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Oropharyngeal Colonization: A Risk Factor of Ventilator-Associated Pneumonia in Critically Ill Children.

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ABSTRACT

Ventilator- Associated Pneumonia (VAP), is defined by the Centers for Disease Control and prevention (CDC) as pneumonia that occurs in a patient receiving mechanical ventilation that develops 48 hours or more after initiation of ventilation. We aimed in this study to evaluate the effect of oropharyngeal colonization on the occurrence and outcome of VAP in critically ill children. This prospective, cohort, study was performed in pediatric intensive care unit, Ain Shams university hospital in Cairo, Egypt. A total of 50 children were selected: 30 children acquired VAP (VAP group) and 20 children didn't acquire VAP (non-VAP group). Assessment of oropharyngeal colonization was done by using a microbiological culture of samples from the tonsillar area at day (0), (2), (4) of admission. VAP was clinically confirmed by Clinical Pulmonary infection Score (CPIS) score and bacterial endotracheal culture. Most of studied cases were admitted due to chest diseases (Asthma and COPD). Duration of mechanical ventilation did not vary significantly among both groups. The actual mortality rate was higher among the patients who acquired VAP (46.7%). There was a significant relationship between microbiological growth of the endotracheal culture of patients of VAP group and the oropharyngeal swab done for those patients on day 2 intubation $p < 0.05$ and that done on day 4 intubation $p < 0.01$. Conclusion: VAP resulted in considerable increase in mortality rate. Oropharyngeal colonization was a significant risk factor for development of VAP.

Keywords: Oropharyngeal colonization, Ventilator-Associated Pneumonia, pediatric intensive care unit

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INTRODUCTION

Ventilator-associated pneumonia (VAP) is among the most frequently occurring nosocomial infections in critical care patients. Patients receiving mechanical ventilation suffer decreased salivary secretions, and oral cavity hygiene worsens, resulting in bacterial overgrowth. (1).

VAP is defined as a hospital acquired pneumonia developing in patients treated with mechanical ventilation for 48 hours or longer with no signs or symptoms of lower respiratory tract infection before intubation (2).

VAP develops when bacteria colonize the pulmonary parenchyma of the lower respiratory tract of patients receiving mechanical ventilation (Coffin et al., 2008). Aspiration of secretions, colonization of the aerodigestive tract, or the use of contaminated equipments results in colonization of the lower respiratory tract of patients with mechanical ventilation (3).

Risk factors for VAP can be divided into three categories: host related, device related, and personnel related. Risk factors for VAP in children currently include: Use of enteral nutrition, reintubation, gastroesophageal reflux (4). Other important risk factors are immune deficiency, bronchoscopy, and medications, specifically steroids, H2 receptor antagonists, immunosuppressants, prior use of antibiotics and sedation (5).

Oropharyngeal colonization with potentially pathogenic microorganisms is crucial in the pathogenesis of VAP (6). Oropharyngeal colonization of ventilated patients can occur due to alterations of immunological mechanisms, reduction of oral secretions and dryness of oral cavity due to tracheal and enteral tubes (7).

As there is no enough data in the literature about the relation between the oropharyngeal colonization and ventilator associated pneumonia especially in the pediatric age group, so we aimed to study the effect of oropharyngeal colonization on the occurrence and outcome of VAP in critically ill children.

Patients and Methods ;

This prospective, cohort, study was performed in pediatric intensive care unit (PICU), Ain Shams university hospital in Cairo, Egypt.

All patients admitted to PICU from 11/ 2013 to 1/ 2016 of more than 1 months age were included, while patients were excluded if they were newborns, already pneumonic at the time of PICU admission, stayed in the PICU for less than 48 hours, or if informed consent was not feasible.

The study was approved by the Medical Research Ethical Committee of NRC and of Ain Shams university hospital and written informed consent was obtained from both parents.

Study Methodology:

Fifty children were selected for participation in this study. Thirty critically ill ventilated patients acquired VAP during their PICU stay either early or late (patient group) and the other twenty ventilated patients did not acquire VAP during their PICU stay (control group)

Data collected included each patient's age; sex; nutritional status (normal, malnutrition, overweight); previous hospital length of stay; presence of infectious or chronic disease at admission; use of antibiotics; type of admission (elective or emergency); diagnoses (clinical or surgical); use of drugs that as central nervous system suppressors, salivary secretion modifiers, immunological suppressors, gastric pH modifiers; characteristics of intubation, including indication (emergency and elective), type (oral or nasal), and type of endotracheal tube (cuffed or uncuffed) and outcome (discharge or death). All patients were followed up until discharge from the PICU.

