INTRODUCTION

Faecal contamination of water bodies is a serious public health problem due to the introduction of waterborne human pathogens. Monitoring environmental microbiological water quality by standard culturing and isolation of human pathogens is impractical, given resources required to surveil a diversity of waterborne human pathogens (Harwood et al., 2014). High throughput sequencing techniques used in metagenomics have been validated and applied in simultaneous detection of diverse waterborne human pathogens including viruses (Bibby et al., 2011; Escobedo-Hinojosa & Pardo-López, 2017), yet such approaches do not address infectivity and are still comparatively unaffordable for routine use in monitoring. Faecal indicator bacteria (FIB) including total coliforms, faecal...
coli, Escherichia coli and enterococci have been used as indicators of microbiological quality of environmental waters for over a century due to their high abundance in faeces and easy detection. However, FIB are found broadly within faeces of warm-blooded animals, and so do not specifically reveal human faecal contamination which is of greatest public health concern due to host specificity of pathogens. Further, as FIB can persist and multiply in environments such as sediments, soils and aquatic vegetation (Field & Samadpour, 2007; Harwood et al., 2014), FIB may not indicate recent faecal pollution. These issues can result in elevated FIB levels being associated with relatively low human health risks (Ferguson et al., 1996). To assess microbial risk and implement appropriate water quality management practices, faecal contamination sources should be accurately assigned and located via microbial source tracking (MST).

In MST, host-specific faecal DNA markers from human and animal-associated micro-organisms such as Bacteroidales – using amplification by polymerase chain reaction (PCR), quantitative PCR (qPCR) and droplet digital PCR (ddPCR) – have been widely applied (Boehm et al., 2013; Field & Samadpour, 2007; Harwood et al., 2014; Meays et al., 2004; Reischer et al., 2013; Scott et al., 2002; Stoeckel & Harwood, 2007). Numerous human-associated markers are used to track human faecal contamination (Harwood et al., 2014; Hughes et al., 2017; Roslev & Bukh, 2011). Based on its occurrence in human faecal samples, HF183 – termed for a particular partial 16S rRNA gene sequence highly specific to human-associated Bacteroides including Bacteroides dorei – has been used to detect and quantify human faecal contamination worldwide (Bakir et al., 2006; Bernhard & Field, 2000a; Bernhard & Field, 2000b; Haugland et al., 2010; Kirs et al., 2016). Diverse PCR and qPCR-based methods use the HF183 gene sequence as the basis for the forward primer, paired with reverse primers and probes targeting broader phylogenetic groups such as Bac708R, BFDRev and BFDFAM – all targeting the entire Bacteroidales family (Bernhard & Field, 2000b; Haugland et al., 2010), as well as BacR287 and BacP234MGB designed based on an alignment of Bacteroidetes 16S rRNA gene sequences containing HF183 (Green et al., 2014). High specificity and sensitivity of HF183 to human faeces compared to other DNA markers have defined HF183 as one of the best performing bacterial MST markers (Boehm et al., 2013). Human faecal contamination based on HF183 detection has been widely documented in various environmental waters including treated wastewater, urban stormwater, rivers, streams, lakes, marine waters and groundwater (Bradshaw et al., 2016; Diston et al., 2015; Jardé et al., 2018; Mayer et al., 2015; Molina et al., 2014; Sidhu et al., 2013; Staley et al., 2016; Staley et al., 2018). HF183 was also consistently observed in beach waters along the California coast in many previous studies associated with sampled drainage, creek and river waters (Cao et al., 2017; McQuaig et al., 2012; Russell et al., 2013; Sercu et al., 2009). In recent studies of several beaches, swimmers were found to be sources of chronic, low concentration, HF183 markers in beach surf zone waters (Li, Van De Werffhorst, Steets, Ervin, Murray, Blackwell, et al., 2021; Li, van De Werffhorst, Steets, Ervin, Murray, Devarajan, & Holden, 2021; Hou et al., 2021). These results raised the question: could aquatic HF183 detections originate from other than human faecal releases or faecal shedding into the environment?

