Analysis of seawater microbiological quality data in Greece from 1997 to 2006: association of risk factors with bacterial indicators

Panagiotis Papastergiou, Varvara Mouchtouri, Maria Karanika, Elina Kostara, Foteini Kolokythopoulou, Nikos Mpitsolas, Angelos Papaioannou and Christos Hadjichristodoulou

ABSTRACT

This study deals with the examination of quality of seawater bathing areas in Greece over a 10-year period and identifies risk factors for high bacteria indicator organism concentrations. Qualitative descriptive analysis was applied and the microbiological test results of 231,205 water samples were associated with pollution markers and other parameters. Measurements of *Escherichia coli* (99.6%) and enterococci (100%) were found to be in accordance with the mandatory value guidelines set by the new European Directive. An increasing trend for the yearly mean value of faecal streptococci was noted. Using logistic regression analysis, phenolic smell (OR = 2.10, CI = 2.04–2.16), rainfall the day before sampling (OR = 1.67, CI = 1.64–1.74), high seas (OR = 1.42, CI = 1.39–1.46) and rainfall on the day of sampling (OR = 1.27, CI = 1.20–1.33) were positively independently associated with high levels of bacterial indicators (total coliforms, faecal coliforms, faecal streptococci and *E. coli*). The highest risk, absolute risk value 42.8% (RR = 3.17, CI = 2.97–3.38), was measured when previous day rainfall, phenolic smell and high seas were simultaneously recorded. Such parameters should be further investigated as predetermining factors for the assessment of beach bathing water quality, providing a timely indication of water risk assessment.

Key words | bacterial indicators, Mediterranean, public health, risk assessment, water quality

Panagiotis Papastergiou
Varvara Mouchtouri
Maria Karanika
Elina Kostara
Foteini Kolokythopoulou
Nikos Mpitsolas
Christos Hadjichristodoulou (corresponding author)
Department of Hygiene and Epidemiology,
School of Medicine,
University of Thessaly,
6 Lapithon Street,
Larissa 41221,
Greece
Tel.: +30 2410 565259
Fax: +30 2410 258197
E-mail: *xhatzi@med.uth.gr*

Angelos Papaioannou
Technological Education Institute of Larissa,
Larissa 41110,
Greece

INTRODUCTION

Greece is located in the eastern Mediterranean with over 2,500 islands and strong maritime tradition. The coastal length of Greece totals approximately 16,000 km. Half of this length surrounds the thousands of Greek islands, while the rest extends along the mainland. Every year, Greece and its coastal bathing areas welcome millions of foreign tourists in addition to its inlanders. The bathing season in Greece lasts between five and six months, depending on the geographical location and climate, beginning between the months of May and June, and ending in October.

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Owing to its geographical position, Greece and its islands have mild winters and warm summers, cooled by different kinds of seasonal wind. The summers are characterized by sunshine and very little rainfall while the mean ambient air temperature in Greece during May, June, July, August, September and October is 20.2°C, 24.6°C, 27.0°C, 26.6°C, 23.3°C and 18.3°C, respectively (www.meteo.noa.gr/ENG/climat-bulletin.pdf, accessed 5 March 2009).

A number of microbiological and epidemiological studies have been carried out since 1953 (Stevenson 1953), in an attempt to define the levels of risk following exposure to different concentrations of bacteria in bathing waters. Standards based on exposure to bacterial indicators have been established through epidemiological studies of recreational waters (Ferley *et al.* 1989; Fleisher *et al.* 1993; Kay *et al.* 1994; Pruss 1998). Such studies support the idea that the rate of infection and disease among bathers increases steadily with increasing concentrations of indicator microorganisms of faecal pollution in a dose-response relationship (Ferley *et al.* 1989). Hence, the survival of human enteric bacteria in the aquatic environment has attracted much interest in view of its public health significance. Based on risk assessments from the World Health Organization (WHO) and academic research sources, studies suggest that millions of gastrointestinal and severe respiratory diseases are caused by swimming and bathing in wastewater-polluted coastal waters (Shuval 2003).

Indicator bacteria including total coliforms, faecal coliforms, *Escherichia coli* and streptococci/enterococci have been used over time for the assessment of water quality and risk assessment in the prediction of water microbial pollution. Total coliform bacteria are commonly found in the environment and are considered natural inhabitants of waters; their presence may be tolerated in a dose-response relationship. Faecal coliform bacteria are a sub-group of the total coliform bacteria. They appear in great quantities in the intestines and faeces of humans and animals; hence the presence of faecal coliforms in water samples often indicates recent faecal contamination. The mere presence of faecal coliforms triggers alarms, as it indicates a concern for the presence of other pathogens. Hence the detection of faecal coliforms is of greater concern than the presence of total coliforms. *E. coli* is a sub-group of the faecal coliform group and its presence almost always indicates recent faecal contamination and therefore a greater risk for the presence of other pathogens.

The presence of streptococci/enterococci always indicates recent human or warm-blooded animal faecal contamination creating a greater risk for the presence of other pathogens. They appear in great quantities in the intestines and faeces of humans and hence their presence goes without doubt as evidence for increased risk. Besides their limitations, these indicator bacteria have been used successfully in many countries as a monitoring tool for microbiological impairment of water and prediction of the presence of pathogens (WHO 2000, 2003; Chandran *et al.* 2008).

European Union regulations require regular sampling of seawater from defined bathing locations and specific bathing water quality standards, based on bacterial indicators, by each Member State. The 1976 EU Bathing Water Directive was adopted by Greece with the Ministerial Decision 46399/1352/86, which had in most part identical quality requirements for bathing water to that of the 1976 EU Bathing Water Directive. However, some quality requirements were sterner than those of the 1976 EU Directive. The Hellenic Ministry for the Environment, Physical Planning and Public Works is the main competent authority for the implementation of this Directive. The number of frequently monitored coastal bathing areas in Greece has steadily increased from 1,731 beaches in 1997 to 1,901 beaches in 2006.

