International Journal of Infectious Diseases 17 (2013) e422–e427

Contents lists available at SciVerse ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Polyclonal endemicity of *Acinetobacter baumannii* in ventilated patients in an intensive care unit in Uruguay

Julio Cesar Medina-Presentado [a,1,*], Verónica Seija [b,1], Rafael Vignoli [c], Julio Pontet [d], Luciana Robino [c], Nicolás F. Cordeiro [c], Inés Bado [c], Virginia García-Fulgueiras [c], Maximiliano Berro [d], Cristina Bazet [b], Eduardo Savio [a], Gloria Rieppi [d]

[a] *Cátedra de Enfermedades Infecciosas, Instituto de Higiene, Facultad de Medicina, Universidad de la República, Av. Alfredo Navarro 3051, Montevideo, 11600, Uruguay*
[b] *Departamento de Laboratorio Clínico, Área Microbiología, Hospital de Clínicas, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay*
[c] *Departamento de Bacteriología y Virología, Instituto de Higiene, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay*
[d] *Unidad de Cuidados Intensivos del Hospital de Clínicas, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay*

ARTICLE INFO

Article history:
Received 14 June 2012
Received in revised form 21 November 2012
Accepted 31 December 2012

Corresponding Editor: Andy Hoepelman, Utrecht, The Netherlands

Keywords:
Acinetobacter baumannii infection
Ventilator-associated pneumonia
Epidemiology

SUMMARY

Objectives: To identify the mechanisms responsible for respiratory infections by *Acinetobacter baumannii* in intubated patients and risk factors for digestive colonization and infection by *A. baumannii*.
Methods: We conducted a prospective study in an intensive care unit (ICU) between May 2005 and November 2006, including 175 consecutive patients at the beginning of invasive ventilation (day 1). We performed pharyngeal and rectal swabs on days 1, 4, 7, 10, 13, and 16. Respiratory samples were taken on days 1 and 7, or on suspicion of ventilator-associated pneumonia (VAP).
Results: We detected 62 patients with *A. baumannii* digestive colonization and 20 cases of *A. baumannii* lower respiratory infection (14 VAP and six purulent tracheobronchitis (PTB)). Digestive colonization by *A. baumannii* was an independent risk factor for lower respiratory tract infections with that microorganism ($p < 0.0001$; relative risk 8.71, 95% confidence interval 2.73–27.77). Respiratory and rectal *A. baumannii* isolates from the same patients were compared by enterobacterial repetitive intergenic consensus (ERIC)-PCR; in 9/11 cases (eight VAP and one PTB) results suggested events of exogenous pneumonia with previous colonization, whereas the remaining two cases (two PTB) were suggestive of exogenous infection without previous colonization.
Conclusions: In our unit the pathogenesis of VAP by *A. baumannii* is mixed, most cases corresponding to exogenous pneumonia with previous colonization.

1. Introduction

Intensive care unit (ICU)-acquired infections are an independent risk factor for hospital mortality even after adjustment for severity scores and age.[1] *Acinetobacter baumannii* infections have become increasingly common among critically ill patients in ICUs worldwide, causing mainly ventilator-associated pneumonia (VAP).[2–4]

In some hospitals, epidemic infections become endemic, yet the clinical and microbiological epidemiology of these infections remains unclear.[5]

Although *A. baumannii* can be detected in the airway without previous colonization,[6] colonization of the digestive tract often precedes VAP. The importance of this event and the subsequent entry of oropharyngeal content into the trachea in the pathogenesis of nosocomial pneumonia have already been suggested.[7] Additionally this previous colonization is the basis for strategies aimed at preventing infections, such as selective decontamination of the digestive tract (SDD).[8]

The goals of this work were to identify the proportion of respiratory infections by *A. baumannii* preceded by previous colonization in intubated patients and to investigate the risk factors for *A. baumannii* digestive colonization and infection.

2. Methods

We conducted a prospective cohort study at the Hospital de Clínicas, a university referral hospital with a 12-bed medical–surgical ICU.

* Corresponding author. Tel.: +598 2487 6981.
E-mail addresses: jcmedina@fmed.edu.uy, jcmedina1@gmail.com (J.C. Medina-Presentado).
[1] M.P.J.C and S.V contributed equally to the experimental work and to the elaboration of this report.

