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ORIGINAL ARTICLE

IDENTIFICATION OF BACTERIA CAUSING DIARRHEA IN UNDER-FIVES CHILDREN USING CULTURE METHODS IN BIMA, INDONESIA

Identifikasi Bakteri Penyebab Diare pada Balita Menggunakan Metode Kultur di Bima, Indonesia

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ABSTRACT

Background: Diarrhea is still a world health issue which is one of the leading causes of morbidity and mortality, with a mortality rate of close to more than 1.30 million deaths globally in 2015. The prevalence period of diarrhea in Indonesia for under-five children in 2018 is 12.30%. The West Nusa Tenggara province is third of the ranks based on doctor's diagnosis (13.45%) and clinical symptoms (15.12%). **Purpose:** This study aims to identify the bacterial infection that causes diarrhea among under-five children in Bima District, West Nusa Tenggara Province. **Methods:** The cross-sectional study was conducted on under-five children brought to Bolo and Wawo's Public Health Center with diarrhea and diagnosed with acute gastroenteritis. **Results:** The samples involved in this study were 102 samples and the results obtained were 1 to 2 types of bacteria in 1 sample. The order of bacteria from the most is E. Coli (29.53%), Klebsiella, sp (25,50%), Shigella, sp (18,79%), Staphylococcus aureus (16,78%), Salmonella Thypii (8,73%), Proteus, sp (0,67%), male (61.76%) had more diarrhea than female (38.24%), with an age range of 1-3 years (53.92%), 0-1 years (37.26%), and 3 until <5 years (8.82%). **Conclusion:** In this research, it was proven that E. coli bacteria are the leading cause of diarrhea in under-five children.

ABSTRAK

Latar Belakang*: Diare masih menjadi masalah kesehatan dunia*

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yang merupakan salah satu penyebab utama morbidiitas dan mortalitas dengan jumlah angka kematian mendekati 1.30 juta jiwa tahun 2015. Periode Prevalence diare di Indonesia untuk kelompok umur balita tahun 2018 adalah 12,30%, Provinsi NTB menempati urutan ketiga berdasarkan diagnosis dokter sebesar 13.45% dan gejala klinis sebesar 15,12% dari seluruh provinsi di Indonesia. ***Tujuan****: Penelitian ini bertujuan untuk mengetahui infeksi bakteri penyabab diare pada balita di daerah Kabupaten Bima Nusa Tenggara Barat.* ***Metode****: Studi cross-sectional dilakukan pada balita yang dibawa ke puskesmas bolo dan puskesmas wawo dengan keluhan utama diare dan terdiagnosis gastroenteritis akut. Semua sampel yang setuju untuk mengikuti penelitian ini harus memberikan persetujuan. Sebanyak 102 sampel tinja dikumpulkan sejak Januari 2020 hingga Maret 2020 dan dianalisis di Laboratorium kesehatan daerah Kota Bima, NTB..* ***Hasil****: Sampel yang dilibatkan dalam penelitian ini sebanyak 102 sampel, dan didapatkan hasil 1 sampai 2 jenis bekteri dalam 1 sampel dengan bakteri terbanyak secara berurutan adalah E. Coli (29,53%), Klebsiella, sp (25,50%), Shigella, sp (18,79%), Stafilokokus aureus (16,78%), Salmonella Thypii (8,73%), Proteus, sp (0,67%). Penderita berjenis kelamin laki-laki (61,76%) lebih banyak dari perempuan (38,24%) dengan rentang usia terbanyak yaitu 1-3 tahun (53,92%), 0-1 tahun (37,26%), dan 3 sampai dengan <5 tahun (8,82%).* ***Kesimpulan****: Bakteri E. coli terbukti menjadi penyebab utama diare pada Balita dalam penelitian ini.*

INTRODUCTION

A diarrhea disease is still a world health issue, especially in developing countries. Globally, diarrhea is the second leading cause of death among under-fives children (Kotloff, 2017). Based on Liu et al (2016) globally in 2015, we estimated that diarrhea was the leading cause of death at all ages (1.31 million deaths). Diarrhea is a common cause of death in under-five children, 499.000 death. Although the global diarrhea mortality rate has decreased from 11% in 2010 to 9% in 2015, this disease still has become the second cause of death in under-fives children. It is estimated that in 2010 there were 1,213 million cases of diarrhea in under-fives children, and in 2015 there were 509,000 cases of death. Diarrhea accounts for one in eight deaths among children younger than five years per annum (Keddy, Smith, & Page, 2016). The research conducted by Chakravarty, Bhattacharya, & Das (2017) showed that diarrhea could negatively impact childhood growth and cognitive development.

