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**CALCULATING COLONY OF *Candida spp.* AMONG CHILDREN WITH INTELLECTUAL DISABILITY IN CIREBON****Dhini Oktaviani\*, Amanah Amanah , Eni Suhaeni, Risnandya Primanagara***Faculty of Medicine, Universitas Swadaya Gunung Jati, Cirebon, Indonesia*

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**ABSTRACT**

**Background:** Children and adults with Intellectual Disability and Autism Spectrum Disorders (ASD) have decreased intestinal permeability and enzyme activity, causing gastrointestinal (GI) problems that are more frequent and more severe than in children from the general population. The immune system which is part of the intestinal barrier in children with Intellectual Disability (ID) experiences various disorders and becomes more susceptible to fungal invasion, such as *Candida spp.* This present study aims to calculate the colony of *Candida spp.* in the digestive tract of Children with Intellectual Disability (ID) in the city of Cirebon.

**Method:** This is an observational study with cross-sectional design using purposive sampling. The study was conducted in November 2018-February 2019. The sample (ID) group consists of 31 individuals from Special Needs School and 60 individuals from National elementary school (non-ID) in the city of Cirebon. The inclusion criteria were children ages 6-12 years and the exclusion criteria were children under antifungal treatment and children under cytotoxic or immunosuppressive drugs. The fecal samples from the ID group and non-ID were examined at the Laboratory of the Faculty of Medicine, Universitas Swadaya Gunung Jati Cirebon, Indonesia using Digital Colony counter. Collected data were stored and analyzed using computer aided Statistical program. Mann-Whitney analysis of variance techniques were used to test the hypotheses.

**Results:** The results showed that there are significant differences in the colonies of *Candida spp.* between the ID group and non-ID group. Median colony of *Candida spp.* in the ID group is 39.00 with mean colony of 126.90 while the median colony of non-ID group is 3.00 with mean colony of 40.93 ( $p < 0.001$ ).

**Conclusion:** There are significant differences between the *Candida spp.* colonies in the ID group with the non-ID.

**Keywords:** Intellectual Disability (ID), *Candida spp.*, Gastrointestinal

**INTRODUCTION**

Children and adults with Intellectual Disability and Autism Spectrum Disorders (ASD) they are extremely likely to be related biochemically and molecularly [1]. They were reported have decreased intestine permeability and enzyme activity, including gastrointestinal (GI) problems that are more frequent and more severe than in children from the general population. Prospective reports from pediatric gastroenterology have described GI symptoms in 46–84% of patients with ID and autism [2]. Intellectual Disability (ID) is a developmental disorder that includes intellectual function deficits which are below the average (IQ <70) and adaptive functions in aspects of academic, social, and independence. These limitations occur before the age of 18 years old [3].



WHO estimates the number of children with special needs in Indonesia 7-10% of the total number of children. In 2016/2017 the number of Special Need School in Indonesia was 1.525 schools, in West Java province there were 329 schools, in Cirebon city there were 5 schools Special Need School and in Cirebon there were elementary school inclusion to ID the government's efforts to assist the children with ID in Cirebon where students with special needs receive education in accordance with their potential so that both students with special needs or students regularly to jointly develop the potential of each [4]. Intellectual disability (ID) caused by many genetic disorders, It can be associated with a genetic syndrome, such as Down syndrome and Fragile X syndrome include autism. The immune system which is part of the intestinal barrier in ID and ASD children experiences various disorders, thus the individual becomes vulnerable to the invasion of bacteria, viruses, and fungi, such as *Candida spp* [5,6,7].

