African Journal of Biotechnology Vol. 10(10), pp. 1926-1931, 7 March, 2011
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Application of a nano-antimicrobial film to prevent ventilator-associated pneumonia: A pilot study

W. Li[1], X. Ma[1], Y. Peng[1], J. Cao[1], W. T. Y. Loo[2,3,4]*, L. Hao[5], M. N. B. Cheung[4], L. W. C. Chow[4] and L. J. Jin[3]

[1]ICU, Shenzhen People's Hospital, Second Clinical Teaching Medical Centre, Medical College of Jinan University.
[2]School of Chinese Medicine, University of Hong Kong.
[3]Faculty of Dentistry, University of Hong Kong, Hong Kong.
[4]UNIMED Medical Institute and Organisation for Oncology and Translational Research Hong Kong.
[5]State Key Laboratory for Oral Diseases and Department of Prosthodontics, West China Hospital of Stomatology, Sichuan University, PR China.

Accepted 3 February, 2011

Ventilator-associated pneumonia (VAP) is one of the most common hospital-associated infections and has accounted for approximately 15% of all hospital-associated infections. In 76% of the VAP cases, the same bacteria colonize the oral cavity and lungs. Oral care interventions may play a role in the prevention of VAP, yet more than half of the hospitals do not have specific policies for the oral care of intubated patients. Oral cavity interlinks with respiratory tracts and digestive tracts. After surgery has been performed in these areas, aerobic and anaerobic bacteria frequently induce operative wound infections in teeth, gingiva and supporting tissues of the teeth and tonsils. This study investigates the effects of a nanotechnology antimicrobial spray (JUC) on the incidence of VAP. 320 patients diagnosed with VAP were randomly divided into treatment and control groups. After using chlorhexidine mouthrinse, the treatment group used a nanotechnology antimicrobial spray to the nose and mouth. The control group was given normal saline. The incidence rate of VAP was significantly lower in the treatment (8.38%) than control group (54.24%) ($p<0.01$). A physical antimicrobial film is formed on the surface of oral and nasal mucosa after using the JUC spray which effectively reduces the microbial colonization in the sprayed areas, thus reducing and delaying the incidence of VAP.

Key words: Ventilator-associated pneumonia, oral care, nanotechnology antimicrobial spray, bacterial colonization.

INTRODUCTION

Ventilator-associated pneumonia (VAP) is one of the most common hospital-associated infections and has accounted for approximately 15% of all hospital-associated infections. It has been the second most common hospital-associated infection following its occurrence in the urinary tract, for which the mortality ranges from 1 to 4%. The mortality rate for VAP which is defined as pneumonia occurring more than 48 h after endotracheal intubation and initiation of mechanical ventilation, ranges from 24 to 50% and can reach 76% in some specific settings or when lung infection is caused by high-risk pathogens (U.S. Department Of Health And Human Services Public Health Service Centers for Disease Control, 1997; Haley et al., 1981; Centers for Disease Control and Prevention, 2000; National Nosocomial Infections Surveillance (NNIS) System, 1999; Bell et al., 1983; Celis et al., 1988; Chastre et al., 1998; Chevret et al., 1993; Craven et al., 1986; Craven and Steger, 1996; Cross and Roup, 1981; Delclaux et al., 1997; Fagon et al., 1989; Haley et al., 1981; Langer et al., 1989; Markowicz et al., 2000; Rello et al., 1993; Rello et al., 1997; Torres et al., 1990; Vincent

*Corresponding author: E-mail: tyloo@hku.hk. Tel: 852-9074-9468. Fax: 852-2861-1386.

Abbreviations: VAP, Ventilator-associated pneumonia; **WBC,** white blood cell; **CFU,** colony formation unit; **BAL,** bronchoalveolar lavage; **CPIS,** clinical pulmonary infection score; **ICU,** intensive care unit.

et al., 1995). VAP is the most common infectious complication among patients admitted to intensive care units (ICUs) and accounts for up to 47% of all infections among ICU patients (Charitos et al., 2009; Leroy et al., 2001). ICU patients are at high risk of infection with *Staphylococcus aureus*, whereas *Haemophilus influenzae* and *Streptococcus pneumoniae* usually dominate in postsurgical trauma patients. VAP prolongs ICU's stay and increases treatment costs as well as the risk of death in critically ill patients (Carolyn et al., 2007; Chevret et al., 1993; Vincent et al., 1995). In 76% of the VAP cases, the same bacteria colonize the oral cavity and lungs (Chastre and Fagon, 2002; Doré et al., 1996). Oral care interventions may play a role in the prevention of VAP, yet more than half of the hospitals do not have specific policies for the oral care of intubated patients (Carolyn et al., 2007; Doré et al., 1996; Marra et al., 2009). Oral cavity interlinks with respiratory tracts and digestive tracts. After surgery has been performed in these areas, aerobic and anaerobic bacteria frequently induce operative wound infections in teeth, gingiva and supporting tissues of the teeth, tonsils, etc. (Salam et al., 2001; Senpuku et al., 2002; 2006). These infected areas generally offer beneficial environment, i.e. suitable temperature and humidity for bacterial proliferation leading to frequent infections.

In general, infections are commonly found in oral cancer patients after surgical excision of the tumor (Senpuku et al., 2003; Senpuku et al., 2006; Tada and Tanzawa, 2002; Tada et al., 2002; Zeng et al., 2008). This could be due to exposure of wounds during and after the operation. Patients, who received oral surgery often appear to have complications relating to bacterial infections. Colonization of pathogenic bacteria in oral cavity is thought to increase the risk of infections such as pneumonia and bacteremia (Costerton and Greenberg, 1999; Gosney et al., 1999). Therefore, it is of high importance to prevent bacteria from entering the lungs orally or nasally.

Currently, the systemic applications of antibacterial drugs have shown better results in curing diseases than local application, which may induce drug-resistant bacteria in the particular area (Belusic-Gobic et al., 2007; Cloke et al., 2004). A nanotechnology antimicrobial spray, JUC, physical antimicrobial dressing was applied to some affected areas of oral cancer patients after surgery and proved to be a new physical antimicrobial method that does not have the tendency to lead to drug resistance (Zeng et al., 2008).

In this study, JUC spray was applied to the oral and nasal cavities of intubated patients in ICU to compare the incidence of VAP with conventional oral care.

MATERIALS AND METHODS

Actions and the quality control of JUC

The antimicrobial effect and quality of JUC spray were monitored and controlled by NMS Technologies Company (Nanjing, China). When water-soluble liquid of JUC was sprayed on skin surfaces or mucosal areas, it immediately solidifies and forms an invisible antimicrobial layer with dual overlapping structure; the bonded film and the positive charge film. The bonded film is composed of macromolecular agents, securely boned to the body surface by means of chemical bonds. This bonded film has a long acting effect to prevent microbial growth. The positive charge film is composed of cationic activators to form a reticulate film with positive charge of the skin surface or mucosal area. The positive film strongly absorbs the pathogenic microorganism with a negative charge, such as bacteria, fungi, and viruses. If the pathogenic microorganisms' respiratory enzyme on which they rely for existence is out of action, they will die due to a lack of oxygen supply (Zeng et al., 2008).

