Original Article

Characterization and sensitivity to antibiotics of bacteria isolated from the lower respiratory tract of ventilated patients hospitalized in intensive care units

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ABSTRACT

Objective: This observational study described the characterization of bacteria isolated from the lower respiratory tract of ventilated patients hospitalized in intensive care units. The demonstration of isolated microorganism resistance to antibiotics and a time-trend analysis of infection comparing a 48-month period were also other objectives.

Method: Semi-quantitative assays of 1254 samples taken from 741 ventilated patients were performed, while pathogens were identified using the Enterotube II assay and VITEK 2 Compact equipment. Bacterial resistance to antibiotics was assessed by the Kirby-Bauer disc diffusion method and time-trend analysis of infection was based on data recorded by hospital microbiology laboratories.

Results: The most prevalent isolated bacteria from the patient's lower respiratory tract were with Gram-negative bacteria (67.8%) mostly represented by: *Acinetobacter* spp. (25.2%), *Pseudomonas* spp. (18.3%) and *Klebsiellas* spp. (9.4%). *Acinetobacter* spp. showed moderate high to very high resistance to ceftriaxone (CRO), gentamicin (CN), amikacin (AK), meropenem (MRP), aztreonam (ATM) and piperacillin/tazobactam (TZP). Some isolates of *Acinetobacter* spp. resistant to colistin (CS) were identified in this patient population. *Pseudomonas* spp. and *Klebsiella* spp. were very highly resistant to ampicillin/sublactam (AMS) and with moderate or low resistance to CRO, ATM, MRP, AK, CN and TZP. A decrease in the *Pseudomonas* spp. prevalence rate was observed, whereas an increase in *Acinetobacter* spp. and *Klebsiella* spp. prevalence rates were observed in a 48-month period.

Conclusion: This research corroborated that these nosocomial infections are a relevant medical problem in our context. The most prevalent bacterial infections in the lower respiratory tract of ventilated patients were by *Acinetobacter* spp., *Pseudomonas* spp. and *Klebsiella* spp. The panel of antibiotics used as preventive therapy was not the solution of infections and probably induced drug-resistance mechanisms in these isolated microorganisms.

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Introduction

The study of nosocomial infections has its origin in the 18th century thanks to the work of Sir John Pringle. As conclusion of this work, these infections were shown to be the most severe consequence of the massive use of hospitals.[1] In connection with this conclusion, three major causes are involved in the generation of nosocomial infections: (1) the use of antimicrobial drugs; (2) some hospital personnel not following basic infection control procedures; and (3) patients in hospitals are increasingly immune-compromised. Nevertheless, organ transplantation, blood supply and hospital infrastructure renovations are also recognized as other causes of nosocomial infections.[2] Among them and not surprisingly, nosocomial pneumonia is currently reported as the second higher cause of nosocomial infections, corresponding to about 18% of the total of nosocomial infections.

Since then (18th century), *Staphylococcus aureus*, *Staphylococcus epidermidis*, Gram-negative bacilli, *Enterococcus* spp., *Candida* spp., *Klebsiella pneumoniae*, *Enterobacter* spp. and *Pseudomonas* spp. have been the most frequently identified causative agents of nosocomial infections.[3,4] On the other hand, in the 1990s, the three most common Gram-positive pathogens *S. aureus*, coagulase-negative *Staphylococci*, and *Enterococci* accounted for about 34% of nosocomial infections, while the four most common Gram-negative pathogens were *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *K. pneumoniae* accounting for about 32%.[3,4]

In parallel, bacterial antimicrobial resistance is another remarkable issue that challenges the efficacy of nosocomial infection treatment.[5] In that sense, the development of the anti-microorganism drug families between 1950 and 1980 stimulated the idea that the medical industry will always be one step forward of pathogenic microorganisms.[6] However, it seems that the generation of new antibacterial drugs to fight bacterial pharmaco-resistance is stagnated.[5] To exemplify this, the National Nosocomial Infection Surveillance System of the United States reported that methicillin resistant *S. aureus* (MRSA) was involved in 55% of nosocomial infections and about 26% of nosocomial infections were also associated with infections caused by *Enterococcus* resistant to vancomicin (VRE). Resistance to third generation cephalosporins was also reported for *E. coli*, *K. pneumoniae* and *Enterobacter* spp.[6,7]

All these data provide evidence on the importance of this relevant clinical problem. However, it is also noteworthy to emphasize that these nosocomial infections are not only the cause of a large number of deaths, but also their control is extraordinarily expensive. To illustrate this feature, it is estimated that the annual cost of nosocomial infections ranged from US$ 4 to 11 billion in the United States.[8]

Objectives

Considering this information, this observational study describes a characterization of bacterial infections in the lower respiratory tract of patients admitted in the ICUs at the Hermanos Ameijeiras Hospital with presumed nosocomial pneumonia. In addition to the resistance characterization of isolated microorganisms to antibiotics, a time-trend analysis of infection comparing a 48-month period was also conducted.

