Validity of a screening instrument for the detection of microbes (diarrheagenic *Escherichia* Coli) in elementary school children

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Abstract

Background. The study of school children in Surabaya, in 2018, has produced a prediction model in the form of a mathematical formula set forth in the screening instrument for Diarrheagenic Escherichia coli (DEC) within feces via DEC transmission media. This model has not been validly tested. Therefore, a validity test must be carried out before applying this screening instrument.

Objective. The study aims to carry out a validity test on the screening instrument for the detection of microbe DEC in elementary school children.

Materials and Methods. This is an observational study with a cross-sectional design. In this study, the sample taken was 109 children. The sample collection method used was simple random sampling. The results of the laboratory test were used as the gold standard for the validity test of IS-DEC.

Results. The majority of the children (94.5%) stated that they tended to buy snacks. 56% of the children bought food and/or drinks that were highly at risk of getting contaminated by bacteria. The laboratory test of the feces samples shows that 13.8% of children were confirmed to have DEC in their feces (DEC-positive). A sensitivity value of 93.3% was obtained from the calculation, meaning that the capability of IS-DEC to predict the presence of DEC-positive within children's feces is at 93.3%. It shows that IS-DEC has a good criterion validity. The specificity of IS-DEC is only 8.5%.

Conclusions. The result indicates that the sensitivity of the Screening Instrument (IS-DEC) to predict the presence of DEC within children's feces is already good.

Introduction

Foodborne Diseases (FBDs) are caused by the consumption of food and/or drinks contaminated by pathogenic microbes. Among others, the types of FBDs are diarrhea, food poisoning, dysentery, typhoid fever, and cholera.[1] FBD still causes a substantial public health, economic and social burden worldwide.[2] FBDs are common in regions where the local residents disregard cleanliness and sanitation in preparing food. More cases of FBDs are found in developing countries, including Indonesia, where the practice of food preparation is not carried out adequately.[3] FBDs are one of the causes of illness and death in the community.[1,4] Various attempts have been made to combat foodborne bacterial pathogens, but the number of foodborne infections remains high worldwide.[5] FBDs affect the health and vitality of students as 36% and 29% of the daily energy and protein of the students, respectively, are derived from school snacks.[6] The type of FBDs contracted the

most by children is diarrhea.[7]

Diarrheagenic Escherichia coli (DEC) is the main cause of diarrhea, one of the most common causes of morbidity and mortality in infants and young children in developing countries.[8–11] Diarrhea is a public health problem and the main cause of morbidity and mortality in infants and children. Low and middle-income countries in Africa, Asia, and Latin America are affected the most by diarrhea. Cases of diarrhea in such countries are often fatal, especially as the living conditions there are poor (inadequate water supply, poor environmental cleanliness and sanitation, insufficient education).[12–15]

Several research results signify that the annual episodes of diarrhea in children are quite high, namely 3.2 – 4.6 episodes, while 70% of the cause is contaminated food.[7] Risky behavior in elementary school children is generally related to personal hygiene and snacking habit at school. Based on the data from the Health Office of the East Java province in 2012, the predicted number of diarrhea cases handled was 72.43%. In Surabaya, such a number was 79.97%. In 2015, a study conducted in Sidoarjo, East Java, found that sausages and rolled noodles sold at schools contained E. coli and Salmonella, rendering them unsafe.[16] The results of a 2011 study conducted in five state elementary schools indicate that 59.7% of students always bought snacks at school, while 36.1% of students sometimes bought snacks at school.[17] Based on the results of a 2013 survey on consumer awareness of food safety conducted by BPOM (National Agency of Drug and Food Control), it is apparent that: i) 83% of respondents stated that unsafe food is caused by microbial poisoning; ii) 36% of extraordinary occurrences happened in hajatan (special celebration) areas, 35% happened at school, and only 4% happened at home.[18]

Food microbiology is a field of study observing microorganisms that contaminate food and are related to foodborne diseases. The food we consume contains microbes and is rarely sterile. Food can contain microbes with various compositions. Microorganisms come from the commensal microflora of raw material and can also get contaminated during the production, storage, and distribution processes.[19]