This spray had been tested by Food & Drug Analytical Services Limited (Approval no: 9083481, USA) against *Acinetobacter baumanii* on a range of surfaces. JUC had passed all the tests on floor, metal handle, perspex, plastic handle and steel surfaces. Also, JUC had been tested by the University of New Brunswick (CE approval No: 153038905) on the zeta potential and hydrodynamic size of the dress sample. JUC demonstrated high zeta-potential values over a broad range of pH and the hydrodynamic size of the sample was 2.57nm in 0.5% aqueous solution.

Selection of subjects

From January 2009 to March 2010, 320 ICU patients requiring mechanical ventilation were recruited from Shenzhen People's Hospital. Each patient was numbered and those of odd numbers were assigned to the treatment group (167 cases) and even-numbered were the control group (153 cases). Patients satisfying the following conditions were excluded: Under 18 years of age, history of using mechanical ventilation, pregnancy or lactating, pneumonia, bronchiectasis, hemoptysis, pulmonary cyst or pulmonary fibrosis (Munro et al., 2009). Both treatment and control groups had teeth, oral mucosa, tongue and palate cleansed by chlorohexidine mouthrinse every 8 h, 3 times daily for 5 days. Suction of 0.2 bar was used to withdraw mouthrinse from patients' mouth. The treatment group was sprayed with JUC spray orally and nasally after mouthrinse. The studied protocols were approved by the Ethics Committee of Shenzhen People's Hospital.

Sample Collection

Tracheal secretion together with oral, nasal and throat swabs of the patients were collected every 4 h for 5 days for bacterial culture and identification after 24 h of intubation. Deep sputum samples were collected by protected specimen brush under bronchoscopy.

Criteria for diagnosis of VAP

The diagnosis of VAP must include persistent radiographic infiltration over 48 h, temperature over 38.5°C, total white blood cell (WBC) count $\geq 10\times10^9$/L and colony formation unit (CFU) test results over 10^3cfu/ml on protected specimen brush or bronchoalveolar lavage (BAL) fluid over 10^4cfu/ml (Elie et al., 2006). According to the onset time, there are two clinical types of VAP, the early and late-onset VAP. The early-onset VAP is pneumonia that occurred within 48-96 h after intubation and mechanical ventilation while the late-onset VAP occurred more than 96 h after mechanical ventilation (Qinhua and Lixian, 2004).

Statistical analysis

The SPSS 11.0 software package was used to collect and analyze

Table 1. General information of 320 patients ($\bar{x} \pm s$).

Parameter	Treatment group (n=167)	Control group (n=153)	*t* value	*p* value
Age(years)	57.4 ±15.2	55.1 ±14.8	1.371	>0.05
Observation days	8.41 ±2.10	8.27 ±2.07	0.596	>0.05
APACHE ii score.	21.62 ±6.78	22.47 ±6.27	1.164	>0.05
CPIS scores	3.85 ±1.58	4.03 ±1.62	1.006	>0.05

Table 2. Incidence of early-onset VAP patients.

Group	Number of cases	Incidence VAP (%)	X^2 p value	Early-onset VAP (%)	X^2 p value
Control	153	83 (54.24)	79.51	42 (50.60)	46.41
Treatment	167	14 (8.38)	*p*<0.01	2 (14.29)	*p*<0.01

Table 3. Pathogens found in pharynx oralis (strains).

Group	A	B	C	D	E	F	G	H	I	J	K	Total strains
Control	63	50	38	36	30	22	18	20	277*	22	25	324**
Treatment	8	7	5	4	3	2	1	2	32*	2	3	37**

A, Klebsiella pneumonia; B, *Pseudomonas aeruginosa*; C, *Acinetobacter*; D, *Pseudomonas maltophilia*; E, *Escherichia coli*; F, *Enterobacter cloacae*; G, *Streptococcus pneumonia*; H, *Staphylococcus aureus*; I, total number of VAP caused bacteria; J, *Candida tropicalis*; K, *Candida albicans*. *Significant difference between both groups at *P*<0.01; **significant difference between the treatment group and the control group at *P*<0.01.

the clinical data expressed as $\bar{x} \pm$SD. The Q-test in analysis of variance was used to compare data between two groups. The t test in paired design was used to compare data collected at different time points within the groups. The rank test was used to compare the rate and constituent ratio of VAP.

RESULTS AND DISCUSSION

General information of patients

There were no significant differences in age, gender, reasons for ICU admission, acute physiology and chronic health evaluation (APACHE) II, clinical pulmonary infection score (CPIS) or days of admission to ICU between both groups prior to recruitment for this study (Table I).

Incidence of VAP

VAP occurred in 14 patients (8.38%) of the treatment group and 83 patients (54.24%) of the control group. Statistically, significant difference (p<0.01) was observed between the two groups (Table 2). Early-onset VAP was observed in 2 patients (14.29%) of the treatment group and 42 patients (50.60%) of the control group with significant difference (p<0.01) (Table 2).

Bacterial culture

10 types of pathogens were collected from 320 patients. 324 strains were isolated in the control group and 37 strains in the treatment group. The isolated strains were mainly composed of Gram negative bacteria including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter*. There were significant difference (p<0.01) between the two groups (Table 3).

Deep sputum culture

10 types of pathogens were cultured in 320 patients. 268 strains were isolated in the control group and 33 strains in the treatment group. The sputum cultures were mainly composed of *Klebsiella pneumoniae* and *pseudomonas aeruginosa* with significant difference (p<0.01) between the groups (Table 4).

Bacterial colonization rate

There was statistically significant difference (p<0.01) between two groups for endotracheal colonization less than 96 h, while no difference was observed for over 96 h. The opposite is applied to oropharyngeal colonization as no

Table 4. Pathogens found in deep phlegm (strains).

Group	A	B	C	D	E	F	G	H	I	J	K	Total strains
Control	56	56	30	30	26	26	7	13	244*	14	10	268**
Treatment	8	7	3	3	4	4	2	0	31*	2	0	33**

A, Klebsiella pneumonia; B, *Pseudomonas aeruginosa*; C, *Acinetobacter*; D, *Pseudomonas maltophilia*; E, *Escherichia coli*; F, *Enterobacter cloacae*; G, *Streptococcus pneumonia*; H, *Staphylococcus aureus*; I, total number of VAP caused bacteria; J, *Candida tropicalis*; K, *Candida albicans*.
*Significant difference between both groups at $P<0.01$; **significant difference between the treatment group and the control group at $P<0.01$.

Table 5. The rate of bacterial colonization in trachea and pharynx oralis between the two groups.