Our objective was to estimate the general quality of the seawater bathing areas in Greece during a ten year period (1997–2006) by using a descriptive analysis of the microbiological quality test results data and, moreover, to formulate a risk factor assessment by associating the different pollution markers (tar, garbage, phenols, oils, floating materials) and other parameters (jellyfish, sea condition, rain, seaweed, wind direction) with microbiological bacterial indicator concentrations of seawater sampling test results.

METHODS

Study areas and sampling programmes

Beaches were sampled on a regular basis with a mean average of 13 samples collected from each beach per year, from predetermined points specified by the competent department of the Hellenic Ministry for the Environment, Physical Planning and Public Works.

Sample collection and testing

Water samples from regularly monitored beaches were taken from the most densely occupied areas of beaches crowded with bathers. The beaches are visited by bathers mainly during mid-June to mid-September with the highest counts of visitors noted during July and August. During the other months there is minimal to zero visiting of the seawater bathing areas for bathing purposes. As a result, between the months of October and April only a few water samples were collected from the coastal bathing areas. Water samples were therefore collected from May through to November. The time of sampling was almost the same for the same beach each time. The majority of samples were taken between 10.30 and 17.30 as this was considered to be the time at which the majority of people engaged in water activities. A volume of 450 ml of water was collected in sterile bottles of 500 ml (or 250 ml) capacity. Samples were taken 20–30 cm below the water surface level at a sea depth of 0.8–1.3 metres. Samples were transferred to the laboratory on the same day of collection in a closed Esky cooler, thereby avoiding any disinfecting effect of sunlight and changes to microbial presence. All samples were processed within 24 hours of collection. Furthermore, visual parameters such as sea colour (normal/abnormal colour), wind direction and general sea condition (high, medium, low waves), the presence or absence of mineral oils, surface-active substances in seawater, phenolic smell, tar, floating materials, garbage, seaweed and jellyfish were all assessed and recorded. Parameters recorded can be seen in Table 1.

The majority of the water samples were collected and analysed by a main contracted private laboratory. Owing to the vast load of samples, over 40 public and private authorities were involved in the sampling operation and 11 public and private laboratories including the main contracted laboratory were involved in the testing of the samples. All laboratories processed samples for microbiological analysis in accordance with standard ISO methods for the detection and enumeration of *E. coli*, faecal coliforms, total coliforms and faecal streptococci/enterococci.

Data collection and validation

Data included in the study were gathered from the archives of the Hellenic Ministry for the Environment, Physical Planning and Public Works and comprised microbiological test results and relevant information recorded during sampling of the regularly monitored coastal bathing areas.

Table 1 | Database of the main variables for the regular monitoring of coastal bathing areas

Variables	Explanation
DATE PAR	Date departure
TIME PAR	Time departure
SEA	Sea condition
RAIND	Rainfall same day
RAINPD	Rainfall previous day
WIND	Wind direction
TRANS	Transparency
COLOUR	Colour
PIS_SEA	Tar in seawater
OIL_SEA	Mineral oils in seawater
PHEN	Phenols (phenolic smell)
APRO_SEA	Surface-active substances in seawater
EPIP	Floating materials
FYK_SEA	Wrack in seawater
TSOUX	Jellyfish
PIS_AKT	Tar on beach
OIL_AKT	Mineral oils on beach
SKOUP	Garbage
FYK_AKT	Wrack on beach
TOL	Total coliforms
FCOL	Faecal coliforms
E.COL	*E. coli*
STREPT	Faecal streptococci
CODE_AKT	Code number of the beach
DATE	Date of sampling
TIME	Time of sampling

All data entries were subjected to data validation and any inaccuracies found in the database, due to data entry errors, were crosschecked with result transcripts and corrected.

Microbiological parameter analysis

In order to assess possible risk factors of seawater contamination, certain pollution markers and other parameters (Table 1) were associated with microbiological indicator (total coliforms, faecal coliforms, faecal streptococci/enterococci, and *E. coli*) test results.

The analysis results showed that an extremely high percentage of beaches conformed to the EU mandatory requirements for bathing water (Table 2); hence it was

Table 2 | Percentages of microbiological test results of the water samples collected from bathing areas of Greece satisfying EU Directives values and the Greek Ministerial Decisions

	Microbiological (indicator) parameters (%)			
Directives	***E. coli***	**Faecal coliforms**	**Streptococci/enterococci**	**Total coliforms**
Directive 76/160/EEC (Guide requirements)	–	95.6	99.9	98.6
Directive 76/160/EEC (Mandatory)	–	99.9	–	100
Ministerial Decision 46399/1352/86 (Guide requirements)	–	95.6	99.9	98.6
Ministerial Decision 46399/1352/86 (Mandatory)	–	99.4	–	100
Directive 2006/7/EU (Excellent quality)	99.2	–	99.9	–
Directive 2006/7/EU (Good quality)	99.6	–	100	–

necessary to create new cut-off levels for the microbial indicators, which would allow the association of qualitative and quantitative values.

For the analysis purposes of this study, a newly derived cut-off level for total coliforms was set at 150 cfu/100 ml, while cut-off levels for *E. coli* and faecal streptococci/enterococci were set at 80 and 30 cfu/100 ml, respectively. An exception was made for faecal coliforms where the cut-off level was left unchanged (100 cfu/100 ml), in accordance with the mandatory values of the Ministerial Decision (Directive 76/160/EEC). The decision not to change the cut-off level of faecal coliforms was made with the consideration that the cut-off level of faecal coliforms could not be lower than that of its sub-group *E. coli*. In general, cut-off levels for the microbiological parameters were decreased by about one-third of the mandatory values of the quality requirements for bathing water for streptococci/enterococci and *E. coli* as set out in the new directive 2006/7/EU. However the mandatory value for faecal and total coliforms was respected without alteration according to the set mandatory values of the Ministerial Decision 46399/1352/86 (Table 3).

