PLATFORM PRESENTATION PROCEEDINGS

The 10th International Symposium On Southeast Asian Water Environment

November 8-10,2012
Hilton Hotel, Hanoi, Vietnam

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The 10th International Symposium on Southeast Asian Water Environment

November 8-10,2012 Hanoi, Vietnam

The 10th International Symposium On Southeast Asian Water Environment

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The 10th International Symposium on Southeast Asian Water Environment

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[B4] Health-related microbiology of the water environment

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Microbial Source Tracking of *Salmonella* sp. and *Escherichia coli* by Antibiotic Resistance Analysis in Upper Citarum River, West Java, Indonesia

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Abstract

Increment of water pollution from domestic activities has worsened the water quality of Upper Citarum River. Targeted prevention efforts can only be performed if the source of pollutant has been identified. Microbial Source Tracking (MST) method can trace domestic contaminants to their source. Antibiotic Resistance Analysis (ARA) can distinguish bacteria from different sources based on the diversity of bacterial resistance to various antibiotics. In this study, the Upper Citarum River was selected as a study area which was divided into nine segments based on the presence of main tributaries. The results showed that the Ciwidey River that resides in Cilampeni Village, Katapang District was the main source of *Salmonella* sp.. Meanwhile, the sources of *Escherichia coli* from nine segments were tracked down using ARA method with ten types of antibiotics. Sources of *Escherichia coli* were divided into human, chicken, goat and cow faeces. The results showed that *Escherichia coli* of human origin were predominant in Margaasih and Rancasari segments, whereas *Escherichia coli* of cows were prevalent at Baleendah segment. *Escherichia coli* originated from chicken and goat faeces were found relatively high in Margaasih segment. The distribution of *Escherichia coli* was probably influenced by the number of livestock and percentage of population served with sanitation facilities, termed as sanitation coverage. In addition, the distribution of *Escherichia coli* as a fecal indicator bacteria from animal and human sources were moderately correlated with the number of species of pathogenic bacteria, *Salmonella* sp.. This study suggested that there is a need to find new indicator bacteria that could specifically trace the pathogenic bacteria and the review of masterplan of domestic wastewater treatment installation.

Keywords Antibiotic Resistance Analysis, Upper Citarum, *Escherichia coli*, Microbial Source Tracking, *Salmonella* sp.

1. Introduction

Various problems that occured in Citarum watershed includes increment of critical land and water pollution from industrial, agricultural and domestic activities. However, up to this moment the peoples around the Citarum River still use the water for irrigation, home industry and domestic use (Cita-Citarum, 2009). Utilization of poor water quality may raise various health risks for the people; therefore restoration of the river quality is an urgent necessity.

Pollution prevention programs could be done effectively when the source of pollution had been identified (Bitton, 2005). The standard method of Most Probable Number (MPN) has some difficulties to identify the source of microbiological pollution originated from domestic, agricultural, livestock and fisheries activities. These pollutants often considered as non-point source and only their dispersion can be determined. MST may trackdown fecal pollution to their source and changes the paradigm into point source.

Two main methods of MST are the conventional and modern method. The conventional method uses cultur characterization and biochemical tests for identification of bacteria, while the modern method use phenotyping and genotyping techniques (Ferguson & Griffith, 2010). Genetic marker detection of *uidA Escherichia coli* genes and *Bacteroidetes* in Lake Michigan was proved succeed to identifiy the source of human fecal pollution and the results was compatible with standard microbiological method (Bower *et al.*, 2005).

ARA is one of phenotyping method which is able to distinguish same bacteria that originated from different sources based on their diversities of antibiotic resistance profile. It could be possible due to the difference of antibiotic consumption pattern between different sources. Utilization of ARA had succeeded to identify the source of fecal pollution in Moores Creek, Virginia (Wiggins, 2001).

Escherichia coli (fecal coliform) dispersion is often used as pathogen indicator. However, some studies suggests low correlation appear between this indicator and pathogenic bacteria existence (Bitton, 2005). *Salmonella* sp. is one of the important pathogen in West Java because its high prevalence of typhoid fever and watery diarrhea. Therefore, understanding of the source and dispersion of *Salmonella* sp. along with its correlation with indicator bacteria in Upper Citarum River is also required.

