Microbiological and host features associated with corynebacteriosis in cancer patients: a five-year study

CAS Martins[1, 3], LMD Faria[1], MC Souza[3], TCF Camello[3], E Velasco[1], R Hirata Jr[3], LCS Thuler[2], AL Mattos-Guaraldi[3]/+

[1]Instituto Nacional do Câncer, Ministério da Saúde, Rio de Janeiro, RJ, Brasil [2]Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil [3]Disciplina de Microbiologia e Imunologia, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Av. 28 de Setembro 87 fundos, 3º andar, 20551-030 Rio de Janeiro, RJ, Brasil

During a five-year period, 932 clinical isolates from cancer patients treated in a Brazilian reference centre were identified as corynebacteria; 86% of the cultures came from patients who had been clinically and microbiologically classified as infected and 77.1% of these patients had been hospitalised (71.1% from surgical wards). The adult solid tumour was the most common underlying malignant disease (66.7%). The univariate and multivariate analyses showed that hospitalised patients had a six-fold greater risk (OR = 5.5, 95% CI = 1.15-26.30 p = 0.033) related to 30-day mortality. The predominant species were Corynebacterium amycolatum *(44.7%),* Corynebacterium minutissimum *(18.3%) and* Corynebacterium pseudodiphtheriticum *(8.5%). The upper urinary tracts, surgical wounds, lower respiratory tracts, ulcerated tumours and indwelling venous catheters were the most frequent sources of* C. amycolatum *strains.* Corynebacterium jeikeium *infection occurred primarily in neutropenic patients who have used venous catheters, while infection caused by* C. amycolatum *and other species emerged mainly in patients with solid tumours.*

Key words: *Corynebacterium amycolatum* - *Corynebacterium* infection - cancer

Corynebacterium species have recently been recognised as important pathogens that infect immunocompromised patients. Despite the increase in the number of reports of severe infection caused by non-diphtheria corynebacteria, these organisms are still frequently dismissed as contaminants. *Corynebacterium* spp have been cited with an increased frequency as pathogens of nosocomial infections associated with septicaemias, endocarditis, infections of surgical wounds, prostheses and infections related to the central venous catheter. Geographical variations in the frequency of isolated species and variations in the natural and acquired antimicrobial resistance have been described (Osterwalder et al. 1986, Rozdzinski et al. 1991, van der Lelie et al. 1995).

Recent advances in *Corynebacterium* identification have shown that the genus exhibits considerable taxonomic complexity and that the phenotypic markers used in the past for its identification can be ambiguous (Barreau et al. 1993, Brandenburg et al. 1996, Oteo et al. 2001, Camello et al. 2003, Meyer & Reboli 2005, Otsuka et al. 2006). When these infections are linked to multiresistant species, they are difficult to treat (Bodey 1995, Funke et al. 1996, Camello et al. 2003, Funke & Bernard 2003). Multiple resistance to antimicrobial agents has been described among some species (Lagrou et al. 1998, Zalas et al. 2004); for example, mutations in the gyrase genes are determinants of the resistance to quinolones in *Corynebacterium striatum* and *Corynebacterium amycolatum* strains (Sierra et al. 2005).

In Rio de Janeiro, Brazil, recent studies conducted at a teaching hospital showed that 68% of the clinical isolates of corynebacteria species corresponded to *Corynebacterium pseudodiphtheriticum, C. amycolatum, Corynebacterium propinquum* and *Corynebacterium minutissimum*. The urinary tract and the venous access were the sites most commonly affected. In the blood and the respiratory tract, there was a predominance of *C. pseudodiphtheriticum* and *C. propinquum*, while *Corynebacterium xerosis* and *C. amycolatum* were the most commonly observed species in central venous catheters. *C. propinquum* and *C. minutissimum* were the most frequently observed species in surgical wounds. Data indicated the occurrence of multi-resistant phenotypes and the possibility of severe infections due to *C. pseudodiphtheriticum*, a pathogen usually overlooked in emerging countries (Camello et al. 2003, 2009).

Infection has been recognised as one of the major obstacles to the successful management of patients with malignant diseases. Few studies have described non-diphtheria corynebacteria species as emerging pathogens in patients with solid tumours, with the exception of those that highlighted the importance of *Corynebacterium jeikeium* (Rozdzinski et al. 1991, van der Lelie et al. 1995). Vancomycin has been demonstrated to be the primary treatment option for *C. jeikeium*. The choice of using glycopeptides to empirically treat patients with neutropenic fever and persistent fever has been criticised since the advent of the vancomycin-resistant *Enterococcus* and the heterogeneous vancomycin-intermediate *Staphylococcus aureus* (Furtado et al. 2006). The new options for therapy

Financial support: INCA, FAPERJ, CNPq, CAPES, SR-2/UERJ, PRONEX
+ Corresponding author: guaraldi@uerj.br
Received 15 May 2009
Accepted 20 August 2009

are also not completely safe for the treatment of gram positive life-threatening infections partially due to the emergence of microbial resistance (Boucher et al. 2000, Dobbs et al. 2006, Schoen et al. 2009).

The aim of this descriptive study was to assess the microbiological and clinical aspects as well as factors related to 30-day mortality in cancer patients with corynebacteriosis.

PATIENTS, MATERIALS AND METHODS

Study setting - In this descriptive study, we retrospectively reviewed clinical and microbiological data over a five-year period (from January 2000-December 2004). The patient information originated from one of the units (Cancer Hospital I with 200 beds, Cancer Hospital II with 90 beds and Cancer Hospital III with 60 beds) of the National Cancer Institute (INCA) in Rio de Janeiro, Brazil.

