Enteric pathogen among children under five years old with diarrheal diseases in Indonesia

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Abstract. Diarrheal diseases are the second cause of the high morbidity and mortality in children under five years old. According to the Basic Health Survey 2018 conducted by the Ministry of Health, the prevalence of diarrheal diseases among children under five years old that were diagnosed by healthcare workers was 11.0%. The aim of this study was to describe the enteric pathogen isolated from children with diarrhea. The study was conducted in five cities in Indonesia: Jakarta, Serang, Denpasar, Makassar, and Mataram. The inclusion criteria were children aged one month to five years old, with diarrhea that was diagnosed by a healthcare worker. The rectal swabs were sent to the Centre for Research and Development for Biomedical and Basic Health Technology, National Institute of Health Research and Development, Ministry of Health in Jakarta. Virus and Enterotoxigenic Escherichia coli (ETEC) identification by using multiplex PCR from Seegene, meanwhile bacteria identified by conventional method. As many as 2626 children under five years old participated in this study. The highest viral pathogen that causes diarrhea is viral 1.807 (68.81%) and 486 (18.56%). The virus etiology was Rotavirus 982 (54.34%) cases, followed by Adenovirus 916 (50.69) cases, Norovirus II 444 (24.57%) cases, meanwhile the bacteria pathogen were Enterotoxigenic Escherichia coli detected in 262 (9.98%) cases followed by Campylobacter jejuni and Shigella spp. This study described Rotavirus is the prevalence etiology of diarrhea among children under five years old followed by Adenovirus and Norovirus, some other cases reported the cause of diarrhea were bacteria ETEC E. coli followed Campylobacter jejuni, Shigella spp, etc.

1. Introduction
Diarrheal diseases are the second cause of the high morbidity and mortality in children under five years old [1]. As many as 525,000 children under five years old are killed by this disease. As many as 1.7 billion children have diarrhea every year [1].

According on the Basic Health Survey 2018 conducted by Ministry of Health, the prevalence of diarrheal diseases among children under 5 five years old that diagnosed by healthcare workers was 11.0%, and based on diagnosed and symptom was 12.3% with the highest prevalence occurred in age group 12-23 month old, around 16.6% [2]. On the other hand, diarrhea caused malnutrition that one of
the risk factor caused stunting [1][3]. WHO defined diarrheal disease as the passage of loose or watery stools as many as three or more frequent times a day[1][4].

There are several factors that might cause diarrhea: infection, malnutrition, source (water and food contamination) and other causes. In terms of infection, there are several pathogens that can cause diarrhea including bacteria, viruses and parasitic organisms. the most common pathogen is rotavirus [1]. Several countries reported the change of etiology pattern when the Rotavirus vaccine become a program, since rotavirus vaccination programs have decreased the prevalence of diarrhea cases caused by Rotavirus [4]. This article will describe the bacteria and virus that causes diarrhea in children under five years old. The data obtained from the study of diarrhea among children under five years old in 2009-2012 in Indonesia.

2. Method

The data used in this article are some of the data obtained from the study of identification and resistance patterns of microorganisms that cause diarrhea among children under-five years old in 2009-2012. The study was conducted in several hospitals and primary healthcare in 5 cities in Indonesia: Jakarta, Serang, Denpasar, Makassar, and Mataram (Figure 1). The Inclusion criterias were children aged 1 month to 5 years old, with diarrhea that was diagnosed by a healthcare worker. Consent from parents requested prior to enrollment. Samples that were not completed with identity and questionnaires were excluded from this study.

Figure 1. The location of study of identification and characterization of microorganism pathogen that caused diarrheal diseases among children under five years old in 5 provinces in Indonesia

The samples collected were two rectal swabs. The first rectal swab was put into Cary Blair for enteropathogen bacterial isolation and identification, meanwhile another rectal swab was put into a viral transport medium that would be used to detect viral enteric pathogen. The clinical samples were stored in a refrigerator at a temperature of 2-4°C before sent to Bacteriology Laboratory, Centre for Research and Development for Biomedical and Basic Health Technology, National Institute of Health Research and Development, Ministry of Health in Jakarta. The samples should be sent to the Bacteriology
Laboratory within 24-48 hours. Rectal swabs were cultured on to XLD, SS, and MacConkey medium, and then incubated in an incubator at 35°C for 18-24 hours.

