An Outbreak of Achromobacter Bacteremia in Pediatric Clinic 1 Tîrgu Mureș in 2010

Man Lidia[1], Baghiu Despina[1,3], Székely Edit[2,4], Man A[2]

1 Pediatric Clinic I, County Emergency Clinical Hospital, Tîrgu Mureș, Romania
2 Microbiology Department, University of Medicine and Pharmacy, Tîrgu Mureș, Romania
3 Pediatric Department I, University of Medicine and Pharmacy, Tîrgu Mureș, Romania
4 Central Clinical Laboratory, Department of Bacteriology, County Emergency Clinical Hospital, Tîrgu Mureș, Romania

Introduction: *Achromobacter* is a less common pathogen, with implications in patients with immune deficiency. The purpose of this paper is to evaluate the *Achromobacter* spp. bacteremia outbreaks in the hematology department of Pediatric Clinic I Tîrgu Mureș and to establish the involvement of this species as an etiologic agent of bacteremia/septicemia or solely as a contaminant of blood culture samples.
Material and methods: We analyzed an outbreak of *Achromobacter* infection from 2010's summer-autumn season in Pediatric Clinic I. Bacteriological (blood culture, antibiotic susceptibility), laboratory (white cells count, inflammatory tests) and clinical aspects (underlying disease, body temperature, treatments, age, sex) were followed.
Results: A total of 26 blood cultures collected from nine children admitted in this period were positive for *Achromobacter* spp., other 28 were negative. In febrile patients with positive blood cultures, the leukocyte and neutrophil count was increased. In non-febrile patients with positive blood cultures, changes in the total number of leukocytes and granulocytes did not show significant variations. The antibiotic susceptibility test for *Achromobacter* strains identified a 100% resistance to ticarcillin, gentamicin, and trimethoprim-sulfamethoxazole, sensitivity to most classes of antibiotics, but an OXA-114 β-lactamase producing phenotype.
Conclusions: An association between the inflammatory syndrome and *Achromobacter* spp. bacteremia was established. *Achromobacter* spp. isolated from blood sampled through catheters is most likely a contaminant. The antibiotic susceptibility testing of *Achromobacter* spp. revealed sensitivity to most classes of antibiotics.

Keywords: *Achromobacter*, bacteremia, neutropenia, pediatrics, endemic outbreak

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Introduction

In 1971, Yabuuchi and Ohyama described a Gram-negative, peritrichous rod that was isolated from purulent ear discharges of seven patients with chronic otitis media. They proposed the name *Achromobacter (Alcaligenes) xylosoxidans* [1]. It is a non-fermentative, aerobic, oxidase and catalase-positive, non-lactose fermenting rod [2]. The genera *Achromobacter* include many species, but the clinically important ones are: *A. xylosoxidans*, with two subspecies (xylosoxidans and denitrificans) and *A. denitrificans*; *A. faecalis*, a less common pathogen but with a distinctive antimicrobial susceptibility pattern; *A. piechaudii*, which has been isolated from clinical specimens, but its significance is doubtful [3].

The purpose of this paper is to evaluate the *Achromobacter* spp. bacteremia outbreaks in hematology department of Pediatric Clinic I Tîrgu Mureș and to establish the involvement of this species as an etiologic agent of bacteremia/septicemia or solely as a contaminant of blood culture samples.

Material and method

From the data of the Central Clinical Laboratory of the County Emergency Clinical Hospital Tîrgu Mureș, all positive blood cultures for *Achromobacter* spp. cases between 05/21/2010 to 12/31/2010 were identified. The clinical information was retrospectively collected from the case report forms of patients admitted to Pediatric Clinic I, hematology-oncology department of the County Emergency Clinical Hospital in that period. From the medical records of patients from whom *Achromobacter* spp. was identified in blood cultures, we recorded the following data: underlying disease, age, sex, number of leucocytes and granulocytes during the blood culture sampling day, as well as during the two days before and after, changes to body temperature, inflammatory tests, blood sampling site, number of blood cultures, antibiotic treatment before blood culture and antibiotic susceptibility.

The blood count, blood cultures, inflammatory tests and antibiotic susceptibility tests (AST) were processed in the Central Laboratory of the County Emergency Clinical Hospital. The blood cultures were performed in the automated systems BacT/ALERT and BacT/ALERT 3D. The identification of species and AST were performed in the Vitek 2 Compact system.

Inclusion criteria: The study included patients hospitalized in the hematology-oncology department of Pediatric Clinic I, between 05/21/2010 and 12/31/2010. The main criterion for choosing the two working groups was the presence or absence of fever in the day of blood culture sampling. If the same patient had multiple blood cultures collected in feverish spurt, in order to avoid calculation errors by duplication of results, we analyzed only the first blood culture/first hospitalization from which *Achromobacter* spp. was identified.

