New Strategies to Control Vascular Catheter-Related Bloodstream Infection with Emphasis on Neonatal Intensive Care Unit

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The infection associated with the use of intravascular devices represents from 10 to 20% of the total nosocomial infections and is one of the most frequent causes of morbidity and mortality, which represents a source of bacteremia and sepsis in hospitalized patients and increases the costs of hospital admission and the time of hospital stay. In Neonatal Intensive Care Units the rate of infection is inversely proportional to the newborn birth weight. Approximately 20% of the premature neonates with very low birth weight (less than 1,500g) experience a systemic infection during their stay in hospital. The aim of this paper was to review the several aspects of catheter-related bloodstream infections as to pathogenesis, etiology, diagnosis and new prevention strategies based on the knowledge of mechanisms of bacterial adhesion and biofilm formation. A new way to prevent the colonization of catheters and reduce infections related to these devices is to interfere with this cell-cell communication that gives microorganisms the capacity of organizing themselves in complex biofilms that protect them from the host immune system and provide more resistance to antimicrobials.

Keywords Catheter; infections.

1. Introduction

Catheters have been very useful in clinical practice since they allow rapid access to the bloodstream and can be used for numerous purposes such as the administration of endovenous fluids and medication, as well as for total parenteral nutrition and hemodialysis. However, these devices are not free from risks of mechanical and infectious complications.

Catheter-related infections constitute one of the main causes of primary nosocomial bacteremia [1]. Catheter-related bloodstream infections (CRBSI) are responsible for over 60% cases of nosocomial bacteremia in European hospitals. The high rates of CRBSI associated with the increasing resistance rates make these infections particularly worrying [2]. Both cases of local infection, evidenced by the catheter colonization, and cases of systemic infection, which occur as a direct result of the presence of catheter, are considered to be related to venous catheter.

Several conditions have been indicated as risk factors for the development of CRBSI, such as catheterism duration, cutaneous colonization at the catheter introduction site, frequent handling of the venous line, its utilization to measure central venous pressure, type of used bandage, basic disease, and severity of the clinical condition [3]. Associated with those factors, immature immunological mechanisms and use of invasive life support systems make newborns (NB), especially premature ones, particularly susceptible to these infections. Approximately 20% premature neonates with very low birth weight (inferior to 1,500g) experience a systemic infection during their initial stay in the hospital [4].

Pessoa-Silva et al. [5] carried out an epidemiological study about nosocomial infections related to neonates in Brazil and reported that, similarly to pneumonia, bloodstream infections constitute the main nosocomial infections affecting neonates, which becomes further worrying for neonates with very low birth weight. Bloodstream infections related to the use of central venous catheters (CVC) reached in that study the rate of approximately 35% diagnosed cases.

According to Pessoa-Silva et al. [5], 60% deaths among children in their first year occur during the neonatal period. Mortality is three-fold higher among neonates and premature children with very low birth weight who develop sepsis than among those who do not develop it. In fact, sepsis is responsible for approximately half of the deaths of neonates and premature babies with very low birth weight on average in the second week of life [4].

Based on the importance of CRBSI, this literature review aims to address the pathogeny, etiology, diagnosis and prevention of such infections, with emphasis on catheter-related infections in neonates.

2. Pathogeny and etiology of vascular catheter-related bloodstream infections

The four routes by which microorganisms can access intravascular catheters are: extraluminal, intraluminal, hematogen or by contaminated infusions. The skin is the main source for short-term catheter colonization and infection. The bacteria that are in the patient’s skin migrate over its surface, colonizing the distal extremity, which results in infection [6]. However, these microorganisms can also colonize the catheter inner surface, to which they adhere and can be incorporated in a biofilm that allows the support of local infection and hematogenic spread. This intraluminal colonization can occur through the handling of the connection and opening of the system by the patient or by health professionals. When catheters are used for long periods, intraluminal colonization is higher than the
extraluminal colonization. On the other hand, contamination by hematogenic route or by infusion of contaminated substances is relatively uncommon [7, 8].

