ORIGINAL ARTICLE

ENTEROCOCCI VS COLIFORMS AS A POSSIBLE FECAL CONTAMINATION INDICATOR: BASELINE DATA FOR KARACHI

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ABSTRACT

Fecal contamination of drinking water is the major cause of water borne illnesses. For long time coliforms are exploited as fecal contamination indicator. However, recent studies indicate low survival rate of coliforms in stress conditions, hence it's use as indicator of fecal pollution is being abandoned in many parts of the developed world. Implementation of such strategy demands availability of local data in the cities like Karachi. The present study provides a comparison between coliforms and enterococcal load and its variation in sewage samples collected (June, August and November, 2006) from eighteen towns of Karachi. All the diluted samples were selective media to obtain colony-forming units (CFU) mainly for coliforms and enterococci. The bacteria isolated were identified on the basis of conventional microbiological methods. Observations thus obtained were subjected to rigorous statistical analysis. The total load of enterococci was found in range of 1.27-8.47 X 10^7 as compared to coliforms (3.03-13.9 X 10^7). However, segregation of data reveals greater inter town variability in CFU/ml both in coliforms and enterococci as suggested by their cumulative standard deviation $\pm$1.5 X10^7. Furthermore, CFU/ml of both coliforms and enterococci also varies to variable scale when collected at different time intervals and at intra town level. Conclusively, the studies suggest high survival rate and lower variability of Enterococci compared to escherichia hence indicating its potential advantage to be used as fecal contamination indicator.

Keywords: Fecal contamination indicator, coliforms, enterococci, escherichia, Karachi towns.

INTRODUCTION

Over population and urbanization leads to immense pressure on the availability of safe drinking water all over the world. World health organization survey revealed that 1.1 billion people all over the world do not have access to improved source of drinking water. Moreover, world wide urban population, with an access to safe drinking water, has dwindled with teeming pace from 770 million in 1994 to 700 million in 2004. In Pakistan the situation is even worse, as household availability of safe water has been reduced from 60% to 40% primarily due to increased urbanization from 31% to 34%. Such grim situation encourages prevalence of a number of water borne illnesses. Indeed, unsafe water compounded with lack of basic sanitations is the direct cause of 1.6 million mortalities and uncountable morbidities annually only in children (under the age 5) most of which belong to the developing countries like Pakistan (WHO; UNICEF, 2004). Monitoring microbiological status of the drinking water is one and perhaps most important step towards rectification of the mentioned problem. In this connection several microbes' ranging from bacteria (Thurston-Enriquez *et al*., 2005) to even coliphages (Luther and Fujoika, 2004) were proposed as indicators of fecal contamination/pollution and thus a health hazard. Despite the traditional use of fecal coliforms as fecal contamination indicator, numerous recent studies suggest that fecal coliforms are more susceptible to a variety of geographical, physico-chemical factors and/or stresses (Sinton *et al*., 1999; Gauthier and Archibald, 2001; Lee *et al*., 2006; Zhang and Lulla, 2006). Among physical factors sunlight causes to greater inactivation of fecal coliforms than enterococci, followed by F-RNA coliphages and somatic coliphages (Davies-Colley *et al*., 1994; Luther and Fujoika, 2004). Geographical and meteorological factors e.g., storm activity, temperature and water flow rate also exert substantial influence on the prevalence of fecal coliforms (Skraber *et al*., 2002; Evanson and Ambrose, 2006; Lee *et al*., 2006). Additionally, sensitivity to sewage treatment process (Luther and Fujioka, 2004) salinity level (Shibata *et al*., 2004) and broad host range (Ørskov, 1984; Wheeler *et al*., 2002) of fecal coliforms especially *Escherichia coli* results in false negative and false positive results. In contrast to this, numerous reports indicate that low geographical impact, narrow host range and stress tolerant ability of enterococci (a subset of fecal streptococci), make it a more suitable fecal contamination indicator when compared to fecal coliforms (Davies-Colley *et al*.,

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Table 1: Total enterococci and coliforms mean load distribution in 18 towns of Karachi along with seasonal variability

