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Letter to Editor

Microbiological Beach Sand Quality in the Gaza Strip in Comparison to Seawater

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Abstract

Gaza beach is the only recreational area available for the local inhabitants of Gaza, Palestine. It is heavily polluted with treated, partially treated and untreated sewage from point and non-point sources. The majority of the population is below the age of 15, an age group vulnerable to gastrointestinal diseases and that usually restricts its activities to beach sand at the swash zone. A total of 5 sampling points along the Gaza beach were selected and monitored for one year (fortnightly). Microbial sand content was evaluated for faecal coliforms (FC) and faecal streptococci (FS) as well as *Salmonella*, *Shigella* and *Vibrio*. Seawater samples were subjected to similar evaluation. *Pseudomonas*, yeast and mold count were performed for all sand samples as possible sand pollution indicators.

Higher faecal indicators (both FC and FS) were obtained in sand rather than in water almost in all locations. The frequency of *Salmonella* and *Vibrio* isolation was also higher in sand than in water despite the fact that only 10 grams of sand were used while one liter of seawater was collected. Statistically significant correlations were found between faecal coliform and streptococci on one side of the beach and *Salmonella* and *Vibrio* on the other side. Similar correlation was also detected between *Pseudomonas* levels and the isolation of *Salmonella* from sand samples.

Keywords: beach quality, microbiological assessment, faecal indicators, Gaza, sand.

Introduction

Recreational water generally contains a mixture of pathogenic and non-pathogenic microbes derived from sewage effluents, industrial process, farming activities, and wildlife in addition to any truly indigenous microorganisms. This mixture can present a hazard to bathers where an infective dose of pathogen colonizes a suitable growth site in the body and leads to disease [1].

The extent of seawater pollution varies according to the quantity and quality of pollutant. However, the problem of seawater pollution is acknowledged worldwide. As a result of recreational activities, many individuals may contract diseases that range from self-limiting gastrointestinal disturbances to severe and life-threatening infections. The disease incidence is dependent on several factors: the extent of water pollution, time and type of exposure, the immune status of users and other factors [2].

Seawater and beach quality monitoring and assessment are considered vital parts of any integrated coastal management program [3]. Extensive research with the aim of establishing guidelines and standards for recreational water quality has been conducted all over the world. In this context, social, cultural, environmental and economic factors should be taken into consideration because of the great variation from one area to another.

The microbiological quality of sediments at the sediment-water interface in bathing waters is receiving in-

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creased attention [4]. There is evidence that faecal indicators and pathogenic bacteria survive in sediments no longer than in the overlying water and it has been proposed that sediments serve as sinks for faecal bacteria with the potential to pollute the overlying bathing waters [5, 6, 7,8].

Stream sediments have been shown to contain faecal coliform at concentrations higher than those observed in the overlying water column. Sediments may contain 100 to 1,000 times the number of faecal indicator bacteria contained in the overlying water [9, 5].

FC was analyzed in water and sediment samples from Oak Creek, Arizona in the United States [10]. They found sediment samples with up to 2,200 times the FC counts of the water column. Results showed that resuspension of sediments due to agitation by recreational activities and storm events during summer season negatively impacted water quality.

Laws and legislations emphasize microbiological indicators level in seawater and almost ignore the fact that many beach visitors may not use water but sand, most especially children. The swash zone of the bathing beach is the interface area that is washed over by waves. This region is a popular play area for young children. Bacteria capable of causing human disease may contaminate the sand in this part of the shoreline. Accordingly, concern has been expressed that beach sand or similar materials may act as a reservoir or vector of infection [11].

Gaza beach is considered the only recreational site for a population of more than one million inhabitants of the Gaza Strip. It is usually very crowded in the summer season mostly with local inhabitants. At the local level, few studies have been conducted by the Environmental and Rural Research Center [12], but they show heavily contaminated recreational seawater along the seashore of the Gaza Strip.

This study would provide the first original data about the microbiological content of sand along the Gaza Strip as well as seawater microbiological quality. This work addresses the issue of sand contamination, which has long been neglected by researchers and policy makers in assessing the quality of beaches. Hence, it is expected to assist local authorities in developing plans and policies and in implementing actions to reduce the pollution to acceptable levels. It may prove helpful in setting standards and guidelines. Finally, this work will add to the accumulating literature on sand and seawater which could change our global view of beach monitoring policies.

Material and Methods

Sample Site Selection

A total of 5 sampling locations were selected based on visual inspection of the beach and the amount of sewage disposed. These sites were identified by land marks and by GPS. The following table illustrates the selected locations.