1201-9712/$36.00 – see front matter
http://dx.doi.org/10.1016/j.ijid.2012.12.025

2.1. Study population

All patients admitted from May 1, 2005 to November 31, 2006 with a predicted need for invasive mechanical ventilation of more than 48 h were included in a successive manner. HIV-positive patients and solid organ transplant recipients were excluded from the study.

2.2. Study design

All patients included in this study were followed until death or discharge from the ICU. The following parameters were recorded: sex, age, admission diagnosis, severity of underlying disease,[9] severity of illness (according to the Acute Physiology and Chronic Health Evaluation (APACHE) II score) at 24 h after admission and at the moment of VAP diagnosis, co-morbidities, stress ulcer therapy, need for reintubation, airway aspiration, previous surgery, cranial trauma, reasons for the need to mechanically ventilate,[10] duration of mechanical ventilation, and use of systemic antibiotics for more than 48 h during the previous 15 days and during ICU stay.

2.3. Microbiological processing

In the context of an ongoing program aimed at the surveillance of dissemination of multiresistant Gram-negative rods within this ICU, throat and rectal swabs were collected by specialized personnel upon patient admission to the unit and on days 4, 7, 10, 13, and 16, or until death or discharge.

In addition, a sample was taken from the inspiratory limb of the ventilator on day 1. Tracheal aspirates were taken on days 1 and 7, or when VAP was suspected. Bronchoalveolar lavage (BAL) was performed when VAP was suspected.

Surveillance samples were plated on sheep blood agar and MacConkey agar with and without 1 mg/l of ceftazidime. Acinetobacter isolates were identified as *A. calcoaceticus–baumannii* complex using standard microbiological methods; identification of *A. baumannii* was further confirmed by PCR for $bla_{\text{OXA-51}}$, which according to Woodford et al.[11] is species-specific.[12]

Colonization on admission was considered when *A. baumannii* was isolated from any of the admission samples. Colonization was considered ICU-acquired only when there was no colonization on admission but *A. baumannii* was detected in at least one sample obtained from day 4 onwards.

Definitions: A confirmative diagnosis of VAP was established if the following criteria were present: (1) new and persistent infiltrate on chest X-ray; (2) two or more of the following criteria: rectal temperature $\geq$38.5 °C or $\leq$36 °C, leukocytosis $\geq 12 \times 10^9$/l or $\leq 4 \times 10^9$/l, purulent respiratory secretions; and (3) one or more of the following: (a) isolation of a potential pathogen in BAL fluid with a bacterial count of at least 10 000 cfu/ml; (b) clinical pulmonary infection score (CPIS) >6 plus positive semiquantitative tracheal aspirates;[13,14] (c) recovery of identical potential pathogens from blood cultures and respiratory secretions, in the absence of other sources of infection; (d) isolation of a potential pathogen from pleural fluid without previous pleural instrumentation.

Cases of purulent tracheobronchitis (PTB) were diagnosed following the US Centers for Disease Control and Prevention definition.[15]

In accordance with Hu et al.,[16] lower respiratory tract infections (VAP or PTB) were considered: (1) endogenous when caused by *A. baumannii* strains clonally unrelated to *A. baumannii* isolates colonizing or infecting other patients; and (2) exogenous when caused by strains already isolated from other patients, either from clinical samples or from surveillance cultures (rectal or pharyngeal swabs). We also registered if patients showed digestive colonization with the same strain. In this sense, lower respiratory tract infections were classified as endogenous with or without previous colonization, or exogenous with or without previous colonization.

The presence of *A. baumannii* isolates in tracheal aspirates in the absence of VAP or PTB was considered as respiratory colonization.

Acute respiratory distress syndrome, severe sepsis, and septic shock were diagnosed using standard published criteria.[17]

2.4. Genotyping

A. baumannii isolates obtained from respiratory and digestive samples belonging to the same patient were compared by enterobacterial repetitive intergenic consensus (ERIC)-PCR, in accordance with Silbert et al.[18]

2.5. Statistical analysis

Numeric variables were expressed with their standard deviation. We performed Chi-square or Fisher's exact test accordingly. A p-value of <0.05 was considered significant. Relative risks (RR) and 95% confidence intervals (CI 95%) were calculated using standard methods. In order to identify independent input variables favoring the presence of *A. baumannii* in the respiratory tract, as well as the digestive system, we performed a multivariate analysis using a logistic regression model (Enter method) with the aid of SPSS 12.0 software (SPSS Inc., Chicago, IL, USA).

