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Relationship Between Isolating Multi-Drug Resistant *A. Baumannii* and *K. Pneumoniae* in Bronchial Aspirate and Subsequently in Blood Cultures: Evaluation of Colistin Aerosol Therapy in Intensive Care Patients

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Abstract

Klebsiella pneumoniae (*K. pneumoniae*) and *Acinetobacter baumannii* (*A. baumannii*) are multiresistant pathogens most frequently found in patients hospitalized in the Intensive Care of our hospital. We found the highest rate of positivity in the bronchial aspirates, often followed by a similar observation in blood culture. We carried out a study to evaluate the reduction of sepsis that developed after the detection of these microorganisms in bronchial aspirate, following the introduction of treatment with colistin aerosol in combination with conventional therapy. From 2012 to the first half of 2014, 533 patients were admitted to the ICU of our hospital. Since May 2013, colistin was administered by aerosol along with conventional therapy. In 43 (78.1%) cases *K. pneumoniae* KPC was isolated from bronchial aspirate and in 30 (54.5%) from blood culture; MDR *A. baumannii* was isolated in 89 (96.7%) cases of bronchial aspirate and 45 (48.9%) from blood culture. From patients treated with added colistin aerosol, *K. pneumoniae* KPC was isolated from bronchial aspirates in 15 cases and in 4 (26.7%) of those was found sepsis. MDR *A. baumannii* was isolated from bronchial aspirate in 41 cases, and in 10 (24.4%) it developed sepsis due to the same germ. The patients admitted to intensive care for more than 7 days were positive for MDR *A. baumannii* and MDR *K. pneumoniae* in bronchial aspirates and subsequent relief of blood culture. The treatment with colistin aerosol resulted in a reduction of the spread of microorganisms from the lower respiratory tract to the blood.

Introduction

The incidence of nosocomial respiratory infections is particularly relevant in ICU patients undergoing assisted ventilation [1]. In these patients, the abolition of the physiological defense mechanisms (glottis, cough reflex, mucociliary clearance, and nasal filter in the upper respiratory tract), as well as the mechanical irritation of the mucous membrane along with the alteration in the microbial ecosystem of the digestive tract [2] may contribute to the onset of pneumonia caused by assisted ventilation, which represents one of the risk factors for the development of sepsis [3,4]. This study was inspired by the observation that the number of positive blood culture results due to MDR *Klebsiella pneumoniae* (*K. pneumoniae*) and MDR *Acinetobacter baumannii* (*A. baumannii*) has decreased since May 2013. We have verified that the reduction of positivity coincided with the introduction, for these patients, of treatment with colistin aerosol, therefore we conducted a study to assess whether the combination of colistin aerosol with the conventional treatment is able to reduce the incidence of sepsis as a complication of pneumonia due to MDR *K. pneumoniae* and MDR *A. baumannii* in patients admitted to the ICU of our hospital.

Patients

Patients: From January 2012 to June 2014, 533 patients were admitted to the ICU of our hospital. 71/533 (13.3%) for a period > 48h and < 7 days and 213/533 (40.0%) for a period > 7 days, whereas 249/533 were present in ICU for a period < 48 h. On 238/284 (83.8%) patients hospitalized for > 48 h a rectal swab was performed on their admission to the ward to detect MDR *K. pneumoniae*. On the basis of the length of their stay in ICU, patients were divided.

In the following groups: from January 2012 to April 2013 128 (44.8%) patients admitted in our ICU for a period < 48 h, 41(14.3%) patients admitted for a period > 48 h and < 7 days, 117 (40.9%) patients admitted for a period > 7 days; from May 2013 to June 2014 121 (49.0%) patients admitted for a period < 48 h, 30 (12,1%) patients admitted for a period > 48 h and < 7 days, 96 (38.9%) patients admitted for a period > 7 days [5,6] (Table 1) Patients hospitalized for < 48 hours were not enrolled in the study: this shorter stay is caused by the transfer to another department or by the patient's death. Patients hospitalized for > 48 hours were enrolled in the study. Patients hospitalized in the period January 2012 - April 2013, following the positivity for MDR *K. pneumoniae* and/or MDR *A. baumannii*, were treated with the conventional therapy, which consists of intravenous

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Table 1: Number of hospitalized patients

	January 2012-April 2013	May 2013- June 2014	TOTAL
Hospitalized patients < 48 h	128 (44.8%)	121 (49.0%)	**249**
Hospitalized patients > 48 h < 7 gg	41 (14.3%)	30 (12.1%)	**71**
Hospitalized patients > 7 gg	117 (40.9%)	96 (38.9%)	**213**
TOTAL	286	247	**533**

Figure 1: Device for the administration of colistin aerosol

colistin injections, because of the unavailability of the device necessary to perform the therapy with aerosolized colistin. Patients hospitalized in the period of May 2013 - June 2014 have received, as a result of microbiological findings, the combined therapy comprising aerosolized colistin and intravenous colistin.

