

# Identification of bacterial diversity in the feces of children with diarrhea

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Research Article


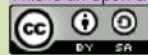


**Identification of bacterial diversity in the feces of children with diarrhea**

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Article Information	ABSTRACT
Submitted: 2021-10-20 Accepted: 2022-07-21 Published: 2022-07-21	<p>Diarrhea diseases collectively constitute a serious public health challenge globally, especially as the leading cause of death in children. This study aims to identify the diversity of bacteria that cause diarrhea in children. This type of research is cross-sectional with a prospective descriptive approach. Sampling of 50 children with diarrhea in several health centers in Makassar City. The instrument used in this study was to use a list of questions related to the suitability of the desired sample and a descriptive chart of gram-negative bacteria and adapted to Bergey's Manual of Determinative Bacteriology to identify the bacteria obtained. The process of testing specimens with the culture method by isolation and inoculation of samples for further biochemical tests including Triple Sugar Iron Agar (TSIA), Indole Motility Sulfite (SIM), Methyl Red (MR), Vogas Proskauer (VP), and carbohydrates tests were carried out at the Microbiology Laboratory of Hasanuddin University Hospital. The results showed that the Escherichia coli bacteria were 15 (30%), Klebsiella sp as many as 14 (28%), Enterobacter sp as many as 12 (24%), Proteus mirabilis as much as 4 (8%), Proteus vulgaris as much as 4 (8%), and the bacteria Alcaligenes faecalis only 1 (2%). The conclusion of the study was that the most common type of bacteria found was Escherichia coli.</p>
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**INTRODUCTION**

Diarrhea is an endemic disease in Indonesia that has the opportunity to become a case of Extraordinary Events (EE) which is usually accompanied by death. This disease is usually characterized by the occurrence of defecation three or more times in one day with liquid stools (Brandt et al., 2015) In 2017, the prevalence of diarrhea cases was 4,274,790 cases, and in 2018 there were 229,792 cases added to 4,504,524 people or 62.93%. A total of 8 provinces and 8 regencies/cities in 2018 experienced outbreaks of cases more than 5 times with a total of 756 cases and 36 deaths. The case fatality rate or CFR for diarrhea cases in 2018 experienced a spike in cases from the previous year, which was 4.76%,

whereas the CFR for diarrhea cases was expected to be 1% (Kementerian Kesehatan Republik Indonesia, 2018).

In developing countries, child morbidity and mortality are mostly caused by five diseases, namely ISPA, diarrhea, measles, malaria, and malnutrition (Farthing et al., 2013). Diarrhea is an infectious disease that is the main cause of death in infants and toddlers (Saputri & Astuti, 2019). Toddler age is a period that is quite difficult for children because the child's immunity is still not stable so that they are susceptible to infectious diseases. The causes of diarrhea can be divided into three factors, namely environmental factors, personal factors and attitude factors. Environmental factors such as the use of dirty water, poor sanitation and lack of drinking water supply facilities. Self factors such as poor nutrition and behavioral factors such as lifestyle and diet (Utami & Luthfiana, 2016).

Etymologically, diarrhea can be caused by pathogen attack, mal-absorption, allergies, chemical or food poisoning, and immunodeficiency (Sukut et al., 2015). Pathogenic bacteria that cause diarrhea are very diverse yaitu *Enterotoxigenic E. coli*/ETEC, *Enteropathogenic E. coli*/EPEC, *Enteraggregative E. coli*/EAggEC, *Enteroinvasive E. coli*/EIEC, *Enterohemorrhagic E. coli*/EHEC), *Shigella spp.*, *Campylobacter jejuni*/*Helicobacter jejuni*, *Vibrio cholerae* 01, dan *V. cholerae* 0139, *Salmonella*/*non-typhoid* (Bellini & Dumoulin, 2018). Bacteria that cause diarrhea in children are: *Eschericia coli*, *Salmonella typhi*, dan *Shigella sp* (Muttaqin et al., 2016).

