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Microbiological Examination of Sebeel Water

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Water samples from clay storage jugs ("zeers") located in homes and at public watering stands ("sebeels") at streets, mosques, and schools were examined. Coliforms, fecal coliforms, and fecal streptococci were detected in 100, 69.88, and 91.56% of the samples, respectively. The general microbiology of the water and some factors affecting microbial load were studied. The predominant bacterial genera of sebeel water were found to be *Staphylococcus, Aerococcus, Micrococcus, Streptococcus, Bacillus, Listeria, Lactobacillus*, and *Arthrobacter*. A simple modification of zeer construction was suggested to help improve sanitation.

The Sudanese commonly keep their drinking water in jars (capacity 30 to 40 liters) made of baked clay called "zeers." These keep the water cool and refreshing due to evaporation through the walls of the jars. "Sebeels" are public, street-side watering stands which may consist of one, two, or up to six zeers. They are normally provided by able individuals in the community in accordance with Islamic teachings. Water from the sebeel is normally served by one cup which is dipped into the zeer whenever one wants to drink.

There are many sebeels in Khartoum—at least one sebeel in every street, mosque, school, hospital, and market place. These sebeels serve a large sector of the population of Khartoum and, at present, are indispensable. In recent years however, the hygienic fitness of the sebeel or zeer has been questioned. The little research (6, 9) carried out to date concerned itself specifically with disease transmission and was confined to zeers found in private homes.

This paper deals with the microbiology of sebeels from a broader viewpoint in an attempt to reveal some of the general as well as the hygienic aspects of the subject.

MATERIALS AND METHODS

Tap water quality. The water used to fill the zeers is clean, filtered, chlorinated tap water from the Central Electricity and Water Administration of Khartoum. Colony counts of tap water ranged from 8 to 20 cells/ml, whereas repeated trials gave negative presumptive tests for both coliforms and streptococci. Its residual chlorine content was between 0.1 and 0.5 mg/liter, and its optical density ranged between 0.005 to 0.015 (using a Hilger colorimeter, blue filter, 430 nm, distilled water as blank). In comparison, zeer water turbidity was 0.01 to 0.04, whereas the chlorine level of 21 samples ranged between 0 and 0.1 mg/liter using the DPD method (1).

Sampling. Water samples were collected in 4-ounce (ca. 120 ml) sterile bottles containing 0.1 ml of 10% sodium thiosulfate solution. Sterile beakers were used to take equal portions of water from each zeer in the sebeel so that these portions together filled the bottle. While taking a sample, care was taken not to disturb the water at the bottom of the zeer. Eighty-three such composite samples, each from a different sebeel, comprising some 350 zeers from all over Khartoum were analyzed. Composite samples were preferred to sampling individual zeers since the level of contamination of zeers in a particular sebeel is expected to be roughly the same due to the fact that lids are usually lacking and one cup normally serves all zeers. Samples were collected between 8:00 and 11:00 a.m., rushed to the laboratory within 20 min, and immediately analyzed.

Microbiological methods. Total colony count was done by the pour plate method using plate count agar (Difco Laboratories, Detroit, Mich.). Phosphate buffer was used as diluent, and plates were incubated at 37°C for 48 h. Counting was carried out with the aid of an Astell colony counter (Laboratory Service Co., Ltd., London, England).

The three-tube procedure using lactose broth (Difco) was used for estimating the most probable number (MPN) of coliform organisms. Tubes were incubated at 37°C for 48 h and the MPN was obtained according to *Standard Methods for the Examination of Water and Wastewater* (1). The confirmed coliform test was done by culturing the positive tubes into brilliant green bile broth and incubating at 37°C for 48 h. Differentiation of fecal coliforms was done by plating positive tubes from the brilliant green bile broth cultures on eosin methylene blue agar. Colonies from these plates were then differentiated by the indole, methyl red, Voges-Proskauer, citrate (IMVic) tests.

The MPN of fecal coliforms was done by transferring three loopfuls from each positive confirmed coliform test tube to brilliant green bile broth tubes and incubating at 44.5 ± 0.2°C for 24 h. Tubes showing any amount of gas production were considered positive, and MPN was obtained from tables in reference 1 as mentioned above.