Sample Collection

Sampling was performed according to the World Health Organization Manual for Recreational Water and Beach Quality Monitoring and Assessment [13]. The sampling frequency was fortnightly. Sample collection lasted from May 2002 to May 2003. Polyethylene (500ml) bottles were used to collect water samples while 100 ml sterile bottles were used to collect sediments. For *Salmonella* isolation from water, 1 liter bottles were used.

Seawater Sample Collection

Water samples were collected while the sampler was standing in water at chest level (about 1.3 m), the lid of the bottle was removed without touching the mouth of the bottle. The bottle was turned upside down and lowered approximately 20-30 cm below the surface with a smooth movement (to avoid collecting 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 [13].

Sediment Sample Collection

Sterile, wide mouthed, disposable plastic containers (100 ml) were used to collect sediment samples from the

Table 1. Sampling site identification information.

Location	City/Address	Prominent mark	GPS locations	
			N	E
1	South Deir Elbalah	Resort	31.25.03.6	34.19.43.0
2	North Deir Elbalah	Elementary school	31.25.50.6	34.20.34.7
3	Al-Zawida	Resort	31.26.37.8	34.21.22.2
4	South Wadi Gaza	Army Station	31.27.40.7	34.22.23.7
5	North Wadi Gaza	Life Guard Station	31.27.56.2	34.22.37.9

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.

Faecal Coliform and Faecal Streptococci

A membrane filter technique was used for the detection and identification of faecal coliform and faecal streptococci according to the standard methods for water and wastewater [14].

The membrane filtration technique was modified for use with sediments. A suspended sediment (SS) fraction was produced by adding 100 ml of 0.85% (w/v) sterile saline to each sample, vigorously shaking for 30 seconds and then allowing brief settlement of the larger stream aggregates. Two 10-ml aliquots of the resultant supernatant (the SS fraction) were collected for analysis. The first aliquot was placed in a graduated centrifuge tube and allowed to settle overnight at 25°C to measure the volume of sediment in an individual sample (sediment load) and to allow accurate comparison between samples. The second 10-ml SS fraction was added to a Warring blender containing 90 ml of sterile saline and mixed on low for 5 min. Appropriate dilutions were then enumerated according to the technique used for water samples **[**10**].**

Salmonella and *Shigella*

Water samples

One liter of seawater sample was filtered through a 0.45 µ membrane filter. The membrane filter was placed in enrichment medium (Selenite-F broth) overnight. Subcultures were made to XLD and SSA agars [14].

Sand samples

Ten grams of sand sample were inoculated into 90 ml of selenite-F broth and incubated at 37°C overnight and subcultured onto XLD and SSA plates. Suspect colonies were identified biochemically using API20 E strips **[**15**].**

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 PseudoSel Agar. Colonies were identified biochemically using API 20 E Strips.

Vibrio Species

Seawater sample

One liter of seawater sample was filtered through a 0.45 µ membrane filter. The membrane filter was placed in enrichment medium (alkaline peptone water) overnight. Subcultures were made to Thiosulfate Citrate Bile Sucrose agars (TCBS) [16].

Sand sample

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 TCBS agar and further incubated for 24 h at 37°C. Yellow or blue colonies growing on TCBS were picked for identification. The exact identity was identified using API20E (API system, France) [16].

Yeast and Mold 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 plate count method, using Dichloran Rose Bengal Chloramphenicol (DRBC)Agar [17].

Results

All locations exhibited variation in both sand and seawater content of faecal coliform and streptococci; however, when comparing the ratio of sand/seawater, it would be clear that the ratio is below one in locations of low pollution (1 and 3), while it is high (ranging from 5.4 to 29.1) in locations with high pollution levels (e. g., 2, 4, and 5). This may suggest the accumulation of these indicators on the sand surface. The ratio of FS is much higher than that of FS in such locations. This may be due to the longer survival rates exhibited by FS. Table 2 illustrates the difference in FC and FS content of both seawater and sand during the monitoring period.

All locations were evaluated using the European Community (EU) standards for Faecal coliform and faecal streptococci and the following tables include the number of failures of a location to meet the required criteria.

It can be observed from Table 3 that the general failure percentage of any of the five locations is more frequent due to failure to comply with faecal streptococci rather than faecal coliform. Failure of both contaminated and relatively clean locations was mainly during winter (no bathers were observed).

Although locations 4 and 5 are of about similar distance from the discharging point of Wadi Gaza, the fail-

ure percentage was higher in location 5. This may be due to the dominant current direction which is usually from south to north, carrying more pollutants toward location 5, which is situated north of Wadi Gaza.