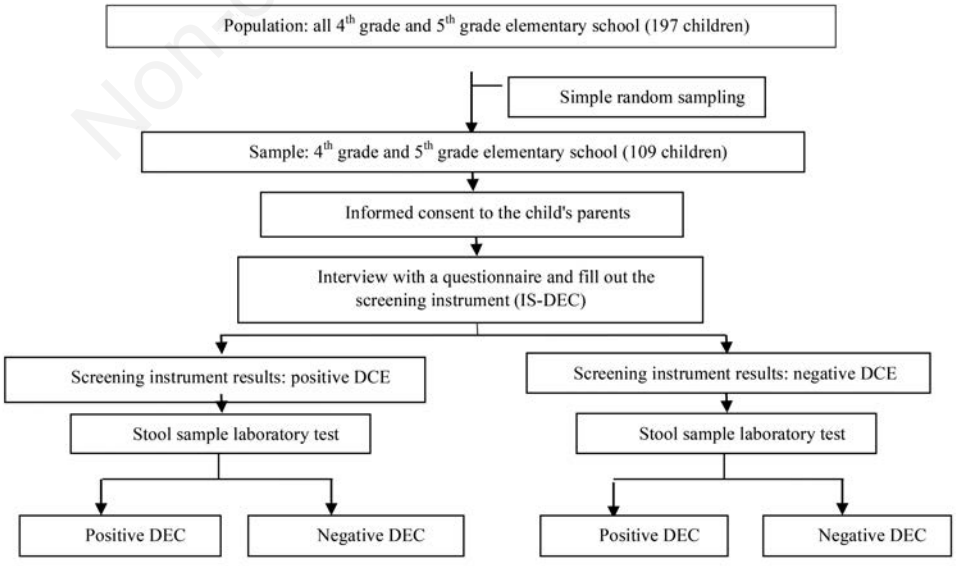
Based on the magnitude of the problem and findings of previous studies, school children are at risk of contracting FBDs, particularly diarrhea. The findings of a 2018 study on school children in Surabaya have produced a prediction model in the form of a mathematical formula set forth in the screening instrument for DEC within feces via DEC transmission media in elementary school children. DEC infection in a child can be predicted by paying attention to their snacking behavior, *i.e.*, snacking frequency and condition of the snacks they often buy at school. This model has not been validity tested. Therefore, a validity test must be carried out before this screening instrument is applied.[17] The general purpose of this study is to carry out a validity test on the screening instrument for the detection of microbe DEC as part of the early prevention effort of FBDs in elementary school children.

Materials and Methods

This is an observational study with a cross-sectional design. The population of the study is every 4th and 5th graders of an elementary school in the Bulak sub-district, Surabaya city. The minimum size of the sample for a cross-sectional study is 96.7 children.[20,21] In this study, the sample taken was 109 children. The sample collection method used was simple random sampling, while the sampling frame is the list of 4th and 5th graders. The size of the sample in this study was calculated using the large formula of the sample that is suitable for case-control research.

$$n = \frac{Z^{2}_{a/2} \cdot p \cdot (1-p)}{d^{2}} \quad \text{with } \alpha = 0.05 \text{ and } d = 0.1$$

A minimum of 96.04 samples were obtained. In this study, the large sample was 109, already meeting a minimum of large samples. Data were obtained through interviews with the children and the collection of feces samples (Figure 1). The research instrument



Note: DEC, Diarrheagenic Escherichia coli; IS-DEC, screening instrument.

Figure 1. Flow chart of laboratory test.

used in this study were questionnaires and screening instruments/IS-DEC (the instrument whose validity would be tested). The contents of the questionnaire are the respondent's characteristics, eating and snacking habits, hand washing habits, bowel habits, history of illness and types of snacks for three days before stool sampling.

The development of IS-DEC based on the prediction model of DEC obtained from the transmission of Foodborne Diseases in feces, which is simple and applicable, an index formula (Z), namely: Presence of DEC = -1.172+1.502 snacking frequency often ≥4times/week+1.307 the high-risk snack. This formula is the result of dissertation research with a nested case-control design. The research population is elementary school children in public elementary schools in Surabaya. The research variables were the habit of washing hands, the habit of using an antiseptic liquid, the condition of the fingernails, the frequency of snacks, the habit of bringing lunch, and the condition of the snacks. Formulation of a predictive model for the presence of DEC in feces through the transmission of FBDs using logistic regression analysis. After the six independent variables were included in the logistic regression analysis, there were only two variables that could be included in the formula, namely the frequency of snacks and the condition of snacks.

