Bloodstream infections in the Cardiovascular Surgery Clinic Iași, Romania

Infecțiile sistemice în Clinica de Chirurgie Cardiovasculară din Iași, România

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Abstract

Objectives: to analyze the aetiology, the origin, epidemiologic features and outcome of bloodstream infections in patients that underwent cardiovascular surgery. Methods: A retrospective study of bloodstream infections was conducted between 27th April 2001 – 26 June 2010 in the Cardiovascular Surgery Clinic of Iasi, Romania. Results: We detected 114 consecutive bloodstream infections in a number of 4304 hospitalizations. Bacteraemia was secondary to an infectious body site in 53.50% of the episodes. Cell-wall deficient forms (52.63%) were the most prevalent microorganisms (78 strains, out of which 53 reverted to the classical state and 25 were L – stable), followed by Gram-positive cocci, Gram-negative bacilli, Gram-positive bacilli, fungi, HACEK group and anaerobes. Conclusions: We found a high proportion of cell wall deficient forms, rarely reported before. This may be due to blood culture collection under antimicrobial therapy (p=0.016), especially β-lactams, and/or the use of more adequate blood culture methods (acridine orange stain for microscopy of the blood culture and reversion media). The therapy of the infections with cell wall deficient forms raises particular problems. For systemic infections associations of chloramphenicol + spiramycin, erythromycin + cephalotin, chloramphenicol + tetracyclin could be adequate treatment, but currently we are collecting more data in order to establish therapeutic protocols.

Keywords: nosocomial bloodstream infections, L-forms, cell wall deficient forms, acridine-orange stain, blood culture, cardiovascular surgery, bacteremia, infective endocarditis.

Rezumat


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coci gram-positivi, bacili gram-negativi, funghi, grupul bacterian HACEK și anaerobi. Concluzii: am depistat o proporție crescută de bacterii cu perete defectiv, rar comunicate în studiile de specialitate. Acest fapt se poate datora recolțării hemoculturilor sub antibioticoterapie (p = 0,016), în special cu β-lactamine, și/sau folosirii unor metode mai adecvate pentru hemocultură (microscopia din hemocultură utilizând acridine orange și utilizarea mediilor de reversie). Terapia infecțiilor sistematice cu bacterii cu perete defectiv ridică probleme particulare; pentru infecțiile sistemice asocierele cloramfenicol + spiramicina, eritromicina + cefalotin, cloramfenicol + tetraciclină ar putea reprezenta opțiunile terapeutice, dar sunt necesare mai multe date pentru a stabili protocoale terapeutice adecvate.

Cuvinte cheie: infecții sistemice, forme L, bacterii cu perete defectiv, colorația cu acridine orange, hemoculturi, chirurgie cardiovasculară, bacteriemie, endocardită.

Introduction

Debilitating conditions of the patients undergoing cardiac surgery predispose these patients to life threatening bloodstream infections (1-3). The treatment of bloodstream infections and especially nosocomial ones has become a challenging task due to the spread of multiresistant bacteria. The profile of nosocomial bloodstream infections varies between institutions and also between various wards of the same hospital. It is important to know the pathogens involved in nosocomial bacteremia and their antimicrobial sensitivity to formulate appropriate treatment guidelines. Since information available from our country on the subject is limited, we conducted this study to obtain data on pathogens causing bloodstream infections in a cardio-vascular surgery ward of a regional hospital in north Romania.

We made a retrospective study of the bloodstream infections in the Cardiovascular Surgery Clinic (50 beds), which includes an Intensive Care Unit as well (12 beds), of the Cardiovascular Diseases Institute Iasi, Romania. Clinical, epidemiological and laboratory data were recorded in standardized files and saved in an electronic database.

Materials & Methods

Definitions

Nosocomial bacteremia: according to CDC criteria (Center for Disease Control and Prevention from 1988 (4), nosocomial bacteremia is defined as microbiologically confirmed bacteraemia that occurs after 72 hours from admission, excepting readmitted cases (that occurred in 72 hours from a previous discharge).

Infective endocarditis: Cases were classified according to the new diagnostic criteria for infective endocarditis proposed by Durack et al (12) and only probable or definite cases of endocarditis were included.