The colony of *Candida spp.* can be found as a normal flora, but they are grouped into opportunistic fungi when there is an imbalance of the normal flora of the intestine, this condition is also called dysbiosis. When things happen to upset this delicate natural balance, candida can grow rapidly and aggressively, causing many unpleasant symptoms to the host [8]. the population of *Candida spp.* themselves which will worsen the behavior of Intellectual Disability (ID) children and their quality of life. [9]. *Candida albicans*, when present in excess has been hypothesized to be correlated with ID and autism, it produces ammonia (NH<sub>3</sub>) as a metabolite. Propionic acid is in the presence of ammonia metabolites in the GIT, could be converted to beta-alanine, which is structurally comparable to the inhibitory neurotransmitter GABA. In recent years a minority of physicians have begun to try to persuade their colleagues, and the public, that *Candida* may present consequences far more devastating to human well-being than vaginitis and thrush [10]. They cite Japanese studies showing that *Candida* is able to produce toxins which cause severe long-term disruption of the immune system and may also attack the brain. In extreme cases, they claim, severe disorders, totally resistant to conventional treatment, can occur as a result of candidiasis. These include depression, schizophrenia and in some cases ID and autism [11]. Excessive growth of *Candida spp.* shows increased irritability, aggressive behavior, and sleep disorders. In addition to other gastrointestinal manifestations, there are diarrhea, constipation, vomiting, abdominal pain, and foul-smelling faeces It can be associated with unusual eating patterns and the selection of the preferred dishes. Which is the cause of major morbidity in the population Intellectual Disability (ID) [12].

This study concerns on calculating the number of colonies of *Candida spp.* on the digestive system of Children with Intellectual Disability (ID) and non ID of children as controls through fecal examination.

## METHODS

This study is an observational study with a cross sectional design using purposive sampling. The study was conducted in November 2018-February 2019. The parents of students who were invited 65 were totally and agreed to informed consent as 31 parents from Special Need School and National elementary school students as the socialization non ID to 70 parents and 60 parents who participated. The inclusion criteria were children ages 6-12 years and the exclusion criteria were children under antifungal treatment, children under cytotoxic or immunosuppressive drugs. Variable dependent is *Candida spp.* and variable independent is Intellectual Disability (ID). This research was conducted in several stages, first stage is introduction stage with the teacher and the environment in the school chosen as the subject of the study. The second stage is to socialize how important this research is to parents and education of stool collection procedures after agreeing to informed consent. Then socialization was carried out by explaining the sampling procedure and giving the kit to the parents of the subject. The faecal sample is examined at the Laboratory of the Faculty of Medicine, University Swadaya Gunung Jati, Cirebon, Indonesia.

Data collection procedure all tools that will be used in this research must be sterilized. Tools made of glass such as petri dishes, Eelenmeyer flasks, measuring cups, and test tubes must be washed and dried before they are used. Sterilization of the equipment is conducted using a sterilizer at 120 ° C for 1-2 hours and making SDA media. The methods of collecting the faecal samples are as follows: faecal collection and its analysis instructions are given to parents / family in charge. Then, they collect fresh faecal samples in the morning into sterile specimen containers and labeled them and use applicator sticks provided for each subject. Samples are taken to the laboratory and examined at the Microbiology Laboratory of the Swadaya Gunung Jati Medical Faculty. *Candida spp.* obtained from Intellectual Disability (ID) children are bred in SDA media. Single colony on SDA plate media is taken aseptically using sterile cotton swabs and streaked on SDA media. Then, breed it in incubated at room temperature 37° C. Re-identification of *Candida spp.* can be conducted macroscopically and microscopically and colonies are calculated using Digital *colony count*.

Collected data stored and analyzed using computer aided Statistical program was used to analyse the data. Analysis of variance techniques were used to test the hypotheses use Mann-Whitney test because the data distribution is not normal. Ethical Clearance Agreement No. 57/EC/FK/XI/2018 on 21 November 2018 from the Ethics Committee of the Faculty of Medicine, Swadaya Gunung Jati University, Cirebon, Indonesia.

## RESULT

The results show that there is significant difference  $P = 0.001$  ( $P < 0.05$ ) in cases of heavy growth of *Candida spp.* in ID group and non ID. from the 31 ID respondents group colonies of *Candida spp.* the highest number is 679 colonies with code sample c23, the average number is 411 colonies with code sample c1 and the smallest number of calculations is 2 colonies with code sample c30 mean value is 126.90. While non ID shows that, from the 60 respondents, the highest number of non ID is 541 colonies with sample code k3, the average number of calculations is 221 colonies with sample code k14 and the smallest number of calculations for 1 colonies is in the sample code k52 mean value is 40.93. Furthermore, based on sex of Intellectual Disability children, the highest number of *Candida spp.* is in male Intellectual Disability, namely 8 or 78%. It is more than female Intellectual Disability children, who are only 5 or 22%.

