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Prospective, randomised, controlled study evaluating early modification of oral microbiota following admission to the intensive care unit and oral hygiene with chlorhexidine

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ABSTRACT

Objectives: Chlorhexidine (CHX) is the most commonly used oral hygiene product for the prevention of ventilator-associated pneumonia (VAP) in patients undergoing mechanical ventilation (MV). The change in dental plaque (DP) microbiota following CHX use in patients under MV has not been described previously. The aim of this study was to evaluate the incidence of pathogenic bacteria associated with VAP and the coverage of DP within the oral cavity in patients administered CHX.
Methods: This was a prospective, randomised, controlled, double-blind study in patients ($n = 16$) under MV who were mouth-rinsed with either CHX or placebo. Microbiology samples were collected from the oral mucosa (OM) and DP after admission to the ICU and on Days 3, 5, 7 and 10. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of CHX were determined.
Results: Upon admission, the occurrence of multidrug-resistant (MDR) bacteria, including carbapenem-resistant *Klebsiella pneumoniae*, was reported. The CHX group had a lower incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) compared with the placebo group for the OM (RR = 0.51, 95% CI 0.27–0.98; $P = 0.011$). There was high agreement between the culture results of OM and DP ($\kappa = 0.825$). VAP developed in six patients. The species identified following tracheal aspiration of VAP patients were similar to those found in the OM for four cases. The strains showed low MICs and MBCs for CHX (<0.039 mg/mL).
Conclusions: Although DP is rapidly colonised by MDR bacteria, use of 2% CHX reduced the incidence of *S. aureus* colonisation.

1. Introduction

Ventilator-associated pneumonia (VAP) is the most clinically significant infection in patients admitted to the intensive care unit (ICU) [1]. It is associated with prolonged hospitalisation, increased treatment costs, and increased patient morbidity and mortality. Pathogen entry into the lower respiratory tract of patients undergoing mechanical ventilation (MV) is facilitated by aspiration of oropharyngeal and nasopharyngeal secretions accumulating over the orotracheal tube balloon [2].

The oral microbiota of patients in the ICU differs from that of the healthy population. For instance, after 48 h of hospitalisation there is a shift in the composition of the oral microbiota from one showing a predominance of normal, Gram-positive bacilli to one that has a prevalence of Gram-negative bacilli associated with VAP [3]. In addition, the normal dental plaque will also act as a natural reservoir for these bacteria [4].

Several studies have shown that maintenance of oral hygiene in patients under MV can reduce the rate of VAP [5]. Chlorhexidine (CHX) has been the most commonly used product for oral hygiene. Previous studies have validated its efficacy in reducing VAP rates [6]. However, the effects of CHX on the dental plaque microbiota of patients under MV have not been described. The aim of this study

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was to evaluate the coverage of dental plaque and the incidence of pathogenic bacteria associated with VAP in the oral cavity of patients mouth-rinsed with either CHX or placebo during their admission to the ICU. The secondary objective was to evaluate the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of CHX among the identified bacteria.

2. Methods

2.1. Study design

This was a prospective, randomised, controlled, double-blind study. The study was carried out at a 600-bed university hospital in Curitiba (Brazil) from June 2014 to March 2015. The study was approved by the Ethics Committee of Hospital Evangélico de Curitiba (Curitiba, Brazil). Clinical data of patients, microbiological samples and the progression to VAP were evaluated.

2.2. Inclusion and exclusion criteria

Inclusion criteria for patients consisted of the following: hospital admission followed by MV; age ≥18 years; patients identified as having a high probability of MV for >48 h; and permanent teeth (anterior and posterior). The following factors were considered for patient exclusion: failure to provide written informed consent; hospitalisation >24 h; recent use of antibiotics (<1 week); recent admission to another hospital or emergency room; suspected infection in the upper or lower respiratory tract; and less than four culture samples as described below.

2.3. Clinical data

Clinical data were evaluated, including age, sex, antibiotic use during the study period, co-morbidities (Charlson comorbidity index) and VAP progression as determined from the onset of the study period to 7 days after the last culture. The VAP criteria were those defined by the US Centers for Disease Control and Prevention (CDC) [7].

2.4. Intervention

Patients included in the study were randomised into two groups, CHX mouth-rinsing or placebo. In the CHX group, patients were subjected to oral washing with 15 mL of 2% CHX digluconate by a trained nursery team. The CHX solution was gently brushed into the gum, oral mucosa and tongue two times daily until ICU discharge. In the placebo group, patients underwent mouth-washing with a 0.9% NaCl solution.

