Scientific Research

Survival of Epidemic, Clinical, Faecal and Recreational Beach Enterococci Strains with Putative Virulence Genes in Marine and Fresh Waters

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Abstract

Culturable faecal coliform, epidemic, clinical, faecal and recreational beach enterococci strains possessing putative virulence genes were enumerated over the course of 5 weeks to comparatively assess their persistence in tropical marine and fresh waters. For the clinical and epidemic strains tested, it took 2.38 ± 0.45 days for a 1-log reduction (T_{90}) in marine water. A higher T_{90} average of 2.51 ± 0.08 was observed for the commensal and environmental strains. Generally, lower T_{90} values of 2.14 ± 0.26 and 2.15 ± 0.16 days respectively were observed for hospital and community acquired enterococci strains in fresh water mesocosms subjected to tropical ambient temperature. Beach water enterococci and enterococci recovered from faeces of humans survived for up to 20 days and 23 days respectively in fresh and marine waters. The epidemic strain, MMH594, an esp-positive clinical bacteremia isolate that previously caused multiple infections in a hospital ward outbreak fares favourably well in tropical marine and fresh aquatic environments. For enterococci, the decay rate was approximately 13% higher in fresh water than was observed for marine water. On the contrary, for *E. coli*, the decay rate was approximately 17% lower in fresh water than was observed in marine water. Generally, the whole, the population trends of *E. coli* and enterococci in fresh and marine water mesocosms did not reveal any evidence of growth. Our findings suggest that potentially pathogenic bacteria can resume active growth after three weeks of being harboured by the reservoir-beach sand and still pose threat to public health.

Keywords

Clinical Enterococci Die-Off, Beach Enterococci Die-Off, Faecal Enterococci Die-Off, Tropical Water

Temperature, Marine and Fresh Water, Virulence

1. Introduction

Microbial contamination of water resources remains a key problem in many parts of the world. Untreated or only partly treated wastewaters and wastes are frequently released into rivers and sea, which affect surface water quality [1]. Traditionally, the presence of indicator organisms in water is used to decipher if the water has been contaminated by faecal material; implying the potential presence of pathogens [2] [3]. The use of *Escherichia coli* and members of the genus Enterococcus, the enterococci (ENT) have been recommended for the assessment of hygienic safety of recreational waters [1] [4] [5]. Nonetheless, the choice of which particular indicator to utilize in monitoring recreational water bodies has been a source of considerable debate among the public health officials [6]. One striking point in these debates is the ability of indicator organisms to grow in the environment, a decisive factor which questions their position as indicator organisms. A number of studies have been conducted on the survival of indicator organisms in the environment but are mainly restricted to temperate and subtropical settings. There is a paucity of published information for such studies in tropical settings.

Apart from being indicator organisms, enterococci also cause diseases [7]. The severity of these diseases is mediated by the presence of one or more virulence markers previously described. Among these are gelatinase, enterococcal surface protein (*Esp*), aggregation substance (*asa*), cytolysin (*cyl*) and hyaluronidase (*hyl*) [7]-[9]. Although only few studies have focused on pathogenicity of environmental strains, there seems to be a general consensus that clinical strains are more pathogenic than their clinical counterparts. Given the permeable barriers in waste handling, generation and transport in the environment of developing nations as argued by Dada *et al.* [10], there is the need to know the fate of pathogenic strains of enterococci should they find their way into the environment. To the best of our knowledge, there is currently no published information on a comparative assessment of the survival of epidemic and environmental strains of enterococci in fresh and marine water at a temperature of $29°C \pm 1°C$ which is common at tropical beaches all year round. The examination of the comparative survival of epidemic and environmental strains of these organisms in recreational beach environment is important for an accurate exposure assessment and risk characterization of potentially pathogenic enterococci. The current study thus aims to assess the persistence of culturable faecal coliform vs. epidemic, clinical, faecal and recreational beach enterococci strains possessing putative virulence genes over the course of 5 weeks in tropical marine and fresh waters.