The MPN for fecal streptococci was determined by the multiple tube method, as shown above, using azide dextrose broth. Tubes were incubated at 37°C and checked for turbidity after 48 h. Confirmation of the

TABLE 1. Average $\log_{10}$ counts of important microbial groups in sebeel water

Source of sample	No. of composite samples examined	Colony count/ml	Coliforms per 100 ml	Fecal coliforms per 100 ml	Fecal streptococci per 100 ml
Streets	51	5.30	2.53	1.09	1.56
Mosques	15	5.04	2.58	1.38	1.81
Homes	10	5.08	3.13	2.34	2.65
Schools	7	5.51	2.41	1.87	1.65

presence of fecal streptococci was done by inoculating samples from the positive tubes into ethyl violet azide broth and incubating at 37°C for 24 h. Formation of a purple button at the bottom of the tube constituted a positive test.

Identification of bacterial genera was done according to *Bergey's Manual of Determinative Bacteriology* (5). Eight isolates of predominant bacteria from each of 40 sebeels were subjected to this identification.

RESULTS AND DISCUSSION

Extent of sebeel water contamination. Table 1 is a summary of the counts of important microbial groups in sebeel water obtained from streets, mosques, schools, and homes. It can be seen that the colony count was of the order of 10^5 cells/ml in each group of sebeel. In general the counts were higher for school and street sebeels than for mosques and homes. On the other hand, counts of coliforms, fecal coliforms, and fecal streptococci were much higher for home sebeels than for other types of sebeel.

Dust, frequency of use, and lack of cleaning undoubtedly all contributed to the colony count. In addition, regrowth of some microorganisms could have taken place. Since coagulants or taste-improving materials are not added to zeer water in the Khartoum area and since no slime films were detected on the inside surface of zeers, these factors could not have biased our results.

The fact that home sebeels scored the highest counts for the three indicator groups of bacteria came as a surprise in light of the popular belief that street sebeels are the most dangerous of all. The possibility that detection of indicator organisms in the other three sebeels was partially prevented by antagonistic action of other microbes cannot be completely ruled out, but it does not seem to explain this situation since all types of sebeels studied scored high colony counts. There is no reason why antagonism should work in one type of sebeel and not in the other. We believe that the high indicator counts of home zeers truly reflected a higher degree of fecal contamination. Unclean children no doubt contributed much to this contamination.

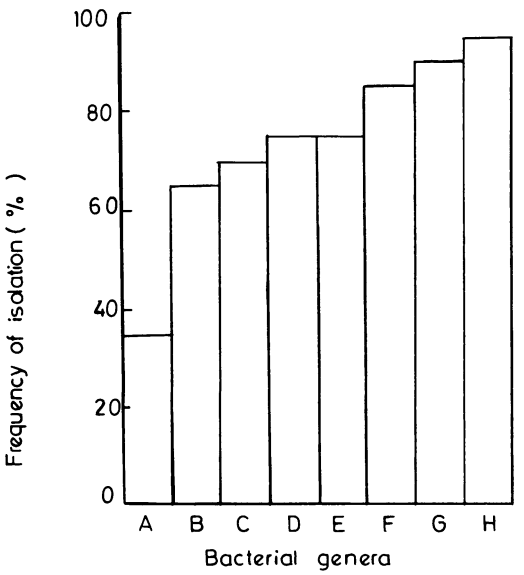

FIG. 1. Predominant bacterial genera found in sebeel water. Abbreviations: A, *Arthrobacter*; B, *Lactobacillus*; C, *Listeria*; D, *Bacillus*; E, *Streptococcus*; F, *Micrococcus*; G, *Aerococcus*; H, *Staphylococcus*.

Predominant bacterial genera in sebeel water. The eight most dominant genera of bacteria in sebeel water were gram positive (Fig. 1). An examination of the natural habitats of these genera (5) shows that they reflect the expected sources of contamination. The genera *Arthrobacter*, *Bacillus*, and *Aerococcus* are typically found in soil and air. *Micrococcus*, although commonly found in soil and fresh water, is also frequently found on the skin of man and other animals. *Lactobacillus*, widely distributed in nature, is also parasitic in the mouth and intestinal tract of many warm-blooded animals including man. *Streptococcus* is found in dental plaques, mouth, throat, upper respiratory tract, and feces of man and animals. *Listeria* is parasitic on and is found in the feces of warm-blooded animals including man. In this regard, birds and goats are regularly seen watering at sebeels.

