Children's oropharyngeal colonization upon admission at a pediatric intensive care unit*

Colonização orofaríngea de crianças à admissão em uma unidade de cuidados intensivos

Colonización de la orofaringe de niños admitidos en una unidad de cuidados intensivos pediátricos

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ABSTRACT

Objectives: This study aimed to identify the microbiological oropharyngeal colonization pattern in children admitted at a Pediatric Intensive Care Unit (PICU) and to verify the influence of children's and implemented therapies' characteristics before admission at the UCIP on this pattern. Prospective study realized at a PICU of a university hospital. **Methods:** Samples of oropharyngeal secretion were obtained in the first 24 hours of the children's admission. Variables related to the children's and the therapies' characteristics were investigated. **Results:** The majority of the children had normal nutritional state, admitted in the hospital in emergencies situations, with chronic diseases and without alterations in the clinical conditions of the oral cavity. **Conclusion:** The children who were colonized by pathogenic microorganisms presented a longer hospital stay (p=0,020) than those with cultures with normal flora species. The investigated characteristics of the children did not exert any significant influence the microbiological oropharyngeal colonization pattern.

Keywords: Pediatric nursing; Cross Infection; Pediatric intensive care unit; Oropharynx; Pneumonia

RESUMO

Objetivos: Estudo prospectivo que objetivou identificar o padrão microbiológico da colonização da orofaringe de crianças à admissão em uma Unidade de Cuidados Intensivos Pediátricos (UCIP) e verificar a influência de características relativas à criança e terapêutica implementada antes da admissão sobre o padrão de colonização orofaríngeo. **Métodos:** As 55 crianças que compuseram a amostra foram submetidas a coleta de secreção de orofaringe nas primeiras 24 horas de internação. **Resultados:** A maioria das crianças eram eutróficas, admitidas no hospital em situação de emergência, portadoras de afecções crônicas, sem alterações clínicas da arcada dentária e mucosa da cavidade oral. **Conclusão:** As características das crianças e terapêutica não interferiram significantemente no padrão microbiológico da colonização orofaríngea das crianças na admissão na UCIP, contudo verificou-se que crianças portadoras de microorganismos patógenos apresentaram tempo de internação hospitalar maior (p=0,020) do que aquelas colonizadas somente por espécies de flora normal.

Descritores: Enfermagem pediátrica; Infecção hospitalar; Unidades de terapia intensiva pediátrica; Orofaringe; Pneumonia

RESUMEN

Objetivos: Estudio prospectivo que tuvo como objetivo identificar el patrón microbiológico de la colonización de la orofaringe de niños admitidos en una Unidad de Cuidados Intensivos Pediátricos (UCIP) y verificar la influencia de las características relativas al niño y terapéutica implementada antes de la admisión sobre el patrón de colonización orofaríngeo. **Métodos:** Los 55 niños que conformaron la muestra fueron sometidos a la recolección de secreción de la orofaringe en las primeras 24 horas de internamiento. **Resultados**: La mayoría de los niños eran eutróficos, admitidos en el hospital en situación de emergencia, portadores de afecciones crónicas, sin alteraciones clínicas de la arcada dentaria y mucosa de la cavidad oral. **Conclusión:** Las características de los niños y la terapéutica no interfirieron significantemente en el patrón microbiológico de la colonización orofaríngea de los niños en la admisión a la UCIP, con todo se verificó que los niños portadores de microorganismos patógenos presentaron tiempo de internamiento hospitalario mayor (p=0,020) que aquellos colonizados solamente por especies de la flora normal.

Descriptores: Enfermería pediátrica; Infección hospitalaria; Unidades de cuidados intensivos pediátrico; Orofaringe; Neumonía

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INTRODUCTION

Critically ill children are at high risk of developing hospital infections, as the physiological defense mechanisms may be impaired by the disease itself, as well as by the therapy and invasive procedures[1].

Hospital infection rates in Pediatric Intensive Care Units (PICU) range from 3% to 27%, with an attributed mortality rate of about 11%, depending on the characteristic of the hospital studied[2]. Among them, mechanical pulmonary ventilation pneumonia (MPVP) stands out. In adult Intensive Care Units (ICU), it is responsible for about 20% to 30% of the total of infections, ranging between 6-30 pneumonia episodes per 1000 days of MPV, according to the National Nosocomial Infecction Surveillance System classifications. In children, the estimated incidence is 20%, occurring more frequently among those from two months to one year old[3].