The catheter material and the intrinsic virulence of the infecting microorganism are important pathogenic factors. Catheters made of PVC and polyurethane are less resistant to microbial adherence than those made of Teflon® or silicon. Irregularities on their surface favor the adherence of several microorganisms such as Coagulase-Negative Staphylococci (CNS), Acinetobacter calcoaceticus and Pseudomonas aeruginosa [9]. In addition, certain materials are more thrombogenic than others, which can also predispose to catheter colonization. S. aureus can adhere to fibrinectin, produced by the patient, which recovers the device. Furthermore, the extracellular polysaccharide (biofilm) produced by CNS and Candida species can act as a barrier to phagocytosis and block the penetration of antibiotics [9].

Gram-positive bacteria are the main agents isolated in these infections and CNS prevail, according to a survey performed by the National Nosocomial Infections Surveillance (NNIS) between 1992 and 1999 [10], with 37% of the cases, followed by Enterococcus with 13.5%, of which 25.9% were resistant to vancomycin. S. aureus is also important, corresponding to 12.6%, and more than half of them are resistant to oxacillin. Gram-negative bacilli correspond to 14% of the isolated microorganisms, highlighting the increasing participation of enterobacteria that produce extended-spectrum β-lactamase (ESBL). Candida spp. represents 8% of the findings and C. albicans, 48% cases, while C. parapsilosis, C. glabrata and C. krusei have shown increasing resistance to fluconazole, which was also observed for 10% of the isolated C. albicans.

The data shown in Table 1 indicate the etiology of bloodstream infections (BSI) from 1986 to 1989 and from 1992 to 1999, with higher frequency of CNS in both periods.

<table>
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<tr>
<td>CNS</td>
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<td>Staphylococcus aureus</td>
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<td>Enterococcus</td>
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<td>Gram-negative bacilli</td>
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<td>Klebsiella pneumoniae</td>
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<td>Candida spp.</td>
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Source: CDC [9].

The other types of intravascular catheters are: peripheral venous catheter (PVC), peripherically inserted central catheter (PICC), non-tunneled central venous catheter (this is the most frequently used CVC type), tunneled central venous catheter, totally implanted catheter (TIC), phlebotomy, pulmonary artery catheter and umbilical catheter. CVCs can be classified as to time of use, type of used material, type of implantation, presence or not of valves, and number of lumens and routes.

Over 90% CRBSI are associated with central vascular catheters [6]. An estimated 248,000 bloodstream infections occur in hospitals of the United States and a large part of these infections are believed to be associated with the presence of a CVC [11]. Approximately 20% to 40% CVC patients develop local infection, and 3% to 10% develop bacteremia. A large number of bacteremias are not considered to be related to catheter because CVC is not cultured and the physician, probably due to having personally experienced few cases of this entity, is not aware of this possibility [12]. CVC inserted into the jugular vein are associated with two and half-fold more infection than catheters inserted into the subclavian vein. The mortality rate attributed to the use of central venous catheter is 12% to 25%, prolonging hospitalization for 10 to 40 days and increasing the treatment costs by around US$ 33,000-35,000 per patient [13].
3. CRBSI in neonatal ICU

Early sepsis is defined as that manifesting within 72 hours of life and late sepsis as that manifesting after 72 hours. The higher susceptibility to infection during the neonatal period is due to both local and systemic deficiencies in the development of the NB defense system [14, 15].

The acquisition of neonatal microbiota is influenced by the maternal genital flora, the breast-feeding type, the people in contact, the environment where the child was born and kept, and the flora of objects and other NB in that environment. The normal colonization of NB starts during the birth with the contact with the mother and the environment until a balance is reached and the normal endogenous neonatal flora is established [16].

The skin is only mature at around 32 to 34 weeks of gestation; therefore, pre-term newborns (PTNB) are more susceptible to infections. Epithelial surfaces of the skin and mucosa of the gastrointestinal and respiratory tract can lose their integrity with the routine and aggressive handling in neonatal intensive therapy units (NITU) by means of venous punctures, installation of invasive devices, nasogastric tubes, endotracheal tubes and others, facilitating the entrance of microorganisms into the bloodstream. Due to the increased permeability and colonization by pathogens, the umbilical cord can also be the entrance to the infectious agent, especially because of its proximity to the circulatory system. Therefore, critically ill children who receive care in NITU have increased risk of acquiring nosocomial infection due to their immunological immaturity and a series of invasive diagnoses and therapeutic procedures [17].

