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Prevalence of *Acinetobacter baumannii* infections during the SARS-CoV-2 pandemic in the SS. Antonio e Biagio e Cesare Arrigo Hospital of Alessandria

Prevalenza di infezione da *Acinetobacter baumannii* presso l'Ospedale SS. Antonio e Biagio e Cesare Arrigo di Alessandria durante la pandemia di SARS-CoV-2

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Key words: *Acinetobacter baumannii*, antibiotic resistance, SARS-CoV-2.

ABSTRACT

Aims: 2020 was marked by the emergence of SARS-CoV-2. The dramatic growth of hospitalized patients infected by SARS-CoV-2 may have led to an increase in secondary infections and/or healthcare-associated infections. It is evident that the need for high-spectrum antibiotic therapy in patients with severe illness contributed to the spread of Multi Drug Resistant (MDR) bacteria. The aim of this work was to evaluate the trend of *Acinetobacter baumannii* infections during the SARS-CoV-2 pandemic in Santi Antonio e Biagio and Cesare Arrigo Hospital in Alessandria.
Materials and Methods: all the isolates of *A. baumannii* and positive swabs for SARS-CoV-2 detected in hospitalized patients were included in the analysis.
Results: between November 2020 and March 2021, an increase of the *A. baumannii* isolation occurred compared to the period November 2019 - March 2020, especially in the Intensive Care Units. A reduction of the *A. baumannii* isolation from November 2021 to March 2022 was observed.
Conclusions: the differences observed are probably due to the administration of vaccines and consequently to the lower severity of COVID-19 cliniacl pictures.

Obiettivi: il 2020 è stato caratterizzato dalla comparsa del SARS-CoV-2. Il drammatico aumento dei pazienti ricoverati infettati da SARS-CoV-2 potrebbe aver portato a un aumento delle infezioni secondarie e/o delle infezioni associate all'assistenza sanitaria. È evidente che la necessità di una terapia antibiotica ad ampio spettro nei pazienti con malattia grave da COVID-19 ha contribuito alla diffusione di batteri multiresistenti ai farmaci. Lo scopo di questo lavoro è stato quello di valutare l'andamento delle infezioni da *Acinetobacter baumannii* durante la pandemia da SARS-CoV-2 nell'Ospedale SS. Antonio e Biagio e Cesare Arrigo di Alessandria.
Materiali e Metodi: tutti gli isolati di *A. baumannii* e i tamponi positivi per SARS-CoV-2 prelevati da pazienti ricoverati sono stati inclusi nell'analisi.
Risultati: da novembre 2020 a marzo 2021 si è osservato un aumento degli isolamenti di *A. baumannii* rispetto al periodo novembre 2019 - marzo 2020, soprattutto nelle Unità di Terapia Intensiva. Da novembre 2021 a marzo 2022 è stata osservata una riduzione dell'isolamento di *A. baumannii*.
Conclusioni: le differenze osservate sono probabilmente dovute alla somministrazione dei vaccini e di conseguenza alla minore gravità dei quadri clinici da COVID-19.

INTRODUCTION

Acinetobacter baumannii is an opportunistic pathogen that, due to its low nutritional requirements, exists in nature but also in the hospital setting,[1] where it is responsible for numerous infections, especially among immunocompromised patients. Strains belonging to this bacterial species are among the most important and fearsome pathogens causing healthcare-associated infections.[2]

The genus *Acinetobacter* includes Gram-negative, aerobic, nonmotile, oxidase-negative, non-fermenting coccobacilli with a C+G DNA content between 39% and 47%. Within the genus *Acinetobacter*, the species *A. baumannii* is of great clinical importance, due to its capability to induce serious outbreaks of nosocomial infection in hospitals. Strains of *A. baumannii* can survive on both wet and dry surfaces for long periods. In fact, they can be isolated from samples taken from sinks, mattresses, pillows, bed rails, steel, rubber, PVC, door handles, *etc*.[2]

Thus, procedures aiming at containing the infectious risk associated with healthcare practices are mandatory. In particular, it is useful to carry out an environmental microbiological control of the surfaces to identify any reservoirs of *A. baumannii* and proceed with disinfection interventions and monitoring of the infectious risk in case of positivity. Contact plates for total microorganism counts and swabs can be used for surface sampling. The samples taken are inoculated onto routine laboratory media such as blood agar and MacConkey agar and incubated at 37°C. Once the colonies have grown, a subculture is made on a non-selective medium. Finally, the identification and susceptibility test of the bacterial strain is performed.