In assessing the cut-off value of faecal streptococci/enterococci, recommendations from the 1986 published recommended water quality criteria for recreational waters from the US Environmental Protection Agency (US EPA) were considered. This agency recommended regulatory levels based on geometric means of at least five samples over a 30-day period with microbial cut-off values of 35 cfu/100 ml for enterococci in marine water (US Environmental Protection Agency 1986). This was near to our derived cut-off level of 30 cfu/100 ml for streptococci/enterococci.

In the statistical analysis only samples with counts of streptococci/enterococci <30 cfu/100 ml and *E. coli* <80 cfu/100 ml, faecal coliforms <100 cfu/100 ml and total coliforms <150 cfu/100 ml were considered as clean (negative) beaches. Samples with counts ≥ 30 streptococci/enterococci cfu/100 ml, ≥ 80 cfu/100 ml *E. coli*, ≥ 100 cfu/100 ml faecal coliforms and ≥ 150 cfu/100 ml total coliforms was considered as positive. This was a necessary step to ensure that no confounding factor or biases were included in the analysis.

Following the outcome of our amended cut-off values, and in order to extract more significant results, it was

Table 3 | Cut-off levels (cfu/100 ml) according to directives, newly defined cut-off values and percentages of satisfactory microbiological test results according to new cut-off levels

Microbiological parameter	Directive values (cfu/100 ml)	New cut-off level (cfu/100 ml)	% of samples satisfying new cut-off levels
Streptococci/enterococci	100*	30	89.8
E. coli	250*	80	94.5
Total coliforms	500†	150	93.8
Faecal coliforms	100†	100	95.6

*According to the excellent values of the 2006/EU Directive.
†According to the excellent values of the 46399/1352/86 Ministerial Decision.

also necessary to define a new variable, 'microball', solely for the purpose of analysis. We defined this variable as being positive when *at least one* of the above-mentioned microbiological parameters was positive with respect to its new modified cut-off value and negative when all microbial counts (cfu/100 ml) were less than their respective cut-off values.

Statistical analysis

Data consisting of 231,205 microbiological test results together with their associated pollution markers and other parameters collected from the coastal bathing areas were built into a database. The database main variables of the regular monitored bathing areas can be seen in Table 1. Univariate analysis was performed between each of the database variables, the bacterial indicators (faecal streptococci/enterococci, *E. coli*, faecal coliforms and total coliforms) and the new parameter 'microball'.

For qualitative data testing, chi square testing was applied, while for quantitative data, the t-test or ANOVA test was used. The 95% confidence intervals were calculated for arithmetic variables. Statistically significant differences were considered when the p value was <0.05. Those variables found to have statistical significance were included in a conditional logistic regression model. For autocorrelation analysis, autoregression with exact maximum likelihood was used. The statistical analysis was performed using EPI-INFO software, version 3.4.3 and SPSS version 16.0.

RESULTS

A total of 231,205 samples were collected from the coastal bathing areas throughout Greece. As seen in Table 2, all samples (100%) complied with the mandatory requirement values set for total coliforms in the Directive 76/160/EEC and the vast majority (98.6%) complied with the more stringent guide requirement values of the same Directive. For faecal coliforms 99.9% of the samples complied with the mandatory requirement values in Directive 76/160/EEC and 95.6% complied with the guide requirement values. As seen in Table 2, 99.4% complied with mandatory requirement values for faecal coliforms as set by the Greek Ministerial Decision 46399/1352/86. For *E. coli* 99.6% of the samples complied with the good quality values and 99.2% complied with the excellent quality values set in the new Directive 2006/7/EU. For streptococci/enterococci all sample results (100%) complied with the good quality values and 99.9% complied with the excellent quality values set in the new Directive 2006/7/EU (Table 2). Furthermore, for streptococci/enterococci, only a very small proportion of only 1,069 (0.1%) water samples failed to comply with the guide requirements values of the Directive 76/160/EEC (excellent quality).

On analysis of the data shown in Table 2, it was apparent that practically all the coastal bathing areas complied with EU mandatory values as set by the 76/160/EEC EU Bathing Water Directive. Moreover a vast majority were also in compliance with the more stringent Greek Ministerial Decision 46399/1352/86 guide values.

During the study period 1997–2006 we noted a statistically significant rise in the yearly mean value of faecal streptococci/enterococci (cfu/100 ml) whereas no significant change in the yearly mean value of *E. coli*, faecal coliforms or total coliforms was noticed (Figure 1). Furthermore we found no significant changes in the mean values of pollution and other recorded parameters. Although no significant change was noticed for *E. coli*, faecal coliforms and total coliforms, it is apparent from Figure 1 that this group (*E. coli*, faecal and total coliforms), are consistently higher in mean value than are faecal streptococci, as would be expected.

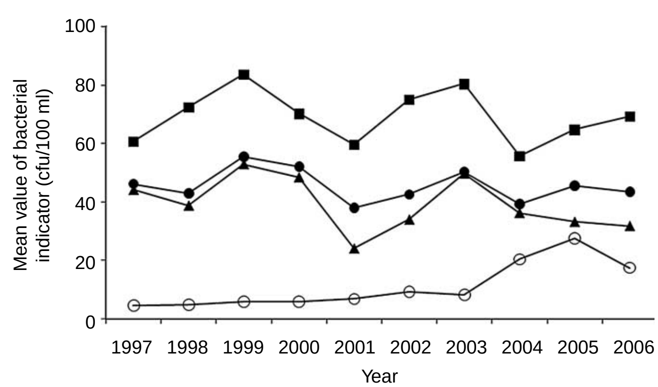

Figure 1 | Yearly mean value of total coliforms (closed squares), faecal coliforms (closed circles), *E. coli* (closed triangles) and streptococci (open circles) during the period 1997–2006. Values are means in cfu/100 ml.