The analysis included bacteriological data from 88,541 cultures and 1,100 irregular gram-positive rods isolated from cancer patients. The operational units included the Laboratory of Microbiology and the Hospital Infection Control Committee (HICC) at the INCA and the Laboratory of Diphtheria and Corynebacteria, State University of Rio de Janeiro (UERJ), Brazil.

During the period of the study, there were no changes in the technical team who processed the material for bacteriological cultures or in the medical team who assisted the patients.

Ethics - This paper was submitted and approved by the Ethical Research Committee at INCA (CEP 008/06) and complies with the Brazilian Government's Ethical Guidelines for research involving human beings (resolution of the National Health Council/Ministry of Health).

Diagnostic measures - The study analysed the clinical characteristics of 315 patients with *Corynebacterium* isolates who had malignant diseases or underwent bone marrow transplantation in the last two years of the study. Data assessment was performed on the basis of a medical records review of patients.

The hospitalised patients were monitored by at least one member of the HICC as a part of the antimicrobial vigilance routine through daily microbiology laboratory charts. Ambulatory patients were identified by the dressing nurses committee, catheter ambulatory (both for children and adults) and bacteriological charts.

In addition to the physicians' experiences of treating patients with cancer in INCA, the diagnosis of bacterial infection was staged according to the Centers for Disease Control and Prevention (CDC) classification (CDC 1992).

The patients with positive cultures were interpreted as infected when these cultures were derived from a normally sterile site associated with a febrile illness. Alternatively, patients with positive cultures were interpreted as infected when a *Corynebacterium* spp was isolated from two or more non-sterile sites in which there was a suspicion of infection and the physician considered it clinically significant to immediately start a specific antimicrobial therapy. A positive urinary culture was considered as significant in the presence of local (dysuria, polyuria) or systemic signs of infection; pyuria was only required in non-neutropenic patients. Tracheal secretions in intubated patients, sputum or tracheal aspirations in non-intubated patients were considered as positive if they were associated with clinical or radiological signs that indicated an infection. Protected sampling was not performed in bronchoscopy (Garner et al. 1988, Hughes et al. 1996, Berghmans et al. 2003).

Bacterial strains and identification - We retrospectively reviewed 932 strains identified as *Corynebacterium* spp that were recovered from representative clinical sites of cancer patients with signs and symptoms of bacterial infection. *Corynebacterium*-like colonies were selected for further identification when they were grown in any quantity from normally sterile body fluid or when they were isolated in significant numbers or in pure culture from other specimens obtained at clinical sites in which infection was suspected (Funke & Bernard 2003). All clinical samples yielding more than three organisms were regarded as contaminated and discarded (Thomson 2007).

The Maki's semi-quantitative method was used to distinguish infection (> 15 colonies) from contamination of catheter-tips (Maki et al. 1977). For quantitative BAL fluid cultures, a colony count > 10^3 colony-forming units (CFU) mL^{-1} of potential pathogens was considered positive. Isolation of two or three species of microorganisms was regarded as a polymicrobial infection for catheter tips and the lower respiratory tract, respectively. Microorganisms were identified from the urine cultures in cystine lactose electrolytes deficient agar (CLED; Merck, Darmstadt, Germany) and were considered to be potential pathogens when the growth was > 10^4 CFU mL^{-1} as the only isolate or > 10^5 CFU mL^{-1} as the predominant isolate; > 10^3 CFU mL^{-1}, in cases of nephropathies, was also considered a potential pathogen. Blood cultures were always obtained in pairs, wherein at least one of the samples was collected through the central venous catheter, if present. Blood specimens were inoculated in Bactec Plus anaerobic/aerobic vials and processed in a Bactec 9240 continuous-monitoring system (Becton-Dickinson Microbiology System, Cockeysville, MD, USA). Other clinical specimens were inoculated onto a Columbia agar base with the addition of 5% sheep's blood and incubated at 37°C in 3-5% CO_2 atmosphere and monitored for 72 h.

In addition to Gram staining, colonial morphology, pigmentation and haemolysis, *Corynebacterium*-like colonies were characterised using the API-Coryne System (BioMérrieux, Lyon, France) (Freney et al. 1991). Microorganisms were also submitted to the following conventional biochemical assays: catalase, pyrazinamidase, lypophilic activities, motility, nitrate reduction, hydrolysis of urea and esculin, acidification of glucose, maltose, sucrose, mannitol and xylose, as well as oxidation-fermentation and CAMP reaction tests (Camello et al. 2003, Funke & Bernard 2003).

Susceptibility testing - Antimicrobial susceptibility testing was performed by the Kirby Bauer's disk diffusion method, using an inoculum of 0.5 McFarland stan-

dard (150 x 10^6 CFU mL^{-1} by direct colonial suspension), adjusted for optical density at λ = 550 nm (Vitek colorimeter Durham, NC, USA). The plates were incubated at 35ºC in ambient air for 24 h and reconfirmed at 48 h in a cation-adjusted Mueller-Hinton Agar with the addition of 5% sheep's blood (Funke et al. 1997). As there is not yet a defined standard by the Clinical and Laboratory Standards Institute for interpreting the results of disk diffusion tests (CLSI 2007), we used the breakpoint for penicillin suggested for *Staphylococcus*.

For the other antimicrobial agents, we used the breakpoints for other microorganisms but not *Haemophilus* spp or *Neisseria gonorrhoeae*, which had been validated by previous studies (Martinez-Martinez et al. 1995, Weiss et al. 1996, Zinkernagel et al. 1996, Funke et al. 1997).

