Calculation of Total Colonies and Coliform Group Counts, and Detection of Enteric and Related Bacteria from Drinking Water in Indonesia

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Abstract: Total colonies and coliform group from 75 samples of drinking water were counted, and the enteric and the related bacteria were detected in samples from regions of Indonesia in June and July, 1982.

We carried out a quantitative test of total colonies and coliform group counts, using URICULT set that houses CLED and MacConkey media. The results of total colonies counts were positive (more than 10^3/ml) in 43 samples. Eighteen samples out of these 43 samples were taken in Kalimantan, Sulawasi and Sumatra. Fifty-seven samples taken from Jakarta, showed the greatest positive rates: 11 out of 28 samples (39%) from tap water and 14 out of 29 samples (48%) from well water. On the other hand, coliform group counts were positive (more than 10^2/ml) in 37 samples (49%). Eighteen out of 37 samples were from Kalimantan, Sulawasi and Sumatra. Fifty-seven samples from Jakarta showed positive in 6 out of 28 samples (21%) from tap water and in 13 out of 29 samples (45%) from well water.

Enteric bacteria and related bacteria found in sample water were primarily *Enterobacter cloacae* and *Citrobacter freundii*. Both species were idetified in 16 samples. The next frequently identified bacteria were *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and they were found in 6 samples. *Escherichia coli*, important bacteria in relation to fecal matter contamination, was found in 5 samples, including 2 samples of tap water taken in Jakarta city. The most noticeable points in this investigation are that *Salmonella* C_1 group was detected from one well in Jakarta city; *Salmonella* E_1 group from another well in Jakarta city, and each type was also found in wells in Kalimantan, Sulawasi and Sumatra.

INTRODUCTION

We have been investigating the quality of drinking water in many regions in Indonesia for the past 15 years, beginning in 1968. We have paid attention to drinking water that plays an important role in the occurrence or transmission of infections, in order to prevent and control tropical diseases. We reported earlier the bacteriological and chemical study of the drinking water in Indonesia (Okuwaki *et al.*, 1982).

To know the quality of drinking water in Indonesia, we presented here more detail report and showed the presence of enteric and related bacteria in many samples. We discussed about these bacteria as the indicative of fecal contamination in drinking water.

PERIOD, AREA, MATERIALS AND METHODS

The investigation was done from June 24 th. through July 30 th. of 1982. Total 75 water samples were collected in many regions of the cities of Java, Kalimantan, Sulawasi and Sumatra (Table 1).

Water samples were directly placed into gas-sterilized polyethylene containers. Approximately 1 ml of test water droped on the URICULT set (Daiichi Kagaku, Co. Ltd., Tokyo) immediately and left in a place (about 30-40°C) to run a culture.. On the next day, the colony counts, developed on each culture medium, were calculated, and total colonies and coliform group counts in 1 ml were studied each other.

Furthermore, 0.5 ml of each test water was injected into 3 ml of semisolid medium to which a purified agar powder was added in a proportion of 0.2% to brain heart infusion broth. Upon returning to Japan, we ran a culture, using agars of SS, MacConkey, modified Drigalski, TCBS, and NAC for the purpose of isolating enteric and related bacteria. Identification was made to determine the species of gram negative rods (Okuwaki *et al.*, 1982).

Table 1. Drinking waters classified by regions and water sources

	JAVA	KALIMANTAN	SULAWASI	SUMATRA	Total
Tap water	28	4	2	0	34
Well water	29	1	2	4	36
Rain water	0	1	0	4	5
Total	57	6	4	8	75

RESULTS

Seventy-five samples of drinking water were collected in many Indonesian regions. Total colonies and coliform group counts were done, using URICULT set, and the following results were obtained. As to total colonies counts, more than 10^3/ml of living bacteria were found in 43 out of 75 samples (57%) and these were hereinafter defined as positive. Table 3 shows a distribution of the positive samples classified by regions of drinking water. According to this result, those in Kalimantan, Sulawasi and Sumatra, were all positive irrespective of source. Approximately half, namely 25 out of 57 samples (44%) were positive in Jakarta. As shown in Toble 3, 11 were positive out of 28 samples (39%) of tap water and 14 out of 29 (48 %) of well water.

In reference to coliform group counts, more than 10^2/ml of bacilli were seen in 37 out of 75 samples. Coliform group should not be detected at all in drinking water (World Health Organization, 1971; Takeuchi, 1980). In this research, it was defined to be positive when it is more than 10^2/ml according to URICULT's detection sensitiveness. Classifying these by region (Table 4), 18 samples taken in Kalimantan, Sulawasi and Sumatra were positive. Nineteen of 57 samples (33%) in Jakarta were positive. Classifying these by sources (Table 5), six out of 28 tap water samples (21%) were positive, 13 out of 29 well water samples (45%) were positive. Four samples of tap water in Kalimantan and 2 samples of tap water in Sulawasi were positive.