Towns Name	Bacterial Load in 10^7 CFU/ml			
	Enterococci	Seasonal Variation (Standard Deviation)	Coliforms	Seasonal variation (Standard Deviation)
Baldia	7.88	3.9	3.83	0.3
Bin Qasim	1.27	1.1	5.05	1.3
Gadap	3.38	2.6	3.08	1.1
Gulberg	4.87	2.7	3.71	1.3
Gulshan-e-Iqbal	4.24	0.7	3.83	1.3
Jamshed Town	5.55	2.0	5.21	1.8
Keamari	5.07	0.1	3.83	1.6
Korangi	5.21	2.6	3.94	0.7
Landhi	5.88	1.8	13.9	10.4
Liaquatabad	6.98	4.9	3.65	1.9
Lyari	8.47	6.4	3.03	1.0
Malir	4.91	1.0	4.48	0.9
NewKarachi	5.17	7.2	3.21	0.8
North Nazimabad	4.20	1.3	2.93	1.5
Orangi	5.28	1.4	4.80	0.9
Saddar	4.71	1.4	3.14	1.5
Shah Faisal	4.63	3.5	11.8	12.6
SITE	5.67	3.5	3.46	1.0
Entire Karachi (Mean)	5.19	1.5	4.83	1.5

1994; Sinton *et al*., 1999; Wheeler *et al*., 2002; Shibata *et al*., 2004; Ahmed *et al*., 2006). Guidelines based on enterococci as fecal contamination indicator, have already been proposed or adopted in a number of states in the United States (Chasis *et al*., 1992), in Canada (Canadian Council of Resource and Environment Ministers, 1992), in Australia (Australia and New Zealand Environment and Conservation Council, 1992) and in New Zealand (McBride *et al*., 1992). However, adaptation of such strategy requires presence of base line indigenous data.

In order to validate the replacement of fecal coliforms to Enterococci as a fecal contamination indicator, the present study is designed in which total loads of Enterococci, and fecal coliforms from sewage samples of all eighteen towns of Karachi were enumerated and analyzed statistically.

MATERIALS AND METHODS

Sample collection

Sewage samples were collected from five different stations of main sewage lines of all eighteen towns of Karachi (fig.1: a-f) in pre-autoclaved wide mouth jars during June, August and November 2006. All samples were stored in ice filled bucket till further processing. The time of further processing ranged from 2-5 hours. pH values of each sample were noted using pH strips (Merck) both at the time of sampling and the processing.

Sample processing

All samples were serially diluted and 100ul of each diluted sample was dispensed over Bile Esculin Agar (BE; Merck), Levine Eosin-Methylene Blue Agar (EMB; Oxoid) and MacConkey Agar (Mac; Oxoid) plates. All the samples were immediately spread using sterile glass spreader till they were completely absorbed in agar plates. All plates were then incubated at 37ºC for 24 to 36 hours.

Load determination

Black colonies on BE Agar and pink color colonies on EMB and Mac Agar directly indicate the number of enterococci and fecal coliforms respectively. The colonies bearing green metallic sheen on EMB agar were considered as of *Escherichia coli* (fig.1.g-i). Colony counts were used to deduce CFU/ml of every sample.

