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Case Report

BACTERIAL COLONY GROWTH IN THE VENTILATOR CIRCUIT OF THE INTENSIVE OBSERVATION UNIT AT RSUD DR. SOETOMO SURABAYA

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ABSTRACT

Ventilator-associated pneumonia (VAP) remains a problem with the highest cos, morbidity and mortalityt in the Intensive Care Unit (ICU). The correlation between mechanical ventilation and pneumonia is considered as common sense, yet scientific evidence to support this statement is still needed. This research aims to analyze the bacterial colony grows in mechanical ventilation circuit and those grew in the patient's sputum culture. We performed an observational study. Samples for bacterial culture were taken from ventilator circuit and patient sputum on Day-0, Day-3 and Day-7.. Sputum samplings are collected using double catheter tracheal aspiration technique; Results are then analyzed with Chi-square test. While the similarity of bacteria species in ventilator circuit to patient's sputum is analyzed with Binomial test. Two samples are dropped out immediately due to the rate of bacterial growth on Day-0. Bacterial colony growth in ventilator circuit shows a significant difference on Day-3 and Day-7 at 50% and 92% respectively (p = 0.05). A comparison for the bacterial similarity of the ventilator circuit and patient's sputum shows that the bacterial growth on Day-3 is 7 out of 14 (50%) and 3 with more than 10^5 CFU/ml colony; while on Day-7, there are 13 out of 14 positive bacterial growth, both in the circuit and the patient's sputum. Among them, 5 out of 14 (35%) of the bacterial colony which grow in the circuit have the same species as those grow in patient's sputum. The recent study shows that there is bacteria colony growth in the ventilator circuit after Day-3 and a significant increase on Day-7. Almost half of the colony illustrates similar species from both ventilator circuit and patient's sputum. This suggests that the bacterial growth on Day-7 in the ventilator circuit might be related to those growth in patient's sputum.

Keywords: *Ventilator Circuit, VAP, Bacterial Colony, Bacteria species*

ABSTRAK

Ventilator-associated pneumonia (VAP) masih menjadi problematik perawatan pasien di ICU dan menghabiskan biaya yang besar. VAP menyebabkan morbiditas dan mortalitas yang tinggi. VAP spesifik untuk infeksi nosokomial yang terjadi pada pasien yang mendapat ventilasi mekanik. Hubungan antara sirkuit ventilasi mekanik dengan terjadinya infeksi paru sudah dianggap sebagai suatu fakta, walaupun tanpa bukti ilmiah. Penelitian ini bertujuan untuk menganalisa pertumbuhan koloni bakteri pada sirkuit ventilator yang digunakan pada pasien di ruang observasi intensif RSUD Dr. Soetomo Surabaya. Penelitian ini menggunakan analisis observasional. Kultur bakteri diambil dengan menggunakan swab pada bagian inspirasi dari sirkuit ventilator pada 16 pasien yang dirawat dengan ventilasi mekanik di ruang observasi intensif RSUD Dr. Soetomo Surabaya. Sirkuit ventilator dilakukan swab pada hari ke-0, ke-3 dan ke-7, kemudian dilakukan perhitungan . pengambilan sampling sputum menggunakan tehnik double catheter tracheal aspiration. Hasil kemudian dianalisa menggunakan uji Chi square dan kesamaan spesies bakteri pada sirkuit dengan sputum pasien dianalisa dengan uji binomial. 2 sampel drop out karena terdapat pertumbuhan koloni bakteri pada hari ke-0. Terdapat perbedaan yang signifikan pertumbuhan koloni bakteri pada sirkuit ventilator hari ke-3 dan ke-7, 50% dan 92% dengan nilai p=0,05. Terdapat pertumbuhan koloni bakteri pada sirkuit ventilator 7 dari 14 sampel (50%) pada hari ke-3 dan 3 dengan jumlah koloni > 10^5 CFU/ml, sedangkan pada hari ke-7 terdapat pertumbuhan koloni 13 dari 14 sampel baik pada sirkuit ventilator maupun pada sputum pasien. Dan 5 (35%) diantaranya memiliki kesamaan spesies bakteri yang tumbuh pada sirkuit ventilator maupun pada sputum pasien. Penelitian ini menunjukkan ada nya pertumbuhan koloni bakteri setelah hari ke-3, dan terjadi peningkatan jumlah koloni yang signifikan pada

