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Colonizing Flora Dynamics in Intubated Surgical ICU Patients

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Abstract

The main cause of the occurrence of nosocomial infections are Gram-negative bacteria. Colonized medical devices (tubes, cannulas, drains, catheters) are an existing risk factor. The aim of this study was to determine bacterial colonization of endotracheal tubes and nasal cannulas in intubated patients at the Clinic of Anesthesiology, Reanimation and Intensive Care (CARIC), which represents a pre-stage to nosocomial pneumonia, characterized by a high mortality rate (up to 70 %). A five-year retrospective study (from 2010 to 2014) was performed at the CARIC, Skopje, Republic of Macedonia. Laboratory data for bacterial isolates from endotracheal tubes and nasal cannulas were obtained through computer software for daily routine work of the Institute of Microbiology and Parasitology. The rate of isolation and Student's t-test were performed. Bacteria of the genus *Acinetobacter spp*. (46.5%) were the most frequently isolated microorganisms from endotracheal tubes and from nasal cannulas - bacteria of the genus *Pseudomonas spp*. (38,4%). *Pseudomonas spp*., *Acinetobacter spp*., *Klebsiella spp*. and *S. aureus* (MRSA strains in particular) are the most frequent colonizers medical devices. Continuous microbial control of hospital environment and systematic monitoring of the hospital ecosystems are necessary to reduce the risk of intra-hospital transmission and infection.

Keywords: nosocomial infections, intensive care, tube, cannula, microbionta, bacteria

Резюме

Основните причини за появата на вътреболнични инфекции са Грам-отрицателните бактерии. Колонизираните медицински устройства (тръби, канюли, канали, катетри) са съществуващ рисков фактор. Целта на това изследване е да се определи бактериалната колонизация на ендотрахеални тръби и назални канюли при интубирани пациенти от Клиника по анестезиология, реанимация и интензивно лечение (КАРИЛ), която представлява предшестващ етап на нозокомиална пневмония, и в която има висока смъртност (до 70%). В КАРИЛ в Скопие, Република Македония е извършено петгодишно ретроспективно проучване (от 2010 до 2014 г.). Чрез компютърен софтуер са получени лабораторни данни за бактериални изолати от ендотрахеални тръби и назални канюли, използвани в ежедневната рутинна работа на Института по микробиология и паразитология. Установена и скоростта на изолиране и е приложен Student's t-test. Най-често изолираните микроорганизми от ендотрахеални тръби са бактерии от рода *Acinetobacter spp.* (46,5%), а от назалните канюли - бактерии от рода *Pseudomonas spp*. (38,4%). *Pseudomonas spp*., *Acinetobacter spp*., *Klebsiella spp*. и *Staphylococcus aureus* (по-специално щамове на MRSA) са най-честите колонизатори на медицински устройства. Непрекъснатият микробиологичен контрол на болничната среда и систематичният мониторинг на болничните екосистеми са необходими, за да се намали рискът от вътреболнична трансмисия и инфекция.