Materials and methods

General information of hospital

The Hermanos Ameijeiras Hospital located in Cuba is a 625-bed hospital with 15 medical wards, 12 surgical wards and 3 intensive care units (ICUs). It provides assistance only for adults.

Patient sampling

A total of 1254 endotracheal secretion samples obtained from the lower respiratory tract of 741 ventilated patients were assessed. Samples of 2 mL were directly taken from patients by traqueal aspiration and subsequently inoculated in sterile tubes (13 mm x 100 mm) containing 5 mL of 0.9% saline solution. These samples were then immediately sent to the hospital's microbiology laboratories.

Microbiological diagnosis

First, Gram staining was performed to detect polimorfonuclear leukocytes as prerequisite to then confirm the existence of bacterial infection. The culture of samples was performed using blood agar (BIOLIFE - Milano, Italy) supplemented with 5% goat blood and MacConkey agar (BIOLIFE - Milano, Italy). All samples were processed according to methods described by the Institute of Clinical and Laboratory Standards of the United States. Next, plates were manually read after 24h and samples were considered positive when the concentration of bacteria was higher than 10^4 cfu/mL. Because of the obvious growth of multiple strains of bacteria, the majority of cultures required subsequent isolation on MacConkey agar (Gram-negative) and on blood agar (Gram-positive). Visualization was done using a stereo-microscope SZ71 (Olympus - Tokyo, Japan).

After that, another Gram staining was performed to isolate cultures and to definitively corroborate the presence of Gram-negative bacilli and to assess purity of each culture. The isolated bacteria was then identified and tested for resistance to several antibiotics.

Assays for microorganism identification

A NN-dimethyl-p-phenylenediamine-oxalate assay was performed to separate Gram-negative microorganisms in two different groups. Then, the Enterotube II procedure [dexcarboxilase assay (Lysine and Ornitine)] was applied to Gram-negative and negative oxidase bacilli. Other Enterotube II assays such as carbohydrate fermentation (adonitol, arabinose, sorbitol, lactose, glucose, dulcitol), production of sulfidric acid, production of urease, citratase, and phenylalaline deaminase were performed. In addition, non-Enterotube II assays such as salted manitol, maltose, arginine, glucose oxidase were done. In some cases of non-fermenter bacilli, the use

of the Vitek 2 Compact equipment was absolutely necessary (bioMérieux Inc. – France). All positive oxidase bacilli were non-fermenter bacilli and from this group, the group of *Pseudomonas* spp. was considered only as pathogen. Assays performed to classify microorganisms within the group of *Pseudomonas* spp. were glucose oxidation, pigment production, growing at 4°C and 42°C, respectively.

Assay for assessing microorganism resistance to antibiotics

All isolates were subjected to antimicrobial resistance testing against a panel of antibiotics using the Kirby-Bauer disc diffusion method[9] whereby the antibiotic will diffuse from a paper disc into the Müller-Hinton medium containing microorganisms and allowing the plate to dry up for 5 min. Growth inhibition was interpreted as a failure of microorganisms to grow in the antibiotic region. Paper discs containing known concentrations of antibiotics were applied to the surface and then incubated at 36 ± 1°C. The concentration of antibiotics used were as follows: 30 µg amikacin (AK), 20 µg ampicillin/sublactam (AMS), 30 µg aztreonam (ATM), 30 µg amoxicillin/clavulanate (AUG), 75 µg azlocillin (AZL), 10 µg colistin (CS), 30 µg ceftriaxone (CRO), 30 µg cefoxitin (FOX), 30 µg chloranphenicol (C), 5 µg ciprofloxacin (CIP), 10 µg meropenem (MRP), 30 µg gentamicin (CN), 85 µg ticarcillin/clavulanic acid (TIC), 50 µg sulfamethoxazole (SMX), 110 µg piperacillin/10 µg tazobactam (TZP). All microorganisms were diluted in 5 mL of Tryptic Soy Broth up to 0.5 McFarland. The appearance of the inhibition zone surrounding the discs indicated resistance of microorganisms, which was measured by comparing the diameter of the inhibition zones to a standard table. *E. coli* ATCC25922 and *K. pneumoniae* ATCC700603 served as reference samples (controls) in these assays.