hari ke-7. 50% dari pertumbuhan koloni ini menunjukkan kesamaan spesien bakteri antara sirkuit ventilator dan sputum pasien. Hal ini menunjukkan ada kemungkinan pertumbuhan koloni bakteri pada harike-7 berkorelasi dengan kultur sputum pasien.

Kata kunci: *Sirkuit ventilator, VAP, Koloni Bakteri, Spesies bakteri*

INTRODUCTION

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection which occurs at the Intensive Care Unit (ICU) and plays an important role in increasing the number of mortality and morbidity. VAP specifically occurs in patients who are treated with mechanical ventilation. VAP which occurs in the first 48-72 hours after intubation is categorized as an early onset of VAP. It might be caused by aspiration as a complication during endotracheal tube insertion. VAP which occurs for more than 72 hours is identified as the late onset of VAP. It is directly correlated with mechanical ventilatory support.[1–4]

VAP as the most commonly occurs as nosocomial infection with 9%-40% incidence rate might prolong hospitalization (2-3x longer), prolongs the length of stay in ICU for 5-7 days, increases health cost, and increases mortality rate to 15-45%. VAP's cost is estimated to increase about $40,000 per patient and around $1.2 billion annually. Previous researches showed that pneumonia infection rate obtained in the hospital (nosocomial) was 15% of all hospital nosocomial infection and 24-27% of it occured at the ICU.[1,2]

Various researches about risk factors related to VAP (such as age, sex, trauma, COPD) and the use of mechanical ventilation have been done several times. Understanding the pathogenesis and epidemiology of VAP is important to formulate a preventive strategy to fight this infection. The most common source for VAP endemic is oropharynx colonization which is caused by endogenous flora or even pathogen flora from ICU environment; especially from the hand of the healthcare workers, contaminated mechanical ventilation, and the air and the water at the ICU. The digestive tract is also a potential source of secondary colonization and gram-negative of nosocomial bacteria storage. Microorganism aspiration from the oropharynx, gaster, and tracheal secretion around balloon of endotracheal tube towards lower respiratory tract, which are supposed to be sterile in normal condition, are the most common endemic source of VAP. Epidemically, VAP is mostly caused by contaminated ventilation, bronchoscopy, inhalation drugs, water (eg: Legionella sp.), and air (eg: Aspergillus sp.). A strategy to combat microbes from the oropharynx and digestive tract is by using chlorhexidine as an oral treatment, antimicrobial prophylaxis for inhalation drugs, or sucralfate as prophylaxis stress ulcer. Moreover, aspiration prevention through patient positioning and continuous suction in the subglotic area are also proven to decrease VAP risks. Disinfection of mechanical ventilation circuit and bronchoscopy, legionella-free hospital water, and infection control from the medical aerosol are important for VAP prevention. Routine inspection towards VAP as an early detection is important.[1,3–5]

The correlation between mechanical ventilation circuit and pulmonary infection is stated as a fact, however, there is no scientific explanation about it. Ting-Chang Hsieh did a cohort of observational study towards 96 patients who were divided into 2 groups; Group 1 underwent circuit replacement every 3 days, while Group 2 underwent circuit replacement every 7 days. Though there was no statistically significant difference, an increasing number of VAP incidence was observed. There was 13% in Group 1 and around 16% in Group 2; besides, there was an increase in mortality rate at 22% in Group 1 and 36% in Group 2. Other researches showed that the main cause of pneumonia in mechanical ventilation users was the colonization of the gastrointestinal tract, followed by aspiration around balloon of the endotracheal tube, and bacteria-transmitted health products.[5,6]

Although there is no research about VAP number in Indonesia, based on the worldwide database, it is stated that VAP incidence is high. The correlation between the mechanical ventilation and the occurrence of VAP is stated as a fact, because the mechanical ventilation circuit is proven to be contaminated by pathogen bacteria, especially from the patient's secretion.