Healthcare setting requires extended desiccation periods and a routine disinfection regime, but *A. baumannii* adapteded to these stressful conditions. Resistance to desiccation is the ability to maintain viability in dry conditions and has been found to be strain-specific, with some isolates remaining viable for nearly 100 days and others being more sensitive. The ability of *A. baumannii* to form biofilms on different abiotic surfaces contributes to infections associated with medical devices.[3]

Possible causes of *A. baumannii* infection could be a long hospitalization, particularly in Intensive Care Units (ICUs), the use of catheters, endotracheal or nasogastric tubes, mechanical ventilation or previous antibiotic therapy.[4]

The most common sites of infection by *A. baumannii* are: exposed skin and soft tissue due to wounds, blood for the use of intravenous catheters, urinary tract in patients with urinary catheters, respiratory tract associated with the use of mechanical ventilation and central nervous system.[5]

Antibiotic resistance is among the greatest challenges of medical therapy, and due to its resistance through multiple different mechanisms, *A. baumannii* is recognized as one of the most difficult Gram-negative bacteria to treat and control. Some strategies used by *A. baumannii* are the inactivation of antibiotic molecules, the alteration of target sites or functions, point mutations, enzymatic modifications, and the limitation of entry into the cell by decreasing the permeability of the outer membrane or the up-regulation of pumps that excrete antimicrobials.[6] Genomic sequencing of Multi-Drug Resistant (MDR) *A. baumannii* isolates allowed the identification of regions called genomic islands, which contain genes for antibiotic resistance, virulence factors, genes coding for phage-derived proteins and for enzymes used for the detoxification of xenobiotic compounds.[6]

To date, antibiotic resistance by *A. baumannii* against several antibiotics, including carbapenems, fluoroquinolones, aminoglycosides and sulfonamides is well known. Due to the quick development of antibiotic resistance, the most effective treatment for MDR *A. baumannii* infections requires a combined antibiotic therapy.[6]

The COVID-19 pandemic that officially broke out on 11th March 2020[7] led to an overuse of antimicrobials in critically ill patients,[8] resulting in a spread of the MDR bacterial isolates.[9] Furthermore, there has also been an exponential increase in patients in the ICU, many of whom required respiratory support.[10]

These two factors, together with the increase in the number of hospitalized patients in other units, may have facilitated the spread of healthcare-associated infections and/or secondary infections.[11]

The extensive use of breathing support devices may have led to an increased incidence of ventilator-associated pneumonia, of which *A. baumannii* is usually a leading cause.[12]

MATERIALS AND METHODS

Design of the study

The project took place in November-March of the years 2019/2020, 2020/2021 and 2021/2022.

All bacterial strains were isolated from biological samples processed for diagnostic purposes.

Inclusion criteria

A. baumannii isolates from biological samples such as: i) blood cultures; ii) respiratory samples; iii) urine cultures; iv) biopsies; v) wound/ulcer swabs; vi) body fluids from sterile anatomical sites; vii) vascular catheters; viii) rectal swabs to check for a possible colonization by MDR bacteria. Isolation from patients admitted to the SS. Antonio e Biagio e C. Arrigo Hospital of Alessandria in the period described above.

Exclusion criteria

Multi-resistant isolates of other bacterial *genera*. *A. baumannii* isolates from biological samples taken from patients admitted to outpatient facilities or other hospitals were considered only once for each patient.

Variables studied and samples processing

The isolation frequency of *A. baumannii* was evaluated in relation to the period considered, the requesting department and the susceptibility to antibiotics. For the processing of the samples, the standard procedures according to each type of biological material were followed. The samples were seeded manually with the use of sterile loops or through the automated Copan Walk Away Specimen Processor (WASP®) on routine laboratory media. The colonies of interest were isolated by subculture and subsequently identified by VITEK MS®[13] and VITEK 2®[14,15] (bioMérieux, Marcy l'Etoile, France). The latter was also used for antimicrobial susceptibility tests.