Resistance of *Acinetobacter* spp. to CS was calculated by the use of the VITEK 2 Compact equipment estimating the minimum inhibitory concentration.

Time-trend analysis of lower respiratory track nosocomial infection

In the period studied for the time-trend analysis of lower respiratory tract infections of patients, there were 6,087 cumulative samples of endotracheal secretion samples taken from patients admitted at the Hermanos Ameijeiras Hospital's ICUs.

Statistical analysis

A single factor-ANOVA test was performed to compare the number of samples infected with *Acinetobacter* spp., *Psedeudomonas* spp. and *Klebsiella* spp. in the time-trend analysis of infections (from 2007 to 2010). The Tukey's test was employed to discriminate statistical differences among the analyzed variables. The significance level applied was 0.05 and the Statgraphics Plus version 5.0 (2000) from Statistical Graphics Corp. was used as tool for the statistical analysis.

Results

As it was mentioned in the material and methods section and in Table 1, this study was carried out evaluating a total number of admitted patients and endotracheal secretion samples equal to 1060 and 1254, respectively. Evidently, the total number of patients admitted at the ICUs of the hospital and samples did not match because more than one sample was taken from some patients, at different moments, to confirm results. From this number of admitted patients, 741 (68.9%) received mechanical ventilation; and accounted to 125 deaths (11.8%) by sepsis and 58 deaths (5.5%) by causes not associated with infections. However, sepsis was also confirmed in these 58 cases by means of a rigorous anatomopathological examination.

Table 1 - Main data of the number of patients and infection rates in the lower respiratory tract of hospitalized patients in the ICUs

Items	Number of patients and samples	Proportions (%)
Total number of patients admitted at the ICUs	1060	100.0
Total number of patients with mechanical ventilation	741	68.9
Total number of assessed samples from ventilated patients	1254	100.0
Patients with positive results to Gram-negative bacteria	503	67.8
Patients with positive results to Gram-positive bacteria	23	3.1
Patients with negative results	215	29.0
Assessed samples contaminated during the sampling	7	0.5
Assessed samples contaminated during the work in microbiology laboratories	7	0.5
Total of patients dead by generalized sepsis	125	11.8
Total of patients dead by unrelated causes but with generalized sepsis	58	5.5

As result of the microbiological assessments, 503 patients (67.8% of ventilated patients) were positive for Gram-negative bacteria whereas only 23 patients (3.1% of ventilated patients) were positive for Gram-positive bacteria. There was no lower respiratory track infection in the remaining 215 (29.0%) ventilated patients. In favor of the execution of this intensive microbiologic investigation, a very low level of contamination (1.1%) due to laboratory errors was observed (Table 1).

We also observed that 53.6% of infections were caused by bacteria of the Enterobacteriaceae family, where the most represented strains were *Klebsiella* spp. (35.7%). Likewise, we detected 43.5% of infections with non-fermenter bacilli, represented by *Acinetobacter* spp. (25.2%) and *Pseudomonas* spp. (18.3%) infections (Fig. 1).

Fig. 2 summarizes resistance rates of *Acinetobacter* spp., *Pseudomonas* spp. and *Klebsiella* spp. to the panel of antibiotics mentioned above. This panel of antibiotics represents, perhaps, the most widely used antibiotics in the hospital during and/or before the studied period.

Data gathered in our study of bacterial resistance also corroborated the very high resistance of isolated *Acinetobacter* spp. to CRO (93%), CN (87%), AK (84%), MRP (81%), ATM (81%) and TZP (78%). However, the most remarkable findings of this study, something of greatest concern, was the isolation of 8 (2%) samples of *Acinetobacter baumannii* resistant to CS and highly resistant to MPR (Fig. 2A).