Extended identification

A few colonies were picked from BE, EMB and Mac

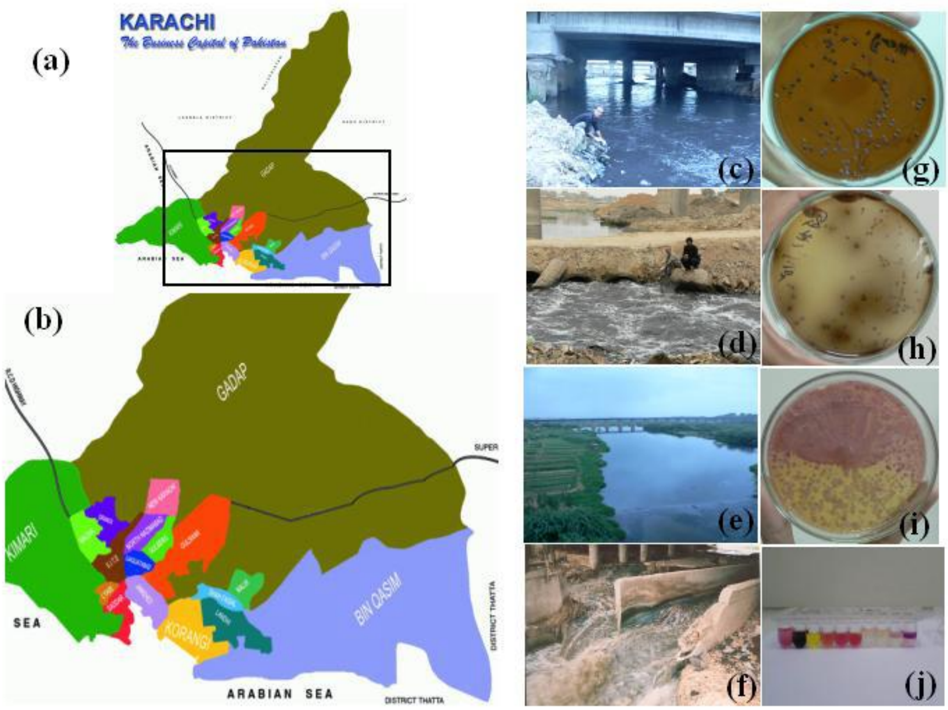

Fig. 1: Towns of Karachi and fecal contamination indicators: Entire Karachi map with subdivision of towns (1. a,b) sewage collection sites Lyari, Gulshen-e-Iqbal, Malir and SITE (1. c-f respectively). Pink color colonies of coliforms on EMB and McConkey Agar (1. g,i, respectively), black color colonies of Enterococci on bile esculin agar and rapid identification test results (1. h, j respectively).

plates with reference to their different colonial characteristics and geographical location and patched over Brain Heart Infusion (Oxoid) agar. Gram staining and catalse, IMViC tests further validated colonies of enterococci, coliforms and escherichia. Taxonomically, unorthodox strains of enterococci were further identified using Rapid identification kit for Streptococci (Remel) (fig.1. j).

RESULTS AND DISCUSSION

pH variability

pH of most sewage samples ranged between 6.5 to 7.0 during all collection events with almost no variations when collected at different stations of the same town and in different months. However, samples from stations of Gadap town exhibited pH 8.5. This high alkaline pH might be the function of extreme wild vegetation and subsequently eutrophication, which was observed in the vicinity of sewage pathway. Interestingly, minor variations of pH (0.5) in collected sewage were observed during the sample transport time (Town stations to Laboratory) during all collection months in all towns except Gulberg and Nazimabad where pH fluctuation was even higher, pH (1.0). However, homogeneity in variations in majority of cases despite of being processed at different times suggests that chemical and/or microbial activities in confined state (collection vessel) may result in pH fluctuations. Minor laboratory variations akin to this are also observed in some reports (Griffith *et al*., 2006).

Load

Total average loads in all stations of each town and in all three collection months indicate that enterococci are more abundant than escherichia and coliforms in fifteen towns of Karachi (fig. 2). Deviation from the mentioned normal trend is confined to only three towns of Karachi where coliforms were more prevalent than Enterococci. In particular, coliform numbers in Bin Qasim, Landhi and Shah Faisal Town ($5.05x10^7$, $1.37x10^8$, $1.18x10^8$ respectively) are considerably higher than enterococci ($1.27x10^7$, $5.88x10^7$, $4.63x10^7$ respectively). The deviated discrepancy from the normal trend of high load of enterococci in comparison to coliforms and Escherichia is due to intra town variation as appeared in case of Bin Qasim where intra town fluctuation of coliforms is higher especially in months of June ($\pm 1.5x10^7$) relative to enterococcal load ($\pm 0.25x10^7$). The same reason could be extended for higher coliforms loads in Landhi and Shah Faisal Town. Thus, it seems plausible to describe that elevated counts of coliforms are likely due to highly diverse load in one and/or few stations of respective town. Such localized variations in mentioned organism are also