Data of Indonesia Basic Health Research 2018, the prevalence of diarrhea in under five years in Indonesia is 12.30%. West Nusa Tenggara Province is third of the ranks based on doctor's diagnosis of 13.45% and clinical symptoms 15.12%. The incidence of diarrhea in under-fives children in the Bima district is 11.87% based on the doctor's diagnosis and 16.07% based on clinical symptoms. Bima district is the third rank after Dompu district (21.16%) and Central Lombok (17.2%) with diarrheas incidence in under-fives children (Ministry of Health RI, 2018).

Diarrhea is more common in tropical areas with poor hygiene and sanitation and limited access to getting clean water. Other conditions, such as malnutrition, can increase the risk of contracting diarrhea in developing countries (Chakravarty, Bhattacharya, & Das, 2017). These factors can cause significant disease burdens and negative economic impacts, resulting from medical costs, work productivity loss, and lower quality of life (Buono, Carson, & Flores, 2017). Diarrhea is one of the reasons why parents bring their children to visit a health center or hospital for treatment. It is because the knowledge of diarrhea disease is still limited (Burokienė, Raistenskis, Burokaitė, Čerkauskienė, & Usonis, 2017).

The infectious diarrheal disease causes most the children under five to die. However, Liu et al

(2016) said that the cause of this disease is usually unknown, so mistakes often occur in administering antibiotics (Hassan, 2021). Therefore, this study aims to identify the bacteria that cause diarrhea in Bima, West Nusa Tenggara, Indonesia.

METHODS

This research is a cross-sectional study. The population in this study was all infants and children aged <5 years who were brought to Bolo and Wawo's Public Health Center with diarrhea and diagnosed with acute gastroenteritis. All samples who agree to participate in this study must give informed consent. Sampling in this study using purposive sampling. A total of 105 stool samples were collected from January 2020 to March 2020, and 2 samples could not be analyzed because the samples were too small, so a total of 102 samples were successfully examined at the Bima City Health Laboratory, NTB. The exclusion criteria in this study were subjects did not take a stool sample, and the stool samples were too small to be analyzed. Ethical permission was granted by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya (No.15/EC/KEPK/FKUA/2021).

The subjects were interviewed to get information about age, gender, and frequency of diarrhea. Stool sampling was carried out during the first visit to the Public Health Center was assisted by medical personnel and research assistants in the process of taking stool samples. Stool samples were stored in a ±10ml vial provided. Then, the sample is taken to the regional health laboratory for culture examination. Identification was carried out on bacterial isolates grown on isolation media by microscopic and macroscopic examination (characteristics of culture results on bacterial and biochemical test results). The data from the questionnaire will be analyzed using descriptive analysis. The data will be presented in the table containing the frequency and percentage of objects analyzed.

RESULTS

This research was conducted from January 2020 to March 2020 in 2 public health centers in Bima district. There were 105 subjects obtained, but two were excluded. The total of under-fives children taken for stool culture was 102 samples. The frequency distribution of respondents based on gender and age of under-five children can be seen in table 1.

Based on Table 1, males (61.76%) had more diarrhea than females (38.24%), with an age range of 1-3 years (53.92%), 0-1 years (37.26%), and 3-<5 years (8.82%). Of the 102 samples involved in this study, the results obtained were 1 to 2 types of bacteria in 1 sample, the order of bacteria from the most is *E. Coli* (29.53%), *Klebsiella sp.* (25.50%), *Shigella sp.* (18.79%), *Staphylococcus aureus* (16.78%), *Salmonella thypii* (8.73%), *Proteus sp.* (0.67%) (see table 2). *E. coli* mainly infects toddlers aged between 1-3 years by 26 samples (17.5%), while *Klebsiella sp.* mostly infects toddlers aged 0-1 years (12.08%) (see table 3).