2. Materials and Method

2.1. Description of Clinical, Faecal and Recreational Beach Enterococci Strains

Eight strains of enterococci were examined in this study. These included clinical strains isolated from catheters of hospital patients with urinary tract infections in 2012 (n = 2), strains recovered from faecal samples (n = 2), strains carrying virulence genes recovered from recreational beach water (n = 2), a strain that previously caused hospital wide epidemic (n = 1) and a vancomycin resistant strain. Phenotypic confirmation of isolates was done following previously published guides [11] [12]. Isolates were tested to confirm if they hydrolyse bile esculin and grow in 6.5% NaCl and in brain heart infusion agar (BHIA) at 45°C. Other tests were to determine motility on SIM agar (Oxoid, UK) and fermentation of a 1% concentration of mannitol, sorbitol, arabinose, raffinose, sucrose, lactose and inulin.

Identities of selected isolates were also confirmed using ddl gene sequencing as described by Dutka-Malen *et al.* [13]. Sequencing was performed with an ABI 3130XL 20 genetic analyzer (Applied Biosystems). Polymerase chain reaction (PCR) assays were applied to check for the presence of virulence determinants: aggregation substance (*asa*), cytolysin (cylA), enterococcal surface protein (esp), gelatinase (gelE) [14]. Details of primers are listed in **Table 1**. Multiplex PCR conditions used in this study included an initial activation step at 95°C for 4mins followed by 30 cycles of denaturation at 94°C for 1min, annealing at 55°C for 1min, extension at 72°C for 1min and a single cycle of 7 mins at 72°C. PCR products were checked using 1% agarose gel electrophoresis.

2.2. Marine and Fresh Water Mesocosm Preparation

Marine and fresh water samples were collected in sterile 5-L polypropylene containers from two marine beaches

Table 1. Primers used during the study.

Gene	Primer name	Sequence (5'–3')	Product (bp)
D-alanine:D-alanine ligase (ddl)	ddlF	ATCAAGTACAGTTAGTCTT	941
	ddlR	ACGATTCAAAGCTAACTG	
RAPD Primer	D8635	GAG CGG CCA AAG GGA GCA GAC	
Aggregation substance (asa+)	ASA 11	GCACGCTATTACGAACTATGA	375
	ASA 12	TAAGAAAGAACATCACCACGA	
Gelatinase (gel+)	GEL 11	TATGACAATGCTTTTTGGGAT	213
	GEL 12	AGATGCACCCGAAATAATATA	
Cytolysin (cyl+)	CYT I	ACTCGGGGATTGATAGGC	688
	CYT IIb	GCTGCTAAAGCTGCGCTT	
Enterococcal surface protein (esp+)	ESP 14F	AGATTTCATCTTTGATTCTTGG	510
	ESP 12R	AATTGATTCTTTAGCATCTGG	

in Malaysia. Fresh water samples were collected from rivers that drain into the receiving marine waters. Pooled samples of the fresh and marine water were sterilised by autoclaving. Quality assurance tests to determine the effectiveness of the sterilization was by way of sterility tests by culturing 5 ml water of each water sample in Brain Heart Infusion (BHI) agar (Oxoid, UK) and incubated at 37˚C for 48 h. One hundred microlitres portion of these tubes were subsequently plated on Columbia Blood Agar (Oxoid CM0331) supplemented with 5% (v/v) sheep blood an incubated for 24 h at 37˚C. Measurements for dissolved oxygen, pH, salinity and temperature of the water samples were taken using YSI model 600QS (YSI, Yellow Springs). The pooled fresh water had a salinity of 0.31 ppt and pH of 7.39 and the pooled marine water had a salinity of 28.53 ppt and pH 7.41.