2.6. Ethics

The study did not intervene in the treatment (clinical or paraclinical) of the patients. Data were handled and results are presented without disclosing the identity of the patients.

3. Results

3.1. Patients and demographic data

One hundred seventy-five patients (101 men and 74 women) were enrolled. The mean age (± standard deviation) of these patients was 49.7 ± 17.6 years. The median APACHE II score at admission was 22 (interquartile range 20–26). Demographic and clinical data of the included patients are shown in Table 1.

3.2. Digestive tract colonization

During the study period, 1306 surveillance samples were taken. Colonization by *A. baumannii* was found in 62 patients (35.4%). Twelve (20%) were already colonized at ICU admission: four were community-acquired, whereas eight were acquired on other hospital wards. The remaining 50 patients (80%) became colonized during their ICU stay, 23 of whom were colonized from day 4, 20 from day 7, three from day 10, three from day 13, and one from day 16 (Figure 1). None of the patients colonized upon admission developed lower respiratory tract infections. No *A. baumannii* isolates were recovered from samples of tracheal aspirates or inspiratory limbs taken on day 1.

3.3. Respiratory infections

A. baumannii isolates were recovered from respiratory samples (tracheal aspirate or BAL) in 37 patients: 20 from lower respiratory tract infections (14 VAP and six PTB) and 17 from cases of respiratory colonization. Table 2 shows the colonization/infection pattern of those patients with respiratory infection.

Molecular biology tools could be applied to strains obtained from 11 out of 20 patients with lower respiratory tract infection. We detected nine cases of respiratory infections with previous colonization by strains that were either infecting or colonizing

Table 1
Demographic and clinical data of included patients

Variable	*N* = 175
Male gender, *n* (%)	101 (57.7%)
Age, years, mean (SD)	49.7 ± 17.6
APACHE II score, median (interquartile range)	22 (20–26)
McCabe and Jackson criteria	
Rapidly fatal disease, *n* (%)	1 (0.6%)
Ultimately fatal disease, *n* (%)	18 (10.3%)
Non-fatal disease, *n* (%)	156 (89.1%)
Pre-existing comorbidities:	
Chronic alcoholism, *n* (%)	24 (13.7%)
Immunosuppression, *n* (%)	9 (5.1%)
Hospitalized 3 months before, *n* (%)	17 (9.7%)
Cardiovascular disease, *n* (%)	10 (5.7%)
Diabetes, *n* (%)	14 (8%)
Liver disease, *n* (%)	4 (2.3%)
Diagnosis at admission:	
Non-traumatic ABI, *n* (%)	31 (17.7%)
Severe CAP, *n* (%)	11 (6%)
COPD exacerbation, *n* (%)	4 (2.3%)
Severe trauma, *n* (%)	43 (24.5%)
Severe sepsis, *n* (%)	35 (20%)
Cardiovascular disease, *n* (%)	5 (2.9%)
Cardiac arrest, *n* (%)	4 (2.3%)
Thoraco-abdominal surgery, *n* (%)	28 (16%)
Miscellaneous, *n* (%)	14 (8%)
Previous antibiotic treatment, *n* (%)	145 (82.9%)
VAP *Acinetobacter baumannii*, *n* (%)	14 (8%)
Mechanical ventilation, mean (SD)	12.2 ± 14.8 days
Crude ICU mortality, *n* (%)	62 (35.4%)
Length of ICU stay, mean (SD)	14.8 ± 15.6 days

APACHE II, Acute Physiology and Chronic Health Evaluation II; ABI, acute brain injury; CAP, community-acquired pneumonia; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; SD, standard deviation; VAP, ventilator-associated pneumonia.

other patients, thus representing episodes of exogenous pneumonia with previous colonization (see Table 2 and Figure 2). The other two patients were assessed as cases of exogenous infection without previous colonization (patients 87 and 102, see Table 2 and Figure 2). In both patients the strains colonizing their digestive tracts were genetically unrelated to the infecting strains.

Regarding the remaining nine patients, we detected previous colonization in four cases, whereas the other five corresponded to events of infection without previous colonization.