The intermediate results were included as resistant. Microorganisms were tested against 15 antimicrobial agents, according to the clinical criteria for empirical therapy in patients with underlying malignancy and infection. The in vitro antimicrobial susceptibility test associated with the analysis of clinical relevance used the interpretation criteria suggested by the Sanford Guide (Gilbert et al. 2006): (+) usually clinically effective or > 60% susceptible, (±) clinical trials lacking or 30-60% susceptible and (0) not clinically effective or < 30% susceptible.

Statistical analysis - The variables were compared using a Pearson Chi square or Fisher exact test. For statistical significance, the value was established at $p < 0.05$. A logistic regression model was developed to identify the variables independently associated with the 30-day mortality. This model included every variable that showed a statistically significant association ($p < 0.05$) in the univariate analysis. The softwares used for the statistical analysis were Epi-Info 2000 for Windows, version 3.3.2 and SPSS, version 14.

RESULTS

During a five-year period (from January 2000-December 2004), the clinical microbiology laboratory at INCA conducted 88,541 cultures, of which 25,173 (28.4%) were positive (n = 36,199). Gram-positive rods represented 1,436 (4%) of the clinical isolates. The genus *Corynebacterium* corresponded with 932 (84.7%) of the total (1,100 strains) number of irregular gram-positive rods (Table I).

The *Corynebacterium* species isolated from cancer patients presenting signs and symptoms of infection are displayed in Table II. Most of the isolates (44.7%) were recognised as *C. amycolatum*, followed by *C. minutissimum* (18.3%) and *C. pseudodiphtheriticum* (8.5%). *C. jeikeium* was the sixth in frequency among the isolates (4.7%). The species *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis*, which are usually associated with zoonoses, were absent or observed in a very low incidence in our population, respectively.

Taking into account the retrospective analysis of our study from 2000-2004, the isolation of *C. amycolatum/ xerosis* increased from 10.4-72.8%. On the other hand, *C. minutissimum* decreased from 31.3-1.3%; the percentage of *Corynebacterium* spp (unidentified species) went from 11.5-0% and *C. striatum* from 12.5-0.8%. *C. xerosis* comprised 20 isolates in 2000 and six in 2001 and was absent from new isolates in the subsequent years.

During the second phase of the study (from January 2003-December 2004), 10 *Corynebacterium* species were observed in 425 clinical isolates obtained from 315 cancer patients. *C. amycolatum* was the most frequent clinical isolate, followed by *C. pseudodiphtheriticum, C. jeikeium and C. minutissimum* (Table III). When only bloodstream infections were considered, catheter-related infections were frequently observed. *C. amycolatum* was widely distributed in all topographies. The upper urinary tract, surgical wounds, lower respiratory tract, ulcerated tumours and indwelling venous catheters were the most

TABLE I

Strains of irregular gram-positive rods isolated from cancer patients between 2000-2004

Genus	2000 n(%)	2001 n(%)	2002 n(%)	2003 n(%)	2004 n(%)	Total n(%)
Corynebacterium	192 (80.3)	158 (83.2)	152 (78.8)	191 (88.0)	239 (91.6)	932 (84.7)
Arcanobacterium	16 (6.7)	4 (2.1)	8 (4.1)	8 (3.7)	5 (1.9)	41 (3.7)
Brevibacterium	13 (5.4)	1 (0.5)	7 (3.6)	7 (3.2)	4 (1.5)	32 (2.9)
Actinomyces	6 (2.5)	5 (2.6)	11 (5.7)	1 (0.5)	2 (0.8)	25 (2.3)
Aureobacterium	2 (0.8)	10 (5.3)	8 (4.1)	1 (0.5)	2 (0.8)	23 (2.1)
Arthrobacter	-	7 (3.7)	3 (1.6)	4 (1.8)	7 (2.6)	21 (1.9)
Leifsonia	8 (3.3)	4 (2.1)	3 (1.6)	1 (0.5)	1 (0.4)	17 (1.5)
Propionibacterium	1 (0.5)	1 (0.5)	-	3 (1.3)	1 (0.4)	6 (0.6)
Rothia	1 (0.5)	-	1 (0.5)	1 (0.5)	-	3 (0.3)
Total	239 (100)	190 (100)	193 (100)	217 (100)	261 (100)	1,100 (100)

the strains identified as gram-positive regular rods [*Lactobacillus* (213), *Bacillus* (94), *Rhodococcus* (17), *Listeria* (9), *Nocardia* (2) and unidentified irregular gram-positive rods (1)] were not included in this study.