Assessment of oropharyngeal colonization was done by using a qualitative microbiological culture assay of samples from the tonsillar area and the upper posterior part of the oropharynx. Samples were collected during

the first 24 hours of PICU admission (day 0), at 48 hours (day 2), at 96 hours (day 4), and at the time of discharge from the PICU.

Microbiological assessment of lung aspirate in ventilated patients was done for all ventilated patients to detect incidence of VAP, samples were collected during the first 24 hours of PICU admission (day 0), on suspecting VAP within 48 hours and 96 hours of ventilation for detecting early and late VAP respectively, and at time of discharge from the PICU . In the microbiological laboratory, both oropharyngeal and endotracheal samples were cultured on blood agar, chocolate agar, and Sabouraud agar, and incubated according to controlled atmosphere, temperature, time, and humidity parameters for qualitative microbiological identification. After incubation, growth of colonies was evaluated and analyses of microbial resistance to antibiotics were done.

Clinical pulmonary infection score (CPIS) which is a diagnostic algorithm that relies on clinical, radiographic, and microbiological criteria (tracheal aspirate cultures) and is considered as a practical objective tool for diagnosing ventilator-associated pneumonia was also applied on all ventilated patients with every culture of lung aspirate. The score varies from 0 to 12 points with a CPIS of more than 6 being associated with a high likelihood of pneumonia (8)

Statistical analysis

The data were coded, entered and processed on computer using SPSS (version 20). The level $p< 0.05$ was considered the cut off value for significance. For qualitative data, an analysis for independent variables was done using Chi-square test. Fischer exact test was performed in table containing value less than 5. Wilcoxon signed-rank test considers information about both the sign of the differences and the magnitude of the differences between pairs. It is more powerful than the sign test. The Mann-Whitney U test (non parametric test) was used to assess the statistical significance of the difference between two population means in a study involving independent sample.

RESULTS

Characteristics of the Sample

Overall, 86 critically ill ventilated patients were enrolled in the study: only 50 (58.1%) patients completed the study, 14 patients (16.2%) were extubated and discharged in less than 4 days and 22 (25.5%) patients died in less than 4 days of intubation. The study shows no significant difference between cases and controls regarding sex, BMI, and age (Table 1).

Table (1): Comparative study between cases and controls regarding sex, BMI and Age.

		Control group (n=20)		Patients group (n=30)		Chi square test	
		No.	%	No.	%	X^2/t^*	P-value
Sex	Male	10	50.0%	15	50.0%	0.000	1.000
	Female	10	50.0%	15	50.0%		
BMI	Underweight	6	30.0%	4	13.3%	2.095	0.351
	Normal	10	50.0%	19	63.3%		
	Overweight	4	20.0%	7	23.3%		
Age (months)	Mean ± SD	14.60 ± 12.12		12.93 ± 13.34		0.449*	0.656

Chest diseases were the most common cause of admission in PICU followed by CVS and CNS related morbidity (Fig 1). At baseline, both groups had similar demographic characteristics, pharmacological and nutritional support. Most of the patients had infectious disease at the time of PICU admission. By comparing the clinical characteristics of the examined population, we found that patients who developed VAP showed significantly higher emergency admission to PICU and was highly significant among patients with medical emergency or conditions compared to surgical condition (Table 2).

