International Journal of Molecular and Clinical Microbiology 2 (2014) 424-427

International Journal of
Molecular and Clinical Microbiology

Microbial colonization of endotracheal tube in intensive care unit patients

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ARTICLE INFO

Article history:
Received 7 may 2014
Accepted 23 Agust 2014
Available online 1 December 2014

Keywords:
Nosocomial,
Endotracheal Tubes,
Biofilm

ABSTRACT

Microbial biofilms has remained a major complication of tracheal intubation in patients requiring ventilator equipment. The aim of this study was to characterize bacterial and fungal biofilms in endotracheal tubes from intensive care unit (ICU) patients in Ahwaz, Iran. In this cross-sectional descriptive study, patients admitted to ICU that required mechanical ventilation for at least 24 hours were evaluated. Specimens were collected from tracheal tubes of patients with endotracheal aspiration, when they had clinical manifestation of pneumonia. The specimens were microbiologically investigated and the bacterial and fungal isolates were identified by using standard cultural and biochemical tests. In total, 350 cases had tracheal tube aspirate positive cultures. The most of isolates are known to cause colonization of endotracheal tube included: Coagulase negative staphylococci (18.2%), *E.coli* (18%), *Enterobacter* spp. (16.2%), *Pseudomonas* spp. (14.6%), *Acinetobacter* spp. (9.7%), *S.aurous* (8.1%), *Klebsiella* spp. (6.7%), and *Serratia* spp. (0.4%). 7.4% were colonized with *Candida* spp. that the most common species was *C.albicans* (42.3%). The coagulase negative staphylococci species identified by mass spectrometry were: *S.epidermidis* (64%), *S.haemolyticus* (17.1%), *S.lugdune* (3.1%), *S.warnerii* (6.25%), *S.hominis* (6.25%), *S.pasteur* (3.1%). There was significant association between duration of being intubated and *S.aurous*, *Enterobacter spp*. (*P*=0.002). The presence of bacterial and fungal biofilms of endotracheal tube suggests that it may be important in biofilm development and may provide a therapeutic target for the prevention of ventilator-associated pneumonia.

1. Introduction

Nosocomial infection is an important health-care problem in hospitals worldwide, accompanied by high rate of morbidities and mortality among hospitalized patients, especially in ICU ward (George, 1995). Hospital staff and the environment can be the microbial source, and because of the overuse of antimicrobial

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agents, they have become multiple drug resistance organisms (Gross, 1987). Invasive medical procedures in the ICU remarkably increase the risk of such infections (Ruffell and Adamcova, 2008). Intubation with mechanical ventilation increases the risk of pneumonia 6 to 20 folds more among ICU patients ventilator-associated pneumonia (VAP). VAP is the most frequent nosocomial infection in the ICU occurring in 8–28% of mechanically ventilated patients and is associated with crude mortality rates of 20% to 40% (Chastre and Fagon, 2002). The presence of an endotracheal tube is an independent risk factor for developing VAP (Amin, 2009). Significantly, the endotracheal tube may also act as a reservoir for pathogens by providing a surface to which they can adhere and form biofilms (Pneumatikos et al., 2009). The dissemination of tracheal tube biofilm into the mechanically ventilated lung has been proposed as a contributory factor in the pathogenesis of ventilator associated pneumonia (Larson, 1970). Bacterial and fungal biofilms has been observed universally on the surface of endotracheal tubes in mechanically ventilated patients (Inglis et al., 1989). Microbial colonization of the inner surface of tracheal tubes has been examined after prolonged use in the critically ill, and dislodgement of this biofilm layer during suction catheterization has been proposed as an etiological factor in VAP (Sottile et al., 1986). The aim of this study was to characterise microbial biofilms in endotracheal tubes from ICU patients.