TABLE II

Corynebacterium species isolated from cancer patients between 2000-2004

Species	2000 n(%)	2001 n(%)	2002 n(%)	2003 n(%)	2004 n(%)	Total n(%)
Corynebacterium xerosis[a]/*Corynebacterium amycolatum*	20 (10.4)	40 (25.3)	65 (42.8)	118 (61.8)	174 (72.8)	417 (44.7)
Corynebacterium minutissimum	60 (31.3)	54 (34.2)	37 (24.3)	17 (8.9)	3 (1.3)	171 (18.3)
Corynebacterium pseudodiphtheriticum	9 (4.7)	10 (6.3)	7 (4.6)	16 (8.5)	37 (15.5)	79 (8.5)
Corynebacterium spp	22 (11.5)	23 (14.6)	5 (3.3)	2 (1.0)	-	52 (5.6)
Corynebacterium striatum	24 (12.5)	10 (6.3)	4 (2.6)	9 (4.7)	2 (0.8)	49 (5.3)
Corynebacterium jeikeium	3 (1.6)	7 (4.4)	8 (5.3)	17(8.9)	9 (3.8)	44 (4.7)
Corynebacterium propinquum	5 (2.6)	5 (3.2)	9 (5.9)	3 (1.6)	6 (2.5)	28 (3.0)
Corynebacterium afermentans	10 (5.2)	2 (1.3)	1 (0.7)	2 (1.0)	4 (1.7)	19 (2.0)
Corynebacterium urealyticum	6 (3.1)	3 (1.9)	3 (2.0)	-	2 (0.8)	14 (1.5)
Corynebacterium Group G	2 (1.0)	3 (1.9)	4 (2.6)	2 (1.0)	1 (0.4)	12 (1.3)
Corynebacterium diphtheriae	4 (2.1)	-	4 (2.6)	2 (1.0)	1 (0.4)	11 (1.2)
Corynebacterium argentoratense	-	-	2 (1.3)	3 (1.6)	-	5 (0.5)
Corynebacterium pseudotuberculosis	1(0.5)	-	-	-	-	1 (0.1)
Corynebacterium accolens	-	-	1 (0.7)	-	-	1 (0.1)
Others[b]	26 (13.5)	1 (0.6)	2 (1.3)	-	-	29 (3.2)
Total	192 (100)	158 (100)	152 (100)	191 (100)	239 (100)	932 (100)

a: 20 strains in 2000 and six in 2001 and no isolates in the following years; *b*: includes species previously classified as Group I (1 isolate), Group A (2), Group B (12), Group F (7), Group F1 (3), Group GI (3) and Group G2 (1).

frequent sources of *C. amycolatum* strains. *C. pseudodiphtheriticum* strains were mainly isolated from the lower and upper respiratory tract and *C. jeikeium* from the intravenous sites and skin lesions. The antimicrobial spectra of microorganisms are exhibited in Table IV.

Clinical data of the 315 cancer patients with fever and/or other signs of infection were retrieved. The epidemiological characteristics, the topographies that were involved and the factors that predisposed cancer patients to corynebacteria infection are depicted in Table V. The findings were interpreted as infection in 86% of patients and specific treatments were initiated. *Corynebacterium* strains were observed as the only microorganism in 46% of the cultures. Bacterial colonisation was confirmed in 44 cases (14%), 35 of which originated in the respiratory tract (25 rhinosinusal, 3 lower respiratory and 7 upper respiratory), four cases were in central venous catheters (Maki method negative), two cases were in skin/tumours and one case was in a surgical wound; two cases were isolated from the digestive tract.

The main underlying malignant diseases were 66.7% adult solid tumours, followed by tumours of the central nervous system and paediatric solid tumours (10.8% and 9.2%, respectively). The patients from surgical wards (71.1%) were previously submitted to head and neck (25.1%), gynaecological (13.3%) and abdominal-pelvic (9.5%) surgeries. Surgical wound infections comprised 32.7% of these patients. The patients from clinical wards were from pediatrics (28.9%), oncology (12.1%) and haematology (7.3%). The majority of patients had been hospitalised (77.1%) and exposed to a hospital environment for a long term (median = 13 days).

The clinical-epidemiological characteristics of 315 patients with 30-day mortality involved a uni and multivariate analysis of risk factors for corynebacteria-associated infection (Table IV).

DISCUSSION

Identification of non-diphtheria bacteria at the species level is often problematic. Recent advances in identification have shown that the genus exhibits considerable taxonomic complexity and the phenotypic markers that have been used in the past for its identification can be ambiguous. Even when sent to a reference laboratory, 30-50% of coryneform bacteria isolates cannot be reliably identified at the species level. Consequently, there is a low rate of identification from clinical isolates (Schiff et al. 2004).

Variations in the occurrence of *Corynebacterium* species during the course of the study were probably due to improvements in the taxonomy and laboratory diagnosis of the genus *Corynebacterium*. The substantial increase in the number of *C. amycolatum* isolates was partially due to the disappearance of *C. xerosis* and the significant reduction of *C. striatum* and *C. minutissimum,* which reflects the progress in the diagnosis of *Corynebacterium* species and consequently, the variability in antimicrobial sensitivity patterns. The former *Corynebacterium* CDC F-2 and CDC I-2 groups have been reclassified as *C. amycolatum*.

Due to imprecise diagnosis at the species level, data from the first three years (507 strains) were not included in the analysis of the bacteriological and clinical characteristics of cancer patients with corynebacteriosis.

TABLE III

Strains of nondiphtherial *Corynebacterium* species isolated from varied clinical sources in cancer patients (n = 315)

Organism	Total n (%)	Respiratory tract infections	Surgical wound infections	Urinary tract infections	Intravenous sites		Skin/ tumour	Rhino sinusal	Misc
		U/L		U/L	Catheter	Blood			
Corynebacterium amycolatum	230 (73.0)	7/48	56	38/0	19	4	9/22	3	24
Corynebacterium pseudodiphtheriticum	39 (12.4)	3/11	4	0/0	0	0	0/1	19	1
Corynebacterium jeikeium	13 (4.1)	1/1	2	0/0	4	1	1/3	0	0
Corynebacterium minutissimum	12 (3.8)	0/2	1	0/3	1	0	2/1	0	2
Corynebacterium propinquum	8 (2.5)	0/3	0	1/0	1	0	0/0	2	1
Corynebacterium striatum	6 (1.9)	1/3	2	0/0	0	0	0/0	0	0
Corynebacterium afermentans	3 (1.0)	0/1	0	0/0	1	1	0/0	0	0
Corynebacterium argentoratense	2 (0.7)	0/0	0	0/0	1	1	0/0	0	0
Corynebacterium Group G	1 (0.3)	0/0	0	0/0	0	0	0/0	1	0
Corynebacterium urealyticum	1 (0.3)	0/0	0	1/0	0	0	0/0	0	0
Total	315 (100)	12/69	65	40/3	27	7	12/27	25	28