At the Intensive Observation Unit of RSUD Dr. Soetomo, there is no procedure about the continual replacement of ventilation mechanic circuit, thus, it might become a primary cause of VAP infection there. This study is conducted to understand the bacterial colony growth in mechanical ventilation circuit at the Intensive Observation Unit of RSUD Dr. Soetomo and expected to standardize the procedure of mechanical ventilation circuit replacement at the Intensive Observation Unit of RSUD Dr. Soetomo.

METHOD

This study employs observational analysis design in time series (3x measurement) with all patients at the Intensive Observation Unit of RSUD Dr. Soetomo as the research subject. The inclusion criteria of this research are the patients who are using mechanical ventilation and exhibit no pneumonia sign and symptom previously based on CPIS criteria. The research consents are obtained from the family members to comply with the Medical Research Ethical regulation from RSUD Dr.Soetomo. While the exclusion criteria are patients with underlying diseases (COPD, ARDS, neuromuscular disease) and those who refuse to participate in this experiment. The drop out criteria are the patients with less than 7 days of extubation, died

in less than 7 days, and develop bacterial growth within 48 hours.

The methods exercised in this research aim to understand the growth of bacteria using bacterial colony measurement. Measuring bacterial colony can be done using pour plate method. Colony Counter is also used to facilitate the accurate calculation of the colony. The sample is taken in ventilator circuit on Day-0 before the circuit is connected to the patient, then it is re-done on Day-3 and Day-7 after the ventilator application. After bacterial colony is being calculated, it will be scored. Besides calculating the bacterial growth in different days, the bacterial culture is performed to study the bacterial species in patient's sputum. All subjects are treated with usual standardized VAP prevention. T-test analysis is also conducted to understand the difference between various colony growth on different days (Day-0, Day-3, and Day-7) for the patients who undergo mechanical ventilation treatments.

RESULT AND DISCUSSION

From 16 samples, 2 samples are dropped out due to bacterial growth on Day-0. The characteristic analysis and data distribution illustrate that male respondents are recorded in 42.9% and female in 57.1%.

Various colony growth is obtained from the sample. The data is categorized into 3 groups, namely a sterile group, a group with the colony number less than 105 CFU/ml, and a group with colony number more than 105 CFU/ml. Table 2 explains about the observed bacterial growth on Day-3 in which the number of sterile group is 7 (50%), while the less than 10^5 CFU/ml bacteria colony has 7 samples (50%). On Day-7, the number of sterile group is 1 sample (7.1%); the less than 10^5 CFU/ml bacterial colony has 10 samples (71.5%); and the more than 10^5 CFU/ml bacterial colony has 3 samples (21.4%).

Table 2 and Figure 1 illustrate the bacteria species growing in ventilator circuit on Day-3 are derived from 5 samples of Acinetobacter spp. (35.7%); 1 sample of Pseudomonas aeruginosa (7.1%); 1 sample of Klebsiella pneumonia (7.1%); and 7 samples with no bacteria growth (50%). On the 7th day, 7 samples (50%) of Acinetobacter spp.; 3 samples (21.4%) of Klebsiella pneumonia; 1 sample of Pseudomonas aeruginosa (7.1%); 2 samples of enterobacter aerogenes (14.3%); and 1 sample with no bacteria colony growth.

Table 1. Bacterial colony growth of the ventilator circuit on day 3 and day 7

Counted Bacterila Colony in the Ventilator Circuit	Observation Time	
	Day 3 (%)	Day 7 (%)
Steril	7 (50,0)	1 (7,1)
< 10^5 CFU/ml	7 (50,0)	10(71,5)
≥ 10^5 CFU/ml	-	3(21,4)

Table 2. Bacterial species in the ventilator circuit and sputum culture of the patient on day 3 and day 7