The two most commonly found genera in sebeel water were *Staphylococcus*, a typical

TABLE 2. Percent of samples in which fecal coliform or fecal streptococci were detected

Sample source	Samples in which organisms were detected (%)	
	Fecal coliforms	Fecal streptococci
Streets	60	80
Mosques	75	80
Homes	100	100
Schools	67	86

skin organism, and *Aerococcus*, a typical air organism. No doubt the main source of staphylococci was the human hand which almost always touches the water when a person takes a drink. Individuals with the habit of nosepicking understandably played an important role here. The extent of contamination with *Staphylococcus* can be said to reflect the frequency of use of the particular sebeel by humans. In this respect, contamination of sebeel water with this organism is likened to the body pollution of swimming pools (7) where staphylococci are suggested as useful indicator organisms

Coliforms, fecal coliforms, and fecal streptococci. Our results show no relationship between total colony count and fecal contamination, indicating that the factors controlling each of these parameters were different. In some instances the colony count could be more than 10^6/ml whereas the same sample was found to be free from fecal contamination. It is possible, however, that detection of index organisms in such samples has been prevented by antagonistic action of some of the organisms in the viable count. Coliforms, fecal coliforms, and fecal streptococci were detected in 100, 69.88, and 91.56% of all 83 composite samples, respectively. In the 69 sebeels in which both fecal coliforms and fecal streptococci were detected, the latter outnumbered the former in 62.3% of the samples. Fecal streptococci were detected in the absence of fecal coliforms in 13% of the samples. Samples showing fecal coliforms but not fecal streptococci constituted only 4.8%.

With respect to the groups of sebeel, fecal coliforms and fecal streptococci were detected in 60 and 80% of the street samples, 75 and 80% for mosques, 100 and 100% for homes, and 67 and 86% for schools, respectively (Table 2).

The presence of coliforms in all samples and their high counts relative to fecal coliforms meant that most of these organisms were of nonfecal origin. Regrowth of some coliforms might have taken place, particularly since zeer water is a little turbid and its temperature is normally above 20°C. In any case, these high counts should cause concern.

The four most commonly encountered low-temperature (37°C) coliforms were found to be, in descending predominance, *Klebsiella ozaenae, Enterobacter aerogenes, Enterobacter cloacae*, and *Serratia marcescens*. The first organism occurs in ozaena and other chronic diseases of the respiratory tract. It is conceivable that it gets access to sebeel water from the fingers of ozaena patients who pick their noses. The two *Enterobacter* species and *S. marcescens* occur primarily in soils and water. Therefore the predominant coliform species in sebeel water do not necessarily indicate fecal contamination.

Even the fecal coliform estimate at 44.5°C does not reflect the true count of these organisms. Only 44.4% of the fecal coliforms were found to be *Escherichia coli* type I as indicated by the IMViC tests (Fig. 2). According to Bardsley (2, 3), *E. coli* type I is the most numerous type of coliform in feces. *E. aerogenes* constituted 30.5% of the high-temperature (44.5°C) coliforms. This finding agrees well with reports from India (8) which showed that 50% of aerogenes-like organisms produced acid and gas at 44°C. Also, Boizot (4), testing organisms isolated from waters in Singapore, found that out of 49 aerogenes-reacting strains, 13% gave acid and gas in MacConkey broth at 44°C.