2.3. Fresh and Marine Water Inoculation

Overnight cultures from Brain Heart Infusion (BHI) agar (Oxoid, UK) supplemented with 5% sterile sheep blood for each strain. These were added to the sterile marine and fresh water such that the initial concentration was approximately 10^7 cfu/ml of each water sample. One kilogram of autoclaved soil was weighed into a sterile plastic bag and added to each of three mesocosms with stirring. In each case, vigorous stirring was employed to ensure even distribution of the inoculum. All samples (marine and fresh water microcosms) were kept for an incubation period of 5 weeks in the dark at ambient temperatures (28˚C ± 2˚C) in the dark. Mesocosms were sampled within 15 mins after inoculation (DO, t = 0). The sediment was minimally disturbed during sampling to avoid artificially elevated enterococci concentrations in the water column. Mesocosms were sampled in triplicates daily in the first week and on alternate daily intervals in the second week up to and including 18 days duration. This involved aseptic plating of each water microcosm on Brain Heart Infusion agar and subsequent incubation at 35˚C for 24 hours. Quality assurance tests to determine the sterility of the plates included the use of dry BHI agar plates, representatives (10%) of each batch were incubated without incubation at 35˚C for 24 hours and at ambient temperature for 3days. This period was sufficient to allow growth of any fungal or bacterial contaminant on the plate.

2.4. RAPD-PCR Typing

D8635 primer was used for the RAPD-PCR reaction. DNA extraction was achieved by repeated washing, harvesting and resuspension of cells in sterile TE buffer before subsequent boiling in TE buffer for 10 mins at 95˚C. PCR amplifications were performed in 0.2 ml reaction tubes each with 25 μl of mixtures composed of 1uM of primer D8635, 200 μM of dNTPs, 1× of PCR Buffer, 2.5 mM of MgCl$_2$, 2U of Taq polymerase and 1 μl of extracted solution of enterococcal DNA. PCR condition adopted involved an initial cycle of 94˚C for 2 min; 35 cycles of 94˚C for 1 min, annealing at 46.9˚C for 1 min, 72˚C for 1.5 min and a final extension step of 72˚C for

10 min. A 10ul volume of each PCR product was mixed with 2 μl of loading dye and subjected to a 1% TAE buffer electrophoresis system. In each run, a molecular weight marker (1 kb PCR ladder, Invitrogen) was included. Following photography using a UV transilluminator, images were processes using ImageJ and Phython PyElPh version 2.6.5.

2.5. Determination of Virulence Markers Distribution in Enterococci

Polymerase chain reaction (PCR) assays were applied targeting the virulence determinants aggregation substance (*asa*), cytolysin (*cylA*), enterococcal surface protein (*esp*), gelatinase (*gelE*) [14]. Details of primers are listed in **Table 1**. Multiplex PCR conditions used in this study included an initial activation step at 95°C for 4 mins followed by 30 cycles of denaturation at 94°C for 1min, annealing at 55°C for 1min, extension at 72°C for 1 min and a single cycle of 7mins at 72°C. PCR amplicons of *asa*, *cylA*, *esp*, *gelE* from the separate recovered strains were confirmed by DNA sequencing with an ABI 3130XL 20 genetic analyzer (Applied Biosystems). The DNA sequences were blasted for sequence similarity to annotated sequences at http://www.ncbi.nlm.nih.gov.

2.6. Resuscitation Experiments

Following an observation of CFU/ml values of enterococcal counts lower than 0.1 for any of the mesocsms, resuscitation experiments was carried out after 5weeks. As described by leo *et al.* [15], ten 1-ml of each 10-fold dilution of microcosm were placed in 9 ml of TSB medium and the suspensions incubated at volumes of undiluted microcosm and ten 1-ml volumes room temperature for five days. All cultures in which visible turbidity appeared, were spread onto S&B agar and colonies subsequently confirmed to detect any possible contaminant. All culture tubes with visible growth were scored and a probability table [16] was used to calculate the MPN. For all mesocsms that was subjected to resuscitation experiments, sand samples were also analysed to check if occurrence of VBNC bacteria were substrate dependent (beach sand) or existed largely unbound but suspended in the water column. Sediment samples were processed by adding 10 g (wet weight) of sediment to 100 ml of buffered water followed by sonication of the mixture for 30 s at 200 rev/s. The sample was then allowed to settle for 10 min before subjecting the supernatant to membrane filtration.