BSI are the most frequent nosocomial infections among NITU patients [18]. The accumulated incidence increased as a result of the higher survival rate among newborns and the more frequent use of central venous catheters [19]. These infections in NITU significantly contribute to morbidity in the hospital and increased costs due to the prolonged hospitalization, constituting therefore the most common and severe complication related to intravenous catheters [18]. From 1992 to 2004, the incidence of bloodstream infections associated with catheters in pediatric ICUs, according to NNIS [20], was 6.6 per 1000 catheters/day. In NITUs, the infection rate is inversely proportional to the newborn birth weight, ranging from 9.1 per one thousand catheters/day in children with birth weight <1000 g to 3.5 per 1000 catheters/day in children with birth weight >2500 g (Table 2).

<table>
<thead>
<tr>
<th>Weight (g)</th>
<th>Average rate 1000 catheters/day</th>
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<tr>
<td>≤1000</td>
<td>9.1</td>
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<tr>
<td>1001 - 1500</td>
<td>5.4</td>
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<td>1501 - 2500</td>
<td>4.1</td>
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Source: NNIS [20].

The availability of a safe venous access that can remain during a prolonged period is very important for ICU children. CVC are preferably used in NITU for a longer period, differently from PVC, which is indicated for a short period and has limitations as to the type of medication that can be infused. Thus, in NITU, CVC is preferred since it allows the infusion of a broad range of medicines, as well as the infusion of parenteral nutrition, and can also be used for hemodynamic monitoring [21].

PICCs have been used in the treatment of neonates for almost three decades and have been the most frequently used devices due to their following advantages: rapid proceeding, the vein keeps visible after catheterization, irritating medication or that of high osmolarity can be safely administered and changed, allowing a better control of contamination risk. It must be also highlighted that it decreases the need of several peripheral puncture attempts, eliminating pain and psychological instability, and are recommended for patients who require access for more than seven days and less than three months [22].

On the other hand, central vascular access through umbilical vessels in many cases still remains the first option. The use of this catheter is frequently limited by the severe complications that can result, including thrombosis, vascular perforation, liver necrosis, cardiac perforation, necrotizing enterocolitis [23, 24]. However, they have their indications and can be very useful, especially in emergency situations (resuscitation) and for newborns of very low birth weight in the first days of life. Surgically inserted CVCs (phlebotomy) show higher risk of infection compared to the remaining types of catheters [23, 25].

Data about the risk of PICC-related bloodstream infection in hospitalized patients are limited. A prospective study carried out by Safdar et al. [26] shows that PICCs used in high-risk patients are associated with a CRBSI rate similar to that of conventional CVC placed at the internal jugular or subclavian veins (2 to 5/1,000 catheters-day), rather higher than the infection rates with PICCs used exclusively at outpatient clinics (around 0.4/1,000 catheters-day) and also superior to CRBSI rates with tunneled CVCs (around 1/1,000 catheters-day).

Using heparin and vancomycin infusion into CVC, Schwartz et al. [27] decreased the frequency of catheter-related bacteremia by vancomycin-sensitive microorganisms, which colonize the catheter lumen. In a study carried out by
Garland et al. [28], the prophylactic use of vancomycin-heparin to prevent CRBSIs in NITU markedly reduced the incidence of CRBSI in high-risk neonates and did not promote resistance to this antimicrobial but was associated with asymptomatic hypoglycemia. However, the routine use of antimicrobials in catheterized patients is not indicated by CDC since this method is limited by some factors such as the concern about selecting vancomycin-resistant Gram-positive cocci and the instability of vancomycin-heparin solution [9].

CNS are the agents frequently isolated in CRBSI in neonates, followed by S. aureus and Candida parapsilosis. Among CNS species, S. epidermidis has been most frequently isolated from infections in neonates and its main virulence factor is the capability of adhering to medical instruments and forming biofilms [29, 30, 31]. S. haemolyticus is the second most frequently isolated species among CNS, followed by the species S. hominis, S. lugdunensis, S. warneri and S. capitis [32, 33, 34, 35].

