

Isolation of enterobacteriaceae bacteria species from feces of Sumatran Orangutans (*Pongo abelii*)

¹Darmawi, ¹Erdiansyah Rahmi, ¹Maryulia Dewi, ¹Joharsyah Hutabarat, ¹Mahdi Abrar and ¹Fakhrurrazi

¹Laboratory of Microbiology, Faculty of Veterinary Medicine of Syiah Kuala University, Banda Aceh 23111, Indonesia

Corresponding Author: d_darmawi@yahoo.com

Abstract. The purpose of this research aimed to isolate enterobacteriaceae bacteria from feces of *Pongo abelii*. The samples of feces were collected from 15 captive orangutans in Orangutan Sumatera Batu Mbelin Sibolangit Quarantine, North Sumatra. Of each sample was cultured in nutrient broth media using sterile cotton swabs or Pasteur pipettes, and incubated at 37°C temperature for 24 hours. Culture was spread on Methylene Blue Agar (Oxoid), examined by Gram staining, and tested by biochemically. The result showed that significantly more common appear *Escherichia sp.* (93,33%) and fewer *Edwardsiella sp.* (66,67%) were isolated from feces samples of *P. abelii*. Others enterobacteriaceae found in feces of *P. abelii* were *Shigella sp.* (46,67%), *Klebsiella sp.* (33,33%), *Citrobacter sp.*, and *Salmonella sp.* (13,33%), respectively.

Keywords: *Pongo abelii*, enterobacteriaceae, bacteria.

Introduction

Pongo abelii (Sumatran orangutans) are found only on the island of Sumatra, Indonesia, in primary tropical lowland forests, including mangrove, swamp forests, and riparian forests in Aceh and North Sumatra. They live almost completely in the trees, building nests in which they nap or sleep for the night (Urban, 2008). The massive destructions of tropical rain forest, which is the natural habitat of *P. abelii* caused decrease number of this key species population. To increase the survivability, Orangutan Sumatera Batu Mbelin Sibolangit Quarantine, North Sumatra mainly focused in quarantine of the *P. abelii* previously held illegally as pet. In the quarantine condition, we hypothesize that enterobacteriaceae possibility appearance in the feces of *P. abelii*.

Here, we investigate enterobacteriaceae bacteria in the feces of *P. abelii*. We hypothesized that not only normal bacteria of less virulence but also occasionally and pathogenic bacteria would be found in the feces. These data may potentially shape the empiric choice of antimicrobial agents to adequately control contamination and to prevent future infection.

Materials and Methods

The samples of feces were collected from 15 captive orangutans in Orangutan Sumatera Batu Mbelin Sibolangit Quarantine, North Sumatra. The samples were sent for examination to the Microbiology Laboratory of Veterinary Faculty of Syiah Kuala University. The samples were cultured to nutrient broth media and incubated at 37°C temperature for 24 hours.

Bacteriological procedures

Methylene Blue Agar (Oxoid) are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical sources. Eosin Methylene Blue Agar is used for isolating and differentiating gram-negative enteric bacilli

(Duytschaever *et al.*, 2013). Cultures are incubated in humid air at 36°C for 48 hours. Cultures are examined each day for growth and any colonies are Gram stained and subcultured (i.e., transferred) to appropriate media.