Correspondence to Lidia Man
Email: lidia.man@gmail.com

Table I. Changes in leukocytes and neutrophils number in febrile patients

	Cell number (x10³)									
	2 days before		1 day before		Sampling day		1 day after		2 days after	
	L	N	L	N	L	N	L	N	L	N
Patient 1	–	–	1.89	0.5	21.9	19.3	–	–	3.2	1.7
Patient 2	2.94	2.09	2.57	1.38	2.07	1.62	1.34	0.71	0.8	–
Patient 3	5.53	2.12	4	2.9	3.34	2.04	3.8	3.03	3.8	–
Patient 4	–	–	4.24	3.52	5.6	5.3	–	–	5.6	–
Patient 5	–	–	4.5	2.12	6.73	5.37	6.17	5.3	3.33	2.07
Patient 6	–	–	9.3	3.09	5.65	3.44	7.22	5.28	2.9	0.9
Mean	4.23	2.1	4.41	2.25	7.54	6.17	4.63	3.58	3.27	1.55

L – leukocytes; N – neutrophils

Exclusion criteria: patients that did not have blood counts performed at least once before, once after and once during the blood culture sampling day were not included, as they would have altered the statistical results.

All the given positive blood cultures from a certain patient during admission were considered as being part of a single infectious episode, so only the first result was studied. To test the resistance spectrum of *Achromobacter* spp., the first isolate from each patient was examined.

All data were collected in an Excel 2007 table and the data was processed in GraphPad Instat 3 software. We used descriptive statistics, comparison of means and Chi-square test, with a confidence interval of 95%.

Results

Between 21 May 2010 – 31 December 2010, 101 blood cultures were generally collected from patients hospitalized in the Pediatric Clinic I, of which 36 (35.64%) were positive for several bacterial species. From these, *Achromobacter* spp. (followed in our study, due to its endemic burst in a short period) was identified in 26 blood cultures (24.75%). Other bacteria (which do not make the purpose of this study) were coagulase-negative staphylococci in 9 cases (8.91%) and Micrococcus spp. in 2 cases (1.98%).

The 26 blood cultures positive for *Achromobacter* spp. derived from 9 patients, of which 2 girls (22.22%) and 7 boys (77.78%). The average age of these patients was 8.66 years with a median of 6, with limits ranging from 2 to 17 years. Four of them (44.4%) were hospitalized for the first time in July, three (33.33%) in August, one (11.11%) in September and one (11.11%) in November 2010.

Table II. Mean number of leukocytes in febrile and non-febrile patients

	Nr of leukocytes (x10³) in the day previous to the BC day	Nr of leukocytes (x10³) In the BC day
Febrile	4.41	7.54
Non febrile	2.24	2.43
	p <0.0001	

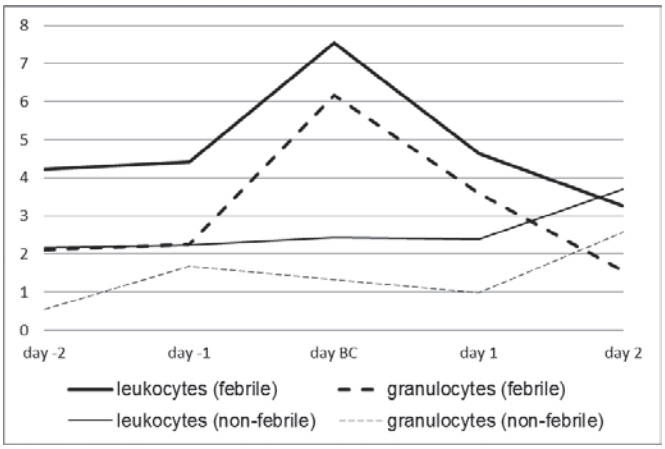

Fig. 1. Changes in leukocytes and granulocytes number in febrile and non-febrile patients

Some of the nine patients had repeated admissions, so a total of 26 blood cultures which revealed *Achromobacter* spp. were collected from 20 different days. One blood culture/day was sampled in 16 cases (80%), but there were cases when the blood cultures were collected 2 times/day (three cases; 15%) or even 3 times/day (in one case; 5%). Twenty-eight blood cultures from the same patients were negative.

Nineteen blood cultures (73.07%) of the 26 positive for *Achromobacter* spp. were sampled in febrile spurt, the remaining 7 (26.93%) being sampled from non-febrile patients.

Achromobacter spp. was identified in febrile spurt in 8 of 9 patients (*Achromobacter* spp. was isolated from one feverless patient). Data of the 8 febrile patients are considered below.