L: lower; Misc: miscellaneous [genital tract: 8 (*C. amycolatum*), gastrointestinal tract: 5 [*C. amycolatum*: 4; *C. minutissimum*: 1), nephrostomy: 3 (*C. amycolatum*), eye: 3 (*C. amycolatum*: 2; *C. propinquum*: 1), bone: 2 (*C. amycolatum*), oropharynx: 2 (*C. amycolatum*: 1; *C. minutissimum*: 1), abscess: 2 (*C. amycolatum*); sinus: 2 (*C. amycolatum*: 1; *C. pseudodiphtheriticum*: 1), biopsy: 1 (*C. amycolatum*); U: upper.

Many non-diphtheria corynebacteria-caused access infections can be effectively treated by antibiotics and local care. Susceptibility testing of corynebacteria is highly recommended to establish a specific therapy (CLSI 2007). Except for the unvarying activity of vancomycin against corynebacteria, the variability in resistance to other classes of antimicrobial agents emphasises the need for the continuous surveillance of their resistance patterns. Resistance to oxacillin was observed for all corynebacteria species isolated from cancer patients at INCA. The data emphasise the relevance of susceptibility testing of *Corynebacterium* isolates during the diagnosis and treatment of infections in cancer patients.

A growing number of reports have confirmed the importance of *C. amycolatum* in the aetiology of a variety of infectious processes. In the reviewed literature, many *C. amycolatum* clinical isolates were at first identified in different laboratories as *C. xerosis* (Funke et al. 1996) and *C. minutissimum* (Zinkernagel et al. 1996). After the mid-1990s, with the advent of the taxonomical characterisation of *C. amycolatum*, this species has been described as a causal agent of infections with high morbidity and mortality rates. Multi-resistant *C. amycolatum* strains were isolated from patients with septicaemia (Berner et al. 1997, de Miguel et al. 1999, Camello et al. 2003), septic arthritis after vascular transplants (Clarke et al. 1999), cardiac electrode implantation (Vaneechoutte et al. 1998), infections in vascular prostheses and open fractures (von Graevenitz et al. 1998), peritonitis in patients undergoing peritoneal dialysis (Chiu et al. 2005), infectious endocarditis accompanied by aorto-atrial fistula (Daniels et al. 2003), nosocomial endocarditis (Knox & Holmes 2002) and septicaemia in leukemic patients (de Miguel et al. 1999). These reports drew attention to the clinical significance of *C. amycolatum* strains in different clinical materials (Esteban et al. 1999).

The clinical characteristics of the patients with favourable outcomes and documented corynebacteriosis could not be compared with those patients who died (controls). Due to the nature of the present research, which was a retrospective study, we could not investigate the pathogenicity of the isolates in relation to 30-day mortality in cancer patients. For the same reason, it was impossible to establish prognostic factors in these patients considering their descriptive characters. Another limitation of this study was the exclusion of isolates from 2000-2002 in the complete analysis due to major changes in taxonomical classification. Nevertheless, the sampling procedures adopted in this study allowed us to detect some patterns and characteristics of the population under investigation.

The univariate and multivariate analyses showed that hospitalised patients had a six-fold greater risk (OR = 5.5, 95% CI = 1.15-26.30 p = 0.033) related to 30-day mortality. Also of statistical significance were patients bedridden for longer than 50% of the day with neoplastic disease in progress or diabetes mellitus.

The study revealed that corynebacteria are increasingly being recognised as a cause of infections in cancer patients. These organisms have been underreported, but

TABLE IV

Comparison of antimicrobial spectra of *Corynebacterium* species (315 patients/425 strains)

Species (samples)	Vancomycin	Amikacin	Tobramycin	Cefazolin	Ceftriaxone	Ceftazidime	Cefepime	Imipenem	Penicillin G	Ampicillin	Oxacillin	Ciprofloxacin	Ofloxacin	Erythromycin	Chloramphenicol
Corynebacterium amycolatum (292)	+	+	+	+	+	0	+	+	+	+	0	±	±	±	±
Corynebacterium pseudodiphtheriticum (53)	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+
Corynebacterium jeikeium (26)	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corynebacterium minutissimum (20)	+	+	+	+	+	0	+	+	±	±	0	±	±	±	±
Corynebacterium striatum (11)	+	+	+	+	+	0	+	+	+	+	0	±	±	+	0
Corynebacterium propinquum (9)	+	+	+	+	+	±	+	+	+	+	±	+	+	±	+
Corynebacterium afermentans (6)	+	+	+	+	+	±	+	+	±	±	±	+	+	±	+
Corynebacterium Group G (3)	+	+	+	+	+	0	+	+	+	+	0	+	+	+	+
Corynebacterium argentoratense (3)	+	+	+	+	+	±	+	+	+	+	0	+	+	+	+
Corynebacterium urealyticum (2)	+	±	±	±	±	±	±	±	±	±	±	+	+	0	0

+: > 60%; ±: 30-60%; 0: < 30% (% sensitivity).