CNS are capable of remaining viable in the hospital environment and in medical equipment for weeks to months. The increased incidence of nosocomial bacteremia by CNS in neonates during the last decades has been associated with the increased survival of neonates with very low birth weight, as well as with their long permanence in the hospital environment and the increased development of resistance to antibiotics [28, 31, 36]. The higher frequency of this microorganism is due to its predominance in the flora of newborns. Currently, CNS are considered essentially opportunistic microorganisms that prevail from a number of organic situations to produce severe infections. However, the interpretation of positive hemocultures for CNS is particularly difficult because these microorganisms colonize the skin and mucous membranes during blood collection. Researchers have used a variety of clinical and laboratorial criteria to distinguish between contamination and bacteremia. Thus, the diagnosis of bacteremia has been done based on the clinical data of patients and on the isolation of identical microorganisms in two or more hemocultures.

### 4. Diagnosis of CRBSI

The diagnosis of CRBSI may be difficult since the inflammation at the catheter site is not always due to the infection and this local sign is absent in 70% of CVC-related bloodstream infections [37]. Although this infection is generally manifested by fever in ICU patients, from 80 to 90% of these fevers are not due to catheter-related infection. In many cases, the diagnosis of CRBSI is overestimated, resulting in unnecessary removal of the catheter and in abusive use of antimicrobials. It is estimated that 75 to 85% catheters are unnecessarily removed by adopting only clinical criteria [37].

Clinical markers show a poor correlation and are sometimes insufficient to establish the diagnosis due to their low sensitivity and specificity. Fever, associated or not with chills, is a highly sensitive but little specific marker, especially in severe patients, to predict whether the infection is related to vascular catheter in ICUs. Thus, microbiological tests can help in this diagnosis [38].

When the patient has bacteremia, the question is whether the catheter is a primary source of microorganisms present in the blood. The incapability of obtaining cultures and dealing with this issue can lead to a false-negative or false-positive diagnosis. A false-negative diagnosis can certainly increase morbidity, whereas a false-positive diagnosis can result in unnecessary removal of the catheter or inappropriate prescription of antibiotics. This result is particularly dangerous since it promotes the emergence of pathogens resistant to the antibiotics and generates exceeding costs [39].

To diagnose infections related to the use of catheter, some categories must be defined for the different infectious phenomena [9]:

- **Catheter colonization:** significant growth of a microorganism in quantitative (≥ 1000 CFU/ml) or semiquantitative (≥ 15 CFU) culture of the catheter tip, subcutaneous segment or connection; this phenomenon does not imply bacteremia or require treatment.

- **Local infection:** isolation or not of the microorganism and can be associated with erythema, edema and purulent exudate, together with other symptoms and signs of infection such as fever, with or without the presence of infection of the concomitant bloodstream.

- **Catheter-associated systemic infection:** Bacteremia or fungemia in patients with catheter showing one or more positive peripheral hemocultures, with clinical manifestation of infection (fever, hypothermia, apnea, bradycardia or shock signs) and without other apparent source of infection besides the catheter, showing the same microorganism in the catheter and in the hemoculture in the microbiological diagnosis.

The reference of CRBSI diagnosis methods is based on catheter tip culture techniques after its removal. However, only 15-25% CVC removed due to a suspect infection are proven to be really infected, and the diagnosis is always retrospective. This is the reason why new diagnostic techniques that do not require catheter removal were developed [40].
4.1 Diagnosis methods based on catheter removal

The most common microbiological techniques are those based on catheter removal: qualitative culture, semiquantitative culture (Maki) and quantitative culture. The qualitative procedure is based on the catheter tip culture in liquid medium, in which turbidity indicates a positive result. This method is not in current use due to its low specificity (75%) in spite of its high sensitivity (100%) [41, 42].

The semiquantitative method proposed by Maki et al. [43] is still an international reference of diagnosis and has been used as standard in any study assessing different diagnosis methods. This method consists in rolling the distal tip of the catheter (3-5 cm) on the blood agar plate surface at least three times. Growth ≥ 15 colony forming units (CFU) indicates significant catheter colonization [44]. Assessing the best cutoff point for CRBSI diagnosis in neonates at the Neonatal Unit of the Teaching Hospital of Botucatu Medical School, Marconi et al. [45] reported that the cutoff point of 122 CFU shows 91% sensitivity, 81.1% specificity, 41.7% positive predicted value (PPV) and 98.4% negative predictive value (NPV), compared to 91.0% sensitivity, 71.6% specificity, and 32.3% PPV of the cutoff point of 15 CFU recommended by CDC [9].