Concerning the analysis of *Pseudomonas* spp. (Fig. 2B) and *Klebsiella* ssp. (Fig. 2C) resistance to antibiotics [there was moderate resistance (< 50%)] of *Pseudomonas* spp. to CN (45%), AK (40%), MRP (44%), ATM (51%). Furthermore,
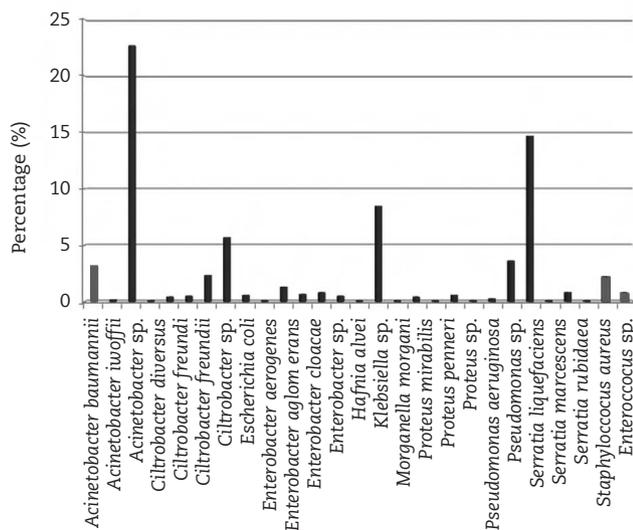
we also found a very high resistance of *Pseudomonas* spp. to AMS (92%), low to TZP (20%) and moderately high to CRO (68%). From this study, it can also be extrapolated that *Klebsiella* spp. were also moderately resistant to CN (45%), AK (38%), MRP (42%), ATM (34%). This group of bacteria showed low resistance to TZP (28%), high resistance to CRO (82%) and very high resistance to AMS (93%).
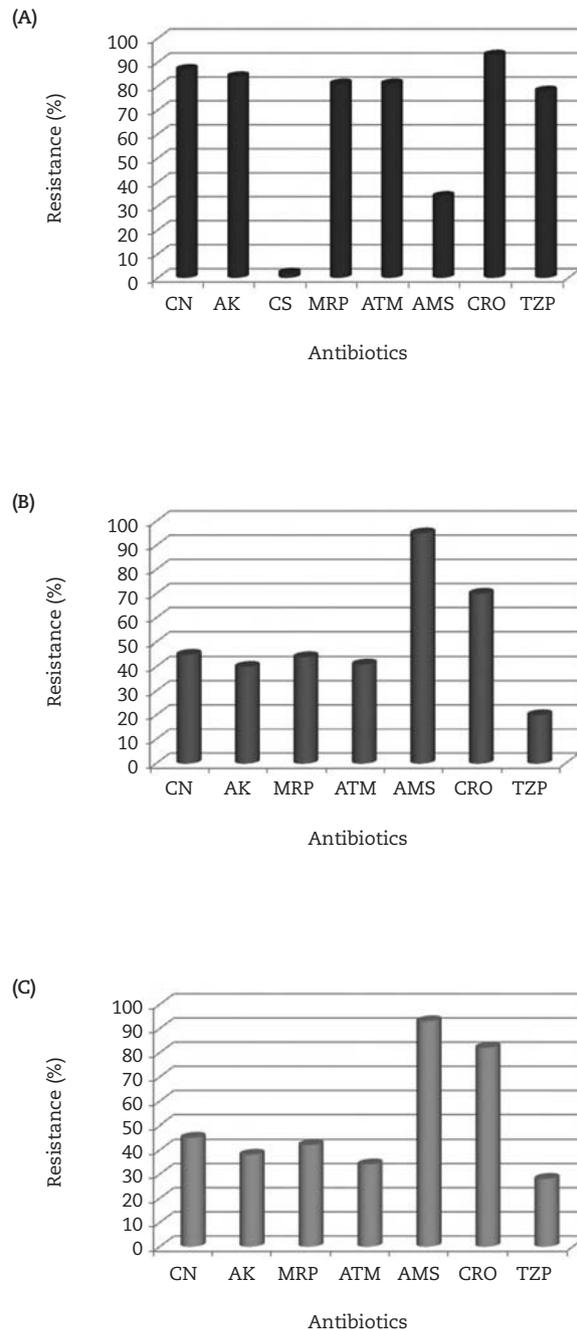
(A)

(B)

(C)

Fig. 1 - **Collection of Gram-negative bacteria isolated from the lower respiratory tract of patients hospitalized in the ICUs. Gram-negative bacteria (darker) and Gram-positive bacteria (lighter).**

Fig. 2 - **Antibiotic resistance patterns of (A)** *Acinetobacter* **spp., (B)** *Pseudomonas* **spp. and (C)** *Klebsiella* **spp. isolated from the lower respiratory tract of patients hospitalized in the ICUs.**

Finally, Fig. 3 illustrates a time-trend analysis of infections of the lower respiratory tract of patients admitted to the ICUs of the hospitals with *Acinetobacter* spp., *Pseudomonas* spp. and *Klebsiella* spp. infections. This comparison was done using recorded data of the last four consecutive years and the number of samples studied per year was as follows: 1626 (2007), 1747 (2008), 1462 (2009), and 1254 (2010).

