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Microbiological quality of some Recreational Beaches along the Shoreline of Lagos State, Nigeria

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Abstract

Water and sand samples from selected recreational beaches in Lagos shoreline from East to west axis, Eleko (ELK), Lekki (LEK), Takwa Bay (TAK) and Badagry (BDG) as well as Marina and Badagry Jetties were purposely collected using standard microbiological methods. Fecal indicator bacteria (FIB) - *E. coli* and *Enterococcus*, were isolated using USEPA methods 1603 and 1600 respectively. Pathogens (*Vibrio parahaemolyticus*, *V. vulnificus*, *Salmonella*, *Citrobacter*, *Serratia*, *Pseudomonas* and *Aeromonas*) as indicative bacteria of public health significance were isolated using membrane filter techniques and plating on appropriate selective media. Higher counts of FIB were isolated from sand samples compared to beach water sampled while Marina and Badagry Jetties recorded highest faecal counts. *V. parahaemolyticus* was detected in all beaches sampled while *V. vulnificus* and *Salmonella* were detected in 4 sampling sites. *Aeromonas* was detected from both water and sand samples of 6 sampling sites while *Citrobacter* was detected in seawater samples from all beaches sampled. Detection of *E. coli*, *enterococci*, *Vibrio*, *Salmonella*, *Pseudomonas*, *Serratia*, *Citrobacter* and *Aeromonas* in this survey showed that there is environmental health risk to the beach goers and suggest the importance of routine microbiological monitoring of the beaches for precautionary and public health measures.

Keywords: sand, seawater, recreational beach, fecal indicator bacteria

1. Introduction

High rate of coastal development and increased recreational use of beaches have resulted in greater threats of water contamination and the associated public health hazard. While indicator bacteria may be present in the swimming water, sediment and sand along the water's edge may also be a significant source of microbes (US EPA, 1999).

It is estimated that the polluted coastal waters, contaminated with fecal matter, cause more than 120 million cases of gastrointestinal illness and 50 million cases of respiratory disease, each year, worldwide (Halliday and Gast, 2011). Most countries implement water quality monitoring programs to protect the bathers' health. Analyses are performed to quantify fecal indicators and verify the compliance in accordance with threshold limits established by legal regulations. Currently, no Federal or State guideline is available for assessing risk of illness from water and sand at recreational beaches in Nigeria.

The microbiological quality of bathing water is one of the indicators available to users, regarding the beach's environmental and sanitary quality. However, sands and sediments of the coastal zone may also provide a habitat where microorganisms can persist, and even thrive upon the right conditions (Halliday and Gast, 2011). Although the water contact surface with the human skin and mucosa is greater than that of the sand, many swimmers remain longer in contact with the sand than with the water.

Beach sands provide a habitat for a wide range of organisms whose activity is strongly influenced by the physical and chemical properties they are exposed to (Heritage *et al*., 1999). Most of the microorganisms present in the sand are of environmental origin, but also of animal or human origin, among which bacteria, fungi, parasites and viruses are included. In this diversified group, some organisms are potential pathogens by direct contact. Therefore, concern with the sand as a reservoir or vector of infection has been already admitted by the

World Health Organization (World Health Organization, 2003). Pathogens on beaches can have several origins, but the majority comes from exogenous sources, normally associated with human activity (Halliday and Gast, 2011; Heaney *et al*., 2009).

Centers for Disease Control reported that the incidence of infections associated with recreational water have steadily increased over the past several decades, as a result of emerging pathogens, increases in aquatic activities and better disease reporting (Halliday and Gast, 2011). Many microorganisms have been isolated from the sand as well. A number of species and genera of these microorganisms are potential pathogens and feasibly can come into contact with humans through sand (Emmanuel *et al*., 2014).

Recreational uses of inland and marine waters are increasing in many countries worldwide. These uses range from whole-body water contact sports, such as swimming, surfing, and slalom canoeing, to noncontact sports, such as fishing, walking, bird watching and picnicking. The hazards that are encountered in recreational water environments vary from site to site, as do the nature and extent of exposure. Most available information relates to health outcomes arising from exposure through swimming and ingestion of water. Indicators are information sets which are formally selected to be used on a regular basis to measure changes that are of importance for tourism development or management (UNWTO, 2004).

Lagos State, Nigeria (latitudes 6°23'N and 6°41'N and longitudes 2°42'E and 3°42'E) is located in South-western part of the country on the West Coast of Africa. It is flanked from the north and east by Ogun State, to the west by the Republic of Benin and bounded southward by the Atlantic Ocean (Gulf of Guinea). Lagos Tarkwa-Bay is a key location that opens the Atlantic Ocean as a source of salt water incursion to the Lagos lagoon, Apapa and Tin can island through the Lagos harbor. Fronting the Atlantic Ocean / Gulf of Guinea, the spectacular sandy coastline of Nigeria stretches for more than 700 km / 435 miles and is home to both the Slave Coast and the Niger Delta.

The study provides data about microbiological quality of some marine beach water and sand along Lagos shoreline. This may be helpful for government and local authorities in developing monitoring, assessment plans and policies for safer and heathy beach for beach goers.

2. Materials and Methods

2.1 Sample Site Selection

A total of 10 sampling locations were selected for this study, this was based on their geographical location in the state (South, West and Central). Table 1 describes the selected locations. pH, salinity and water temperature were measured using hand held YSI Professional Plus Multiparameter Instrument (YSI Incorporated, Yellow Spring, Ohio, USA). Dry and wet sand samples temperature were measured with hand held thermometer.