To prevent the lack of detection of microorganisms present in the catheter lumen, a culture system was developed by Cleri et al. [46] so that quantitative cultures are obtained by washing the inner part of the catheter with culture medium. The number of colony forming units is determined by the seeding of dilutions of this broth and the counting of the number of colonies. Those authors defined the value ≥ 10^3 CFU/ml as significant based on the comparison of these results with the evidence of catheter-related bacteremia. This procedure was modified by Brun-Buisson et al. [42] for routine use, which consists in washing the inner surface of the catheter tip with 1.0 ml sterile water, followed by agitation in vortex for 1 minute and seeding of a 100 µl aliquot onto the surface of a blood agar plate. The counting of 100 colonies (cutoff point ≥ 10^3 CFU/ml) at 37°C for up to 72h indicates significant catheter colonization. This test showed, in a meta-analysis, 100% sensitivity, 92% specificity, 45% PPV and 100% NPV [26]. The predictive value of these cultures may vary according to the type and the site of the endovenous device and the colonization source. For those inserted for over one week, in which the bacterium intraluminal dissemination occurs from the catheter connection, the semiquantitative method is less sensitive compared to quantitative methods that detect samples from the inner and outer surfaces of the catheter [47].

A disadvantage of these methodologies of semiquantitative and quantitative culture is the need of catheter removal to prepare the culture; thus, other CRBSI diagnosis methodologies that do not require removal of the device must be considered. The most promising methods are those allowing the access maintenance. This advantage is particularly marked in critically ill patients, with difficult access, and those with long-term catheters. This has led to the use of new techniques for CRBSI diagnosis without catheter removal.

4.2 Methods based on the catheter maintenance

The procedures for CRBSI evaluation without catheter removal include the intraluminal brushing of the catheter proposed by Markus et al. [48], in which a sterile brush is passed on the intraluminal part of the catheter. This brushing can capture biofilm, microorganisms and fibrin thrombi. The few studies about this technique have shown results that disagree as to sensitivity, specificity and risks for the patient [49].

Rusforth et al. [50] described a new technique in 1993, in which approximately 1 ml blood is aspirated by the catheter, placed inside a tube containing 1.2 ml hypotonic saline solution and subjected to centrifugation. Then, the supernatant is discarded, stained in a slide with acridine orange (0.001%) and analyzed under ultraviolet light for visualization of microorganisms. This is a simple, rapid (30 min) and cheap technique that shows sensitivity, specificity, PPV and NPV of 87%, 94%, 87% and 93%, respectively, according to a meta-analysis done by Safdar et al. [26].

Differential quantitative hemocultures from samples simultaneously collected from the catheter and a peripheral vein are also used for CRBSI diagnosis. This method is considered positive when cultures are positive in both samples and the concentration of microorganisms present in the culture of the sample collected from the device is 3-to-5-fold higher than the culture of the peripheral sample. On the other hand, when the source of bacteremia is not the catheter, the count of microorganisms is similar in both samples. The sensitivity of this method varies from 79 to > 80% and its specificity, from 94 to 100% [51, 52]. This technique may facilitate the monitoring of the antibacterial treatment efficacy, which must be done if the catheter is left at the site; however, hospitals generally do not have methodologies for quantitative hemocultures and this technique is not routinely used in the clinical practice [53].

Another methodology that can be adopted without catheter removal was described by Blot et al. [54], in which the differential time to positivity (DTP) of paired samples, collected through the catheter and by venous puncture, indicates CRBSI. DTP is obtained from methods of continuous monitoring of microorganism growth in the blood (BACTEC; BactAlert). This method is considered positive for CRBSI when the blood sample collected from the catheter becomes positive 2 hours before the hemoculture collected by the peripheral vein. The value of this technique is probably higher in long-term CVC patients since the endoluminal route is the predominant route of colonization of these catheters, whereas in short-term CVCs the catheter outer surface prevails as the colonization and infection route. Another frequent issue is the need of collecting samples from all routes of the CVC when it shows multiple lumens. Dobins et al. [55] studied triple-lumen CVs and noted that random collection of only one of the lumens was detected in only 60%
colonized and CRBSI-associated CVCs. Therefore, for those authors, each lumen should be considered a possible source of CRBSI. In a study carried out by Safdar et al. [26], this method showed sensitivity and specificity of 94% and 99%, respectively, and PPV and NPV of 94% and 91%, respectively.