Table 1
Frequency Distribution of under-five children by Gender and Age in Bolo and Wawo's Public Health Center Area, Bima District, March 2020

Child Characteristics	n	%
Gender		
Male	63	61.76
Female	39	38.24
Age (years)		
0–1	38	37.26
1–3	55	53.92
3–<5	9	8.82
Total	102	100.00

Examination of bacterial stool cultures that cause acute diarrhea was carried out on 102 samples, and the results were 1 to 2 types of bacteria in 1 sample. Therefore, the children <5 years old were infected by more than 1 type of bacteria and 149 total bacteria-infected 102 under-five children (see Table 2).

Table 2
Stool Culture Examination Results in Bolo and Wawo's Public Health Center Area, Bima District, March 2020

Result of Stool Culture	n	%
E. Coli	44	29.53
Klebsiella, sp	38	25.50
Shigella, sp	28	18.79
Staphylococcus aureus	25	16.78
Salmonella Thypii	13	8.73
Proteus, sp	1	0.67
Total	149	100.00

Based on Table 4, on microscopic examination found two samples of worm eggs parasites (*Trichuris trichiura*) (1.96%) and two samples of Ascaris I (1.96%). Macroscopic examination found the consistency of solid (49.02%), semi-

solid (21.57%), and liquid (29.41%). Most samples obtained blood (83.33%) (see Table 4).

DISCUSSION

Based on data of child characteristics, it was found that there were more diarrhea sufferers in males (61.76%) than females (38.24%), almost the same as research from Nirwati et al (2017), were male (67.31%) more than female (32.69%). The research conducted by Adam et al (2018) and Saeed, Abd, & Sandstrom (2015) obtained the same results, which males get more diarrhea than females. In another study conducted by Mekonnen, Mengistie, Sahilu, Kloos, & Mulat (2019), it was found that females (55.20%) had more diarrhea than males (44.80%). It is not yet clearly known what caused this, but in several studies, it was reported that the difference in the incidence rate for males and females did not significantly differ (Al-abbas, 2018).

Based on age, children aged 1-3 years have more frequent diarrhea (53.92%), children aged 0-1 years (37.26%), while children aged 3-<5 years (8.82%) less than the age of other children. It is almost the same as the research conducted by Muttaqin, Hartoyo, & Marisa (2016). The incidence of acute diarrhea in Ulin Hospital showed males (66.67%) more than females (33.33%). Then, the largest age group was 1-2 years (36.11%), at least 2-5 years (8.3%), because children aged 3-5 years already had a better immune system, so they were more resistant to diarrhea. Also, the children aged 0-1 years still getting exclusive breastfeeding can increase immunity so that children aged 0-1 years who get exclusive breastfeeding get sick less often. Exclusive breastfeeding can protect children from infections caused by bacteria, viruses, etc.

In this study, children aged 1-3 years had more diarrhea because, at that age, the children had been given additional food so that if the child is given inappropriate food, it will cause the digestive system not to develop properly. Consequently, the children will get diarrhea (Seyedian, Nokhostin, & Malamir, 2019). Children aged 1-3 years generally get breast milk with additional food or food alone without breastfeeding again, which makes it easy for children of this age to eat contaminated food. The hygiene factor of baby feeding food tools and lack of cleanliness of food prepared by mothers are the causes of increased diarrhea in children. Children at this age can become infected with bacteria that cause diarrhea when playing in dirty environments and through unsanitary ways of living (Chidziwisano et al., 2019). Saeed, Abd, & Sandstorm (2015), said that contaminated hands are one of the most common routes of transmission of food-borne infections. It may be one reason why diarrhea is high in the age group over 2 years due to the practice of washing hands less in this age group.