Evolution of the total number of leukocytes and granulocytes in febrile patients can be followed in Table I. We mention that only the cases where the blood counts were performed at least once before, once after and once during the blood culture sampling day have been taken into account, so two cases were excluded. In some cases, the blood counts were not performed, while in other cases, the number of neutrophils was too small and it could not be read.

During the blood culture (BC) sampling day in febrile patients, the mean leukocytes count increased by 70.97% (or 1.7 times), (p=0.3274) and that of neutrophils by 174% (or 2.7 times), (p=0.1764). In non-febrile patients, during the blood culture sampling day, changes in the total number of leukocytes and granulocytes did not show significant variations (p=0.488 for leukocytes and p=0.778 for neutrophils) (Figure 1). There is a statistically significant difference (p <0.0001) between the mean number of leukocytes in the BC sampling day and in the previous day, in febrile vs non-febrile patients (Table II).

Of the 8 patients that presented fever during the blood culture sampling day and positive for *Achromobacter* spp., 3 of them had fever on the day before, one with 2 days before, 5 the day after and 3 on two days after sampling (Figure 2).

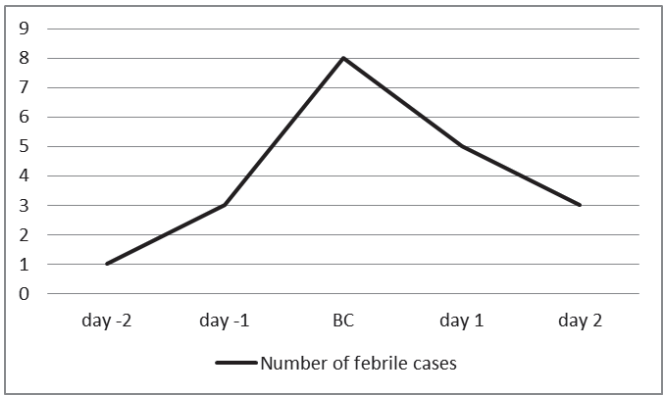

Fig. 2. The febrile cases number 2 days before and after blood culture (BC) sampling day

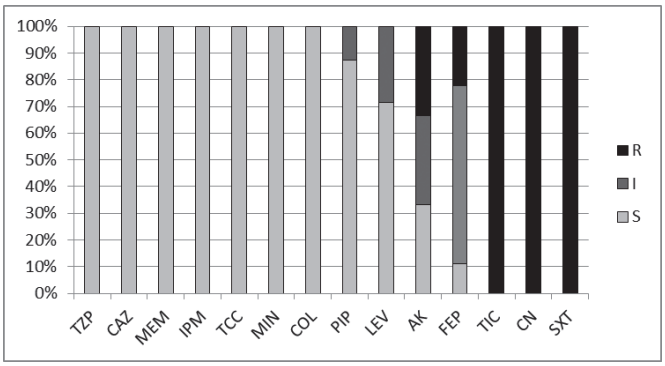

Fig. 3. Antibiotic susceptibility of *Achromobacter* spp.

In febrile patients during the blood culture sampling day, the Erythrocyte Sedimentation Rate (ESR) was increased in 4 of 8 patients (a mean of 51 mm/hour) and in normal limits in one patient (5 mm/hour); 3 patients were not tested for ESR. C-reactive protein (CRP) was increased in all 8 patients (with a mean of 39 mg/dl). Three blood cultures (37.5%) were double (collected both through the catheter and by venous puncture), 3 (37.5%) by venous puncture and only 2 (25%) through the catheter.

In the group of non-febrile patients during the blood culture sampling, the inflammatory markers were higher than normal in all five patients (mean ESR = 39 mm/hour and mean CRP = 29 mg/dl). In four of the five patients (80%), the blood cultures were collected by venous catheter, and in one (20%) by venous puncture.

The antibiotic susceptibility test for *Achromobacter* strains identified a 100% resistance to ticarcillin, gentamicin, and trimethoprim-sulfamethoxazole. Marked and intermediate resistance was identified for 4th generation cephalosporins and amikacin, and an intermediate resistance to fluoroquinolones. Sensitivity was preserved for piperacillin, piperacillin-tazobactam, carbapenems, ceftazidim, ticarcillin-clavulanate, colistin and minocycline (Figure 3).

Among febrile patients during the blood culture sampling, most were on chemotherapy treatment for acute lymphoblastic leukemia (7 patients) or acute myeloid leukemia (1 patient), some of them being in neutropenic stage. Four patients (50%) were under antibiotic treatment with colistin, ceftriaxone and ceftibuten. Two patients received associated therapy with trimethoprim-sulfamethoxazole as the Clinic's internal protocol requires for the prophylaxis of Pneumocystis jirovecii infection.