In Makassar City, the incidence of diarrhea is still quite high. Based on data from the South Sulawesi Provincial Health Office, in 2017 the number of diarrhea cases in South Sulawesi Province was 230,048 cases where the highest incidence of diarrhea was at Antang Health Center. The incidence of diarrhea in Makassar City itself in 2012 was 29,265 cases. In 2013-2014 there was a decrease of 28,908 cases and 26,485 cases. However, in 2015 the number of diarrhea cases increased by 28,223 cases and decreased again in 2016 as many as 22,052 cases (Hamzah et al., 2020). Causes of morbidity and mortality due to diarrhea can not be known specifically. This is because the diagnosis by medical personnel does not pass through the examination in the laboratory, but the diagnosis is made only by looking at the symptoms that exist in the patient. If this is allowed, then diarrhea cases in Makassar City will increase again.

An alternative that can be done to reduce the number of diarrhea cases in Makassar City is to carry out supporting examinations in the laboratory, namely by identifying the factors that cause diarrhea cases. A similar study conducted a laboratory examination of body fluids (blood) using the Widal test method. This test aims to determine the presence of agglutinins against the bacteria *Salmonella typhi* that causes diarrhea in the patient's blood by reacting a person's serum with O, H, and Vi antigens (Sinaga & Sembiring, 2016). This test is easy to do so it is often used to establish a diagnosis. However, this test has weaknesses, namely low sensitivity and specificity and difficulty in interpretation. In this study, laboratory examination of feces was carried out using the culture method. Examination of stool samples by culturing bacteria is the gold standard to determine the pathogenic bacteria that cause diarrhea. Identifying the factors that cause diarrhea cases is very important as a support in the management of diarrhea cases. Because the pathogenic bacteria that cause diarrheal infections are very diverse, this study was conducted to identify the diversity of bacteria in the stools of children with diarrhea to reduce diarrhea cases, especially in Makassar City.

## RESEARCH METHODS

This type of research is a descriptive study with a cross-sectional design to determine the diversity of bacteria that cause diarrhea in children based on the culture method. The study was conducted in 2



different locations, namely sampling test and sample testing. The collection of test samples was carried out at Puskesmas in Makassar City, namely Antang, Bara-baraya, Pampang, and Tamangapa Health Centers. Bacterial observations were carried out at Hasanuddin University Hospital.

The population in this study were all diarrhea patients who were examined at 4 predetermined sample collection locations with a total sample of 50 people. Eligible samples were taken by looking at the inclusion criteria, namely, children aged 0-59 months, defecating more than 3 times a day in liquid form, not taking antibiotics, and being willing to sign the informed consent. The tools and materials used in this study include test tubes, Erlenmeyer, measuring cups, Petri dishes, ose rods, tube racks, Bunsen, sterile cotton, laminar air flow, analytical balances, analytical balances, refrigerators, and rectal swab tips. The materials used include Cary-Blair Agar, BHIB, NA, Mac Conkey Agar, Methyl Red (MR) test medium, Vogas Proskauer (VP) test medium, Sulfate, Indole, Motility (SIM) test medium, medium urease test, Simmon citrate test medium, Triple Sugar Iron Agar (TSIA), and carbohydrate test medium. The scheme of this research is shown in Figure 1.

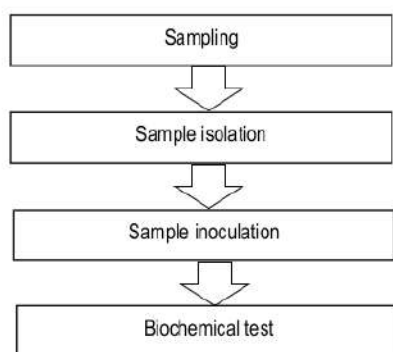


Figure 1. Research Scheme

The data collection technique used in this study was to isolate and culture stool samples from children with diarrhea. Stool collection is done using an anal swab technique. The patient is placed on the left side. Open the anal canal by hand, then insert a cotton swab into the anus, rotate it 360° previously soaked in NaCl solution. The swab tip is then inserted into the carrier vehicle, the CaryBar. After arriving the laboratory, the rectal swab sample was dipped into the fertilization medium, namely BHIB, and incubated 37°C for 2 hours. Rectal swab samples were inoculated on nutritional media, specifically BHIB and incubated at 37 °C, 2 hours, 1 dose loop was collected and inoculated on Nutrient Agar and Mac Conkey Agar. Then the samples were incubated for 2 hours at 37°C.