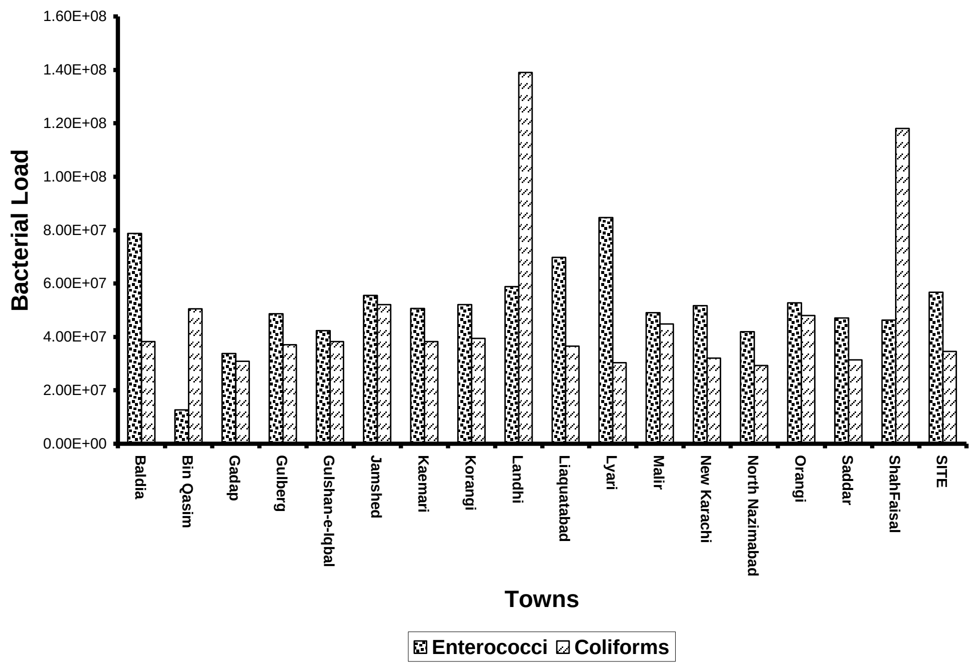

Fig. 2: Cumulative Histogram of Total of Enterococci (dotted bars) and Coliforms (dashed bars) mean load distribution in 18 towns of Karachi: Note the normal trend of greater load of enterococci in most towns.

reported in previous studies conducted on same lines at different geographical coordinates (Kirschner *et al*., 2004; Evanson *et al*., 2006; Ahmed *et al*., 2006). Several physico-chemical factors like UV radiation (Sinton *et al*., 1999; 2002), osmotic stress (Kirschner *et al*., 2004) and laboratory-associated factors (Griffith *et al*., 2006) are attributed to the mentioned intra locality variability in primarily escherichial and coliform loads.

Further, cumulative inter town microbial load standard deviation of enterococci ($\pm 1.5X10^7$) is found less than coliforms [($1.6\pm X10^8$) (table1)]. Aside from the mentioned reasons responsible for intra town variation population density of a particular area (Moe *et al*., 1991; Evanson *et al*., 2006) and extended host range (Ørskov, 1984; Wheeler *et al*., 2002) of escherichia (a major contributor of coliforms) also are likely to be the cause of this inter locality variations.

Enterococci appeared more responsive to seasonal variations as their load varies significantly more than coliforms in eleven out of eighteen towns during collection events. Sewage sample from only Gulshan-e-Iqbal, Kemari, Korangi and Shah Faisal Town showed more changes in coliforms load compared to enterococci. Remaining three towns showed same enterococcal load variations as that of coliforms. These inter locality seasonal variation tendency of enterococci could be exploited as marker for microbial source tracking for point and otherwise (non-point) contamination (Simpson *et al*., 2002; Wheeler *et al*., 2002; Garcia-Armisen and Servais, 2006). Conversely, the cumulative load per collection month was not changed significantly in case of enterococci but varied greatly with reference to coliforms (table 1). Precisely, the total cumulative mean load of enterococci of entire Karachi during June, August and November was $5.97\ X10^7$, $5.66\ X10^7$ and $3.94\ X10^7$ respectively compared to coliforms load of $1.41X10^8$, $6.83\ X10^7$ and $8.15\ X10^7$. The suggestive reason for these seasonal fluctuations in coliforms load is waterfall and subsequent salinity level that hampered the growth of coliforms in particularly the Escherichia (Kirschner *et al*., 2004; Evanson *et al*., 2006; Noble *et al*., 2006). Although the over all load of coliforms in Karachi seems higher but inter town data analysis showed that this high load is merely the function of high one or few stations of three towns (it has already been stated else where).

CONCLUSIONS

On the basis of over all data analysis it is plausible to state that enterococci are more prevalent in sewage than the coliforms. In addition to this, their low inter locality variations at fixed time period and relatively more vulnerability to seasonal factors at a particular locality extend enterococci an advantage over coliforms as not only fecal contamination indicator but also as microbial source tracking bacteria.

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