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Introduction

Nosocomial infections are one of the leading causes of illness in the world and therefore represent a significant public health problem (Centers for Disease Control and Prevention, 2014). Infection acquired in a hospital environment is the most common type of complication that occurs in hospitalized patients. In Europe, 10% of the population is hospitalized each year, and it is assumed that 5% of hospitalized (3.8% of patients in general departments and 15.3% of patients in intensive care units) will acquire at least one intra-hospital infection. In developed countries, although the number of patients treated in a hospital environment and the average length of stay in hospital have decreased over the last few decades, the rate of nosocomial infections has increased (European Science Foundation, 2005). The most significant infections acquired in a hospital environment, according to the frequency and severity of the infection, are bacteremia and sepsis, surgical wounds infections and infections associated with the use of medical devices (Guggenbichler *et al*., 2011). At least half of all intra-hospital infections are associated with the use of medical equipment. Of special importance are medical devices (catheters, tubes) that are categorized as critical equipment for the occurrence of infection. The presence of a medical device greatly facilitates the occurrence of infection. Their use is massive in intensive care units (Locci *et al*., 1981; Vincent, 2003; Lorente *et al*., 2005; Safdar *et al*., 2011). Hospital treatment in intensive care centers involves the use of mechanical ventilation equipment (respirators), intravenous and urinary catheters, which perform mechanical depression of the already weakened defense mechanisms of patients, thereby contributing to the easier development of infection. Sometimes, despite the low virulence of colonizing microorganisms, defense mechanisms are not able to prevent infection, even when the host is completely immunocompetent (Guggenbichler *et al*., 2011; Safdar *et al*., 2011). According to statistics made in hospitals in the United States, the death rate in intensive care units is four times higher in patients who have had nosocomial infection than patients who did not develop a hospital infection (World Health Organization, 2010). Ventilator-associated pneumonia (VAP) is an infection associated with the use of tubes (endotracheal or tracheostomy) and nasal cannulas and is the most common hospital infection acquired in intensive care centers with an incidence of 9% to 70% - an average of 20-25% (Locci *et al*., 1981; Vincent, 2003; Diaz *et al*., 2005; Lorente *et al*., 2005; Safdar *et al*., 2011). The main causes of the occurrence of VAP are microorganisms that colonize the intubation devices. These microorganisms are part of the nasopharyngeal and oropharyngeal endogenous flora of patients, or exogenously acquired microorganisms from the hospital environment: contaminated respiratory equipment, hands of hospital workers and air (Cunnion *et al*., 1996; Fagon *et al*., 1996; Cook and Kollef 1998; Adair *et al*., 1999; Rello *et al*., 2003; Diaz *et al*., 2005; Hurley, 2005; Safdar *et al*., 2005). Colonization precedes nosocomial infection. Colonization of patients in hospital conditions occurs within the first 3-5 days after admission to the hospital, whereby the normal microflora is replaced by a hospital colonizing microflora. This colonization occurs firstly in the oral cavity and then in the tracheobronchial stem. Colonization of the tracheobronchial stem is a pre-stage of infection on the lower respiratory tract (Johanson *et al*., 1972). The most common antibiotic treatment in intensive care units is the use of broad-spectrum antibiotics, which results in a dramatic change in the normal oral microflora of patients and dominance of multiresistant Gram-negative bacilli and *Staphylococcus aureus*. The massive use of drugs in these departments, antibiotics and proton pump inhibitors leads to alkalization of the gastric acid and the stomach acid becomes a reservoir of multiresistant Gram-negative bacilli (Bonten *et al*., 1997; Schierholz *et al*., 2001). These facts emphasize the need for continuous monitoring of nosocomial infections through microbiological control of medical equipment, as well as by monitoring the microflora of ecosystems in the intensive care centers. This paper will investigate the condition of the ecosystem and the colonizing microflora in the largest ICU in the Republic of Macedonia.

The aim of this paper was to determine the bacterial colonization of endotracheal tubes and nasal cannulas in patients at the Clinic for Anesthesia, Resuscitation and Intensive Care (CARIC) and to determine if there are changes in this ecosystem over a longer period of time.

Material and Methods

In this retrospective study, laboratory data for 1007 bacterial isolates from endotracheal tubes and nasal cannulas from CARIC were used as material, sent for microbiological analysis at the Institute of Microbiology and Parasitology in the period from 2010 to 2014. The current laboratory data were obtained by a computer search method

using the laboratory software for daily routine work (LabIS-Codex) at the Institute of Microbiology and Parasitology. The isolation rate of the most isolated bacterial species from both types of specimens (medical devices) was determined annually. For the statistical analysis, Student's t-test was used to determine the statistical significance of the most frequently isolated bacteria and to track the changes in their isolation rate during the examined period. The obtained results were compared with literary data for previously monitored periods.

Results

Bacterial strains were isolated from 690 examples (68.5%). A total of 748 endotracheal tubes were microbiologically examined. Bacterial isolates were found in 468 (62.6%). The most common isolated microorganisms were Gram-negative non-fermentative bacteria: *Acinetobacter spp.* (60.3%) and *Pseudomonas spp.* (22.4%). The Gram-positive bacterium most frequently isolated from the endotracheal tubes was *S. aureus* (9.6%). Methicillin resistant *S. aureus* (MRSA) strains were 70%. The most common bacterial isolates from the endotracheal tubes in the five year period are shown in Table 1.

In the examined five-year period, from a total of 259 microbiologically examined nasal cannulas, 222 (85.7%) were positive (with bacterial isolates). The most commonly isolated microorganisms were Gram-negative bacteria of the genera: *Acinetobacter spp*. (37.4%) and *Pseudomonas spp*. (49.5%). The most common Gram-positive colonizer was *S. aureus* with a low isolation percentage (11.7%). *S. aureus* isolates were 84.6% MRSA strains. The isolation rate of these bacteria annually is shown in Table 2.

The most statistically significant ($p < 0.0001$) are the Gram-negative bacteria of the genus *Acinetobacter spp*. (52.9%) and *Pseudomonas spp*. (31.2%) relative to other isolated microorganisms (*Klebsiella spp*., *S. aureus*, *Enterobacter spp*., *Serratia spp*., *Enteroccocus spp*, *Proteus spp*., *Escherichia coli*, *Coagulase-negative staphylococci* etc.). Changes in their isolation rate during the examined period from year to year were not statistically significant. According to the isolation rate, the most frequently isolated bacteria after *Acinetobacter spp*. and *Pseudomonas spp*. are: *S. aureus* (10.3%) and *Klebisella spp*. (9.7%).