2.2 Sample Collection

Beach water and sand samples were collected according to World Health Organization Manual for Recreational Water and Beach Quality Monitoring and Assessment (WHO, 2000). Sterile 500ml Nalgene polyethylene bottles were used to collect water and sand samples. Wet sand samples were collected from intertidal zone while dry sand sample were collected along the sampling transect above the high-tide line. All samples were transported to the Lab for analysis on ice.

Water samples were collected while the sampler was standing at chest level (about 1.3m), the lid of the bottle was removed without touching the mouth of the bottle. The bottle was turned upside down and lowered below the surface with a smooth movement to avoid sediments. The bottle was then turned so that the mouth was pointing upward, and when the bottle was approximately 2/3 filled, it was lifted above the surface and the lid placed back on the bottle (WHO, 2000).

Sterile, wide mouthed, disposable plastic containers (500 ml) were used to collect sand samples from the swash zone. The lid of the bottle was carefully removed, and the bottle was inverted and forced into the sand. In order to ease the removal of the bottle with the sample, a large spatula was used to remove the surrounding sand. The bottle was then pulled together with the samples. Samples were stored on ice until analyzed.

Table 1: Sample Location and Coordinates

Location	**Coordinates**
Eleko Beach 1	6° 26' 16.94" N
	3° 51' 53.38" E
Eleko Beach 2	6° 26' 18.49" N
(Obadore)	3° 51' 19.84" E
Lekki Beach 1	6° 25' 21.70" N
(Mayegun)	3° 30' 58.88" E
Lekki Beach 2	6° 26' 19.58" N
(Mayegun)	3° 30' 25.88" E
Takwa Bay 1	6° 24' 05.14" N
	3° 23' 45.91" E
Takwa Bay 2	6° 23' 37.58" N
(Light House)	3° 23' 46.12" E
Marina Jetty	6° 25' 29.90" N
	3° 24' 24.75" E
Badagry 1	6°23'26.85"N
	2°49'55.83"E
Badagry 2	6° 23' 28.75" N
	2° 50' 23.15" E
Badagry Jetty	6° 24' 25.43" N
	2° 52' 55.32" E

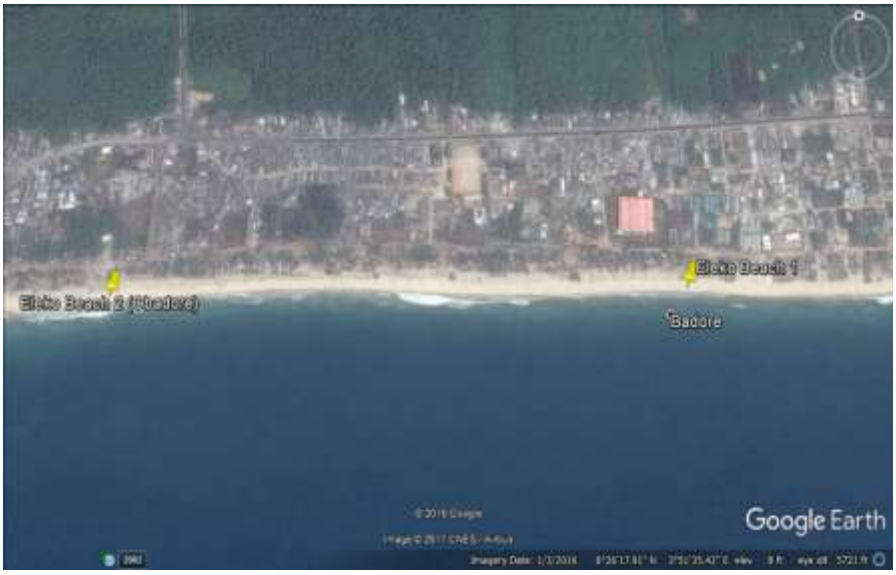

Fig 1: Eleko Beach Sampling Stations 1 and 2. Maps not to scale. Image was accessed through Google Earth.

Fig 2: Lekki Beach Sampling Stations 1 and 2. Maps not to scale. Image was accessed through Google Earth.

Fig 3: Takwa Bay Beach sampling stations 1 and 2 as well as Marina Jetty. Maps not to scale. Image was accessed through Google Earth.

Fig 4: Badagry Beach sampling stations 1 and 2 as well as Badagry Jetty. Maps not to scale. Image was accessed through Google Earth.

2.3 Methods Used to Analyze Microorganisms

Water and sand samples collected during this study were analyzed for level of *E. coli*, enterococci, *Vibrio*, *Salmonella*, *Citrobacter*, *Pseudomonas*, *Aeromonas* and *Serratia*. For all assay, microorganisms from sand aliquots were eluted using ratio of eluant volume to sand mass of 10:1 (Boehm *et al*., 2009). The mass of sand eluted was 10g. Sand was eluted by a previously described method of shaking by hand (Boehm *et al*., 2009), using sterile saline (0.85% w/v). In summary, 10 g of sand was placed in a presterilized 250-ml polypropylene bottle, 100 ml of eluant was added and shake for 2 minutes by hand over an arc of about 10 cm. Following a 30-

seconds settling time, the eluant was decanted into a second sterile bottle by pouring, taking care to leave the sand behind. Eluant was membrane filtered through 0.45µm pore size EZHAWG filters (Millipore, Bedford, MA, USA).