5. Genotypic methods in CRBSI diagnosis

To elucidate CRBSI, it is essential to determine the similarity among microorganism strains isolated from catheter tips and hemocultures. Biochemical tests can determine the microorganism genus and species, and the antibiogram can determine the similarity between samples isolated from the catheter and the hemoculture based on the resistance profile of the bacterium present in the samples, helping not only in the treatment of CRBSI, but also in the diagnosis of these infections. In a study comparing molecular techniques and microbiological methods in the determination of sources of nosocomial infections, Martin-Lozano et al. [56] assessed the utility of the antibiogram for epidemiological studies and concluded that the diagnosis of the source of bacteremia using conventional clinical and/or microbiological criteria, including antibiogram, are not always accurate and sufficient.

The search for methodologies with more refined specificity and sensitivity capable of establishing genetic relationships between isolates from catheters and hemocultures for CRBSI diagnosis has led to the emergence of molecular typing techniques that can represent an additional discriminating potential, especially for infections in which the pathogen may be part of the normal microbiota. Furthermore, the understanding of relationships between these microorganisms is essential to elucidate CRBSI.

Molecular typing systems can be employed for outbreak investigation, confirmation and design of patterns of transmission of one or more clones, tests of hypothesis about the origin and vehicles of transmission of these clones and monitoring of their reservoirs. Molecular analysis is useful to perform epidemiological surveys and evaluation of control measures by means of documentation, occurrence of certain strains over time and circulation of clones among infected populations [57, 58].

The application and the interpretation of microbial typing tools in epidemiological studies require the understanding of the limitations of techniques. In addition to reliability, a technique is considered valid when its capability of discriminating between strains is satisfactory, allowing a biological basis for grouping strains with apparently distinct types [59]. There are several molecular techniques based on the differentiation of patterns of genotypic profiles analyzed in total DNA samples or digested through enzymes that fragment the DNA in different parts and that can be analyzed by means of their different restriction profiles. However, the profile patterns are clearly complex and the isolates need to be separated by electrophoresis [60].

Based on the principles of polymerase chain reaction (PCR), the advance of molecular typing was facilitated, and several other techniques arose: RAPD-PCR (Random Amplified Polymorphic DNA-based PCR), REP-PCR (Repetitive Extragenic Palindromic Sequence-based PCR), ERIC-PCR (Enterobacterial Repetitive Intergenic Consensus Sequence PCR), AFLP (Amplified Fragment Length Polymorphism), and most recently the technique of Sau-PCR (Restriction Endonuclease Sau3AI Fragment Polymorphism), which can be used in the analysis of bacterial polymorphism.

In 1984, Schwartz and Cantor [61] described pulsed-field gel electrophoresis (PFGE) by introducing a new method of separation of DNA, so that the limiting size for DNA separation in agarose was extended from 30-50 kb to more than 10 Mb (10,000 kb). Instead of cloning a large number of small DNA fragments, PFGE allows the cloning and analysis of a smaller number and great dimensions of parts of a genome. Pulsed-Field Gel Electrophoresis (PFGE) is the most used tool for molecular typing in the world and is considered the method of choice for several microorganisms such as methicillin-resistant S. aureus (MRSA) [62]. In general, PFGE is the most reproducible and discriminatory molecular typing technique available. The greatest difficulties related to PFGE are its difficult implementation and the initial cost of the equipment. The preparation of a suitable genomic DNA sample requires on average 1 to 3 days, depending on the species analyzed by means of their different restriction profiles. However, the profile patterns are clearly complex and the isolates need to be separated by electrophoresis [60].