Location 3 passed the mandatory standards for FC and failed the Guidelines 5 times during the monitoring period, while it failed 8 times to comply with the guidelines for streptococci.

Salmonella, Shigella, and *Vibrio*

Salmonella and *Shigella* are definitely pathogens that are of worldwide importance and transmitted mainly through food and water. Their presence in all types of water, including recreational waters, render that water unfit for human use. *Salmonella* was isolated only one time from water samples taken from location 4 and an-

Table 2. Comparison of the levels of faecal coliform and streptococci in both seawater and sand (cfu/100 ml seawater and 100g sand).

Location no.		Average count Sand	Average count Seawater	Sand/Sea ratio
1	FC	74	37	2
	FS	133	181	0.7
2	FC	13996	2585	5.4
	FS	94566	8216	11.5
3	FC	37	71	0.52
	FS	152	184	0.83
4	FC	1134	73	15.4
	FS	4080	140	29.1
5	FC	5455	4742	1.1
	FS	1813	1502	1.2

Table 3. The percentage failure of the studied location when compared to the EU bathing directive (76/160/EEC).

Location	% failure when compared to EU standards (N=26)		
	FC Mandatory	FC Guidelines	FS Guidelines
1	0	8	23
2	31	73	84.6
3	0	19	30.8
4	0	42	34.6
5	15	53	57.7

Table 4. Number of isolated *Salmonella, Shigella,* and *Vibrio* from sand and seawater.

Location no	No. of isolation incidents out of 26 sampling occasions for each location					
	Salmonella		*Shigella*		*Vibrio*	
	Sand	Seawater	Sand	Seawater	Sand	Seawater
1	0	0	0	0	1	0
2	1	0	0	0	10	2
3	0	0	0	0	0	0
4	4	1	0	0	9	3
5	4	1	0	0	9	5
Total	9/130	2/130	0/130	0/130	29/130	10/130

other from location 5. *Shigella* was not isolated from any sample during the monitoring program.

The *Vibrio* genus includes several species, the most important of which is *Vibrio cholera*. Non-cholera species were isolated on several occasions in locations 2, 4, and 5. Seawater samples from locations 1 and 3 were free from any of these three pathogens.

Salmonella was isolated from locations 2, 4, and 5 during the period 10/10/2002 to 27/2/2003 while *Vibrio* spp. was isolated during the period 26/9/2002 to 27/2/2003 in locations 4 and 5. *Vibrio* isolation showed no seasonality in location 2.

The Chi square test was used to detect any possibly significant correlation between *Salmonella* presence and the level of faecal indicators (FC and FS), *Pseudomonas* count, and yeast and mold counts. A similar test was used for *Vibrio*. Table 5 shows that at all instances when the level of FC and FS were below 200 cfu/100 g, *Salmonel-*

Table 5. Statistical analysis (Chi square test) for the relation between microbial indicators and *Salmonella* (*N*=130).

Indicator parameter	*Salmonella*		Significance level
	Negative	Positive	
Faecal streptococci range			0.009
0-200	54	0	
> 200	67	9	
Faecal coliform range			0.003
0-200	62	0	
> 200	59	9	
Pseudomonas range			0.005
0-100	92	3	
>100	29	6	
Yeast and mold range			0.184
0-100	68	3	
>100	53	6	

Correlation is significant at the 0.05 level.

Table 6. Statistical analysis (Chi square test) for the relation between microbial indicators and *Vibrio* in sand (N=130).

Indicator parameter	Vibrio		Significance level
	Negative	Positive	
Faecal coliform range			0.000
0-200	60	2	
> 200	41	27	
Faecal streptococci range			0.000
0-200	51	3	
> 200	50	26	
Pseudomonas range			0.000
0-100	85	10	
>100	16	19	
Yeast and mold range			0.013
0-100	61	10	
>100	40	19	

Correlation is significant at the 0.05 level.

la was not isolated. All incidences of *Salmonella* isolation were associated with counts higher than 200 cfu/100 g. Chi square test results show a very high significance for both FC and FS. With regard to *Pseudomonas* counts, only in 3 out of 62 instances when the level was lower than 100 cfu/100 g was *Salmonella* isolated. *Pseudomonas* also correlated significantly with *Salmonella*. Yeast and mold counts did not show significant correlation with *Salmonella.*

All measured microbiological parameters correlated significantly with *Vibrio* isolation. Table 6 presented the statistical analysis of the studied microbial indicators and *Vibrio* in sand.