Bacterial Species	Ventilator Circuit		Sputum Culture	
	Day 3 (%)	Day 7 (%)	Day 3 (%)	Day 7 (%)
Steril	7(50,0)	1(7,1)	8(57,1)	1(7,1)
Acinobacter spp	5(35,7)	7(50,0)	5(35,7)	11(78,6)
Klebsiella pneumonia	1(7,1)	3(21,4)	-	-
Pseudomonas aeruginosa	1(7,1)	1(7,1)	1(7,1)	2(14,3)
Enterobacter aerogenes	-	2(14,3)	-	-

Furthermore, Table 2 and Figure 2 also displays the bacterial culture results of patient's sputum on Day-3, namely 5 samples of Acinetobacter sp (35.7%); 1 sample of Pseudomonas aeruginosa culture (7.1%); and 8 samples with no bacteria growth (57.1%). On the 7th day of evaluation, 11 samples of Acinetobacter sp. (78.6%); 2 samples with Pseudomonas aeruginosa culture (14.3%); and 1 sample with no bacterial growth (7.1%) are obtained.

Table 3 demonstrates data recapitulation about bacterial colony growth in ventilator circuit on Day-3 and Day-7. According to the data, Chi-square statistic test is done to understand the significant difference in bacterial colony growth in different days.

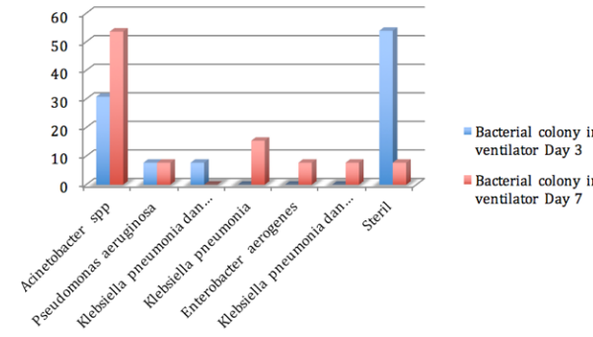

Figure 1. Bacterial species in the ventilator circuit on day 3 and day 7

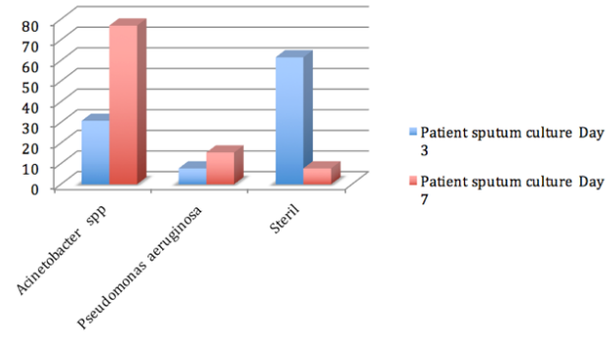

Figure 2. Patient Culture on Day 3 and Day 7

Table 3. Comparison test bacterial colony growth in the ventilator circuit on day 3 and day 7

Day 3	Day 7		Kappa	Harga p
	Positive Result (%)	Negative Result (%)		
Positive Result (%)	6 (42,9)	1 (7,1)	0,429	0,049
Negative Result (%)	3 (21,4)	4 (28,6)		

Table 4. Binomial test for similarity bacterial species between ventilator circuit and patient's sputum culture on day 3

Ventilator Circuit	Sputum Culture		Kappa	Harga p
	Positive Result (%)	Negative Result (%)		
Positive Result (%)	4 (28,6)	1 (7,1)	0,689	0,010
Negative Result (%)	1 (7,1)	8 (57,1)		

Table 5. Binomial test for similarity bacterial species between ventilator circuit and patient's sputum culture on day 7

Ventilator Circuit	Sputum Culture		Kappa	Harga p
	Positive Result (%)	Negative Result (%)		
Positive Result (%)	4 (28,6)	1 (7,1)	0,689	0,010
Negative Result (%)	1 (7,1)	8 (57,1)		

Table 6. Correlations between bacterial colony growth in the circuit ventilator with CPIS >6

CPIS 3 Day 3	CPIS 7 Day 7		CPIS 3 Day 3 and CPIS 7 Day 7
	Negative	Positive	
Negative	4	7	13
Positive	0	2	0.016

The statistic shows that there is a significant difference between bacterial growth in Day-3 and Day-7 (p = 0.049).