In addition, a laboratory test on the feces samples was conducted. To collect the feces samples, the children were given a plastic pot and other supplementary items (an instruction to collect feces, plastic glove, spoon to collect feces, mask, and name label) to contain the feces when they defecated. The pots containing the feces were then taken by the enumerator and put into a styrofoam box that had been filled with ice gel to prevent damage to the feces samples. After that, the enumerator delivered the box to the laboratory. The length of the stored stool sample is an average of not half a day, for example, if the enumerator takes a stool sample in the morning, the enumerator will deliver it to the laboratory officer in the afternoon.

The screening instrument (IS-DEC) includes questions concerning the snacking frequency and types of snacks (snacks and beverages) that are often bought by the children. Snacking frequency is divided into the categories of often (≥4 times/week), sometimes (1-3 times/week), and never. Food or drinks whose condition was assessed were food or drinks bought the most by the children at school and food/drinks bought during the filling of the questionnaire. Food and drinks categorized as high-risk are food and drinks that are highly likely of getting contaminated by E. coli. In Table 1, it can be seen how snacks are categorized into high-risk and low-risk based on their risk of getting contaminated by E. coli.

This research has received ethical approval from Health Research Ethics Committee, Faculty of Public Health Universitas Airlangga with no: 24/EA/KEPK/2021. The results of the laboratory test were used as the gold standard for the validity test of IS-DEC in predicting microbe DEC within the feces.

Data analysis was carried using the calculation of prevalence (%), sensitivity (%), and specificity (%) for the validity test of the IS-DEC in predicting microbe DEC within the feces of school children. Furthermore, the calculation of the positive predictive value (PPV) and Negative Predictive Value (NPV) of IS-DEC was also conducted. The MacConkey-Sorbitol agar media was used in the laboratory test.[22] This media was used in the screening of E. coli as a gold standard.[20] DEC and E. coli pathogens will be distinguished by using antisera tests. Antisera tests can specifically identify E. coli types EPEC, ETEC, Enterohemorrhagic E. coli (EHEC), including type 0157 H7.

Result

Characteristics of the children

This study was carried out in an elementary school located in the Kenjeran urban village, Bulak sub-district, Surabaya. The respondents were the 4th (fourth) and 5th (fifth) graders. The size of the sample was in accordance with the calculation of the minimum sample, namely 109 children. The average age of the children was 10.35 years with a range of 8.5 – 12 years. The majority (80.7%) of the children were aged 10-11 years, and the proportion of sex was almost equal between boys and girls. The details can be seen in Table 2.

Description of snacking habit

The children bought snacks at school before the lessons started, during the break, and after school. Table 2 presents the distribution of snacking frequency at school. The results of filling out the IS-DEC show that all children bought snacks at school but at different frequencies. Most of the children bought snacks at school ≥4 times/week, which is 86.2%. The detailed results of IS-DEC are presented in Table 2. Moreover, the majority of the children (70.6%) stated that they often bought snacks while at home. Only six children never bought snacks while at home.

The school children's snacks

The condition of food and/or drinks refers to the condition of the food and/or drinks bought at school that is at risk of getting contaminated by E. coli. From the results of filling out the IS-DEC, it is apparent that a number of children bought food and/or drinks that were highly at risk of getting contaminated by bacteria. Most of the children (56.0%) buy high-risk snacks for E. coli (Table 2).

The prevalence of the presence of diarrheagenic escherichia coli within feces

In this study, DEC within feces was identified using the

Table 1. Snacks categories based on their risk of getting contaminated by escherichia coli.