In parallel, the isolation frequency of SARS-CoV-2 in the same periods was evaluated. The detection of SARS-CoV-2 was performed using two devices: GeneFinder® COVID-19 Plus RealAmp Kit on the ELITe InGenius® platform (ELITechGroup, Inc., Bothell, WA, and Osang Healthcare, Anyang, Korea) and Cobas® SARS-CoV-2 kit on the Cobas® 6800 System (Roche Diagnostics GmbH, Mannheim, Germany).

The Gene Finder® COVID-19 Plus RealAmp Kit, based on a reverse Real-Time PCR technique, detects the SARS-CoV-2 nucleic acids in samples taken from nasopharyngeal, oropharyngeal, nasal swabs, bronchoalveolar lavage fluids, sputum, *etc*.

Cobas® SARS-CoV-2, is a RT-PCR test for the qualitative research of SARS-CoV-2 nucleic acids from oropharyngeal, nasopharyngeal, anterior nasal swabs. Cobas SARS CoV-2® is based on fully automated sample preparation, followed by PCR amplification and detection.

Statistical methods

Categorical variables are expressed as absolute numbers and percentages, and continuous variables are expressed as median. A chi-square was used to compare the frequencies of patients positive for *A. baumannii* among the groups. Microsoft Excel 2010 (Microsoft Corporation, Redmond, USA; available from: https://office.microsoft.com/excel) was used for all the analyses.

RESULTS

Colonization and infection by *A. baumannii*

Colonization/infection by *A. baumannii* was assessed in 135 patients (median age of 73.5 years). The requesting departments are described in Figure 1. During the entire period considered, the Intensive Care Units required the highest number of analyses: 62/135; 46%.

Most of the *A. baumannii* strains were isolated from rectal swabs (39; 29%) and respiratory materials (22; 16%), followed by bronchoaspirate (18; 13%) and urine culture from catheter (18; 13%), blood culture (11; 8%), blood culture from central line (7; 5%), ulcer swab (4; 3%), urine culture (4; 3%), sputum (3; 2%), surgical wound swab (3; 2%), wound swab (2; 2%), pressure sore swab (2; 2%) and finally tracheal aspirate (1; 1%) and vascular catheter (1; 1%).

The majority of patients with *A. baumannii* colonization or infection were male (94 of 135; 70%).

Regarding the resistance profile to the five main drugs tested according to EUCAST guidelines, Figure 2 shows the interpretative categories of the sensitivity tests *in vitro* (Sensitive, Intermediate, Resistant) performed on the strains. The majority of the isolates were MDR, the percentages of resistance to each drug tested were: ciprofloxacin 121/128, 94.5%; amikacin 115/128, 89.8%; cotrimoxazole 119/128, 92.9%; meropenem 121/128, 94.5%. No resistance to colistin was observed.

Table 1. Comparison of frequencies between the ICU patients (Intensive Care Unit and Cardiac Surgery Intensive Care Unit) and patients hospitalized in other wards according to time periods.

	Time periods			
Wards	2019-2020	2020-2021	2021-2022	Total
ICUs	3	48	11	62
Other wards	13	31	29	73
Total	16	79	40	135

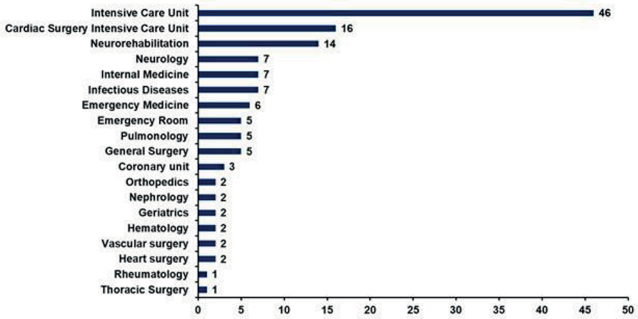

Figure 1. Units requesting microbiological tests for the detection of *A. baumannii* (absolute numbers).

In the period 2020/2021, an increase in the isolations of *A. baumannii* occurred; in particular, from November 2019 to March 2020, the number of isolates was 16/135 (11.9%), from November 2020 to March 2021, 79/135 (58.5%) and finally from November 2021 to March 2022 40/135 (29.6%).