Some factors controlling microbial populations in sebeel water. Of the possible factors which

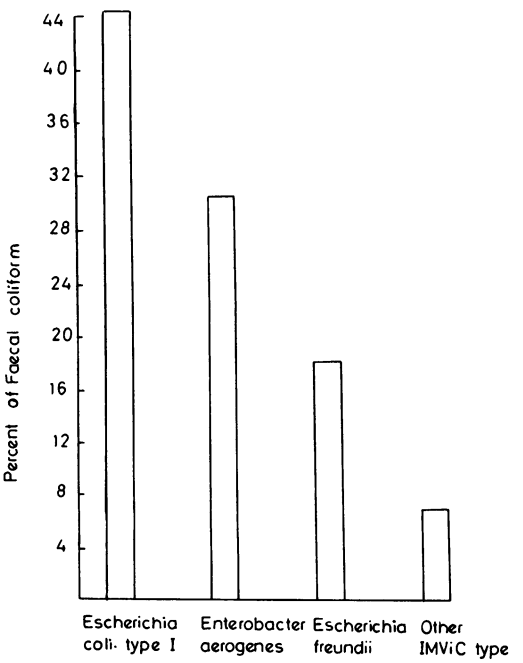

FIG. 2. Relative abundance of the groups constituting the fecal coliforms of sebeel water.

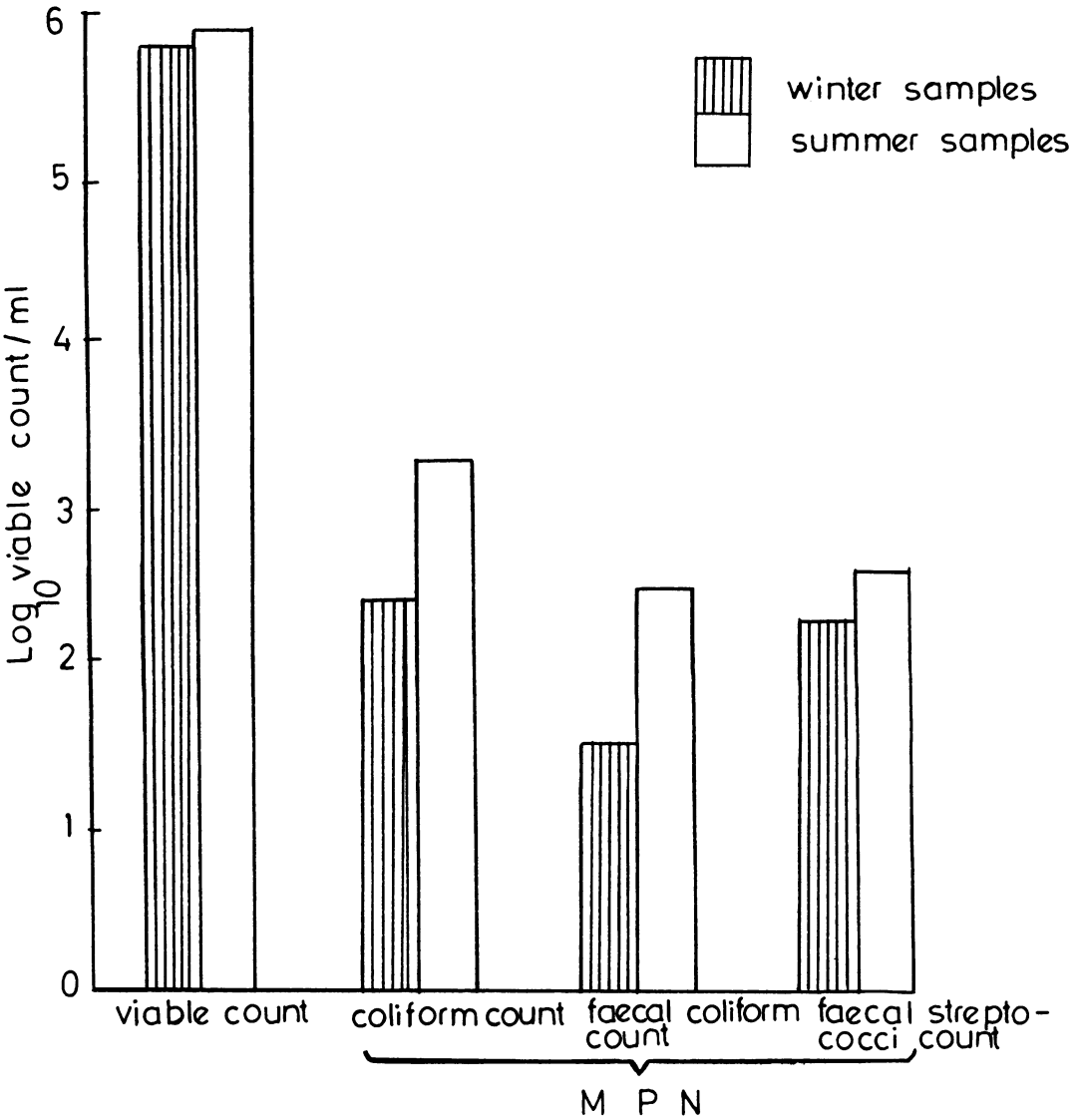

FIG. 3. Effect of season on microbial indicator groups.