Univariate analysis

Clean (negative) beaches, as defined above, accounted for 160,895 out of the 231,205 samples investigated. While 25,730 samples were positive for streptococci/enterococci, 10,903 samples were positive for *E. coli*, 10,143 samples were positive for faecal coliforms and 17,616 samples positive for total coliforms. As shown in Table 4, a descriptive analysis of all the parameters showed that the presence of phenolic smell, raining on the previous day, raining on the same day and high seas were all statistically significantly associated with increasing concentrations of bacterial indicators (total and faecal coliforms, *E. coli* and streptococci/enterococci) including the new parameter 'microball'.

More specifically, it was noticed that the presence of phenolic smell was significantly positively associated with increasing values of total coliforms (Relative Risk, RR = 2.88), of faecal coliforms (RR = 1.98), of *E. coli* (RR = 1.82) and of streptococci/enterococci (RR = 1.75) as well as for the parameter 'microball' (RR = 1.80).

Previous day rainfall was significantly associated with increasing mean values of total coliforms (RR = 2.09), of faecal coliforms (RR = 2.17), of *E. coli* (RR = 2.01), of streptococci/enterococci (RR = 1.81) and 'microball' (RR = 1.65). Same day rainfall was significantly associated with increasing mean values of total coliforms (RR = 2.11), of faecal coliforms (RR = 2.07), of *E. coli* (RR = 1.95), of streptococci/enterococci (RR = 1.74) and of 'microball' (RR = 1.60).

High sea was significantly associated with increasing values of total coliforms (RR = 1.55), of faecal coliforms (RR = 1.32), of *E. coli* (RR = 1.28) and of streptococci/enterococci (RR = 1.54) as well as for the parameter 'microball' (RR = 1.39).

The presence of tar on beaches was significantly associated with low levels of total coliforms (RR = 0.47), of faecal coliforms (RR = 0.33), of *E. coli* (RR = 0.41) and of streptococci/enterococci (RR = 0.45) as well as for the parameter 'microball' (RR = 0.56).

The presence of tar in seawater was significantly associated with low levels of total coliforms (RR = 0.52), of faecal coliforms (RR = 0.27), of *E. coli* (RR = 0.63) and of streptococci/enterococci (RR = 0.37) as well as for the parameter 'microball' (RR = 0.56).

The presence of garbage was significantly associated with increasing values of faecal coliforms (RR = 1.21, CI = 1.15–1.28, $p < 0.001$), of *E. coli* (RR = 1.14, CI = 1.08–1.20, $p < 0.001$) and of streptococci/enterococci (RR = 1.13, CI = 1.09–1.17, $p < 0.001$).

The presence of mineral oils in seawater was positively associated with increasing concentrations in seawater of total coliforms (RR = 1.31, CI = 0.99–1.74, $p = 0.044$), of faecal coliforms (RR = 1.67, CI = 1.19–2.33, $p = 0.004$) and of *E. coli* (RR = 2.12, CI = 1.61–2.80, $p < 0.001$). An exception was noticed for streptococci/enterococci where there was a negative association with the presence of mineral oils (RR = 0.63, CI = 0.44–0.91, $p = 0.004$).

The presence of wrack was positively associated with increasing concentrations only of faecal coliforms (RR = 1.18, CI = 1.12–1.24, $p < 0{,}001$), of *E. coli* (RR = 1.13, CI = 1.08–1.19, $p < 0.001$) in seawater and with increasing concentrations of faecal coliforms (RR = 1.15, CI = 1.10–1.19, $p < 0{,}001$) and of *E. coli* (RR = 1.19, CI = 1.14–1.24, $p < 0.001$) on the beaches.

Furthermore the presence of jellyfish in seawater was statistically significantly associated with low levels of faecal coliforms (RR = 0.87, CI = 0.58–1.30, $p = 0.287$) and *E. coli* (RR = 0.88, CI = 0.60–1.30, $p = 0.295$) but, surprisingly, positively associated with total coliforms (RR = 2.27, CI = 1.94–2.65, $p < 0.001$), streptococci/enterococci (RR = 1.66, CI = 1.42–1.94, $p < 0.001$) and the parameter 'microball' (RR = 1.54, CI = 1.36–1.75, $p < 0.001$).

In addition we found that the simultaneous presence of previous day rainfall, phenolic smell and high seas was positively associated with the presence of indicator bacteria with a relative risk of 3.17, (CI = 2.97–3.38) as seen in Table 4. The simultaneous presence of previous day rainfall and garbage was also positively associated with the presence of indicator bacteria (RR = 2.04, CI = 1.93–2.15, $p < 0.001$). Similarly, the presence of previous day rainfall and high seas (RR = 2.10, CI = 2.03–2.18, $p < 0.001$), previous day rainfall, garbage, phenolic smell and high seas (RR = 3.17, CI = 2.54–3.97, $p < 0.001$), previous day rainfall and phenolic smell (RR = 2.48, CI = 2.37–2.61, $p < 0.001$), previous day rainfall, same day rainfall, phenolic smell and high seas (RR = 2.99, CI = 2.72–3.29, $p < 0.001$) all showed a positive association.

