Contamination analysis of antimicrobial resistance in escherichia coli in the domestic environment

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Abstract. Antimicrobial resistance (AMR) is one of the top 10 global public health. In Indonesia, little is known about the prevalence of Extended-Spectrum Beta-Lactamase (ESBL)-producing E. coli in the environment increases the risk of contamination of groundwater sources because of the potential for rapid colonization through the water. A study validation in AMR E. coli was carried out in the rainy season (March 2020). The results showed that 33.3% (20/60) of groundwater samples were positive for resistance to the antibiotic cefotaxime. This research aimed to determine the significance of seasonal variations in contamination of AMR and the correlation AMR present with faecal sources. The results showed that 22.2% (12/54) of groundwater samples were positive for cefotaxime antibiotic resistance with comparison test on seasonal variations shows a significant difference in E. coli contamination (p = 0.045), but not in AMR E. coli contamination (p = 0.234). The correlation relationship of the contamination from faecal sources on AMR E. coli in water sources shows a significant correlation (p-value 0.041) because of the use of on-site sanitation but no correlation between livestock ownership (p-value 0.891).

1 Introduction

Antimicrobial resistance (AMR) is a growing and significant threat to public health on a global scale [1]. The World Health Organization has declared that antibiotic-resistant bacteria (AMR) is one of the top 10 global public health threats facing humanity. Lack of clean water and sanitation and inadequate infection prevention and control increase the use of antibiotics and the spread of bacteria, some of which are resistant to antibiotic treatment. Thus, antibiotics are becoming increasingly ineffective as drug resistance spreads globally which makes it more difficult to treat infections and increases mortality [2]. The spread of AMR is currently fast because antibiotic resistance genes can be transferred from resistant bacteria to other bacteria as vectors through a horizontal gene transfer (HGT) mechanism [3]. The antibiotic gene attached to E. coli produces -lactams that form Extended Spectrum Beta-Lactamases (ESBLs). This enzyme then makes negative bacteria such as E. coli resistant to third-generation cephalosporin antibiotics such as cefotaxime [4].

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This research aimed to determine the significance of seasonal variations in contamination of AMR, knowing the contamination of AMR in faecal sources, analyzing the correlation of AMR in local groundwater, and identifying strategies to prevent AMR to meet groundwater supply needs.

2 Methodology

2.1 Sampling and survey data

The samples used in this study were samples from clean water sources and samples from faecal sources such as animal faeces and effluent from septic tanks. Water samples were taken as much as 2 x 100 ml using whirl-pack 118 ml. To complete the analysis needs, questionnaire data was given to respondents by interviews and filled the answers in the online survey application. The questions given included personal data, source of drinking water, type of sanitation facilities and livestock ownership.

2.1.1 Adapting AMR E. coli with IDEXX trays

The adaptation of the IDEXX Quanti Tray in detecting antibiotic bacteria was initiated by North Carolina State University to test the quantification and accuracy of ESBL-producing E. coli. The IDEXX protocol used is to prepare two water samples of 100 ml each. The first 100 ml aims to estimate the amount of E. coli produced in the test sample. While other 100 ml is used to test the antibiotic bacteria of suspected ESBL-producing E. coli using cefotaxime solution. The standard procedure for AMR testing is the same as for E. coli, but modifications were made by giving 80 μL of cefotaxime solution (solubility of 5 mg/ml) after the addition of colilert 18 packages. Then sealed using an IDEXX sealer. After 18 hours, place the trays in a UV light chamber and count. To support the accuracy of tests on faecal sources, for AMR confirmation test used 10^{-3} and 10^{-4} dilutions for samples from animal faeces and 10^{-2} and 10^{-3} dilutions for samples from septic tanks. Meanwhile, the EC confirmation test used 10^{-5} and 10^{-6} dilutions for samples from animal faeces and 10^{-5} and 10^{-6} dilutions for samples from septic tanks.

3 Results and discussion

3.1 Sampling rain intensity

The research was carried out at the end of the dry season at the beginning of October 2021. Sampling and testing prosess were run for 2 weeks starting on October 5th 2021, until October 14th 2021. To find out the rainfall during the sampling period, direct sampling was carried out every 24 hours. The results of the subsequent rainfall measurements are presented in Table 1.

Meteorology, Climatology, and Geophysical Agency of Indonesia stated that the start of the dry season can be determined based on the amount of rainfall in 10 days (Indonesia: dasarian) is less than 50 mm and followed by several subsequent on the next 10 days [5]. Based on results sampling, the amount of rainfall on the third dasarian in September was 15.84 mm and followed by 0.89 mm on the next dasarian. Even though there is rain, the intensity of the rain shows a low value so that it is included in the dry season category.