Gori et al. [64] carried out a comparative study between the techniques RAPD (Random Amplified Polymorphic DNA) and PFGE for the typing of Klebsiella pneumoniae ESBL strains and verified that both techniques showed concordance of results and discrimination of epidemiologically related groups of these strains. More subclonal variants between the epidemic clones were determined by PFGE than by RAPD. Thus, both are useful for typing K. pneumoniae strains in epidemiological investigations; however, RAPD showed to be more efficient as to viability and cost. These results corroborate those obtained for P. aeruginosa strains by Renders et al. [65], who concluded that RAPD can be reliably used as the first triage in epidemiological studies and not as the only microbial typing technique; however, the capability of evaluating molecular evolution rates depends on the choice of restriction enzymes and primers for both RAPD and PFGE. Other studies have recommended the use of both RAPD and PFGE techniques in order to increase the discriminatory potential [66].

The REP-PCR method employs specific primers that bind to multiple non-codi fying repetitive sequences (generally 30 to 500bp) spread over the bacterial genome, constituting a strategy established for the classification of subspecies and for the design of bacterial strains. The multiple DNA amplicons of different sizes and of several quantities (intensities) are generated during PCR [67, 68].
Studies comparing the methods RAPD-PCR and REP-PCR have concluded that the validity of the molecular epidemiology method depends on the studied microorganism; according to Cartelle et al. [69], for *Acinetobacter baumannii* samples, the best typing technique was REP-PCR.

SAU-PCR is a technique based on the digestion of genomic DNA with the restriction endonuclease Sau3AI and subsequent amplification with a sequence of primers that is based on the recognition of Sau3AI. The method was tested in lactic bacterial strains but could be proposed for almost all culturable organisms. SAU-PCR can be considered a possible alternative for RAPD when reproducibility and polymorphism levels are not satisfactory and a more economically viable alternative when the approach is the use of restriction and amplification enzymes, relative to the technique AFLP. Its binding site, GATC, is of 4 bp of length, with G+C percentage values of 50, which makes it capable of producing a large number of small fragments of total DNA digestion for most microorganisms. This enzyme generates sticky ends with 4 base pairs at extremities. In addition, all primers have 7 long-chain nucleotides CCGCGGC at extremity 5’, which does not participate in the initial annealing (low stringency phase) with the aim of providing, through the use of C and G only, an annealing temperature conveniently high for the amplification of successive cycles of high stringency [70].

The technique ERIC-PCR is based on the analysis of repeated chromosomal sequences and has been used for the clonal characterization of different enterobacteria species and for the study of genetic relationship among isolates. However, ERIC-PCR has been successfully used for the clonal differentiation of the strains Shigella sonnei, Salmonella typhimurium and Vibrio cholerae [71, 72], as well as for the study of genetic variability of *E. coli* pathogenic to birds [73].

Studies comparing the methods RAPD-PCR, REP-PCR and ERIC-PCR have concluded that the validity of the molecular epidemiology method depends on the studied microorganism; according to Cartelle et al. [69], although ERIC-PCR and RAPD-PCR show the same numbers of genotypes for *Klebsiella pneumoniae*, ERIC was the best PCR-based method for typing this microorganism; however, for *Acinetobacter baumannii* samples, the best typing technique was REP-PCR. The technique REP-PCR has great discriminatory and reproducible capacity relative to PFGE for several enterobacteria species and also for some yeasts [67, 69, 74].

An excellent correlation was obtained between the results of analyses with RAPD-PCR and ERIC-PCR for the typing of *Aeromonas* spp., and all strains had the same profile in both RAPD-PCR and ERIC-PCR analyses, confirming the good discriminatory potential of these techniques for this species. However, those authors do not recommend REP-PCR for *Aeromonas* spp. typing since it did not allow analyzing all strains, showing the presence of a small number of amplified products, which suggests that repetitive extragenic palindromic sequences may not be widely distributed over *Aeromonas* spp. genome [75].

6. New perspectives in the control of catheter-related infections

The biofilm is responsible for most bacterial infections and for 65% vascular catheter-related infections. Biofilm formation was considered a result of adhesion, formation of microcolonies and production of extracellular polysaccharides by the microorganisms. After 24 hours of insertion, most CVC can become colonized by microorganisms involved in a biofilm over the catheter surface and after eight days of permanence, inside the catheter lumen. In some cases, microorganisms will proliferate at a sufficient number, resulting in CRBSI. In this context, the determination of biofilm production by microorganisms isolated from these hemocultures collected from the catheter can contribute to deciding whether to remove the device [76].