2.7. Decay Rate Calculations

Decay rates of culturable enterococci concentrations were calculated as described by Anderson *et al.* (2005) using the standard exponential growth/decline equation (Equation (1)):

$$r = \left[In(N_t) - In(N_o) \right] / t \qquad (1)$$

where r = decay rate, N_t = $\log_{10}$ (CFU 100 ml^{-1}) at time t, N_0 = $\log_{10}$ (CFU 100 ml^{-1}) at time zero, and t = time (in days). Time (t) was determined by the days between the first sampling event and either the last sampling event or when culturable cells could no longer be detected. On the whole, a negative r depicts a decrease in CFU (net cell die off), while a positive r connotes an increase in CFU (growth). An absolute value of the decay rate was taken as the magnitude of change in culturable concentrations.

2.8. Statistical Analysis

For each microcosm, the average log-transformed spread plate counts were analyzed by linear regression to calculate the time elapse for a 1-log removal (T$_{90}$). Multivariate linear regressions was used to model the significance of association between water type (marine vs fresh), isolate type (enterococcus vs *E. coli*) and strain classification (environmental vs. clinical) on survival using Stata (Stata Corp. USA). $P < 0.05$ was considered statistically significant.

3. Results and Discussion

Culturable faecal coliform (FC), epidemic, clinical, faecal and recreational beach enterococci strains possessing putative virulence genes (**Table 2**) were enumerated over the course of 5 weeks to comparatively assess their persistence in tropical marine and fresh waters. The intentional addition of epidemic and potentially virulent

Table 2. Description of Strains used for Mesocosm inoculation.

Organism	Strain	Collection date	Source	Enterococci virulence marker			
				esp	*asa*	*gel*	*cyl*
Pathogenic							
E. faecalis	MMH594	1999*	Clinical	+	+	+	+
E. faecalis	VRBW23	2013	Clinical	+	+	+	+
E. faecalis	UT27	2013	Urinary catheter	+	+	+	+
E. faecalis	UT44	2013	Urinary catheter	−	+	+	+
Commensal							
E. faecalis	FA7	2013	Human faeces	+	−	+	−
E. faecalis	FA12	2013	Human faeces	+	−	+	−
E. faecalis	BWE16	2013	Marine beach water	+	+	+	−
E. faecalis	BWE24	2013	Marine beach water	+	−	+	−
E. coli	FAE12	2013	Human faeces	NA	NA	NA	NA

NA: not applicable; *although collected as laboratory stock culture in 2013.

strains to natural waters could pose considerable risks to human health. Additionally, assessment of differential survival of these strains would present considerable challenges especially with continued diffuse source inputs of indicator organisms. For these reasons, the study was conducted using a mesocosm setting.

All tested *E. faecalis* isolates were confirmed by ddl gene sequencing (**Figure 1**). All the mesocosms were inoculated with exponentially grown enterococci cells at a final concentration of 10^7 cfu/ml. This was in line with previously reported published information that starved and stationary cells have superior survival abilities and multi-stress resistance compared to growing cells [17]. The RAPD fingerprint of the enterococci strains by the second week up to the 23rd day had disappeared with only a maximum of two bands remaining. This was in concert with Liu *et al.* [18]. However, morphological features, detection of ddl and virulence gene carriage of the enterococci strains didn't change up till the 23rd day of storage. Repeated subculturing or long-term storage has been documented to cause significant variations in PFGE profiles of the strains, but the variations does not invalidate epidemiological lineage of the tested strains [19].

