Bacterial Contamination Analysis on Children Toys in the Early Childhood Education Program of Makassar

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Abstract. Bacteria can easily move from nature and contaminate children toys. Contaminated toys could infect children and put them in a risk to get a disease. The study aims to determine the presence of bacteria on toys in a childhood education program and to evaluate hygiene knowledge of teachers. This study conducted in Makassar city applying purposive sampling that 40 children toys was taken out to be analysed. Swab method was used to analyse bacterial presence on toys. The analysis showed that 38 of 40 toys samples contained Gram-positive and Gram-negative bacteria. Teacher's knowledge on personal hygiene was quite good but no implementation. Most bacteria grown in children's toys were *Bacillus sp, Klebsiella sp, Enterobacter aglomerans, Enterobacter aurogenes, Enterobacter cloacae, and Enterobacter hafniae*. In conclusion, bacteria were found in almost all types of toys sampled. Teachers work in both early childhood education program is suggested to improve and implement the correct hygiene practices to children, pay attention to the management of toys by closing toys after use, and clean up the toys based on the type.

Keywords: Bacteria, Children Toys, Early Childhood Education Program.

1 Introduction

Early age (pre-school age) is a golden age period for all aspects of human development, whether physical, emotional, and social cognition. Children develop through interaction with their environment. One component that plays a significant role is a school environment that provides education outside the home for early childhood. In Indonesia, it is called Early Childhood Education (PAUD) and Kindergarten (TK). PAUD is vital because it is hoped to builds a strong character at the earliest age [1],[2] Pre-school children are children aged 2-5 years. At this time, the child is prepared for school, senses, and receptor system of stimulation and memory process must be ready so that children will be able to learn well. Learning process of this period is by playing [3]. Pre-school children almost spend most of their time playing activities. Children play with toys specially made for them, such as dolls, balls, cooking toys, crafts toys, doctor toys, toy cars, etc.[4],[5]

Toys are not just tools to play, but they are a part of the children's environment. Children's toys are also closely related to children's health. It could be media transmission of disease agents. According to World Health Organization (WHO), the environment was associated with illness in children aged 0-14 years, such as diarrhoea [6]. It may come from toys that contain pathogenic bacteria. This also may result in itching when the kids were exposed in the short time and illness when they were contaminataed within a long time [7].

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Bacteria is one of the microorganisms found in many places, such as soil, dust, air, water, food, or tissue surfaces. The existence of these microorganisms have benefits for human life, but also have disadvantage such can cause various infectious diseases even can cause damage due to contamination. The effects of multiple bacterial infections can lead to various illnesses to the children [8].

Research conducted in the Philippine highlights the importance of attention to children suffering from whooping cough caused by Bordetella pertussis with severe pneumonia. High mortality rates in pertussis cases are mostly in children younger than three months. B. pertussis is the most common bacterial pathogen among children admitted to hospital. However, the effects of pertussis are less concern in developing countries [9]. Diarrhea is still the main problem in developing countries such as Indonesia, due to their high morbidity and mortality. Diarrhea is the 13th leading cause of death with a proportion of 3.5%. Diarrhea occurs in all age groups, with the highest prevalence of 16.7% found in children aged 1-4 years old [10].

Factors that cause disease transmission includes people who cough or sneeze and not cover their nose and mouth. Small droplets containing bacteria will be spread through the air and can infect people who are close (less than one meter) with the patient. Infected people have bacteria in their hands after wiping their eyes or nose, coughing, or sneezing. If they touch the hands of another person or an object, the bacteria can be abandoned. Bacteria can infect the next person when a person touches his eyes, nose, or mouth. Some bacteria can remain alive and attached to surfaces such as door handles, taps, telephones, and toys for hours. People who work and interact with children (caregivers) who help clean up child's equipment such as toys, food places, duster, and so on. When those things contaminated with nasal and throat secretions from an infected child, bacteria can easily pass into caregiver’s hand as they touch the equipment [8].

This study aimed to describe bacterial contamination, knowledge of individual hygiene of the teachers, and the presence of bacteria on toys in early childhood education (PAUD).

2 Materials and Method

2.1 Sampling

This study was observational with a descriptive approach. The research was conducted in two PAUD in Makassar, consisted of Rama and UNM Teratai Kindergarten. Children's toys in the two PAUD was included as samples as many as 40 samples, represented of toys type (hard and soft toys).