2.5. Microbiology samples

Samples were collected from the oral mucosa (OM) and dental plaque (DP) by curettage with a brush. The first sample was collected immediately after admission to the ICU and subsequent samples were collected on Days 3, 5, 7 and 10. Samples were collected 6 h after the routine oral hygiene and were immediately plated for bacterial identification. After collection, samples were immediately inoculated on MacConkey and blood agar; only pathogenic bacteria were included for identification. Further identification of bacteria and susceptibility tests were performed using an automated method (VITEK®2; bioMérieux, Marcy-l'Étoile, France) or disk diffusion, with the Clinical and Laboratory Standards Institute (CLSI) used as reference [8]. The resistance rating (multidrug resistance, extensive drug resistance and pandrug resistance) was adapted from Magiorakos et al. [9]. Carbapenem-resistant Enterobacteriaceae were tested for carbapenemase production by the modified Hodge test; if positive, a molecular test for detection of the bla_{KPC} gene was performed as previously described [10]. In brief, isolates with a positive modified Hodge test were submitted to PCR for bla_{KPC} using EasyQ KPC (bioMérieux), with *Escherichia coli* ATCC 25922 and bla_{KPC}-carrying *Klebsiella pneumoniae* strain ATCC BAA-1705 as negative and positive controls, respectively. The amplified product was purified using Exonuclease I and Shrimp Alkaline Phosphatase (ExoSAP-IT; USB Corp., Cleveland, OH) for DNA sequencing. Sequencing reactions were performed using BigDye® v.1.1 Sequencing Kits (Applied Biosystems, Foster City, CA). Sequence data were acquired on an ABI 3100 Genetic Analyzer (Applied Biosystems). Gene sequences were compared with entries in databases queried by NCBI BLAST (nucleotide sequence database).

2.6. Determination of chlorhexidine minimum inhibitory concentrations and minimum bactericidal concentrations

A concentrated solution of 50 mg/mL (5% m/v) CHX digluconate (Sigma-Aldrich, St Louis, MO) was prepared with ultrapure water. This solution was diluted to obtain volumes of 100 mL at 2.5 mg/mL (0.25%), 1.25 mg/mL (0.12%), 0.65 mg/mL (0.065%), 0.31 mg/mL (0.031%), 0.15 mg/mL (0.015%), 0.078 mg/mL (0.0078%) and 0.039 mg/mL (0.0039%).

Bacteria were aerobically grown on Mueller–Hinton agar (HiMedia Laboratories, Mumbai, India) at 37 °C for 24 h. Characteristic colonies were obtained and were suspended in sterile 145 mM NaCl solution until a turbidity reading equivalent to 0.5 on the McFarland scale (ca. 1.5×10^8 CFU/mL). Bacterial suspensions were diluted at a ratio of 1:20 in sterile 145 mM NaCl solution.

Then, 50 μL aliquots of each bacterial suspension were transferred to 1.5 mL microtubes (Eppendorf AG, Hamburg, Germany) and were centrifuged at 10 000 × *g* for 5 min at room temperature. The supernatants were discharged by tube inversion and then 500 μL aliquots of different CHX concentrations were added to each tube. Pellets were suspended by vortexing for 10 s and the final suspensions were incubated at 37 °C. After 1 h and 12 h, the microtubes were centrifuged (10 000 × *g*, 5 min) and the pellets were washed with 145 mM NaCl. This procedure was repeated more than two times for the removal of residual CHX. Following a final centrifugation, the pellets were suspended with 300 μL of Mueller–Hinton broth (HiMedia Laboratories). The volumes were transferred to 96-well 'U' bottom microtitration plates (K30-5096U; Kasvi Produtos Laboratoriais, Curitiba, Brazil) and were incubated aerobically at 37 °C for 24–48 h.

The absence of bacterial sediments was indicative of inhibitory effects. The CHX MIC was the lowest concentration of the solution required to achieve these effects. The suspensions were spun and 10 μL aliquots were plated onto Muller–Hinton agar surfaces. Dishes were incubated for 48 h. The absence of bacterial growth was also indicative of bactericidal effects, whereby the lowest concentration of CHX required for these effects was referred to as the MBC. The procedures were performed in triplicate, in three independent moments. Negative controls (145 mM NaCl) were conducted in parallel.

2.7. Statistical analysis

Statistical analyses were performed with PASW Statistics for Windows v.18.0 (SPSS Inc., Chicago. IL) and statistical tests were performed according to the variables. Clinical data were used to evaluate the similarities between the patient treatment outcomes of CHX and placebo. Bivariate analysis was performed separately for each variable. *P*-values were calculated using the χ^2 test or Fisher's exact test for categorical variables, and Student's *t*-test or Wilcoxon rank-sum test for the continuous variables. The level of