Figure 2 presents the survival pattern of a total of eight strains of enterococci and a strain of faecal *E. coli* over a period of 23 days in fresh and marine water. For the clinical and epidemic strains tested, it took 2.38 ± 0.45 days for a 1-log reduction (T_{90}) in marine water. A higher T_{90} average of 2.51 ± 0.08 was observed for the commensal and environmental strains. It is difficult to make comparisons of the T_{90} obtained as there is a dearth of published information on the survival of pathogenic enterococci in tropical fresh or marine waters. Lleo *et al.* [15] reported survival of a single clinical strain of *E. faecalis* 56R but did not highlight the T_{90} value for the tested strain. In line with other survival studies however, the reduction rate in the absence of light followed the first order kinetics (y = mx + b) [20] [21]. Generally, lower T_{90} values of 2.14 ± 0.26 and 2.15 ± 0.16 days respectively were observed for hospital and community acquired enterococci strains in fresh water mesocosms subjected to tropical ambient temperature in our study.

Beach water enterococci and enterococci recovered from faeces of humans survived for up to 20 days and 23 days respectively in fresh and marine waters (**Figure 2(c)** and **Figure 2(d)**). Comparing cell counts for day 1, *E. coli* isolated from human faeces survived longer in fresh water than in marine water. Also, clinical strains isolated from urinary tract infection patients (UT1 and UT44) could no longer be cultured at days 17 and day 20 respectively when inoculated into fresh and marine waters (**Figure 2(a)**). The epidemic (MMH594) and the vancomycin resistant (VRBW23) strains were no longer culturable at 20 days when inoculated in fresh and marine waters (**Figure 2(b)**). The similar number of days of survival as was observed for environmental strains tested suggest that the epidemic strain, MMH594, an esp-positive clinical bacteremia isolate that previously caused multiple infections in a hospital ward outbreak [22] fares favourably well in marine and fresh aquatic environments in tropical settings.

Presented in **Table 3** is the correlation coefficient (R^2) and die off rate for all tested strains of enterococci. For enterococci, the decay rate was approximately 13% higher in fresh water than was observed for marine water. On the contrary, for *E. coli*, the decay rate was approximately 17% lower in fresh water than was observed in marine water. The observation of higher decay rate of *E. coli* in marine water as opposed to the comparatively lower decay rate of enterococci in marine water is in agreement with previous studies [23].
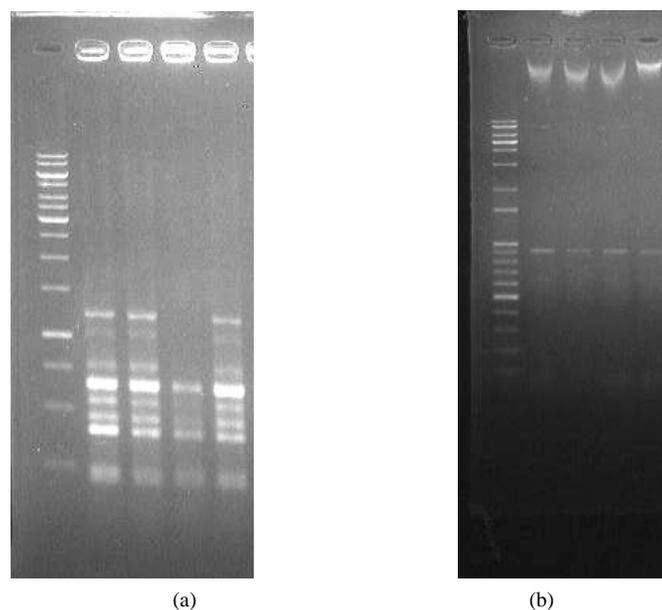

(a)   (b)

Figure 1. Panel (a) Molecular typing of an isolate from each group of *E. faecalis* by RAPD-PCR. Reference: Gel 1% agarose in buffer TAE 1X buffer. Panel (b) Identification of *E. faecalis* using ddl gene (900 bp). Reference: Gel 1% agarose in buffer TAE 1X buffer. Panel (a) and (b) Lane 1: 1 kb DNA ladder, Lane 2: *E. faecalis* FAE7, Lane 3: *E. faecalis* BWE24, Lane 4: *E. faecalis* UTI44 and Lane 4: *E. faecalis* UTI44 and UTI27.

