. It is important to understand what these other bacteria are and how they may contribute to the Bacterisk data. To accomplish this, 3 water samples from a single location including an OP and two TP were DNA-sequenced to determine the bacterial flora composition. The OP sample contained a larger proportion of Psedomonas than the TP samples (Figure 3).

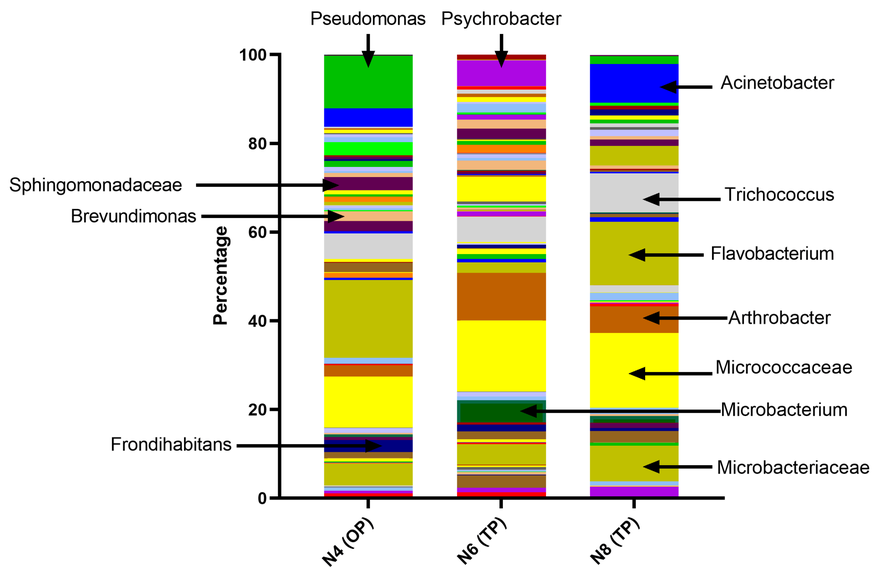

Figure 3. 16S RNA sequencing data from water samples showing the different genera present in typical samples. OP = off-target positives; TP = True positives based on *E. coli* >500CFU/100mL.

4 Discussion & Conclusion

- This study has shown that Bacterisk can rapidly and accurately assess water quality for bacterial contamination.
- Bacterisk can provide results in 15 minutes compared with 48 hours for conventional culture results. This will allow for rapid pro-active decision making for the use of recreational water.
- By detecting bacteria other than *E. coli,* ('off-target positives') Bacterisk can alert to the presence of potentially harmful pathogens that would otherwise be missed when only testing for FIB.

References

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2. Good CR, White A, Brandao J, Jackson S. Endotoxin, a novel biomarker for the rapid risk assessment of faecal contamination of coastal and transitional waters. J Water Health. 2024 Jun;22(6):1044-1052.