Pseudomonas, Yeast & Molds

Table 7 summarizes the results of minimum, maximum, median, geometric mean and average of sand samples contents of *Pseudomonas* and yeast and molds in the various locations, while Figure 1 showed the average values of FC, FS, *Pseudomonas* and yeast and molds of the studied locations.

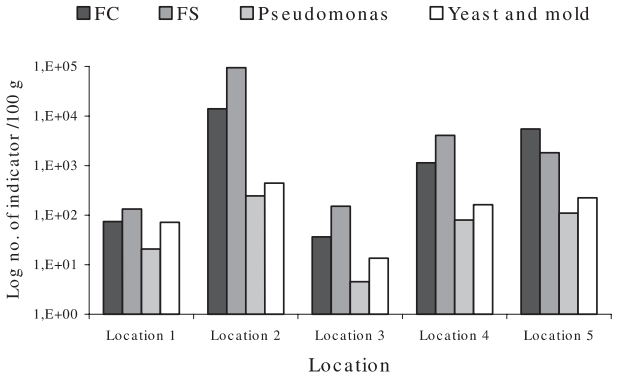

Fig. 1. Average sand microbiological parameters in all locations

Fungi were implicated as a cause of skin infection and thought to be transmitted from contact with infested sands and soil. In this research, as shown in Table 7, locations 2 and 5 showed the highest levels of yeast and mold counts. *Pseudomonas* could be a good indicator of faecal pollution in sand since it correlated with FC in three locations; with yeast in 4 locations; *Vibrio* in three locations and with *Salmonella* in one location. Yeast showed a similar pattern to that of *Pseudomonas*, but linear correlations were fewer.

Discussion

The wide difference between locations 4 and 5 with regard to the percentage of compliance failure despite the fact that the two locations are only about 300 meters apart could only be interpreted by the fact that current direction was toward location 5 during most of the monitoring period. A similar interpretation was provided [18]. This finding suggests that any future monitoring program should take into consideration a daily record for current direction and, if possible, current speed.

The highest concentration of faecal indicators was found in an area receiving land runoff during the rainy season. In another study and during the summer period, no *E. coli* were isolated from all sampling points, whereas in autumn, the organism was isolated in most of the sampling points used in this study [19].

In a study by [20], the impact of heavy rains on the microbiological quality of water persisted for a few days and depended on the amount and intensity of rain and weather conditions after the rain episode.

When faecal coliform and faecal streptococci results were compared to the EU standards, a general higher compliance failure percentage was associated with faecal streptococci than that of faecal coliform. Failure to comply the faecal streptococci guidelines showed that locations 2 and 5 exhibited the highest percentage (84.6% and 57.7%, respectively). A similar pattern, yet lower

Table 7. Summary results of sand *Pseudomonas*, Yeast and molds (cfu/100 g).

Location no.		Mini	Max	Median	Geometric mean	Average
1	Pseudo	1	200	10.5	8	21
	Yeast	8	800	24.5	32	72
2	Pseudo	1	900	160	106	245
	Yeast	1	2300	330	243	444
3	Pseudo	1	21	1	2	5
	Yeast	1	42	12	9	14
4	Pseudo	11	400	30.5	44	80
	Yeast	11	820	121	86	162
5	Pseudo	15	310	65	74	110
	Yeast	12	420	205	173	225

percentage (73% and 53%) of failure to comply the faecal coliform guideline, was obtained. Location 3 complied with the mandatory standards of faecal coliform and failed the guidelines 4 times, but failed the guidelines 8 times for *Streptococci.* Similar results were achieved with a higher failure frequency using enterococci compared with *E. coli* [21]. Others found that 99% of failures were due to enterococci during a storm study and 60% of failure during a summer study [22].

One possible explanation for the consistently higher rate of enterococci standard failures is that enterococci survive longer in the marine environment than TC or FC. While *E. coli* survival in marine water was 0.8 day while enterococci survival was 2.4 days [23]. Also, *E. coli* degraded more rapidly with increased sunlight intensity than did enterococci, a finding that was recently confirmed for bacterial samples from southern California [24, 25].

This differential survival hypothesis seems to be supported by the greater consistency in standard failures among indicators in the storm study than in the dry weather studies [22]. During wet weather, land-based runoff is distributed to the beach more quickly and represents a "fresher" source of contamination, providing less time for differential degradation to occur.

Generally, faecal indicator concentration was higher in sand than in the corresponding water column. The highest ratio obtained in this study was for faecal streptococci in location 4 (1:29.1). This is considered a small ratio compared to other findings, where they identified sites with high faecal coliform counts averaging 2,200 times the faecal coliform counts in the water column [10]. This may be due to different types of samples in which they obtained bottom samples while we obtained intertidal sand samples.