Table 3. Regression results, die off rate and T90 removal time for the tested enterococci and *E. coli* strains in fresh and marine water at tropical temperature condition.

Isolate	Temp (˚C)	Marine water			Fresh water		
		R^2	T_{90} (days)	k (day^{-1})	R^2	T_{90} (days)	k (day^{-1})
MMH594	29.5	0.83	1.88	0.53	0.62	2.13	0.47
VRBW23	29.5	0.69	2.41	0.41	0.49	2.49	0.40
UT27	29.5	0.71	2.97	0.34	0.89	1.87	0.53
UT44	29.5	0.78	2.25	0.44	0.87	2.07	0.48
T_{90} Average + SD			2.38 ± 0.45			2.14 ± 0.26	
FA7	29.5	0.83	2.55	0.39	0.79	2.12	0.47
FA12	29.5	0.78	2.59	0.39	0.69	2.01	0.50
BWE16	29.5	0.80	2.51	0.40	0.62	2.09	0.48
BWE24	29.5	0.79	2.40	0.42	0.68	2.38	0.42
T_{90} Average + SD			2.51 ± 0.08			2.15 ± 0.16	
E. coli FAE12	29.5	0.91	2.14	0.47	0.64	2.58	0.39

R2—regression correlation coefficient, T_{90} is the time needed or 1-log reduction, k—die off rate.
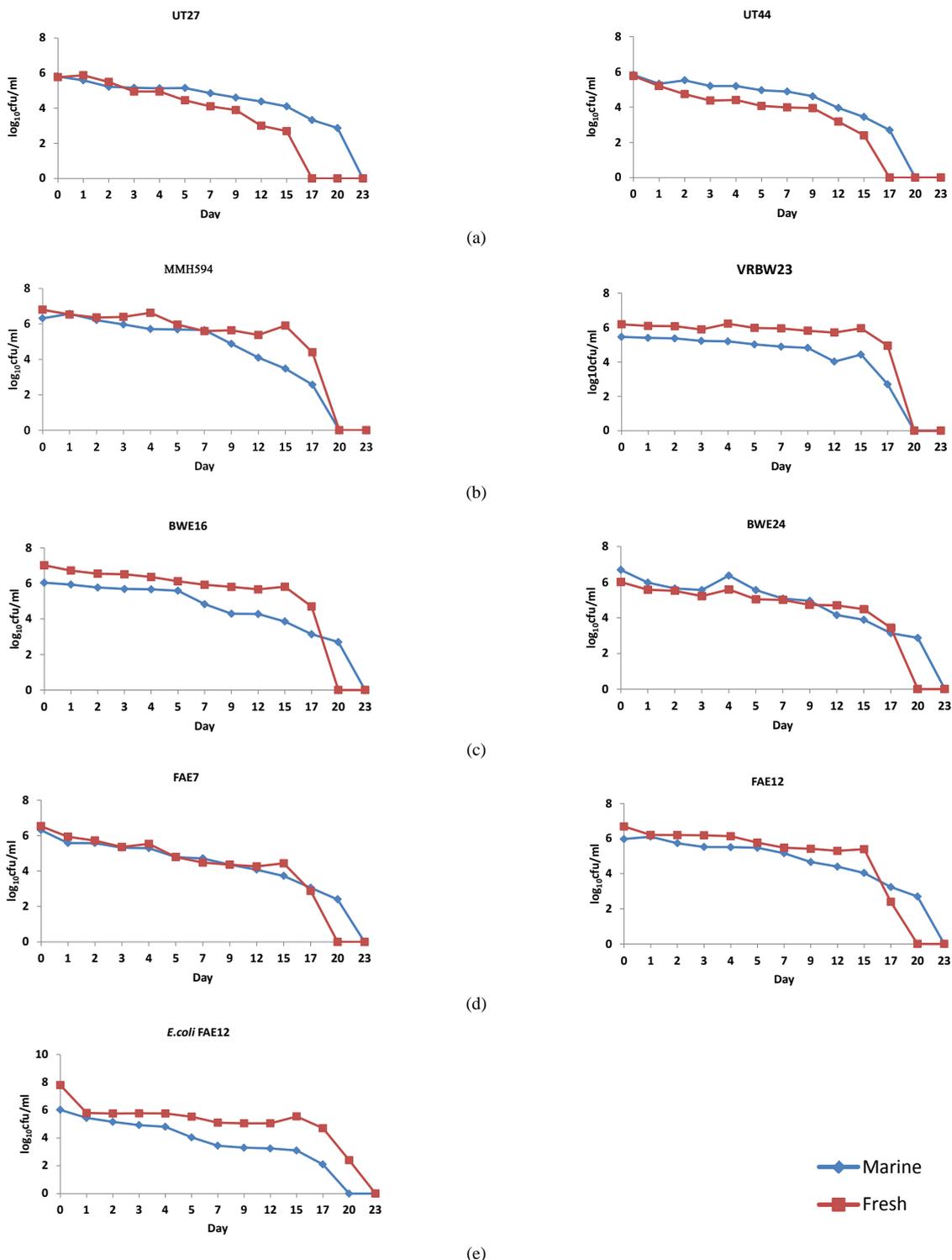
Figure 2. (a) Survival of enterococci isolates recovered from urinary tract infections in marine and fresh water at 29 C; (b) Survival of epidemic and vancomycin resistant enterococci isolates in marine and fresh water at 29 C; (c) Survival of recreational beach water enterococci isolates in marine and fresh water at 29 C; (d) Survival of enterococci isolates recovered from human faeces in marine and fresh water at 29 C; (e) Survival of *E. coil* isolate recovered from human faeces in marine and fresh water at 29 C. Spread plate counts obtained for each time point days were averaged and log-transformed. A linear regression was calculated for each of the mcrocosms to assess the time sufficient to achieve a 1-log reduction.