TABLE V

Risk factors to mortality related to *Corynebacterium* spp infection in 315 cancer patients

Predictors	Patients who survived n = 271 (%)	Patients who died n = 44 (%)	OR (95% CI)	p value
Age < 65 years	197 (72.7)	29 (65.9)	1.38 (0.66-2.85)	0.354
Age ≥ 65 years	74 (27.3)	15 (34.1)		
Gender (female)	154 (56.8)	18 (40.9)	0.52 (0.27-1.00)	0.049
Central venous catheter used	31 (11.4)	7 (15.9)	1.46 (0.60-3.56)	0.398
Central venous catheter infection	22 (8.1)	6 (13.6)	1.78 (0.68-4.69)	0.251[a]
Corynebacterium minutissimum	7 (2.6)	5 (11.4)	4.83 (1.46-15.98)	0.016[a]
Hospitalized patients	201 (74.2)	42 (95.5)	7.31 (1.72-31.00)	0.002
Infection (*versus* colonization)	232 (85.6)	39 (88.6)	1.31 (0.48-3.53)	0.591
Diabetes mellitus (plasma glucose > 200 mg/dL)	19 (7.0)	8 (18.2)	2.94 (1.20-7.22)	0.036[a]
Renal insufficiency (creatinine > 2.0 mg/dL)	20 (7.4)	10 (22.7)	3.69 (1.59-8.54)	0.004[a]
Hematopoietic stem cell transplantation	5 (1.8)	1 (2.3)	1.23 (0.14-0.84)	0.598[a]
Co-infection	102 (37.6)	26 (59.1)	2.39 (1.25-4.58)	0.007
Undergo surgery	156 (57.6)	16 (36.4)	0.42 (0.21-0.81)	0.009
Surgical wound infection	63 (23.2)	2 (4.5)	0.15 (0.03-0.66)	0.004
COPD	14 (5.2)	2 (4.5)	0.87 (0.19-3.98)	1.000[a]
Confined to bed more than 50% of waking hours	42 (15.5)	20 (45.5)	4.54 (2.30-8.95)	< 0.001
Solid tumors	240 (88.6)	34 (77.3)	0.43 (0.19-0.97)	0.039
Length of stay > 13 days	165 (60.9)	27 (61.4)	1.02 (0.53-1.96)	0.952
Neutropenia (< 1000 µL/cells)	14 (5.2)	4 (9.1)	1.83 (0.57-5.85)	0.294[a]
Isolated in pure culture	125 (46.1)	20 (45.5)	0.97 (0.51-1.84)	0.934
Isolated in association	23 (8.5)	6 (13.6)	1.70 (0.65-4.45)	0.266[a]
Isolated in co-infection not related	22 (8.1)	5 (11.4)	1.45 (0.51-4.05)	0.559[a]
Under nutrition (weight loss > 10%)	43 (15.9)	13 (29.5)	2.22 (1.07-4.59)	0.028
Body temperature > 38°C	19 (7.0)	4 (9.1)	1.32 (0.42-4.10)	0.543[a]
Mucositis (NCI scale > 3)	4 (1.5)	0 (0.0)	0.85 (0.82-0.89)	1.000[a]

↳

Predictors	Patients who survived n = 271 (%)	Patients who died n = 44 (%)	OR (95% CI)	p value
Karnofsky index < 70%	87 (32.1)	28 (63.6)	3.70 (1.90-7.19)	< 0.001
Corticotherapy (prednisone > 40 mg/day)	27 (10.0)	3 (6.8)	0.66 (0.19-2.28)	0.781[a]
Arterial hypotension (systolic < 90 mm/hg)	18 (6.6)	10 (22.7)	4.13 (1.76-9.69)	0.002[a]
Graft-*versus*-host disease	2 (0.7)	1 (2.3)	3.12 (0.27-35.24)	0.364[a]
Isolated in persistent infection	33 (12.2)	8 (18.2)	1.60 (0.68-3.74)	0.272
Radiotherapy	94 (34.7)	12 (27.3)	0.70 (0.34-1.43)	0.334
Tracheotomy	48 (17.7)	13 (29.5)	1.94 (0.94-3.99)	0.065
Progressive disease	108 (39.9)	33 (75.0)	4.52 (2.19-9.34)	< 0.001
Chemotherapy	76 (28.0)	13 (29.5)	1.07 (0.53-2.16)	0.837
Others *Corynebacterium* growing in association	58 (22.1)	13 (30.2)	1.52 (0.74-3.11)	0.244
Ascites	5 (1.8)	0 (0.0)	0.85 (0.82-0.89)	1.000[a]
Tumoral embolization	1 (0.4)	1 (2.3)	6.27 (0.38-102.27)	0.260[a]
Pulmonary thrombo-embolism	11 (4.1)	2 (4.5)	1.12 (0.24-5.25)	0.700[a]
Instrumentalization	14 (5.2)	3 (6.8)	1.34 (0.37-4.87)	0.716[a]

a: Fisher exact test

TABLE VI

Multivariate analysis of independent risk factors for 30-day mortality

Variable	OR	95% CI		p value
Hospitalized patients	5.500	1.150	26.304	0.033
Confined to bed more than 50% of waking hours	3.621	1.173	11.179	0.025
Corynebacterium minutissimum	3.560	0.681	18.625	0.133
Progressive disease	3.167	1.346	7.453	0.008
Diabetes mellitus (plasma glucose > 200 mg/dL)	3.106	1.015	9.504	0.047
Renal insufficiency (creatinine > 2.0 mg/dL)	2.710	0.951	7.722	0.062
Arterial hypotension (systolic < 90 mm/hg)	2.429	0.851	6.934	0.097
Undernutrition (weight loss > 10%)	1.842	0.710	4.779	0.209
Isolated in co-infection no related	1.260	0.572	2.773	0.566
Solid tumors	0.762	0.268	2.169	0.610
Gender (male)	0.656	0.295	1.457	0.301
Undergo surgery	0.441	0.192	1.012	0.053
Karnofsky < 70	0.835	0.287	2.425	0.740