Discussions

Patients with cancer, patients undergoing hematopoietic and organ transplantation, patients with hypo-γ-globulinemia, patients with HIV infection/AIDS and premature infants are all at increased risk, and in such individuals, *Achromobacter* infections occasionally present as life-threatening illnesses. Most *Achromobacter* infections are acquired during hospitalization and frequently a common source can be traced. Possible sources include intravascular catheters, contaminated dialysis fluid, deionized water, mechanical ventilators, chlorhexidine solution, and incubators, as well as normal stool matter colonized by *Achromobacter* spp. [4,5].

The outbreak developed between July–November 2010, some of the patients returning for several times, and in every admission *Achromobacter* spp. was identified from the blood culture. This could mean that they are carriers for the *Achromobacter* strain.

In patients who were febrile during blood culture sampling, although they presented neutropenia due to chemotherapy for hematological disorders, an increase in the number of leukocytes and granulocytes, and elevated ESR and CRP could be observed. Thus, an association between the inflammatory syndrome and *Achromobacter* spp. bacteremia can be established. In the same patients, during the non-febrile periods, but in which the blood cultures were positive for *Achromobacter* spp., although ESR and CPR values were increased, there was no evidence of increased number of leukocytes. This raises the suspicion of blood culture contamination, especially as most of them were collected through the catheter and not by venous puncture.

The small number of febrile cases in the days before and after the blood culture sampling suggests rather a transient bacteremic episode than sepsis.

The literature describes that most of the strains of *Achromobacter* spp. present in vitro susceptibility to trimethoprim-sulfamethoxazole, antipseudomonal penicillins (piperacillin, ticarcillin) and carbapenems (meropenem), and a high resistance to aminoglycosides (amikacin, gentamicin, tobramycin) and fluoroquinolones [6].

Phenotypically, in terms of resistance spectrum to antibiotics, all strains of *Achromobacter* were practically identical, with minor differences for cefepime, amikacin, levofloxacin and piperacillin, suggesting a clonal distribution. To clarify the degree of similarity between the isolated strains, a molecular typing method (PFGE – pulse field gel electrophoresis) will be performed in a future study.

The antibiotic susceptibility testing revealed a less common phenotype of resistance, which involves sensitivity to 3rd generation cephalosporins (ceftazidime), but resist-

ance to the 4th generation (cefepime), this being achieved by some kind of β-lactamase. Among the four β-lactamase molecular classes, class D β-lactamases are the most diverse enzymes. Class D β-lactamases are usually not affected by β-lactamase inhibitors like clavulanic acid (in our cases, *Achromobacter* spp. presented resistance to ticarcillin, but sensitivity to ticarcillin-clavulanate), this property not being present in other classes of β-lactamases [7]. The resistance mechanism could be achieved primary by the production of a narrow-spectrum class D beta-lactamase, but with a secondary contribution of an OXA-114 like enzyme. The studies identified OXA-114 as a naturally-occurring beta-lactamase of *A. xylosoxidans*. The blaOXA-114 gene, responsible for the synthesis of OXA-114 β-lactamase was cloned from the genome of *Achromobacter xylosoxidans* (formerly *Alcaligenes denitrificans* subsp. *xylosoxydans*). This β-lactamase has an amino-acid sequence similarity of other β-lactamases (56% with those produced by *Burkholderia cepacia* and 42% with β-lactamases OXA-9 and OXA-18). OXA-114 has a narrow-spectrum profile, and a very low level of activity against imipenem. Also, in contrast with other blaOXA genes, the expression of blaOXA-114 is not inducible [8].

Conclusions

1. An association between the inflammatory syndrome (fever, high leukocyte count, positive inflammatory tests) and *Achromobacter* spp. bacteremia was established, sustained by:
   - Isolation of *Achromobacter* spp. from blood cultures, sampled mostly by venous puncture;
   - An increase of leukocyte count in febrile patients by 1.7 times and of neutrophils by 2.7 times, on neutropenic background, during the blood culture sampling day;
   - A statistically significant difference between the mean number of leukocytes in the blood culture sampling day and in the previous day, in febrile vs non-febrile patients;
   - Persistence of fever several days before and after the blood culture sampling day;
   - High levels of inflammatory markers (ESR, CRP).
2. Most cases consisted in transient bacteremic episodes with *Achromobacter* spp.
3. The phenotypic similarities of the isolated *Achromobacter* strains suggest a clonal spreading.
4. *Achromobacter* spp. isolated from blood sampled through catheters is most likely a contaminant or a catheter colonizer, fact sustained by the lack of association with clinical and laboratory signs of infection.
5. The antibiotic susceptibility testing of *Achromobacter* spp. revealed sensitivity to most classes of antibiotics, but an OXA-114 β-lactamase producing phenotype.

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