Effectiveness of Autoclave Combination Treatment with Andosol Soil to Decrease the Number of Bacillus Cereus

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ABSTRACT
Strict regulations and permits in managing medical waste create dependency on third party. This has an impact on medical waste especially syringe medical waste which must be piled up waiting for the collection process. Improper handling medical waste may cause accidents and disease transmission. Soil has been known to be used as an antibacterial in medicine, but there is still little in the process of application in medical waste management. This study aims to determine the effectiveness of the use of autoclaves combined with andisol soils to decrease the number of bacterial colonies of Bacillus cereus. This study uses a true experiment design using a completely randomized design. The research was carried out starting from the isolation of bacteria from syringe medical waste and rejuvenation on sterile media to test the effect of the application treatment on a laboratory. Anova Repeated Measure Test (Ranova) at alpha 0.05 was used to determine differences in the number of bacteria that grew in the media before and after treatment. The results of this study indicate the combination treatment of autoclaves in the variation of contact time with andosol soils at variations in concentration produced the difference (p <0.001) with the number of bacterial colonies Bacillus cereus between 290,000-340,000 CFU / ml and the effectiveness of all treatments reaches 100%. At the end of this study, a combination of autoclave treatment at a temperature of 121 ^{0}C was taken for 15 minutes with andosol soil treatment at a concentration of 45% with a contact duration of 2 minutes that could be applied independently at the hospital.

Keywords: Autoclave, Soil, Andosol, Bacillus cereus

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INTRODUCTION

Hospital is a public facility that functions as a place for examination, treatment, care, health recovery and among others it serves as a place for education, training and research. From 2012 to April 2018 there was an increase in the number of Hospitals by 5.2%.[1] The increase in the number of hospitals in addition to having a positive impact in the form of increased access to health services also has a negative impact in the form of an increase in the amount of waste it generates.

The increase in the number of hospitals has not been matched by an increase in the waste treatment facilities it generates. The impact is the pile of medical waste due to the constrained treatment of waste in hospital as the operation of the incinerator is constrained by the technical requirements.[2] Apart from being unable to operate the incinerator, medical waste buildup also occurs because some hospitals do not have waste treatment facilities.[3,4]

Based on the status of the Health Service Facility Waste Management Report in Southeast Asia Region issued by WHO in 2017, the total medical waste produced by Indonesia is 225 tons / day with the average waste generated is 0.68 kg / TT / day.[5] Further, the production of solid waste generated in the form of domestic waste was 76.8% and the remaining 23.2% was infectious waste.[6] In 2017, the percentage of hospitals managing medical waste according to standards in Central Java Province only reached 25.61%.[7]

Improper management of hospital medical waste can trigger the risk of occupational accidents and disease transmission. Every year there are 385,000 occurrences of injuries caused by sharp objects contaminated with blood on health workers in America (American Nurses Association).[8] Research conducted by WHO shows that around 8.70% of 55 hospitals in 14 countries in Europe, The Middle East, Southeast Asia

and the Pacific indicates the presence of HAIs. Prevalence of HAIs most in the Eastern Mediterranean and Asia Southeast namely 11.80% and 10% while in Europe and the Western Pacific they were 7.70% and 9%, respectively.[9] Research conducted in 11 hospitals in Jakarta showed that 9.80% of inpatients suffered from Health care-Associated Infections (HAIs).[10]

The most commonly used technology is incineration, which is able to reduce the volume of medical waste. Side effects of the incineration process are pollutants produced like dioxins, furans, several types of heavy metals (lead, mercury and cadmium), acid gases (hydrogen chloride and sulfur dioxide), carbon monoxide and nitrogen oxides.[11] Emissions from the incineration process can cause serious problems in public health and the environment. The incineration process also produces waste in the form of flying ash and bottom ash which also contains dioxin, toxic chemicals and heavy metals. Another technique that can be used in treating syringe waste is wet heating with an autoclave. An increase in contact duration and temperature can cause a decrease in the number of bacteria after treatment. Optimal experimental conditions measured by the degree of bacterial inactivation are 121 ° C for 15 minutes for gram-negative bacteria, 121 ° C and 131 ° C for 60 and 30 minutes for gram-positive bacteria.[12]

Another method that can be used to treat syringe waste is chemical disinfection. Chemical compounds that can be used as disinfectants include chlorine.[13] Sodium hypochlorite is one of the inexpensive disinfectants and is commonly found on the market, besides, this disinfectant is also effective in reducing bacterial populations. Sodium hypochlorite with a concentration of 100 ppm has the greatest effect in reducing the population of bacteria *Bacillus cereus* to> 5 CFU / ml.[14]

The most typical pathogenic bacteria in solid medical waste are the genera *Bacillus, Staphylococci* and *Streptococci*.[6,15] The

most common bacteria found in solid medical waste are *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus* and *Candida albicans*.[16] The regrowth of bacteria in solid medical waste treated by thermal techniques was evaluated by Hossain.[12] Regrowth of bacteria after inactivation by thermal techniques, successively after the second to sixth day of the bacterial species *Bacillus subtilis, Staphylococcus aureus, Streptococcus pyrogenes* and *Acinetobacter baumannii* and *Escherchia coli* and *Psudomonas aeroginosa*.[12]

Based on previous studies, it is known that pathogenic bacteria can still grow back after inactivation with thermal sterilization technology. Pathogenic microorganisms found in infectious waste have diverse characteristics so that in sterilizing or disinfection with a single method can only kill or inactivate certain bacteria or viruses, not as a whole.

