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Full Length Research Paper

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

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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

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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.

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