Methods

The patients admitted to our ICU were subjected to, on admission, rectal swabs, bronchial aspirates and blood cultures. The rectal swabs were performed at admission to detect carrier status while other tests were carried out to verify the initial microbiological condition of the patient. Patients admitted to the ICU were subjected to bronchial aspirates and blood cultures every week or if their internal body temperature was found to be > 38˚C. Rectal swabs were not repeated during the hospital stay. On 238/284 (83.8%) patients hospitalized for > 48 hours a rectal swab was performed on their admission to the ward to detect MDR *K. pneumoniae* The search of MDR *K. pneumoniae* from rectal swab was performed by radial diffusion of a meropenem diskette on agar Mac Conkey [7,8]. In samples found positive for MDR *K. pneumoniae,* identification and susceptibility testing were performed with the system Vitek2 (bioMérieux-Marcy l'Etoile, France) and the carbapenem MICs between 0.5 and 2 mg/ml were tested using E-test (bioMérieux-Marcy l'Etoile, France). The mechanisms of resistance of multiresistant *K. pneumoniae* and *A. baumannii* (MDR *K. pneumoniae*, MDR *A. baumannii*) were detected by synergistic effect between boronic acid and carbapenems (meropenem, imipenem and ertapenem) on Müller-Hinton agar [9,10] and using the modified Hodge test [11,12]. The bronchial aspirate was inoculated on enriched chocolate agar, sheep blood agar, Columbia CNA agar, Mac Conkey agar, mannitol salt agar, Sabouraud agar with CAF. The cultures were incubated at 37˚C and measured at 24h and 48h. The blood for culture is collected in bottles containing the culture medium: the levy provides 3 vials for aerobic germs and one bottle for anaerobic germs that are incubated for 7 days at 37˚C in Bact Alert (Bio-Merieux) system. The definition of multi-resistant microorganism has been given on the ground of resistance to beta-lactam antibiotics, carbapenems and chilonons. Since May 2013, when the device was available (Figure 1), in patients whose blood was isolated in the bronchial aspirated MDR *K. pneumoniae* with number of colonies > 10^6 CFU/ml, colistin was administered by aerosol (1-2 × 10^6 UI every 8 h) with Aeroneb® Solo System (Aerogen- Dangan, Ireland), along with conventional therapy (colistin 10^5 UI/kg of body weight divided into 3 doses/day with minimum dosage of 9 × 10^6 UI + meropenem 2 g/3 times/day + tigecycline 100 mg followed by 50 mg/twice/day) [13-16]. For cases in which MDR *A. baumannii* was found in bronchial aspirate, with number of colonies > 10^6 CFU/ml, treatment with aerosol-administered colistin was performed in addition to the conventional treatment (colistin 10^5 UI/Kg of body weight divided into 3 administration/day with minimum dosage of 9 × 10^6 UI + rifampicin 10 mg/kg/day ± meropenem 2 g/3 times/day) [17].

Results

Rectal swabs were performed in 238 patients. 5 of these 238 rectal swabs (2.1%) were found to be positive for *K. pneumoniae* KPC. Of those patients who were found to be positive for *K. pneumoniae* KPC in rectal swabs: one was found positive in bronchial aspirates, one was found positive in blood culture, one was found positive from urine and, in one case, we observed positivity in blood culture, bronchial aspirates and urine at the same time. The positivity for *K. pneumoniae* KPC appeared after an average of 9.8 days (range: 3-15 days). One patient died within 8 days from admission without positive feedback from other materials. In 1/71 patients hospitalized for > 48 hours and < 7 days *K. pneumoniae* KPC was isolated, while in no instance MDR *A. baumannii* was found. In 55/213 (25.8%) patients hospitalized for > 7 days *K. pneumoniae* KPC was isolated, and in 92/213 patients (43.2%) MDR *A. baumannii* (Table 2) was isolated. In 43 (78.1%) patients *K. pneumoniae* KPC was isolated from bronchial and in 30 patients (54.5%) from blood culture; MDR *A. baumannii* was isolated in 89 (96.7%) cases from bronchial aspirates and in 45 patients (48.9%) from blood culture (Table 3). We also observed that in patients positive for *K. pneumoniae* KPC the appearance of positivity for *K. pneumoniae* KPC in blood is always preceded by the appearance of positivity in bronchial aspirate with an average of 10.2 days (range 1-30 days). However, for patients positive for MDR *A. baumannii*, in 19 cases the positive blood followed the positivity of the bronchial appearance after an average of 6.7 days (range 1-29 days) and in 9 patients the positive blood preceded the positivity of the bronchial appearance with an average of 7.7 days (range 1-21 days. In the group of patients treated with conventional therapy alone, 28 were found positive for *K. pneumoniae* KPC in bronchial aspirate, and 13 patients (46.4%) of these developed sepsis caused by the same organism; in 48 patients MDR *A. baumannii* was isolated from bronchial aspirates and in 22 patients (45.8%) MDR *A. baumannii* was isolated from blood culture. In the time elapsed since the aerosol therapy was introduced in May 2013, 15 cases were found positive for *K. pneumoniae* KPC in bronchial aspirate and 41 tested positive for MDR *A. baumannii*. Of the patients positive for *K. pneumoniae* KPC, 4 patients (26.7%)