Table 2. Results of total colonies counts classified by regions

	No. of samples	Positive samples (%)
JAVA	57	25 (44)
KALIMANTAN	6	6 (100)
SULAWASI	4	4 (100)
SUMATRA	8	8 (100)
Total	75	43 (57)

Table 3. Results of total colonies counts classified by water sources

	JAVA	KALIMANTAN	SULAWASI	SUMATRA
Tap water	11/28 (39)	4/4 (100)	2/2 (100)	0
Well water	14/29 (48)	1/1 (100)	2/2 (100)	4/4 (100)
Rain water	0	1/1 (100)	0	4/4 (100)

/ : Positive samples / Total samples
( ) : (%)

Table 4. Results of coliform group counts classified by regions

	No. of samples	positive samples (%)
JAVA	57	19 (33)
KALIMANTAN	6	6 (100)
SULAWASI	4	4 (100)
SUMATRA	8	8 (100)
Total	75	37 (49)

Table 5. Results of coliform group counts classified by water sources

	JAVA	KALIMANTAN	SULAWASI	SUMATRA
Tap water	6/28 (21)	4/4 (100)	2/2 (100)	0
Well water	13/29 (45)	1/1 (100)	2/2 (100)	4/4 (100)
Rain water	0	1/1 (100)	0	4/4 (100)

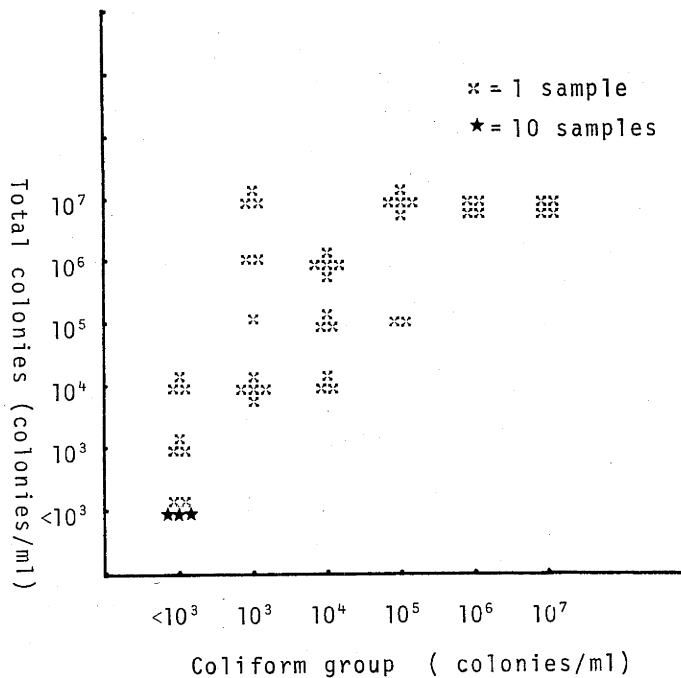/ : Positive samples / Total samples
( ) : (%)

Fig. 1. Correlation between total colonies and coliform group counts.

Figure 1 shows the positive correlation between total colonies and the coliform group counts obtained from this study. A good correlation was observed between total colonies and coliform group counts from drinking water in Indonesia.

Table 6 summarizes the species of bacteria that was isolated from the test water. The most frequently detected species of bacteria were *Enterobacter cloacae* and *Citrobacter freundii*, which were detected in 16 samples, respectively. The next frequently detected bacteria was *Pseudomonas aeruginosa* in 6 samples. *Escherichia coli* which was important in relation to fecal contamination was isolated in 5 samples, ofwhich 2 samples from the tap water in Jakarta are included. Each one of samples had a *Salmonella* C_1 group and *Salmonella* E_1 group from well water in Jakarta city. Moreover, the *Salmonella* E_1 group was detected in well water in Kalimantan, Sulawasi and Sumatra.

Table 6. Isolated strains from drinking waters

	JAVA		KALIMANTAN			SULAWASI		SUMATRA	Total number
	Tap water	Well water	Tap water	Well water	Rain water	Tap water	Well water	Well water	
Salmonella C_1 group		*							1
Salmonella E_1 group		*	*				*	*	4
Escherichia coli	**	**	*						5
Klebsiella pneumoniae	*	**						*	4
Serratia marcescens		*	*			*		**	5
Enterobacter cloacae	*	*** *** ***	**	**		*		*	16
Enterobacter aerogenes	*								1
Citrobacter freundii	**	* *** *	**		*		**	** **	16
Citrobacter diversus	*	*	*	*				*	5
Pseudomonas aeruginosa		* *** *			*				6
Pseudomonas putida		*							1
Pseudomonas putrefaciens		*							1
Aeromonas puncteta		*							1
Aeromonas hydrophila	*	**				*	*		5

DISCUSSION

Nothing is more closely related to life than water. However, the water in the tropical zones occasionally becomes contaminated with the pathogenic microorganisms and works as an important carrier in transmitting the causative microorganisms of the infectious diseases.