2.4 Microbial Analyses

EPA methods 1603 (US EPA, 2006a) and 1600(US EPA, 2006b) were used to enumerate *E. coli* and *Enterococci* respectively. Three, 30 and 50ml volume of sand eluant was used to ensure countable number of colonies on all assay. The EPA method 1603 for isolation of *Escherichia coli*, is a single-step method that uses one medium, modified mTEC Agar, and does not require the transfer of the membrane filter to another medium or other substrate. The modified medium contains a chromogen (5-bromo-6-chloro-3-indolyl-β-D-glucuronide), which is catabolized to glucuronic acid and a red-or magenta-colored compound by *E. coli* that produce the enzyme β-D-glucuronidase. Preparation of modified mTEC agar was according to manufacturer instruction. Also, CHROMagar *E. coli* (CHROMagar, Paris, France) was also used for confirmation of *E. coli* isolates. Plates were incubated at 44.5^{o}C°C for 18-24hr.

Method 1600 describes a membrane filter (MF) procedure for the detection and enumeration of the enterococci bacteria in water. This is a single-step method that is a modification of EPA Method 1106.1 (mE-EIA). Unlike the mE-EIA method, it does not require the transfer of the membrane filter to another medium. The modified medium has a reduced amount of triphenyltetrazolium chloride (TTC) and includes indoxyl β -D-glucoside, a chromogenic cellobiose analog used in place of esculin. In this procedure, β -glucosidase-positive enterococci produce an insoluble indigo blue complex which diffuses into the surrounding media, forming a blue halo around the colony. mEI agar was used for enterococci enumeration following EPA method 1600. Plates were incubated at 41^{o}C for 18-24hr.

2.5 Salmonella and Citrobacter

One hundred milliliter (100ml) of seawater sample was filtered through a 0.45 µ membrane filter. The membrane filter was placed in enrichment medium (Selenite-F broth) and incubated at 35 ±2°C overnight. Subcultures were made to CHROMagar Salmonella Plus base agars (APHA, 2012). Ten grams of sand sample were inoculated into 90 ml of selenite-F broth and incubated at 35 ±2°C overnight and subcultured onto CHROMagar Salmonella Plus base plates (CHROMagar, Paris, France). Suspect colonies were identified biochemically using API20 E strips (Baron and Finegold, 1990).

2.6 Pseudomonas Count

A 10^{-1} w/v suspension of sand sample based on wet weight was prepared in 0.1% buffered peptone water, thoroughly mixed and serial dilutions were made. Counts were estimated using the plate count method (spread plate method), using Cetrimide Agar. Colonies were identified biochemically using API 20 E Strips (Abdallah *et al*., 2005).

2.7 Vibrio species

One hundred milliliter (100ml) of seawater sample was filtered through a 0.45 µ membrane filter. The membrane filter was placed on selective media (CHROMagar Vibrio and in enrichment medium, alkaline peptone water) incubated at 35 ±2°C overnight. Subcultures were made to onto Vibrio selective agar, CHROMagar *Vibrio* (CHROMagar, Paris, France) (Dumontet *et al*., 2000). Ten grams of sand samples were inoculated in 90 ml of Alkaline Peptone Water (Oxoid), the pH of which was adjusted to 8.6. After incubation at 37°C for 24 h, cultures were streaked onto Vibrio selective agar, CHROMagar Vibrio (CHROMagar, Paris, France) and further incubated for 24 h at 35 ±2°C. The plates were incubated at 35 ±2°C overnight. *V. parahaemolyticus* appeared as round, mauve colonies on CHROMagar. *V.vulnificus* colonies appeared as green blue to turquoise blue. Suspected *V. vulnificus* were subcultured onto mCPC agar (modified cellobiose-polymyxin B -colistin agar). The exact identity was identified using API20E (API system, France) (Dumontet *et al*., 2000).

2.8 Detection of E. coli 0157:H7

E. coli O157:H7 rapidly ferments lactose and is indistinguishable from most other *E. coli* on traditional lactose-containing media. However, unlike approximately 80% of other *E. coli*, nearly all isolates of *E. coli* O157:H7 ferment D-sorbitol slowly, or not at all. Sorbitol-MacConkey (SMAC) agar was developed to take advantage of this characteristic by substituting the carbohydrate sorbitol for lactose in MacConkey agar and is the medium of choice for isolation of *E. coli* O157:H7 (March and Ratnam, 1986). *E coli* isolates were inoculated onto SMAC and incubated for 18-24 hours at 35±2°C. Sorbitol-negative colonies appeared colorless on SMAC. Sorbitol-negative colonies selected from SMAC were tested with E. coliPRO O157 kit (Hardy Diagnostics, Santa Maria, CA 93455, USA) antiserum or latex reagents (O157 antibody-coated latex and control latex) according to the procedures recommended by the manufacturer (March and Ratnam, 1989).

2.9 Detection of Aeromonas and Serratia species

Ten grams of sand samples were transferred into 90 ml of sterile phosphate-buffered saline (pH 7.2), then they were shaken vigorously by hand for 2 min to suspend bacteria. Following 30 min sedimentation, 5 ml and 10 ml of the supernatant were filtered through 0.45µm pore size EZHAWG filters (Millipore, Bedford, MA, USA). 10 ml, 20 ml and 30 ml of collected samples of water were also filtered through 0.45µm pore size EZHAWG filters (Millipore, Bedford, MA, USA). The filters were then aseptically transferred to dish containing Aeromonas Isolation Medium (Himedia) with 30 µg/ml ampicillin as a selective agent to reduce the growth of non-Aeromonas. The plates were incubated for 48 h at 37°C (Mudryk *et al*, 2015).