Anti-bacterial testing methods using soil have also been carried out in previous studies where the addition of bentonite and kaolin soils in soap can increase the ability of soap to inhibit bacterial growth.[17] The addition of soil in soap made in previous studies has proven to be effective in removing bacteria, soils contained in soap increase the level of interface tension so that it can attract dirt and bacteria that stick.[17–25]

Based on the background described above, researchers are interested in finding out the effectiveness of the combination of autoclaves with andosol soils in reducing and eliminating *Bacillus cereus* bacteria even in spores.

MATERIALS AND METHODS

This study uses a true experiment design using a complete random design. Bacteria used in this study were species of *Bacillus cereus* obtained from the results of hospital syringe waste isolation. The research treatment focused on the effectiveness of the combination of autoclave with andosol soil to reduce the number of bacteria in syringe medical waste on a laboratory scale. The treatment was carried out by contaminating sterile syringes on bacterial isolates from rejuvenation that were successfully obtained from hospital syringe waste. In the next stage, an autoclave treatment was carried out at 121^0C with variations in contact time of 1 minute, 5 minutes and 15 minutes followed afterwards andosol soil treatment at variations in the concentration of solution of 15%, 30% and 45% with a contact duration of 2 minutes. The number of repetitions in the treatment of this study was calculated using the Federer formula approach, with the results of 2 repetitions. Data processing was performed to determine differences in the number of bacteria that grow in the media of plate count before and after treatment with the Repeated Measure Anova (Ranova) test at alpha 0.05.

RESULTS AND DISCUSSION

Initial data measurements on the number of *B. cereus* bacterial colonies were performed before the syringe that had been decontaminated with bacterial suspensions treated with a combination of autoclave and andosol soils. Preliminary data obtained by finding the average value of the results of 2 treatment replications and then homogeneity test analysis was carried out on the initial data on the number of bacterial colonies of a combination of autoclave treatment and andosol soils presented in Table 1 below:

Table 1: Initial measurements of the number of bacterial colonies Bacilluscereus in the combination treatment of autoclaves and andosol soil

Variable	n	Mean	Std.Deviation	Minimum	Maximum	p
Control(0)	2	300,000	14,142	290,000	310,000	
Autoclave 1 minute and soil 15%	2	305,000	7,071	300,000	310,000	
Autoclave 1 minute and soil 30%	2	310,000	0	310,000	310,000 310,000	
Autoclave 1 minute and soil 45%	2	310,000	0	310,000	310,000	
Autoclave 5 minutes and soil 15%	2	320,000	0	320,000	320,000	<0,001
Autoclave 5 minutes and soil 30%	2	320,000	0	320,000	320,000	
Autoclave 5 minutes and soil 45%	2	320,000	0	320,000	320,000	
Autoclave 15 minutes and soil 15%	2	335,000	0	330,000	340,000	
Autoclave 15 minutes and soil 30%	2	340,000	0	340,000	340,000	
Autoclave 15 minutes and soil 45%	2	340,000	0	340,000	340,000	

One Way Anova test shows that the analysis of the data difference is significant (p <0.001). This situation shows that in the initial conditions of the study, the number of colony of bacteria *B. cereus* in a state that is not homogeneous.

Therefore, when analyzing the effect of a combination of heating duration variations using autoclave and variations in soil concentration on the number of bacterial colonies, it is necessary to control the data on the number of bacterial colonies before being given treatment.
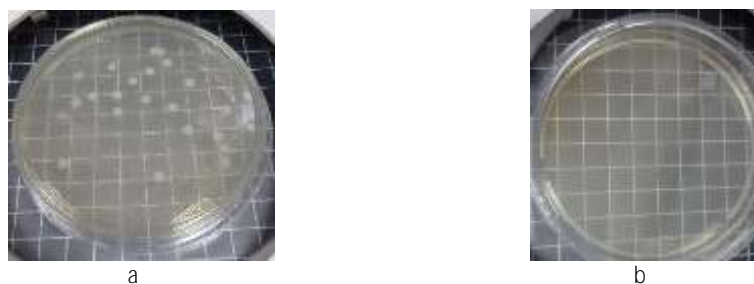
<p style="text-align:center">a   b</p>