Infections occur when there is an inequality between the host's defenses and the microorganisms' virulent potential, i.e. when the pathogens become capable of surpassing the mechanical, humoral and/or cellular defenses of the body, invading, colonizing and establishing the affection in the host[4]. The importance of each potential reservoir of the gastrointestinal tract for colonization by infection-causing microorganisms, whether oropharyngeal or gastric, has been a source of discussion among researchers for many years. A large number of host-related characteristics may contribute to the occurrence of changes in oropharyngeal colonization and, consequently, in the pathogenesis of different infections, such as the disease severity, the need for surgical procedures and previous antibiotic therapy, the use of certain drugs and previous contact with invasive devices and equipment[5].

In ICU hospitalized adults, the colonization of the oropharynx by potentially pathogenic microorganisms may occur very quickly. However, the same cannot be insured for severely ill children, due to the lack of research corroborating this statement[6]. The sequence and mechanisms changing the oropharyngeal colonization of children are still under study, but they are multi-factor and may result from endogenous and exogenous processes, occurring before or after PICU hospitalization[5].

Endogenous colonization results from the proliferation of bacteria peculiar to the body, whether typical members of normal flora or not. These are microorganisms that only express their pathogenic activity when the host provides the appropriate conditions. Such conditions are particularly found in hospitalized patients and are associated, most of the times, to the use of antibiotics and immunosuppressants, surgical procedures, baseline diseases and the use of invasive devices[7]. At the PICU, exogenous colonization may occur as sporadic cases or epidemic outbreaks, caused by microorganisms considered as of hospital origin, which invade the body by means of crossed transmission from another colonized or infected patient, mediated by contact with the healthcare professionals' hands or alternative sources, such as ICU materials and equipment[7].

Literature lacks studies showing the relation between oropharyngeal colonization in children and the development of hospital infections. Thus, this study aimed to identify the microbiological pattern of oropharyngeal colonization in children upon admission at a PICU and to check the influence of characteristics related to the child and the therapy implemented before admission at the PICU on an oropharyngeal colonization pattern.

METHODS

Prospective and correlational study, performed at a PICU of a university hospital with 630 beds, located in São Paulo, from June 2005 to March 2006; the institution's Ethics Committee gave its approval prior to the implementation of the study. This study is characterized as a research sub-project, aimed at checking the influence of oral hygiene in preventing mechanical pulmonary ventilation pneumonia in children.

Within the study period, 146 (100.0%) children were admitted at the unit, 56 (38.3%) integrating the sample, with the following exclusion criteria were: newborn age level, diagnosis of pneumonia upon admission and remaining at the PICU for less than 48 hours. All children were subject to the collection of oropharyngeal cultures. However, due to problems in the processing of one of the cultures, the sample was constituted by 55 (98.2%) children. The sample was composed by all children, when possible, and/or their legal representatives who agreed to participate in the research, as proposed in the informed consent form.

The cultures for the analysis of oropharyngeal colonization were collected by rolling a swab in the tonsilar and posterior pharynx areas, within the first 24 hours of the child's hospitalization at the unit. All collections were performed in the morning by three previously trained nurses. For the performance of the collection, a protocol was developed, specifying the materials to be used, collection technique, sample identification, and conditions established for storage and transportation to the laboratory.

In the microbiological analysis laboratory, qualitative cultures of the oropharyngeal secretion samples was performed, in Agar blood, Agar chocolate, Agar eosine methylene blue and Agar Sabouraud and incubated according to the parameters determined for room,

temperature, time and humidity[8].

After the incubation, the suspected colonies were subject to biochemical tests for the exact identification of the species. Antibiograms were performed for the pathogenic microorganism species isolated in the cultures, and these were classified according to their reaction to certain antimicrobial agents, as sensitive or resistant.

The techniques for the determination of the strains' sensitivity followed the recommendations by the National Committee for Clinical Laboratory Standards (NCCLS), updated in 2005[9]. For the qualitative analysis, techniques of disk diffusion in Agar Mueller-Hinton were used, following the recommendations regarding thickness, ion concentration, temperature, pH, nutritive characteristics of the plate, incubation and application of the antibiotic disks to be tested, with subsequent analysis results as sensitive, intermediate or resistant, defined by the reading of the size of the halos formed and comparison with the cut-off points established by the NCCLS. In the study hospital, the selection of antimicrobial agents to be tested was established in the beginning of 2005, in a consensus obtained by the professionals in charge of the clinical microbiology laboratory, infectious disease specialists, hospital infection board and antimicrobials and drugs use control board.

Variables related to the child, such as gender, age and skin color were investigated, as well as variables related to aspects previous to the admission at the PICU that could change the children's oropharyngeal colonization such as: nutritional status; clinical changes in the oral cavity and mucous membrane; type of pathology, chronic or acute; admission characteristic at the hospital, ER or elective; previous hospitalization time, use of antibiotics upon children's PICU admission and hospitalization.