Table 4 | Association of bacterial indicators with visually inspected parameters

Microbial indicators		Presence of variables: Phenolic smell		Previous day rain		Same day rain		High sea		Tar on beaches		Tar on seawater		Previous day rain, phenolic smell, high seas	
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
T. coliforms (cut-off level = 150 cfu/100 ml)		5,051/ 21,859	12,565/ 156,652	2,725/ 14,353	14,891/ 164,158	1,858/ 9,437	15,758/ 169,074	12,250/ 106,340	5,366/ 72,171	77/1667	17,539/ 176,844	17/329	17,599/ 178,182	460/1,251	3,784/ 6,1480
	%	23.1	8.0	19.0	9.1	19.7	9.3	11.5	7.4	4.6	9.9	5.2	9.9	36.8	6.2
	RR	2.88*		2.09*		2.11*		1.55*		0.47*		0.52*		5.97*	
	CI	2.80–2.97		2.02–2.17		2.02–2.21		1.50–1.60		0.37–0.58		0.33–0.83		5.52–6.47	
F. coliforms (cut-off level = 100 cfu/100 ml)		1,991/ 18,799	8,152/ 152,239	1,554/ 13,182	8,589/ 157,856	1,001/ 8,580	9,142/ 162,458	6,632/ 100,722	3,511/ 70,316	32/1,622	10,111/ 169,416	5/317	10,138/ 170,721	158/949	2,550/ 60,246
	%	10.6	5.4	11.8	5.4	11.7	5.6	6.6	5.0	2.0	6.0	1.6	5.9	16.6	4.2
	RR	1.98*		2.17*		2.07*		1.32*		0.33*		0.27*		3.93*	
	CI	1.89–2.07		2.06–2.28		1.95–2.20		1.27–1.37		0.23–0.47		0.11–0.63		3.39–4.56	
E. coli (cut-off level = 80 cfu/100 ml)		1,997/ 18,805	8,906/ 152,993	1,564/ 13,192	9,339/ 158,606	1,014/ 8,593	9,889/ 163,205	7,050/ 101,140	3,853/ 70,658	43/1633	10,860/ 170,165	13/325	10,890/ 171,473	75/866	2,820/ 60,516
	%	10.6	5.8	11.9	5.9	11.8	6.1	7.0	5.5	2.6	6.4	4,0	6,4	8.7	4.7
	RR	1.82*		2.01*		1.95*		1.28*		0.41*		0.63		1.86*	
	CI	1.74–1.91		1.91–2.12		1.83–2.07		1.23–1.33		0.31–0.55		0.37–1.07		1.49–2.31	
Streptococci/ enterococci (cut-off level = 30 cfu/100 ml)		4,804/ 21,612	20,926/ 165,013	3,563/ 15,191	22,167/ 171,434	2,272/ 9,851	23,458/ 176,774	17,959/ 112,049	7,771/ 74,576	106/1,696	25,624/ 184,929	17/329	25,713/ 186,296	426/1,217	5,792/ 5,792
	%	22.2	12.7	23.5	12.9	23.1	13.3	16.0	10.4	6.3	13.9	5.2	13.8	35.0	9.1
	RR	1.75*		1.81*		1.74*		1.54*		0.45*		0.37*		3.84*	
	CI	1.70–1.80		1.76–1.87		1.67–1.81		1.50–1.58		0.37–0.54		0.24–0.59		3.54–4.16	
Microball		7,483/ 24,291	29,717/ 173,804	4,843/ 16,471	32,357/ 181,624	3,104/ 10,683	34,096/ 187,412	25,203/ 119,293	11,997/ 78,802	186/1,776	37,014/ 196,319	37/349	37,163/ 197,746	592/1,383	9,013/ 66,709
	%	30.8	17.1	29.4	17.8	29.1	18.2	21,1	15,2	10.5	18.9	10.6	18.8	42.8	13.5
	RR	1.80*		1.65*		1.60*		1.39*		0.56*		0.56*		3.17*	
	CI	1.76–1.84		1.61–1.69		1.55–1.65		1.36–1.42		0.48–0.64		0.42–0.77		2.97–3.38	

*p value < 0.001.
RR: relative risk; CI: 95% confidence interval.

Furthermore as shown in Table 4, our analysis showed that in the presence of phenolic smell there was an absolute risk of 23.1% for the presence of total coliforms, 10.6% for the presence of faecal coliforms, 10.6% for the presence of *E. coli*, 22.2% for the presence of streptococci/enterococci and 30.8% for the presence of any bacterial indicator included in the parameter 'microball'. In the presence of previous day rain, there was an absolute risk of 19% for the presence of total coliforms, 11.8% for the presence of faecal coliforms, 11.9% for the presence of *E. coli*, 23.5% for the presence of streptococci/enterococci and 29.4% for the presence of any bacterial indicator included in the parameter 'microball'. In the presence of same day rain there was an absolute risk of 19.7% for the presence of total coliforms, 11.7% for the presence of faecal coliforms, 11.8% for the presence of *E. coli*, 23.1% for the presence of streptococci/enterococci and 29.1 for the presence of any bacterial indicator included in the parameter 'microball'. In the presence of high seas there was an absolute risk of 11.5% for the presence of total coliforms, 6.6% for the presence of faecal coliforms, 7% for the presence of *E. coli*, 16% for the presence of streptococci/enterococci and 21.1% for the presence of any bacterial indicator included in the parameter 'microball'.

In the presence of garbage there was an absolute risk of 9.9% for the presence of total coliforms, 7.0% for the presence of faecal coliforms, 7.1% for the presence of *E. coli*, 15.4% for the presence of streptococci/enterococci and 19.9% for the presence of any bacterial indicator included in the parameter 'microball'.

More significantly, in the presence of all three variables (previous day rainfall, phenolic smell and high seas) the absolute risk showed to be 36.8%, 16.6%, and 8.7%, for total coliforms, faecal coliforms and *E. coli*, respectively, with the greatest significant absolute risk of 35% recorded for streptococci/enterococci and 42.8% for 'microball'.

Conditional logistic regression analysis

Variables that were significant in the univariate analysis were entered into a multiple logistic regression model. The analysis was conducted in relation to the concentration of bacterial indicators (total and faecal coliforms, *E. coli* and streptococci/enterococci) including the new parameter 'microball'.

