Multidrug resistant *Pseudomonas aeruginosa* infection in children undergoing chemotherapy and hematopoietic stem cell transplantation

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ABSTRACT

Pseudomonas aeruginosa is one leading gram-negative organism associated with nosocomial infections. Bacteremia is life-threatening in the immunocompromised host. Increasing frequency of multi-drug-resistant (MDRPA) strains is concerning. We started a retrospective survey in the pediatric hematology oncology Italian network. Between 2000 and 2008, 127 patients with *Pseudomonas aeruginosa* bacteremia were reported from 12 centers; 31.4% of isolates were MDRPA. Death within 30 days of a positive blood culture occurred in 19.6% (25/127) of total patients; in patients with MDRPA infection it occurred in 35.8% (14/39). In the multivariate analysis, only MDRPA had significant association with infection-related death. This is the largest series of *Pseudomonas aeruginosa* bacteremia cases from pediatric hematology oncology centers. Monitoring local bacterial isolates epidemiology is mandatory and will allow empiric antibiotic therapy to be tailored to reduce fatalities.

Key words: *Pseudomonas aeruginosa*, infection-related, local bacterial.

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Introduction

*Pseudomonas aeruginosa (*PA) is an invasive Gram-negative bacterial pathogen, responsible for a wide range of clinical manifestations, including pneumonia, urinary tract infection, and bacteremia, in the immunocompetent patient. In the immune compromised host, PA may behave as an opportunistic pathogen, causing severe invasive diseases, and represents one of the most severe nosocomial pathogens.[1-5] In a European survey of intensive care units, PA was the most frequent bacterial isolate accounting for 29% of the total isolates;[6] furthermore, its multi-resistance also represents an increasing problem.[7-8] The low permeability of its cell wall, together with mutations leading to antibiotic-resistance via overexpression of efflux pumps, decreased expression of porine, or mutations in quinolone targets,[8] make PA a pathogen with high propensity to become multi-resistant to antibiotic therapy.[9] Multi-resistant strains may be responsible for nosocomial outbreaks, especially among populations at risk such as patients with cancer or cystic fibrosis.[8-11] In a retrospective analysis of patients with PA bacteremia at a large university hospital over a 10-year period, 51 out of 358 cases (14.2%) were multi-resistant to ciprofloxacin, ceftazidime, imipenem, gentamicin, and piperacillin.[12] The impact of multidrug resistant PA (MDRPA) on mortality and costs to the health service is illustrated by several studies.[7,13,14] Patients with MDRPA had significantly higher in-hospital mortality than those with more susceptible strains (67% *vs.* 23%; P=0.001). Reported mortality rates in adults with MDRPA range from 20% to 70%, depending on patient- and infection-related factors.[15]

Inadequate antimicrobial treatment[16] has been proposed to be the most important risk factor for mortality among hospitalized patients with bacteremia. Appropriate dosing and

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intervals of administration, were found to be important determinants of clinical outcome that the physician can directly influence.[17]

In spite of the clinical importance of bacteremia due to PA, only few data are available on MDRPA in children.[1-3,18] Therefore, we performed a retrospective study among pediatric hematology-oncology centers of the *Associazione Italiana Ematologia Oncologia Pediatrica* (AIEOP). The aim of the study was to evaluate all cases of PA bloodstream infection to determine their characteristics, and in particular the proportion and outcome of cases associated with multi-resistant strain.

Design and Methods

Study design

AIEOP includes about 90% of Italian centers caring for patients with childhood cancer.[19] A total of 12 AIEOP centers (Catania, Firenze, Genova, Milano San Raffaele, Modena, Monza, Padova, Palermo, Perugia, Roma Bambino Gesù, Torino, and Trieste) agreed to retrieve from the bacteriology data-base all cases of PA bacteremia diagnosed in children receiving antineoplastic chemotherapy or hematopoietic stem cell transplantation (HSCT) between January 2000 and October 2008. Data on patient demographics, cancer type, chemotherapy administered, as well as details on strain isolates and antibiotic *in vitro* sensitivity, clinical presentation, treatment and outcome (death within 30 days of a positive blood culture) were retrospectively retrieved from data bases and charts, and collected on specific forms. Only the first PA bacteremia from each patient during the study period was included. Given the nature of the data collection, IRB approval was not required. The presence of PA bacteremia was defined by the isolation of this pathogen in a blood culture specimen.