The quantitative variables were represented by mean, standard deviation (sd) and median and the qualitative variables by absolute (f) and relative (%) frequency. For statistical analysis, the Chi-Square Test, Fisher's Exact Test, Mann-Whitney Non-parametric Test and the T-test were used, adopting the significance level of 0.05[10].

RESULTS

Table 1 shows the demographics of the children in the sample. Most children were male (63.6%), white (67.3%) and with a mean age of 4.4 years, raging from 29 days to 16 years. Of the 55 children, 38.2% used antibiotics before PICU admission, for a mean time of 22.68 ±18.13 days. â-lactams, glycopeptides and aminoglycosides were the most frequent ones.

The results of the oropharyngeal secretion cultures obtained from children upon PICU admission showed that 32 (58.2%) had normal flora species colonizing the oropharynx, while and 23 (41.8%) had pathogenic microorganisms. The normal flora and pathogenic bacteria species isolated in the cultures are shown in Table 2.

Table 1 - Demographics of the children admitted at the Pediatric Intensive care Unit, São Paulo 2006.

n=55

Demographics		*f(%)*
Sex		
Male		35 (63,6)
Female		20 (36,4)
Skin color		
White		37 (67,3)
Brown		16 (29,1)
Black		2 (3,6)
Previous use of antibiotics		
Yes		21 (38,2)
No		34 (61,8)
Age		
Median	2.3 years old	
Mean	4.4 years old	
Standard Deviation	±4.5 years old	

Of the 23 children showing pathogenic microorganisms in the oropharyngeal secretion culture, in 22 (95.6%), these bacteria were antibiotic-resistant. Of the seven isolated strains of *Staphylococcus aureus*, 42.8% were methycillin-resistant. Four strains of *Klebsiella pneumoniae* were identified, half of which produced extended spectrum â-lactamase (ESBL), and four strains of *Enterobacter spp*, all from the group *Citrobacter, Enterobacter, Serratia and Providencia* (CESP), i.e., chromosomal â-lactamase producing bacteria, two of which were second and third generation cephalosporin-resistant.

Table 2 - Normal and pathogenic flora in the oropharyngeal secretion culture from children upon admission at a Pediatric Intensive Care Unit, São Paulo, 2006.

n=55

Microorganisms	f (% of total cultures)
Normal flora	
Streptococcus from the group viridans	43(78,1)
Negative-coagulase Staphylococcus	19(34,5)
Moraxella spp	17(30,9)
Pathogenic flora	
Staphylococcus aureus	7(12,7)
Acinetobacter baumanii	4(7,2)
Klebsiella pneumoniae	4(7,2)
Enterobacter spp	4(7,2)
Pseudomonas aeruginosa	2(3,6)
Escherichia coli	2(3,6)
Pseudomonas stutezeri	1(1,8)
Proteus mirabillis	1(1,8)
Morganella morganii	1(1,8)

Regarding the study of aspects prior to PICU admission, a predominance of eutrophic children was found (50.9%), admitted at the hospital in emergency

situations (54.5%), with chronic health problems (70.9%), without clinical changes in the dental arches (92.7%) and oral cavity mucous membrane (89.0%). These children showed a mean hospitalization time, previous to PICU admission, of 5.23±12.55 days and hospitalization time of 16.0±20.53 days. The study results on the relation between characteristics and aspects previous to hospitalization, with the identification of normal flora and pathogenic microorganisms, are shown in Table 3.

It was seen that most children colonized with potentially pathogenic microorganisms had chronic diseases, were malnourished and showed a longer hospitalization time before PICU admission and significantly longer hospitalization time (p=0.020) than the children whose oropharyngeal secretion cultures showed only normal flora species. It is further emphasized that, in children colonized only with normal flora species, a relatively longer time of previous antibiotics use was seen (Table 3)

DISCUSSION

The oropharyngeal colonization by potentially pathogenic microorganisms has been associated to different systemic diseases, including cardiovascular, pulmonary, renal disorders, and others. In the PICU, children constitute a patient population that may have an appropriate oral health or pre-existing diseases. The composition of dental plaque, presence of tartar and the number and severity of decay are factors that might influence the amount of bacteria colonizing the oropharyngeal site[11].