Figure 1 a: The number ofbacterial colonies Bacillus cereus before treatment (320,000 CFU / ml); b. after treatment and observation until the fifth day (0 CFU / ml)

Figure 1.a shows the number of bacterial colonies in the observation before the autoclave combination treatment with andosol soil, with an average colony number of 320,000 CFU / ml. Figure 1.b shows the observations until the fifth day that there was no growth of bacteria *Bacillus cereus* (0 CFU / ml) Analysis of the combination treatment of autoclave and andosol soil on the effect of decreasing the number of bacteria before and after treatment with pre –controlled data using the *General Linear Model* (GLM) indicated the decrease in the group that was given a combination of heat treatment (autoclave) at various concentrations and andosol soil treatment at various concentrations compared to the group that was not given the combination of the treatment (control). The General Linear Model in the Anova Repeated Measure (Ranova) test by controlling the average number of bacterial colonies before being given a combination treatment showed an interaction of decreasing the average number of bacterial colonies between groups that were given combination and control treatments ($p < 0.001$).

Table 2: Estimation of the effect of the combination of autoclaves and andosol soils on various variations on the decrease in the number of bacteria B. cereus in syringe waste

Combination autoclave and andosol soil	ColonyColony Bacteria		β	p	CI 95%	Eta Square (%)
	Pre	Post				
Control	320,000	320,000				
Autoclave 1 minute and Andosol soil 15%	320,000	0.00	320,000	<0.001	-320,000; -320,000	100.00
Autoclave 1 minute and Andosol soil 30%	320,000	0.00	320,000	<0.001	-320,000; -320,000	100.00
Autoclave 1 minute and Andosol soil 45%	320,000	0.00	320,000	<0,001	-320,000; -320,000	100.00
Autoclave 5 minutes and 15% andosol soil	320,000	0.00	320,000	<0,001	-320,000; -320,000	100.00
Autoclave 5 minutes and andosol soil 30%	320,000	0,00	320,000	<0,001	-320,000; -320,000	100,00
Autoclave 5 minutes and andosol soil45%	320,000	0.00	320,000	<0,001	-320,000; -320,000	100.00
Autoclave 15 minutes and andosol soil15%	320,000	0,00	320,000	<0,001	- 320,000; -320,000	100.00
Autoclave 15 minutes and andosol soil30%	320,000	0.00	320,000	<0,001	-320.0 00; -320,000	100.00
Autoclave 15 minutes and andosol soil 45%	320,000	0.00	320,000	<0.001	-320,000; -320,000	100.00

The result of Eta Squared test in Table 2 shows that the combination treatment of autoclave contact duration at various concentrations and treatments of andosol soils at various concentrations can significantly reduce the number of bacterial colonies (100%). Test estimation of the combination treatment duration of the autoclave contact at various concentrations and the treatment of andosol soils at various concentrations can reduce the number of *B. cereus* bacterial colonies. When treated with all types of combinations the duration of contact autoclave at various concentrations and andosol soil treatments at various concentrations colonies of *B. cereus* bacteria were 320,000 lower than in the control group ($β = -320,000$).

The mean difference of *B. cereus* bacterial colonies between these combination treatments tested using a Significantly Different Least (LSD), were significantly different ($p < 0.05$). The non-different combination pairs ($p > 0.05$) were found in autoclave 1 minutes and 15% soil with autoclave 1 minute and soil 30%, and 15%, autoutoclave 1 minute and soil 45%, and 30%. The difference also existed in the combination of Autoclave 5 minutes and soil 15% ,30%, and 45%.

Previous studies of variations in the use of autoclaves with time (0, 5, 15, 30 and 60 minutes) and temperature (111℃, 121℃ and 131℃) at adjusted pressures automatically led to an increase in bacterial death. At 121℃ with contact variations of 1. 5 and 15 minutes with temperatures, bacterial colonies were still found.[12] Gram-positive bacteria are able to survive during the autoclave sterilization process because they have a thicker layer of peptidoglycan walls when compared to gram negative.[12.26] The results of the study explain that bacteria are still able to regenerate or regrow after 72 hours on medical solid waste.[27] Based on the above explanation, it is concluded that the autoclave was not able to eliminate 100% of the bacteria that the combination of treatments is needed to increase the effectiveness in killing bacteria. Using autoclaves in hospitals are inefficient because the amount of is syringe waste is more than the capacity of autoclaves to eliminate.

Autoclaves can damage bacterial cell structures, including the outer membrane and cytoplasm. Damage to bacterial cell structure that occurs depends on the mechanism of bacteria, bacterial cell structure, temperature and duration of heat exposure.[12,28] The combination of autoclave treatment with soil will further reduce the number of *Bacillus cereus* bacteria because minerals contained in clay such as *silicate tetrahedral* (SiO_4) and *octahedra* (consisting of Al, Mg, and Fe) are building minerals that have mineral builders ability to exchange cations and be able to release metals that encourage antibacterial reactions.