Table 1. Study location rainfall data.

No	Day	Date	Rainfall (mm)	Dasarian (mm)
1	Tue	9/21/2021	0	15.84
2	Wed	9/22/2021	0	
3	Thu	9/23/2021	0	
4	Fri	9/24/2021	0	
5	Sat	9/25/2021	0	
6	Sun	9/26/2021	0	
7	Mon	9/27/2021	8.94	
8	Tue	9/28/2021	149.49	
9	Wed	9/29/2021	0	
10	Thu	9/30/2021	0	
11	Fri	10/1/2021	0	0.89
12	Sat	10/2/2021	0	
13	Sun	10/3/2021	0	
14	Mon	10/4/2021	0	
15	Tue	10/5/2021	8.94	
16	Wed	10/6/2021	0	
17	Thu	10/7/2021	0	
18	Fri	10/8/2021	0	
19	Sat	10/9/2021	0	
20	Sun	10/10/2021	0	
21	Mon	10/11/2021	0	
22	Tue	10/12/2021	0	
23	Wed	10/13/2021	0	
24	Thu	10/14/2021	0	
Average (mm)			6.97	

3.2 Seasonal variation in contamination of AMR

Tests for ESBL-producing E. coli contamination later referred to in this study as AMR E. coli, were carried out in Sumur Batu Village. This research was previously carried out by a research team from the University of Indonesia and a research team from North Carolina State University during the rainy season in March 2020. In the research conducted at that time, the total number of groundwater samples for the research was 60 samples from 49 houses because 11 of them have double water sources. 45 water sources in the rainy season still use water from the same house in this dry season. By adding 9 new water sources, the total sample of water sources tested in this dry season amounted to 54 data.

Based on the results of the AMR E. coli test against the antibiotic used, cefotaxime, it showed that in 60 samples of clean water tested in the rainy season, 44 samples (73.3%) were positive for confirmation of E. coli contamination and 20 samples (33.3%) showed positive confirmation of resistance to cefotaxime antibiotics. Meanwhile, in this dry season study, 33 samples (61.1%) were confirmed positive for E. coli and 12 samples (22.2%) were confirmed to be resistant to the antibiotic cefotaxime. Then it was found that there was a decrease in the amount of E. coli contamination followed by a decrease in AMR E. coli contamination in the dry season. Details of the number of samples with confirmation of E. coli and AMR of E. coli on samples by season at each groundwater source can be seen in Table 2.

Table 2. Sample number of research by season.

Source	Rainy season (2020) n = 60			Dry season (2021) n= 54			Same number in both season (n=45)
	Number of samples	*E. coli*	AMR *E. coli*	Number of samples	*E. coli*	AMR *E. coli*	
Artesian	20	15	6	20	12	2	13
Borehole	36	25	12	32	19	9	30
Unprotected well	3	3	2	1	1	0	1
Protected well	1	1	-	1	1	1	1
Total Sample	60	44	20	54	33	12	45
Sampel positive		73.3%	33.3%		61.1%	22.2%	

The comparison of groundwater quality according to the seasonal variation was analyzed on 45 from the same water source in the same household as the previous study. Based on the test, it was found that during the rainy season, 32 samples (71.1%) confirmed positive for E. coli and 15 samples (33.3%) were positive for E. coli which is resistant to the antibiotic cefotaxime. Meanwhile, in this dry season, 30 samples (66.7%) were positive for E. coli and 12 samples (26.7%) were positive for AMR E. coli. High rainfall intensity is correlated with soil flow velocity [6] so that the transmission of E. coli can reach the water sources, causing contamination of E. coli and AMR of E. coli to be higher in the rainy season.

Comparison of the concentrations of E. coli and AMR E. coli were also analyzed in the study of the same water source (n=45). The results showed that the average positive AMR E. coli concentration in the rainy season was 78 MPN/100 mL, while the average positive AMR E. coli concentration in the dry season was 139.5 MPN/100 mL. Thus, according to the season, the average concentration of AMR E. coli in the dry season is higher than in the rainy season. Increased concentrations of E. coli in the dry season have also been reported in a publication by O'Dwyer et al. [7], who stated that drier summers and drought conditions would increase their frequency due to the absence of infiltration or infiltration for microbial transport. In addition, drier periods allow higher concentrations of antimicrobial residues below the surface, thereby leading to increased exposure of bacteria to sub-therapeutic levels of antimicrobial residues [8]. Visualization of the distribution of E coli and AMR Ecoli contamination data according to season can be seen in Figure 1.