Table 4 shows that the observation on Day-3 found 7 samples with no bacterial colony in ventilator circuit or in patient's sputum (50%); 3 samples with similar bacterial species (21.4%); and 4 samples with no similar bacteria species in both ventilator circuit and patient's sputum (28.6%). Based on the statistical analysis using binomial test, there is a significant difference between both groups on Day-3 (p = 0.01).

Table 5 shows the observation on Day-7, it proves that there are 7 samples containing similar bacterial species in the ventilator circuit and patient's sputum (50%) and 7 samples without similar bacterial species in both locations. After being statistically tested using binomial test, the bacterial species in ventilator circuit and the patient's sputum on Day-7 show no significant difference (p = 0.515).

The correlation analysis of bacterial colony in ventilator circuit (CPIS> 6) is also tested in this research. Table 6 shows that during the Day-3 observation, there are 2 patients with CPIS more than 6; while during the Day-7 observation, there are 12 patients with CPIS more than 6. Using binomial test, it is proven that there is no significant difference in the amount of bacterial colony in ventilator circuit (CPIS> 6) on patients who undergo mechanical ventilation treatment.

Based on this research's comparative study, it is found that there is a significant difference between bacterial colony growth on Day-3 and Day-7 (p = 0.049). Bacterial colony growth in ventilator might be caused by many factors, such as humidifier which produces microorganism-carrying water drops, thus, the water inside the humidifier might be contaminated as well. Therefore, the water refill in humidifier should be done aseptically and use sterile fluid. The condenser in ventilator circuit might also contaminate the ventilator circuit, if the drainage is conducted thoroughly. Around 1980, there was a high risk of infection related to the contaminated nebulizer reservoir. A drug used by nebulizer might contaminate the ventilator circuit and penetrate into patient's respiratory tract. The contamination suffered by some health workers may also play a role in ventilator circuit contamination; therefore, all treatments (such as sterilization, ventilator circuit formation, and ventilator circuit maintenance) must be performed aseptically.[1,6–8]

The increase of bacterial colony significantly on Day-3 to Day-7 in this research still cannot predict the main role of ventilator circuit in VAP contamination, because of the VAP's complex pathogenesis and various factors possibly cause VAP. However, the bacterial colony growth in ventilator circuit still cannot be ignored, because the colony growth in ventilator circuit might penetrate the lung tissue through inhalation and further cause pneumonia. Based Long and Fink's researches, the suspension of maintenance and ventilator circuit replacement from once a day to twice a day might decrease VAP occurrence. While CDC and The Healthcare Injection Control Practices Advisory Committee have issued some guidelines for preventing health care-associated pneumonia, ventilator circuit replacement is recommended to be conducted only if the circuit is dirty or malfunctioned. Other research shows that ventilator circuit replacement over 48 hours is safe and might not increase VAP prevalence .