2.2 Bacterial Analysis

Bacteria sample of toys were obtained by rubbing the surface of the toy with swab tool. After wiping, the swab was put in flask then taken immediately to the laboratory for incubation within 1 × 24 hours. The bacteria assessment used Total Plate Count (TPC) method using Plate Count Agar (Oxoid LTD, England) and MacConkey Agar (Oxoid LTD, England). The bacterial development medium used Plate Count Agar (PCA) and MacConkey agar. PCA serves as a solid growth medium (containing agar) for calculating a total number of microorganisms present in toys samples. MacConkey agar was one types of media used for

microorganisms identification. This medium inhibits the growth of Gram-positive bacteria, so the bacteria grow on this agar are only Gram-negative.

The incubated sample was mixed in a tube containing NaCl (Merck KGaA, Darmstadt, Germany) using vortex (Vibrofix VF1 Electronic, IKA-Werk). As much as a 0.5 ml sample was pipetted into dilution reaction tube I (10^{-1}) (Pyrex IWAKI-YENACO) containing NaCl. From the dilution tube I, it was pipetted again as much as 0.5 ml to the dilution tube II (10^{-2}) and again from the dilution tube II (10^{-2}) to the dilution tube III (10^{-3}). From the dilution tube III (10^{-3}) 0.5 ml sample was taken and put into a sterilized petri dish (ANUMBRA). Agar plate count that had been heated in an autoclave (WEBECO GmbH-Germany) for 2 hours was poured into the petri dish until covering the petri dish surface then homogenized by making number '8' movement 12 times. The same procedure was done for MacConkey agar. Each petri dish was allowed for 10 minutes. The Petri dishes were incubated for 24 hours in the temperature of 37^{o} with up-down side position. After that, the bacteria colony was counted.

2.3 Bacteria Identification

Bacteria colony from petri dish was flattened on glass object contained NaCl. This was performed until all types of colonies contained in Petri dishes have been leveled on the glass objects. The glass objects were fixed on bunsen until the bacterial smear dry. The smear surface on the glass object was covered with Crystal violet (Merck KGaA, Darmstadt, Germany) stain for 2-3 minutes, then rinsed with flowing water, lugol for 20 seconds, flowing water, 96% alcohol (OneMed, Indonesia) until the color fades, flowing water, Fuchsin alkali (Merck KGaA, Darmstadt, Germany) for 2 minutes, then finally with clean flowing water, respectively. It was drained on filter paper. After drying, the cell morphology was observed using a microscope.

2.4 Biochemistry Assay

The bacterial biochemical test is a way or treatment performed to identify a pure culture of bacterial isolation through its physiological properties in reagents [11]. In this biochemical assay, nine agar was used as a food source for bacteria: Triple Sugar Iron Agar (TSIA) (Merck KGaA, Darmstadt, Germany), Sulfide Indol Motility Agar (SIM) (Basingstoke, Hampshire, England), Methyl Red-Vogues Proskauer Agar (MRVP) (Merck KGaA, Darmstadt, Germany, citrate agar (Basingstoke, Hampshire, England) and urea agar (Merck KGaA, Darmstadt, Germany), glucose (Merck KGaA, Darmstadt, Germany), lactose (Merck KGaA, Darmstadt, Germany), sucrose (Merck KGaA, Darmstadt, Germany), and mannitol agar (Merck KGaA, Darmstadt, Germany).

3 Results

Gram-positive bacteria and gram-negative bacteria was found in the sample of children toys. Gram-positive bacteria were observed in 18 toys (85%) and gram-negative bacteria were found in 15 toys (75%) of Rama's kindergarten. In UNM Teratai Kindergarten, 12 (60%) gram-positive and 17 (85%) gram-negative bacteria were observed as well on children toys surfaces. Of 40 toys, 75% toys contained gram-positive bacteria, Bacillus sp., that grew on Plate Count Agar medium and 34 toys (85%) contained gram negative bacteria that grew on MacConkey agar medium. Klebsiella was the most common gram-negative bacteria found on toys, on 8 toy samples in Rama and also in UNM Teratai Kindergarten. Enterobacter

aurogenes was observed only on one toy each in Rama and UNM Teratai Kindergarten (Table 1).