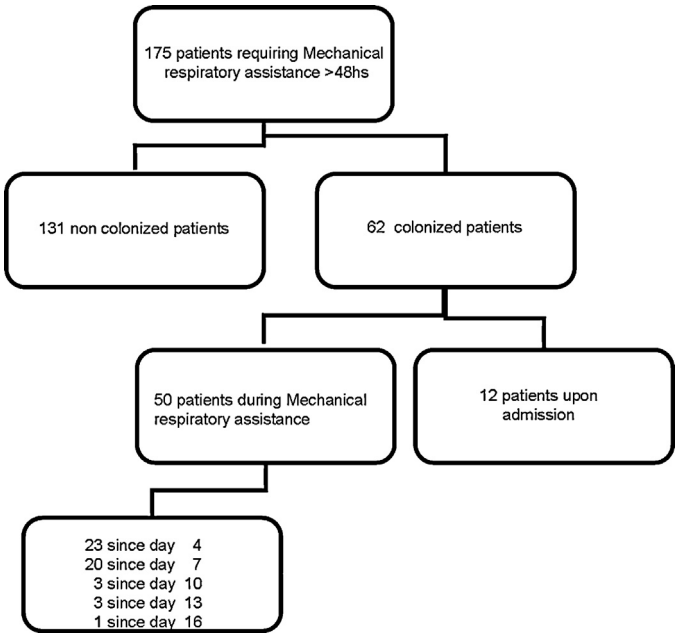

Figure 1. Distribution of digestive colonization by *Acinetobacter baumannii*.

As shown in Table 2 and Figure 2, we found four different *A. baumannii* clones. Clone 1 was present in patients 9, 11, 87 (rectal sample only), and 110. The first two patients were in the unit at the same time, but patients 87 and 110 were admitted at 8 and 9 months, respectively, after patient 11 was discharged.

Clone 2 was present in patients 33 and 102 (pharyngeal sample only). Clone 3 was present in four patients: patients 39, 71, 81, and 97. Clone 4 was found in three patients: tracheal aspirates from patients 87 and 102 and digestive and respiratory samples from patient 105.

3.4. Risk factors

The association between the presence of *A. baumannii* in the digestive tract and the variables under study are shown in Table 3. Logistic regression analysis pointed to the prior use of oxyimino-cephalosporins ($p = 0.014$; RR 2.35, 95% CI 1.18–4.68) and diabetes ($p = 0.036$; RR 3.78, 95% CI 1.09–13.08) as the only independent risk factors for digestive colonization with *A. baumannii*.

Table 4 indicates the link between respiratory infections by *A. baumannii* and the variables under study. In this case, logistic regression showed digestive colonization with *A. baumannii* as an independent risk factor for the development of respiratory infections with *A. baumannii* ($p < 0.0001$; RR 8.71, 95% CI 2.73–27.77).

4. Discussion

There are few studies analyzing the relationship between digestive colonization and respiratory infections by *A. baumannii*, and the existing information is somewhat incomplete.[19,20] To the best of our knowledge this is the first prospective study in Latin America that has included molecular biology tools to investigate this matter.

Our main finding was the demonstration of the occurrence, in an apparent endemic scenario, of mixed pathogenic mechanisms responsible for respiratory infections by *A. baumannii*, most of them being exogenous with previous pharyngeal and/or rectal colonization.

We were also able to demonstrate the presence of different clones co-circulating during the study period, not only within the ICU, but also among the patients. Regardless of such diversity, all cases were dubbed as exogenous respiratory infections, most of them with previous colonization, since the four *A. baumannii* clones were detected for prolonged periods of time (e.g., clone 1 was recovered again a year after it was first detected). In this sense, even the first isolate of each clone (supposedly index cases) could have been circulating within this ICU prior to the beginning of our work.

Our findings indicate that within our unit *A. baumannii* displays a polyclonal endemic behavior, in which those patients developing infectious processes by this microorganism were previously colonized with it (14/20). On the other hand a significant proportion of patients (6/20) showed no evidence of previous colonization. This situation could have at least two probable explanations: (1) rectal and/or pharyngeal swabs could have a low sensitivity for the detection of colonization events with a small bacterial count, which has already been demonstrated for *A. baumannii*;[21] or (2) direct inoculation of the microorganism into the respiratory tract, as has been documented for, e.g., *K. pneumoniae*.[22]

Irrespective of this, the digestive tract of patients seems to be a likely reservoir of strains responsible for infectious processes, as demonstrated by infections involving clone 1 (Table 2). Unfortunately, we were unable to analyze all the colonizing strains during the study period in order to thoroughly back up this hypothesis.