The same analysis, stratified by the requesting Unit, is described in Figure 3. In the period 2020/2021, the Intensive Care Unit

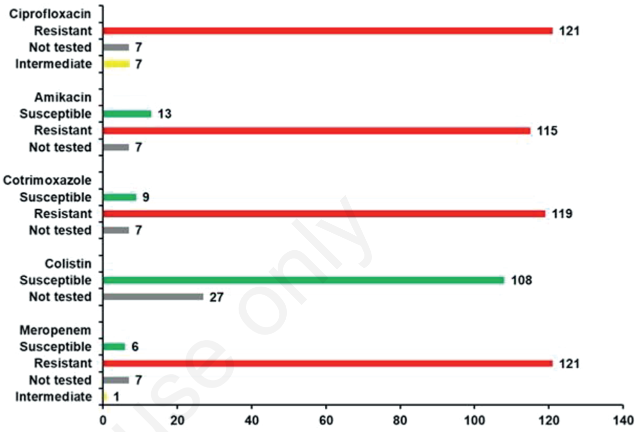

Figure 2. Resistance profile to the five main drugs tested according to EUCAST guidelines (absolute numbers).

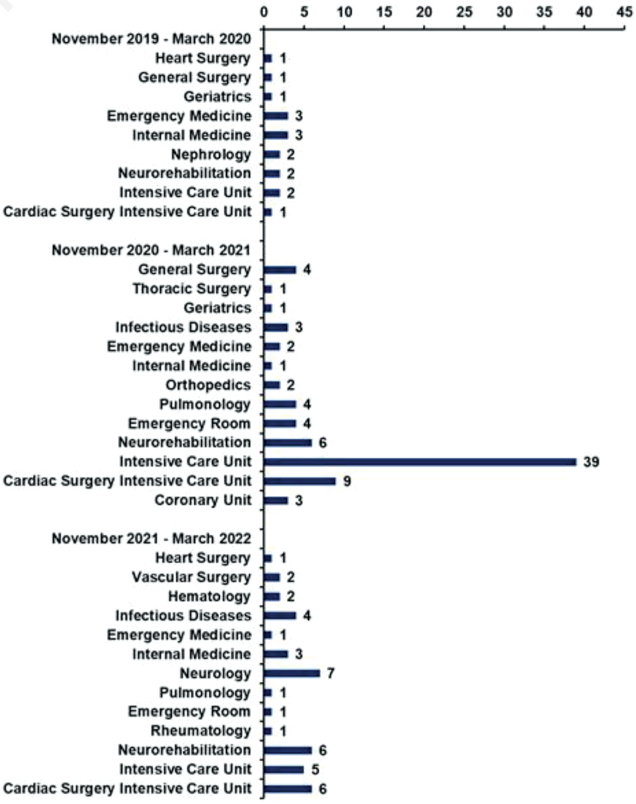

Figure 3. Isolation frequency in relation to the three different periods considered and the requesting Unit (absolute numbers).

required most of the culture tests, which then resulted positive for A. baumannii 48/135 (35.6%); a substantial reduction was observed in the period 2021/2022: 11/135 (8.1%). The difference of frequencies between the ICU patients (Intensive Care Unit and Cardiac Surgery Intensive Care Unit) and patients hospitalized in other wards according to time periods was statistically significant: Chi-Square 17.23, p=0.0002 (Table 1).

SARS-CoV-2 infection

During the entire period considered, a total of 9986 molecular tests from nasopharyngeal swabs were performed in the Emergency Unit of the Civil Hospital and Pediatric Hospital, of which 2446 (22.5%) were positive. The median age of the included patients was 68.4 years. Male patients were 5420/9986 (54.3%), of which 1386 were negative (26%). On the other hand, among the female patients, 1060 (23%) were infected by SARS-CoV-2. The proportion of patients who tested positive was slightly higher in male than in female patients. The number of molecular swabs performed in relation to the period considered is described in Figure 4. A smaller number of swabs was expected in the period between autumn 2019 and winter 2020 (664/9986; 6.6%) as the pandemic hit Italy in 2020. The same analysis performed by stratifying the data by result is described in Figure 5. The percentage of positives out of the total is reduced from 59.3% in the 2019-2020 period to 20.6% in the 2021/2022 period.