2. Materials and methods

This study is divided into two main phases i.e. (1) tracking of *Salmonella* sp. along Upper Citarum River and finding its correlation with their physical and chemical parameters, and (2) tracking the source of *Escherichia coli* along Upper Citarum River by ARA.

In the first phase, water samples were taken from 10 sampling points in the Upper Citarum River system. These samples were then analyzed their microbiological (*Salmonella sp.* and coliform) and physico-chemical (pH, temperature, DO, BOD, COD, nitrate, nitrite, ammonium, phosphate) characteristics. The correlation between dispersion of pathogenic bacteria with their chemical, physical and microbiological properties were then analyzed statistically by using Spearman Correlation.

In the second phase, the source of *Escherchicia coli* was divided into human, cow, goat and chicken. Antibiotic resistance pattern from these groups were named as the library characteristic. The library isolates was originated from fecal matter of each source in Upper Citarum watershed. Human fecal samples were obtained from four septic tanks in Katapang, Bojongsoang, Dayeuhkolot and Margaasih Sub Districts. Cow fecal samples were taken from slaughter house in Cibolerang and cow farm in Majalaya Sub District. Chicken fecal samples were taken from chicken farm house in Margaasih Sub District, while goat fecal samples were originated from livestock in Katapang Sub District. The fecal sampling locations were determined based on the distance to Upper Citarum River, antibiotic consumption pattern and its potency of fecal run off onto the river. The isolation was performed both for the library and river isolates from the sampling points. A total of 130 isolates in the Main Upper Citarum

River were subjected to ARA by disk diffusion method in Mueller Hinton Agar (Lalitha, 2004; Coyle, 2005). The antibiotics used in this study are shown in Table 1. The inoculation is performed on 260 Mueller Hinton agars. The agar was then incubated on 35°C for 18-24 hours.

Table 1 Antibiotics used in the study.

No.	Antibiotics	Concentration	Unit
1	Kanamycine	30	μg/ml
2	Tetracycline	30	μg/ml
3	Ampicillin	10	μg/ml
4	Chloramphenicol (*double dose*)	60	μg/ml
5	Cotrimoxazole (*quarter dose*)	100	μg/ml
6	Streptomycine	10	μg/ml
7	Cotrimoxazole	25	μg/ml
8	Erythromycin	15	μg/ml
9	Chloramphenicol	30	μg/ml
10	Oxytetracycline	30	μg/ml

Clear zone diameter was noted and compared with diameter of breakpoint standard of NCCLS/ACLS as shown in Table 2.

Table 2 Clear zone diameter of NCCLS/ACLS breakpoint standard.

Antibiotic	Concentration (μg/ml)	Sensitive	Intermediate	Resistant
Ampicillin[a]	10	≥ 14 mm	12-13 mm	≤11 mm
Chloramphenicol[d]	30	≥18 mm	13-17 mm	≤12 mm
Kanamycin[b]	30	≥18 mm	16-17 mm	≤15 mm
Oxytetracycline[c]	30	≥19 mm	15-18 mm	≤14 mm
Streptomycin[a]	10	≥15 mm	12-14 mm	≤11 mm
Tetracycline[d]	30	≥19 mm	15-18 mm	≤14 mm
Erythromycin[b]	15	≥21 mm	19-20 mm	≤18 mm
Cotrimoxazole[a]	23.75/1.25	≥16 mm	11-15 mm	≤10 mm

Source: [a]hhmi.org; [b]eucast.org; [c]jmilabs.com and conservancy.umn.edu; [d]Harley and Prescott (2002)

Finally the determination of *Escherichia coli* source was performed by logistic regression analysis.

3. Results and discussion

3.1 Tracking of Salmonella sp. in Upper Citarum River

Results of MPN and *Salmonella* sp. enumeration are shown in **Figure 1**. Cisanti Spring had very small number of total coliforms and fecal coliforms. Increment of total coliforms and fecal coliforms appeared in Rancasari was caused by livestock waste comes from Majalaya to Cijeruk.