ISSN: 0975-8585

Table (2): Comparative study of Baseline clinical characteristics of the studied cases

Clinical characteristics		Control group(n=20)		Patient group(n=30)		Chi square test	
		No.	%	No.	%	X^2	P-value
Emergency admission to hospital	No	10	50.0%	8	26.7%	2.836	0.092
	Yes	10	50.0%	22	73.3%		
Emergency admission to PICU	No	12	60.0%	6	20.0%	8.333	**0.004***
	Yes	8	40.0%	24	80.0%		
Pre-existing chronic condition	No	10	50.0%	18	60.0%	0.487	0.485
	Yes	10	50.0%	12	40.0%		
Underlying condition	Surgical	12	60.0%	2	6.7%	16.93	**<0.001***
	Medical	8	40.0%	28	93.3%		
Presence of infection at admission	No	14	70.0%	13	43.3%	3.435	0.064
	Yes	6	30.0%	17	56.7%		
Antibiotic therapy before admission	No	14	70.0%	17	56.7%	0.905	0.341
	Yes	6	30.0%	13	43.3%		

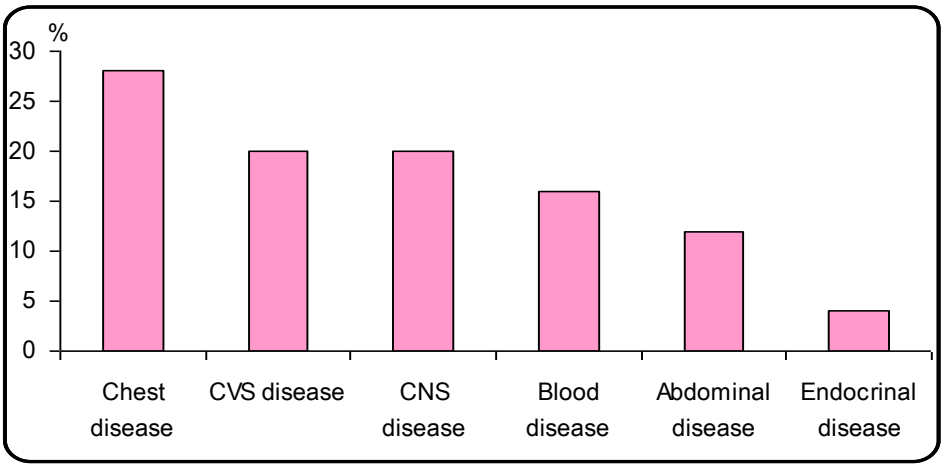

Figure (1): The distribution of studied cases regarding the cause of admission to PICU.

Because of discharge and death, sequential samples of oropharyngeal secretions were obtained from 50 patients on day 0, day 2, day 4, and only 32 patients at PICU discharge. The pattern of colonization of the oropharynx of both patient group and control groups is represented in (table 3).

On admission, overall 60% of oropharyngeal samples were negative, and 40% contained pathogenic organisms. The frequency of oropharyngeal colonization differs significantly between patient group and control group regarding the oropharyngeal swab done on day 2 (P=0.001), day4 (P=0.003) and on extubation (P<0.001). During the first 48 hours of PICU admission, the number of children colonized with pathogenic microorganisms increased both in the patient and control groups. Acintobacter species was detected in most of the samples in both groups. Acintobacter species were detected more often in the patient group than in the control group. Overall, gram-negative bacteria were the predominant species (Table 3).

Table (2): Comparison between the two groups regarding microbiological profile of oropharyngeal secretions.

		Control group		Patients group		Chi square test	
		No.	%	No.	%	X^2	P-value
Swab D0	Acintobacter	2	10.0%	6	20.0%	5.417	0.367
	Klebsiella	2	10.0%	2	6.7%		
	Mixed	2	10.0%	0	0.0%		
	Negative	12	60.0%	18	60.0%		
	Pseudomonas	2	10.0%	2	6.7%		
	Staphylococus	0	0.0%	2	6.7%		
Swab D2	Acintobacter	0	0.0%	12	40.0%	24.053	**0.001**

ISSN: 0975-8585

	Klebsiella	6	30.0%	3	10.0%		
	Mixed	4	20.0%	0	0.0%		
	Negative	6	30.0%	5	16.7%		
	Candida	0	0.0%	2	6.7%		
	Pseudomonas	2	10.0%	6	20.0%		
	Staphylococus	2	10.0%	2	6.7%		
Swab D4	Acintobacter	6	30.0%	4	13.3%	22.906	**0.003**
	Candida	2	10.0%	2	6.7%		
	Klebsiella	4	20.0%	9	30.0%		
	Mixed	0	0.0%	10	33.3%		
	Negative	4	20.0%	0	0.0%		
	Pseudomonas	2	10.0%	4	13.3%		
	Staphylococus	2	0.0%	1	3.3%		
Swab on extubation	Acintobacter	4	20.0%	2	6.7%	29.444	**<0.001**
	Candida	2	10.0%	0	0.0%		
	Died	2	0.0%	16	53.3%		
	Klebsiella	0	20.0%	4	13.3%		
	Mixed	4	0.0%	2	6.7%		
	Negative	6	40.0%	2	6.7%		
	Pseudomonas	0	0.0%	2	6.7%		
	Staphylococus	2	0.0%	2	6.7%		