HF183 and human-associated Bacteroides including B. dorei were previously identified and isolated from human faeces (Bakir et al., 2006; Bernhard & Field, 2000a), and HF183 sequences were occasionally detected in chicken and dog faeces in low quantities (Feng & McLellan, 2019; Harwood et al., 2014). However, little is known about the distribution of HF183 and B. dorei beyond the human gastrointestinal tract and faeces. In the last decade, numerous studies have been performed to reveal human skin and urine microbiomes (Fouts et al., 2012; Grice et al., 2009; Lehtimäki et al., 2017; Lewis et al., 2013; Meisel et al., 2016; Oh et al., 2014; Siddiqui et al., 2011; Turnbaugh et al., 2007; van Rensburg et al., 2015). Considering the possibilities for contamination of human body skin and the urinary tract by HF183 and human-associated B. dorei, as well as the potential for any HF183 populations on humans to be sources to environmental waters during bather shedding (Li, Van De Werffhorst, Steets, Ervin, Murray, Blackwell, et al., 2021; Li, van De Werffhorst, Steets, Ervin, Murray, Devarajan, & Holden, 2021; Hou et al., 2021), it is worth determining whether HF183 and human-associated B. dorei are within sequences associated with human skin and urine microbiomes. In this study, we examine this issue based on secondary examination of previously published human skin and urine microbiome gene sequence data. The results reveal previously undiscussed sources of HF183 and human-associated Bacteroides in human microbiomes outside of faeces, and add new understanding useful to interpreting HF183 detections in water bodies such as recreational waters.

**MATERIALS AND METHODS**

**Human body skin and urine microbiome data sets**

The HF183 marker is located in the V2 region of the 16S rRNA gene sequence, thus only published studies utilizing primers of nearly full length (27F and 1492R) or the V1-V3 region (27F and 534R) of the 16S rRNA gene...
for sequencing were selected. Five independent previously published studies (S1–S5) focusing on human body skin microbiomes and three studies (U1–U3) focusing on human urine microbiomes were selected for downloading sequencing data sets (Fouts et al., 2012; Grice et al., 2009; Lehtimäki et al., 2017; Lewis et al., 2013; Meisel et al., 2016; Oh et al., 2014; Siddiqui et al., 2011; van Rensburg et al., 2015). A part of the sequencing data sets for human skin microbiomes was also downloaded from the HMP project (https://hmpdapcc.org/). Based on the sampling information provided in these studies, all of the human skin samples were collected by study personnel or nurses. An exception may be the HMP project for which the sampling information is unavailable. All of the urine samples were collected following a similar protocol, by which either volunteers were instructed to provide a clean-catch, mid-stream voided, urine sample into a sterile container or urine was obtained directly via a catheter in subjects utilizing sterile catheters. The details of all nine data sets and the sampling locations on human skin or in urine are listed in Table S1. These nine data sets were sequenced by Sanger, 454 or Illumina sequencing. In brief, 71.3 Gb of data representing 2943 skin samples from more than 15 human body sites were acquired, and 1.5 Gb of data from 77 urine samples were downloaded. Due to the remarkable variation in the amount of sequencing data obtained for each sample across the nine data sets, the sequencing data were not normalized in this study.

**Bioinformatic analyses**

To confirm the source of HF183 as *B. dorei*, the human faeces specific sequence HF183 (5’-ATCATGAGTTCACATGTCCG-3’) used as the forward primer in HF183 assays (Table S2) was aligned against the NCBI NT and SILVA SSU r132 RefNR database (https://www.arb-silva.de/) using blastn as well as the RDP database using Probe Match (https://rdp.cme.msu.edu/). HF183 was then searched in all six data sets from the human skin microbiome studies and three data sets from human urine microbiome studies using blastn with criteria of 100% match and 100% coverage. The HF183 containing sequences from skin and urine microbiome data sets were further used to generate a phylogenetic tree together with all *B. dorei* reference 16S rRNA sequences as well as all Bacteroides genus type strain 16S rRNA sequences archived in the RDP database. The sequences were aligned using MAFFT (Katoh et al., 2002), and the phylogenetic tree was constructed using MEGA X by the neighbour-joining algorithm and the Jukes–Cantor distance estimation method with bootstrap analyses for 1000 replicates (shown in Figure 1) (Kumar et al., 2018). Additionally, all reverse primers and probes (Table S2) were aligned against the HF183 containing sequences with nearly full length (using primers 27F and 1492R) using blastn with criteria of 100% match and 100% coverage. The nearly full-length sequences were selected considering the variable loci of reverse primers and probes.