Nosocomial infective endocarditis: The acquisition of IE was considered nosocomial if the diagnosis was made 72 h after hospital admission and if there was no evidence that IE was present at the time of admission. Diagnoses made within 60 days of a previous hospital admission were also classified as nosocomial, or when a risk procedure for bacteremia was performed, or when any predisposing factor for IE was present during hospitalization.

Revertant: cell wall deficient form that is capable of reverting to the classical stage. Sometimes only microscopic reversion occurs and the bacterium remains uncultivable on usual solid media (5).

Stable L-form: cell wall deficient form that is not capable of reverting to the classical stage and cannot grow on usual solid media (5).

Negative blood culture: the lack of microscopic evidence of growth and the absence of colonies on solid media.

Clinical significance of positive blood cultures was assessed after considering several factors: the identity of the microorganism (6-8), the number of positive blood culture sets (7, 9, 10), the time necessary for microbial growth to occur (10, 11), documented infection with the
same microorganism at another body site (12, 13), clinical state of the patient (including predisposing risk factors, inflammatory variables - plasma C-reactive protein level, plasma procalcitonin level and therapeutic proof) and imagistic tests (X-ray, computed tomographic scan).

Microbiological examination of blood cultures

Blood culture media. We used biphasic media (Hemoline Performance Duo bioMérieux, France) with visual growth control.

Blood cultures examination. We incubated blood culture bottles at 37°C for 48 hours and examined visually for evidence of growth (haemolysis, gas production, turbidity or detection of certain bacterial colonies on agar slide) during the first 48 hours. Visually positive blood culture bottles were subcultured on Columbia agar (5% sheep blood) and Sabouraud-dextrose agar. We identified the isolates based on preliminary data of bacterial isolates and then we used API bioMérieux system for identification. Antibiotic susceptibility tests were done by disk diffusion method, according to recommendations of the National Committee for Laboratory Standards/Clinical and Laboratory Standard Institute (NCCLS/CLSI): 2001-2010 and miniAPI bioMérieux kits. Screening for methicillin resistance in staphylococci, vancomycin resistance (breakpoints method, using ATB STAPH) and high level aminoglycoside resistance (HLAR) in enterococci and extended spectrum β-lactamase (ESBL) production in Gram-negative bacteria was done according to NCCLS/CLSI guidelines (2001-2010).

If there were no macroscopic evidence of growth in the first 48 hours, we made blind subcultures and acridine orange staining. When L-forms were detected on wet mount with acridine orange (Figures 1, 2, 3, 4) we used 2 reversion media (13, 27) as follows: “medium 1” (brain heart broth – 3.5g, yeast extract – 0.5g, NaCl – 0.2g, distilled water – 100mL) and “medium 2” (tryppticase soy broth – 3g, yeast extract – 0.2g, sucrose – 10g, agar – 0.05g, distilled water – 87mL). We transferred 1 mL from the blood culture to medium 1. After 48-72 hours we made subcultures from “medium 1” into “medium 2” (200µL per tube) and Columbia agar with 0.2% NaCl.

Blood cultures were incubated for 14-21 days and on the final day Columbia agar (with 5% sheep blood) was used for terminal subculture.

Results

During the 10 years of the study period, there were 4304 admissions in our hospital. A total of 662 patients presented signs for bloodstream infection. From these patients 1335 blood culture sets were collected (2 sets on average per patient). The positivity rate was 25.61%.

We detected 114 bloodstream infections out of which 105 were nosocomial and 9 were community acquired based on epidemiological criteria. The mean age of patients was 50 years (range between 2 and 70), involving 71.05% males. Systemic infections were secondary to an infectious body site in 53.5% of the cases: catheter-related (25 cases, 21.92%), wounds (12 cases, 10.52%), lower respiratory tract (11 cases, 9.64%), lower respiratory tract (11 cases, 9.64%), infective endocarditis (10 cases, 8.77%, 4 nosocomial infective endocarditis and 6 community acquired), urinary tract infections (3 cases, 2.63%). From the wound infections, only 2 cases (1.75%) represented infections contracted post-surgery in the Cardiovascular Surgery Clinic; the rest (10 cases, 8.77%) were pre-surgery infections (gangrenes of the legs, trophic ulcers, post-amputation wounds or other interventions in different services). The mortality rate was 25.33%.