Table 2
Pattern of colonization/infection in 20 patients infected by *Acinetobacter baumannii*

Patient number	Date of admission	Date of discharge	Colonization at admission	ICU-acquired colonization (clone)[a]	Day of ICU-acquired colonization	Type of infection (clone)[b]	Day of acquired infection	Molecular relatedness	Mechanism involved[c]
9	15/05/05	2/06/05	No	Yes (Clone 1)	7	VAP (Clone 1)	8	Yes	Exogenous W/colonization
11	24/05/05	26/08/05	No	Yes (Clone 1)	4	VAP (Clone 1)	8	Yes	Exogenous W/colonization
33	24/09/05	06/11/05	No	Yes (Clone 2)	16	PTB (Clone 2)	19	Yes	Exogenous W/colonization
39	30/09/05	09/11/05	No	Yes (Clone 3)	10	VAP (Clone 3)	14	Yes	Exogenous W/colonization
46	04/11/05	21/11/05	No	Yes	7	PTB	15	-	Pres. exogenous W/colonization
54	28/11/05	07/12/05	No	No	-	VAP	9	-	Pres. exogenous WO/colonization
55	26/11/05	12/12/05	No	No	-	PTB	7	-	Pres. exogenous WO/colonization
71	06/01/06	23/01/06	No	Yes (Clone 3)	4	VAP (Clone 3)	5	Yes	Exogenous W/colonization
73	16/01/06	26/01/06	No	No	-	VAP	4	-	Pres. exogenous WO/colonization
81	30/03/06	07/07/06	No	Yes (Clone 3)	4	VAP (Clone 3)	7	Yes	Exogenous W/colonization
87	11/04/06	24/04/06	No	Yes (Clone 1)	10	PTB (Clone 4)	9	No	Exogenous WO/colonization
97	04/05/06	22/06/06	No	Yes (Clone 3)	7	VAP (Clone 3)	15	Yes	Exogenous W/colonization
102	09/05/06	28/05/06	No	Yes (Clone 2)	4	PTB (Clone 4)	7	No	Exogenous WO/colonization
105	14/05/06	07/06/06	No	Yes (Clone 4)	4	VAP (Clone 4)	16	Yes	Exogenous W/colonization
110	29/05/06	02/07/06	No	Yes (Clone 1)	4	VAP (Clone 1)	14	Yes	Exogenous W/colonization
140	16/07/06	14/09/06	No	Yes	7	VAP	5	-	Pres. exogenous WO/colonization
148	08/08/06	28/08/06	No	Yes	7	VAP	8	-	Pres. exogenous W/colonization
157	20/09/06	31/10/06	No	No	-	VAP	11	-	Pres. exogenous WO/colonization
166	22/09/06	09/10/06	No	Yes	4	VAP	12	-	Pres. exogenous W/colonization
172	14/10/06	12/11/06	No	Yes	4	PTB	5	-	Pres. exogenous W/colonization

ICU, intensive care unit; PTB, purulent tracheobronchitis; VAP, ventilator-associated pneumonia.
[a] Colonizing clone.
[b] Infecting clone.
[c] With (W), without (WO), presumptively (Pres).

Regarding digestive colonization, we detected the presence of *A. baumannii* in 62 patients (35.4%), a proportion similar to that previously reported by other authors.[19,20] Of these, 88.7% (n = 55) were detected within the first 7 days of admission to the ICU, which suggests that in our unit *A. baumannii* colonizes patients faster than previously reported.[5]

Logistic regression identified the previous use of oxyiminocephalosporins as a risk factor for digestive colonization by *A.*