Observations [26, 27] of lower enteric bacteria survival rates in natural seawater as compared to sterile seawater suggest involvement of biological processes. This hypothesis was supported by measuring earlier and at faster declines of *E. coli* viability (CFU) in seawater and sediment during a 13-day period when indigenous seawater flora were present as compared to sterile conditions [28]. Predation [22, 29-35], competition [28] and bacteriophages [32, 36] have been implicated in reducing enteric bacterial concentrations in seawater.

Variation in the numbers of bacteria in sand and seawater from the five locations in the middle camp beach may be a result of varying sources of pollution. Another factor that may have produced a dramatic effect in location 4 was the height of the sand at the intersection between water and land, where intertidal waves have a shorter contact time than in other locations.

Salmonella is commonly present in sewage effluent that can contaminate recreational waters. Water microbiology quality standards for recreational waters are based on coliform indicators as predictors of the presence of pathogenic microorganisms [37, 38]. While epidemiological studies constituted the basis of water quality standards in the USA [38], such studies were not used for standard development in Europe. The objectives of the European Community Bathing Water Directive are to protect the environment and public health [37]. Their principal microbiological parameters are total and faecal coliform (TC and FC).

The highest incidence of *Salmonella* isolation (15.4%) was obtained from sand samples in locations 4 and 5, and in comparison, seawater samples had only 3.8%. This finding agreed with [39], where all studied microorganisms were found to be higher in sediments than the overlying water. During a 12-month survey, no *Salmonella* were isolated from sand samples collected from northwest England [40]. This may be attributed to variations in climate and quantity and quality of sewage. *Salmonella* was isolated with a frequency of 3.8% from seawater samples from locations 4 and 5. This percentage agrees with findings by [41, 42].

The isolation of *Salmonella* from sand on several occasions during this study and the failure to do so from seawater favors the use of sand samples rather than seawater samples in cases where *Salmonella* is suspected. *Salmonella* is clearly associated with contaminants from human origin. This assumption is due to the isolation of *Salmonella* from locations that are exposed to sewage (e. g., 2, 4, and 5).

The aim of the present study is to assess possible associations between the presence of *Salmonella* and concentrations of indicator organisms in relation to established standards. A significantly higher incidence of *Salmonella* isolation (P=0.003) was found in samples containing FC levels higher than the recommended standards (>200 cfu/100 ml). Similar findings were produced by [43]**.** A similarly significant correlation (P=0.009) was found between *Salmonella* and FS levels higher than 200 cfu/100 ml. *Pseudomonas* levels were grouped into less than 100 cfu and higher than 100 cfu and tested using Chi square, where a significant (P=0.05) correlation was obtained. No significant correlation between *Salmonella* and the levels of yeast and mold count was obtained when subjected to grouping in a similar manner to that of *Pseudomonas*.

Several studies have been conducted for the purpose of correlating the densities of faecal indicators with the presence of *Salmonella.* In the Mediterranean Sea [44], a better correlation of the coliform group with *Salmonella* isolation compared with the other indicators was recorded. Faecal coliform and *C. perfringens* were most closely related to *Salmonella* spp. [41]. While in polluted marine areas, faecal coliform, faecal streptococci and coliphage correlated well with *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and *Candida albicans* [45]. The total and faecal coliform correlated well with the presence of *P. aeruginosa* in bathing beaches [46]. A moderate positive relationship between the three indicators and the pathogen *Staphylococcus aureus* in Croatia was also described [47]. On the other hand a significant correlation between the occurrence of coliform bacteria and fae-

cal streptococci as well as *Vibrio vulnificus* [48]. While it was found that faecal coliform were good predictors of *Candida albicans* in moderately polluted areas [49]. In addition, studies often have found poor correlations between *E. coli* and, in particular, pathogens [50, 51, 52, 53, 54]**.** Yet one may expect that *E. coli* may still serve as an indicator of health risk, rather than as an indicator of particular pathogens.

The isolation of *Pseudomonas aeruginosa* from almost all sand samples should be considered an alarming factor. *Pseudomonas aeruginosa* showed a die-off rate similar to that of faecal streptococci and slower than that of faecal coliform [55]. A probability of correspondence between the presence of this bacterium and the secondary gastrointestinal infection diagnosed was found, pointing towards a need for the inclusion of other microorganisms, one of which may be *P. aeruginosa*, as indicators of health risk associated with drinking waters in Mexico [56].

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