Archives of Oral Biology 85 (2018) 64–69

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Research Paper

Colonization of oropharynx and lower respiratory tract in critical patients: Risk of ventilator-associated pneumonia

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ARTICLE INFO

Keywords:
Ventilator-associated pneumonia
Intensive care unit
Oral cavity
Bacteria

ABSTRACT

Objective: To investigate the microbial diversity existing in oral cavity and respiratory tract samples (from mini-bronchoalveolar lavage (BAL), endotracheal aspirate, and orotracheal tube) of patients on mechanical ventilation by using the checkerboard DNA-DNA hybridisation. Also, the study aimed to evaluate whether the microbial profile in the oral cavity is found in respiratory tract samples, at different periods of mechanical ventilation time (12 h, 48 h, 96 h) in attempt to identification of relationship between VAP (ventilator-associated pneumonia) and bacterial species studied. The last objective was to analyses correlation between blood cultures and VAP.
Design: The samples were collected from ten patients in intensive care unit with medical prescription of orotracheal intubation and mechanical ventilation. Clinical data were tabulated and blood cultures were performed according medical indication. For checkerboard samples collection, chosen sites were the dorsal side of the tongue and gingival sulcus at 12 h, 48 h, 96 h, BAL, at 12 h, endotracheal aspirate at 48 and 96 h, and orotracheal tube at extubation time, when feasible.
Results: It was possible to identify the presence of bacterial species in mouth and in the BAL/endotracheal aspirate. The data demonstrated an increase in the quantity of bacterial associated with prolonged use of mechanical ventilation (48 and 96 h).
Conclusions: Bacterial species may migrate rapidly from mouth and upper airways during orotracheal intubation which contributes to the pathogenesis of VAP. There were associations between VAP and Enterococcus faecalis, Fusobacterium periodonticum, Gemella morbillorum, Neisseria mucosa, Propionibacterium acnes, Prevotella melaninogenica, Streptococcus oralis, Streptococcus sanguinis, Treponema denticola, Treponema socransckii, and Veillonella parvula.

1. Introduction

Pneumonia is the second most common hospital infection and the main cause of mortality among the hospital-acquired infections. Aspiration of microorganisms from the oropharynx into the lower respiratory tract, followed by bacterial proliferation and invasion of parenchyma, is the most important way by which ventilator-associated pneumonia (VAP) develops. Also, it may be occurs due to inhalation of contaminated aerosols or, less frequently, by haematogenic dissemination from a remote focus (Cavalcanti, Valencia, & Torres, 2005).

The ventilator-associated pneumonia (VAP) is characterised by the development of lung infection after 48 h of orotracheal intubation (OTI) and within 48 h of invasive mechanical ventilation (IMV) following extubation. Critical patients in intensive care unit (ICU) who are submitted to OTI and IMV present a VAP incidence above 78%, with mortality rate ranging from 24% to 76% depending on the population studied and technique used for pneumonia diagnosis (Bahrani-Mougeot et al., 2007).

The aetiology of VAP is variable and depends on time elapsed after initiation of mechanical ventilation, hospitalisation length, population, and hospital proceedings. Bahrani-Mougeot et al. (2007) analysed microorganisms from oral cavity and BAL by using the method of molecular cloning and sequencing to identify the most common species, namely: *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus influenzae*, which are predominant in the initial stage of VAP. Gram-negative aerobic bacteria, including species of enterobacteria

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http://dx.doi.org/10.1016/j.archoralbio.2017.09.029
Received 28 October 2016; Received in revised form 20 June 2017; Accepted 24 September 2017

Table 1
Strains used for the development of bacterial DNA probes.

Species	Strain ATCC	Species	Strain ATCC
Actinomyces gerecseriae	238060	*Leptotrichia bucallis*	14201
Actinomyces israelii	12102	*Neisseria mucosa*	19696
Actinomyces oris	43146	*Parvimonas micra*	33270
Actinomyces odontolyticus	17929	*Porphyromonas gingivalis*	33277
Aggregatibacter actinomycetemcomitans	43718 + 29523	*Prevotella intermedia*	25611
Campylobacter gracilis	33236	*Prevotella melaninogenica*	25845
Campylobacter rectus	33238	*Prevotella nigrescens*	33563
Campylobacter showae	51146	*Propionibacterium acnes*	11827 + 11828
Capnocytophaga gingivalis	33624	*Selenomonas noxia*	43541
Capnocytophaga ochracea	33596	*Streptococcus anginosus*	33397
Capnocytophaga sputigena	33612	*Streptococcus constellatus*	27823
Eikenella corrodens	23834	*Streptococcus gordonii*	10558
Enterococcus faecalis	29212	*Streptococcus intermedius*	27335
Eubacterium nodatum	33099	*Streptococcus mitis*	49456
Eubacterium saburreum	33271	*Streptococcus oralis*	35037
Fusobacterium nucleatum spp. Polymorphum	10953	*Streptococcus sanguinis*	10556
Fusobacterium nucleatum ssp. Nucleatum	25586	*Tanerella forsythia*	43037
Fusobacterium nucleatum ssp. Vicentii	49256	*Treponema denticola*	B1
Fusobacterium periodonticum	33693	*Treponema socranscki*	S1
Gemella morbillorum	27824	*Veillonella parvula*	10790

(*Enterobacteriaceae* family), are reported in both initial and advanced stages of the disease. *Pseudomonas aeruginosa* and species belonging to the *Acinobacter* and *Enterobacter* classes are more predominant in the more advanced stages only. *H. influenzae* and *S. pneumoniae* are more predominant in polytraumatized patients during the post-operatory period (Bahrani-Mougeot et al., 2007).