control microbial populations in sebeel water, three factors were tested: the seasonal factor, dusty winds, and sebeel design. The count of each microbial group was higher in the summer than it was in the winter (Fig. 3). Warm temperatures, increased dust storms, more frequent use by humans, and regrowth of bacteria could explain these high counts in the summer. The percent increases in colony, coliform, fecal coliform, and streptococcal counts were 12.5, 530.8, 937.4, and 124%, respectively. Clearly the colony count showed relatively little tendency to vary with the season. This could mean that the colony count is not determined by frequency of human use of the sebeel or by growth in water; rather it is largely determined by natural factors like wind and dust which are present in both seasons.

In the hot summer season these sebeels see frequent use by many humans. This is reflected in the observed substantial increase in the counts of the three indicator groups. In particular, there was a very big increase in the fecal coliform count. In part, this increase could be due to coliforms of nonfecal origin such as the aerogenes-like group, which could also be responsible for the increase in the coliform count.

The second factor assessed was the "haboub" (dusty wind) factor. This is an important factor since Sudan is riddled with such winds. Counts

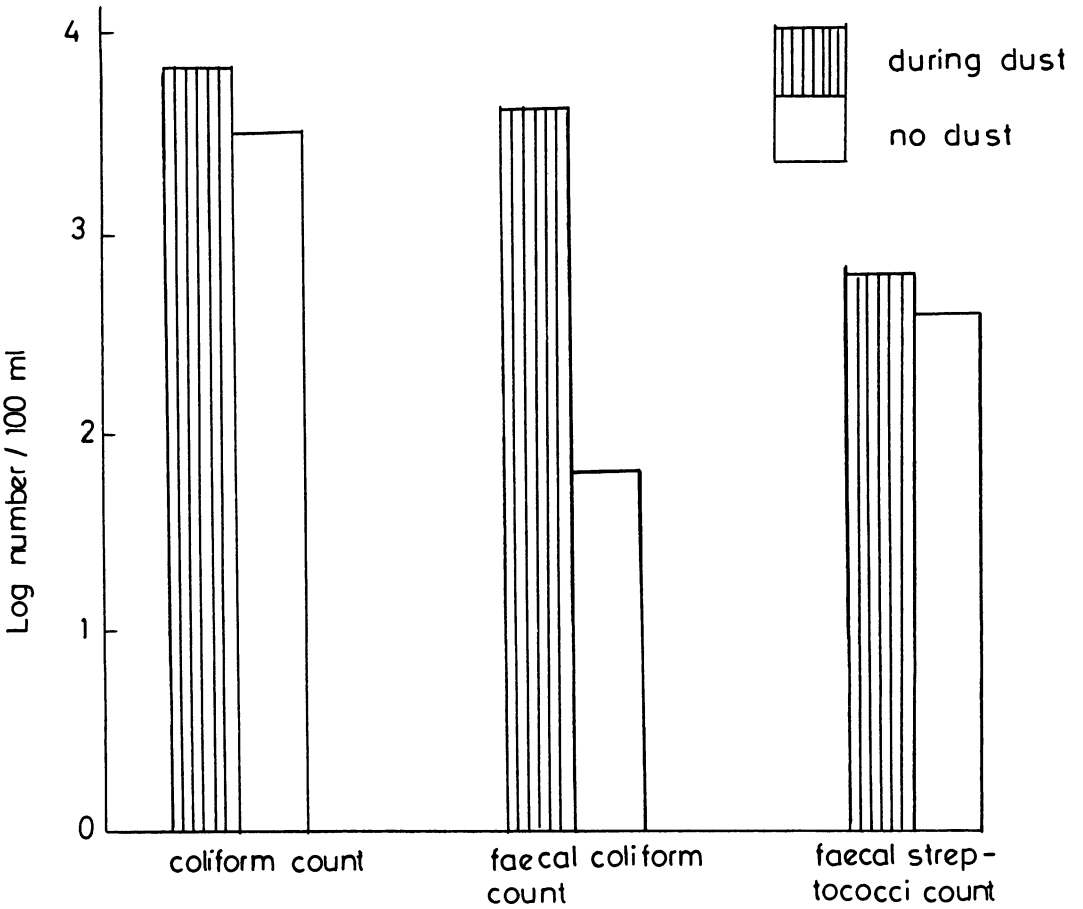

FIG. 4. Effect of dust storm on the count of indicator groups.

of all microbial groups were measured before and after a wind storm in five sebeels (Fig. 4). The results look similar to those obtained for the seasonal variation experiment. Again here, an extremely high rise in the count of fecal coliforms can be seen.

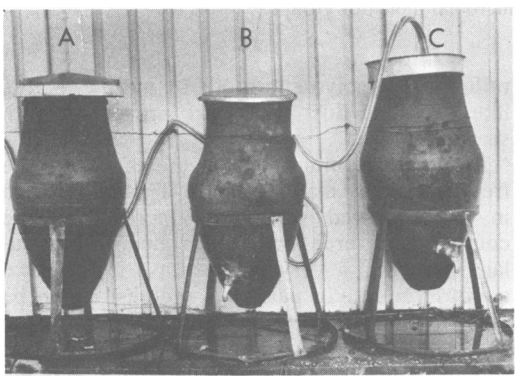

FIG. 5. Three designs of zeer: A, has no faucet; B, has faucet and movable lid; C, has faucet and firmly fitted lid.