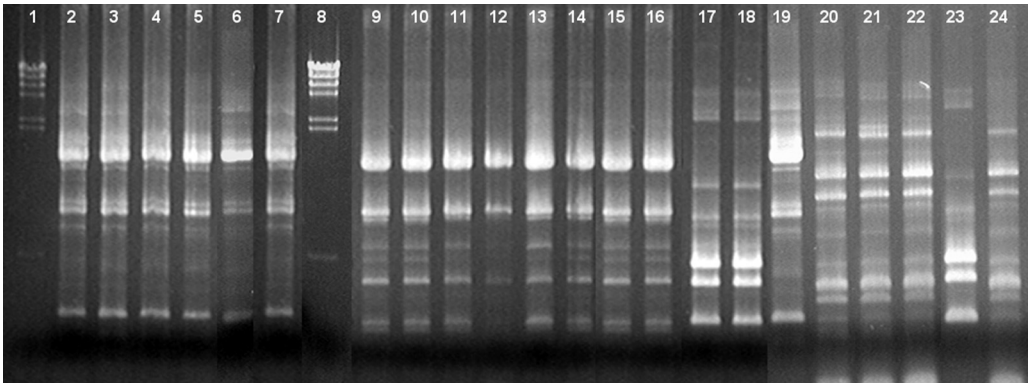

Figure 2. Comparison of *Acinetobacter baumannii* strains obtained from patients that yielded this microorganism in digestive and respiratory samples. ERIC-PCR assays were performed for strains from 11 cases. 1, ladder; 2, 9.3R; 3, 9TA; 4, 11.2P; 5, 11TA; 6, 110.2R; 7, 110TA; 8, ladder; 9, 39.4P; 10, 39TA; 11, 71.2R; 12, 71BAL; 13, 81.3P; 14, 81TA; 15, 97.2R; 16, 97TA; 17, 33.6P; 18, 33TA; 19, 87.4R; 20, 87TA; 21, 105.5P; 22, 105TA; 23, 102.2P; 24, 102TA.

Table 3
Relationship between the occurrence of *Acinetobacter baumannii* in the digestive tract and different variables. Logistic regression analysis

Variables	Digestive colonization $n=62$	No digestive colonization $n=113$	Univariate analysis		Multivariate analysis	
			RR (95% CI)	*p*-Value	RR (95% CI)	*p*-Value
Neurocritical	29 (46.8%)	41 (36.3%)	1.54 (0.82–2.89)	0.19	1.82 (0.92–3.59)	0.08
Reintubation	9 (14.5%)	7 (6.2%)	2.57 (0.90–7.28)	0.09	2.16 (0.68–6.86)	0.19
Previous use of oxyiminocephalosporins[a]	28 (45.2%)	27 (23.9%)	2.62 (1.35–5.08)	0.006	2.35 (1.18–4.68)	0.014
Hemodialysis	4 (6.5%)	1 (0.9%)	7.72 (0.84–70.7)	0.05	4.37 (0.38–50.33)	0.23
Diabetes	9 (14.5%)	5 (4.4%)	3.66 (1.17–11.4)	0.037	3.78 (1.09–13.08)	0.036

RR, relative risk; CI, confidence interval.
[a] Oxyiminocephalosporins: cefuroxime, ceftriaxone, ceftazidime, cefotaxime.

Table 4
Relationship between respiratory infection by *Acinetobacter baumannii* and different variables. Univariate analysis and logistic regression

Variables	Respiratory infection $n=20$	No respiratory infection $n=155$	Univariate analysis		Multivariate analysis	
			RR (95% CI)	*p*-Value	RR (95% CI)	*p*-Value
Occurrence of *A. baumannii* in digestive tract	16 (80%)	46 (29.7%)	9.47 (3.00–29.89)	<0.001	8.71 (2.73–27.77)	<0.001
Previous use of oxyiminocephalosporins[a]	12 (60%)	43 (27.7%)	3.90 (1.49–10.21)	0.009	3.27 (0.90–11.89)	0.071
Reintubation	5 (25%)	11 (7.1%)	4.36 (1.33–14.24)	0.022	-	-

RR, relative risk; CI, confidence interval.
[a] Oxyiminocephalosporins: cefuroxime, ceftriaxone, ceftazidime, cefotaxime.

baumannii. These antibiotics have already been pointed out as predisposing factors for digestive colonization by this microorganism,[23,24] as well as by oxyiminocephalosporin-resistant *Enterobacteriaceae*.[25]

We could thus formulate the following chain of events: once admitted to the ICU, those patients treated with oxyiminocephalosporins become a risk group for digestive colonization with *A. baumannii*, which in turn constitutes a predisposing factor for the colonization of the lower respiratory tract and further development of infections. Our group has already demonstrated that previous use of ceftriaxone and ciprofloxacin are independent risk factors for the development of *A. baumannii* VAP,[26] and that the restricted use of both antibiotics helps to decrease the incidence of that pathogen.[27]

This theory supports the finding of other authors concerning the importance of patients as 'reservoirs' for this microorganism.[28] In this sense, several authors have pointed out that clinical infections are more frequent in patients with *A. baumannii* digestive colonization.[28,29]

In our ICU, respiratory infections by *A. baumannii* can be abated only by multimodal strategies that take into consideration both the pathogenesis and epidemiology of that microorganism.[8] Concerning SDD, several studies have demonstrated that selective decontamination regimes are associated with a decrease in VAP and mortality among patients in ICUs.[30,31] SDD could be considered as a reasonable additional control measure, in view of the high rates of fecal carriage observed in *A. baumannii* outbreaks.[32]

In conclusion, in our particular setting, gut colonization precedes respiratory infections in up to 70% of cases, on account of different endemic clones of *A. baumannii*. Further studies are needed to evaluate the impact of multi-intervention strategies to control this endemic situation.