In a literature review, Paju and Scannapieco (2007) found an important role played by oral bacteria in the respiratory infections, suggesting that pathogens existing in the oral cavity such as *Streptococcus pneumonia, Haemophilus influenza* and *Mycoplasma pneumoniae* colonise the lower respiratory tract (Paju & Scannapieco, 2007).

Beraldo and Andrade (2008) reported that oral microbiota undergoes changes following intubation as there is an increase in microorganisms and formation of biofilm after 48 h (Beraldo & Andrade, 2008). Gram-negative bacteria are frequently isolated from both dental biofilm and pulmonary secretion, with the former consisting of multiple bacteria such as *Pseudomonas aeruginosa*, *Proteus spp.*, *Acinetobacter spp.* and *Staphylococcus aureus* (Bassin & Niederman, 1995).

One of the methods used for exploring the microbial diversity is the checkerboard DNA-DNA hybridization, which allows the investigation of multiple bacteria species by using a large number of samples containing microorganisms. It is a rapid and relatively viable technique in terms of economic perspective (Socransky et al., 2004).

Therefore, the present study aimed identify the microbial diversity existing in the oral cavity and their presence in both bronchoalveolar lavage (BAL) and endotracheal aspirate, including the microbial profile in ICU patients on IMV at different periods of OTI and the association between VAP and bacterial load obtained by DNA probes of the species studied.

2. Materials and methods

2.1. Study design and participants

This research was approved by the Institutional Ethics Committee (protocol # 283.047). For this clinical trial, samples were collected from ten patients recruited from the Intensive Care Unit at the POLICLIN hospital, São José dos Campos, São Paulo, Brazil. The laboratorial study was conducted at the Institute of Science and Technology, São Paulo State University, UNESP.

Prior to obtain the initial sample, all the patients were evaluated to collect clinical data: personal information, general health conditions and oral cavity situation of all individuals were recorded. Before the inclusion and exclusion criteria been applied, ICU responsible doctor evaluated each case and had selected a list of patient for this research. Ten critical ICU patients who were on invasive mechanical ventilation through orotracheal tube were included in the study, according inclusion (orotracheal intubation, presence of at least one tooth, and no severe or terminal lung disease) and exclusion criteria (Terminal and severe conditions, total teeth absence, acute oral infection, BAL with purulent aspect, and informed consent form not signed by the patient responsible relative or the patient).

Initial samples were obtained from bronchoalveolar lavage, two places in mouth: gingival sulcus (collected with paper cone), and dorsal side of the tongue (collected with swab), 12 h after the orotracheal intubation. After 48 h and 96 h, samples were collected from endotracheal aspirate, gingival sulcus, and dorsal side of the tongue. Finally, the last samples were gathered from the inner part of the orotracheal tube, with swab, after extubation.

The collection of endotracheal aspirate was performed according to standard procedure, using a 12 siliconized polyvinyl chloride (PVC) tracheal aspiration probe and the aspirated secretion was put into a sterile polypropylene collector tube (Eppendorf).

BAL was proceeded using telescoping catheters manually guided. The catheter were placed, locked and 20 ml of saline were instilled. Sample was obtained aspirating with vacuum to 40–60 mm Hg and immediately placed in sterile tube.

All the samples obtained were transferred to VMGAIII medium at temperature of up to 2 °C and kept at −20 °C for maintenance of their viability for further laboratorial processing.

For DNA Checkerboard analysis, samples and DNA standards representing 10^5 and 10^6 cells for target species were fixed on a membrane in thin lanes, then simultaneously cross-hybridized with 40 labelled, whole genome probes.

DNA from selected bacteria for probes, was isolated and purified as described previously (Smith, Socransky, & Smith, 1989). The genomic probes were prepared for each one of the 40 species (Table 1) by conjugating 1 mg of bacterial DNA with digoxigenin, by the random primer labelling kit Digoxigenin (Roche Diagnostics', Indianapolis, IN, USA) according to the method described previously (Feinberg & Vogelstein, 1983).

After hybridisation with such probes, the membranes were removed from the Miniblotter 45 apparatus treated according as previously described (Smith et al., 1989; Socransky et al., 2004) and placed in a radiographic film cassette (Kodak® X-OMAT, Kodak Brasileira Com. e Ind. Ltda, São José dos Campos, SP, Brazil) for approximately 40 min