The oral colonization by pathogens may occur especially in patients admitted in emergency situations or due to chronic disease. Children with chronic pathologies are frequently seen in outpatient services and eventually require hospital care, many times showing impaired oral health due to difficulties to keep up appropriate oral hygiene and regular visits to the dentist[12-13]. Regarding emergency care, it may predispose to the breach of aseptic techniques established for the performance of procedures, due to the critical nature of the situation. The use of contaminated materials, devices or equipment could cause a higher colonization of the oral cavity by potentially virulent microorganisms[14]. However, the child's hospitalization status was a characteristic that did not significantly change the identification of normal flora or pathogen species among children.

The standard of oropharyngeal colonization is different between healthy and sick children. In this research, a large part of the malnourished children showed pathogen species colonizing the oropharynx.

Table 3 - Characteristics before hospitalization, according to oropharyngeal secretion colonization by normal flora or pathogenic microorganisms. São Paulo, 2006.

Characteristics	Normal Flora	Pathogenic Flora	Total	*p*
	f(%)	*f(%)*		
Type of hospitalization				
Emergency	17(56,6)	13(43,4)	30 (100,0%)	0,823[a]
Elective	15(60,0)	10(40,0)	25 (100,0%)	
Type of pathology				
Chronic	21(52,5)	19 (47,5)	40 (100,0%)	0,276 [a]
Acute	11(7303)	4 (26,7)	15 (100,0%)	
Clinical conditions of dental arch				
Change	3 (50,0)	3 (50,0)	6 (100,0%)	0,682 [b]
No change	29 (59,2)	20 (40,8)	49 (100,0%)	
Clinical conditions of gums and oral mucous membrane				
Change	-	3 (100,0)	3 (100,0%)	0,067[b]
No change	32 (61,5)	20 (38,5)	52 (100,0%)	
Nutritional Status				
Eutrophy	19 (67,8)	9 (32,1)	28 (100,0%)	0,141
Malnutrition	11 (44,0)	14 (66,0)	25 (100,0%)	
Overweight	2 (100,0)	-	2 (100,0%)	
Previous hospitalization time (days)				
Mean	4,48	6,14	5,23	
Standard Deviation	±13,64	±11,15	±12,55	0,321 [c]
Previous time of antibiotics use (days)				
Mean	21,14	11,5	22,68	
Standard Deviation	±26,69	±13,53	±18,13	0,191 [c]
Total hospitalization time (days)				
Mean	18,75	28,40	16,0	
Standard Deviation	±18,93	±15,7	±20,53	0,020 [c]

Key: [a] Chi-Square Test, [b] Fisher's Exact Test, [c] t-test.

Similarly, when evaluated by other investigators, the oropharyngeal colonization of severely malnourished children revealed an increased incidence of gram-negative microorganisms, inversely associated to the children's nutritional status and age[15].

The children showing the presence of pathogenic microorganisms showed a significantly longer hospitalization time, when compared to those colonized by normal flora species. The colonization by pathogenic bacteria, which might occur in long-lasting hospitalization and/or children subject to intensive care, represents a risk to the child, as they are associated to more severe infections and increased mortality rates.

Some children used systemic antibiotics during the hospitalization period before PICU admission, and those showing only normal flora species had received antibiotics previously for a relatively longer mean time than that of children in which pathogens were isolated. Even though there was no evidence of statistically significant differences, such results indicate the need for further investigation to determine the action of antibiotic therapy in the oropharyngeal colonization of hospitalized children.

The oropharyngeal normal flora species isolated in children's cultures were *Streptococcus* from the group viridans, coagulase-negative *Staphylococcus,* and *Moraxella* spp, findings similar to those showed in epidemiological studies developed to evaluate the incidence of the oropharyngeal colonization by gram-negative aerobial bacilli[16].

Regarding the potentially pathogenic microorganisms seen in this study, a higher number of gram-negative species was isolated, the most important of which *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumanii.* A single gram-positive bacteria was identified, *Staphylococus aureus*. A study regarding oropharyngeal colonization and the lower airways infection of 45 children, showed the presence of microorganisms uncommon to the normal oropharyngeal flora in 37.7%, and the main microorganisms identified were: *Pseudomonas aeruginosa* (50%), methycillin-sensitive *Staphylococcus aureus* (37%), *Haemophilus influenza* (11%), *Streptococcus pneumoniae* (9%) and *Moraxella catarrhalis* (9%)[17]. The proportion of children with pathogenic microorganisms in the above mentioned study was lower when compared to that obtained in this investigation (41.2%), and the pathogens identified had a different frequency and type of species.