**RESULTS**

By aligning against the RDP, SILVA and NCBI NT databases, HF183 was only matched to sequences affiliated with *B. dorei* (29, 18 and 15 sequences respectively) and all other matches belonged to uncultured bacteria without definite species names (Table S3). The matching results of HF183 to human skin and urine microbiome data sets are listed in Table S1 and summarized in Table 1. HF183 was detected in all six skin data sets, and the ratios of positive samples ranged from 0.5% to 36.3%. Study S1 collected skin microbiome samples from healthy adults and showed the lowest detection of HF183, possibly due to less sequencing depth (less than 1000 sequences per sample) obtained by cloning and sequencing compared to the other studies which utilized high-throughput sequencing methods. Study S5 investigated the microbiome on volar forearm of 275 children of ages ranging from 2 months to 14 years (Lehtimäki et al., 2017) and showed the highest overall ratio of HF183 detection as 36.3%. This value was significantly higher than the ratios of other studies, suggesting that children might have more HF183 associated bacteria in their skin microbiome compared with adults. Studies S2 (Meisel et al., 2016) and HMP both characterized the microbiome in healthy adults and have similar overall HF183 detection ratios (4.7% and 3.1% respectively). The HF183 detection level was also similar (4.7%) in study S3 which analysed the skin microbiome before, during and after experimental inoculation of the arm with *Haemophilus ducreyi* in adult volunteers who subsequently resolved the infection or formed abscesses (van Rensburg et al., 2015). HF183-associated bacteria on skin were not obviously influenced by infection (data not shown) (van Rensburg et al., 2015). Study S4 has the largest number of samples (1542 samples) obtained from healthy volunteers of 23 to 39 years of age without any chronic skin diseases (Oh et al., 2014). For that study, the highest ratio of HF183 positive samples came from popliteal fossa (37.6%), following by inguinal crease (18.8%), volar forearm (13.3%) and back (12.5%). Not all body skin sites were characterized in the six skin microbiome data sets, but there were several skin sites where HF183 seemed to be consistently present in the different studies, such as cubital fossa, volar forearm, palm,
HF183 ON HUMAN SKIN AND IN URINE

Comparing to the other skin sites, popliteal fossa, volar forearm and inguinal crease were hot spots for HF183 sequences, which might be from faecal contamination during showering, or defecation. Meanwhile, HF183 was also detected at some unique body sites such as nare and retroauricular creases where there would seem to be less likelihood of contamination from faecal sources. The detection ratios still ranged from 3.1% to 12.2%, indicating that the presence of HF183 at these unique sites was not occasional.

Human faecal marker HF183 was also detected in 2 of 3 human urine microbiomes. The ratios of positive samples ranged from 0% to 37.5%. No HF183 was present in any microbiome of voided urine from asymptomatic adults in study U3. However, HF183 was detected in three of eight healthy adult female urine microbiomes in study U1 and present in a urine sample of one female with neuropathic bladder due to spinal cord injury in study U2, with all urine specimens culturing negative.

As shown in Figure 1, all HF183 containing sequences obtained from nine data sets used in this study were grouped with B. dorei reference 16S rRNA sequences as well as Bacteroides genus type stain 16S rRNA sequences archived in the RDP database determined by the neighbour-joining method. Bootstrap values of >50% (obtained with 1000 resamplings) are shown at nodes. Methanosphaera cuniculi type strain DSM 4103 (HE582783) was used as an outgroup. GenBank accession numbers are in parentheses.

**DISCUSSION**

Although it is possible that the HF183 marker sequence could match sequences that are not related to *Bacteroides* spp. at all, the HF183 containing sequences found in the microbiome data sets of this study were all affiliated with *B. dorei* as shown in Figure 1 by aligning with all *B. dorei* reference 16S rRNA sequences as well as all *Bacteroides* genus type strain 16S rRNA sequences archived in the RDP database. This confirmed the high specificity in this study of HF183 marker sequence to *B. dorei*. 