Table 1. Bacteria observed on children toys in Rama and Teratai UNM Kindergarten

Bacteria Types	Rama Kindergarten		Teratai Kindergarten		Total	
	n	%	N	%	N	%
Gram Positive Bacteria	18	85	12	60	30	75
Gram Negative Bacteria	15	75	17	85	32	80
Plate Count Agar Medium *Bacillus* sp	18	85	12	60	30	75
MacConkey Agar Medium						
Enterobacter aglomerans	2	10	4	20	6	15
Enterobacter aurogenes	1	5	1	5	2	5
Enterobacter cloacae	2	10	2	10	4	10
Enterobacter hafniae	2	10	2	10	4	10
Klebsiella sp	8	40	8	40	16	40

Table 2. Total colony and bacteria type on children toys in Rama Kindergarten

Type of toys	Total colony*		Bacteria types
	PCA Medium	MacConkey Medium	
Hard Toys Groups			
Popsicle	6	0	*Bacillus* sp
Bowling ball	1	17	*Bacillus* sp and *Klebsiella* sp
Plastic toys	0	0	
Cutlery			
Spoons	1	0	*Baciilus* sp
Inner side of glass	6	10	*Bacillus* sp and *Klebsiella* sp
Outer side of glass	42	110	*Bacillus sp* and *Klebsiella* sp
Cutting boards	86	10	*Bacillus* sp and *Enterobacter cloacae*
Knives	29	78	*Bacillus* sp and *Enterobacter hafniae*
Plates	1	0	*Bacillus* sp
Lego block	21	75	*Bacillus* sp and *Enterobacter cloacae*
Small balls	43	45	*Bacillus* sp and *Enterobacter aglomerans*
Groceries			
Carrots	35	36	*Bacillus* sp and *Klebsiella* sp
Eggs	6	32	*Bacillus* sp and *Klebsiella* sp
Pizza	78	23	*Bacillus* sp and *Klebsiella* sp
Bananas	3	2	*Bacillus* sp and *Enterobacter aglomerans*
Wood materials			
Wood Cubes	13	20	*Bacillus* sp and *Enterobacter hafniae*
Cresent-shaped woods	1	0	*Bacillus* sp

Wood blocks	81	13	*Bacillus* sp and *Enterobacter aurogenes*
Soft Toys Groups			
Paper cubes	0	15	*Klebsiella* sp
Plastic	45	81	*Bacillus* sp and *Klebsiella* sp

*CFU, colony-forming unit.

Table 3. Total colony and bacteria type on children toys in Teratai UNM Kindergarten

Type of toys	Total colony*		Bacteria types
	PCA Medium	MacConkey Medium	
Hard Toys Groups			
Balls			
Small green balls	0	1	*Klebsiella* sp
Small orange balls	0	0	
Bowling balls	0	12	*Enterobacter hafniae*
Lego blocks	0	2	*Enterobacter aglomerans*
Cobek tools			
Cobek	0	1	*Enterobacter aglomerans*
Ulekan	11	8	*Bacillus* sp and *Enterobacter aglomerans*
Cutlery			
The inner side of the glass	1	0	*Bacillus* sp
The outer side of the glass	23	55	*Bacillus* sp and *Enterobacter agglomerates*
Cutting boards	203	51	*Bacillus* sp and *Enterobacter cloacae*
Knives	0	2	*Enterobacter hafniae*
Small plates	156	38	*Bacillus sp* and *Klebsiella* sp
Wood materials			
Triangle-shaped woods	3	0	*Bacillus* sp
Cube-shaped woods	0	2	*Klebsiella* sp
Groceries			
Biscuits	0	12	*Enterobacter aurogenes*
Mango	163	207	*Bacillus* sp and *Klebsiella* sp
Cabbages	58	17	*Bacillus* sp and *Klebsiella* sp
Fishes	24	54	*Bacillus* sp and *Enterobacter cloacae*
Soft Toys Groups			
Plastacin	15	6	*Bacillus* sp and *Klebsiella* sp
Cork materials			
Cork blocks	217	10	*Bacillus* sp and *Klebsiella* sp
Triangle-shaped cork	6	49	*Bacillus* sp and *Klebsiella* sp

*CFU, colony-forming unit.

Tables 2 and 3 shows that hard surface toys resulted in more bacteria colony grew than soft surface toys.

Table 4. Toys management practice of teachers

Toys Management practice	Rama Kindergarten n	%	Teratai Kidergarten n	%	Results N	%
Clean toys						
Yes	15	100	9	100	24	100
Toys cleaning in a month						
Once	0	0.0	1	11.1	1	4.1
Twice	1	6.7	0	0.0	1	4.1
Three times	0	0.0	3	33.3	3	12.5
Four times	6	40	1	11.1	7	29.1
Others (depend on needs, cleaned if necessary)	8	53,3	4	44.4	12	50
Cleaning way						
Wipe cloth (wet)	15	100	9	100	24	100
Cleaning Materials						
Water	9	60	9	100.0	18	75
Special cleaning solution	6	40	0	0.0	6	25

The frequency of toys cleaning in a month depended on the need. Toys were washed when they were dirty. All teachers in the kindergartens stated that they cleaned the toys using cloth. The toys firstly separated based on how they are cleaned, then washed with clean water and wiped with a clean cloth.