Acknowledgements

This project was partially funded by the Comisión Sectorial de Investigación Científica (CSIC), Universidad de la República, Uruguay.

Conflict of interest: No competing interest to declare.

References

1. Ylipalosaari P, Ala-Kokko T, Laurila J, Laurila J, Ohtonen P, Syrjälä H, et al. Intensive care acquired infection is an independent risk factor for hospital mortality: a prospective cohort study. *Crit Care* 2006;**10**:R66.
2. Landman D, Quale JM, Mayorga D, Adedeji A, Vangala K, Ravishankar J, et al. Citywide clonal outbreak of multiresistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Brooklyn, NY: the preantibiotic era has returned. *Arch Intern Med* 2002;**162**:1515–20.
3. Trouillet JL, Chastre J, Vuagnat A, Joly-Guillou ML, Combaux D, Dombret MC, et al. Ventilator-associated pneumonia caused by potentially drug resistant bacteria. *Am J Respir Crit Care Med* 1998;**157**:531–9.
4. Rocha Lde A, Vilela CA, Cezário RC, Almeida AB, Gontijo Filho P. Ventilator-associated pneumonia in an adult clinical–surgical intensive care unit of a Brazilian university hospital: incidence, risk factors, etiology, and antibiotic resistance. *Braz J Infect Dis* 2008;**12**:80–5.
5. Corbella X, Montero A, Pujol M, Domínguez MA, Ayats J, Argerich MJ, et al. Emergence and rapid spread of carbapenem resistance during a large and sustained hospital outbreak of multiresistant *Acinetobacter baumannii*. *J Clin Microbiol* 2000;**38**:4086–95.
6. de La Cal MA, Cerda E, Garcia-Hierro P, Lorente L, Sanchez-Concheiro M, Diaz C, et al. Pneumonia in patients with severe burns: a classification according to the concept of the carrier state. *Chest* 2001;**119**:1160–5.
7. Safdar N, Crnich CJ, Maki DG. The pathogenesis of ventilator-associated pneumonia: its relevance to developing effective strategies for prevention. *Respir Care* 2005;**50**:725–39. discussion 739–41.
8. Zandstra DF, Van Saene HK. Selective decontamination of the digestive tract as infection prevention in the critically ill. A level 1 evidence-based strategy. *Minerva Anestesiol* 2011;**71**:212–9.
9. McCabe WR, Jackson GG. Gram-negative bacteremia: I. Etiology and ecology. *Arch Intern Med* 1962;**110**:847–55.
10. Zwillich CW, Pierson DJ, Creagh CE, Sutton FD, Schatz E, Petty TL. Complications of assisted ventilation: a prospective study of 354 consecutive episodes. *Am J Med* 1974;**57**:161–70.
11. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter spp*. *Int J Antimicrob Agents* 2006;**27**:351–3.
12. Héritier C, Poirel L, Fournier PE, Claverie JM, Raoult D, Nordmann P. Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2005;**49**:4174–9.
13. Bergmans DC, Bonten MJ, De Leeuw PW, Stobberingh EE. Reproducibility of quantitative cultures of endotracheal aspirates from mechanically ventilated patients. *J Clin Microbiol* 1997;**35**:796–8.
14. Flanagan PG, Findlay GP, Magee JT, Ionescu A, Barnes RA, Smithies M. The diagnosis of ventilator-associated pneumonia using non-bronchoscopic, non-directed lung lavages. *Intensive Care Med* 2000;**26**:20–30.
15. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008;**36**:309–32.
16. Hu HB, Huang HJ, Peng QY, Lu J, Lei XY. Prospective study of colonization and infection because of *Pseudomonas aeruginosa* in mechanically ventilated patients at a neonatal intensive care unit in China. *Am J Infect Control* 2010;**38**:746–50.

17. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992;**101**:1644–55.
18. Silbert S, Pfaller MA, Hollis RJ, Barth AL, Sader HL. Evaluation of three molecular typing techniques for nonfermentative Gram-negative bacilli. *Infect Control Hosp Epidemiol* 2004;**25**:847–51.
19. Ayats J, Corbella X, Ardanuy C, Dominguez MA, Ricart A, Ariza J, et al. Epidemiological significance of cutaneous, pharyngeal, and digestive tract colonization by multiresistant *Acinetobacter baumannii* in ICU patients. *J Hosp Infect* 1997;**37**: 287–95.
20. Garrouste-Orgeas M, Chevret S, Arlet G, Marie O, Rouveau M, Popoff N, et al. Oropharyngeal or gastric colonization and nosocomial pneumonia in adult intensive care unit patients. A prospective study based on genomic DNA analysis. *Am J Respir Crit Care Med* 1997;**156**:1647–55.
21. Marchaim D, Navon-Venezia S, Schwartz D, Tarabeia J, Fefer I, Schwaber MJ, et al. Surveillance cultures and duration of carriage of multidrug-resistant *Acinetobacter baumannii*. *J Clin Microbiol* 2007;**45**:1551–5.
22. Aumeran C, Poincloux L, Souweine B, Robin F, Laurichesse H, Baud O, et al. Multidrug-resistant *Klebsiella pneumoniae* outbreak after endoscopic retrograde cholangiopancreatography. *Endoscopy* 2010;**42**:895–9.
23. Park YS, Lee H, Lee KS, Hwang SS, Cho YK, Kim HY, et al. Extensively drug-resistant *Acinetobacter baumannii*: risk factors for acquisition and prevalent OXA-type carbapenemases—a multicentre study. *Int J Antimicrob Agents* 2010;**36**: 430–5.
24. Tacconelli E, Cataldo MA, De Pascale G, Manno D, Spanu T, Cambieri A, et al. Prediction models to identify hospitalized patients at risk of being colonized or infected with multidrug-resistant *Acinetobacter baumannii calcoaceticus* complex. *J Antimicrob Chemother* 2008;**62**:1130–7.
25. Vignoli R, Calvelo E, Cordeiro NF, Lucero R, Ingold E, Quintana A, et al. Association of broad-spectrum antibiotic use with faecal carriage of oxyiminocephalosporin-resistant *Enterobacteriaceae* in an intensive care unit. *J Hosp Infect* 2006;**63**:306–15.
26. Medina J, Formento C, Pontet J, Curbelo A, Bazet C, Gerez J, et al. Prospective study of risk factors in patients with ventilator-associated pneumonia caused by *Acinetobacter spp*. *J Crit Care* 2007;**22**:18–27.
27. Medina Presentado JC, Paciel López D, Berro Castiglioni M, Gerez J. Ceftriaxone and ciprofloxacin restriction in an intensive care unit: less incidence of *Acinetobacter spp*. and improved susceptibility of *Pseudomonas aeruginosa*. *Rev Panam Salud Publica* 2011;**30**:603–9.
28. Corbella X, Pujol M, Ayats J, Sendra M, Ardanuy C, Dominguez MA, et al. Relevance of digestive tract colonization in the epidemiology of nosocomial infections due to multiresistant *Acinetobacter baumannii*. *Clin Infect Dis* 1996;**23**: 329–34.
29. Thom KA, Hsiao WW, Harris AD, Stine OC, Rasko DA, Johnson JK. Patients with *Acinetobacter baumannii* bloodstream infections are colonized in the gastrointestinal tract with identical strains. *Am J Infect Control* 2010;**38**:751–3.
30. Urban C, Segal-Maurer S, Rahal JJ. Considerations in control and treatment of nosocomial infections due to multidrug-resistant *Acinetobacter baumannii*. *Clin Infect Dis* 2003;**36**:1268–74.
31. De Jonge E, Schultz MJ, Spanjaard L, Bossuyt PM, Vroom MB, Dankert J, et al. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomized controlled trial. *Lancet* 2003;**362**:1011–6.
32. Stoutenbeek CP, van Saene HK, Little RA, Whitehead A, Working Group on Selective Decontamination of the Digestive Tract. The effect of selective decontamination of the digestive tract on mortality in multiple trauma patients: a multicenter randomized controlled trial. *Int Care Med* 2007;**33**:261–70.