After incubation, green colonies on plates represent *Aeromonas* spp and were selected for further identification and further analysis. Aeromonas isolates were identified using API 20 E strips (API System, BioMerieux, Paris, France). To discriminate *Aeromonas sobria*, *Aeromonas caviae* and *Aeromonas hydrophila*, two additional tests: hydrolysis of esculin and gas production from D-glucose fermentation were carried out (Trakhna *et al*., 2009). Also, resistance/sensitivity to vibriostatic agent (0/129) was carried out to distinguish *Aeromonas* from *Vibrio.*

Colonies that were pink compared to blue color of *E. coli* on CHROMagar *E. coli* after incubation at 35±2°C overnight were picked for Identification using API 20E system they were identified as *Serratia* species.

3. Results

Sample locations, coordinates and physico-chemical analysis results are summarized in Table 2. Surface water temperature averaged 28.56 (range 26.8 - 31.4^{o}C), wet sand temperature ranged from 30.3^{o}C to 35.5^{o}C (mean 32.5^{o}C), dry sand temperature ranged from 29.6^{o}C to 35.1^{o}C (mean 32.4^{o}C), pH value ranged from 6.8 to 8.0 (average 7.71), and salinity ranged from 16.24ppt to 35.02ppt (mean 30.10ppt). Highest water temperature was recorded at Lekki beach 1 (31.4^{o}C) while the lowest was recorded at Eleko beach 1 (26.8^{o}C). For wet sand temperature, the highest value was found to be 35.5^{o}C at Eleko beach 2 site and the lowest was at Takwa Bay 1 site with the temperature of 30.3^{o}C. Dry sand sample temperature was highest at Eleko beach 1 with the temperature of 34.9^{o}C while the lowest was recorded at Takwa Bay 2 (Light House) beach with the temperature of 30.0^{o}C). Wet sand and dry sand temperature showed no significant differences. The pH of the beach water samples showed no significant difference. The highest pH value was recorded at Badagry beach 2 with the value of 8.0 and the lowest values of 7.8 were recorded at Eleko beach 2, Lekki beaches 1 and 2 as well as Takwa Bay 1. The pH of 7.4 and 6.8 were recorded for Marina Jetty and Badagry Jetty respectively. The highest value of 35.02ppt salinity was recorded at Takwa bay 2 (Light House) beach while the lowest value of 16.24ppt was recorded at Marina Jetty.

Results of bacteria isolated from seawater and sand were summarized in Table 3 and Fig. 5. All study locations exhibited variation in both sand and seawater content of *E. coli* and enterococci counts (Table 3). For *E. coli*, the highest count of 2.0 x 10^4 cfu/100ml was found in water sample from Marina Jetty followed by 1.0 x 10^4 cfu/100ml from Badagry Jetty. Water sample from Eleko Beach 1 has 309 cfu/100ml while dry sand recorded 1,130 cfu/100g and 580 cfu/100g for wet sand. Eleko beach 2 showed counts of 363 cfu/100ml and 3,100 cfu/100g from water and dry sand respectively while 800 cfu/100g was recorded for wet sand. Lekki beach 1 showed the count of 10 cfu/100ml and 465 cfu/100g from water and dry sand respectively, also, 40 cfu/100g was

recorded for wet sand. In contrast, Lekki beach 2 recorded 40 cfu/100ml and 60 cfu/100g from water and dry sand respectively and non-was detected from wet sand. Water, dry and wet sand samples from Takwa Bay 1 recorded 1,272 cfu/100ml, 500 cfu/100g and 705 cfu/g respectively. For Takwa Bay 2 (Lighthouse beach), Water sample showed 50 cfu/100ml while 415 cfu/100g was recorded for dry sand, no viable *E. coli* was detected in wet sand sample. Results of *E. coli* detection from Badagry 1 and 2 locations showed that 20 cfu/100ml was isolated from Badagry 1 site while 38 cfu/100ml was recorded for Bdagry 2. Dry sand samples showed 200 cfu/100g and 106 cfu/g from Badagry 1 and 2 locations respectively. However, for wet sand samples, the counts were 20 and 10 cfu/100g for locations 1 and 2 respectively.

The highest count of enterococci was 2.5 $x10^4$ cfu/100ml from Marina Jetty, this was followed by 3.9 $x10^3$ cfu/100ml for Badagry Jetty. Water samples from Eleko beach 1 and 2 has 577 and 700 cfu/100ml respectively while dry sand showed 620 and 2760 cfu/100g respectively. Counts of 1,240 cfu/ 100g was recorded for Eleko beach 2 wet sand in contrast to 300 cfu/100g from Eleko beach 1. Counts form Lekki beach 1 and 2 locations showed that water samples have 10 and 120 cfu/100ml enterococci count respectively. In addition, Dry sand sample recorded 35 and 380 cfu/100g for locations 1 and 2 respectively. On the other hand, wet sand samples showed 15 and 260 cfu/100g for site 1 and 2 respectively. Results of enterococci from Takwa Bay 1 and 2 locations showed that wet sand sample from Takwa Bay 1 has the highest count of 630 cfu/100g while no viable enterococci was detected in wet sand sample from Takwa Bay 2. Dry sand also showed similar pattern with 580 cfu/100g from Takwa Bay 1 site compared with 220 cfu/100g from location 2. However, 543 cfu/100ml was recorded for water sample form location 1 while 45 cfu/100ml was observed in location 2. Enterococci was also detected in all samples collected at 2 locations in Badagry, counts from water samples from location 1 showed 28 cfu/100ml while 70 cfu/100ml was recorded for location 2. Dry sand samples showed 95 cfu/100g for location 1 and 50 cfu/100g in location 2. Counts for wet sand samples from both location showed 35 cfu/100g.