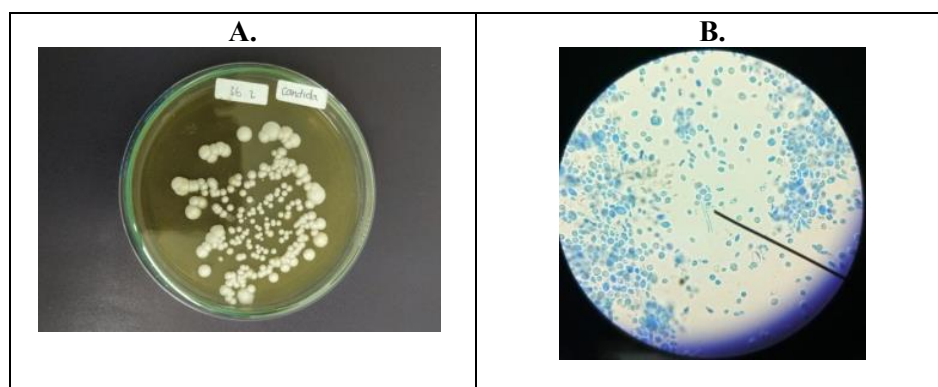


Figure 1. Identification of *Candida spp.*

Note : A = Macroscopic identification  
B = Microscopic Identification

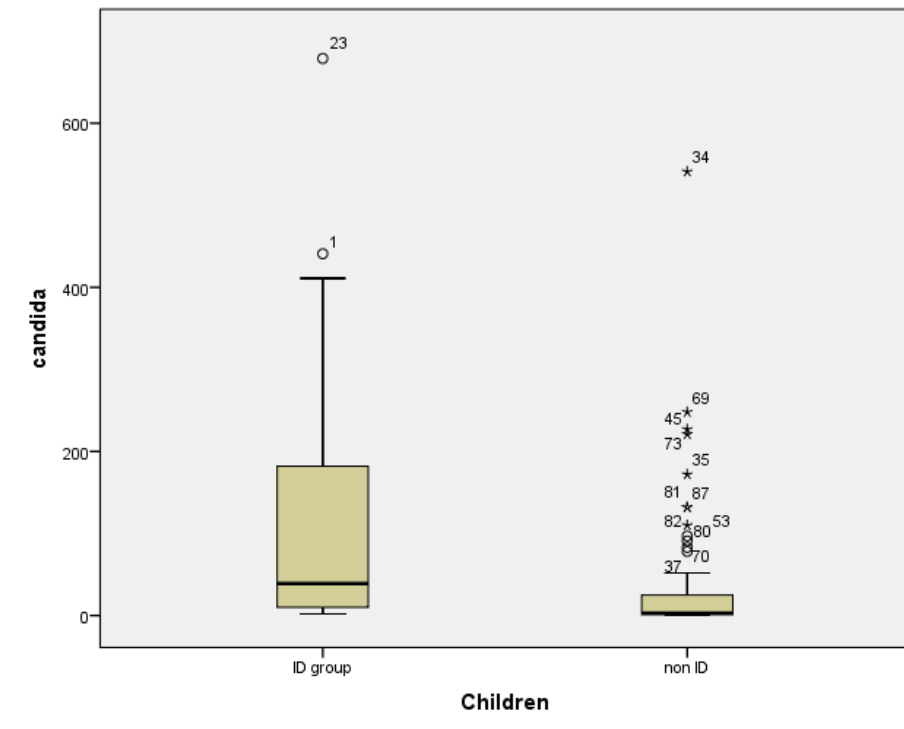


Figure 2. Presentation Graph of the Number of *Candida spp.* in the ID and Non ID.

The graph shows there are difference *Candida spp.* colonies in ID group and non ID. The results colonies of *Candida spp.* in the ID group with 679 colonies while in non ID the number of *Candida spp.* is only 541 colonies.

Table 1. The results of the Mann-Whitney test analysis

Groups	Median (Minimum- Maximum)	Mean	Value p
ID Groups	39.00 (2-679)	126.90	0.001
Non ID	3.00 (1-540)	40.93	

Based on table 1 the results show that the median value of ID group is 39.00, mean value is 126.90 and the median value in the non ID is 3.00, mean value is 40.93 with the value of  $p = 0.001$ . It means, there are significant differences in the ID group and non ID.