Region	Time of colony formation (h)	Treatment group (n=167)		Control group (n=153)		χ^2	P value
		No. of cases	Bacterial colonization (%)	NO. of cases	Bacterial colonization (%)		
Endotracheal	<96h	4	2.40	5	3.27	0.22	$P>0.05$
Mouth	>96h	7	4.19	34	22.22	23.24	$P<0.01$
Nose	<96h	5	2.99	7	4.58	0.55	$P>0.05$
Pharyngeal cavity	>96h	8	4.79	50	32.68	41.85	$P<0.01$

statistically significant difference exists between the two groups under 96 h, but there was significant difference ($p<0.01$) over 96 h (Table 5).

VAP is defined as pneumonia in patients receiving mechanical ventilation and it is also a leading cause of sepsis with acute respiratory failure and a significant contributor to morbidity and mortality in intensive care unit patients (Leroy et al., 2001; Tejerina et al., 2010). During 1992 to 2004, NNIS report reveals a median rate of VAP associated with mechanical ventilation to be 2.2 to 14.7 cases per 1000 patients per day in adult ICUs. The estimated mortality rate is between 20 and 70% (Cuellar et al., 2004).

VAP can be categorized into:

Early-onset VAP (EOP) occurs during the first 4 days of trachea cannula and artificial airway establishment and accounts for 50% of VAP, most often caused by pharyngeal parasitic bacterium (such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*).

Late-onset VAP occurs at least 5 days after intubation and is most often caused by gram negative bacterium (for example, *Enterobacter*, *Acinetobacter,* and *Pseudomonas aeruginosa*) (Badia and Torres, 2008; Niederman and Craven, 2005; Diaz et al., 2009; Medford et al., 2009).

There is a wide range of bacteria in the mouth, nose and pharynx, including various potential pathogenic bacteria. The barriers of these areas plus lower respiratory tract of patients receiving mechanical ventilation are directly destroyed. Transient pressure decreased by air sac, change in posture or airway diameter cause the secretion to pass to the lower respiratory tract through the gap between endotracheal wall and catheter (Marra et al., 2009).

Contemporary oral hygiene for ICU patients mainly uses normal saline or chlorhexidine mouth rinse to clean the oral cavity but they have no or short term disinfection effect. Antibiotic solution may increase the risk of resistance for pathogenic bacteria and is not recommended (Díaz et al., 2010). Many international scholars are exploring effective oral hygiene methods to reduce the incidence of VAP (Gastmeier and Geffers, 2007; Heyland et al., 2002; Keenan et al., 2002; Livingston, 2000). JUC Spray Dressing can provide the antimicrobial effect for 8 h and produces no drug resistance, providing an innovative solution to prevent the incidence of VAP.

The mechanism of JUC in reducing the incidence rate of VAP by killing and inhibiting pathogenic microorganism by electrostatic force. JUC spray has little irritation to mucous membrane and does not cause drug resistance after long term usage.

In this study, it was found that patients who had JUC spray applied to oral and nasal cavities had lower incidence rates of VAP, the proportion of early-onset VAP and bacterial colonization in trachea, mouth, nose and pharyngeal portion was compared to the control group. JUC is a safe and effective physical antimicrobial spray dressing for mouth, nose and pharyngeal cavity. Although there was no significant difference in the incidence of bacterial colonization in mouth, nose, pharyngeal cavity and trachea between the two groups of early-onset VAP (<96h), the colonization rate of early onset VAP (<96h) in the treatment group was lower than that in the control group.

JUC is a physical antimicrobial agent that can replace contemporary disinfectants for oral care and alleviate the setback of clinical drug resistance. It is a new method for preventing the incidence of VAP safely and effectively.

ACKNOWLEDGEMENTS

We would like to thank JUC-NMS Technologies Company Nanjing, China, for providing nanotechnology antimicrobial spray (JUC) in this pilot clinical study. We also appreciate Miss Wei Li, the chief of critical care nursing group at ICU of Shenzhen People's Hospital for sacrifice in the observation of patients and data entry.

REFERENCES

Badia JR, Torres A (2008). Ventilator-Associated Pneumonia. In: Mechanical Ventilation (First Edition). P Peter J, Md, Fccm, L Burkhard, PhD, A Editorial. Philadelphia: W.B. Saunders, pp. 645-649.

Bell RCCJ, Smith JD, Johanson WG (1983). Multiple organ system failure and infection in adult respiratory distress syndrome. Ann Int. Med. 99: 293-298.

Belusic-Gobic MCM, Juretic M, Cerovic R, Gobic D, Golubovic V (2007). Risk factors for wound infection after oral cancer surgery. Oral Oncol. 43: 77-81.

Carolyn L, Cason TT, Sue Saunders (2007). Nurses' Implementation of guidelines for ventilator-associated Pneumonia from the centers for disease control and prevention. 16: 28-37.

Celis RTA, Gatell JM, Almela M, Rodriguez-Roisin R, Agusti-Vidal A (1988). Nosocomial pneumonia. A multivariate analysis of risk and prognosis. Chest. 93: 318-324.

Charitos T, van der Gaag LC, Visscher S, Schurink KAM, Lucas PJF (2009). A dynamic Bayesian network for diagnosing ventilator-associated pneumonia in ICU patients. Expert Syst. Applications, 36(2, Part 1): 1249-1258.

Chastre J, Fagon J (2002). Ventilator-associated pneumonia. Am. J. Respir. Crit. Care Med. 165: 867-903.

Chastre JTJ, Vuagnat A, Joly-Guillou ML, Clavier H, Dombret MC, Gibert C (1998). Nosocomial pneumonia in patients with acute respiratory distress syndrome. Am. J. Respir. Crit. Care, 157: 1165-1172.

Chevret SHM, Carlet J, Langer M (1993). Incidence and risk factors of pneumonia acquired in intensive care units. Results from a multicenter prospective study on 996 patients. European Cooperative Group on Nosocomial Pneumonia. Intensive Care Med. 19: 256-264.

Cloke DJGJ, Khan AL, Hodgkinson PD (2004). McLean NR. Factors influencing the development of wound infection following free-flap reconstruction for intra-oral cancer. Br. J. Plast Surg. 57: 556-560.

Costerton JWSP, Greenberg EP (1999). Bacterial biofilms: a common cause of persistent infections. Science, 284: 1318-1322.

Craven DE KL, Kilinsky V, Lichtenberg DA, Make BJ, McCabe WR (1986). Risk factors for pneumonia and fatality in patients receiving continuous mechanical ventilation. Am. Rev. Respir. Dis. 133: 792-796.

Craven DE, Steger K (1996). Nosocomial pneumonia in mechanically ventilated adult patients: epidemiology and prevention in 1996. Semin Respir. Infect. 11: 32-53.

Cross AS, Roup B (1981). Role of respiratory assistance devices in endemic nosocomial pneumonia. Am. J. Med. 70: 681-685.