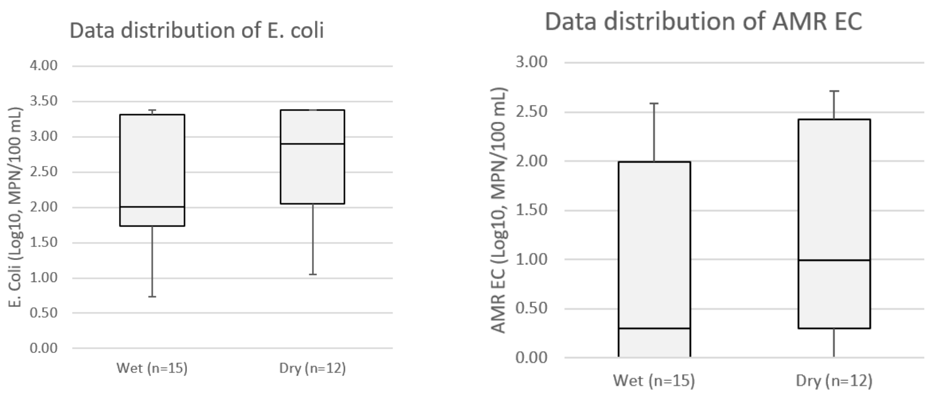

Fig. 1. Data distribution contamination of E. coli and AMR E. coli.

Statistical tests were carried out to determine the difference in seasonal variations in contamination (MPN/100 mL) of E. coli and AMR of E. coli by using the Mann Whitney non-parametric comparative test. Based on the calculation results can be seen in Table 3, the value of Asymp. Sig for contamination of E. coli against seasonal variations is 0.045 ($p < 0.05$), then the hypothesis (H0) is accepted, there is a statistical difference in E. coli contamination measured from the concentration of E. coli (MPN/100 mL) against seasonal variations. However, on AMR E. coli contamination with seasonal variations, the Asymp Sig value is 0.234 ($p>0.05$). So with these results, hypothesis H0 is rejected, there is no significant difference in the concentration of E. coli (MPN/100 mL) between the rainy season and the dry season on AMR E. coli contamination in the sample water sources used. The results of this statistical calculation can be seen in Table 3.

Table 3. Statistical results of the Mann-Whitney U test on comparison of AMR E. coli in the rainy season (n=60) and the dry season (n=54).

Variable	*E. coli*	AMR *E. coli*
Mann-Whitney U	1273.000	1454.000
Wilcoxon W	2758.000	2939.000
Z	-2.005	-1.189
Asymp. Sig. (2-tailed)	**0.045***	0.234

*) Significant

The Mann Whitney method is used to compare the mean in the independent population. So, to find out the significance between paired samples from the same water sources coming in both seasons, the Wilcoxon test is used [9]. This test was carried out on 45 samples from the same water source to show confirmation of AMR E. coli in the rainy and dry seasons in pairs. The calculation results of the Wilcoxon test on E. coli contamination showed the Asymp sig value was 0.738 ($p < 0.05$). These results indicate that there is no difference in pairwise E. coli contamination in the same water source concerning seasonal variations. Similar results were obtained in the calculations for AMR E. coli. Asymp Value. Sig is 0.530. The results of the statistical calculation of the Wilcoxon test for seasonal variations in the same water source in pairs can be seen in Table 4.

Table 4. Wilcoxon test statistical results on comparison of AMR E. coli paired in dry season - rainy season (n=45).

Variable	*E. coli* **Dry - Rainy**	AMR *E. coli* **Dry - Rainy**
Z	-.335	-.628
Asymp. Sig. (2-tailed)	0.738	0.530

The finding that there was no significant difference in results, especially in AMR E. coli contamination both with the calculation of the Mann Whitney test and in pairs with the Wilcoxon test on seasonal variations, indicated that there were other factors that caused AMR E. coli contamination in water sources. This is because there is no definite consensus regarding the spread of AMR contamination which can be driven by various environmental risk factors and other specific sources such as water source design, location and maintenance, local hydrogeology, climate change, land use patterns, permutations and spatiotemporal distributions [8].

3.3 Correlation of AMR E. coli in groundwater sources with faecal source

Analysis of water sources used in this analysis is using local water sources. The correlation between the presence of AMR E. coli in terms of local faecal contamination from local sanitation and animal ownership was calculated statistically using the chi-square correlation test method. In the analysis of the use of local sanitation, 18 out of 34 data were identified. Based on the results of this calculation, the Asymp sig value is 0.041 ($p<0.05$). Thus, it is known that there is a correlation between the use of local sanitation (septic tank/pit latrine) with positive confirmation of AMR E. coli in the local groundwater sources (borehole/protected well/unprotected well) used.