Risk category	Snack food	Snack drinks
Low-risk	Packaged snacks Snacks wrapped by producers (*e.g.*, bread, donuts, chips) Main dish (*e.g.*, fried rice, *nasi kuning* (turmeric rice), fried chicken with rice, etc.)	Packaged drinks Warm drinks
High-risk	Snacks eaten with peanut sauce, shrimp paste, and tomato sauce (*e.g.*, *cilok* (meatball with sauce), *cireng* (fried tapioca flour), meatball, *batagor* (fried fish dumpling) sauce, shrimp paste for fritters, meatball soup sauce, *etc.*)	Iced drinks (For example ice tea, *es degan* (young coconut ice), iced syrup, *es kacang ijo* (iced mung bean), etc.)

Source: Results of the laboratory test of primary data and the Ministry of Health, the Republic of Indonesia, 2015.

MacConkey-Sorbitol media. The results of the laboratory test on the feces samples show that out of 109 children, 15 (13.8%) children had DEC within their feces (DEC-positive). Therefore, the prevalence of children who were infected with DEC was 13.8%. The results of the study show that the majority (66.7%) of DEC-positive results were found in boys, while the majority (52.1%) of negative results were found in girls (Table 3).

The sensitivity and specificity of the screening instrument

The sensitivity of IS-DEC to predict microbe DEC. The sensitivity value was calculated using the following formula:

Sensitivity value = (14 / (14+1)) x 100 = 93.3 %

A sensitivity value of 93.3% was obtained from the calculation, meaning that the capability of IS-DEC to predict the presence of DEC-positive within children's feces is at 93.3% (Table 4). It shows that IS-DEC has a good criterion validity.

The specificity of IS-DEC to predict microbe DEC. The specificity value was calculated using the following formula:

Specificity value = (8 / (8+86)) x 100 = 8.5%

A specificity value of 8.5% was obtained from the calculation, meaning that the capability of IS-DEC to predict the absence of DEC-negative within children's feces is at 8.5%. The calculation results showed that the PPV = (14/100) x 100 = 14% and the Negative Predictive Value (NPV) = (8/9) x 100 = 88.9%. The sensitivity of instrument screening is very high (93.3%). Factors (snacking frequency and condition of the snacks) that contribute to high sensitivity are allegedly due to assessment aspects in instrument screening is a variable that has been statistically proven to have a significant relationship with the presence of DEC in child feces, while the specificity is low (8.5%), likely because most elementary school children have snack habits.

Discussion

The results of the study indicate that all children bought snacks at school but at different frequencies. 86.2% of the children bought snacks at school ≥4 times/week. The school children's habit of consuming snacks at school, whose nutritional values and cleanliness are unknown, will affect their health and can result in health problems.[23]

Based on the results of the study, it was found that 56% of children bought food and/or drinks that were at high risk of being contaminated with bacteria. Food can get contaminated by pathogens in various ways. Contamination can occur during the production, packaging, and serving processes. Food can get contaminated through the unclean hands of the sellers. There are also foodborne diseases caused by food exposed to pathogen-contaminated water used during the production, processing, and preparation of food.[24] Foodborne diseases frequently occur in regions where the local residents disregard cleanliness and sanitation in preparing food.[3] In developing countries, street food is currently fulfilling the snacking needs of people, especially children, due to affordable prices, accessibility of various food options that suit different communities and social classes.[25] However, hygiene practices among food handlers, most food vendors and catering services have been reported to be substandard.[26]

Escherichia coli is an opportunistic pathogen. If it is situated in the right location within the human body, it can cause health problems such as minor abdominal pain, diarrhea, urinary tract infection, sepsis, and meningitis.[27] The proportion of children whose feces contained DEC-positive was 13.8%. Diarrheagenic E. coli is one of the causes of Foodborne Diseases such as diarrhea. While its proportion is low, the virulence of DEC is quite high. Certain strains like EHEC and Enteroaggregative E. coli can cause hemolytic-uremic syndrome, kidney failure, meningitis, and bacteremia.[28–30] The proportion of 13.8 % found in this study is higher than the result of the 2017 study conducted by Syahrul, F in Surabaya of 6.88%.[28]

The following is an example of one of the results of the laboratory test on the stool samples: colorless colonies on the surface of Mac Conkey Sorbitol agar after incubation at 37□C for 24 hours. Children's stool samples that are positive for DEC contain 3-5 bacterial colonies. The infective dose of DEC is very low, *i.e.*, 1-10 bacteria, so that the results of this study are in accordance with the infective dose range. The infective dose of DEC is very

Table 2. Characteristic of the children.