For the purposes of the present study, data regarding susceptibility to antibiotics were retrieved for piperacillin, ceftazidime or cefepime, imipenem or meropenem, amikacin, ciprofloxacin, cotrimoxazole. Over the study period, the methods of evaluating antibiotic susceptibility shifted at different time points, according to individual center organization, from the Kirby-Bauer to the automated blood culture systems (VITEK 2 – BioMérieux, E-test, BACTECR or BacT/ALERTR) with calculation of minimum inhibitory concentrations. Thus, data about susceptibility to the different antibiotics were categorized as "susceptible" or "resistant". All cases indicated as intermediate, were re-classified as resistant.

Multidrug resistant PA (MDRPA) was defined in the presence of resistance to at least three of the following antibiotic classes: penicillin, cephalosporin, carbapenem, aminoglycoside, cotrimoxazole, and fluoroquinolones.[20] Initial empirical antimicrobial therapy was defined as "adequate" if the initial antibiotics, which were administered within six hours after acquisition of a blood culture sample, included at least one antibiotic that was active *in vitro* against the causative microorganisms, and when the dosage and route of administration conformed with current medical standards.[21] "Inadequate" initial antimicrobial therapy referred to the administration of antimicrobial agents to which the causative microorganisms were resistant *in vitro* or to the lack of an antimicrobial therapy for a known causative pathogen.[21] Infection-related mortality was recorded as event.

Statistical analysis

Continuous variables such as age were summarized as median, range and interquartile difference; qualitative variables were summarized in frequency tables describing absolute numbers and percentage. Univariate associations between MDRPA infection, age, gender, neutropenia, cancer type, were estimated by univariate Odds Ratio, and reported with 95% confidence intervals. The impact of MDRPA on infection-related mortality was evaluated by adjusting for presence of neutropenia, gender, and type of cancer, and evaluated using multiple logistic regression models. In all analyses a probability value less than 0.05 was considered statistically significant. Data were analyzed using the statistical software STATA 10.0 (Stata Co.,College Station, TX, USA).

Results

Patients' characteristics

Main features of the 127 patients are summarized in Table 1. A total of 127 patients with PA bacteremia, documented by isolates from blood, were reported from the 12 participating centers. Of them, 90 had received chemotherapy alone, while 37 also underwent HSCT.

Antibiotic susceptibility

Three patients were excluded from the analysis of the *in vitro* susceptibility to antibiotics because of insufficient data. Table 2 reports the results of *in vitro* antibiotic susceptibility tests of the 124 isolated PA strains. Since not all the antibiotics were tested in all strains, the number of isolates tested was slightly different for each antibiotic. Overall, 27% of strains were resistant to an anti-pseudomonas penicillin, and 33% were resistant to an anti-pseudomonas cephalosporin. The lowest proportions of resistant strains were observed for ciprofloxacin (18%) and amikacin (11%). An MDRPA was identified in 39 (31%) strains, with one single strain which was sensitive only to one antibiotic (colistin).

Patients' outcome

Infection-related mortality occurred in a total of 25 (19.6%) patients with PA bacteremia; when considering multidrug resistance, death within 30 days of a positive blood culture occurred in 14 of 39 patients with bacteremia caused by MDRPA (35.8%), *versus* 11 of 88 patients with non-MDR isolates (12.5%).

From the unadjusted analysis the odds of death in patients with MDRPA bacteremia was 3.92 (95% CI 1.42-10.78, P=0.002) times higher than that observed in non-MDRPA. After adjusting for gender, type of cancer and presence of neutropenia, the estimated odds ratio was still significant and increased to 4.3 (95% CI 1.67-11.07, P=0.002).