Highest count of 140 cfu/100ml of *Vibrio parahaemolyticus* was recorded for Lekki beach 2 while 80 cfu/100g were recorded for Eleko beach 1 wet sand, Lekki beach 2 wet sand, Takwa Bay 2 dry sand and Badagry 2 wet sand (Fig. 6). For *Vibrio vulnificus*, highest count was found in Eleko beach 2 with the count of 50 cfu/100ml. *Vibrio parahaemolyticus* was detected at all the beaches studied. *V. parahaemolyticus* was detected in 7 of the 8-marine beach water tested, representing 87.5% while 6 of the dry and wet sand was found to habour the same bacteria, representing 75%. However, *V. vulnificus* was only detected in 4 water samples and 2 dry sand sample representing 50 and 25 % respectively. *V. vulnificus* was not detected in any of the samples collected form Eleko beach 1, Lekki beach 2, Badagy 1 and Badagry 2 beaches. Also, *V. parahaemolyticus* and *V. vulnificus* were not detected from any of the Jetty studied.

Table 2: Sample Location and Description

Location	Coordinates	Water Temp. (°C)	Wet Sand Temp. (°C)	Dry Sand Temp. (°C)	pH (Water)	Salinity (ppt)
Eleko Beach 1	6° 26' 16.94" N 3° 51' 53.38" E	26.8	32.0	34.9	7.9	33.32
Eleko Beach 2 (Obadore)	6° 26' 18.49" N 3° 51' 19.84" E	30.0	35.5	35.1	7.8	33.29
Lekki Beach 1 (Mayegun)	6° 25' 21.70" N 3° 30' 58.88" E	31.4	34.4	33.9	7.8	33.67
Lekki Beach 2 (Mayegun)	6° 26' 19.58" N 3° 30' 25.88" E	31.1	34.3	33.8	7.8	34.01
Takwa Bay 1	6° 24' 05.14" N 3° 23' 45.91" E	28.0	30.3	29.6	7.8	29.93
Takwa Bay 2 (Light House)	6° 23' 37.58" N 3° 23' 46.12" E	27.1	30.5	30.0	7.9	35.02
Marina Jetty	6° 25' 29.90" N 3° 24' 24.75" E	28.6	NA	NA	7.4	16.24
Badagry 1	6°23'26.85"N 2°49'55.83"E	26.9	31.4	31.1	7.9	33.98
Badagry 2	6° 23' 28.75" N 2° 50' 23.15" E	27.0	31.4	31.0	8.0	34.09
Badagry Jetty	6° 24' 25.43" N 2° 52' 55.32" E	28.7	NA	NA	6.8	17.48

NA= Not Applicable

Table 3: Bacteria Isolated from Seawater and Sand (cfu/100ml water and cfu/100g sand)

Location	Sample	*E. coli*	Enterococci	*V. paraheamolyticus*	*V. vulnificus*	*Salmonella*	*Serratia*	*Pseudomonas*	*Aeromonas*
Eleko Beach 1	Water	309	577	40	ND	ND	20	ND	ND
	Dry Sand	1,130	620	20	ND	ND	ND	ND	ND
	Wet Sand	580	300	80	ND	ND	ND	80	ND
Eleko Beach 2	Water	363	700	60	50	ND	ND	ND	40
(Obadore)	Dry Sand	3100	2760	ND	10	ND	ND	ND	ND
	Wet Sand	800	1240	10	ND	20	ND	ND	ND
Lekki Beach 1	Water	10	10	ND	30	ND	20	ND	30
(Mayegun)	Dry Sand	465	35	ND	10	ND	ND	20	60
	Wet Sand	40	15	60	ND	ND	ND	ND	ND
Lekki Beach 2	Water	40	120	140	ND	ND	ND	ND	40
(Mayegun)	Dry Sand	60	380	40	ND	ND	80	ND	10
	Wet Sand	ND	260	80	ND	ND	ND	ND	ND
Takwa Bay 1	Water	1272	543	60	10	ND	ND	ND	60
	Dry Sand	500	580	70	ND	20	40	ND	40
	Wet Sand	705	630	10	ND	ND	ND	ND	ND
Takwa Bay 2	Water	50	45	40	20	ND	ND	ND	20
(Light House)	Dry Sand	415	220	80	ND	10	ND	ND	ND
	Wet Sand	ND	ND	40	ND	ND	ND	ND	ND
Marina Jetty	Water	20,000	25,000	ND	ND	ND	ND	ND	ND
Badagry 1	Water	20	28	40	ND	ND	ND	ND	ND
	Dry Sand	200	95	60	ND	20	ND	ND	ND
	Wet Sand	20	35	ND	ND	ND	ND	ND	ND
Badagry 2	Water	38	70	60	ND	ND	ND	ND	ND
	Dry Sand	106	50	80	ND	ND	ND	ND	ND
	Wet Sand	10	35	ND	ND	ND	ND	ND	ND
Badagry Jetty	Water	10,000	3900	ND	ND	ND	40	ND	20