Table 7. Result of ventilator circuit and sputum culture

	Day 0			Day 3			Day 7		
	Bacterial colony growth in the circuit ventilator	**Bacterial Species in the circuit ventilator**	**Patient's Sputum culture**	**Bacterial colony growth in the circuit ventilator**	**Bacterial Species in the circuit ventilator**	**Patient's Sputum culture**	**Bacterial colony growth in the circuit ventilator**	**Bacterial Species in the circuit ventilator**	**Patient's Sputum culture**
Patient 1	Steril	-	Steril	> 10^5 CFU/ml	Acinetobacter sp	Acinetobacter sp	> 10^5 CFU/ml	Acinetobacter sp	Acinetobacter sp
Patient 2	Steril	-	Steril	45 CFU/ ml	Acinetobacter sp	Acinetobacter sp	10^5 CFU/ ml	Acinetobacter sp	Acinetobacter sp
Patient 3	Steril	-	Steril	Steril	-	Steril	10^5 CFU/ ml	Klebsiella pneumonia	Pseudomonas aeruginosa
Patient 4	Steril	-	Steril	75 CFU/ ml	Acinetobacter sp	Acinetobacter sp	> 10^5 CFU/ml	Acinetobacter sp	Acinetobacter sp
Patient 5	Steril	-	Steril	100 CFU/ ml	Acinetobacter sp	Pseudomonas aeruginosa	10^5 CFU/ ml	Acinetobacter sp	Acinetobacter sp
Patient 6	Steril	-	Steril	Steril	-	Steril	10^5 CFU/ ml	Acinetobacter sp	Acinetobacter sp
Patient 7	Steril	-	Steril	Steril	-	Steril	10^5 CFU/ ml	Klebsiella pneumonia	Acinetobacter sp
Patient 8	Steril	-	Steril	Steril	-	Steril	25 CFU/ ml	Enterobacter aerogenes	Steril
Patient 9	Steril	-	Steril	Steril		Steril	Steril	-	Acinetobacter sp
Patient 10	Steril	-	Steril	10^5 CFU/ ml	Klebsiella pneumonia	Acinetobacter sp	12.2 × 10^5 CFU/ ml	Klebsiella pneumonia	Acinetobacter sp
Patient 11	Steril	-	Steril	Steril	-	Steril	100 CFU/ ml	Acinetobacter sp	Acinetobacter sp
Patient 12	Steril	-	Steril	100 CFU/ ml	Pseudomonas aeruginosa	Steril	12.5 × 10^5 CFU/ ml	Pseudomonas aeruginosa	Acinetobacter sp
Patient 13	Steril	-	Steril	Steril	-	Steril	10^5 CFU/ ml	Acinetobacter sp	Pseudomonas aeruginosa
Patient 14	Steril	-	Steril	10^5 CFU/ ml	Acinetobacter sp	Acinetobacter sp	> 10^5 CFU/ml	Enterobacter aerogenes	Acinetobacter sp

CFU : Colony Form Unit

Table 7 is expected to inform the readers about the treatment and ventilator circuit replacement at the ICU. The similarity test, which is conducted to analyze bacteria growth in ventilator circuit and patient's sputum on Day-3, indicates a significant difference (p = 0.010). It might be caused by the complex pathogenesis of VAP. Therefore, the bacteria growth might be caused by many factors. The bacteria in patient's sputum might come from the digestive tract colonization of the pathogenic microorganism and increase the chance of contaminated secrete aspiration to lower respiratory tract; further, it might also colonize the oropharynx mucosal surface.[1,3,4] Optic fiber bronchoscopy, tracheal mucous suction, and respirator might induce pathogen bacteria contamination into the lower expiratory tract. The bacterial colony in ventilator circuit might be coming from the healthcare worker's hand contamination, water, air, or even patient's own secrete. Bacteria test similarity shows that the bacteria growth in ventilator circuit and patient's sputum culture on Day-7 has no significant difference (p = 0.515). From the Day-7 of observation, there is no proof whether the bacterial colonization of the patient's respiratory is coming from the ventilator circuit or not. The bacterial colony growth in patient's sputum reaches 50% of the total sample. It might be caused by the microorganism mapping at the ICU itself. Meanwhile, the bacteria commonly found in the ventilator circuit, humidifier, and respirometer are *Acinetobacter calcoaceticus, Burkholderia cereus,* and *Pseudomonas aeruginosa*.[1]

In this research, there is no conclusion can be drawn on whether bacterial colonization in patient's sputum is related to

the bacterial colonization in the ventilator circuit. Those factors may affect this research's results. This research also analyzes the correlation of the bacterial colony in ventilator circuit with CPIS criteria more than 6 on the patient with mechanical ventilation help and diagnosed as pneumonia. This research shows no significant correlation between bacterial colony size in ventilator circuit with CPIS > 6 with the help of mechanical ventilation, stated by p=0.016.