Endotracheal tube aspirate culture was done on three sessions: just after intubation, on suspecting VAP and on extubation of the patient. There was no significant difference between VAP and non- VAP groups regarding the microbiological profile done on intubation and on suspecting VAP, while VAP group was significantly higher compared to controls regarding the microbiological profile of the endotracheal aspirate culture done just before extubation (p=0.005) (Table 4)

Table (3): Comparison between the two groups regarding microbiological profile of endotracheal secretions.

		Control (n=20)		Patient (n=30)		Chi square	
		N	%	N	%	X^2	p-value
Endotracheal culture on intubation	Acintobacter	3	15.0	4	13.3	7.918	0.161
	Klebsiella	2	10.0	1	3.3		
	Mixed	0	0.0	4	13.3		
	Negative	13	65.0	19	63.3		
	Pseudomonas	0	0.0	2	6.7		
	staphylococcal	2	10.0	0	0.0		
Endotracheal culture on suspecting VAP	Acintobacter	3	15.0	4	13.3	10.283	0.113
	Candida	1	5.0	2	6.7		
	Klebsiella	4	20.0	7	23.3		
	Mixed	1	5.0	8	26.7		
	Negative	7	35.0	5	16.7		
	Pseudomonas	1	5.0	4	13.3		
	staphylococcal	3	15.0	0	0.0		
Endotracheal culture just before extubation	Acintobacter	3	15.0	4	13.3	18.585	**0.005***
	Klebsiella	1	5.0	5	16.7		
	Died	2	10.0	16	53.3		
	Negative	9	45.0	5	16.7		
	Candida	1	5.0	0	0.0		
	Pseudomonas	1	5.0	0	0.0		
	Mixed	0	0.0	0	0.0		
	staphylococcal	3	15.0	0	0.0		

ISSN: 0975-8585

Table 5 shows that There was no significant association between the endotracheal aspirate culture showing VAP and the Oropharyngeal swab done on intubation while there was significant association between the endotracheal aspirate culture showing VAP and the Oropharyngeal swab done on day 2 (P= 0.044*) and the oropharyngeal swab done on day 4 (P <0.001).

Table (5): Relation between microbiological growth of endotracheal culture on suspecting VAP and Oropharyngeal swabs done on day 0, day 2 and day4 of intubation.

	Endotracheal culture on suspecting VAP													
	Negative		acintoacter		klebsiella		candida		pseudomonas		Mixed		Chi-square	
	N	%	N	%	N	%	N	%	N	%	N	%	X^2	P-value
Swab day 0														
Negative	5	16.7	1	3.3	5	16.7	0	0.0	3	10.0	4	13.3	30.893	0.057 NS
Acintoacter	0	0.0	3	10.0	2	6.7	0	0.0	0	0.0	1	3.3		
Klebsiella	0	0.0	0	0.0	0	0.0	1	3.3	0	0.0	1	3.3		
Pseudomonas	0	0.0	0	0.0	0	0.0	0	0.0	1	3.3	1	3.3		
Staph	0	0.0	0	0.0	0	0.0	1	3.3	0	0.0	1	3.3		
Swab day 2														
Negative	2	6.7	1	3.3	2	6.7	0	0.0	0	0.0	0	0.0	38.211	**0.044* S**
Acintoacter	1	3.3	3	10.0	3	10.0	0	0.0	1	3.3	4	13.3		
Klebsiella	0	0.0	0	0.0	1	3.3	1	3.3	0	0.0	1	3.3		
Candida	2	6.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0		
Pseudomonas	0	0.0	0	0.0	1	3.3	0	0.0	3	10.0	2	6.7		
Staph	0	0.0	0	0.0	0	0.0	1	3.3	0	0.0	1	3.3		
Swab day 4														
Acintoacter	0	0.0	4	13.3	0	0.0	0	0.0	0	0.0	1	3.3	47.817	**<0.001* HS**
Klebsiella	2	6.7	0	0.0	5	16.7	1	3.3	0	0.0	1	3.3		
Candida	2	6.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0		
Pseudomonas	0	0.0	0	0.0	1	3.3	0	0.0	2	6.7	1	3.3		
Mixed	1	3.3	0	0.0	1	3.3	1	3.3	2	6.7	5	16.7		
Acintoacter	1	3.3	3	10.0	3	10.0	0	0.0	1	3.3	4	13.3		
Klebsiella	0	0.0	0	0.0	1	3.3	1	3.3	0	0.0	1	3.3		
Candida	2	6.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0		
Pseudomonas	0	0.0	0	0.0	1	3.3	0	0.0	3	10.0	2	6.7		
Staph	0	0.0	0	0.0	0	0.0	1	3.3	0	0.0	1	3.3		