Cuellar Ponce de Leon L, Rosales R, Rosenthal VD (2004). Prospective Study To Evaluate Mechanical Ventilator-Associated Pneumonia Rate in Intensive Care Units in a Peruvian Public Hospital: Benchmark with NNIS American Rates. Am. J. Infect. Control, 32: E114-E115.

Díaz LA, Llauradó M, Rello J, Restrepo MI (2010). Prevención no farmacológica de la neumonía asociada a ventilación mecánica. Archivos de Bronconeumología, 46: 188-195.

Delclaux CRE, Blot F, Brochard L, Lemaire F, Brun-Buisson C (1997). Lower respiratory tract colonization and infection during severe acute respiratory distress syndrome: incidence and diagnosis. Am. J. Respir. Crit. Care Med. 156: 1092-1098.

Diaz E, Ulldemolins M, Lisboa T, Rello J (2009). Management of Ventilator-Associated Pneumonia. Infect. Dis. Clin. North Am. 23: 521-533.

Doré PRR, Grollier G, Rouffineau J, Lanquetot H, Charrière JM, Fauchère JL (1996). Incidence of anaerobes in ventilator-associated pneumonia with use of a protected specimen brush. Am. J. Respir. Crit. Care Med. 153: 1292-1298.

Elie A, Jean-Francois T, Muriel T (2006). Candida Colonization of the Respiratory Tract and Subsequent Pseudomonas Ventilator-Associated Pneumonia. Chest. 129: 110-117.

Fagon JYCJ, Domart Y, Trouillet JL, Pierre J, Darne C, Gibert C (1989). Nosocomial pneumonia in patients receiving continuous mechanical ventilation. Prospective analysis of 52 episodes with use of a protected specimen brush and quantitative culture techniques. Am. Rev. Respir. Dis. 139: 877-884.

Gastmeier P, Geffers C (2007). Prevention of ventilator-associated pneumonia: analysis of studies published since 2004. J. Hospital Infect. 67: 1-8.

Gosney MAPA, Corkhill J, Millns B, Martin MV (1999). Pseudomonas aeruginosa septicaemia from an oral source. Br. Dent. J. 187: 639-640.

Haley RWHT, Culver DH, Stanley RC, Emori TG, Hardison CD, Quade D, Shachtman RH, Schaberg DR, Shah BV (1981). Nosocomial infections in US hospitals, 1975-1976: estimated frequency by selected characteristics of patients. Am. J. Med. 70: 947-959.

Qinhua He, Iixian He (2004). Progress of Study in Preventing Ventilator-Associated Pneumonia. Foreign Medicine, Fascicule of Respiratory System. 6: 122-124.

Heyland DK, Cook DJ, Dodek PM (2002). Prevention of ventilator-associated pneumonia: Current practice in Canadian intensive care units. J. Crit. Care, 17: 161-167.

Keenan SP, Heyland DK, Jacka MJ, Cook D, Dodek P (2002). Ventilator-associated pneumonia: Prevention, diagnosis, and therapy. Crit. Care Clin. 18: 107-125.

Langer M MP, Cigada M, Mandelli M (1989). Long-term respiratory support and risk of pneumonia in critically ill patients. Intensive Care Unit Group of Infection Control. Am. Rev. Respir. Dis. 140: 302-305.

Leroy O, Sanders V, Girardie P, Devos P, Yazdanpanah Y, Georges H (2001). Mortality due to ventilator-associated pneumonia: Impact of medical versus surgical ICU admittance status. J. Crit. Care, 16: 90-97.

Livingston DH (2000). Prevention of ventilator-associated pneumonia. Am. J. Surg. 179(Sup 1): 12-17.

Markowicz PWM, Djedaini K, Cohen Y, Chastre J, Delclaux C, Merrer J, Herman B, Veber B, Fontaine A (2000). Multicenter prospective study of ventilator-associated pneumonia during acute respiratory distress syndrome. Incidence, prognosis, and risk factors. Am. J. Respir. Crit. Care Med. 161: 1942-1948.

Marra AR, Cal RGR, Silva CV, Caserta RA, Paes ÂT, Moura Jr DF (2009). Successful prevention of ventilator-associated pneumonia in an intensive care setting. Am. J. Infect. Control, 37: 619-625.

Medford ARL, Husain SA, Turki HM, Millar AB (2009). Diagnosis of ventilator-associated pneumonia. J. Crit. Care, 24: 473.e471-473.e476.

Munro CL, Grap MJ, Jones DJ, McClish DK, Sessler CN (2009). Chlorhexidine, toothbrushing, and preventing ventilator-associated pneumonia in critically ill adults. Am. J. Crit. Care, 18: 428-437.

National Nosocomial Infections Surveillance (NNIS) System (1999). National Nosocomial Infections Surveillance (NNIS) System report, data summary from January 1990-May 1999. Am. J. Infect. Control, 27: 520-532.

Niederman MS, Craven DEC (2005). Guidelines for the Management of Adults with Hospital-acquired, Ventilator-associated, and Healthcare-associated Pneumonia. Am. J. Respir. Crit. Care Med. 171: 388-416.

Prevention CfDCa (2000). Monitoring hospital- acquired infections to promote patient safety: United States, 1990- 1999. MMWR. 49: 149-153.

Rello JAV, Ricart M, Castella J, Prats G (1993). Impact of previous antimicrobial therapy on the etiology and outcome of ventilator-associated pneumonia. Chest. 104: 1230-1235.

Rello JRM, Jubert P, Muses G, Sonora R, Valles J, Niederman MS (1997). Survival in patients with nosocomial pneumonia: impact of the severity of illness and the etiologic agent. Crit. Care Med. 25: 1862-1867.