As seen in Table 5, the presence of phenolic smell was significantly associated with increased values of total coliforms (odds ratio, OR = 3.42), of faecal coliforms (OR = 2.13), of *E. coli* (OR = 1.98), of streptococci/enterococci (OR = 1.86) and the parameter 'microball' (OR = 2.10). Previous day rain was significantly associated with increased values of total coliforms (OR = 1.85), of faecal coliforms (OR = 1.92), of *E. coli* (OR = 1.81), of streptococci/enterococci (OR = 1.78) and 'microball' (OR = 1.67). Same day rainfall was significantly associated with increasing values of total coliforms (OR = 1.52), of faecal coliforms (OR = 1.40), of *E. coli* (OR = 1.39), of streptococci /enterococci (OR = 1.27) and the parameter 'microball' (OR = 1.27). High seas was significantly associated with increasing mean values of total coliforms (OR = 1.46), of faecal coliforms (OR = 1.25), of *E. coli* (OR = 1.21), of streptococci/enterococci (OR = 1.59) and 'microball' (OR = 1.42). The presence of garbage was significantly associated with increasing mean values of total coliforms (OR = 1.07), of faecal coliforms (OR = 1.21), of *E. coli* (OR = 1.10), of streptococci/enterococci (OR = 1.29) and 'microball' (OR = 1.15). The only parameter noticed to be negatively associated with the concentration of bacterial indicators was the presence of tar. We found that the presence of tar in beaches was significantly associated with decreasing mean values of total coliforms (OR = 0.50), of faecal coliforms (OR = 0.34), of *E. coli* (OR = 0.39), of streptococci/enterococci (OR = 0.46) and the parameter 'microball' (OR = 0.54).

Similarly we found that the presence of tar in seawater was associated with decreasing mean values of total coliforms (OR = 0.61), of faecal coliforms (OR = 0.40), of *E. coli* (OR = 0.77), of streptococci/enterococci (OR = 0.48) and the parameter 'microball' (OR = 0.61).

The presence of mineral oils in sea was positively associated with increasing concentrations in seawater of total coliforms (OR = 1.55, CI = 1.06–2.24, $p = 0.022$), of faecal coliforms (OR = 2.35, CI = 1.56–3.53, $p < 0.001$) and of *E. coli* (OR = 2.47, CI = 1.71–3.57, $p < 0.001$). Streptococci/enterococci however exhibited a negative trend with respect to mineral oils in sea (OR = 0.65, CI = 0.43–1.01, $p = 0.054$).

The presence of wrack on beaches was positively associated with increasing concentration in seawater for

Table 5 | Odds ratio and *p*-values after conditional logistic regression analysis of the variables phenolic smell, rain, high seas, garbage and tar

	Phenolic smell		**Rain**				**High seas**		**Garbage**		**Tar**			
			Previous day		**Same day**						**On beaches**		**In seawater**	
	OR	***p*-value**	**OR**	***p*-value**	**OR**	***p*-value**	**OR**	***p*-value**	**OR**	***p*-value**	**OR**	***p*-value**	**OR**	***p*-value**
Bacterial indicator	**CI**		**CI**		**CI**		**CI**		**CI**		**CI**		**CI**	
T. coliforms	3.4230	<0.001	1.8540	<0.001	1.5222	<0.001	1.4649	<0.001	1.0742	<0.001	0.4985	<0.001	0,6148	0.069
(cut-off level = 150 cfu/100 ml)	3.2967–3.5541		1.7550–1.9586		1.4260–1.6249		1.4152–1.5163		1.0145–1.1375		0.3923–0.6334		0.3641–1.0383	
F. coliforms	2.1253	<0.001	1.9149	<0.001	1.4039	<0.001	1.2519	<0.001	1.2114	<0.001	0.3390	<0.001	0.4023	0.049
(cut-off level = 100 cfu/100 ml)	2.0161–2.2405		1.7873–2.0516		1.2911–1.5266		1.1995–1.3067		1.1312–1.2973		0.2373–0.4841		0.1627–0.9946	
E. coli	1.9837	<0.001	1.8132	<0.001	1.3868	<0.001	1.2084	<0.001	1.0956	0.008	0.3910	<0.001	0,7659	0.379
(cut-off level = 80 cfu/100 ml)	1.8825–2.0904		1.694–1.9407		1.2770–1.5061		1.1597–1.2592		1.0243–1.1718		0.2853–0.5358		0.4230–1.3868	
Streptococci	1.8644	<0.001	1.7766	<0.001	1.2675	<0.001	1.5906	<0.001	1.2913	<0.001	0.4590	<0.001	0.4761	0.004
(cut-off level = 30 cfu/100 ml)	1.7985–1.9327		1.6942–1.8631		1.1959–1.3434		1.5453–1.6372		1.2326–1.3528		0.3744–0.5625		0.2861–0.7922	
'Microball'	2.0992	<0.001	1.6732	<0.001	1.265	<0.001	1.4202	<0.001	1.1537	<0.001	0.5403	<0.001	0.6050	0.007
	2.0358–2.1645		1.6048–1.7446		1.202–1.3313		1.386–1.4552		1.108–1.2013		0.4608–0.6335		0.4202–0.8710	

total coliforms (OR = 1.08, CI = 1.04–1.13, $p < 0.001$), for faecal coliforms (OR = 1.13, CI = 1.07–1.20, $p < 0.001$), for *E. coli* (OR = 1.25, CI = 1.19–1.32, $p < 0.001$) with the exception once again for streptococci/enterococci, where there was a slight negative association with wrack in beaches (OR = 0.80, CI = 0.77–0.83, $p < 0.001$) and for 'microball' (OR = 0.96, CI = 0.93–0.99, $p = 0.007$).