ND= Not Detected

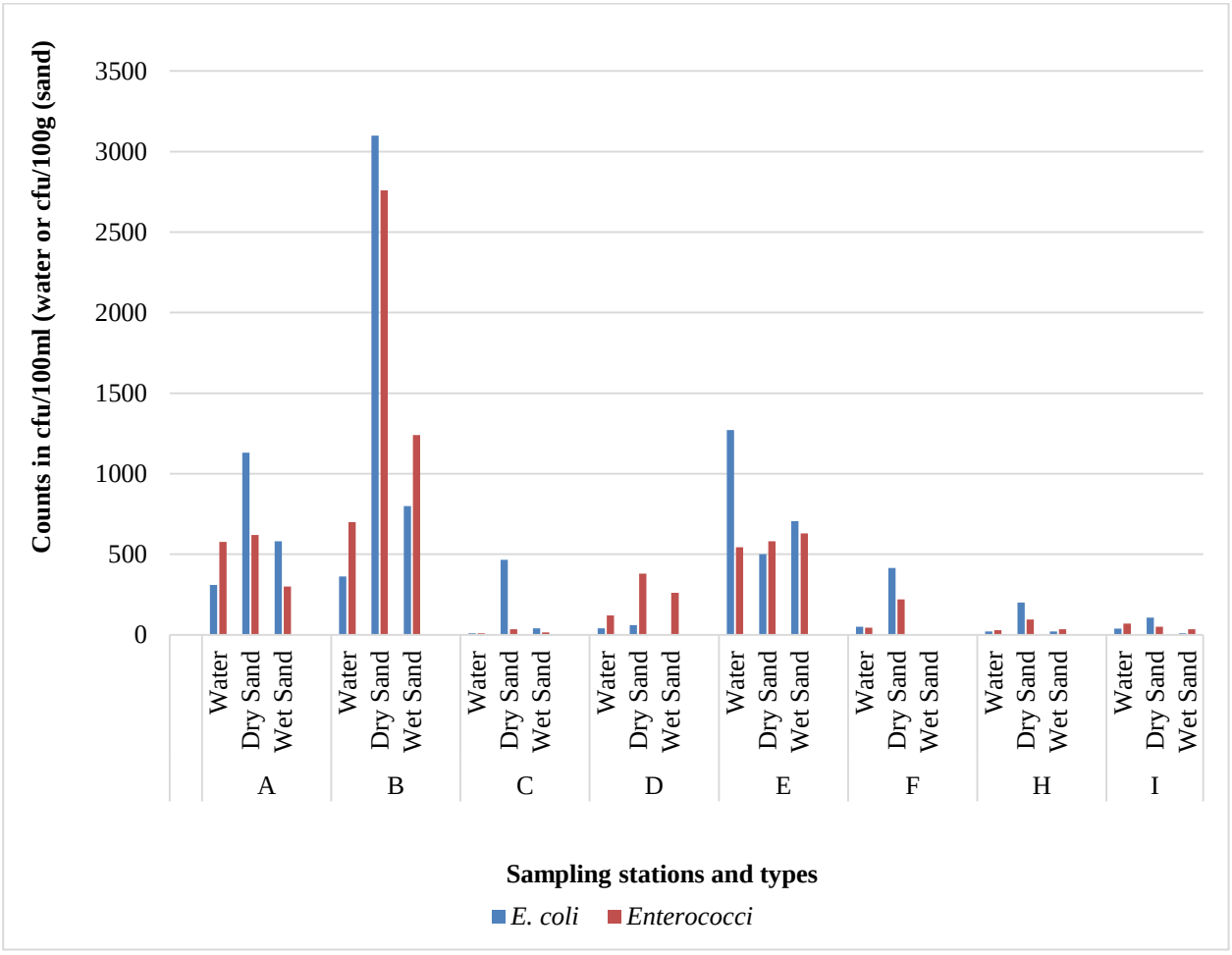

Fig 5: *E. coli* and *Enterococci* counts from beach samples.

Sampling Station key:

A = Eleko Beach 1 B = Eleko Beach 2 (Obadore)

C = Lekki Beach 1 (Mayegun) D = Lekki Beach 2 (Mayegun)

E = Takwa Bay 1 F = Takwa Bay 2 (Lighthouse)