Salam MASH, Nomura Y, Matin K, Miyazaki H, Hanada NM (2001).Isolation of opportunistic pathogens in dental plaque, saliva and tonsil samples from elderly. Jpn. J. Infect. Dis. 54: 193-195.
Senpuku H SA, Inoshita E, Tsuha Y, Miyazaki H, Hanada N (2003). Systemic disease in association with microbial species in oral biofilm from elderly requiring care. Gerontology, 49: 301-309.
Senpuku H TA, Takada M, Sato Y, Hanada N (2002). Reproducibility of oral bacterial isolation in the elderly. Jpn. J. Infect. Dis. 33: 61-62.
Senpuku H TA, Uehara S, Kariyama R, Kumon H (2006). Postoperative infection by pathogenic micro-organisms in the oral cavity of patients with prostatic carcinoma. J. Int. Med. Res. 34: 95-102.
Tada AHN, Tanzawa H (2002). The relation between tube feeding and pseudomonas aeruginosa detection in the oral cavity. J. Gerontol. Biol. Sci. Med. Sci. 57: M71-72.
Tada A WT, Yokoe H, Hanada N, Tanzawa H (2002). Oral bacteria influenced by the functions status of the elderly people and type and quality of facilities for the bedridden. J. Appl. Microbiol. 93: 487-491.
Tejerina E, Esteban A, Fernández-Segoviano P, Frutos-Vivar F, Aramburu J, Ballesteros D (2010). Accuracy of clinical definitions of ventilator-associated pneumonia: Comparison with autopsy findings. J. Crit. Care, 25: 62-68.
Torres AAR, Gatell JM, Jimenez P, Gonzalez J, Ferrer A, Celis R, Rodriguez-Roisin R (1990). Incidence, risk, and prognosis factors of nosocomial pneumonia in mechanically ventilated patients. Am. Rev. Respir. Dis. 142: 523-528.
U.S. Department Of Health And Human Services Public Health Service Centers for Disease Control (1997). Guidelines for Prevention of Nosocomial Pneumonia. 46: RR-1.
Vincent JLBD, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, Wolff M, Spencer RC, Hemmer M (1995). The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. JAMA. 274: 639-644.
Zeng Y, Deng R, Barry HS, Yeung W, Loo TY, Mary Cheung NB, Chen JP, Bingrong Z, Yifu F, Lanzhu H, Mingxing L, Min W (2008) Application of an antibacterial dressing spray in the prevention of post-operative infection in oral cancer patients: A phase 1 clinical trial. Afr. J. Biotechnol. 7: 3827-3831.

非洲生物技术杂志 Vol. 10(10), pp. 1926-1931

纳米抗微生物膜在预防呼吸机相关性肺炎中的应用：一项初步研究

李威[1]，马晓华[1]，彭粤铭[1]，曹静[1], W. T. Y. Loo[2,3,4*] , L. Hao[5], M. N. B. Cheung[4], L. W. C. Chow[4], L. J. Jin[3]

1 深圳市人民医院重症监护室，暨南大学医学院第二临床教学中心。

2 香港大学中医药学院。

3 香港大学牙医学院。

4 香港 UNIMED 医学研究所及肿瘤与转化研究组织。

5 四川大学华西口腔医院口腔疾病国家重点实验室及修复科。

收稿日期：2011 年 2 月 3 日

摘要：呼吸机相关性肺炎（VAP）是最常见的医院获得性感染之一，约占所有医院感染的 15%。在 76%的 VAP 病例中，口腔和肺部定植的细菌相同。口腔护理干预可能在预防 VAP 中发挥作用，但超过一半的医院未制定针对插管患者口腔护理的具体政策。口腔与呼吸道和消化道相连，这些区域手术后，需氧菌和厌氧菌常引发牙齿、牙龈、牙周组织及扁桃体的手术伤口感染。本研究探讨了一种纳米技术抗微生物喷雾剂（JUC）对 VAP 发病率的影响。320 例诊断为 VAP 的患者随机分为治疗组和对照组。治疗组在使用氯己定漱口水后，使用纳米抗微生物喷雾剂喷洒口鼻；对照组使用生理盐水。治疗组 VAP 发病率（8.38%）显著低于对照组（54.24%）（$p<0.01$）。使用 JUC 喷雾后，口腔和鼻黏膜表面形成物理抗微生物膜，有效减少喷洒区域的微生物定植，从而降低并延迟 VAP 的发生。

关键词：呼吸机相关性肺炎，口腔护理，纳米技术抗微生物喷雾剂，细菌定植。

引言

呼吸机相关性肺炎（VAP）是最常见的医院获得性感染之一，约占所有医院感染的 15%。它是继尿路感染之后第二常见的医院感染，死亡率在 1%至 4%之间。VAP 被定义为气管插管和机械通气开始 48 小时后发生的肺炎，其死亡率范围为 24%至 50%，在某些特定情况下或由高风险病原体引起的肺部感染中，死亡率可高达 76%（美国卫生与公众服务部公共卫生服务中心疾病控制中心，1997；Haley 等，1981）。VAP 是重症监护病房（ICU）患者最常见的感染性并发症，占 ICU 患者所有感染的 47%（Chartios 等，2009；Leroy 等，2001）。ICU 患者感染金黄色葡萄球菌的风险较高，而术后创伤患者通常以流感嗜血杆菌和肺炎链球菌为主。VAP 会延长 ICU 住院时间，增加治疗成本，并提高危重患者的死亡风险（Carolyn 等，2007；Chevret 等，1993；Vincent 等，1995）。在 76%的 VAP 病例中，口腔和肺部定植的细菌相同（Chastre 和 Fagon，2002；Doré等，1996）。口腔护理干预可能在预防 VAP 中发挥作用，但超过一半的医院未制定针对插管患者口腔护理的具体政策（Carolyn 等，2007；Doré 等，1996；Marra 等，2009）。口腔与呼吸道和消化道相连，这些区域手术后，需氧菌和厌氧菌常引发牙齿、牙龈、牙周组织及扁桃体的手术伤口感染（Salam 等，2001；Senpuku 等，2002；2006）。这些感染区域通常为细菌增殖提供了适宜的温度和湿度环境，导致频繁感染。

一般而言，口腔癌患者在手术切除肿瘤后常发生感染（Senpuku 等，2003；Senpuku 等，2006；Tada 和 Tanzawa，2002；Tada 等，2002；Zeng 等，2008）。这可能与手术期间和术后伤口暴露有关。接受口腔手术的患者常出现与细菌感染相关的并发症。口腔内致病菌的定植被认为会增加肺炎和菌血症等感染的风险（Costerton 和 Greenberg，1999；Gosney 等，1999）。因此，防止细菌经口或鼻进入肺部至关重要。

目前，全身应用抗菌药物在治疗疾病方面比局部应用显示出更好的效果，后者可能在特定区域诱导耐药菌的产生（Belusic-Gobic 等，2007；Cloke 等，2004）。一种纳米技术抗微生物喷雾 JUC（物理抗微生物敷料）已被应用于部分口腔癌患者术后感染区域，并被证明是一种新型的物理抗微生物方法，不易导致耐药性（Zeng 等，2008）。

在本研究中，JUC 喷雾被应用于 ICU 插管患者的口腔和鼻腔，以比较其与传统口腔护理方法在 VAP 发病率上的差异。

材料与方法

JUC 的作用与质量控制

JUC 喷雾的抗微生物效果和质量由南京神奇科技开发有限公司监控和控制。当 JUC 的水溶性液体喷洒在皮肤表面或黏膜区域时，会立即凝固并形成具有双重重叠结构的隐形抗微生物层：胶联膜和正电荷膜。胶联膜由高分子制剂组成，通过化学键牢固附着于体表。这种胶联膜具有长效抑菌作用。正电荷膜由阳离子活化剂组成，在皮肤表面或黏膜区域形成带正电荷的网状膜。正电荷膜能强力吸附带负电荷的病原微生物（如细菌、真菌和病毒）。如果病原微生物赖以生存的呼吸酶失活，它们将因缺氧而死亡（Zeng 等，2008）。