The instrument used in this study was to use a list of questions related to the suitability of the desired sample and a descriptive chart of gram-negative bacteria and adapted to Bergey's Manual of Determinative Bacteriology to identify the bacteria obtained. The data analysis technique used in this study is to identify bacteria by biochemical tests. A biochemical test is one of the tests that identify bacteria. This test is used to determine the metabolic activity of microorganisms. Biochemical tests performed include Triple Sugar Iron Agar (TSIA), Indole Motility Sulfite (SIM), Methyl Red (MR), Vogas Proskauer (VP), and carbohydrate test (glucose, sucrose, lactose, maltose).

## FINDING AND DISCUSSION

This research was conducted in the microbiology laboratory of Hasanuddin University Hospital Makassar with a sample of 50 stools of children with diarrhea in several health centers in Makassar city. A total of 50 samples of rectal swabs were obtained which were inoculated on BHIB media, then inoculated on culture media, and further biochemical tests were performed. The results of biochemical tests for the types of bacteria found can be seen in Table 1.

Table 1. Results of Biochemical Tests for Types of Bacteria in Samples

Types of bacteria	Biochemical Reaction												
	TS IA			SIM				M R	V P	Karbohidrat			
	Sla nt	Ba th	H <sub>2</sub> S	G as	Ind ol	H <sub>2</sub> S	M otility			G	L	S	M
<i>Klebsiella sp.</i>	As	As	-	+	-	-	+	+	+	+	-	+	+
<i>Escherichia coli</i>	As	As	-	+	+	-	+	+	-	+	+	+	+
<i>Enterobacter sp.</i>	Alk	As	-	-	-	-	-	-	+	-	+	+	+
<i>Proteus mirabilis</i>	As	As	+	+	-	+	+	+	+	+	-	-	-
<i>Proteus vulgaris</i>	As	As	+	+	+	+	+	+	-	+	-	+	-
<i>Alcaligenes faecalis</i>	Alk	Alk	-	-	-	-	+	-	-	-	-	-	-

Information: TSIA= Triple Sugar Iron Agar; SIM= Sulfat Indol Motility; MR= Metil Red; VP= Vogas Proskauer; G= Glucose; L= Lactose; S= Sucrose; M= Maltose; As= Acid; += Positive; and -= Negative

The results of observations of bacteria using the culture method, found some bacteria found in the feces of children with diarrhea are as follows. The types of bacteria in the feces of children with diarrhea can be seen in Table 2.

Table 2. Types of Bacteria in the Stool of Children with Diarrhea

No	Bacteria names	Sample Code	Frequency (n)	Percentage (%)
1	<i>Escherichia coli</i>	2;4;6;10;12;13;16;28;30;35;36;38;39;44;45	15	30%
2	<i>Klebsiella sp.</i>	1;3;7;8;11;14;19;23;25;29;34;37	14	28%
3	<i>Enterobacter sp.</i>	17;20;21;22;26;27;31;32;33;37;40;43;46;48	12	24%
4	<i>Proteus mirabilis</i>	9;41;42;49	4	8%
5	<i>Proteus vulgaris</i>	15;18;24;50	4	8%
6	<i>Alcaligenes faecalis</i>	5	1	2%
	Total		50	100%