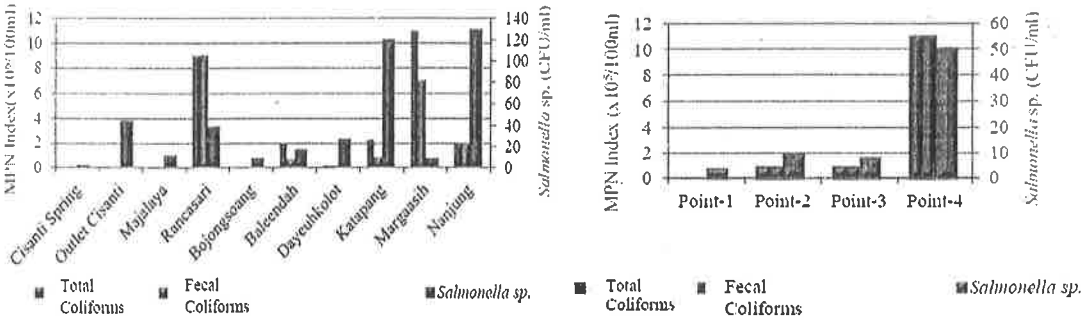


Figure 1 Microbiological parameter examination in the main river of Upper Citarum (a, left panel) and Ciwidey River (highest *Salmonella* sp.) (b, right panel).

As shown in Figure 1a, total coliforms and fecal coliforms in Bojongsoang were decreased significantly. This was influenced by existence of centralized wastewater treatment plant of Bandung City located in Bojongsoang which treated domestic waste from Bandung area. Distribution pattern of fecal coliforms in Katapang and Margaasih was correlated with number of livestock in those areas. *Salmonella* sp. distribution pattern was associated with sanitation coverage in those sub districts. Early segment of Upper Citarum has high sanitation coverage i.e. about 40 %, therefore the number of *Salmonella* sp. was relatively low. Increment of *Salmonella* sp. in the end segment of Upper Citarum (<120 CFU/ml) was affected by low sanitation coverage.

Discrepancies between fecal coliform as pathogen indicator were observed in Rancasari, Katapang, Margahayu and Nanjung. This was caused by difference of persistence between *Escherichia coli* and *Salmonella* sp.. The results of this study showed that the Ciwidey River in Katapang Sub District had highest *Salmonella sp.* abundance. Therefore the tracking was continued in Ciwidey River. The results of bacterial counting in Ciwidey River are shown in Figure 1b. The *Escherichia coli* was the main pollutant. It suggested that human and livestock were the main source of microbial pollution in this area.

The results of chemical and physical examination of water samples are shown in Figure 2 and Figure 3.

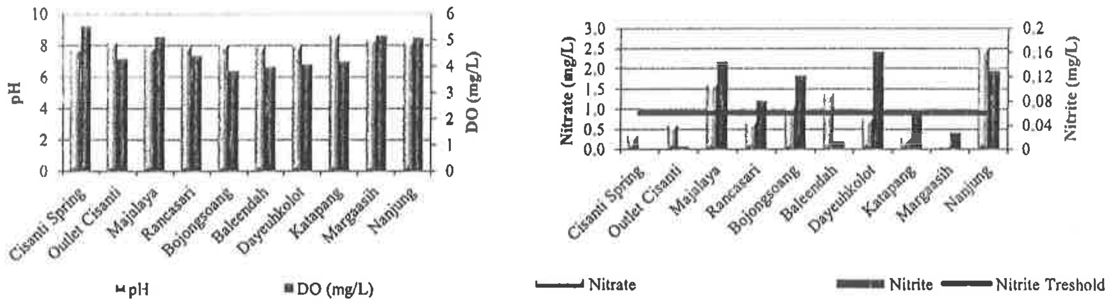


Figure 2 pH, DO, nitrate, and nitrite profiles in Upper Citarum River.

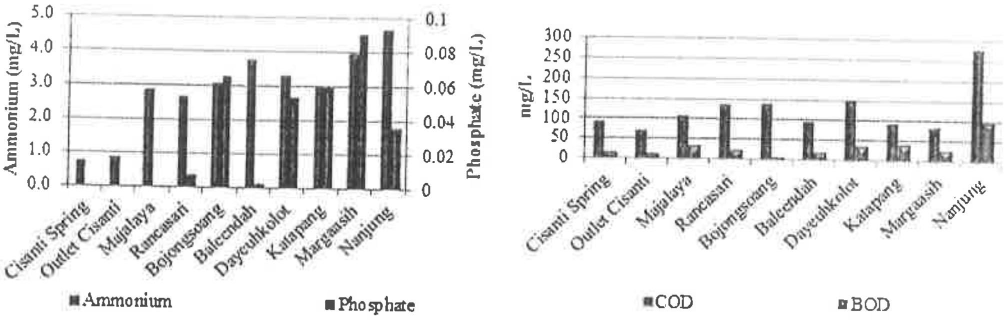


Figure 3 Ammonium, phosphate, COD, and BOD profiles in Upper Citarum River.

