Proceedings of International Conference on Technology and Social Science 2018 (ICTSS 2018)

Adsorption Study of Clinical Bacteria onto Clay : Application of Clay in Islamic Cleansing

Taufikurrahmi[1], Annisa Fitria[2], Siti Zahliyatul Munawiroh[3]

Department of Pharmacy, Universitas Islam Indonesia, Yogyakarta, Indonesia

[1]<taufikkurrahmi@gmail.com>, [2]<annisa.setyabudhi@gmail.com>, [3]<zahlia@gmail.com>

Keywords: *Clay, Time adsorption assay, MTT, Gram-positive, Gram-negative*

Abstract In Islamic law, clay or soil is used for cleansing the religiously-prohibited dirt (*najis*) . A study of clay, as a potential Islamic cleansing's material, was conducted and characterized by its adsorption activity toward bacteria which contain in canine's saliva. Three types of clay were selected: kaolin, bentonite, and talcum. A total of four different microbial species (*Staphylococcus haemolyticus, Micrococcus sp, Klebsiella pneumonia* and *Proteus mirabilis*) were recovered from mouth swab cultures from dog and used as tested microorganism in this study. Comparison of clays adsorption ability onto Gram positive and Gram negative bacteria was analyzed using time adsorption curve. In conclusion, each clay is able to adsorb bacteria with adsorption percentage of 70-90%. The adsorption capacity of clay toward bacteria is influenced by variation of the bacterial surface which was categorized into Gram-positive and Gram-negative.

1. Introduction

Indonesia is one of the countries with the largest Muslim population. On this ground, all the provisions in Islam especially those concerning with religious activities become a matter of great concern. Islam teaches about cleanliness, especially from any dirt that shall be avoided as a requirement for religious activity, which is called *najis*. In Islam, *najis* is divided into three categories which are *mukhaffafah*, *mutawassitah* and *mugallazah* (heavy form of pollutants). One of an example of *mughallazah* is canine's saliva [1]. Indonesian Council of Ulema (MUI) stated that the cleaning of *mugallazah* must be done through seven times of washing, one of which must be from water which contains sand, clay or soil [2].

The use of these materials directly onto skin based on this procedure is time-consuming and inefficient. Therefore, it is necessary to develop a form of Islamic cleanser required by the Moslem community to provide an ease in their daily worship routine. Besides being the requirement for worship puposes, cleaning the skin from *najis* could help eliminate the threat of passing the pathogens for those diseases to humans. Canine's saliva might contain pathogenic bacteria that can cause infection to human through direct contact [3]. This finding leads to the necessity to conduct the study to analyze the role of clay toward adsorption of pathogenic bacteria which isolated from canine's saliva.

Clay as potential material in the development of Islamic cleanser is known for its ability to adsorb bacteria. Many studies found that there are many factors affecting the adsorption activity. According to previous studies, there are different types of clay with different ability in adsorbing bacteria [4,5]. The adsorption capacity of the clay also depends on the species or strain of bacteria which was used as a tested microorganism. The assessment of clay to reduce the number of bacteria is an important aspect of research that relates to the potential applications of these materials in cleanser product.

2. Material and Methods

2.1 Clay

Three selected clays: bentonite, talcum, and kaolin were obtained from PT. Lautan Luas. Further purification of clay was conducted by dry heat sterilization with the utilization of oven at 160°C for 2 hours.

2.2 Tested Microorganism

In order to isolate the bacteria in canine's saliva, the saliva was collected from a healthy dog at Prof. Soeparwi Pets Hospital. This procedure was approved by Ethics Committee in Research of the Islamic University of Indonesia. The sample has been processed using a standard technique for bacteriological culture. The isolated colony was identified on the basis of colony morphology, Gram's stain, and biochemical test at Yogyakarta Health Laboratory Service.