A multivariable linear regression analysis of the bacterial cell count was conducted for each time water type (marine versus fresh water), strain type (clinically versus environmental), organisms type (enterococci versus *E. coli*). The analysis revealed that the survival of all tested strains of enterococci and *E. coli* were significantly associated with the water type (fresh versus marine) (p = 0.0004). On the whole, the population trends of *E. coli* and enterococci in fresh and marine water mesocosms did not reveal any evidence of growth. This is in line with the submission of a recent study in a tropical country setting with reported temperature of 29°C [24]. However this finding is contrary to the findings obtained in previous reports from subtropical environments in the US [25] [26]. The observation is also contrary to another study which reported that *E. coli* and *E. faecium* multiplied in autoclaved river water [27]. Comparison is difficult because this study was conducted using a temperature (15°C) below the ambient temperatures in tropical locations while marine beach water was not included in the study design.

Our observations also revealed that saline water had a negative effect on the persistence of *E. coli*. This was highlighted as the decay rate of *E. coli* in marine water was higher than was observed for freshwater. For *E. coli* and all strains of enterococci tested, persistence appeared to be encouraged in sediments as bacteria cells were cultural two weeks after culturable bacteria was not detected in the water column. The average decay rate of enterococci strains from the clinical and epidemic strains was higher for fresh water than was observed for marine water indicating higher persistence of enterococci in marine water. This was also in agreement with the T_{90} values with higher T_{90} values among clinical strains in marine water as compared to fresh water.

In a previous study, bacteria introduced in the culture medium remained culturable for more than three months at room temperature [15]. According to previous studies [15] [28] [29], during this period of nutrient limiting period, they may activate a survival or starvation mode to resume active division following the emergence of more favourable conditions. This was demonstrated in our recent report as starved cells resumed active growth after restoration of nutrients but produced biofilms at lower efficiency as compared to actively growing cells that were not subjected to starvation [24]. In our study, culturable bacteria were not detectable in samples from all fresh and marine water mesocosms beyond 23 days. There is no published information on a comparative assessment of the survival of epidemic and environmental strains of enterococci at a temperature of 29°C ± 1°C which is common at tropical beaches all year round. As this temperature prevails in Malaysia and a number of countries in the Asian south east region, there is the need to assess location specific scenarios for the survival and persistence of faecal, commensal or clinical enterococci in available surface waters. This is particularly needed in Asia and similar tropical locations where surface water contamination present strong linkages with waterborne diseases [30].