Discussion

Positive samples or samples of isolated bacteria are about 70%-85%, indicating massive colonization of medical respiratory devices in the intensive care unit. Isolated bacteria represent a hospital colonizing microflora of patients at the intensive care center (Johanson *et al*., 1972). The highest isolation rate was observed in bacteria of the genus *Acinetobacter spp*. (46.5%) from endotracheal tubes. The epidemiological characteristics of these bacteria contrib-

Table 1. Most common bacterial isolates from endotracheal tubes (2010-2014)

Year	*Acinetobacter spp.*	*Pseudomonas spp.*	*S. aureus*
2010	28.5%	19.5%	12.3%
2011	45.9%	12.6%	8.2%
2012	37.7%	12.3%	4.8%
2013	47.3%	9.3%	4.7%
2014	24.4%	17.4%	2.6%

Table 2. Most common bacterial isolates from nasal cannulas (2010-2014)

Year	*Acinetobacter spp.*	*Pseudomonas spp.*	*S. aureus*
2010	43.3%	35.6%	15.6%
2011	28.6%	49%	14.3%
2012	28.3%	28.3%	6.5%
2013	31.3%	50%	3.1%
2014	40%	20%	0

ute to their dominance in the hospital ecosystem. *Acinetobacter spp*. can survive in a hospital environment up to several weeks. The ability to endure conditions of high humidity, as well as a dry environment for a longer period of time makes it a very important nosocomial pathogen. This genus has a tendency towards endemicity, as well as tendencies towards seasonal variation. Seasonal variation of *Acinetobacter spp*. isolated from tubes and cannulas from CARIC was proven for the period from 2010 to 2012 (Petrovska *et al*., 2014; Popovska *et al*., 2014). *Acinetobacter spp*. was proven to be an important pathogen in CARIC in the period from 1991 to 1994 (during this period, *Pseudomonas aeruginosa* dominated in the hospital ecosystem), but its isolation rate increased dramatically in the period 2007-2012 while the percentage of MRSA declined. It is interesting that in the period from 1997 to 2007, the rate of isolation of MRSA strains in CARIC was over 80%, and in 2007 this rate was halved (Popovska *et al*., 2009). The results of this paper show that the isolation of MRSA is significantly reduced and *Acinetobacter spp*. has displaced this microorganism from the CARIC ecosystem. Most likely, this is due to widely taken preventive measures against the development of MRSA, an already proven nosocomial pathogen in CARIC for a period longer than 10 years. *Acinetobacter spp*. is a multiresistant bacterium, just like MRSA, but its epidemiology is different. An increase in the isolation rate of *Acinetobacter spp*. from the intensive care unit increases the rate of resistance to carbapenems. Treatment of infections caused by *Acinetobacter spp*. has become more difficult and so is its eradication from the hospital environment (Popovska *et al*., 2014). Bacteria of the genus *Pseudomonas spp*. were most often isolated from nasal cannulas during the examined period (38.4%). *P. aeruginosa* was the most common cause of nosocomial lower respiratory infections and is proven to be an endemic microorganism in intensive care units in 17 countries worldwide. In the period from 1991 to 1994, it was the most widely spread microorganism in the hospital system of CARIC with 41%, and then in the period from 2006 to 2007 its presence in the hospital environment dropped to 14% and again rose to 31.2% during the examined period (Popovska *et al*., 2009). In correlation with literature data, *Pseudomonas spp*., *Acinetobacter spp*., *Klebsiella spp*. and *S. aureus* (especially MRSA strains) are the most common colonizers of the oral microflora, as well as the most commonly isolated microorganisms from the lower airways in cases of hospital-acquired pneumonia in intensive care units (Groeger, 1993; Bonten *et al*., 1997; Friedland *et al*., 2001; Schierholz *et al*., 2001; Khosravi *et al*., 2012; Abdollahi *et al*., 2013). The vicious circle of colonization and infection of the patient, contamination of the inanimate environment and colonization of other patients allowed these bacteria to remain the main components of the ecosystem in the Clinic for Anesthesia, Resuscitation and Intensive Care in the period from 2010 to 2014. By determining the colonizers of the hospital environment and the endemic strains in the hospital ecosystem, it is possible to predict infection and appropriate therapy, as well as to take measures for prevention of nosocomial infections, raising the health of patients at a higher level, reducing hospital days and the cost of treatment.

Conclusions

A significant change in the CARIC ecosystem has been confirmed in relation to the pre-monitored period, which has led to the replacement of MRSA with *Acinetobacter spp*. This is due to the measures undertaken for the eradication of MRSA. Continuous microbiological control of the inanimate hospital environment is necessary as well as systematic monitoring of the microflora in the hospital ecosystems in order to improve the preventive measures.

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