Contaminated blood cultures represented 4.41% out of the total examined blood cultures.

Gram-positive cocci were the most frequent isolates (35 strains, 30.70%), followed by Gram-negative bacilli (30 strains, 26.31%), irreversible L-forms (25 strains, 21.92%), Gram-positive bacilli (11 strains, 9.65%), fungi (9 strains, 7.89%), HACEK group (3 strains, 2.63%), anaerobes (1 strains, 0.87%) (Table 1).

From all isolates, cell-wall deficient forms represented 52.63% - 78 strains, out of
which 53 returned to their typical morphology (16 Gram-negative bacilli, 12 Gram-positive cocci, 6 Gram-positive bacilli, 1 anaerobe) and 25 were L-stable forms, without reversion to the normal bacterial growth.

The proportion of polymicrobial episodes was 2.66% (Staphylococcus aureus + Haemophilus paraphrophilus and H. parainfluenzae + Enterococcus faecalis).

We isolated 13 strains of methicillin-resistant staphylococci representing 50% of all staphylococci. In case of S. aureus 8 strains (44.4%), while in case of coagulase negative staphylococci 5 strains (62.5%) were methicillin-resistant. No staphylococci with reduced susceptibility to vancomycin were detected with the methods we used.

All 5 strains of Enterococcus spp. isolated by us were sensitive to vancomycin. HLAR phenotype was detected in 2 strains.

Among Enterobacteriaceae strains, 41.17% were ESBL producers (7 out of 17), 58.83% were susceptible to fluoroquinolones (10 strains) and 47.05% were susceptible to aminoglycosides (sensitive to both gentamicin and amikacin).

One of the Acinetobacter baumannii strains was susceptible only to colistin, the rest presented susceptibility to carbapenems (4 strains out of 4), fluoroquinolones (1 strain), aminoglycosides (3 strains susceptible to gentamicin and amikacin).

All 3 strains of Pseudomonas aeruginosa were susceptible to carbapenems and fluoroquinolones and only 2 strains were susceptible to ceftazidime.

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**Figure 1.** Acridine orange staining, smear from blood culture (magnification 400x):
A. L-forms; arrows indicate leucocytes. B. Cocci. C. Bacilli

**Figure 2.** Escherichia coli. A. Smear from blood culture (Gram stain, magnification 1000x): 1. Gram negative bacilli; 2. L – forms. B. Smear from culture on solid media (Gram stain, magnification 1000x)
Discussions

Patients undergoing cardiac surgery appear to be at increased risk for the development of nosocomial bacteremia due to the presence of surgical wounds, intravascular devices and the antibiotic therapy/prophylaxis from the post-surgery period (1-3).

According to Kollef and coworkers, approximately 98.5% (1) of patients received antibiotics for surgical wound prophylaxis in the perioperative period. Those who did not receive antibiotics in the perioperative period presented increased risk of developing surgical wound infections. The empirical administration of antibiotics precedes the development of nosocomial infections in 93.6% of the cases (1). In our study, 85.08% of the blood cultures were collected from patients who had already received antibiotics. The consequence of this practice was the increased isolation of cell wall deficient forms. The therapy of nosocomial bacteraemia represents new challenges because of the increased resistance of microorganisms. The proportion of *S. aureus* resistant to methicillin in the USA increased from 22% in 1995 to 57% in 2001 (16), and in Europe a similar increase has been noticed (15). In both studies, the proportion of coagulase-negative staphylococci resistant to methicillin was 75%. The percentage of strains of *Enterococcus faecalis* resistant to vancomycin was 2% and for those of *E. faecium* 60% (15, 16).