they may account for approximately 4% of the cases of infection in patients presenting solid tumours. The data reflects a tendency towards infection or colonisation by *Corynebacterium* in cancer patients who have undergone surgical procedures and a long subsequent hospitalisation period. From the study data, we also emphasise the need for the rigorous identification of *Corynebacterium* species isolates from different sites, especially of invasive strains in patients with clinical conditions of persistent fever and no other attributable site of infection. *C. jeikeium* infection occurs primarily in neutropenic patients who have used venous catheters (Rozdzinski et al. 1991), while *C. amycolatum* infection appears mainly in patients with solid tumours.

REFERENCES

Barreau C, Bimet F, Kiredjian M, Rouillon N, Bizet C 1993. Comparative chemotaxonomic studies of mycolic acid-free coryneform bacteria of human origin. *J Clin Microbiol 31*: 2085-2090.

Berghmans T, Sculier JP, Klastersky J 2003. A prospective study of infections in lung cancer patients admitted to the hospital. *Chest 124*: 114-120.

Berner R, Pelz K, Wilhelm C, Funke A, Leititis JU, Brandis M 1997. Fatal sepsis caused by *Corynebacterium amycolatum* in a premature infant. *J Clin Microbiol 35*: 1011-1012.

Bodey GP 1995. Emerging antimicrobial-resistant pathogens in the immunocompromised host. *Curr Opin Infect Dis 8*: 411-414.

Boucher HW, Wennersten CB, Eliopoulos GM 2000. *In vitro* activi-

ties of the glycylcycline GAR-936 against gram-positive bacteria. *Antimicrob Agents Chemother 44*: 2225-2229.

Brandenburg AH, van Belkum A, van Pelt C, Bruining HA, Mouton JW, Verbrugh HA 1996. Patient-to-patient spread of a single strain of *Corynebacterium striatum* causing infections in a surgical intensive care unit. *J Clin Microbiol 34*: 2089-2094.

Camello TCF, Mattos-Guaraldi AL, Formiga LCD, Marques EA 2003. Nondiphtherial *Corynebacterium* species isolated from clinical specimens of patients in a University Hospital, Rio de Janeiro, Brazil. *Brazilian J Microbiol 34*: 39-44.

Camello TCF, Souza MC, Martins CAS, Damasco PV, Marques EA, Pimenta FP, Pereira GA, Hirata Jr R, Mattos-Guaraldi AL 2009. *Corynebacterium pseudodiphtheriticum* isolated from relevant clinical sites of infection: a human pathogen overlooked in emerging countries. *Lett App Microbiol 48*: 458-464.

CDC - Center for Disease Control and Prevention 1992. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *Morb Mortal Wkly Rep Recomm Rep 41*: 1-19.

Chiu YL, Wu VC, Wun KD, Hsueh PR 2005. Recurrent peritonitis caused by *Corynebacterium amycolatum* in a patient undergoing continuous ambulatory peritoneal dialysis. *Clin Nephrol 63*: 241-242.

Clarke R, Qamruddin A, Taylor M, Panigrahi H 1999. Septic arthritis caused by *Corynebacterium amycolatum* following vascular graft sepsis. *J Infect 38*: 126-127.

CLSI - Clinical and Laboratory Standards Institute 2007. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria. *Approved Standard Document M45-A*, CLSI, Wayne, p. 4-6.

Daniels C, Schoors D, Van Camp G 2003. Native valve endocarditis with aorta-to-left atrial fistula due to *Corynebacterium amycolatum*. *Eur J Echocardiogr 4*: 68-70.

de Miguel I, Rodriguez E, Martin AM 1999. *Corynebacterium amycolatum*: sepsis in hematologic patients. *Enferm Infecc Microbiol Clin 17*: 340-341.

Dobbs TE, Patel M, Waites KB, Moser SA, Stamm AM, Hoesley CJ 2006. Nosocomial spread of *Enterococcus faecium* resistant to vancomycin and linezolid in a tertiary care medical center. *J Clin Microbiol 44*: 3368-3370.

Esteban J, Nieto E, Calvo R, Fernandez-Robals R, Valero-Guillen PL, Soriano F 1999. Microbiological characterization and clinical significance of *Corynebacterium amycolatum* strains. *Eur J Clin Microbiol Infect Dis 18*: 518-521.

Freney J, Duperron MT, Courtier C, Hansen W, Allard F, Boeufgras JM, Monget D, Fleurette J 1991. Evaluation of API Coryne in comparison with conventional methods for identifying coryneform bacteria. *J Clin Microbiol 29*: 38-41.

Funke G, Bernard KA 2003. Coryneform gram-positive rods. In: PR Murray, EJ Barron, JH Jorgensen, MA Pfaller, RH Yolken, *Manual of Clinical Microbiology*, 8th ed., ASM Press Washington DC, p. 472-501.

Funke G, Punter V, von Graevenitz A 1996. Antimicrobial susceptibility patterns of some recently established coryneform bacteria. *Antimicrob Agents Chemother 40*: 2874-2878.

Funke G, von Graevenitz A, Clarridge JE, Bernard KA 1997. Clinical microbiology of coryneform bacteria. *Clin Microbiol Rev 10*: 125-159.