该喷雾已通过 Food & Drug Analytical Services Limited（批准号：9083481，美国）对鲍曼不动杆菌在多种表面上的测试。JUC 在地板、金属把手、有机玻璃、塑料把手和钢表面均通过了所有测试。此外，JUC 还通过了新不伦瑞克大学（CE 批准号：153038905）对敷料样品 zeta 电位和流体动力学尺寸的测试。JUC 在广泛的 pH 范围内表现出高 zeta 电位值，样品在 0.5%水溶液中的流体动力学尺寸为 2.57nm。

研究对象的选择

2009 年 1 月至 2010 年 3 月，从深圳市人民医院招募了 320 例需要机械通气的 ICU 患者。每位患者被编号，奇数编号的患者分配到治疗组（167 例），偶数编号的患者为对照组（153 例）。符合以下条件的患者被排除：年龄小于 18 岁、有机械通气史、怀孕或哺乳期、肺炎、支气管扩张、咯血、肺囊肿或肺纤维化（Munro 等，2009）。治疗组和对照组均每 8 小时使用氯己定漱口水清洁牙齿、口腔黏膜、舌和腭部，每日 3 次，持续 5 天。使用 0.2 巴的吸力从患者口腔中吸出漱口水。治疗组在漱口后使用 JUC 喷雾喷洒口鼻。研究方案经深圳市人民医院伦理委员会批准。

样本采集

患者气管插管 24 小时后，每 4 小时采集其气管分泌物及口腔、鼻腔和咽喉拭子，连续 5 天进行细菌培养和鉴定。通过支气管镜下的保护性标本刷采集深部痰液样本。

VAP 诊断标准

VAP 的诊断必须满足以下条件：持续 48 小时以上的影像学浸润、体温超过 38.5°C、白细胞总数（WBC）≥10×10^9/L，且保护性标本刷的菌落形成单位（CFU）超过 10^6cfu/ml 或支气管肺泡灌洗液（BAL）超过 10^7cfu/ml（Elie 等，2006）。根据发病时间，VAP 可分为两种临床类型：早发型和晚发型。早发型 VAP 指插管和机械通气后 48-96 小时内发生的肺炎，而晚发型 VAP 指机械通气 96 小时后发生的肺炎（Qinhua 和 Lixian，2004）。

统计分析

采用 SPSS 11.0 软件包进行数据收集和分析。计量资料以均数±标准差（X±s）表示，组间比较采用方差分析中的 Q 检验，组内不同时间点数据比较采用配对设计的 t 检验，VAP 发生率和构成比的比较采用秩和检验。

结果与讨论

患者基本信息

两组在年龄、性别、ICU 入院原因、APACHE II 评分、CPIS 评分及 ICU 住院天数上无显著差异（表 1）。

VAP 发病率

治疗组 VAP 发病率为 8.38%（14 例），显著低于对照组的 54.24%（83 例）（p<0.01）。早期 VAP 发病率在治疗组为 14.29%（2 例），对照组为 50.60%（42 例），两组差异显著（p<0.01）（表 2）。

细菌培养结果

在 320 例患者中共分离出 10 种病原体。对照组分离出 324 株病原体，治疗组仅 37 株，主要为革兰氏阴性菌（如肺炎克雷伯菌、铜绿假单胞菌和不动杆菌），两组差异显著（p<0.01）（表 3）。

深部痰培养结果

在 320 例患者中共分离出 10 种病原体。对照组分离出 268 株病原体，治疗组仅 33 株，

主要为肺炎克雷伯菌和铜绿假单胞菌，两组差异显著（p<0.01）（表 4）。

细菌定植率

两组在气管内定植时间小于 96 小时方面存在统计学显著差异（p<0.01），而超过 96 小时则未观察到差异。口咽部定植情况则相反，两组在 96 小时内无统计学显著差异，但超过 96 小时后存在显著差异（p<0.01）（表 5）。

VAP 被定义为接受机械通气患者发生的肺炎，也是导致脓毒症伴急性呼吸衰竭的主要原因，并且是重症监护病房患者发病率和死亡率的重要影响因素（Leroy 等，2001；Tejerina 等，2010）。1992 年至 2004 年间，NNIS 报告显示成人 ICU 中与机械通气相关的 VAP 中位发病率为每 1000 患者日 2.2 至 14.7 例。估计死亡率在 20%至 70%之间（Cuellar 等，2004）。

VAP 可分为：

早发型 VAP（EOP）发生在气管插管和人工气道建立后的前 4 天内，占 VAP 的 50%，主要由咽部寄生菌（如肺炎链球菌、流感嗜血杆菌、金黄色葡萄球菌）引起。

晚发型 VAP 发生在插管至少 5 天后，主要由革兰阴性菌（如肠杆菌、不动杆菌和铜绿假单胞菌）引起（Badia 和 Torres，2008；Niederman 和 Craven，2005；Diaz 等，2009；Medford 等，2009）。

口、鼻和咽部存在大量细菌，包括多种潜在致病菌。这些区域的屏障加上接受机械通气患者的下呼吸道直接受到破坏。气囊压力瞬时降低、体位改变或气道直径变化导致分泌物通过气管导管壁与气管之间的间隙进入下呼吸道（Marra 等，2009）。

目前 ICU 患者的口腔卫生主要使用生理盐水或氯己定漱口水清洁口腔，但它们没有或仅有短期消毒效果。抗生素溶液可能增加致病菌耐药风险，因此不推荐使用（Díaz 等，2010）。许多国际学者正在探索有效的口腔卫生方法来降低 VAP 发生率（Gastmeier 和 Geffers，2007；Heyland 等，2002；Keenan 等，2002；Livingston，2000）。JUC 喷雾敷料可提供 8 小时的抗微生物效果且不产生耐药性，为预防 VAP 发生提供了创新解决方案。

JUC 通过静电力杀灭和抑制病原微生物来降低 VAP 发生率。JUC 喷雾对黏膜刺激性小，长期使用不会导致耐药性。

本研究发现，与对照组相比，使用 JUC 喷雾于口鼻腔的患者 VAP 发生率较低，早发型 VAP 比例以及气管、口腔、鼻腔和咽部的细菌定植率也较低。JUC 是一种安全有效的口鼻腔物理抗微生物喷雾敷料。虽然两组在早发型 VAP（<96 小时）的口腔、鼻腔、咽腔和气管细菌定植发生率方面无显著差异，但治疗组早发型 VAP（<96 小时）的定植率低于对照组。

JUC 是一种物理抗微生物剂，可以取代现有的口腔护理消毒剂，缓解临床耐药性问题。它是一种安全有效预防 VAP 发生的新方法。

致谢

我们衷心感谢中国南京 JUC-南京神奇科技开发有限公司为本项先导性临床研究提供的纳米抗微生物喷雾剂（JUC）。同时，我们要特别感谢深圳市人民医院 ICU 危重症护理组组长李伟女士在患者观察和数据录入工作中所付出的辛勤努力。