Table 5. AMR contamination information on local sanitation use.

		Onsite Sanitation		Total
		Yes	No	
AMR	Positive AMR	8	2	10
	Negative AMR	10	14	24
Total		18	16	34

In reviewing the effect of the relationship between chicken ownership and AMR E. coli contamination in the local groundwater sources used, it was found that 13 out of 34 data had chickens. The correlation test on animal ownership shows the Asymp sig value is 0.891 (>0.05). Thus, it can be determined that there is no correlation between ownership of chickens and positive confirmation of AMR E. coli in the groundwater used.

Table 6. AMR contamination information on livestock ownership.

		Livestock ownership		Total
		Yes	No	
AMR	Positive AMR	4	6	10
	Negative AMR	9	15	24
Total		13	21	34

Although there was no significant relationship between animal ownership and the emergence of AMR E. coli in local groundwater sources, the potential for transmission of antibiotic resistance from animals to humans could occur. Transmission pathways of antibiotic resistance from animals to humans can be in the form of direct consumption, direct contact of animal waste with humans or transmission from environmental contamination by animal faeces [10].

Transmission of AMR E. coli in groundwater can be influenced by anthropogenic and hydrological factors. On anthropogenic factors, faecal sources in terms of livestock ownership and local sanitation use can influence and have a significant linear relationship on E. coli concentrations and confirmation of E. coli AMR. This is as stated by O'Dwyer, et.al., [7] that the role of anthropogenic factors such as the density of the septic tank and animal ownership provides a significant relationship to AMR pollution. While on hydrological factors, the transmission of antibiotic-resistant E. coli in groundwater is influenced by pH, temperature, redox potential, amount and nature of organic matter, soil properties, groundwater depth, salinity and physiological properties of groundwater [11].

4 Conclusion

The contamination of antimicrobial resistance increases the risk of contamination of groundwater sources because of the potential for rapid colonization through the water. Tests for ESBL-producing E. coli contamination are referred to in this study as AMR E. coli as a model indicator for bacteria resistance transmitted on groundwater in Sumur Batu Village. In the rainy season (n=60), 33.3% of water sources were contaminated with AMR E. coli, while the AMR contamination showed 22,2% in the dry season (n=54). In the comparison of seasonal variations, significant results were obtained on E. coli contamination (p = 0.045), but no significant differentiation in AMR E. coli contamination (p = 0.234). Besides, there was a correlation between AMR E. coli contamination on local sanitation use (p=0.041) but not for livestock ownership (p=0.891). Many factors caused AMR E. coli contamination in water sources because of various environmental risk factors and other specific sources.

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References

1. B. Lyimo, J. Buza, M. Subbiah, W. Smith, and D. R. Call, BMC Microbiol. **16**, 1 (2016)
2. WHO, (2020)
3. B. A. Wee, D. M. Muloi, and B. A. D. van Bunnik, Clin. Microbiol. Infect. **26**, 1612 (2020)
4. D. Berendes, A. Kirby, J. Brown, and A. L. Wester, Lancet Planet. Heal. **4**, e246 (2020)
5. BMKG, (2017)
6. M. V. da Silva, H. Pandorfi, A. M. da R. F. Jardim, J. F. de Oliveira-Júnior, J. S. da Divincula, P. R. Giongo, T. G. F. da Silva, G. L. P. de Almeida, G. B. de Albuquerque Moura, and P. M. O. Lopes, Urban Clim. **38**, 100911 (2021)
7. J. O'dwyer, P. Hynds, M. Pot, C. Adley, and M. Ryan, (2017)
8. L. Andrade, M. Kelly, P. Hynds, J. Weatherill, A. Majury, and J. O'Dwyer, Water Res. **170**, 115360 (2020)
9. B. Statistik, *Modul Pelatihan Statistika Dasar: Teori Dan Aplikasinya Dengan Software SPSS v.24, Surabaya* (Biro Statistik Surabaya:, Surabaya, 2021)
10. V. Economou and P. Gousia, Infect. Drug Resist. 49 (2015)
11. A. Larson, S. M. Hartinger, M. Riveros, G. Salmon-Mulanovich, J. Hattendorf, H. Verastegui, M. L. Huaylinos, and D. Mäusezahl, Am. J. Trop. Med. Hyg. **100**, 1363 (2019)