Furtado GH, Mendes RE, Pignatari AC, Wey SB, Medeiros EA 2006. Risk factors for vancomycin-resistant *Enterococcus faecalis* bacteremia in hospitalised patients: an analysis of two case-control studies. *Am J Infect Control 34*: 447-451.

Garner IS, Iarvis WR, Emeri TG, Horan TC, Hughes IM 1988. CDC definitions for nosocomial infections. *Am J Infect Contr 16*: 128-140.

Gilbert DN, Moellering RC, Eliopoulos GM, Sande MA 2006. Comparison of antimicrobial spectra. In DN Gilbert et al. *The Sanford guide to antimicrobial therapy*, 36th ed., Antimicrobial Therapy Inc, Hyde Park, p. 53-55.

Hughes WT, Flynn PM, Williams BG 1996. Nosocomial infection in patients with neoplastic diseases. In: CG Mayall, *Hospital epidemiology and infection control*, Williams & Wilkins, Baltimore, p. 618-631.

Knox KL, Holmes AH 2002. Nosocomial endocarditis caused by *Corynebacterium amycolatum* and other nondiphtheriae corynebacteria. *Emerg Infect Dis 8*: 97-99.

Lagrou K, Verhaegen J, Janssens M, Wauters G, Verbist L 1998. Prospective study of catalase-positive coryneform organisms in clinical specimens: identification, clinical relevance and antibiotic susceptibility. *Diagn Microbiol Infect Dis 30*: 7-15.

Maki DG, Weise CE, Sarafin HW 1977. A semiquantitative culture method for identifying intravenous-catheter-related infection. *N Engl J Med 296*: 1305-1309.

Martinez-Martinez L, Ortega MC, Suarez AI 1995. Comparison of *E-test* with broth microdilution and disk diffusion for susceptibility testing of coryneform bacteria. *J Clin Microbiol 33*: 1318-1321.

Meyer DK, Reboli AC 2005. Others Corynebacteria and *Rhodococcus*. In: GL Mandell, R Dolin, JE Bennet, *Principles and practice of infectious diseases,* Churchill-Livingstone, New York, p. 2465-2478.

Osterwalder B, Frei R, Gratwohl A, Reber H, Speck B 1986. Antibiotic-resistant Corynebacteria - a new problem of infection in immunosuppressed patients. *Schweiz Med Wochenschr 116*: 880-884.

Oteo J, Aracil B, Alós JI, Gómez-Garcés JL 2001. Bacteriemias significativas por *Corynebacterium amycolatum*: un patógeno emergente. *Enferm Infec Microbiol Clin 19*: 103-106.

Otsuka Y, Ohkusu K, Kawamura Y, Baba S, Ezaki T, Kimura S 2006. Emergence of multidrug-resistant *Corynebacterium striatum* as a nosocomial pathogen in long-term hospitalized patients with underlying diseases. *Diag Microbiol Infect Dis 54*: 109-114.

Rozdzinski E, Kern W, Schmeiser T, Kurrle E 1991. *Corynebacterium jeikeium* bacteraemia at a tertiary care center. *Infection 19*: 201-204.

Schiff H, Mücke C, Lang SM 2004. Exit-site infections by non-diphtheria corynebacteria in CAPD. *Perit Dial Int 24*: 454-459.

Schoen C, Unziker C, Stuhler G, Elias J, Einsele H, Grigoleit GU, Abele-Horn M, Mielke S 2009. Life-threatening infection caused by daptomycin-resistant *Corynebacterium jeikeium* in a neutropenic patient. *J Clin Microbiol 47*: 2328-2331.

Sierra JM, Martinez-Martinez L, Vazquez F, Giralt E, Vila J 2005. Relationship between mutations in the *gyrA* gene and quinolone resistance in clinical isolates of *Corynebacterium striatum* and *Corynebacterium amycolatum*. *Antimicrob Agents Chemother 49*: 1714-1719.

Thomson Jr RB 2007. Specimen collection, transport and processing: bacteriology. In: PR Murray, EJ Baron, JH Jorgensen, ML Landry, MA Pfaller, *Manual of clinical microbiology,* ASM Press, Washington DC, p. 485-514.

van der Lelie H, Leverstein-Van Hall M, Mertens M, van Zaanen HC, van Oers RH, Thomas BL, von dem Borne AE, Kuijper EJ 1995. *Corynebacterium* CDC group JK (*Corynebacterium jeikeium*) sepsis in haematological patients: a report of three cases and a systematic literature review. *Scand J Infect Dis 27*: 581-584.

Vaneechoutte M, De Bleser D, Claeys G, Verschraegen G, De Baere T, Hommez J, Devriese LA, Riegel P 1998. Cardioverter-lead electrode infection due to *Corynebacterium amycolatum*. *Clin Infect Dis 27*: 1553-1554.

von Graevenitz A, Frommelt L, Punter-Streit V, Funke G 1998. Diversity of coryneforms found in infections following prosthetic joint insertion and open fractures. *Infection 26*: 36-38.

Weiss K, Laverdière M, Rivest R 1996. Comparison of antimicrobial susceptibilities of *Corynebacterium* species by broth microdilution and disk diffusion methods. *Antimicrob Agents Chemother 40*: 930-933.

Zalas P, Mikucka A, Gospodarek E 2004. Antibiotic sensitivity of *Corynebacterium amycolatum*. *Med Dosw Mikrobiol 56*: 327-334.

Zinkernagel AS, von Graevenitz A, Funke G 1996. Heterogeneity within *Corynebacterium minutissimum* strains is explained by misidentified *Corynebacterium amycolatum* strains. *Am J Clin Pathol 106*: 378-383.