## DISCUSSION

Based on the findings of the study, it is showed that there are difference *Candida spp.* colonies in ID group and non ID. In this study, it could occur because the ability of *Candida* to invade into tissues is strongly influenced by predisposing factors. One of the predisposing factors is a decrease in the body's immune system (Immunocompromised host). Gupta research shows that children with ID and ASD experience immune disorders. The immune system which is an intestinal barrier in autistic children experiences various disorders [13].

Defects in the immune system can lead to increased growth of microorganisms such as fungi in the digestive tract so that there can be an imbalance of normal flora found in the digestive tract which will cause inflammatory reactions in the digestive tract and the entry of foreign substances including food



allergens into various other body parts that cause allergic reactions with manifestations including behavioral disorders [14].

Also, in this study there was statistically significant difference  $P = 0.001$  ( $P < 0.05$ ) in cases of heavy growth of *Candida spp.* in ID group compared with the non ID group and this is similar to the study of Herawati's research which has a result that there significant correlation between the increase in *Candida albicans* colonies count and the incidence of ASD. *Candida albicans* can produce the enzymes aspartyl proteinase, phospholipase, and lipophospholipase which play a role in the process of adhesion to the mucosal epithelium, can increasing the permeability of the intestinal mucosa. In this situation abnormal peptides (caseomorphin and gluteomorphin) will enter the intestinal mucosal epithelial cells through (tight junctions), which are then absorbed in the bloodstream and into the central nervous system. The central nervous system of the peptide molecule will act as a fake neurotransmitter and interfere with brain development. This situation causes disruption of the perception (perception), cognition, feelings (emotions) and behavior of children with ID and ASD [15].

The high number of *Candida spp.* in the ID group could be due to the food consumption. Knivsberg's research reported that exacerbation of GI and behavioral symptoms in autism induced by certain foods, particularly those containing gluten and casein, has been shown through dietary intervention and their removal from the diet. Autistic children on gluten and casein-free diets also showed significantly lower eosinophil infiltrate in intestinal biopsies compared with those on a conventional diet [16]. In this study the results of direct interviews reveal that ID children often consumed foods containing high carbohydrates, such as biscuits, bread, sweets, ice cream, chocolate. The growth of *Candida spp.* in the intestine can lead to the lower absorption of carbohydrates and minerals to higher toxicity. *Candida spp.* can produce enzymes that play a role in the process of adhesion to the mucosal epithelium. In this condition the abnormal peptide will enter the intestinal mucosal epithelial cells which are then absorbed in the bloodstream and into the central nervous system. The peptide molecule will act as a fake neurotransmitter causing changes in the levels of neurotransmitters-glutamate and neurotransmitters-gamma-Aminobutyrate (GABA) and disrupting brain development [16,17,18,19]. Intestinal microbiota can also interact with sex hormones to modulate innate and adaptive immunity, the development of diseases and autoimmune disorders [20,21].

On the contrary, Adams research showed no differences of yeast infection among stools from ASD and healthy controls [2]. Particular conditions like in this study with 8 subjects in non ID having a high number of *Candida spp.* can disrupt their balance, and can cause yeast cells to multiply rapidly, and aggressively which eventually leads to infection. Predisposing factors play a role in increasing the growth of *Candida spp.* and facilitating fungal invasion into the tissues of the human body due to changes in the body's defense system. Variations in the results of this study can also be due to differences in HLA (*Human Leucocyte Antigen*), some individuals are identical and well equipped to deal with attacks from outside. The important role of HLA antigens lies in the control of self-recognition and defense against microorganisms [22,23,24].

This study has limitations on fecal examination only identification of *Candida spp* fungal species was identified. no identification in other species such as bacteria such as *Clostridium histolyticum*, and *Lactobacillus spp.* and limited to the examination of *Candida spp.* the digestive tract is not done in other body systems such as the mouth and vagina.

## CONCLUSION

There are significant differences between the colonies of *Candida spp.* in the ID group and non ID. Future studies could be conducted to verify whether *Candida* elimination therapy is useful to manage GI symptoms. It would also be beneficial to include a simple screening test based on stool culture for

diagnosis of *Candida* overgrowth in ID and ASD children so that parents of infants with yeast sensitivity would know to avoid including products in their children's diets that could produce an overgrowth of gastrointestinal yeast.

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