Most teachers in Rama kindergarten had good knowledge of proper hand washing way. The teacher's knowledge about the frequency of washing hand in a day at Rama kindergarten was mostly 3 times a day and depends on the children need to wash their hands (more than 5 times) with each question answered by 6 (40%) teachers while in Teratai kindergarten was also depending on need answered by 6 (66.7%) teachers. Hand washing materials most used by children answered by 10 (66,7%) teachers in Rama kindergarten was water and antiseptic soap.

Table 5. Knowledge of Teacher about Hygiene in Rama and UNM Teratai Kindergarten

Teacher Hygiene Knowledge	Rama Kindergarten		Tratai Kindergarten		Total	
	n*		n**	%	n	%
Hand washing knowledge						
Yes	14	93.3	6	66.7	20	83.3
No	1	6.7	3	33.3	4	16.6
Frequency of hand washing in a day						
Once	1	6.7	0	0.0	1	4.1
Twice	6	40.0	2	22.2	8	33.3
Four times	2	13.3	1	11.1	3	12.5
Depending on need, can be many times (> 5 times)	6	40.0	6	66.7	12	50
Hand wash materials						
Water and common hand soap	5	33.3	2	22.2	7	29.1
Water and antiseptic hand soap	10	66.7	7	77.8	17	70.8
Wash hands before eating						
Yes	15	100	9	100	24	100
Wash hands after eating						
Yes	15	100	9	100	24	100
Wash hands after urinating/defecating						
Yes	13	86,6	7	77,7	20	83,3
Wash hands after holding toys						
Yes	9	60	2	22,2	11	45,8

n* : Teachers in Rama Kindergarten
n** : Teachers in Teratai UNM Kindergarten

It is showed that the majority of teachers have adequate knowledge about hygiene (Table 5). What they know also was transferred to the children.

4 Discussion

There are many bacteria in the children toys potentially to give a negative impact on children. Children in PAUD perform many activities such as playing and touching or exchanging goods with friends. Bacteria on the toys or tools they play will easily move from one child to another. Hand washing is one of the most effective ways to clean hands and protect ourselves as well as others from illness [12]. Research on the knowledge, attitude, and practices of hand hygiene among parents of preschool children in Malaysia shows that most parents teach hand washing to the child, but do not know the correct technique. Hand washing with the correct technique has been shown to reduce the spread of infectious diseases, such as

diarrhea and respiratory infections. Clean hands are the most important factor in preventing the spread of pathogens [13].

In this study, water and antiseptic soap are used to wash the hands — hand washing using soap and antimicrobial ingredients significantly reducing a number of germs. The substance is useful for killing or reducing a number of microorganisms so that practice using antiseptic soaps containing anti-germs obtained a lower number of germs [14]. Teacher knowledge of hand washing habits is very important to maintain children's health. Although the majority of teachers had good knowledge about personal hygiene, some of them did not practice hand washing after handling toys. Not wash hands after playing will result in various colonies of bacteria grew on the surface of the hand. A bacterial colony consists of trillions of bacteria or fungi plus immature immune defenses system. This is why children are especially vulnerable to illness. Diseases such as tuberculosis, eye infections, skin infections, and nail infections are some of the health problems that can affect children and can be avoided just by simple means such as hand washing. Hand washing preferably before and after meals, after holding the mouth / cleaning the nose/sneezing/ coughing, after holding the animal, after playing inside or outside the room, and from the bathroom.

It was found as well in this study that from 40 samples of toy samples, there are only 2 (5%) of children's toys from both of Kindergarten with unidentified bacteria. Bacteria grow on toys are gram-positive and gram-negative bacteria. Gram-negative bacteria are bacteria that do not retain the violet crystal dye during the Gram staining process the color is red when observed with a microscope while Gram-positive bacteria will be purple. Gram-positive bacteria have only a single plasma membrane surrounded by a thick cell wall of peptidoglycan. On the other hand, gram-negative bacteria have multiple membrane systems. A permeable outer membrane covers the plasma membrane. This bacteria has a thick peptidoglycan cell wall, located between the inner membrane and the outer membrane [15].