retroauricular crease and toe web (Table 1). Comparing to the other skin sites, popliteal fossa, volar forearm and inguinal crease were hot spots for HF183 sequences, which might be from faecal contamination during showering, or
### Table 1  The ratios of HF183 positive human skin and urine microbiome samples in five independent studies S1–S5, HMP project and three independent studies U1–U3\(^a\)

| Site                  | S1\(^b\) | S2\(^c\) | S3\(^d\) | S4\(^e\) | S5\(^f\) | HMP\(^g\) | U1\(^h\) | U2\(^i\) | U3\(^j\) |
|-----------------------|----------|----------|----------|----------|----------|-----------|----------|----------|----------|
| Back                  | 0% (0/10)|          |          |          | 12.5% (2/16) |           |          |          |          |
| Cubital fossa         | 0% (0/10)|          |          | 11.1% (2/18) |          |           |          |          |          |
| Forehead              |          |          |          | 11.1% (2/18) |          |           |          |          |          |
| Inguinal crease       | 0% (0/10)|          |          |          |          | 18.8% (6/32) |          |          |          |
| Manubrium             | 0% (0/10)|          |          |          |          | 11.8% (2/17) |          |          |          |
| Nare                  | 0% (0/10)|          |          |          |          | 12.2% (32/263) |          |          |          |
| Occiput               | 10% (1/10)| 0% (0/16) |          |          |          | 0% (0/17) |          |          |          |
| Palm                  | 0% (0/10)|          |          |          |          | 6.1% (2/33) |          |          |          |
| Plantar heel          | 0% (0/10)|          |          |          |          | 0% (0/36) |          |          |          |
| Popliteal fossa       | 0% (0/10)|          |          |          |          | 37.6% (38/101) |          |          |          |
| Retroauricular crease | 0% (0/10)| 0% (0/18) |          |          |          | 8.1% (15/186) |          |          | 3.1% (4/128) |
| Toe web               | 0% (0/10)|          |          |          |          | 5.6% (1/18) |          |          | 3.0% (1/33) |
| Umbilicus             | 0% (0/10)| 0% (0/18) |          |          |          |          |          |          |          |
| Upper arm             |          |          |          |          |          | 4.7% (9/191) |          |          |          |
| Volar forearm         | 0% (0/10)|          |          |          |          | 13.3% (41/308) | 36.3% (207/571) |          |          |
| Other                 | 0% (0/70)| 0% (0/26) |          |          |          | 0% (0/105) |          |          | 0% (0/65) |
| Urine                 |          |          |          |          |          |          | 37.5% (3/8) | 2.0% (1/51) | 0% (0/16) |
| Total                 | 0.5% (1/200)| 4.7% (7/150) | 4.7% (9/191) |          | 11.8% (182/1542) | 36.3% (207/571) | 3.1% (9/289) | 37.5% (3/8) | 2.0% (1/51) |

\(^a\)The number of positive samples and the total number of samples characterized at each skin site or in urine are shown in parentheses. The sites not characterized in each study are left as blank. Data sets in studies S1–S5 and U1–U3 were fully downloaded and analysed, while 100 sequencing files of human skin microbiome in the HMP project were randomly selected and analysed in this study. The amount of sequencing data for each study is shown in Table S1.

\(^b\)Reference Grice et al. (2009) using Sanger sequencing for 16S rRNA clone library.

\(^c\)Reference Meisel et al. (2016) using Illumina sequencing.

\(^d\)Reference van Rensburg et al. (2015) using 454 sequencing.

\(^e\)Reference Oh et al. (2014) using 454 sequencing.

\(^f\)Reference Lehtimäki et al. (2017) using Illumina sequencing.

\(^g\)The HMP project (https://hmpdacc.org/) with the sequencing techniques unavailable.

\(^h\)Reference Siddiqui et al. (2011) using 454 sequencing.

\(^i\)Reference Fouts et al. (2012) using 454 sequencing.

\(^j\)Reference Lewis et al. (2013) using 454 sequencing.
Multiple HF183 detection assays have been established (Bernhard and Field 2000; Seurinck et al., 2005; Haugland et al., 2010; Green et al., 2014). All of these assays use the same HF183 marker sequence as forward primer, but use different reverse primers and probes with variable specificities either to the whole Bacteroidales family including B. dorei (Bernhard & Field, 2000b; Haugland et al., 2010), or some Bacteroidetes sequences containing HF183 (Green et al., 2014). Thus, these reverse primers and probes are less specific when comparing to the HF183 specific sequence, and the specificities of these HF183 detection assays are determined by the HF183 marker sequence as the forward primer. To validate this, almost full length 16S rRNA gene sequences obtained using 27F and 1492R primers in the human microbiomes of this study were selected and aligned by HF183 sequence and all reverse primers and probes. All reverse primers and probes match almost all 22 sequences containing HF183. Although only one sequence did not match the reverse primers/probes Bac242R (Seurinck et al., 2005), BacR287 and BacP234MGB (Green et al., 2014), this sequence still belonged to B. dorei as confirmed by Figure 1, and matched other reverse primers/probes Bac708 (Bernhard and Field 2000), BFDFRev (Haugland et al., 2010) and BFDFAM (Haugland et al., 2010) which were all designed to match the whole Bacteroidales family. The BacR287 primer and BacP234MGB probe in the Green et al. (2014) method were designed based on an alignment of available Bacteroidetes 16S rRNA gene sequences with 100% similarity to the HF183 primer. Thus, it is not surprising that the BacR287 primer and BacP234MGB probe do not match all of the sequences containing HF183 sequence in this study. However, in real applications of MST, the less specific reverse primers and probes could result in more nonspecific amplifications compared to more specific primers and probes such as the BacR287 primer and BacP234MGB probe (Green et al., 2014).