In a recent study [24] that attempted to highlight an appropriate indicator organism for tropical beach water monitoring, enterococci concentrations negatively correlated with temperature as opposed to the positive correlation reported in some non-tropical countries. Ultraviolet radiations, light exposure in addition to temperature are important drivers of enterococci concentrations in tropical aquatic environments. The effect of ultraviolet radiation was however not tested in our study. Lleo *et al.* [15] reported that when cells in a mesocosm were left at room temperature in a non-tropical environment or at 4°C without direct light, they did not reach the non-culturable state but maintained their culturability for periods as long as two months indicating that they activated starvation instead of the VBNC (viable but not culturable) state. In tropical settings however, our findings suggest that a combination of tropical room temperature without direct light produced the non-culturable state after 20 days indicating that the activated the VBNC state rather than the starvation mode as was observed in the mesocosm study in a non-tropical setting reported by Lleo *et al.* [15].

While a range of temperature (10°C - 18°C) is recognised and reported as ambient temperatures for a number of studies that focused on the survival of indicator organisms in the environment, conditions in humid tropical settings may present different influence on population dynamics of microbes in these locations. Two fundamental issues thus arise: year round humid conditions and the high temperatures. Previous studies [21] [24] assert that temperature differences in tropical climates may influence the survival of indicator organisms in these environments. For instance, a study in Malaysia reported negative correlation of enterococci with temperature [23]. In another study, it took 10.97 + 3.47 days to achieve 1-log reduction (T_{90}) for methicillin resistant *Staphylococcus aureus* strains in marine water kept at 13°C. For a temperature higher by 7°C, a T_{90} value of 7.89 + 1.62 days was recorded. In Malaysia and neighbouring countries in the equator, a much higher ambient temperature of 29°C would undoubtedly exert its influence on the T_{90} values. These are important considerations in the design of beach water quality surveillance programs. The second consideration for similar countries in the equator

is that of all year round humid weather conditions prevalent in these locations. Arguably, more rainfall events in terms of frequency and intensity comes along with increased intensity and frequency of discharge of storm water run offs and combined sewer overflows (CSO) into recreational water available. Increased frequency and intensity of stormwater runoff in addition to pollution from diffuse sources may present a steady supply into recreational water of organic matter-rich nutrients along with non-indigenous faecal indicator organisms which nullifies the diminishing effect of temperature on indicator organism population dynamics in the tropics. This assumption however needs to be cautiously interpreted and validated in future studies particularly bearing in mind the differences in organic carbon constituent of the stormwater runoff in these tropical settings. Nonetheless, Anderson *et al.* [23] reported lowest decay rates (greatest persistence) in mesocosms inoculated with contaminated soil and waste water containing variable amounts of organic matter. Similar findings were reported by Fujioka and Unutoa [31] and Masmoudi *et al.* [32].

A limitation in the current study was the restriction of the variables tested to three viz: strain type, water type and organism type. The survival of bacteria in environmental waters is affected by a complex array of physical, chemical and biological factors that are often difficult to simulate in the laboratory [23] [33].

4. Conclusion

Our results show that epidemic, clinical, faecal and recreational beach enterococci strains containing putative virulent markers survived in marine and fresh waters for up to but not beyond 23 days in tropical temperatures (29˚C) while retaining their virulent markers for this period. Also, cells recovered from the sediment below the water column still retained their virulence markers. Potentially pathogenic bacteria can resume active growth after 3 weeks of being harboured by the reservoir-beach sand and still pose threat to public health.

Acknowledgements

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