2. Materials and Methods

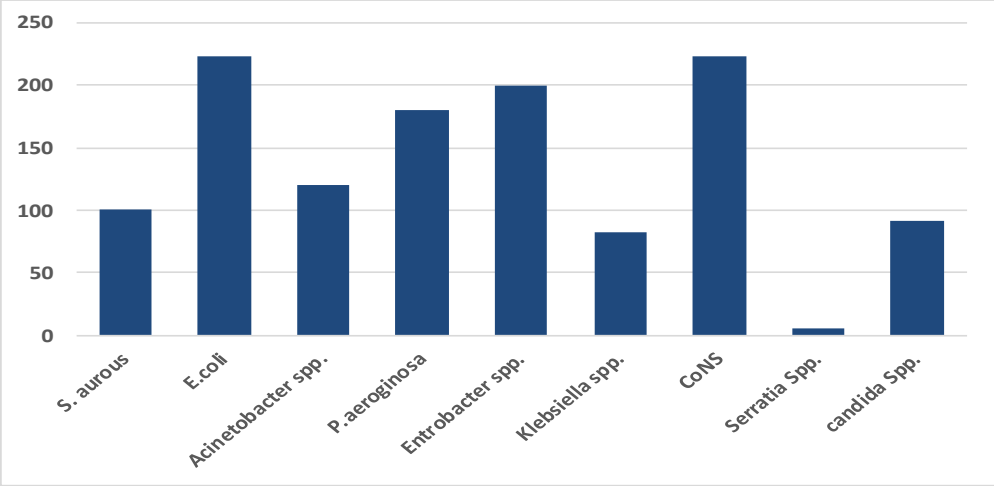
In this descriptive cross-sectional study which was set to determine the prevalence of bacterial and fungal species present in tracheal tubes who were intubated and mechanically ventilated on the ICU of Emam Khomaini and Golestan hospitals, Ahvaz, Iran, from April to December of 2014. The duration of intubation prior to endotracheal tube collection with endotracheal aspiration, when they had clinical manifestation of pneumonia (cough, purulent respiratory secretion, fever and new or progressive infiltration of lung). The length of stay of patients in mentioned wards was at least two weeks prior to sampling. Collected endotracheal tubes were placed in sealed sterile bottles and referred immediately to the laboratory for processing.

From the central region of each endotracheal tube, 1 cm section was cut and processed for quantitative microbial culture. The biofilm was aseptically removed from the endotracheal tube lumen using a sterile scalpel and this was resuspended in 5 ml of sterile normal saline. 50 ml volume of each dilution were deposited on to each agar media. The samples were cultured on chocolate agar, MacConkey agar, blood agar and sabouraud dextrose agar as soon as they were received, and incubated in 37°C for 24 to 48 hours. Then, the shape and color of colonies and gram staining were studied and the bacterial strain were determined according standard identification biochemical tests including oxidase and catalase, reaction in triple sugar iron agar (TSI) medium, indole production and motility, plus MRVP test, SH2 production, lactose fermentation, citract reaction urea util-ization and catalase, coagulase, manitol fermention on manitol salt agar and DNase tests were performed. The coagulase negative staphylococci species identified by mass spectrometry. *Candida* species were identified based methods such as colony color on CHROM agar candida medium (CHROMagar Company, Paris, France), germ-tube tests in serum at 37 ºC for 2–3 h, microscopic morphology on corn-meal agar (DIFCO laboratories, Detroit, Mich., USA) with 1% tween 80.

3. Results

Out of 870 patients tracheal tube aspirate, the bacterial and fungal colonization were recovered from 40.22% samples. The positive specimens were belonged to 249 male and 101 female hospitalized patients. There was no sex related significant difference of contamination in the study subjects (P=0.43). The average age was 36.9±1.05 (between 1 to 89 yr.). The hospital stay duration average was 29±3.68 days and duration of being intubated had a median of 9 days. 87.1% of patients had at least one underlying disease such as diabetes mellitus, hypertension, hyperlipidemia, cardiovascular diseases, pulmonary diseases, and renal diseases. The most of isolates known to cause tracheal tubes colonization was 26.3% *Staphylococcus* spp. and less common was *Serratia* spp. with 0.4%. The coagulase negative staphylococci species included: *S.epidermidi*s (64%), *S.haemolyticus* (17.1%), *S.lugdune* (3.1%), *S.warnerii* (6.25%), *S.hominis* (6.25%),