![Figure 3. Actinomyces pyogenes. A. Smear from blood culture (Gram stain, magnification 1000x); B. Microculture (magnification 30x); C. Smear from microculture (Gram stain, magnification 1000x)](image)

![Figure 4. Haemophilus aphrophilus. A. Smear from blood culture (Gram stain, magnification 1000x); B. Reversion medium; C. Smear from reversion medium (Gram stain, magnification 1000x)](image)
During our study (10 years) we found relatively few systemic infections (only 114) so that we can not make an adequate statistical processing of data on susceptibility to antibiotics, but our results are important for local epidemiological purposes. Generally, antibiotics inhibiting cell wall structures are not active against L-forms. Correspondingly, antibiotics which interrupt protein synthesis are inhibitive to the variant if the par-

### Table 1. Microorganisms with clinical significance isolated from blood cultures

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>No. of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=114</td>
<td></td>
</tr>
<tr>
<td><strong>Gram-positive cocci</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S aureus</td>
<td>18(6)</td>
<td>15.78</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci(^1)</td>
<td>8(4)</td>
<td>6.66</td>
</tr>
<tr>
<td>Enterococcus spp(^2)</td>
<td>5(1)</td>
<td>4</td>
</tr>
<tr>
<td>Oral streptococci(^1)</td>
<td>3(1)</td>
<td>2.63</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>1</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>Gram-negative bacilli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>9(7)</td>
<td>4</td>
</tr>
<tr>
<td>Acinetobacter spp(^4)</td>
<td>6(4)</td>
<td>5.26</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>6(1)</td>
<td>5.26</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>1 (1)</td>
<td>0.87</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
<td>0.87</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>3(1)</td>
<td>2.63</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>3(2)</td>
<td>2.63</td>
</tr>
<tr>
<td><strong>Irreversible L-forms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>21.92</td>
</tr>
<tr>
<td><strong>Gram-positive bacilli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corynebacterium group G</td>
<td>3(1)</td>
<td>2.63</td>
</tr>
<tr>
<td>C. jeikeium</td>
<td>2</td>
<td>1.75</td>
</tr>
<tr>
<td>Actinomyces pyogenes</td>
<td>2 (2)</td>
<td>1.75</td>
</tr>
<tr>
<td>C. afermentans</td>
<td>1(1)</td>
<td>0.87</td>
</tr>
<tr>
<td>C. glucuronolyticum</td>
<td>1(1)</td>
<td>0.87</td>
</tr>
<tr>
<td>Rhodococcus spp</td>
<td>1(1)</td>
<td>0.87</td>
</tr>
<tr>
<td>Unidentified Gram-positive bacilli</td>
<td>1</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>4</td>
<td>3.50</td>
</tr>
<tr>
<td>Candida spp(^5)</td>
<td>3</td>
<td>2.63</td>
</tr>
<tr>
<td>Rhodotorula glutinis</td>
<td>1</td>
<td>0.87</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>1</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>HACEK</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. parainfluenzae</td>
<td>2</td>
<td>1.74</td>
</tr>
<tr>
<td>Haemophilus aphrophilus</td>
<td>1</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>Anaerobes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium tertium</td>
<td>1(1)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

\(^1\) S epidermidis 3, S haemolyticus 1, S warneri 1  
\(^2\) E faecalis 4, E hirae 1  
\(^3\) S oralis 1, S constellatus 1, untypable Streptococcus 1  
\(^4\) A baumannii 2, Acinetobacter johnsonii 4  
\(^5\) C norvegica 1, C valida 1, C inconspicua 1  

\( ) = \) isolates identified through the reversion of the initial L-forms
ent bacterium is sensitive. However, exceptions are numerous. Antibiotics considered metabolic inhibitors, which are exquisitely toxic to the parent bacterium, may be tolerated by the variant, for inexplicable reasons. In the therapy of infections where L-forms are found, it may be necessary to treat both the wall deficient stage and the classical organisms since the suppression of one stage may foster the propagation of the other (17). For systemic infections associations of chloramphenicol + spiramycin, erythromycin + cephalotin, chloramphenicol + tetracycline could be adequate treatment (17).

From the treatment perspective of bacteremia with L-forms, we encountered two particular situations.

In the first case, the 56 year old patient, RV, with a mitral disease was diagnosed with infective endocarditis. All three sets of collected blood cultures yielded cell wall deficient forms, irreversible to the normal growth. At the moment blood cultures were collected, the patient received empirical antibiotic therapy with ceftriaxone. After the laboratory informed the physician of the presence of L-forms, the treatment of the patient was changed to glycopeptide + β-lactam antibiotic, according to recommendations from the literature (18). Within this therapy the clinical state of the patient improved and she was released.