Gram-negative bacteria are a type of pathogenic bacteria because it has a double membrane on the cell wall, endotoxin, and has resistance to the drug. Gram-negative bacteria are the causative agent of respiratory tract infections, sexually transmitted diseases, and gastrointestinal diseases [16] Based on the results of this study, almost all samples of toys grown by 80%. Gram-negative bacteria that grow on a child's toy sample should be the school's attention. These bacteria can cause disease in children with a weak immune system.

There were six types of bacteria in 40 samples of children's toys from the two institutions. Gram-positive bacteria Bacillus sp. is a class of bacteria found in almost all types of toys. Children get microbial contamination while playing in public places through contaminated soil and toys. Children are one of the most vulnerable subpopulations that put them at risk for severe health effects when exposed to pathogenic bacteria. In addition, the faecal-oral transmission pathway possibly exposed children to these pathogens is increased due to potentially microbial handling of toys in contaminated play areas and possible handling of inadequate hand hygiene.6 Similar studies of the presence of E. coli or total coliform found in toys in Honduras (North America) is caused by unwashed child's hands then touching their mouth, and direct contact with caregiver's hand can also affect exposure to these microorganisms [17].

Bacillus sp. are commonly found in soil. Most members of the Bacillus genus are saprophytic organisms common in soil, water, air, and plants. These organisms can sometimes cause illness in children who have a low immune function. Commonly infecting diseases are meningitis, endocarditis, endophthalmitis, conjunctivitis, or gastroenteritis. Enterococcus sp. are also found in a study of dolls in hospital in the UK. Washing of soft-tooted toy proved not

to remove pathogenic bacteria in one of its parts. Bacteria are still present in the legs as many as 72 colonies and pieces of puppet sleeve as many as 90 colonies [18].

The most common Gram-negative bacteria found in children's toys samples in these two PAUDs was Klebsiella sp. as many as 8 colonies (40%). Klebsiella sp. can be found everywhere. It can be found in the skin, esophagus, or digestive tract, even in sterile wounds. These bacteria include the type of bacteria that infect humans. Klebsiella sp. is easily found in mucosal lining, especially in the lungs. This bacterium is also one of the causes of bronchitis or respiratory tract infections originally originating from the lungs. Acquired nosocomial infection during hospitalization in a hospital in Mexico was caused by contamination between the hands of health workers and their patients. The spread of this nosocomial infection 50.45% caused by Klebsiella sp. and 8.25% Enterobacter sp [19].

Four species of Enterobacter bacteria were also found in the samples of toys. One of the species was Enterobacter aerogenes. Enterobacter aerogenes is a bacterium found everywhere, naturally in soil, fresh water, vegetables as well as human and animal waste. Enterobacter aerogenes can cause infection in many parts of the human body. These bacteria are the cause of lower respiratory infections, including pneumonia, urinary tract infections, and skin infections. If the bacteria reach the blood (bacteremia), it can cause sepsis. Bacteria Enterobacter genus is also found in soap bubble toys in Italy. The soap solution present in the toy is good for survival and possibly the growth of microorganisms [20].

The number of bacteria found in children's toys in two early childhood education caused by the lack of awareness to clean the toys and supported by unaccustomed children to wash their hands after holding the toy. The bacteria found in toy samples are also caused lid indirectly or kept and cleaned after every time children play so that many bacteria in the air or from children and teachers suffered from infectious diseases can be directly contaminated on children toys. The identified bacteria from 40 child toy samples were not continued on examination whether they are pathogenic or not, but the presence of bacteria from each of these toy samples should be a concern for increasing attention of toys cleanliness. Pre-school age children are period for children susceptible to infectious diseases

5 Conclusion

The presence of bacteria in 38 of 40 samples of toys in PAUD is Gram-positive and Gram-negative bacteria. The bacteria types of toys in PAUD were Bacillus sp. Enterobacter agglomerates, Enterobacter aurogenes, Enterobacter cloacae, Enterobacter hafniae, and Klebsiella sp. Hand washing habit taught by the teacher had been done by all children before and after eating, after defecation and urinating, but not after holding the toy. Suggestions from this research are to socialize the cleaning of toys routinely in Early Childhood Education in Makassar. For Rama and Teratai kindergarten, it was expected to improve and apply hygiene practices to children, pay attention to toys management by closing lid after use and routinely cleaning toys according to its type.

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