表 1 320 例患者基础情况($\bar{x}\pm s$)

项　目	试验组(n=167)	对照组(n=153)	t 值	P 值
年龄(岁)	57.4 ±15.2	55.1 ±14.8	1.371	>0.05
实验观察天数	8.41 ±2.10	8.27 ±2.07	0.596	>0.05
APACHE Ⅱ评分	21.62 ±6.78	22.47 ±6.27	1.164	>0.05
CPIS 评分	3.85 ±1.58	4.03 ±1.62	1.006	>0.05

表 2　VAP 发生情况比较

组别	例数	发生 VAP	百分率%	早发型 VAP	百分率
对照组	153	83	54.24	42	50.60
试验组	167	14	8.38	2	14.29
X^2 值		79.51		46.41	
P 值		*P*<0.01		*P*<0.01	

表 3　两组口、鼻、咽部细菌群情况比较　(株)

组别	例数	肺炎克雷伯菌	铜绿假单胞菌	不动杆菌	嗜麦芽假单胞	热带念珠菌	大肠埃希菌	阴沟肠杆菌	白色念珠菌	金黄色葡萄球菌	肺炎链球菌	总株数
对照组	153	63	50	38	36	22	30	22	25	20	18	324*
试验组	167	8	7	5	4	2	3	2	3	2	1	37*

* X^2=142.19，*P*<0.01，两组比较有极显著差异。

表 4　深部痰培养细菌种类情况比较　(株)

组别	例数	肺炎克雷伯菌	铜绿假单胞菌	不动杆菌	嗜麦芽假单胞	热带念珠菌	大肠埃希菌	阴沟肠杆菌	白色念珠菌	金黄色葡萄球菌	肺炎链球菌	总株数
对照组	153	56	56	30	30	14	26	26	10	13	7	268*
试验组	167	8	7	3	3	2	4	4	0	0	2	33*

* X^2=120.72，*P*<0.01，两组比较有极显著差异。

表 5　两组气管内细菌及口、鼻、咽部细菌定植率(%)

细菌定植的时间 H		试验组(*n*=167)		对照组(*n*=153)		χ^2 值	*P* 值
		例数	定植率	例数	定植率		
气管内	<96h	4	2.40	5	3.27	0.22	*P*>0.05
	>96h	7	4.19	34	22.22	23.24	*P*<0.01
口鼻咽部	<96h	5	2.99	7	4.58	0.55	*P*>0.05
	>96h	8	4.79	50	32.68	41.85	*P*<0.01

参考文献：

Badia JR, Torres A (2008). Ventilator-Associated Pneumonia. In: Mechanical Ventilation (First Edition). P Peter J, Md, Fccm, L Burkhard, PhD, A Editorial. Philadelphia: W.B. Saunders, pp. 645-649.

Bell RCCJ, Smith JD, Johanson WG (1983). Multiple organ system failure and infection in adult respiratory distress syndrome. Ann Int. Med. 99: 293-298.

Belusic-Gobic MCM, Juretic M, Cerovic R, Gobic D, Golubovic V (2007). Risk factors for wound infection after oral cancer surgery. Oral Oncol. 43: 77-81.

Carolyn L, Cason TT, Sue Saunders (2007). Nurses' Implementation of guidelines for ventilator-associated Pneumonia from the centers for disease control and prevention. 16: 28-37.

Celis RTA, Gatell JM, Almela M, Rodriguez-Roisin R, Agusti-Vidal A (1988). Nosocomial pneumonia. A multivariate analysis of risk and prognosis. Chest. 93: 318-324.

Charitos T, van der Gaag LC, Visscher S, Schurink KAM, Lucas PJF (2009). A dynamic Bayesian network for

diagnosing ventilator associated pneumonia in ICU patients. Expert Syst. Applications, 36(2, Part 1): 1249-1258.
Chastre J, Fagon J (2002). Ventilator-associated pneumonia. Am. J. Respir. Crit. Care Med. 165: 867-903.
Chastre JTJ, Vuagnat A, Joly-Guillou ML, Clavier H, Dombret MC, Gibert C (1998). Nosocomial pneumonia in patients with acute respiratory distress syndrome. Am. J. Respir. Crit. Care, 157: 1165-1172.
Chevret SHM, Carlet J, Langer M (1993). Incidence and risk factors of pneumonia acquired in intensive care units. Results from a multicenter prospective study on 996 patients. European Cooperative Group on Nosocomial Pneumonia. Intensive Care Med. 19: 256-264.
Cloke DJGJ, Khan AL, Hodgkinson PD (2004). McLean NR. Factors influencing the development of wound infection following free-flap reconstruction for intra-oral cancer. Br. J. Plast Surg. 57: 556-560.
Costerton JWSP, Greenberg EP (1999). Bacterial biofilms: a common cause of persistent infections. Science, 284: 1318-1322.
Craven DE KL, Kilinsky V, Lichtenberg DA, Make BJ, McCabe WR (1986). Risk factors for pneumonia and fatality in patients receiving continuous mechanical ventilation. Am. Rev. Respir. Dis. 133: 792- 796.
Craven DE, Steger K (1996). Nosocomial pneumonia in mechanically ventilated adult patients: epidemiology and prevention in 1996. Semin Respir. Infect. 11: 32-53.
Cross AS, Roup B (1981). Role of respiratory assistance devices in endemic nosocomial pneumonia. Am. J. Med. 70: 681-685.
Cuellar Ponce de Leon L, Rosales R, Rosenthal VD (2004). Prospective Study To Evaluate Mechanical Ventilator-Associated Pneumonia Rate in Intensive Care Units in a Peruvian Public Hospital: Benchmark with NNIS American Rates. Am. J. Infect. Control, 32: E114-E115.
Díaz LA, Llauradó M, Rello J, Restrepo MI (2010). Prevención no farmacológica de la neumonía asociada a ventilación mecánica. Archivos de Bronconeumología, 46: 188-195.
Delclaux CRE, Blot F, Brochard L, Lemaire F, Brun-Buisson C (1997). Lower respiratory tract colonization and infection during severe acute respiratory distress syndrome: incidence and diagnosis. Am. J. Respir. Crit. Care Med. 156: 1092-1098.
Diaz E, Ulldemolins M, Lisboa T, Rello J (2009). Management of Ventilator-Associated Pneumonia. Infect. Dis. Clin. North Am. 23: 521-533.
Doré PRR, Grollier G, Rouffineau J, Lanquetot H, Charrière JM, Fauchère JL (1996). Incidence of anaerobes in ventilator-associated pneumonia with use of a protected specimen brush. Am. J. Respir. Crit. Care Med. 153: 1292-1298.
Elie A, Jean-Francois T, Muriel T (2006). Candida Colonization of the Respiratory Tract and Subsequent Pseudomonas
Ventilator Associated Pneumonia. Chest. 129: 110-117.
Fagon JYCJ, Domart Y, Trouillet JL, Pierre J, Darne C, Gibert C (1989). Nosocomial pneumonia in patients receiving continuous mechanical ventilation. Prospective analysis of 52 episodes with use of a protected specimen brush and quantitative culture techniques. Am. Rev. Respir. Dis. 139: 877-884.
Gastmeier P, Geffers C (2007). Prevention of ventilator-associated pneumonia: analysis of studies published since 2004. J. Hospital Infect. 67: 1-8.
Gosney MAPA, Corkhill J, Millns B, Martin MV (1999). Pseudomonas aeruginosa septicaemia from an oral source. Br. Dent. J. 187: 639- 640.
Haley RWHT, Culver DH, Stanley RC, Emori TG, Hardison CD, Quade D, Shachtman RH, Schaberg DR, Shah BV (1981). Nosocomial infections in US hospitals, 1975-1976: estimated frequency by selected characteristics of patients. Am. J. Med. 70: 947-959.
Qinhua He, lixian He (2004). Progress of Study in Preventing Ventilator Associated Pneumonia. Foreign Medicine, Fascicule of Respiratory System. 6: 122-124.
Heyland DK, Cook DJ, Dodek PM (2002). Prevention of ventilator associated pneumonia: Current practice in Canadian intensive care units. J. Crit. Care, 17: 161-167.
Keenan SP, Heyland DK, Jacka MJ, Cook D, Dodek P (2002). Ventilator-associated pneumonia: Prevention, diagnosis, and therapy. Crit. Care Clin. 18: 107-125.
Langer M MP, Cigada M, Mandelli M (1989). Long-term respiratory support and risk of pneumonia in critically ill patients. Intensive Care Unit Group of Infection Control. Am. Rev. Respir. Dis. 140: 302-305.