Based on observations found 50 bacterial samples as shown in Table 2, namely 15 (30%) *Escherichia coli*, 14 (28%) *Klebsiella sp.*, 12 (24%) *Enterobacter sp.*, *Proteus mirabilis*, and *Proteus vulgaris* species respectively. *Escherichia coli* bacteria can be found in the feces of people with diarrhea because *Escherichia coli* lives in the digestive tract. *Escherichia coli* is a bacterium that lives in the human digestive tract and is a normal flora (Zikra et al., 2018). *Escherichia coli* is a species of bacteria in the *Enterobacteriaceae* family, namely enteric bacteria or bacteria that can live and can survive in the digestive tract. *Escherichia coli* can survive at high acidity concentrations inside the human body and



outside the human body. Diarrhea occurs when the number of *Escherichia coli* increases in the digestive tract or when these bacteria are outside the intestine. The number of *Escherichia coli* bacteria can increase in the human digestive tract when consuming food or drink contaminated with *Escherichia coli*. *Escherichia coli* can ferment lactose, so it is called coliform bacteria. Coliform bacteria are good indicators bacteria for fecal contamination, especially in water. Therefore *Escherichia coli* is also known as an indicator bacteria for sanitation and hygiene. A person can get diarrhea when in an environment with poor sanitation and hygiene.

*Escherichia coli* bacteria have a high percentage compared to other bacteria where the percentage of *Escherichia coli* bacteria in the sample is 30%. The high cases of diarrhea due to infection with *Escherichia coli* bacteria are due to the very diverse habitats of these bacteria. These bacteria can be found in water and foodstuffs consumed by humans. Research conducted in the city of Padang found *Escherichia coli* contamination in drinking water samples in restaurants and cafes (Tia et al., 2018). Based on research conducted by Musawir & Arsin, cross-tabulation results between *Escherichia coli* in milk bottles and the incidence of diarrhea showed that as many as 21 infants (77.8%) had diarrhea with milk bottles contaminated with *Escherichia coli* and as many as 10 infants (14.1%) had diarrhea. Diarrhea with a bottle of milk without *Escherichia coli* (Sani et al., 2020).

Furthermore, *Klebsiella sp* bacteria can be found in the feces of diarrhea sufferers because *Klebsiella sp*. lives as a saprophyte in the environment in water, soil, food, and vegetables. *Klebsiella sp* is also a species of bacteria in the family *Enterobacteriaceae*, namely enteric bacteria or bacteria that can live and can survive in the digestive tract. *Klebsiella sp* has been considered part of the normal intestinal flora. *Klebsiella sp* usually does not cause disease and in the intestine plays a role in normal function and nutrition. However, these bacteria become pathogenic if the bacteria are present in tissues outside the normal intestinal tissue or in places where normal flora is rare. Under suitable conditions, these bacteria can cause infections, such as nosocomial infections (hospital-acquired infections) and community-acquired infections (Makalew et al., 2016). The *Klebsiella sp* bacteria found in the feces of diarrhea sufferers were 28% because *Klebsiella sp* is enteric bacteria found in small numbers as normal upper respiratory tract flora. *Klebsiella sp* bacteria colonize up to 9% of healthy subjects (Cheng et al., 2012). These bacteria are gram-negative bacteria, facultative anaerobes, non-motile (immobile) bacteria, and can ferment lactose.

*Enterobacter sp* bacteria can be found in the feces of people with diarrhea because *Enterobacter sp* is present in humans, in soil, and water. *Enterobacter sp* is also included in the *Enterobacteriaceae* family which includes several species, such as *Enterobacter agglomerans*, *Serratia rubidaea*, and *Enterobacter cloacae*. *Enterobacter agglomerans*, also known as *Pantoea agglomerans*, is commonly found and isolated in foods, vegetables, and grains. *Enterobacter agglomerans* can cause diarrhea, nausea, and vomiting. *Serratia rubidaea* is isolated from foods, such as vegetables, tomatoes, and salads. If a person ingests food contaminated with *Serratia rubidaea* bacteria, it can cause gastrointestinal infections. *Enterobacter cloacae* live in a thermophilic environment with an optimal temperature of 37. *Enterobacter cloacae* are commonly found in fruits and vegetables. *Enterobacter cloacae* is a pathogenic bacterium that causes intra-abdominal infection, nausea, vomiting, and diarrhea (Porotu'o et al., 2015). *Enterobacter sp* bacteria found in the feces of diarrhea sufferers by 24% because it is an enteric bacterium that is found in small amounts as normal intestinal flora. *Enterobacter sp* was found to be gram-negative, bacilli-shaped, and facultatively anaerobic (Janasuta et al., 2020). This bacterial culture is motile.