The results of this study indicated that HF183 was widespread on human skin based on the results of the six data sets although these data sets were obtained by different sequencing techniques including Sanger, 454 and Illumina. Compared to Sanger sequencing, 454 and Illumina sequencing can generate much larger amounts of sequence data which clearly increased the chance of finding sequences containing HF183 (Table S2). However, to avoid biasing the findings of this study to certain sequencing techniques, the data sets obtained using different sequencing techniques were retained in this study but not normalized due to the high variance of the sequencing depth. Overall, the results of this study still demonstrated that HF183 was widespread on human skin regardless of sequencing technique. The presence of HF183 was further observed on not only some hot spots but also some unique body sites such as nare and retroauricular creases. While the source of HF183 in these locations is unclear and was not studied here, toilet flushing is known to aerosolize human faecal bacteria (Abney et al., 2021) which could include B. dorei. Human urine within the urinary tract has in general been considered sterile, based on a lack of culturable micro-organisms present in urine specimens obtained by the clean-catch method and by catheterization (Fouts et al., 2012; Lewis et al., 2013; Siddiqui et al., 2011). However, advances in sequencing techniques have proven that a diverse spectrum of bacterial profiles is associated with healthy, culture negative human urine (Fouts et al., 2012; Lewis et al., 2013; Siddiqui et al., 2011). The results of this study demonstrated the occasional presence of HF183 in human urine microbiomes. It should be noted that self-collected urine samples are prone to faecal contamination when proper collection procedures are not followed (Franz & Hörl, 1999), although a sterile sampling protocol was followed based on the sampling description of the studies used here (Fouts et al., 2012; Lewis et al., 2013; Siddiqui et al., 2011). Although B. dorei has been described as strictly anaerobic (Bakir et al., 2006), other obligate anaerobic Bacteroides species such as B. vulgatus have also been found in human skin microbiomes in abundance (Meisel et al., 2016). Considering that B. dorei was widespread on human skin and frequently present in human urine according to the data sets of this study, the human body skin and urinary track might be not only contaminated by human faeces but also be potential niches for B. dorei outside of faeces.

Since the widespread application of HF183 in MST, particularly using qPCR and ddPCR (Steele et al., 2018), human faecal contamination has been documented worldwide in diverse environmental waters (Bradshaw et al., 2016; Diston et al., 2015; Jardé et al., 2018; Mayer et al., 2015; Molina et al., 2014; Sidhu et al., 2013; Staley et al., 2016; Staley et al., 2018). The results of this study suggest possible sources of HF183 in environmental waters could be associated with human skin and urine microbiomes, although HF183 on human skin or in urine might still originate from human faecal contamination. HF183 has been consistently detected in beach waters along California coast (Cao et al., 2017; McQuaig et al., 2012; Russell et al., 2013; Sercu et al., 2009), and public health risks have been estimated based on quantitative microbial risk assessment (QMRA) of HF183 detection levels (Boehm et al., 2015; Brown et al., 2017). Correlations between swimmers and the presence of low levels of HF183 have been reported, suggesting bather shedding as a source of HF183 to recreational waters (Li, Van Der Werfhorst, Steets, Ervin, Murray, Blackwell, et al., 2021; Li, van De Werfhorst, Steets, Ervin, Murray, Devarajan, & Holden, 2021; Toubiana et al., 2021). The
results of this study may inform such correlations by suggesting that B. dorei in skin or urine microbiomes of bathers might contribute to the observed low levels of HF183 in environmental waters.

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CONFLICT OF INTEREST
No conflict of interest declared.

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