DISCUSSION

The most important result that emerges from this work is that even comparing only the periods 2020-2021 and 2021-2022, the number of positive molecular swabs for SARS-CoV-2 was significantly higher in the first period. In the same time range a significant increase in cases of colonization and infection by *A. baumannii* was observed.

This could be related to i) a higher frequency of COVID-19 cases during the period November 2020-March 2021 and ii) the lack of a vaccination in that specific period of time leading to a very high number of hospitalized patients (especially in the Intensive Care Unit), affected by bilateral pneumonia.

The explosive spread of SARS-CoV-2 caused public health crises with comorbidities and high mortality rates, particularly in individuals with severe manifestations of the disease, severe co-infections, and, in general terms, in the elderly population. The overcrowding of hospital facilities, including Alessandria's, due to a significantly higher number of hospitalized patients in relation to the capacity of the hospitals, led to a greater prevalence of co-infections and secondary infections associated with care. During the overcrowding the number isolations of *A. baumannii* increased especially in the Intensive Care Unit, while in the period 2021-2022, after a year of vaccination campaign, decreased.

Although antibiotics are ineffective for the treatment of COVID-19, they have been prescribed to several patients with suspected or documented SARS-CoV-2 infection for a variety of reasons, including the difficulty of ruling out bacterial co-infections and the possibility of a secondary bacterial infection in the course of the disease. The administration of antimicrobials, sometimes even as a preventive method, could have caused further damage, such as the emergence and spread of MDR bacterial isolates.

In addition to the increased use of antibiotics, the high use of intravenous, urinary catheters or mechanical ventilation in hospitalized patients contributed certainly to the spread of co-infections, including those caused by *A. baumannii.*

The screening and knowledge of the proportion of COVID-19 patients showing bacterial co-infection is necessary and critical to ensure optimal clinical outcomes.

Certainly, regular hand hygiene, isolation of suspected or diagnosed patients, and proper sterilization of medical equipment are preventive and control measures against *A. baumannii* infections.

CONCLUSIONS

The evidence supports an association between the spread of the COVID-19 pandemic and the increase of *A. baumannii* colonization and infection. The reduction in severe COVID-19, mainly due to vaccination, contributed to hamper the expansion of *A. baumannii* in hospital.

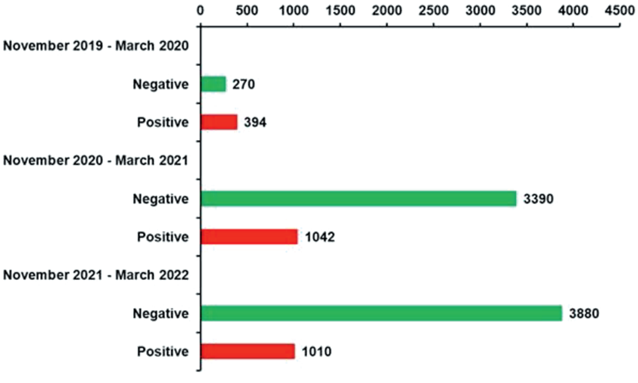

Figure 4. Number of molecular swabs analyzed in the Emergency Unit of the Civil Hospital and the Pediatric Hospital of Alessandria in relation to the period considered (absolute numbers).

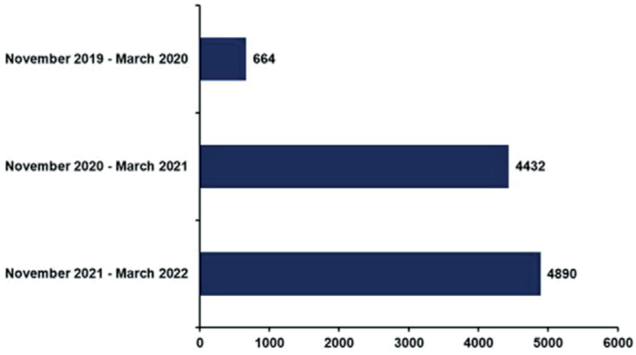

Figure 5. Number of swabs analyzed in relation to the period and the result (absolute numbers).

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