Our research revealed a decrease in the prevalence rate for *Pseudomonas* spp. (-1.19%) when 2007 and 2010 were compared. Nonetheless, an opposite time-trend in the prevalence rate was confirmed for *Acinetobacter* spp. and *Klebsiella* spp. An increase by approximately 4.5% was seen for *Acinetobacter* spp. from 2009 to 2010. However, a linear increase for this nosocomial infection has been noticed since 2007 (y = 6.18x+1.55). The infection rate of *Klebsiella* spp. showed a dramatic increase in the last two years (11.3%).

The comparison of the respective infection rates demonstrated significant differences among the number of samples infected per year with *Acinetobacter* spp., *Pseudomonas* spp. and *Klebsiella* spp. (p = 0.027). The Tukey's test confirmed ANOVA results, showing significant difference between the infection rates of *Acinetobacter* spp. and *Klebsiella* spp. Comparison between *Pseudomonas* spp. and Klebsiela spp. infection rates did not show significant difference (p = 0.053).
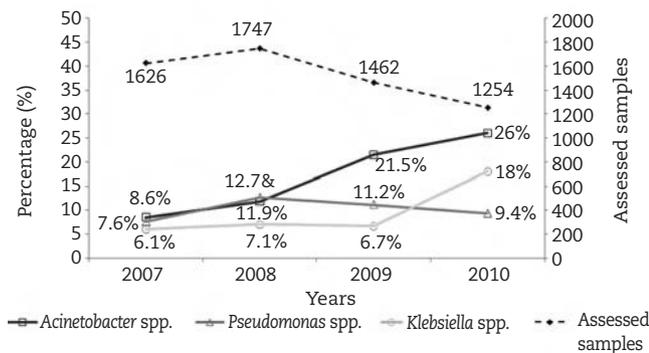

Fig. 3 - Time-trend analysis of infection of *Acinetobacter* spp., *Pseudomonas* spp. and *Klebsiella* spp. isolated from the lower respiratory tract of patients hospitalized in the ICUs.

Discussion

Nosocomial infections are estimated to double mortality and morbidity risks of any hospital admitted patient. It has been estimated that about 1.7 million hospital-acquired infections occurred and almost 99,000 deaths were associated with nosocomial infections, making it the sixth leading cause of death in the United States.[3,4] Similar data have been reported from Europe.[10] In this regard, the most susceptible patients to nosocomial pneumonia

are those who receive assisted (mechanical) ventilation, which is directly associated with the increase in the length of hospital stay. As a general rule, patients that receive assisted ventilation develop pneumonia more frequently and are more likely to have a fatal outcome than those not requiring assisted respiration.[11,12] In this respect, updated data from the U.S. National Healthcare Safety Network indicated that Gram-negative microorganisms (*P. aeruginosa*, and *A. baumannii*) predominate in the cases of nosocomial pneumonia.[13]

Taking into consideration this universal relevant clinical problem, we made a characterization of infection of the lower respiratory tract of 741 ventilated patients admitted to the ICUs with presumed nosocomial pneumonia. From these patients 1254 samples were collected and assessed by several microbiology tests. Some results were also corroborated by anatomopathological examination (Table 1).