Gram stain

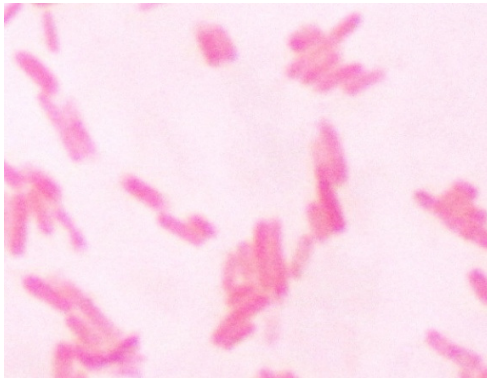
The usual first step in any bacterial identification is the determination of whether or not it is a gram-positive or gram-negative bacterium. Here, we obtain a clean glass slide to prepare a smear of each bacteria to be stained by taking a loopful of the bacteria (with a sterile loop) and spreading it over a small area in the center of the slide. The slide allowed the smear to air dry and then heat fix by passing the slide quickly through a flame. The slide placed on paper towels and add a drop or two of crystal violet to the smear to let set 1 minute. The slide washed gently the stain off with tap water carefully in order to being not to wash off bacteria. Then, Gram's iodine apply to let set 1 minute. To remove any excess stain or stain that has not adhered to the cell, the slide washed gently the iodine off with tap water and then add the decolorizing agent (95% EtOH) drop by drop until it runs clear. The decolorizing reagent washed off with tap water, and counterstain with safranin by adding 1-2 drops and let it set for 45 seconds. Finally, the slide rinsed with tap water, looked at under the microscope, and determined if bacterium is Gram-positive or Gram-negative. Gram-negative cells will pick up the counterstain and appear red or pink as described by Health Protection Agency (2007) as described by Darmawi *et al.* (2011).

Biochemical assayed

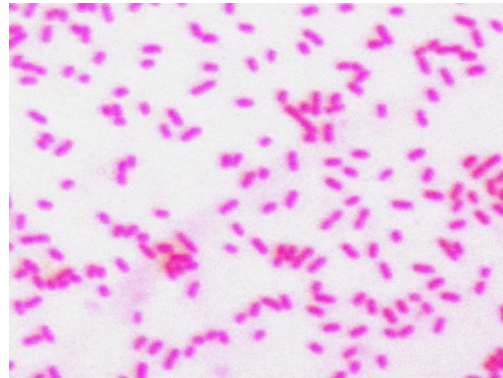
All isolates were identified according to the methods advocated by Edwards and Ewing (1962). The specific methods involved were colonial characteristics on media including size, inability to swarm, ability or inability to ferment lactose. Specific tests such as carbohydrate namely: glucose (Merck), lactose (Merck), sucrose (Merck), and mannitol (Merck) utilization tests, indole formation, Methyl Red (Oxoid), Voges Proskauer (Oxoid), and Simmon's Citrate (Oxoid) tests were done as described by Darmawi *et al.* (2011) with certain modifications.

Results and Discussion

Under microscopic investigation we found that Gram-negative cells pick up the counterstain and appear red or pink colour as shown in the Figure 1. In the present study we found that red or pink colour bacteria in order to Gram stain as shown in Figure 1. Gram-positive cells (those without an outer membrane) stain purple in the procedure, gram-negative cells (which have the outer membrane) stain red or pink. It is well known that most bacteria are characterized by having not only a cell membrane but also a cell wall which lies outside of the cell membrane. This cell wall is composed mostly of peptidoglycan and helps to maintain osmotic pressure and the cell's characteristic shape. Some taxonomic groups of bacteria also have an outer membrane that is attached to the peptidoglycan by small lipoprotein molecules. This difference in outermost cell structure is the basis for classification of bacteria by a differential staining technique known as the Gram stain. The samples were positive for spesies of the Enterobacteriaceae bacteria isolated from feces of *P. abelii* as shown in Table 1.



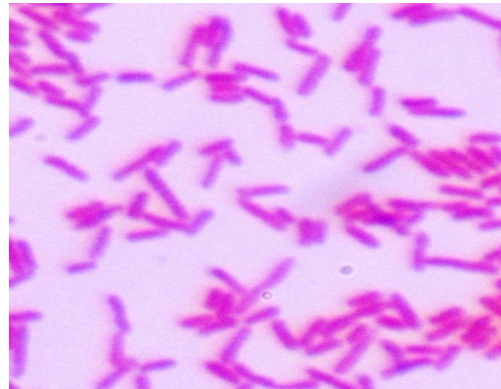
Escherichia sp.



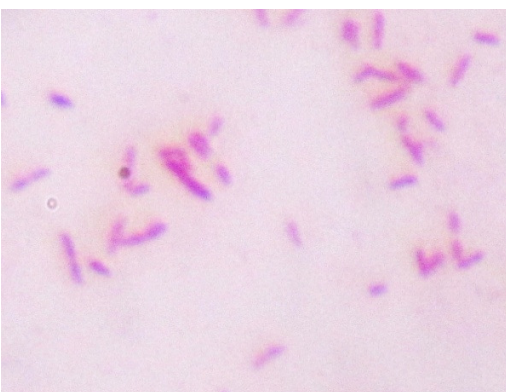
Citrobacter sp.