Table 3 shows that the increment of total coliforms always followed by the increment of fecal coliforms. This shows that fecal coliforms were the main source of total coliforms. Fluctuation and dispersion of *Salmonella* sp. was not associated properly with the fluctuation of indicator bacteria. This indicated that fecal coliforms could represent the presence of *Salmonella* sp. but unable to predict the fluctuation of *Salmonella* sp. This was due to *Salmonella* sp. that originated only from sick person.

Table 3 Correlation of physico-chemical and microbiological parameters using Spearman Correlation (CC: correlation coefficient, Sig.: Significance, two-tailed).

PARAMETERS	Score	pH	Temperature	DO	BOD	COD	Phosphate	Nitrate	Nitrite	Ammonium	Total coliforms	Fecal coliforms	*Salmonella* sp.
pH	CC	1.00	0.28	-0.18	0.10	-0.11	-0.01	-0.21	0.20	0.30	0.52	0.51	0.64
	Sig		0.34	0.54	0.73	0.71	0.98	0.47	0.49	0.29	0.06	0.06	0.01
Temperature	CC	0.28	1.00	-0.55	0.22	0.43	0.31	-0.14	0.46	0.76	0.68	0.60	0.28
	Sig	0.34		0.04	0.45	0.13	0.28	0.63	0.10	0.00	0.01	0.02	0.32
DO	CC	-0.18	-0.55	1.00	0.00	-0.39	0.27	0.33	-0.17	-0.16	-0.10	-0.01	-0.36
	Sig	0.54	0.04		0.99	0.17	0.36	0.25	0.57	0.58	0.72	0.98	0.21
BOD	CC	0.10	0.22	0.00	1.00	0.65	0.13	-0.28	0.44	0.64	0.45	0.44	0.51
	Sig	0.73	0.45	0.99		0.01	0.65	0.33	0.11	0.01	0.11	0.12	0.06
COD	CC	-0.11	0.43	-0.39	0.65	1.00	-0.22	-0.20	0.59	0.62	0.20	0.21	0.33
	Sig	0.71	0.13	0.17	0.01		0.44	0.49	0.03	0.02	0.48	0.48	0.25
Phosphate	CC	-0.01	0.31	0.27	0.13	-0.22	1.00	0.14	-0.21	0.36	0.60	0.51	-0.05
	Sig	0.98	0.28	0.36	0.65	0.44		0.63	0.47	0.20	0.02	0.06	0.87
Nitrate	CC	-0.21	-0.14	0.33	-0.28	-0.20	0.14	1.00	0.13	-0.04	-0.21	-0.06	-0.03
	Sig	0.47	0.63	0.25	0.33	0.49	0.63		0.66	0.89	0.47	0.83	0.92
Nitrite	CC	0.20	0.46	-0.17	0.44	0.59	-0.21	0.13	1.00	0.54	0.33	0.42	0.47
	Sig	0.49	0.10	0.57	0.11	0.03	0.47	0.66		0.04	0.24	0.13	0.09
Ammonium	CC	0.30	0.76	-0.16	0.64	0.62	0.36	-0.04	0.54	1.00	0.69	0.64	0.42
	Sig	0.29	0.00	0.58	0.01	0.02	0.20	0.89	0.04		0.01	0.01	0.13
Total coliforms	CC	0.52	0.68	-0.10	0.45	0.20	0.60	-0.21	0.33	0.69	1.00	0.96	0.50
	Sig	0.06	0.01	0.72	0.11	0.48	0.02	0.47	0.24	0.01		0.00	0.07
Fecal coliforms	CC	0.51	0.60	-0.01	0.44	0.21	0.51	-0.06	0.42	0.64	0.96	1.00	0.56
	Sig	0.06	0.02	0.98	0.12	0.48	0.06	0.83	0.13	0.01	0.00		0.04
Salmonella sp.	CC	0.64	0.28	-0.36	0.51	0.33	-0.05	-0.03	0.47	0.42	0.50	0.56	1.00
	Sig	0.01	0.32	0.21	0.06	0.25	0.87	0.92	0.09	0.13	0.07	0.04	

▨ Strong correlation (>0.60) ☐ Significant correlation

The increase of organic parameters (BOD and COD) was not correlated with the increment of fecal coliforms or *Salmonella* sp. (Table 3). Other parameter seemed to have positive correlation (>0.6) with total coliforms, fecal coliforms and *Salmonella* sp. dispersion.