Identification of colonies uses gram staining, oxidase, catalase, and simple biochemical tests, such as motility indole ornithine (MIO) and Kligler Iron Agar (KIA). Bacteria species identified by using API 20E. Identification of Enterotoxygenic *E. coli* by a commercial multiplex, Anyplex ETEC from Seegene. The RNA Virus isolation was performed by using GeneAll Ribospin vRD* and the viral identification used Seeplex Diarrhea-V-Ace Detection.

The process of entry, coding, editing, and data analysis conducted in the Center for Biomedical and Basic Technology of Health using SPSS software 15.0. The data for this article are shown as a percentage frequency distribution.

3. **Results**

As many as 2626 children under five years old participated in this study. The highest viral pathogen that causes diarrhea is viral 1.807 (68.81%) as described by figure 2.

![Figure 2. Distribution of etiology of diarrhea among children under five years old in Indonesia](image)

The most prevalence virus identified in clinical samples were Rotavirus 982 (54.34%) cases, followed by Adenovirus 916 (50.69) cases, Norovirus II 444 (24.57%) cases, Astrovisor 90 (9.48%) cases, and Norovirus I 42 (2.32%) cases. We detected more than 1 virus detected in the clinical specimen (Table 1).

**Table 1.** The Distribution of enteric pathogen virus isolated from children under five years old with diarrheal disease in 5 provinces in Indonesia.

| Virus         | Number | %    |
|---------------|--------|------|
| Rotavirus     | 982    | 37.40|
| Adenovirus    | 916    | 34.88|
| Norovirus II  | 444    | 16.91|
| Astrovirus    | 90     | 3.43 |
| Norovirus I   | 42     | 1.60 |
| Total         | 1807   | 68.81|
As many as 224 (8.53%) infected with bacterial pathogen enteric other than E. coli pathogen. Among pathogen bacteria, the highest proportion is Campylobacter jejuni, followed by Shigella spp as described in table 2.

**Tabel 2.** The Distribution of enteric pathogen bacteria isolated from children under five years old with diarrheal in 5 provinces in Indonesia.

| Bacteria                   | Number | %   |
|----------------------------|--------|-----|
| Enterotoxigenic E. coli (E. coli Patogen) | 262    | 9.98 |
| Salmonella spp             | 36     | 1.37 |
| Shigella sonnei            | 25     | 0.95 |
| Shigella flexneri          | 21     | 0.80 |
| Shigella boydii            | 1      | 0.04 |
| Shigella spp               | 1      | 0.04 |
| Campylobacter jejuni       | 81     | 3.08 |
| Campylobacter coli         | 7      | 0.27 |
| Vibrio cholera             | 2      | 0.08 |
| Vibrio parahemolyticus Non O1/O139 | 2    | 0.08 |
| Aeromonas hydrophila       | 48     | 1.83 |
| **Total**                  | 486    | 18.51 |

This study also revealed seven diarrhea cases caused by Vibrio cholera. Followed investigation to the clinical specimen revealed that Enterotoxigenic E. coli was detected in 262 (9.98%) cases with mixed ST and LT toxin.

### 4. Discussion

The etiology of diarrhea depends on geographical area, climate change, season and economic status of a country [5]. Surveillance of diarrhea cases is very important to understanding preventive measures, case management including antimicrobial therapy [5]. In this study the causal of diarrheal in less than 20% cases remain unclear. It could happen since diarrhea is also known as symptoms of other diseases, and the cause of diarrhea is not only infection but also lactose intolerance [1][4]. It is also the limitation of study, where the pathogen identification is limited to the major pathogen that caused diarrhea. There also another pathogen that may cause diarrhea such as protozoa and other bacteria such as diarrheagenic E. coli that covered enteropathogenic E. coli (EPEC), enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), enterohemorrhagic E. coli (EHEC) and enteroaggregative E. coli (EAEC), meanwhile in this study only investigate Enterotoxigenic E. coli [6].