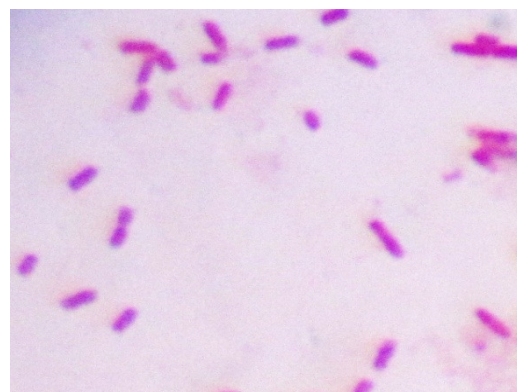
Klebsiella sp.



Shigella sp.



Salmonella sp.



Edwardsiella sp.

Figure 1. Gram stain with 10x100 magnification

Table 1. Enterobacteriaceae bacteria isolated from feces of *P. abelii*

Test	<i>Escherichia</i>	<i>Shigella</i>	<i>Salmonella</i>	<i>Klebsiella</i>	<i>Edwardsiella</i>	<i>Citrobacter</i>
Indol	+	+ / -	-	+ / -	+	-
Citrate	-	-	d	+	-	+
Methyl Red	+	+	+	-	+	+
VP	-	-	-	+	-	-
H ₂ S TSIA	-	-	+	-	+	+ / -
Motility	+ / -	-	+	-	+	+
Glucose	+	-	+	+	+	+
Sucrose	d	-	-	+	-	d
Lactose	+	-	-	+	-	d
Manitol	+	+ / -	+	+	-	+

TSIA = Triple Sugar Iron Agar; VP = Voges Proskauer; d = *dubius*

Table 2. The Percentages of Enterobacteriaceae bacteria isolated from feces of *P. abelii*

Enterobacteriaceae	Percentages
<i>Escherichia</i> sp.	93.33%
<i>Shigella</i> sp.	46.67%
<i>Klebsiella</i> sp.	33.33%
<i>Citrobacter</i> sp.	26.67%
<i>Salmonella</i> sp.	13.33%
<i>Edwardsiella</i> sp.	6.67%

In this study, only six enterobacteriaceae bacteria were detected, *Escherichia* sp., *Shigella* sp., *Klebsiella* sp., *Citrobacter* sp., *Salmonella* sp. and *Edwardsiella* sp. isolated from feces of *P. abelii* (please see Table 1). *E. coli* is a species of fecal coliform bacteria that is specific to fecal material from humans and other warm-blooded animals. Colibacteria are relatively harmless microorganisms, which are present in the intestines of humans and animals in large numbers. They play an important role in food digestion. Faecal colibacteria (enterobacteriaceae) are a subgroup of colibacteria. *E. coli* is the most commonly known species of faecal colibacterium. Faecal colibacteria are different from other species of colibacteria, because they grow under conditions of increased temperature, and because

they are only present in human and animal faeces. When faecal colibacteria are present in aquatic environments, this indicates that water is polluted by human or animal faeces. This generally leads to the conclusion that pathogenic bacteria are present, which come from faeces. These microorganisms can introduce disease in humans and animals that swim in polluted water.

Shigellosis is an infectious disease caused by various species of *Shigella*, transmitted from an infected person to another usually by a fecal-oral route. *Shigella* are present in the diarrheal stools of infected persons while they are ill and for a week or two afterwards. Most *Shigella* infections are the result of the bacterium passing from stools or soiled fingers of one person to the mouth of another person. Patient infected with *Shigella* develop diarrhea, fever and stomach cramps starting a day or two after they are exposed to the bacterium. The diarrhea is often bloody.