*Proteus sp* bacteria such as *Proteus mirabilis* and *Proteus vulgaris* can be found in the feces of diarrhea sufferers because these bacteria are the bacteria that cause diarrhea. *Proteus mirabilis* is a

normal flora in the digestive tract, is widely distributed in the environment, is a facultative anaerobe, and is known to be an opportunistic pathogen. *Proteus vulgaris* is a normal flora found in the human gastrointestinal tract. *Proteus sp* is a bacterium that has fast mobility and can produce urease. 8% of *Proteus sp* bacteria found in the feces of diarrhea sufferers because *Proteus sp* is an enteric bacterium that is found in small amounts as normal gastrointestinal flora. *Proteus sp* is often found in small numbers (Kumar Trivedi, 2015).

Furthermore, *Alcaligenes faecalis* bacteria can be found in the feces of patients with diarrhea because *Alcaligenes faecalis* can be found in soil, water, and in hospital environments such as in respirators, hemodialysis systems, and intravenous solutions (W<sub>10</sub> et al., 2020). *Alcaligenes faecalis* is a bacterial pathogen that has the potential to emerge and cause opportunistic infections in humans. The organism has been isolated from various clinical materials such as feces, urine, blood, wound fluid, cerebrospinal fluid, and respiratory secretions. In addition to causing diarrhea, *Alcaligenes faecalis* can also cause endocarditis, bacteremia, meningitis, endophthalmitis, skin and soft tissue infections, urinary tract infections, otitis media, peritonitis, and pneumonia (Huang, 2020). The bacteria *Alcaligenes faecalis* found in the feces of diarrhea sufferers is 1% because *Alcaligenes faecalis* is generally found in small amounts. This is in line with research conducted by Bakri et al. (2015) which also found 1 isolate of *Alcaligenes faecalis* bacteria in feces. *Alcaligenes faecalis* was found to be a gram-negative, rod-shaped bacterium (bacillus), obligate aerobic, and non-fermenting. *Alcaligenes faecalis* belongs to the family *Achromobacteriaceae*.

Bacteria can be transmitted through water or food media. Transmission of water-borne diseases can be grouped into mechanisms of transmission through water and watersheds. Mechanisms of transmission through water are oral and gastrointestinal transmission. Contamination to humans can occur through drinking alcohol, bathing, and washing clothes. The catchment mechanism is transmission related to water used for personal hygiene and cleaning of utensils such as cooking utensils and cutlery (Muttaqin et al., 2016). Children are very susceptible to various infections such as diarrhea. Therefore, it is important to maintain the availability of clean water facilities, maintain a diet so that the digestive system is in a healthy condition, maintain food sanitation and hygiene, wash hands before eating, and wash cutlery before use.

## CONCLUSION

Pathogenic bacteria that cause diarrheal infections are very diverse. Laboratory tests are needed to determine the causal organism of diarrheal disease so that cases of diarrhea can be suppressed. The bacteria found in 50 stool samples of children with diarrhea included 15 (30%) *Escherichia coli* (30%), 14 *Klebsiella* spp (28%), 12 *Enterobacter* sp (24%), 4 (8%), *Proteus mirabilis* 4 (8%), *Proteus vulgaris* (4%). 8%), and *Alcaligenes faecalis* only 1 (2%). Generally, these bacteria live in the digestive tract and are normal flora. However, these bacteria are pathogenic if their numbers increase in the digestive tract or if they are outside the intestine. So that people are no longer exposed to diarrhea caused by these bacteria, it is recommended to maintain the availability of clean water facilities, maintain food sanitation and hygiene, wash hands before eating, and wash eating utensils before use.

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