The results of a survey of the antibacterial properties of clay show that most contain transition metals such as Fe^{2+}, Cu, Mn, Zn) which can combine and cause toxic conditions to bacteria.[29] The soil type of andosol used as a media treatment in this study has a high cation exchange capacity (CEC) of 21.9 me / 100 gr so that the ability of positive ion bonds on cell walls and negative ions on the surface of the soil are greater,[17] that the soil is able to play a role in reducing the number of bacterial colonies as if the CEC value is greater, the greater the ionic bond between the positive ions on the bacterial cell wall and negative ions on the ground surface.[17] All soil components support the expansion of cation exchange sites, but cation exchange in most soils is centered according to the physical properties of the clay soils and contains organic matter. Cation exchange reactions in the soil occur mainly near clay surfaces such as colloids and humus particles called micelles. Each micelle can have thousands of negative charges neutralized by the adsorption cation.[30] The results of this study are in line with previous research that the physical and chemical properties possessed in soil have cleansing effect.[31]

The results of antimicrobial tests for sterile soil activity showed that sterile soil used as antimicrobials could inhibit bacterial growth with inhibition zone diameters of 11.7 mm. Then in the sterile soil experiment which was inoculated against individual bacteria could form the inhibitory zone of the sterile soil. This proves that sterile soils have substances / compounds that can inhibit bacterial growth.[20]

Antibacterial originating from the ground has been widely studied by scientists. According to one study, clay can eliminate all bacteria in 24 hours.[32] Further confirmed in other studies that there is a chemical content of clay which can become antibacterial in certain types of bacteria pathogenic to humans, including those that are resistant to antibiotics. Clay as a natural antibacterial has a size (<200 mm), and contains elements of Fe which acts as toxic or toxic to certain bacteria.[33]

Gram-positive bacteria do not have an outer membrane that can protect them from the environment like gram-negative bacteria, but gram-positive bacteria have a relatively thicker layer of peptidoglycan when compared to gram-negative bacteria. The relatively thicker peptidoglycan layer is a long anionic polymer or commonly called teichoic acid. Teichoic acid consists mainly of glycerol phosphate, glucocil phosphate, ribitol phosphate. Teichoic acid is a polyol phosphate polymer that has a covalently strong negative charge, teichoic acid is negatively charged due to the presence of phosphate structure in it.[34,35]

Electrostatic interactions occur between soil particles and bacteria. The surface of bacterial cells is dominated by negative charges while the content clay is positively charged. The load on the surface of clay can be adjusted by modifying the mineral composition. Moieties produce positive charges or produce radical or hydroxyl oxygen which can damage bacterial cell walls. The surface of the clay can be regulated by adjusting the pH of the soil. The soil will be negatively charged if the soil's pH is in an alkaline position, otherwise the soil will be positively charged if it has an acidic pH so that with this mechanism clay is able to eliminate gram-negative and gram-positive bacteria efficiently.[36]

Clay clay minerals contain aluminosilicate hydro minerals with a chemical composition of $Al_2Si_4O_{10}(OH)_2$ which can used to reduce the amount of *Eschericia coli* by 95%.[35] One of the concepts in disinfection using clay is the mechanism of adhesion. Adhesion is the absorption of bacterial cells attached to the surface of soil minerals influenced by chemical and physical interactions between the surface of the material and bacteria.[37.38]

This interaction can be explained by DLVO theory (*Boris Derjaguin and lev landau, Evert Verwey and Theodoor Overbeek*) where the aqueous dispersion aggregation illustrates the forces of attraction between charged surfaces that interact through liquid media. Since the two particles are close to one another, ionic begins to overlap between bacterial cells and the surface of the adsorbent.[35.39]

Hydrophobic adsorbents stimulate bacterial absorption, attachment of microbial cells to the surface depending on a number of motion factors, van der waals traction, gravitational force and surface electrostatic charge, which are important factors of cell hydrophobicity.[40]

Cell hydrophobicity can increase depending on the type of surface supporting adhesion, changes in environmental conditions such as temperature of nutrient composition and growth phase affect the hydrophobic response.[41,42] Clay can be used for disinfection by killing or through physical or chemical modification of adhesion which can cause changes in the surface area, structure and function of clay mineral surfaces and changes in mineral composition.[43]

CONCLUSIONS

The results of the study on the capability of the combination of autoclave and andosol soils produce an effectiveness value

of 100.00%. The combination of treatments can be applied in hospitals in the management of syringe medical waste is at an autoclave temperature of 121^0C for 15 minutes and continued with soil treatment at 45% concentration with a contact time of 2 minutes.

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