The finding of the present study supported by previous cases. Munoz *et al.* (2006) explained that more than 80% of fecal samples tested positive for *K. pneumoniae* dairy cows. Fecal shedding of *K. pneumoniae* by a large proportion of dairy cows may explain why *Klebsiella* mastitis occurs in herds that use inorganic bedding material or other bedding material that is free from *Klebsiella* upon introduction into the barn. *Klebsiella* spp. are Gram-negative, nonmotile, usually encapsulated rod-shaped bacteria, belonging to the family Enterobacteriaceae. Fecal shedding of *Klebsiella* by dairy cows contributes to the presence of *Klebsiella* in the environment.

Citrobacter spp., of the Enterobacteriaceae family, are gram-negative, facultative anaerobic bacteria that appear as rods or coccobacilli. *Citrobacter* are rare opportunistic nosocomial pathogens. *Citrobacter* normally cause urinary tract infections, blood stream infections, intra abdominal sepsis. Salmonellosis is a food borne illness caused by the salmonella bacteria carried by some animals, which can be transmitted from water, soil, animal feces. Salmonella infections typically affect the intestines, causing vomiting, and fever. *Edwardsiella*, a member of the family Enterobacteriaceae, exists widely in nature. It is associated with freshwater environments. Janda and Abbott (1993) reported that the most frequently reported manifestation of infection caused by *E. tarda* in humans is gastrointestinal disease. The organism has been cultured from stool samples obtained from patients with diarrheal illness in Asia, Australia, and Central America. In addition, Bennett *et al.* (1986) investigated that fecal bacterial flora of normal newborn infants was associated with growth of *Klebsiella*, *Clostridium difficile* and *Clostridium perfringens*. Duytschaever *et al.* (2013) fecal Enterobacteriaceae from patients with cystic fibrosis and healthy siblings enumerated onto Eosin Methylene Blue agar containing amoxicillin were assigned to *Klebsiella oxytoca* (58.4%), *Escherichia coli* (28.3%), *Klebsiella variicola* (7.5%) or *Citrobacter* sp. (5.8%). Here, important to note that the our finding is indicative that special attention should be given to the prevention and management of enterobacteriaceae bacterial infections from feces of *P. abelii*.

Conclusions

There are some enterobacteriaceae contained in feces of *P. abelii*, namely: *Escherichia sp.*, *Shigella sp.*, *Klebsiella sp.*, *Citrobacter sp.*, *Salmonella sp.* and *Edwardsiella sp.*

Acknowledgements

We wish to thank the Orangutan Republic Foundation (OUREF), and Sumatran Orangutan Conservation Program (SOCP) for cooperation that support this research in order to scholarship for students from Faculty of Veterinary Medicine of Syiah Kuala University.

References

- Darmawi, Darniati, Z.H. Manaf, Syafruddin, and A. Sayuti. 2011. Isolation of Gram-Negative Bacteria From Metacarpal Injury of *Panthera tigris sumatrae* Trapped in Subulussalam. Annual International Conference Syiah Kuala University (AIC Unsyiah), pages: 131-135.
- Bennet, R., Erikson M., Nord C.E., Zetterstrom R.. 1986. Fecal bacterial microflora of newborn infants during intensive care management and treatment with five antibiotic regimens, The Pediatric Infectious Disease Journal, 5(5): Abstract.
- Duytschaever G, Huys G, Boulanger L, De Boeck K, Vandamme P. 2013. Amoxicillin-clavulanic acid resistance in fecal Enterobacteriaceae from patients with cystic fibrosis and healthy siblings. J. Cyst Fibros. Abstract, doi: 10.1016/j.jcf.2013.06.006.
- Janda JM, Abbott SL. 1993. *Infections associated with the genus Edwardsiella: the role of Edwardsiella tarda in human disease. Clin Infect Dis ;17:742-8.*
- Munoz M.A., C. Ahlström, B.J. Rauch, R.N. Zadoks. 2006. Fecal Shedding of *Klebsiella pneumoniae* by Dairy Cows. Journal of Dairy Science. 89(9): 3425-3430.
- Health Protection Agency (2007). *Staining procedures*. National Standard Method Issue 1.
- Urban, K. 2008. "*Pongo abelii*" (On-line), Animal Diversity Web. Accessed September 14, 2013 at http://animaldiversity.ummz.umich.edu/accounts/Pongo_abelii/