A total of 37 patients (29%) developed PA during HSCT. Of them 6 died, 3 with MPA strain infection. Among the 90 patients treated with chemotherapy only, 19 died (21%), including 12 MPA. The risk of either MDRPA or mortality was not higher in patients undergoing HSCT.

Bacteremia associated with perineal involvement, documented by the presence of swelling, abscess or round ulcers with necrotic center (*Ecthyma gangrenosum*), was reported in 18 patients (14%) of whom 6 (33%) died; 4 of these 6 had an MPA isolate.

Details on initial antibiotic therapy and *in vitro* sensitivity were available for 71 (56%) patients; 63 (89%) of them received appropriate empirical therapy. However,

11 (17%) died. Of the remaining 8 (11%) patients receiving inappropriate empirical therapy, 4 died.

Discussion and Results

Recent advances in the cure rate of many types of childhood cancer have been achieved by intensification of chemotherapy (allowing better disease control and prevention of relapses) and treatment of infections. PA bacteremia remains a serious complication of childhood cancer treatment. In this paper we provide the largest series of children with cancer and PA bacteremia.

The most relevant, novel information is that multidrug resistant strains of *P. aeruginosa* accounted for 30% of the total in this large series of unselected, consecutive patients reported from 12 pediatric hematology-oncology centers. This proportion is definitely higher than the 17.2% reported by the 2007 Annual EARRS report.[22] The population we describe includes children treated with intensive chemotherapy and, in 29% of cases, also with HSCT.

Among factors potentially affecting infection-related survival of patients with PA bacteremia, only multidrug resistance had independent value in the multivariate regression analysis. In fact, 35% of patients with multidrug resistant isolates died of PA infection; a proportion which is significantly higher than that of patients with non-multidrug resistant strains.

Although our data point to an independent prognostic value of MDRPA infection compared to non-MDRPA cases, our findings could also be consistent with the possibility that MDRPA infection is a marker for some other variables potentially associated with mortality. Among potential confounders, length of time with cancer, past infection or failed treatment for infection, past antibiotic exposure, type of infection, depth of immunosuppression, unit at which the patient is receiving treatment, or many others could be included. We can only try to address some of them: since this is a pediatric cohort, factors such as comorbidities or long-lasting colonization, possibly observed in some adult patients, would not be frequently observed. In contrast to reports in adults, current therapeutic approaches in pediatric hematology-oncology centers in our cooperative group do not include antibacterial prophylaxis.

Since the methods used for identification of *in vitro* sensitivity to antibiotics in the participating centers varied over the years, in this study we decided to categorize the results as sensitive or resistant. Cases with intermediate sensitivity were grouped together with those with resistant sensitivity. We checked whether this may have modified the evaluation of the resistant cases: only one patient in the MDRPA group had an intermediate sensitivity to amikacin (the drug chosen for treatment), and unfortunately had a fatal outcome.

The proportion of isolates resistant to individual antibiotics in our series appears to be 20% or more, in keeping with that reported by the EARRS,[22] with the only exception of amikacin, which turned to be resistant in 11%.

Are we able to predict a higher risk for MPA in children treated for cancer? While patients undergoing HSCT apparently were not at a higher risk for such an unfavorable event, it may be remarkable that one-third of patients with perineal localization of the infection had a fatal outcome. Of the 8 patients who developed a septic shock, all had perineal involvement and 3 had MPA strains. Since it is well known that perineal infection may persist beyond the healing of PA bacteremia[23] it may be important to know that a patient bears such a colonization; whether reactivation of such a life-threatening pathogen has to be considered a real risk, and thus an adjustment of the therapeutic strategy should be considered, must still be assessed.