Leroy O, Sanders V, Girardie P, Devos P, Yazdanpanah Y, Georges H (2001). Mortality due to ventilator-associated pneumonia: Impact of medical versus surgical ICU admittance status. J. Crit. Care, 16: 90- 97.

Livingston DH (2000). Prevention of ventilator-associated pneumonia. Am. J. Surg. 179(Sup 1): 12-17.

Markowicz PWM, Djedaini K, Cohen Y, Chastre J, Delclaux C, Merrer J, Herman B, Veber B, Fontaine A (2000). Multicenter prospective study of ventilator-associated pneumonia during acute respiratory distress syndrome. Incidence, prognosis, and risk factors. Am. J. Respir. Crit. Care Med. 161: 1942-1948.

Marra AR, Cal RGR, Silva CV, Caserta RA, Paes ÂT, Moura Jr DF (2009). Successful prevention of ventilator-associated pneumonia in an intensive care setting. Am. J. Infect. Control, 37: 619-625.

Medford ARL, Husain SA, Turki HM, Millar AB (2009). Diagnosis of ventilator-associated pneumonia. J. Crit. Care, 24: 473.e471- 473.e476.

Munro CL, Grap MJ, Jones DJ, McClish DK, Sessler CN (2009). Chlorhexidine, toothbrushing, and preventing ventilator-associated pneumonia in critically ill adults. Am. J. Crit. Care, 18: 428-437.

National Nosocomial Infections Surveillance (NNIS) System (1999). National Nosocomial Infections Surveillance (NNIS) System report, data summary from January 1990-May 1999. Am. J. Infect. Control, 27: 520-532.

Niederman MS, Craven DEC (2005). Guidelines for the Management of Adults with Hospital-acquired, Ventilator-associated, and Healthcare associated Pneumonia. Am. J. Respir. Crit. Care Med. 171: 388-416.

Prevention CfDCa (2000). Monitoring hospital- acquired infections to promote patient safety: United States, 1990-1999. MMWR. 49: 149- 153.

Rello JAV, Ricart M, Castella J, Prats G (1993). Impact of previous antimicrobial therapy on the etiology and outcome of ventilator associated pneumonia. Chest. 104: 1230-1235.

Rello JRM, Jubert P, Muses G, Sonora R, Valles J, Niederman MS (1997). Survival in patients with nosocomial pneumonia: impact of the severity of illness and the etiologic agent. Crit. Care Med. 25: 1862- 1867.

Salam MASH, Nomura Y, Matin K, Miyazaki H, Hanada NM (2001).Isolation of opportunistic pathogens in dental plaque, saliva and tonsil samples from elderly. Jpn. J. Infect. Dis. 54: 193-195.

Senpuku H SA, Inoshita E, Tsuha Y, Miyazaki H, Hanada N (2003). Systemic disease in association with microbial species in oral biofilm from elderly requiring care. Gerontology, 49: 301-309.

Senpuku H TA, Takada M, Sato Y, Hanada N (2002). Reproducibility of oral bacterial isolation in the elderly. Jpn. J. Infect. Dis. 33: 61-62.

Senpuku H TA, Uehara S, Kariyama R, Kumon H (2006). Postoperative infection by pathogenic micro-organisms in the oral cavity of patients with prostatic carcinoma. J. Int. Med. Res. 34: 95-102.

Tada AHN, Tanzawa H (2002). The relation between tube feeding and pseudomonas aeruginosa detection in the oral cavity. J. Gerontol. Biol. Sci. Med. Sci. 57: M71-72.

Tada A WT, Yokoe H, Hanada N, Tanzawa H (2002). Oral bacteria influenced by the functions status of the elderly people and type and quality of facilities for the bedridden. J. Appl. Microbiol. 93: 487-491.

Tejerina E, Esteban A, Fernández-Segoviano P, Frutos-Vivar F, Aramburu J, Ballesteros D (2010). Accuracy of clinical definitions of ventilator-associated pneumonia: Comparison with autopsy findings. J. Crit. Care, 25: 62-68.

Torres AAR, Gatell JM, Jimenez P, Gonzalez J, Ferrer A, Celis R, Rodriguez-Roisin R (1990). Incidence, risk, and prognosis factors of nosocomial pneumonia in mechanically ventilated patients. Am. Rev. Respir. Dis. 142: 523-528.

U.S. Department Of Health And Human Services Public Health Service Centers for Disease Control (1997). Guidelines for Prevention of Nosocomial Pneumonia. 46: RR-1.

Vincent JLBD, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, Wolff M, Spencer RC, Hemmer M (1995). The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. JAMA. 274: 639-644.

Zeng Y, Deng R, Barry HS, Yeung W, Loo TY, Mary Cheung NB, Chen JP, Bingrong Z, Yifu F, Lanzhu H, Mingxing L, Min W (2008) Application of an antibacterial dressing spray in the prevention of post-operative infection in oral cancer patients: A phase 1 clinical trial. Afr. J. Biotechnol. 7: 3827-3831.