3.2 Tracking of Escherichia coli in Upper Citarum River

Antibiotic resistance profile of library isolates for each source resulted regression equation as the probability of group member determination, which shown as follows:

- Probability of *Escherichia coli* originated from Chicken (ECFA) :
  y = -19.403 + 39.64 (Tetracyclin) – 20.459 (Cotrimoxazol double dose) -1.723 (Streptomycin)

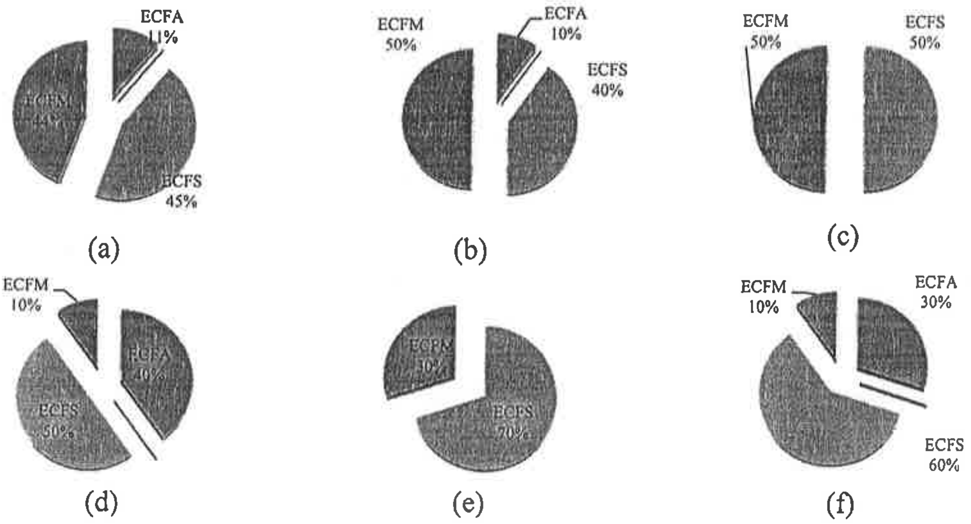
- Probability of *Escherichia coli* originated from Goat (ECFK) :
  y = 0.916 + 20.287 (Streptomycin) – 22.119 (Chloramphenicol)

- Probability of *Escherichia coli* originated from Cow (ECFS) :
  y = -22.589 + 1.386 (Streptomycin) + 20.717 (Chloramphenicol)

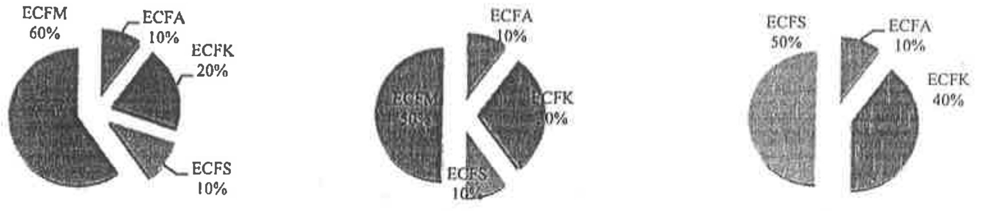
- Probability of *Escherichia coli* originated from Human (ECFM) :
  y = 21.203 – 22.812 (Tetracyclin)

The identification of *Escherichia coli* pollution source in each segment could be accomplished by applying those equations. The results are shown in Figure 4.

Pattern of chicken, goat, cow, and human *Escherichia coli* number in the river was affiliated with *Salmonella* sp. enumeration (Figure 5). ECFA and ECFK increased in Margaasih segment. Moreover, ECFS increased in Rancasari, while ECFM showed relatively high number in Rancasari and Margaasih. Meanwhile, *Salmonella* sp. increased in Rancasari, Katapang, and Nanjung. Therefore, *Salmonella* sp. dispersion pattern was not in line with *Escherichia coli* dispersion pattern which originated from chicken, goat, cow, and human. This also indicated that fecal coliforms could represent the presence of *Salmonella* sp. but was unable to predict the variation of *Salmonella* sp. number in the river body. This might be related with the nature of *Salmonella* sp. that originated only from the person in the sickness.