The presence of jellyfish in seawater were statistically negatively associated with low levels of faecal coliforms (OR = 0.92, CI = 0.60–1.41, $p = 0.709$), *E. coli* (OR = 0.88, CI = 0.58–1.32, $p = 0.535$) but as above in the conditional logistic regression analysis jellyfish were significantly positively associated with total coliforms (OR = 2.35, CI = 1.89–2.92, $p < 0.001$), streptococci/enterococci (OR = 1.96, CI = 1.59–2.41, $p < 0.001$) and the parameter 'microball' (OR = 1.72, CI = 1.43–2.06, $p < 0.001$) (Table 5). All the variables identified to be independently associated with the microbial indicators were also found to be associated by using autocorrelation analysis.

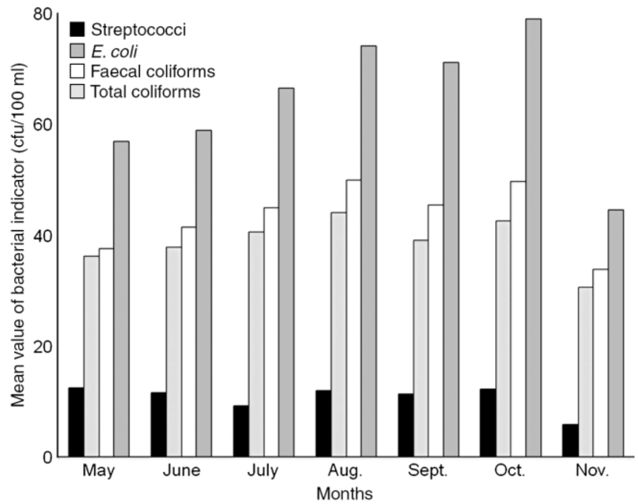

Figure 2 | Means of bacterial indicators by month (1997–2006).

Seasonality impact of bacterial indicators

On examination of the seasonal trends we can see from Figure 2 that there is a significant increasing trend in all bacterial indicators over the months considered. More specifically, we see an increase in total coliforms between the months of August through to October. Faecal coliforms are shown to have increased by about 10 cfu/100 ml from the beginning of the bathing season to the peak of the bathing period in August. Another peak of faecal coliforms and *E. coli* values are noted during the month of October before a decrease in November when all bacterial indicator values decrease. Streptococci values, although relatively lower in comparison with other bacterial indicators, also tend to show more subtle peaks at the beginning of the bathing season (May–June) and then peak again between the months of August and October before decreasing at the end of the bathing season.

DISCUSSION

During the study period almost all of the microbiological test results of samples collected from the Hellenic coastal bathing areas complied with the mandatory requirement values of the Directive 76/160/EEC and those of the Ministerial Decision 46399/1352/86. Furthermore the vast majority complied with the more stringent guide requirement values as well. With the new directive values set by the new European recreational water directive 2006/7/EU it seems that in major part the quality of the Hellenic recreational seawaters has not changed in any significant way.

However, throughout the study period 1997–2006 an increasing trend is noticed of the yearly mean value of faecal streptococci/enterococci (cfu/100 ml) but there was no significant change in the yearly mean value of *E. coli*, faecal coliforms or total coliforms.

According to other studies, streptococci/enterococci are by far the best indicator for recreational seawater quality, representing the strongest trend for increasing relative risk for recreational water-associated illnesses (Fattal & Shuval 1998; Shuval 2003; Wade *et al.* 2003). If this increasing trend of streptococci/enterococci is to continue during subsequent years, then despite the relatively low numbers of streptococci/enterococci recorded at present (fulfilling the EU criteria), it will surely be cause for concern in the near future, requiring further remedial action.

As described above, rainfall on the previous day or the same day of the sampling was significantly associated with increasing concentrations of bacterial indicators. However the association was even stronger for samples of previous day rainfall. This was not a surprising, as rainfall can have a significant effect on indicator densities in recreational water as animal wastes are washed from land. According to other studies, increased incidences of faecal contamination have been found during winters which are characterized by elevated rainfall, while faecal contamination during winters characterized by lower than normal rainfall is significantly reduced when compared with normal conditions (Lipp et al. 2001a,b,c; Boehm et al. 2002; Morisson et al. 2003; Shehane et al. 2005). Increased anthropogenic inputs that may occur within seawaters may also play a significant role during periods of increased beach and sea activity.

The presence of phenolic smell was significantly associated with increasing concentrations of bacterial indicators. Phenol is a colourless or white solid, but usually used as a liquid. It has a strong odour that is sickeningly sweet and irritating, evaporating at a slower rate than water and dissolving fairly well in water. Phenols are involved in many industrial processes and may be released into the environment through industrial discharges and are often found as a natural constituent of animal wastes and organic material. One common use of phenol is in the manufacturing of plastics. Phenol is also used as a slimicide and a disinfectant. Moreover, nitrophenols and chlorophenols occur in the environment as degradation products of the organophosphorus and chlorinated phenoxyalkanoic acid pesticides respectively. Phenol can remain in water for longer than 9 days. Large quantities of phenol have been found in surface waters and surrounding air, having been contaminated when phenol is released from industrial and commercial products, more than is usually found in the environment under natural circumstances (Eco-USA, www.eco-usa.net/toxics/phenol.sht ml, accessed 5 March 2009). In our study, the presence of phenol was recorded by the presence of a subjective phenolic smell. It would be of interest to examine chemically the concentrations of phenols that may be reported in bathing water and correlate these findings with that of phenolic smell recording. Nevertheless, whether bad odour from phenols, or bad odour alone, a strong positive association was noticed with increasing concentrations of bacterial indicators.