The second was the case of the 48 years old patient LP, diagnosed with mediastinitis after cardiac surgery performed abroad. From the mediastinal liquid and the blood cultures methicillin-resistant Staphylococcus aureus was isolated. Beside bacteria with normal morphology, the microscopic examination of blood cultures highlighted bacteria with a defective cell wall. The patient received therapy with vancomycin, but his clinical state did not improve and the S. aureus continued to grow in large amounts from the mediastinal liquid. After communicating the L-forms in blood cultures, the therapy was changed and the patient was treated with linezolid. After this therapeutic decision, the patient progressed well and was released fully recovered.

In a national program of supervising nosocomial systemic infections in Belgium (4), the origin of bacteremia was detected in 63.80% and the main causes for infections were: catheter related (23.5%), urinary tract (11.6%), lower respiratory tract (9.1%), wounds infections (5.6%). In our study a portal of entry for bacteremias was detected in 60% of the cases. The most frequent site was represented by catheters (21.92%), followed by wounds (10.52%; the majority without causal relation with the present surgery), lower respiratory tract (9.69%) and urinary tract (2.63%). The increased frequency of endocarditis as a source for bacteremia can be a consequence of the particularities of our group of patients (cardiovascular surgery patients).

In the case of revertants we detected the origin of bacteremia in 27 patients (catheter-13, lower respiratory tract-5, wounds-5, infective endocarditis-4). In all these situations the patients had received antibiotic therapy before the specimens were collected.

We encountered a particular situation in the case of the patient TM, 49 years old, diagnosed with an infective endocarditis caused by Candida albicans. The fungus was isolated from blood and from the piece of valve. After communicating the isolate from the blood culture, the patient was given fluconazol, according to the antibiotic susceptibility tests and 3 sets of blood cultures collected afterwards isolated cell wall deficient forms irreversible to normal growth. The smears made in the reversion media showed fungi, but these did not grow on solid media, therefore we could not identify them or perform susceptibility testing.

In 2004, a study made in the USA (16) carried out during a period of 7 years the most frequent etiological agents of nosocomial bacteremia were the coagulase negative staphylococci (31%), S aureus (20%), enterococci (9%), Candida spp (9%). The Gram-negative bacteria and
the anaerobe ones were isolated in small percentages: *E. coli* (2.8%), *Klebsiella spp* (2.4%), *Pseudomonas aeruginosa* (2.1%), *Enterobacter spp* (1.9%), anaerobes (1%). Other studies confirmed, as well, the emergence of Gram-positive pathogens (4, 19, 20) and especially of the coagulase negative staphylococci, *S. aureus* and enterococci. In the adult patients post cardiovascular surgery, the frequency of isolates was: Gram-positive cocci, Gram-negative bacilli, *Candida spp* (21, 22). In the group studied by us, the most frequent isolates were also the Gram-positive cocci, but the second place was occupied by Gram-negative bacilli, followed by the irreversible L-forms, the Gram-positive bacilli, fungi, HACEK group and the anaerobes.

We noticed an increased percentage of Gram-positive bacilli (9.65%) isolate and this can be explained through the fact that more than half of these bacilli derive from the reversion of the initial L-forms. Lately, the coryneform bacteria were more and more involved in infections: infective endocarditis on native or prosthetic valves, catheter-related bacteremias, infections of the lower respiratory tract (22-24, 26). In case of Gram-positive bacilli isolated in our study we identified the portal of entry in 7 cases out of 11 (infective endocarditis – 3, catheter-related – 3, lower respiratory tract - 1).

**In conclusion,** we consider that more reproducible methods should be developed for the isolation and identification of L-forms. Many techniques currently used today are classical methods of the past century but these methods no longer satisfy the needs of modern medicine. Future clinical studies should include: the detection of L forms in different pathological samples, the identification of these L forms through fast molecular methods; studying the ongoing changes of the inflammatory response of the host, the antibiotic therapy should eliminate both types of bacterial populations (in normal stage and in L-forms stage).

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**Ethical approval:** not required.

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