The most general results evidenced that the majority of patients showed the presence of Gram-negative bacteria (67.8%) and 11.8% of patients died as consequence of generalized sepsis. Coincidentally, similar observations have been made by other investigators in the world. For instance, in the United States ICUs, infection with Gram-negative bacteria account for about 70% of nosocomial infections, but similar data have been reported from other parts of the world. In that sense, several Gram-negative microorganisms were responsible for those nosocomial infections, but among them, the Enterobacteriaceae family was the most commonly identified group overall.[14] In 2006, it was estimated a death rate of 33% to 50% among patients who developed ventilator-associated pneumonia. Furthermore, mortality was more likely when pneumonia was caused by *Pseudomonas* spp. or *Acinetobacter* spp.[14]

In addition to those findings, 53.6% of infections were caused by bacteria belonging to the Enterobacteriaceae family, but on the contrary the most commonly isolated bacteria within this family were *Klebsiella* spp. (35.7%). In addition, we also detected 43.5% of infections with non-fermenter bacilli [*Acinetobacter* spp. (25.2%) and *Pseudomonas* spp. (18.3%)]. In regard to *Acinetobacter* spp., we would like to emphasize the fact that between 1986 and 2003, *Acinetobacter* species were the only Gram-negative microorganisms that increased markedly as cause of pneumonia in ICUs in the United States[15] and unfortunately, the extraordinary resistance of these microorganisms to a wide spectrum of antibiotics has posed important therapeutic challenges.

Interestingly, our results were also consistent with those reported from the United States in 2010, where the most represented microorganisms found as cause of the nosocomial infections, were *Klebsiella* spp. accounted for about 20%.[14,16,17] Equally, 72.9% of infections with non-fermenter bacilli were reported in Kolar (India).

The high representativeness of these microorganisms in the nosocomial infections might be due to that most of them are found in the soil, water, intestinal tract and pharynx. They are also able to survive in wet places, soaps, mechanical respiration equipments (such as nebulizers, tracheal suction catheter, circuits of respirators) and pipes of difficult access to be washed and/or dried.

The contamination of patients with Gram-negative bacteria represents a problem of a great clinical relevance, because the drug (antibiotic)-resistance mechanisms in these bacteria are increasingly being reported worldwide.[13] Our investigation carried out to measure the resistance of the isolated bacteria to several antibiotics confirmed the high resistance of *Acinetobacter* spp. to CRO, CN, AK, MRP, ATM and TZP. Curiously, part of these results is controversial to those reported by Diomedi[18] and Espinosa et al.[19] Diomedi reported only 19% of *Acinetobacter* spp. resistance to MRP and Espinosa et al.,[19] only observed 60% of resistance of *Acinetobacter* spp. to MRP. Perhaps this difference could be explained by the development of drug resistance mechanisms in these strains. In this regard, it is important to realize that something similar might occur in future with the CS resistant variants of *Acinetobacter* spp. (*A. baumannii*) isolated in our conditions; if specials cares are not taken responsibly.

We also demonstrated a very low level of resistance of *Acinetobacter* spp. to CS, which is one of the last resort antibiotics for multidrug resistant *A. baumannii* and *P. aeruginosa.*[20,21] Analogously, a previous study performed at the Hermanos Ameijeiras Hospital (2002-2006) by other group of researchers, also demonstrated the existence of *Acinetobacter* spp. in the ICUs.[19] Although, the resistance of *Acinetobacter* spp. isolates to CS was not analyzed or reported in that study.

Our results were coincident with those described by Reis et al.[22] This group reported up to 5% of resistance of *Acinetobacter* spp. to CS in a hospital in Brazil. However and surprisingly, none of these isolates were detected by the Kirby-Bauer disc diffusion assay. Nevertheless, something prominent of this work was that they elucidated the mechanism of resistance of these bacteria to CS, which was provoked by the membrane protein OprH-1, which interferes with the anionic linking site of polymyxins with the cell wall lipolysaccaride.

Other researchers have illustrated a similar phenomenon. For instance, Park et al.[23] reported of the isolation of 63 isolates of *Acinetobacter* spp., from which 30.2% were resistant to CS and 19 isolates corresponded to *A. baumannii*. Most of these isolates (84.2%) were collected from endotracheal aspirate, while others (15.8%) were from peritoneal fluid and sputum. Nevertheless, when all these isolates were characterized, all belonged to a single clone, ST22, and all contained the blaOXA-23 and blaOXA-66 genes.

The mechanism of resistance to CS, Col(r), in *A. baumannii* was analyzed by Adams et al.[24] They selected in vitro Col(r) derivatives of the multidrug-resistant *A. baumannii* isolate (AB0057). The DNA sequencing identified mutations in genes encoding proteins PmrA and/or PmrB in each strain and also in a Col(r) clinical isolate.