I = Badagry 2 H = Badagry 1

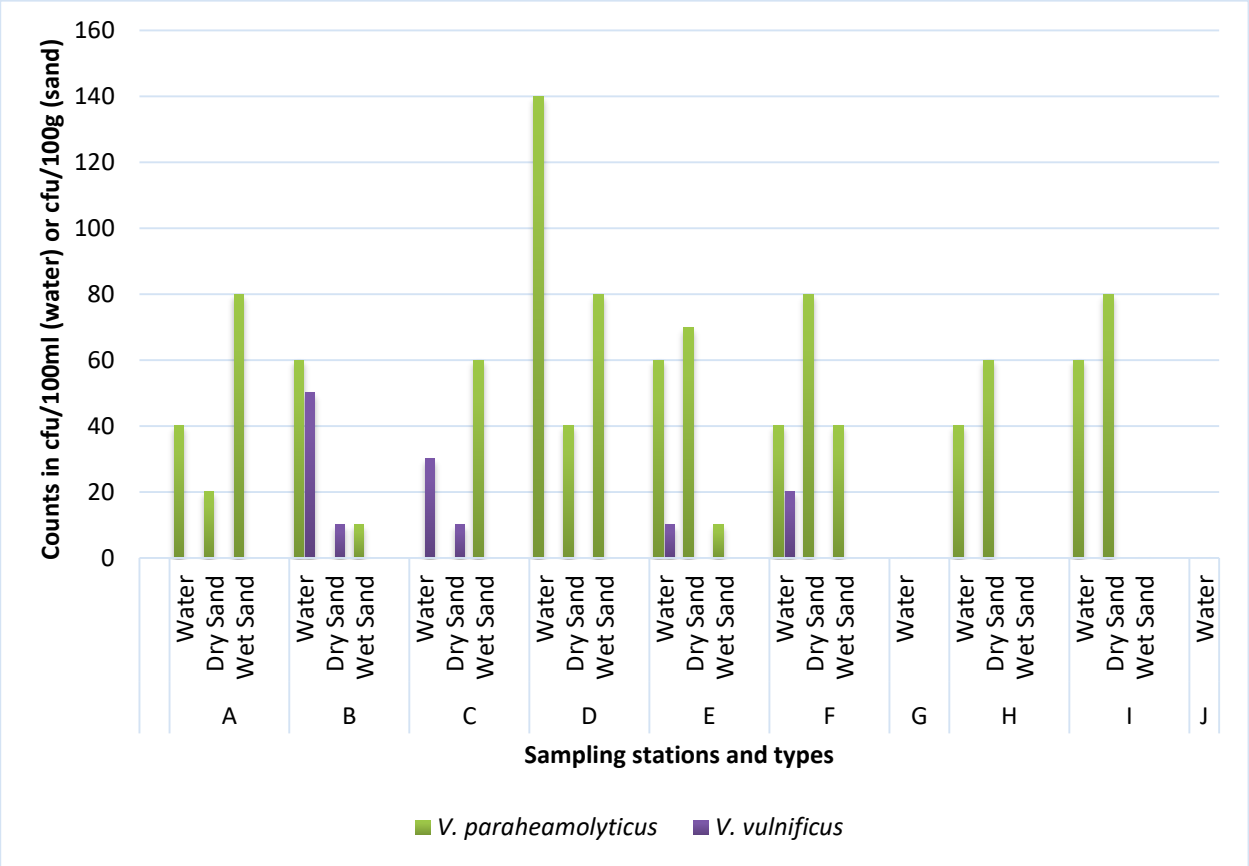

Fig 6: *Vibrio* counts from beach and Jetty samples

Sampling Station key:

A = Eleko Beach 1 B = Eleko Beach 2 (Obadore)

C = Lekki Beach 1 (Mayegun) D = Lekki Beach 2 (Mayegun)

E = Takwa Bay 1 F = Takwa Bay 2 (Lighthouse)

G = Marina Jetty H = Badagry 1

I = Badagry 2 J = Badagry Jetty

Salmonella species were isolated for 4 sampling stations, namely, Eleko beach 2 (wet sand) 20 cfu/100g, Takwa Bay 1 (Dry sand) 20 cfu/100g, Takwa Bay 2 (dry sand) 10 cfu/100g and Badagry 1(dry sand) 20 cfu/100g (Table 3). *Serratia* species was isolated in Eleko beach 1 water sample (20 cfu/100ml), Lekki beach 1 water sample (20 cfu/100ml), Lekki beach 2 dry sand sample (80 cfu/100g), Takwa Bay 1 dry sand sample (40 cfu/100g) and Badagry Jetty water sample (40 cfu/100ml). *Pseudomonas* species was isolated from 2 locations, Eleko Beach 1 wet sand with the count of 80 cfu/100g and Lekki beach 1 dry sand with the count of 20 cfu/100g. *Citrobacter* species was isolated from water samples from Badagry 2, Takwa Bay 1, and Marina Jetty. Also, it was detected from wet sand samples of Takwa Bay 1, Takwa Bay 2, Eleko 1, Eleko 2 and Lekki 1 sites; dry sand samples of Takwa Bay 1, Eleko 1 and Eleko 2 sampling locations.

Aeromonas species was detected from Eleko beach 2 water sample (40 cfu/100ml), Lekki beach 1 water and dry sand samples (30 cfu/100ml and 60 cfu/100g respectively). Lekki beach 2 water and dry sand samples (40 cfu/100ml and 10 cfu/100g respectively). Takwa Bay 1 water and dry sand samples (60 cfu/100ml and 40 cfu/100g respectively), Takwa Bay 2 water 20 cfu/100ml and Badagry Jetty 20 cfu/100ml. Of 150 *E. coli* isolates

screened for shiga toxin-producing *E. coli* (STEC) only one strain from Takwa Bay 1 beach was positive for O157:H7 after confirmation using E. coliPRO O157 kit form Hardy Diagnostics, Santa Maria, CA, USA.

Table 4: Frequency and Percentage Occurrence of Isolates from Water and Sand Samples

Isolates	**Frequency**	**Percentage (%)**
Enterococcus faecalis	23	13.37
Enterococcus faecium	17	9.88
Enterococcus casseliflavus	2	1.16
Enterococcus gallinarum	2	1.16
E. coli	47	27.32
Vibrio parahaemolyticus	26	15.12
Vibrio vulnificus	14	8.14
Salmonella species	4	2.33
Citrobacter freundii	7	4.07
Citrobacter sp	6	3.49
Aeromonas hydrophila	4	2.33
Aeromonas sobria	5	2.91
Aeromonas caviae	4	2.33
Pseudomonas putida	1	0.58
Pseudomonas fluorescens	2	1.16
Pseudomonas sp	2	1.16
Serratia marcescens	5	2.91
Serratia sp	1	0.58
Total	172	100

4. Discussion

The highest counts of fecal indicators were found in Marina and Badagry Jetty (Table 2), These areas are subject to human fecal pollution as a result of open defecation and waste water discharge. Pollution of the Lagos lagoon have been found to be great environmental problems in Lagos Metropolis. Microbiological study carried out by Ajayi and Akonai (2005) reported the isolation of lactose fermenting gram negative bacteria such as *E. coli* form Lagos Lagoon.