Majority of the pathogen caused diarrhea among children under five years old was virus, especially Rotavirus, this results was similar with the several studies [7][8]. A study in Sub-Saharan and Asia, showed the countries prior to implementation of rotavirus vaccination found the majority caused of diarrheal diseases were Rotavirus, Cryptosporidium and Shigella spp [8]. The Rotavirus vaccination decreased the prevalence of diarrhea in countries.

The etiology of viral diarrhea in our study similar with surveillance that conducted in China in 2009 – 2018 that found, Rotavirus, Adenovirus and Astrovirus as the most etiology meanwhile in our study the Rotavirus, Adenovirus and Norovirus as the common viral etiology. Several studies reported that Norovirus is the major cause of diarrhea among adults [5][9]. Norovirus known as the etiology of traveler diarrhea and food borne outbreak. In the country where the rotavirus vaccine already implemented as a program, the Norovirus remains as the etiology of the diarrhea [10].
There were several cases detected with more than one virus in this study. This is one of advantage using multiplex PCR to identify enteric viral pathogen, besides have high sensitivity, may detect an uncultivable pathogen, this method could detect mixed infection [11][12].

The pattern of bacteria as the etiology of the diarrhea cases found in this study is similar with other studies that were concluded in five regions [13]. The most prevalent bacteria that caused diarrhea in this study is Enterotoxigenic *E. coli*. This pathogenic bacteria, is the main cause of diarrhea in developing countries [1][5]. Global Enteric Multicenter Study also found that infant and toddler who died caused by diarrhea, statistically more likely infected with enteropathogenic *E. coli*, Enterotoxigenic *E. coli* and Cryptosporidium [8]. This bacteria also has become a burden for farmer since this bacteria become a problem in broiler, swine, cattle and other farm animals. The interesting findings in this study is that *Campylobacters* spp was the most prevalent bacteria detected in the rectal swab other than *E. coli* pathogen. This pathogen is a foodborne zoonotic pathogen, a common cause in developed and developing countries [4][13][14].

*Shigella* spp is another etiology of diarrhea in this study, commonly mentioned as dysentery that has symptoms, bloody diarrhea. There were several species of *Shigella* spp found, and some species more tend to cause bloody diarrhea than others. *Shigella flexneri*, known as the strongest species causing bloody stool [15]. A meta-analysis study conducted by Kirkby et al, found that the odds of death were higher in diarrhea cases caused by *Shigella* spp than without infection.

WHO reported that diarrhea is the disease from inadequate water, sanitation, and hygiene. Several diarrhea pathogens contaminate the food and drinking water. Salmonellosis cases are usually caused by contaminated eggs, poultry and other food from animal origin. Diarrhea that is caused by *Campylobacter* spp may be caused by raw milk, raw or undercooked poultry and drinking water. Pathogenic *Escherichia coli* usually infected human through unpasteurized meat, fruits and vegetables [1].

Not all diarrhea needs antibiotics therapy, through this study, diarrhea in children under 5 years old caused by virus, therefore the appropriate management based on WHO guidelines is needed, such as rehydration and zinc. Other diarrhea that is caused by bacteria should be considered to be treated by using antibiotics appropriately [1][15]. Therefore, it is important to re-evaluating every diarrhea case to define the case management. Several prevention programs to control diarrhea should be implemented and monitored. Water sanitation, hand hygiene, and vaccination should be done.¹

5. Conclusion
This study described Rotavirus is the prevalent etiology of diarrhea among children under five years old followed by Adenovirus and Norovirus, some other cases reported the cause of diarrhea were bacteria, such as ETEC *E. coli* followed *Campylobacter jejuni*, and *Shigella* spp. The etiology of diarrhea in a countries depend on the geography, season and also the implementation of rotavirus vaccine.

6. References
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