(a) (b) (c) (d) (e) (f)



(g) (h) (i)

Figure 4 Identification of *Escherichia coli* source in each segment of Upper Citarum River (a) Cisanti Outlet, (b) Majalaya, (c) Rancasari, (d) Bojongsoang, (e) Baleendah, (f) Dayeuh Kolot, (g) Katapang, (h) Margaasih, and (i) Nanjung.

Number of livestock did not influence the number of *Escherichia coli* in those areas due to the performance of livestock wastewater treatment installation and existing condition of fecal runoff. Moreover, sanitation coverage should prevent the increment of human *Escherichia coli* in the river. The sanitation coverage in all areas of watershed was still below 50% and generally could be categorized as low coverage.

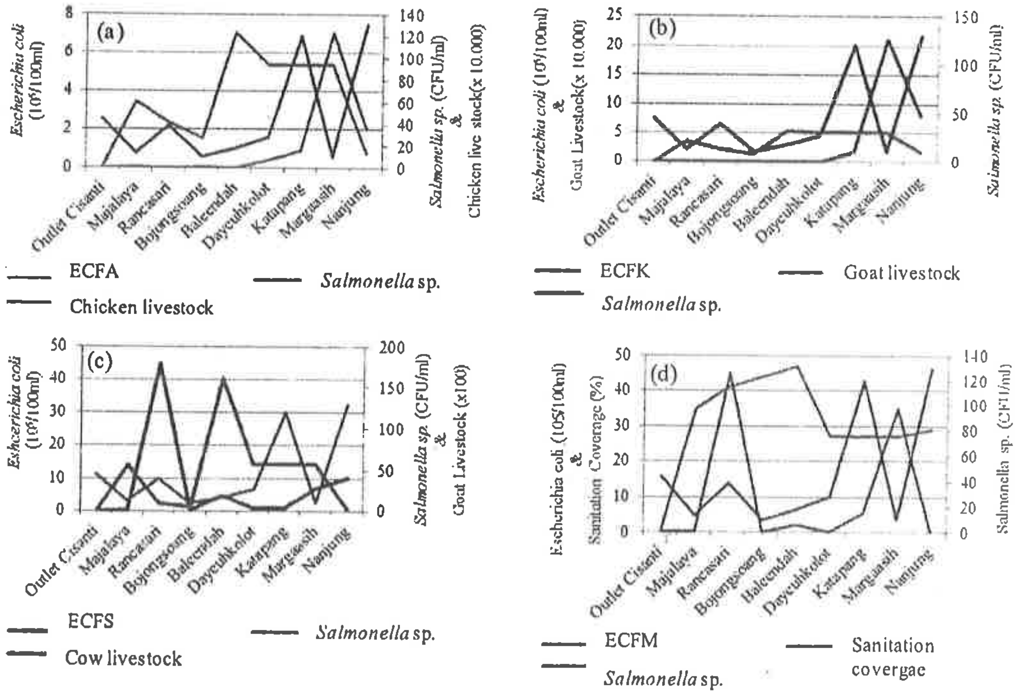


Figure 5 Relationship between numbers of *Escherichia coli* originated from (a) chicken, (b) goat, (c) cow, and (d) human with the number of livestock and sanitation coverage.

4. Conclusions

Based on the experimental results, the following conclusions were drawn:

- *Escherichia coli* dispersion from livestock could be influenced by number of livestock and its wastewater treatment installation. The number of *Escherichia coli* originated from human in Upper Citarum River could be affected by sanitation coverage. However, the increase of *Escherichia coli* originated from human and livestock were not affiliated with the increment of *Salmonella* sp..

- *Escherichia coli* originated from human (ECFM) were dominant in Rancasari and Margaasih. Therefore, this area required more reliable domestic wastewater treatment plant. Meanwhile, *Escherichia coli* from chicken and goat (ECFA & ECFK) were relatively high in Margaasih while from cow (ECFS) was considered to be high in Baleendah.

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