Samples collected from beaches used in this study showed highly variable fecal indicator concentrations, ranging from undetectable to hundreds of CFU/100 mL in water and per 100 g sand samples (Table 2). Of all the beaches sampled, Lekki, Eleko, and Takwa Bay 1 beach failed recreational water quality criteria of >104 CFU/100 mL of *Enterococcus* during a single sampling when compared to EPA Recreational Water Quality Criteria (US EPA, 2012). Dry sand samples have highest occurrence of faecal indicator when compared to water and wet sand. The sanitary condition of Eleko and Lekki beach was not good as human and animal faeces were found littered the sand on the beach. This finding is in agreement with other studies at beaches in Florida, (Abdelzaher *et al*., 2010; Bonilla *et al*., 2007), California, (Yamahara *et al*, 2007), Gaza and along the Great Lakes, (Wheeler *et al*., 2003; Whitman and Nevers, 2003). Beach sands from surveyed beaches in Lagos, Nigeria were enriched in fecal indicator bacteria (*E. coli* and enterococci) relative to the water. There was no fecal indicator organism isolated from Takwa Bay 2 site but the isolation of *Salmonella* species from this site is of concern. These results is in agreement with findings of Yamahara *et al*., 2012, they reported that *Salmonella* species was higher in sand samples than water in the study of beach sand of selected beaches in California.

Although, *Vibrio* species are naturally found in seawater but their ability to cause disease in human is of great environmental health importance. All the beaches sampled had either *V. parahaemolyticus* and/or *V. vulnificus*

isolated from water or sand samples (Fig. 6). The genus *Vibrio*, mainly species of *V. vulnificus* and *V. parahaemolyticus*, is commonly known to cause gastroenteritis, and can also cause ear, nail and skin infections (Kueh *et al*., 1992). *V. parahaemolyticus* infections of the skin may happen when open wounds are exposed to the seawater or the sand containing these bacteria (Viera *et al*. 2001).

Other bacteria isolated in this study include *Salmonella* species, *Serratia marcescens, Pseudomonas* species, *Aeromonas* species and *Citrobacter* species. Although *S. marcescens* was considered to be an innocuous, non-pathogenic organism, over the last two decades they have become an opportunist pathogen causing nosocomial infections (Hejazi and Falkiner, 1997). A broad range of hospital-acquired infections caused by *S. marcescens* include respiratory tract infections, urinary tract infections (UTI), septicaemia, meningitis, pneumonia, conjunctivitis wound and eye infections, osteomyelitis, keratoconjunctivitis, keratitis, endophthalmitis and endocarditis (Kida *et al*., 2007). The isolation of *Serratia* spp from water and dry sand samples of 4 of the beaches surveyed showed that it can be of great environmental health importance especially from Lekki beach 2 with the count of 80 cfu/100g of dry sand. *Pseudomonas* has been shown to play important role in the contamination of recreational seawater as well as outbreak of opportunistic *Pseudomonas* infections (Yoshpe-purer and Golderman, 1987). This finding agrees with the work of Ghinsberg *et al.,* 1994, they reported both seawater and sand on a number of beaches in Israel were found to contain various levels of *Pseudomonas aeruginosa*. The isolation of *P. aeruginosa* and of other *Pseudomonas spp*. was proportionally higher in sand than in seawater samples.

The genus *Aeromonas* are wide spread in environmental habitats such as water and soil and are pathogens of cold-blooded animals and mammals including humans (Harf-Monteil *et al*., 2003). *Aeromonas* species was isolated from water (Eleko beach 2, Lekki beach 1 and 2, Takwa Bay 1 and 2 and Badagry jetty). *Aeromonas* has been implicated in severe human diseases, including gastroenteritis, bacteremia and soft tissue infections associated with traumatic injuries contaminated with water or soil (Janda and Abbott, 1998). The human pathogen, *Aeromonas hydrophila* has been recovered along with pathogenic *Vibrio* spp. from sands along the Tel Aviv coast in the Mediterranean (Ghinsberg *et al.,* 1995). *Citrobacter* species has been isolated from bathing beaches of Adriatic Sea coastal areas (Bonadonna *et al*., 2002).

5. Conclusion

This work represents initial study of water and sand sampled from selected recreational beaches in Lagos shoreline, Nigeria. Some of these beaches are not suitable for human use based on high level of fecal indicator bacteria (*Enterococcus* and *E. coli*) as specified by US EPA guide for recreational water. Prevalence of *E. coli* and *Enterococcus* were generally higher in sand samples when compared to seawater. Other bacteria of public health importance detected from these beaches are *V. parahaemolyticus, V. vulnificus, Salmonella, Serratia, Pseudomonas, Aeromonas* and *Citrobacter*. The findings of the present study suggest that bathing beaches could be source of transmission of pathogenic bacteria. As a result of this, government should establish water and sand microbiological standards to enhance beach quality in Lagos State, Nigeria. Currently, there is no bathing beach quality standards in Nigeria.

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