2.3 Time Adsorption

Time adsorption assay was conducted according to the methods suggested by 0,5,10,15 with a little modification. The standardized bacterial culture (10^5 CFU/ml) and a sterile clay were added to a flask which contains 10 mL of Muller Hinton Broth (MHB). This bacterial culture was incubated at 37°C for 30 minutes in with gentle rotary mixing to ensure the contact with the clay and to prevent sedimentation. The two controls in this experiment were a bacterial culture without clay and media consisted of only clay without inoculum, which was incubated under the same condition. The sample of 1 mL amount from sample flask and control flasks were collected every 5 minutes (0, 5, 10 and 15 minutes) with collecting tube then centrifuged at speed 5000 rpm for 15 minutes. Supernatant with an amount of 200µl was transferred in well microplate then added with 10 µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and incubated for 15 minutes at 37°C [6]. The absorbance was measured at the maximum absorption wavelength of 570 nm and calculated with the equation below:

$$\%\ Adsorption = \frac{Abs.bacteria\ of\ adsoprtion}{Abs.bacteria\ of\ adsoprtion + Abs.bacteria\ of\ not\ adsoprtion} \times 100\% \quad (1)$$

Another step was conducted to confirm the viability of the bacteria cells in a bacteria cell-clay complex. Sample from pellet was subcultured on Muller Hinton agar then incubated at 37°C for 24 hours. The viable bacteria were counted by colony counter. Each sample which went through all the steps of an experiment was analyzed in triplicate [7].

3. Result and Discussion

In order to represent the actual event of skin contacted with dog's saliva as *najis*, the bacteria isolated from canine's saliva was used as tested bacteria in the present study. Four different bacterial species were isolated from dog's saliva. Morphological observation and analysis through various biochemical tests determined that these isolates are *Staphylococcus haemolyticus*, *Micrococcus sp*., *Klebsiella pneumonia* and *Proteus mirabilis*. With Gram staining method it was determined that *Staphylococcus haemolyticus* and *Micrococcus sp*., belong to Gram-positive bacteria, while *Klebsiella pneumonia* and *Proteus mirabilis* belong to Gram negative bacteria. These bacteria classified as opportunistic pathogens that may cause infection in animals and humans with a weakened immune system. In line with the former studies, *Staphylococcus*, *Micrococcus*, and *Proteus* were also found as a common genus among cultivated bacteria from canine oral [8,9]. All of these bacteria were used as

tested microorganism to analyze a different interaction toward clay that may occur because of the variation of its membrane and cell wall structure.

The purpose of time adsorption assay was carried out to determine the ability of clay to adsorb bacteria in a short time. According to this mechanism, MTT was used for determining viable cell number in this assay. Viable cells with active metabolism convert MTT into a purple colored formazan product. This color formation serves as a useful marker to differentiate the viable bacteria cells with clay. This assay could be selected as an effective method to quantify bacteria in a complex form with clay to determine the capability of clay to adsorb bacteria in solution. The adsorption of the bacteria from solution onto the surface of clay will immobilize the cells and prevent them to replicate. Table1. shows that clay acted as an adsorbent. This mode of action was determined by subcultured procedure toward clay-bacteria complex. It was confirmed that all of the tested bacteria still has growth capability after interacting with clay for 15 minutes.

Table 1. Total colony of bacteria absorbed by bentonite, kaolin and talcum.

Clay	Time	Amount of Bacteria in CFU (X ± SD)			
		Staphylococcus haemolyticus	***Proteus mirabilis***	***Micrococcus sp***	***Klebsiella pneumoniae***
Bentonite	0	169.5 ± 194.45	75 ± 12.73	110 ± 14.14	229 ± 29.70
	15	89 ± 100.41	119 ± 123.04	85 ± 63.64	256.5 ± 103.94
Kaolin	0	213.5 ± 194.45	156.5 ± 146.37	170 ± 42.43	258.5 ± 101.12
	15	338.5 ± 432.04	135 ± 35.36	165 ± 120.21	266.5 ± 36.06
Talcum	0	111.5 ± 78.49	64 ± 70.71	120 ± 28.28	328 ± 257.39
	15	112 ± 96.17	95 ± 74.25	165 ± 35.36	112 ± 175.36

The stability of the clay-bacteria complex affected the adsorption activity. Based on the time adsorption profile in Fig. 1., it was revealed that the adsorption activity of kaolin toward four species of bacteria was affected by time. On the other hand, bentonit could be referred to as the most active clay of all clays by its percentage of adsorption. It was apparent that different clay has a different capability to make a complex interaction with bacteria. The results also indicated that the adsorbing power of clay was superior to Gram-positive bacteria compared to Gram-negative bacteria.