According to the criteria, this current research was conducted on 102 samples collected from 2 public health centers, namely children aged <5 years who experience diarrhea more than 3 times a day and have not taken medication / antibiotics. Based on the stool culture examination results, several types of bacteria were found to be the etiology of diarrhea in children, namely *E. coli, Klebsiella sp., Shigella sp., Staphylococcus aureus., Salmonella thypii, Proteus sp.* In this research, *E. coli* was the most common etiology type of diarrhea with 29.53%, followed by *Klebsiella sp.* with 25.50%. The research conducted by Muttaqin, Hartoyo, & Marisa (2016) described the types of aerobic bacteria that cause diarrhea in children aged 1-18 months at Ulin Hospital, Banjarmasin, using the culture method. The types of aerobic bacteria that cause diarrhea in children with a total of 36 samples are E. Coli with 26 (72.22%) samples, *Salmonella typhi* with 7 (19.44%) samples, then *Shigella sp*. 3 (8.33%) samples. As well as research conducted by Saeed, Abd, & Sandstorm (2015) about Microbial etiology of acute diarrhea in children under five years of age in Khartoum Sudan, available of the 437 samples analyzed, 211 (48%) tested positive for diarrhoeagenic *Escherichia coli*, 96 (22%) *Rotavirus a.*, 36 (8%) *Shigella sp.*, 17 (4%), *Salmonella sp.*, 8 (2%) *Campylobacter sp.*, 47 (11%) *Giardia intestinalis* and 22 (5%) *Entamoeba histolytica*.

In their research, Adam et al (2018) found several types of bacteria and viruses that cause diarrhea in under-five children at Khartoum teaching hospitals in Sudan. The types of bacteria identified were *Escherichia coli* (EHEC / EPEC), *Shigella sp., Salmonella sp., Vibrio parahaemolyticus*. Almost the same as the research conducted by Afolabi, Saka, Ojuawo, & Biliaminu (2018), it was found that the types of bacteria identified in the stool samples by culture method were 39 isolates (39.00%) *E. coli*, 28 isolate (28.00%) *Klebsiella sp.*, 4 isolate (4.00%) *Proteus sp.* and 2 isolate (2.0%) *Pseudomonas*.

Table 3
Stool Culture Examination Results based on age characteristics in Bolo and Wawo's Public Health Center Area, Bima District, March 2020

Result of Stool Culture	Public Health Center Bolo & Wawo			Total	
	0–1	1–3	3–<5	n	%
E. coli	7	14	0	21	20.59
E. coli & Shigella sp	2	4	0	6	5.88
E. coli & Klebsiella sp	7	7	1	15	14.71
E. coli & S. Aureus	1	1	0	2	1.96
Klebsiella sp	9	9	1	19	18.63
Klebsiella sp & S. aureus	2	1	1	4	3.92
Shigella sp	4	7	4	15	14.71
Shigella sp & S. aureus	1	3	2	6	5.88
Shigella sp & Proteus sp	0	1	0	1	0.98
Salmonella typhi & S. Aureus	5	8	0	13	12.79
Total	38	55	9	102	100.00

Table 4
Macroscopic and Microscopic Laboratory Examination Results in Bolo and Wawo's Public Health Center Area, Bima District, March 2020

Type of Examination	n	%
Macroscopic Specimens		
Consistency		
Solid	50	49.02
Semi-Solid	22	21.57
Liquid	30	29.41
Color		
Brown	23	22.55
Pale brown	1	0.98
Light brown	6	5.88
Dark brown	6	5.88
Yellow	21	20.59
Pale yellow	6	5.88
Light yellow	2	1.96
Dark yellow	4	3.92
Greenish-yellow	2	1.96
Green	19	18.63
Light green	2	1.96
Pale green	3	2.94
Greenish white	1	0.98
Greenish	6	5.88
Blood and Mucus		
Blood (+)	85	83.33
Blood (-), Mucus (+)	5	4.90
Blood (+), Mucus (+)	12	11.76
Microscopic (parasite)		
No parasite	98	96.08
Worm eggs (Trichuris trichiura)	2	1.96
Ascaris I	2	1.96
Total	102	100.00

Diarrheal disease is a major cause of illness and death in developing countries. It is a public health problem, because of the high morbidity and mortality rates (Farfa´n-Garcı´a et al., 2020). *Escherichia coli* bacteria also becomes one of the causes of diarrhea in children. Many factors cause diarrhea, such as low environmental conditions, contamination of food and beverages, inadequate clean water supply, poverty and low levels of maternal education (Getaneh, Hordofa, Ayana, Tessema, & Regass, 2021). *Escherichia coli* is an opportunistic germ that is commonly found in the human intestines as normal intestinal flora. *E. coli* is a gram – negative bacteria that are facultatively anaerobic, non-spore-forming, flagellated. *E. coli* becomes pathogenic when the amount in the digestive tract increases or is outside the intestine (Mueller & Tainter, 2021).