On the other hand, a low resistance to CN, AK, MRP, ATM and TZP was observed for *Pseudomonas* spp. and *Klebsiella* spp. The results observed with *Pseudomonas* spp., were expected and consistent with previous reports.[25,26]

In the United States, *K. pneumoniae* is important in nosocomial infections among adult and pediatric populations and accounted for approximately 8% of all nosocomial infections. Depending on the study reviewed, they comprise from 3% to 7% of all nosocomial bacterial infections, placing them among the top 8 pathogens in hospitals. *K. pneumoniae* cause as many as 14% of primary bacteremia cases and a significant increased mortality have resulted from infections with it.[25] Thus, the extensive use of broad-spectrum antibiotics in hospitalized patients to treat this strain has probably led to the development of multidrug-resistant strains.[27,28]

According to our results, the most recommended antibiotics to be used as preventive therapy for either *Pseudomonas* spp. or *Klebsiella* spp. is TZP (< 28% of resistance).[29] This antibiotic inhibits the action of bacterial betalactamases and its efficacy is amplified when is enriched by piperacillin, which is an extended spectrum betalactamic antibiotics of the ureidopenicillin class.

As consequence, we hypothesize, that the use of these antibiotics to treat preventively patients infected with this kind of bacteria (*Acinetobacter* spp., *Pseudomonas* spp. and *Klebsiella* spp.) was the non-solution of infections and perhaps the stimulation of drug-resistance mechanisms by these microorganisms. Therefore, the application of most of the antibiotics (the ones mentioned above and others) as preventive therapy is considered unsuitable; mostly when a microbiologic assay employed to identify microorganisms and select the proper antibiotic can be performed in a relative short period of time. In such sense, the administration of an empirical antibiotic therapy should not only be delayed in severely ill patients, on account of the diagnostic procedure; because in any other medical situation the inappropriate use of antibiotics could only increase the morbidity and mortality, contribute to the development of bacteria-drug resistance mechanisms and in conclusion increase health care costs.

Finally, this study team also considered prudent presenting to the specialized readers a trend-time analysis of these three bacterium associated-nosocomial infections. As complementary information we must share that a previous resembling trend-time study was performed in this hospital, 2002-2006. This study also demonstrated the predominance of Gram-negative bacteria in the ICUs, particularly, non-fermentative bacilli such as *Acinetobacter* spp. and *Pseudomas* spp., with almost a generalized growth of the microorganism resistance patterns; but not *Klebsiella* ssp. This study also demonstrated the very high resistance of *Acinetobacter* spp. to CRO (88-96%) and a moderate resistance to MRP (55-62%). In that revision, the group of Gram-positive bacteria was represented by *S. aureus* with an evident resistance to oxacillin, but all were very low resistance (0%) to vancomicyn.[19]

However, the time-trend analysis performed in our research verified a decrease in the infection rate for *Pseudomonas* spp., but a marked increase in the infection rates for *Acinetobacter* spp. and *Klebsiella* spp. Perhaps, these results might be attributable to the fact that *Pseudomonas* spp. were the most susceptible bacteria to the panel of antibiotics employed preventively. As it can be observed, in the last two years, the highest shift in the infection of the patient's lower respiratory tracks was produced by *Klebsiella* spp. reaching a value of 11.6%.

We hardly recommend that such time-trend of infections with *Klebsiella* spp. and *Acinetobacter* spp. has to be decreased by the no treatment of patients in the community with antibiotics, deliberate prescription, before receiving attention in the hospital, modifications in the surveillance strategies, development and/or use of non-invasive

infection-resistant devices and the most importantly health-care workers should urgently improve their work in order to get a better consolidation of prevention practices for nosocomial infections.

In summary, this research corroborated that these nosocomial infections are a relevant medical problem in our context as it is in all over the world. It also shown that the most prevalent infections with bacteria in the lower respiratory track of ventilated patients admitted at the ICUs of the hospital were caused by *Acinetobacter* spp., *Pseudomonas* spp. and *Klebsiella* spp. The use of the panel of antibiotics employed to treat this type of infections, as preventive therapy, was the non-solution of infections and probably the induction of drug-resistance mechanisms in these isolated microorganisms. Some isolates of *Acinetobacter* spp. resistant to CS were identified in our conditions and finally, a decrease in the prevalence rate for *Pseudomonas* spp. was observed, although an opposite time-trend of infections with *Acinetobacter* spp. and *Klebsiella* spp. was corroborated in a 48-month period.

Acknowledgments

Conflict of interest

All authors declare to have no conflict of interest.

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