Different types of clay have a different composition of mineral and compounds, which is responsible for the antibacterial activity of the clay. The different structure of cell wall and composition of Gram-positive bacteria and Gram-negative bacteria also play important role in the interaction with the clay surface. Bacteria can be adsorbed by clay of its negative ion charge on the cell wall [10,11]. Another factor influencing the adsorption power difference in each clay is ionic charge strength, pH level, and zeta potential on the clay [12]. Those factors will influence the electrostatic interactions between clay and bacteria in order to create complex interaction during adsorption [13]. Further study on a chemical and physical characteristic of clay is needed to provide sufficient information related to the factors that influence the role of this material in removing bacteria.

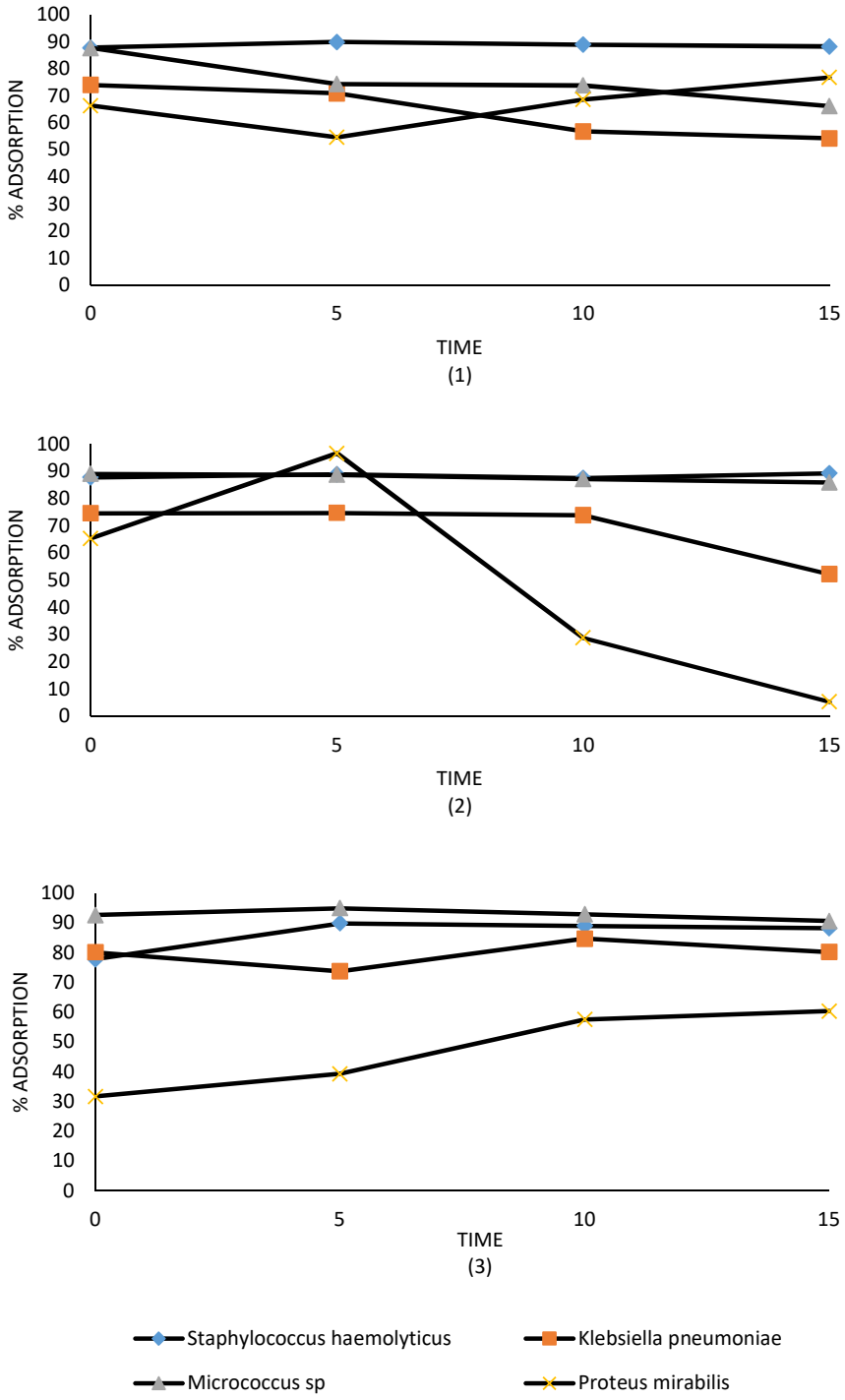

Fig. 1. Time Adsorption of Clay Bentonite, Kaolin, and Talcum, (a)= clay bentonite, (b)= clay kaolin, (c)= clay talcum.