S.pasteur (3.1%). Frequency distribution of microbial species present in tracheal tubes of patients admitted in ICU shown in Figure 1. Results show that *Candida* spp. was isolated in 7.4%, that *C.albicans* (42.3%) was the most frequently isolated species followed by other species included *C.glabrata* (25%), *C. tropicalis* (21.7%), and *C.krusei* (10.8%). There was a significant relationship between microbial colonization of endotracheal tube in ICU patients with duration of hospitalization, duration of being endotracheal tube, and underlying disease ($P \leq 0.05$). The results suggested that *S. aurous* and *Entrobacter* spp. had a significant relation with duration of being intubated (P=0.002). The highest incidences of colonization by *candida* spp. generally occurred at underlying disease such as diabetes mellitus and pulmonary diseases (P=0.04).



* CoNS: Coagulase negative staphylococci

Figure 1. Frequency distribution of bacteria and fungi isolated present in tracheal tubes of patients admitted in ICU of Emam Khomaini and Golestan hospitals.

4. Discussion

The incidence of nosocomial infections have been constantly increasing in parallel with the raising in the number of patients involving in predisposing factors (Wenzel, 1995). Sottile et al. first suggested a link between the endotracheal biofilm and pulmonary infection that this was supported by identical microbe being present in the lung specimen of patients with VAP and the endotracheal biofilm (Sottile et al., 1986). In order to prevent endotracheal tube biofilm formation, determining the sources of the microorganisms involved and the ways of acquisition of infection is essential to prevent both affliction and expansion of the diseases (Wolcott and Ehrlich, 2008). In our study was carried out in ICU, during the routine hospital practices, high rate of contamination was demonstrated with potential nosocomial pathogens (Khodavaisy et al., 2011). In this study which was performed in ICU admitted patient's tracheal tubes suggested that coagulase negative staphylococci and *E.coli* were the most prevalent bacteria isolated from tracheal tubes of the patients. In similar study undertaken by Tulla et al., the most common pathogens was *E.coli* (Tullu et al., 1998). However the study of Andair et al., was against the findings of the present study, which in overall they reported *Enterobacter* spp., *P. aeruginosa*, and *S.aureus* were the most prevalent bacteria isolated from tracheal tubes of the patients (Adair et al., 2002). Moreover, it has been demonstrated that *P.aeroginosa* and *Acinetobacter* spp. were the most common contaminating microorganisms. Interestingly, *Candida* spp., which is also a common colonizer of the oral cavity, was detected in 92 (7.4%) endotracheal tubes, that *C.albicans* (42.3%) was the most frequently isolated species. In the study, *C. albicans* was isolated in 29 patients (45%), while other species of *Candida* grew in 17 patients (26%), that was the same as our findings (Gil-Perotin et al., 2012). The studies showed that *C.albicans* produces filamentous growth forms, which

could readily enhance formation and structural stability of the biofilm and this yeast also demonstrates synergistic and antagonistic interactions with *S.aureus* and *P.aeruginosa* (López-Ribot, 2005, Park, 2005). The present study has demonstrated that colonization of tracheal tube in ICU patients increases with duration of intubation, that the results of some studies were similar to our findings (Amini et al., 2009).

Conclusion

The findings of this study indicate that microbial biofilms can form on the surface of tracheal tubes and increases with duration of intubation .The presence of bacterial and fungal biofilms of endotracheal tube suggests may be important in biofilm development and may provide a therapeutic target for the prevention of ventilator-associated pneumonia.

Acknowledgements

The authors are grateful to Zahra Hasanpor zafarani and Mohammad Reza Safari for his help in part of the analysis. We wish to thanks the staff of Emam Khomaini and Golestan hospitals for help in technical assistance to sampling.

Refereces

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