Staphylococcus aureus is a potential pathogen, and can be found in the nasopharyngeal region as well as on the skin. Severe staphylococcal infections are more frequently acquired in hospitals, and the main route of transmission, the dissemination, is from one sick patient to another by the hands and contaminated objects. The risk factors for acquiring these species in the hospital environment have been related to extended hospitalization, extended antimicrobial therapy, and proximity of colonized or infected patients. Even though *Staphylococcus aureus* may be susceptible to the action of a number of drugs active against gram-positive bacteria, it is also known for its increased ability in developing resistance to many of them. The resistant lines produce â-lactamase, an enzyme inhibiting the action of the drug, codified by plasmidial genes. The use of methycillin and other semi-synthetic penicillins, such as â-lactamase resistant oxacillin, which started in 1959, represented a significant step in anti-staphylococcal therapy. However, resistance to these antibiotics was detected two years after the start of its use. Currently, a large part of the infections caused by *Staphylococcus aureus* are caused by methycillin-resistant lines[3,18-20].

Regarding the enterobacteria, they are gram-negative, non-sporulated, facultative anaerobial bacilli. Most enterobacteria are found in the gastrointestinal tract of human beings, among animals, in water, soil and vegetables. Some are also considered enteropathogenic, as they preferably cause gastrointestinal infections, even though they may also cause infections in other sites. They represent about 80% of all clinically important gram-negative microorganisms, and are responsible for around 70% of urinary infections and 50% of septicemias. In hospital infections, currently predominating enterobacteria are *Escherichia coli, Klebsiella spp,* and *Enterobacter spp*[3,18-20]. In our study, four strains of *Klebsiella pneumoniae* were identified, two of which were extended spectrum â-lactamase producers (ESBL), plasmidial, non-inducible, very potent enzymes that degrade practically all â-lactams, except only for carbapenems and cephamycins[19].

Regarding the *Enterobacter spp*, four strains belonging to group CESP - *Citrobacter*, *Enterobacter, Serratia and Providencia* were isolated. These microorganisms have become more related to hospital infections and antimicrobial-resistance over time. The resistance mechanism responsible for the loss of activity and action spectrum of third generation cephalosporins, monobactans and board-spectrum penicillins, in this species, emerged from the production of inducible chromosomal â–lactamases, called Class 1 or AmpC. Bacterial species from Group CESP have a gene that codifies increased enzyme production. This gene is usually inactive, although the bacteria's exposure to an inducing substance, usually the â–lactams themselves, activates the gene and the bacteria starts to produce a large amount of enzymes, thus becoming resistant to second and third generation cephalosporins[19].

Of the *Acinetobacter baumanii* strains isolated in this research, 87.5% showed carbapenems resistance. The gram-negative bacilli, classified as non-fermenters, are aerobial, non-sporulated microorganisms characterized as unable to use carbohydrates as source of energy by means

of fermentation, degrading them through oxidation. The characterization of the bacteria group is very important in cases of hospital infection, as they usually show resistance to a number of antibiotics and are able to cause severe infections. These bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter baumanii*, colonize and cause infections, especially in severe patients and patients subject to invasive procedures[3,18-20].

It is emphasized that the resistance and sensitivity pattern manifested by the isolated pathogenic bacteria is similar to the bacteria pattern identified at the study hospital. In this pattern, the carbapenems' and other broad spectrum â-lactams' resistance is identified in *Acinetobacter* spp and *Pseudomonas aeruginosa* samples; oxacillin resistance in samples of *Staphylococcus aureus, Escherichia coli,* and ESBL producer *Klebsiella pneumoniae* and inducible chromosomal â-lactamase producer *Enterobacter* spp resistant to ceftazidime and other third generation cephalosporins[19].

This way, due to the importance of upper airway colonization by pathogens in MPV pneumonia and the pathogenesis of other hospital infections, the description of these patients' microbial oral flora profile may be useful to acknowledge children at higher risk for the development of infections by virulent pathogenic microorganisms.

CONCLUSION

The investigation of the microbiological oropharyngeal colonization pattern of PICU admitted children showed that 58.2% had normal flora species colonizing the oropharynx, while 41.8% had pathogenic microorganisms, most of the latter showing antibiotics resistance. The normal flora and pathogenic bacteria species more frequently isolated in the cultures were: *Streptococcus from the group viridans, negative-coagulase Staphylococcus, Moraxella spp, Staphylococcus aureus, Acinetobacter baumanii, Klebsiella pneumoniae, Enterobacter spp , and Pseudomonas aeruginosa.*

The characteristics regarding the child and the therapy implemented before PICU admission did not interfere significantly in the microbiological oropharyngeal colonization pattern upon PICU admission. However, children with pathogenic bacteria showed a significantly longer total hospitalization time when compared to those with predominant colonization by normal flora species.

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