DISCUSSION

Ventilator-associated pneumonia (VAP) is described as the commonest hospital-acquired infection in mechanically ventilated patients. It is defined as nosocomial pneumonia that develops after 48 hours or more of instituting mechanical ventilation (9). It is also associated with significant morbidity (27%) including increased ventilatory days, intensive care unit (ICU) stay and higher medical cost that leads to high mortality rate in ICU (10).

Within 48 hours of hospital admission, the oropharyngeal flora of critically unwell patients undergoes a change from predominantly gram positive organisms to predominantly gram negative organisms, creating a more virulent flora. This bacterial flora may then migrate to the lungs and result in a hospital-acquired pneumonia (11).

We aimed to study the impact of oropharngeal colonization on the occurrence and outcome of VAP in critically ill children admitted to the PICU Ain Shams University. In our study, thirty patients out of fifty developed VAP according to diagnostic categories based on clinical, laboratory and radiological criteria. Nineteen patients developed early VAP and eleven patients developed late VAP. So we studied some clinical characteristics and their impact on occurrence and outcome of VAP and found that emergency admission to PICU, medical underlying emergency, together with history of previous intubation showed significant association with occurrence of VAP.

By analyzing causes of admission of our studied group to the PICU; it was found that chest diseases were the most common illness among the studied group (28%), followed by CNS diseases and CVS diseases

(20%) each, (16%) had blood diseases, (12%) were admitted to PICU due abdominal diseases and (4%) due to endocrinal diseases. In contrast to our study, *Maher and Bateman, 2009* found that the most common primary PICU admitting diagnostic category for children was the cardiovascular system (n=62 (75%)). In contrast, the respiratory system was most common among the three middle age categories (38-42% of children). In the oldest age group, the respiratory and central nervous systems were the most common admitting diagnostic categories (32 and 34% of children, respectively). There was an increasing trend with age in proportion of children whose primary PICU admitting diagnostic category was the central nervous system (12).

There was no significant difference between patient group and control group regarding emergency admission to the hospital, pre-existing chronic condition, presence of infection at admission, and antibiotic therapy before admission. The patient group who developed VAP showed significantly higher emergency admission to PICU and highly significant underlying medical emergency or conditions compared to surgical conditions. In contrast to our study *Joseph and coworkers, 2010* (13) noted that the surgical ICUs have higher rates of VAP compared to the medical ICUs.

According to the Centers for Disease Control and Prevention (CDC) sources, indiscriminate use of broad spectrum antibiotics cause more than doubles an individual chance of acquiring future infection with a resistant organism (14). According to our study the use of triple antibiotic therapy during PICU stay significantly affects the occurrence of VAP. In the control non VAP group only 30% of patients were on triple antibiotic therapy during their PICU stay while in VAP group, 73.3% were on triple antibiotic therapy during their PICU stay.