4. Conclusion

This study confirmed that three selected clay could effectively adsorb bacteria with adsorption percentage of 70-90% within 15 minutes. The adsorption process depends on variation of cell wall and membrane structure of bacteria.

Acknowledgements

The author would like to thank you for Hanida Destriana, Nilam Permata Sari, Siti Asmaliah, Nurvita Permata Sari for their contribution to provide a laboratory data and Ministry of Research, Technology and Higher Education of the Republic of Indonesia.

References

[1] N. Kassim, P. Hashim, M. Hashim, and H. Jol, "New approach of samak clay usage for halal industry requirement," ***Procedia. Soc. Behav. Sci.***, Vol. 121, pp. 186–192, 2014.

[2] Anonim, "*Himpunan Fatwa Halal Majelis Ulama Indonesia",* ***Departemen Agama Republik Indonesia***, 2009

[3] K. Sivakami, B. Ambrin Farizah, T. Aishwarya, B. Vaishnavi, R. Viveka Devi, and Sakthivel, "Isolation and characterisation of salivary microbiota of street dogs", ***J. Biol. Inf. Sci.***, Vol. 4, pp. 2–6, 2015.

[4] Z. Hong, W. Chen, X. Rong, P. Cai, W. Tan, and Q. Huang, "Effects of humic acid on adhesion of Bacillus subtilis to phyllosilicates and goethite", ***Chem. Geol.***, Vol. 416, pp. 19–27, 2015.

[5] Y. Cao, X. Wei, P. Cai, Q. Huang, X. Rong, and W. Liang, "Preferential adsorption of extracellular polymeric substances from bacteria on clay minerals and iron oxide", ***Colloids Surfaces B Biointerfaces***, Vol. 83, No. 1, pp. 122–127, 2011.

[6] P. Herrera, R.C. Burghardt, T.D. Phillips, "Adsorption of *Salmonella enteritidis* by cetylpyridinium-exchanged montmorillonite clays", ***Veterinary. Microbiol***., Vol. 74, pp. 259–272, 2000

[7] M. Burton, E. Cobb, P. Donachie, G. Judah, V. Curtis, and W.P. Schmidt, "The effect of handwashing with water or soap on bacterial contamination of hands", ***Int. J. Environ. Res. Public Health***, Vol. 8, pp. 97–104, 2011.

[8] F.E. Dewhirst, E.A. Klein, E.C. Thompson, J.M. Blanton, T. Chen, L. Milella, C.M Buckley, I.J. Davis, M.L. Bennett, and Z.V.M. Jones, "The canine oral microbiome", ***PloS One***, Vol. 7, e36067, 2012

[9] D.R. Elliott, M. Wilson, C.M.F. Buckley, and D.A. Spratt, "Cultivable oral microbiota of domestic dogs", ***J. Clin. Microbiol***, Vol. 43, pp. 5470–5476, 2005.

[10] E. Burdukova, M. Becker, D.J. Bradshaw, and J.S. Laskowski, "Presence of negative charge on the basal planes of New York talc", ***Journal of Colloid and Interface Science***, Vol. 315, pp. 337-342, 2007.

[11] I. Chakroun, E. Fakhfakh, M. Medhioub, A.L. Galindo, I.C. Viseras, and F. Jamoussi, "Characterization of some tunisian clays to be used as antidiarrhoeic agents", ***Periodico di Mineralogia*** , Vol. 82, pp. 89-100. 2013.

[12] R.A. Chotzen, T. Polubesova, B. Chefetz, and Y.G. Mishael, "Adsorption of soil-derived humic acid by seven clay minerals: A systematic study", ***Clays Clay Miner***, Vol. 64, No. 5, pp. 628-638, 2016.

[13] X. Rong, Q. Huang, X. He, H. Chen, P. Cai, and W. Liang, "Interaction of *Pseudomonas putida* with kaolinite and montmorillonite : A combination study by equilibrium adsorption, ITC, SEM and FTIR", ***Colloids and Surfaces B: Biointerfaces***, Vol. 64,. pp 64:49–55, 2008.