The third factor tested was zeer construction. The three zeer designs tested are shown in Fig. 5. Three newly made zeers were used for this experiment: zeer A, which had a movable lid, had no faucet at the bottom; zeer B had a faucet as well as a movable lid; zeer C had a faucet and a tight-fitting lid which could not be lifted. For fitting the faucet, the zeer was carefully bored and the faucet was inserted and cemented in place. After the three zeers were located where they would definitely be used, they were filled with tap water using a clean hose (shown in Fig. 5), and the degree of contamination was followed regularly. The results (Table 3) show that zeer C showed no signs of fecal contamination even after a whole month has passed, whereas the other two zeers were contaminated within the first 2 days. This simple finding illustrates that, with a little effort, important improvements in the hygienic standards of zeers could be achieved without resorting to costly measures.

TABLE 3. Comparison of three different zeer constructions with respect to microbial water quality[a]

Time lapse	Microbial group	Microbial count (MPN)		
		Zeer A	Zeer B	Zeer C
2 days	Coliforms	2.4×10^3	1.1×10^3	$0. \times 10^1$
	Fecal coliforms	2.4×10^3	0.7×10^1	Nil
	Fecal streptococci	2.4×10^3	0.9×10^1	Nil
3 days	Coliforms	1.1×10^3	1.1×10^3	Nil
	Fecal coliforms	9.3×10^1	9.3×10^1	Nil
	Fecal streptococci	1.1×10^3	9.3×10^1	Nil
1 wk	Coliforms	1.1×10^3	1.1×10^3	1.5×10^1
	Fecal coliforms	1.1×10^3	9.3×10^1	Nil
	Fecal streptococci	1.1×10^3	1.1×10^3	Nil
3 wk	Coliforms	2.4×10^3	2.4×10^3	Nil
	Fecal coliforms	9.3×10^1	9.3×10^1	Nil
	Fecal streptococci	9.3×10^1	9.3×10^1	Nil
1 mo	Coliforms	2.4×10^3	2.4×10^3	2.3×10^1
	Fecal coliforms	9.3×10^1	4.3×10^1	Nil
	Fecal streptococci	9.3×10^1	9.3×10^1	Nil

[a] Zeer A—movable lid, no tap; Zeer B—movable lid, tap; Zeer C—fixed lid, tap.

Our zeer improvements, i.e., faucets and tight-fitting lids, could well be afforded by the Khartoum population.

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