Combined resistance is the dominant threat imposed by invasive PA in Europe.[22] Since resistance in *P. aeruginosa* emerges readily during antibiotic treatment, the time when blood cultures are taken is crucial as any isolate collected after prolonged exposure with antimicrobial chemotherapy will predictably be a multi-resistant phenotype. We tried to address the efficacy of current empirical antibiotic therapy in our setting. At the time *in vitro* sensitivity of the isolate became available, 88% of patients

Table 1. Main presenting features of the 127 study patients.

Category	Total N (%)	MDRPA N (%)	Non-MDRPA N (%)
Patients	127	39 (30.7)	88 (69.3)
Age (years)			
Median	5.5	7.3	4.3
Range	0.04 - 20.5	1.2 - 20.5	0.04 - 18
Quartiles	2.5 ; 12	2.3 ; 13.7	2.4 ; 9.3
Gender			
Female	60 (47.3)	15 (11.8)	45 (35.5)
Male	67 (52.7)	24 (18.9)	43 (33.8)
Neutropenia (degree)			
<100/mm^3	50 (39.4)	17 (13.4)	33 (26.0)
100-500/mm^3	31 (24.4)	5 (3.9)	26 (20.5)
500-1000/mm^3	13 (10.2)	2 (1.6)	11 (8.6)
>1000/mm^3	6 (4.7)	0 (0)	6 (4.7)
Data not available	27 (21.3)	15 (11.8)	12 (9.5)
Diagnosis			
Leukemia/lymphoma	92 (72.4)	28 (22)	64 (50.4)
Solid tumor	24 (18.4)	7 (5.5)	17 (13.4)
Marrow failure	7 (5.5)	3 (2.4)	4 (3.1)
Inborn error	4 (3.2)	1 (0.8)	3 (2.4)
Perineal involvement			
Yes	18 (14.2)	10 (7.9)	8 (6.3)
No	34 (26.8)	8 (6.3)	26 (20.5)
Data not available	75 (59.0)	21 (16.5)	54 (42.5)

MDRPA: cases with multidrug resistant P.aeruginosa.

Table 2. Distribution of *in vitro* resistance to major antibiotic classes of 124 PA strains* isolated from patients treated with chemotherapy and or HSCT.

	N. strains tested	N. susceptible strains	N. resistant strains (%)
Antibiotic			
Piperacillin	95	69	26 (27%)
Ceftazidime	113	79	34 (30%)
Cefepime	54	36	18 (33%)
Imipenem	115	87	28 (24%)
Meropenem	85	63	22 (25%)
Amikacin	105	93	12 (11%)
Ciprofloxacin	113	92	21 18%)

**Data not fully available for 3 of the 127 cases.*

were receiving at least one antibiotic which was seen to be effective *in vitro*. In this subset, the infection-related mortality ratio was 17%, while of the remaining 8 patients with "ineffective" antibiotic therapy, 4 died. Thus, empirical antibiotic therapy, although non-uniform in this multicenter survey, proved to be largely appropriate for *P. aeruginosa* infection.

The frequency of MDRPA strains was apparently higher in some centers (*data not shown*) suggesting a possible "cluster" effect. Whether other factors in these hospitals may have affected the probability of death for some patients included in this series cannot be definitely excluded. *P. aeruginosa* bacteremia in the immunocompromised patient is usually associated with prolonged hospital stay and increased mortality. This may be of special relevance in the case of MDRPA infections. Therefore, altering infection-control practices to limit the dissemination of certain bacterial species may be more effective than attempts to control only antibiotic-resistant isolates.[24]

In conclusion, *P. aeruginosa* infection remains a major concern for children undergoing chemotherapy. Although current empirical antibiotic therapy usually contains at least one active drug, resistance may soon develop and patients with multidrug resistant strains are at an exceedingly high risk for fatal outcome.

Authorship and Disclosures

DC was the principal investigator and takes primary responsibility for the paper. DC, SC, OZ, GZ, RM, SL, MC, GMM, BC, ML, CB, MA, EC recruited the patients. SF participated in the statistical analysis. SC, SL, and EC coordinated the research. DC and MA wrote the manuscript. All co-authors reviewed and approved the final version of the manuscript.

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