From these standpoints, we have been conducting a study of the drinking water in many regions of Insonesia since 1968.

We used URICULT set that housed 2 media, CLED and MacConkey medium. The CLED medium contains lactose, peptone, beef extract, and L-cystine and this is used for etecting the general bacteria. The MacConkey medium contains bile salts that inhibits growth of Gram-positive cocci and Gram-positive bacilli, and this is excellent for isolating enteric bacteria. We performed a quantitative test of total colonies and coliform group counts in 1 ml of drinking water in the field, using URICULT set. The results of this quantitative testing revealed that both total colonies counts and the coliform group counts were positive in all the samples, including 6 samples from tap water in Kalimantan, Sulawasi, and Sumatra. In Jakarta, the total colonies counts of tap water was positive in 39% and coliform group counts in 21%. These results agree with our earlier reports (Fujita *et al.*, 1974; Fujita *et al.*, 1980, Okuwaki *et al.*, 1982; Fujita *et al.*, 1982). This is possibly due to the incomplete disinfection of drinking water as well as defects in the construction of water pipe lines (Japan Association for Tropical Medicine, 1982). This is also inferred from the results of the species of enteric bacteria. Namely, *Escherichia coli* was detected in 2 samples of tap water from Jakarta city. *Escherichia coli* is a representative parasitic bacterium which is excreted from the human and animal intestinal tract. For this reason, the presence of *Escherichia coli* is the most crucial index in determining the level of fecal contamination. In addition, *Klebsiella pneumoniae* and *Enterobacter cloacae* were detected in many water samples. It is noteworthy that the *Salmonella* C_1 and E_1 group were detected from each one sample of well water in Jakarta and *Salmonella* E_1 group from 3 samples taken from other islands in Indonesia. Wells in Indonesia are not dug so deep as in the case in Japan, but dug rather shallow (Japan Association for Tropical Medicine, 1982). In large city such as Jakarta where the population is concentrated, the increased risk of fecal contamination always exsists.

Adesiyun, A.A. *et al.* (1983) conducted a study of 20 specimens of test well water in Katsina, Nigeria. They reported that number of the coliform group more than 2400 was found in 65%, and the remaining 35% were detected in a range of 79 to 920. *Salmonella* sp., *Enterobacter* sp. and *Pseudomonas* sp. were detected in 10% of the samples respectively, and *Citrobacter* sp. in 15% of them. Also, Wright, R.C. (1982) studied the causal relationship between bacteria detected in feces and *Salmonella* sp. found in water from 29 local cities located in Sierra Leone, and he detected *Salmonella* sp. in 13 samples of test water. There are, of course, regional and geographical differences between Africa and Indonesia, but results obtained in Africa will be helpful.

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インドネシア各地の飲料水よりの一般細菌数, 大腸菌群数の算定, および腸内細菌とその類縁菌の検出

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我々は, 過去15年間にわたりインドネシア各地の飲料水を調査し, 衛生学的見地から, 種々の問題点を提起してきている.

今回は, 1982年6〜7月にかけて, インドネシア各地で採取した飲料水75検体についての一般細菌数, 大腸菌群数, および腸内細菌とその類縁菌の検出成績を報告する.

一般細菌数の算定成績では, 43検水 (57%) で陽性 (10^8/ml以上) が示され, このうちカリマンタン島, スラウェシ島, スマトラ島での18検水ではすべてで陽性であった. 一方, ジャカルタ市を中心とするジャワ島での57検水については, 水道水で28検水中11検水 (39%), 井戸水で29検水中14検水 (48%) が陽性であった.

大腸菌群数の算定成績は, 37検水 (49%) で陽性 (10^2/ml以上) が示され, そのうちのカリマンタン島, スラウェシ島, スマトラ島での18検水はすべて陽性であった. ジャワ島での水道水29検水では21%に, また井戸水29検水では45%に陽性がみられた.

これらの飲料水より分離された腸内細菌とその類縁菌は, *Enterobacter cloacae* と *Citrobacter freundii* が最も多く, それぞれ16検水より分離された. 次いで多かったのは, 6検水よりみられた *Pseudomonas aeruginosa* であった. し尿系汚染との関連から重視される *Escherichia coli* は5検水から分離され, そのなかにはジャカルタ市内の水道の2検水から分離されたものが含

74

まれている.

今回の調査成績で特に注目されることは，*Salmonella* C_1 群がジャカルタ市内の1件の井戸水より，また *Salmonella* E_1 群が ジャカルタ市内，およびその他の3島のそれぞれ1件ずつの井戸水からというように，ほとんどインドネシア全島の検水から分離されたことである.