Snacking frequency	n	%
Age (years)		
8-9	14	12.8
10-11	88	80.7
12-13	7	6.4
Sex		
Male	55	50.5
Female	54	49.5
Frequency snacking at school		
Often (≥4 times/week)	94	86.2
Sometimes (1-3 times/week)	15	13.8
Never	0	0
Frequency snacking at home		
Often (≥4 times/week)	77	70.6
Sometimes (1-3 times/week)	26	23.9
Never	6	5.5
Condition of the snacks in the school		
High-risk	61	56.0
Low-risk	48	44.0

Note: If the child buys low-risk and high-risk snacks, it is included in the high-risk category.

Table 3. Distribution of the school children's sex based on the presence of diarrheagenic e. coli within the feces.

Sex	DEC-Positive		DEC-Negative	
	n	%	N	%
Boy	10	66.7	45	47.9
Girl	5	33.3	49	52.1

DEC, Diarrheagenic Escherichia.

Table 4. The calculation of the sensitivity and specificity values of the screening instrument.

Screening instrument	Laboratory test (gold standard)				Total
	Positive		Negative		
	n	%	n	%	
Positive	14	93.3	86	91.5	100
Negative	1	6.7	8	8.5	9

low, namely 1-10 bacteria.[31,32]

The transmission media of the FBDs used to predict the presence of DEC within children's feces were snacking frequency at school and the condition of the snacks they often bought at school. According to a study from Syahrul F, in Surabaya, the more often a student snacks at school, the more likely he or she would have DEC in their feces. A student who snacks four times a week is more likely to consume food and/or beverages that are contaminated with microbes, particularly E. coli. Based on this study too, the risk of feces being detected DEC-positive is 3.696 times greater for children who frequently buy snacks with a higher risk than children who buy low-risk snacks.[33–35] The index that can be used to predict the presence of DEC within the feces of a child can be calculated based on the 2 (two) variables. In the end, the index serves as an early warning for children who are at risk of contracting FBDs, particularly diarrhea. Validity mainly refers to the accuracy of the measuring instrument toward the questions that will be assessed. The types of validity are content validity, construct validity, and criterion validity.[36] In this study, the validity of IS-DEC for the detection of the presence of microbe, particularly DEC, within the feces of school children, can be seen from its sensitivity and specificity values (Table 4). A sensitivity value of 93.3% was obtained from the calculation of validity, meaning that the capability of IS-DEC to predict the presence of DEC-positive within children's feces is at 93.3%. Such a result indicates that the sensitivity of IS-DEC to predict the presence of DEC within children's feces is already good. A specificity value of 8.5% was obtained from the calculation, meaning that the capability of IS-DEC to predict the absence of DEC-positive within children's feces is at 8.5%.

This form is used for elementary school children as a tool for early alertness against infection due to DEC. In a sense, if the child has a frequency of snacks ≥4 times / week with high-risk hawking conditions, then the mother should be vigilant if there is likely to be DEC bacteria in her child's stool. The expected response of the mother is to increase the child's immunity or bring enough food so that the child does not need snacks at school.[37,38]

Conclusions

This study showed that the prevalence of the presence of DEC within feces is 13.8 % and the IS-DEC to predict microbe DEC in school children has a sensitivity value of 93.3% and a specificity value of 8.5%. The author suggests that systematic and disciplined monitoring of the condition of the school children's snacks is required. This can be realized by reactivating the existing School Food Safety Team. In addition, the follow-up study to test the implementation of the IS-DEC on a larger scale to predict the presence of DEC within the feces of children is needed as one of the measures to prevent FBDs.

The limitation of this study is the screening instrument is used to estimate the presence of DEC bacteria in the feces of school children based on the snacking frequency and condition of the snacks; if in the child's stool there is DEC, then the child is more at risk for infection with DEC (where one of the symptoms is diarrhea). So, this IS-DEC is not intended to predict DEC infection.

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