SUMMARY

In Vitro Comparison of Doubling Time between Multiresistant and Non-multiresistant Strain of Methicillin Resistant *Staphylococcus aureus* (MRSA) of Patient's Isolates in Malang

Staphylococcus aureus (*S.aureus*) is one of normal flora of the skin and mucous membrane which sometimes could be a pathogen. The emergence of strain *Methicillin Resistant Staphylococcus aureus* (MRSA), has made treatment of MRSA infection become complicated.

For being a successful pathogen, bacteria have to armed himself with virulence factor that insist them to invade human body. One of the virulence factor is the total amount of bacteria that infect the host , which depend on the growth rate. Faster growth rate will supply more bacteria that ready to invade the host and cause acute infection. Whereas slower growth rate will slowly supply bacteria so the infection will be a chronic infection.

Based on fenotypical characteristic of susceptibility to antibiotics, Methicillin Resistant *Staphylococcus aureus* (MRSA) bacteria could be grouped into 2 groups, which are multiresistant (mr) strain of MRSA and non-multiresistant (nmr) strain of MRSA. There was a growing concern about these two strains whether multiresistant strain doubling slowly than non-multiresistant strain since multiresistant strain has many resistance gene than in non-multiresistant strain. In addition, there is "cost" from the bacteria life that should be sacrificed if a bacteria become resistant as a consequences of having an additional resistance gene from other bacteria via plasmid, transposon, or operon. The bacteria need an "extra energy" in other to maintain the additional virulence genes or resistance genes. The latter compensation would be involved bacterial life cycle, including the time needed to divide (doubling time).Therefore, the multiresistant strain needs more time to duplicate than the non-multiresistant.

The aim of the research was to investigate whether there was differences in doubling time of multiresistant strain of MRSA between non-multiresistant strain of MRSA, to compare the doubling time between multiresistant strain of MRSA and non-multiresistant strain of MRSA, to compare the logarithmic phase between multiresistant strain of MRSA and non-multiresistant strain of MRSA, and to investigate the level of antibiotic's resistance between multiresistant strain of MRSA and non-multiresistant strain of MRSA.

Twenty seven isolates of multiresistant strain of MRSA and four isolates of non-multiresistant strain of MRSA was involved in this study. The samples were subcultured from the microbiology laboratory stocks and tested for susceptibility profiles by Vitek 2 System (bioMerieux, Durham, NC, USA). We obtained susceptibility profile to eight classes of antibiotics.After the susceptibility profiles were obtained, those isolates were tested for doubling time's counting. The calculation of doubling time was performed as an overnight of bacterial cultures were diluted 1 : 100 in 5 mL of BHI medium and grown for 3 hours at 37°C, 180 rpm. These cultures were seeded at a 1:100 dilution in another 5 mL BHI medium and incubated at 37°C,180 rpm for futher 4 hours. Optical density was measured every 30 until 4 hours. Values were converted into log2 values, and the doubling time was calculated as the reciprocal of the slope.

The mean of doubling time of multiresistant strain of MRSA was 27.9 $\pm$ 5.80 minutes, whereas the mean of doubling time of non-multiresistant strain of MRSA was 22.3 $\pm$ 0.99 minutes. Statistical analysis showed that the mean of doubling time from both strain was significantly differ ($p<0.05$). The logarithmic phase also different significantly between groups ($p<0.05$). There was a correlation between the duration of logarithmic phase and doubling time significantly ($p<0.05$). In statistical analysis there was also a significant differences in the resistant antibiotics (aminoglicoside, glikopeptide, macrolide, and rifampisin) between multiresistant and non-multiresistant strain of MRSA, which is multiresistant strain was more resistant than non-multiresistant. It indicate that the consumption of those antibiotic is still limited in hospital setting. In other hand, there is no significant differences in the resistant antibiotics (fluoroquinolon, tetracycline, trimethoprim-sulfamethoxazole, quinapristine – dalfo pristine) between multiresistant and non-multiresistant strain of MRSA. It indicate that the consumption of fluoroquinolon and tetracycline is common in community.

The last two results could be due to:

1. The insufficient number of sample. In this research there were difficulties to gain not only the non-multiresistant strain but also multiresistant strain which has resist to more than six classes of antibiotics. Only one sample in multiresistant strain group which resistance to more than six classes ofantibiotics, so it was not sufficient to make a relationship and conclusion between the number of resistance classes of antibiotic and the doubling time.
2. Perhaps there was a "lost" resistence gene when subculturing and stocking, especially in the group of multiresistant strain of MRSA which most of the samples were fenotypically resistant to vancomycin. The gene which responsible for the resistancy of MRSA to vancomycin therapy is *vanA* gene that acquired from a transposon of *Enterococcus sp*. It was mentioned that induction of van A-Type resistance in MRSA isolates could decrease the fitness cost, but if the induction is absence, the fitness cost would be minimal.
3. There was a subtype/ subpopulation in Vancomycin Intermediate *Staphylococcus aureus* (VISA), which is named as heteroresistanceVancomycin Intermediate *Staphylococcus aureus* (hVISA). In this subtype, there was a small number of cells in any given colony maintain a more resistant phenotype while the vast majority do not. This allows for the overall population of cells to grow more rapidly and infect more readily while still maintaining a subset of resistant cells that are capable of surviving in the setting of vancomycin use. Therefore, although there was a subtype which is resistant to vancomycin, the doubling time was the same as the strain that is susceptible to vancomycin.

The conclusion are there was a significance different between the mean of doubling time from multiresistant and non-multiresistant MRSA by in vitro desain.