In our study, 63.35% of patients acquired VAP were early onset VAP within first 2 days of ventilation. According to *Joseph and coworkers, 2010* study *(13)*, 41.7% of cases were early onset VAP. It was observed that majority of the VAP episodes occurred within the first two days of MV, on the other hand only 11 patients out of 30 acquired late VAP (36.6%) while in contrast to our study, *coffin and coworkers2008* stated that (58.3%) of cases in their study acquired late pneumonia (15)

Thirty patients (60%) patients of our studied group were admitted to PICU without bacterial colonization and twenty patients (40%) were already colonized before time of admission to PICU and this may be explained by the fact that critically ill patients even before admission to ICU are at high risk and great vulnerability to be colonized. A similar finding was noted by *predriera et al. 2009* who studied oral care interventions and oropharyngeal colonization in children receiving mechanical ventilation on admission *(6)*

At time of intubation, an endotracheal aspirate culture was done for all our studied cases, 32 (64%) cases showed free cultures while the cultures of 18 (36%) patients showed growth of organisms mostly Acintobacter baumanii (14%) and mixed infections(8%). The second endotracheal aspirate culture was done on suspecting VAP clinically, radiologically and laboratory, only 12 (24%) of patients were culture free meaning that 20 patients had acquired infection in the PICU. The most common organisms isolated were Klebsiella pneumonaie (22%),and mixed infection with polymicrobes (18%). *Badr* et al. 2011 stated that cultures reported in his study revealed that gram negative bacteria were isolated from the majority of VAP patients (68.6%), with klebsiella organism predominating the positive culture (34.3%). On the other hand, gram positive infection comprised 21.8% of the total cultures with Staphylococcus aureus predominating the positive culture (15.6%) while Candida was positive in 9.3% of samples examined (16)

At time of extubation only 32 patients completed the study and 18 patients died. 92% of the endotracheal aspirate culture of our patients showed positive growth of organisms mainly Acintobacter baumanii and Klebsiella pneumoniae.

There was no significant difference between both groups (VAP and non-VAP) regarding oropharyngeal colonization at day 0 and day 4 while there was significant difference between both groups regarding oropharyngeal colonization at day 2 and at discharge from the PICU.

On intubation 65% of the control group showed free cultures while 63.3% of the control group showed no growth in their tracheal aspirate culture. The most common isolated organisms were Klebsiella pneumonie, acintobacter baumanii and mixed infection.

On suspecting VAP, the endotracheal aspirate cultures of the (VAP) group showed 13.3% acintobacter baumanii, 6.7% candida, 23.3% klebsiella pneumonie, 26.7% mixed,16.7% negative cultures, 13.3% pseudomonas aeuraginosa. Cultures reported in *Badr* et al. 2011 study in neonates revealed that gram negative bacteria was isolated from the majority of VAP patients (68.6%), with klebsiella organism predominating the positive culture (34.3%). On the other hand Candida was positive in 9.3% of samples examined (16).

By time of extubation 10% of non VAP group died and 53.3% of the VAP group died. The most common isolated organisms among the survivors were Klebsiella pneumonie, acintobacter baumanii. There was no significant association between the endotracheal aspirate culture showing VAP and the oropharyngeal swab done on intubation while there was significant association between the endotracheal aspirate culture showing VAP and the Oropharyngeal swab done on day 2 ($p=0.044^*$) and that there was significant relationship between the endotracheal aspirate culture showing VAP and the oropharyngeal swab done on day 4 ($p <0.001$).indicating that VAP in our study was hospital acquired not community acquired.

The overall mortality rate in our study (36%) was high compared to mortality rates in other studies, for example a study done by *Naghib and coworkers 2010* who studied mortality in very long stay pediatric intensive care unit patients; he found that the average PICU mortality rate was only (4.6%) (17). This wide variation of mortality rates between our PICU and other ICUs draws attention to differences in causes of admission, diagnoses of patients at time of admission and protocols of treatment used in different ICUs, together with variables as medical care, financial resources and infection control measures that can affect the mortality rates.

Study Limitations: This study was limited by a small sample for all 4 time points. Conversely, the results do provide unique, preliminary data on the impact of oropharyngeal colonization on critically ill children receiving mechanical ventilation. Future research should address issues such as the effect of oral care on the development of VAP in children.

CONCLUSION

There was significant association between oropharyngeal colonization and acquisition of early VAP among critically ill mechanically ventilated patients.

RECCOMENDATIONS

VAP bundle should be one of treatment mainstay of recent established medicine in pediatric intensive care units. Preventive measures against acquisition of colonization during PICU stay should be considered significantly to decrease rate of nosocomial colonization.

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