On the third day of observation, there are 2 patients with CPIS>16; while on the 7th day of observation, there are 13 patients with CPIS> 6 out of 14 patients in this research's sample. The early onset of VAP might be observed in less than 4 days and the late onset of VAP can be observed over 4 days after the patient is treated with mechanical ventilation. Pathogen which causes pneumonia on the early onset of VAP and late onset of VAP is usually different. The early onset of VAP mostly has a good prognosis. Moreover, the early onset of VAP might be caused by *Haemophilus influenzae* and *Streptococcus pneumonia*; while the late onset of VAP might be caused by high-risk pathogens, such as *Pseudomonas aeruginosa, Acinetobacter spp.,* and *Stenotrophomonas rnaltophilia*. As stated by Kollef, these high-risk pathogens record a high mortality rate (65%) compared to the late onset of VAP which is caused by other pathogen microorganisms (31%). Besides the contaminated ventilator circuit, there are various risk factors causing VAP, such as surgery. Post-surgery patient is exposed to higher rate of VAP occurrence. Based on American Society of Anesthesiologist, the risk factors of pneumonia post surgery include smoking, long inpatient treatment, longer surgery operation, thorax, and upper abdomen surgery.[8–10]

The irrational antibiotic usage also plays a role in the occurrence of nosocomial pneumonia and the infection of antibiotic resistant microbes. Stress ulcer prophylaxis also influences bacterial colonization in the digestive tract. The prophylaxis stress ulcer which is unable to change gastric pH have a lower bacterial colonization rate and in turn, may lower the pneumonia nosocomial rate as well. Endotracheal tube, tracheostomy, and reintubation may also lower patient's immune system and further causes local trauma and inflammation. It can increase the probability of pathogen bacterial aspiration from the oropharynx area, specifically around the cuff. Oropharyngeal secretion's continuous suction, elevated head position, the use of nasogastric tube, and enteral feeding play a part in VAP occurences.[9,10]

CONCLUSION

The recent study shows that there are bacterial colony growth in the ventilator circuit after Day-3 and significant increase in the number of colony on Day-7. Almost half of the colony displays similar species in both ventilator circuit and patient's sputum. This suggests that the growth of bactery on Day-7 in the ventilator circuit might be related to the growth in patient's sputum.

REFERENCES

1. Safdar N, Crnich CJ, Maki DG. The pathogenesis of ventilator-associated pneumonia: its relevance to developing effective strategies for prevention. Respir Care. 2005 Jun;50(6):725-39-41.
2. Hooser T Van. Ventilator-Associated Pneumonia (VAP) Best Practice Strategies for Caregivers By D . Theron Van Hooser. Kimberly-Clark Heal Care. 2002;
3. Kollef MH. The Prevention of Ventilator-Associated Pneumonia. N Engl J Med. 1999 Feb 25;340(8):627–34.
4. Wiryana M. VENTILATOR ASSOCIATED PNEUMONIA. J Penyakit Dalam. 2007 Sep;8(3):1–15.
5. Branson RD. The ventilator circuit and ventilator-associated pneumonia. Respir Care. 2005 Jun;50(6):774-87-7.
6. Hess DR, Kallstrom TJ, Mottram CD, Myers TR, Sorenson HM, Vines DL, et al. Care of the ventilator circuit and its relation to ventilator-associated pneumonia. Respir Care. 2003 Sep;48(9):869–79.
7. Craven DE, Kunches LM, Kilinsky V, Lichtenberg DA, Make BJ, McCabe WR. Risk factors for pneumonia and fatality in patients receiving continuous mechanical ventilation. Am Rev Respir Dis. 1986 May;133(5):792–6.
8. Chastre J, Fagon J-Y. Ventilator-associated pneumonia. Am J Respir Crit Care Med. 2002 Apr 1;165(7):867–903.
9. Garibaldi RA, Britt MR, Coleman ML, Reading JC, Pace NL. Risk factors for postoperative pneumonia. Am J Med. 1981 Mar;70(3):677–80.
10. Rello J, Ausina V, Ricart M, Castella J, Prats G. Impact of previous antimicrobial therapy on the etiology and outcome of ventilator-associated pneumonia. Chest. 1993 Oct;104(4):1230–5.