E. coli bacteria enter through fecal contamination in food and water. Environmental hygiene and sanitation are very influential in the transmission of *Escherichia coli* to the human body (Getaneh, Hordofa, Ayana, Tessema, & Regass, 2021). Exposure to diarrheal disease can occur by consuming food from food vendors with inadequate hygiene or poor environmental sanitation. Another factor is the consumption of animal products is a source of *E. coli* contamination, such as the use of animal products is not cooked to the proper procedures, which increase the number of germs and lead to increased risk of infection, as well as the virulence of the bacteria (Sarowska et al., 2019).

In developing countries, *E. coli* is the most common cause of all diarrhea incidents (Al-abbas, 2018). Transmission of germs can occur through

waterborne or foodborne. Diseases transmitted through water media are categorized as waterborne and watershed mechanisms. The waterborne mechanism is transmitted through the mouth and digestive system. Contamination in humans can be done through drinking, bathing and washing. The water-washed mechanism is transmission related to water used for personal hygiene and water for cleaning household appliances, especially kitchen utensils and cutlery (Procop & Cockerill, 2003). It is under the sample criteria in this study, children with diarrhea who live in Bima, NTB and use well water for their daily needs.

In this study, 25.50% of *Klebsiella sp.* were found, the most after *E. coli* (29.53%). The research conducted by Afolabi, Saka, Ojuawo, & Biliaminu (2018), 28.00% *Klebsiella sp.* bacteria were found from a total of 100 samples examined. In another study, 10.70% of *Klebsiella* bacteria were found of 244 positive samples examined (Al-abbas, 2018). Bacteria of the genus *Klebsiella* are normal flora found in humans and animals' mouth, nose, and gastrointestinal tracts. Still, these bacteria can also be opportunistic pathogens. Bacteria from the *Klebsiella* genus can cause several diseases such as pneumonia, urinary tract infections, meningitis, diarrhea, peritonitis, and soft tissue infections (Ristuccia & Cunha, 1984). *Shigella sp.* were detected in 28 (18.80%) stool samples. In the research conducted by Mekonnen, Mengistie, Sahilu, Kloos, & Mulat (2019), 10.50% *shigella* bacteria were found from a total of 134 samples examined. In another study, 7% were infected with *Shigella* bacteria (Zhu et al., 2016), and in Ghana, there were 5.60% positive *Shigella* from 347 samples examined (Ashie et al., 2017). *Shigella* is a gram-negative bacteria whose habitat is in the digestive tract with infection through the oral phase (Yang, Chen, Xia, & Zhang, 2020). Shigella can cause infection in all age groups. The high-risk group includes very young, elderly, and immune-compromised people (Khalil et al., 2016). *Shigella* will release toxins that stimulate systematic changes in the intestinal mucosa, causing cells to die in the small intestinal epithelial tissue, resulting in small ulcers in the area of invasion (Sanchez-Garrido, Slater, Clements, Shenoy, & Frankel, 2020).

Salmonella thypii was found in 8.73% of stool samples in this research. This percentage is similar to that obtained in another study in Sudan where the prevalence was around 4% (Saeed, Abd, & Sandstorm, 2015), Ghana about 5.80% (Ashie et al., 2017), Iraq about 12.70% (Al-abbas, 2018) and Ethiopia approximately 12.60% (Ameya, Tsalla, Getu, & Getu, 2018). It can be concluded that the incidence of diarrhea caused by *Salmonella sp.* is in the range of 4-12%.

CONCLUSION

This study reported the initial data on bacterial infection in under-five children with acute diarrhea who visited Bolo and Wawo's public health center. The data obtained shows that *E. Coli* bacteria is one of the causes of diarrhea in patients aged <5 years. Bacterial infection must be considered as one of the causes of diarrhea in children, especially in developing countries.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest in this study.

AUTHOR CONTRIBUTION

All authors participate actively in this article . M was in charge of the content of the writing, covering the introduction, method, analysis, and discussion. DH had the contribution to the introduction, writing concept, discussion, analysis, conclusions, Validation and Supervision. BU contributed and provided inputs regarding the writing concept, methods, design, analysis, Validation and Supervision.

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