It can be hypothesized that the presence of waves and high seas can bring sediments containing faecal pollution back into coastal waters from areas of settlement. The increased levels of turbidity due to such actions present an environment for potential bacterial absorption and/or protection from detrimental effects of sunlight (Kibbey et al. 1977; Fujioka et al. 1981; Desmarais et al. 2002; Sinton et al. 2002). In general it is noted that higher turbidity levels usually indicate higher bacterial concentrations. Furthermore there is increasing evidence that sand is a favourable environment rather than a hostile environment for bacteria and therefore a potential reservoir for bacteria (Cinotto 2005; Beversdorf et al. 2007).

The presence of garbage was significantly associated with increasing concentrations of all bacterial indicators but not as strongly as for the previously described parameters. In general it is usually inferred that a highly littered beach has poor water quality (Robens Institute of Industrial & Environmental Health & Safety 1987).

The presence of oil was associated with increasing concentrations of all bacterial indicators with the only exception being that of streptococci/enterococci where a negative association was noticed without any plausible explanation. As for litter described above, the presence of oil is generally also poorly associated with bathing water quality (Robens Institute of Industrial & Environmental Health & Safety 1987). Regardless of the fact that the presence of wrack on beaches showed a positive association in relation to the presence of bacterial indicators, we are unable to justify the significance of its presence as an influencing factor. The most logical conclusion is that the presence of wrack on beaches may be a surrogate for wave action.

Historically, tar and wood tar were some of the first compounds to be used as disinfectants in the distilling industries. The presence of tar on beaches, surprisingly, was significantly associated with low concentrations of bacterial indicators, possibly because tar may be a hostile environment for the survival of bacteria (Greenberg 1956; Hood et al. 1975). One other possible explanation for this result may be that tar on beaches or water may deter bathers, reducing contamination from them.

In a nutrient-enriched sea environment we can expect changes of zooplankton (jellyfish) with varying species composition and increases of their biomass. The presence of jellyfish was positively associated with the presence of total coliforms, streptococci/enterococci and the parameter 'microball' while they were negatively weakly associated with *E. coli* and faecal coliforms. As streptococci/enterococci is considered to be the best bacterial indicator for seawater (Fujioka *et al.* 1981; Sinton *et al.* 2002) and we have found a positive association with jellyfish we can speculate that the presence of jellyfish can be considered a reliable pollution parameter. Furthermore, as similar results were observed with the parameter 'microball' which included the presence of any of the bacterial indicators, this association presents a positive significance and is more likely to be valid.

The greatest risk factor for the presence of bacterial indicators was noticed in combination with the presence of specific parameters: that is, previous day rain in combination with phenolic smell and high seas [absolute risk = 42.8% (RR = 3.18, CI = 2.98–3.38, $p < 0.001$)]. Moreover it was noticed that the presence of previous day rain in combination with other parameters always showed the greatest risk.

From our results, it was observed that in the presence of certain visual parameters, the highest risk was recorded for the presence of streptococci/enterococci [phenolic smell = 22.2% (RR = 1.75, CI = 1.70–1.80), previous day rain = 23.5% (RR = 1.81, CI = 1.76–1.87), same day rain = 23.1% (RR = 1.74, CI = 1.67–1.81), high seas = 16% (RR = 1.54, CI = 1.50–1.58) and garbage = 15.4% (RR = 1.13, CI = 1.09–1.17)] and for the presence of total coliforms [phenolic smell = 23.1% (RR = 2.88, CI = 2.80–2.97), previous day rain = 19% (RR = 2.09, CI = 2.02–2.17), same day rain = 19.7% (RR = 2.11, CI = 2.02–2.21), high seas = 11.5% (RR = 1.55, CI = 1.50–1.60) and garbage = 9.9% (RR = 1.00, CI = 0.96–1.05)]. This result strengthens the value of streptococci/enterococci as the most important microbial indicator in bathing water. More astonishingly, in the simultaneous presence of previous day rain, phenolic smell and high seas an absolute risk of 35% (RR = 3.84, CI = 3.54–4.16) was calculated for the presence of streptococci/enterococci (Table 4). These findings may be utilized in the absence of regular microbiological monitoring as a proxy for the assessment of bathing water quality.

According to our results it seems that seasonality may play a significant role. The increasing means of bacterial indicators from May to August are not a surprising result and for this period it seems that the main factor that adversely influences the counts of bacterial indicators could be bather density, which increases during this period and reaches its peak in August (traditionally the month of vacation in Greece). After that a slight decrease of means of bacterial indicators in September is presumed to be a result of the lower bather densities. The increase in the means of bacterial indicators in October can be attributed to the beginning of the country's first rainfalls. The increase in bather density would also explain why the adverse antibacterial effect of increased hours of sunlight during the summer months does not reduce the concentration of these bacterial indicators.

Since standard culture procedures for water samples have a mean result turnaround time of 3 days, it seems that we should take advantage of the use of visual parameters to assess risk for the presence of bacterial indicators. This can be clearly supported by the significant findings of the absolute risk of 35% (RR = 3.84, CI = 3.54–4.16) for streptococci/enterococci and 36.8% (RR = 5.97, CI = 5.52–6.47) for total coliforms when the three parameters (previous day rain, phenolic smell and high seas) are recorded simultaneously. The use of visual parameters do not demand trained personnel, are quick, efficient, cheap and are supported by absolute risk values in our study as a useful primary tool to assess the possible health risk to recreational seawater bathers.

CONCLUSION

From our analysis of the quality data of seawater we can conclude that the use of visual parameters to assess risk factors for the presence of bacterial indicators proved to be a useful primary tool that can be applied directly without having to wait for time-consuming culture results. The possible association of visually inspected parameters with bacterial indicators should be further investigated in order to minimize the need for water sampling in the assessment of the quality of beach bathing water. This in turn would aid in the assessment of the quality of beach bathing waters and provide a more timely indication of bathing water quality, hence contributing to the immediate health protection of bathers.

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