Table 2: Number of isolation of *K. pneumoniae* KPC and MDR *A. baumannii*

	2012	2013	Jan-Jun 2014	TOTAL
K. pneumoniae KPC	21	22	12	**55 (25.8%)**
MDR *A. baumannii*	34	38	20	**92 (43.2%)**

Table 3: Materials from which were isolated *K. pneumoniae* KPC and *A. baumannii* MDR

	K. pneumoniae KPC	MDR *A. baumannii*	*K. pneumoniae* KPC + MDR *A. baumannii*
Bronchial Aspirate	43	89	31
Blood	30	45	14
Urine	9	8	0
Others	6	6	1

Table 4: Isolation of *K. pneumoniae KPC* and MDR *A. baumannii* from patients with conventional antibiotic treatment vs patients treated with colistin aerosol in addition to conventional antibiotic treatment.

Conventional Treatment		Conventional Treatment+Colistin Aerosol	
K. pneumoniae KPC		*K. pneumoniae* KPC	
Bronchial Aspirate	28	Bronchial Aspirate	15
Bronchial Aspirate + Blood	13 (46.4%)	Bronchial Aspirate + Blood	4 (26.7%)
MDR *A. baumannii*		MDR *A. baumannii*	
Bronchial Aspirate	48	Bronchial Aspirate	41
Bronchial Aspirate + Blood	22 (45.8%)	Bronchial Aspirate + Blood	10 (24.4%)

developed sepsis. Of those patients who were found to be positive for MDR *A. baumannii*, 10 (24.4%) developed sepsis (Table 4). The analysis of the obtained data allows us statistical treatment only for cases in which the result on the positivity for MDR *A. baumannii* was numerically sufficient to calculate the index of probability. In this case, the comparison between patients receiving only conventional therapy and patients who received the combined therapy allows us to detect a statistically significant difference ($p < 0.005$) in the reduction of incidence of sepsis after introducing the colistin aerosol therapy. The statistical treatment was not possible for the groups positive for *K. pneumoniae* MDR: the data available to us, which is numerically insufficient for statistical analysis, only allows for an estimate of the percentage reduction of sepsis in patients with bronchial aspirates positive for *K. pneumoniae* MDR-treated colistin aerosol.

Discussion

We observed that the increase of the duration of hospital stay favors the onset of infection by *K. pneumoniae* KPC and MDR *A. baumannii*. Patients admitted to the ICU for less than 7 days did not present positivity for these microorganisms. The only exception was a patient already presenting with for *K. pneumoniae* KPC at admission to the ICU, condition which then showed on the positive bronchial aspirate. On the other hand, we have observed a high incidence of positivity for *K. pneumoniae* KPC and MDR *A. baumannii* in bronchial aspirates in patients hospitalized for more than 7 days, often followed by the same positivity in blood culture. Following the introduction of treatment with colistin aerosol in combination with conventional therapy, we observed a reduction in the spread of microorganisms from the lower respiratory tract to the blood. The evaluation of samples positive for *K. pneumoniae* KPC has enabled us to confirm this observation with the reduction of positive blood cultures; however, the number of cases at our disposal is insufficient for a statistical evaluation. In contrast, the higher frequency of positivity for MDR *A. baumannii* allows us not only to observe the reduction of positive blood cultures but also to detect a statistically significant difference ($p < 0.05$) among patients treated with conventional therapy alone and those in which it has been added aerosol therapy. Our data allow us, therefore, to assert that the use of treatment with colistin aerosol effectively supports conventional therapy to aid in the elimination of *K. pneumoniae* KPC and MDR *A. baumannii* from the lower respiratory tract. This prevents the spread of these microorganisms to the blood and, consequently, prevents episodes of sepsis.

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