Nowadays, several methods have been used to detect biofilms in *Staphylococcus* besides, as a complement, molecular methods (PCR) which provide direct evidence of genetic bases of biofilm production. Among the available methods, the simplest ones that could be used in the routine of a clinical laboratory are qualitative methods such as that described by Christensen et al. [77] and the Congo Red Agar (CRA) method described by Freeman et al. [78]. The most commonly used method is that of tube adherence described by Christensen et al. [77]. Several authors have used this method, which showed to yield reliable results and to be efficient with excellent sensitivity and appropriate specificity, leading to reliable and suitable diagnosis for routine use [76].

Several regulating systems are involved in the determination of *S. aureus* virulence; of all these existing regulatory systems, the most characterized are *agr* and *sar* [79]. The system *agr* includes two operons transcribed in opposite direction. The operon transcribed from P2 codifies the molecule RNA II, which regulates the expression of genes *agrB, agrD, agrC* and *agrA* which, interacting with the system *sar*, controls the transcription of a mRNA called RNAIII which in turn controls the expression of *S. aureus* virulence genes. In the logarithmic phase of bacterial growth there is the expression of genes that codify adesines, whereas in the stationary phase the RNAIII molecule inhibits the expression of genes that codify adesines and induces the expression of genes that codify toxins. The system *agr* is controlled by the quorum-sensing, a language used by bacteria which, when at high cell density, can “feel” the best moment to express virulence factors and assure an efficient joint attack to the host. The principle of quorum-sensing is simple, when a single bacterium releases an auto-inducer in the environment, its concentration is too low to be detected; however, when a sufficient number of bacteria is present, the concentration of auto-inducers reach a level sufficient to make cells respond to the stimulus, activating or inhibiting target genes.
A new form of preventing colonization and biofilm formation by *S. aureus* and *S. epidermidis* is to interfere with this cell-cell communication which leads to the virulence phenotype. RNAIII synthesis is regulated by a mechanism of quorum-sensing and can be inhibited by a RNAIII-inhibiting peptide (RIP). The latter is a linear peptide of seven amino acids produced by coagulase-negative staphylococci (suggested to be *S. warneri* or *S. xylosus*) and has the sequence YSPXTNF, in which X may be a cysteine, a tryptophan or a modified amino acid [80, 81, 82]. Both natural RIP and a synthetic analogous YSPXTNF are extremely effective in inhibiting *in vitro* and *in vitro* RNAIII synthesis. Studies have shown that when *S. aureus* is pre-incubated in the presence of RIP, the pathogenic potential of the bacterium is markedly reduced. Several studies with animal models have verified strong activity in the prevention of infections by *S. aureus*, including those caused by drug-resistant strains such as methicillin-resistant *S. aureus* (MRSA) and vancomycin intermediate *S. aureus* (VISA) [80, 81, 82].

These results support the hypothesis that RIP could be used to cover catheters in order to prevent colonization and consequent infections related to these devices. The prevention of biofilm formation by RIP can therefore not only decrease the infection rate, but also preserve the susceptibility of bacteria to antibiotics [76].

### 7. Conclusions

CRBSI are responsible for a large number of nosocomial bacteremia cases and constitute one of the main infections affecting premature and low weight neonates. Most vascular catheter-related bloodstream infections (CRBSI) are caused by Coagulase-Negative Staphylococci (CNS), while the pathogenesis of these infections is complex and suggested to be directly related to the adherence of microorganisms, which become capable of colonizing the device, forming the biofilm, which potentiates its pathogenicity.

The definitive diagnosis of CRBSI is established when the catheter is significantly colonized with the same microorganism found in the hemoculture. It must be emphasized that the lack of standardization of clinical criteria, diagnoses and the diversity of concepts of infection compromise the system of epidemiological surveillance of infections and impairs the generalization of results of the performed studies. Clinical markers show a poor correlation in CRBSI and are generally not sufficient to establish the diagnosis; thus, the use of microbiological techniques is of great importance to confirm this type of infection. Molecular typing can represent an additional discriminatory potential, especially for infections in which the pathogen can be part of the normal microbiota, and can provide the understanding of relationships between these microorganisms, which is essential to elucidate CRBSI.

The training and education of the health team, approaching techniques in the passage, care in the maintenance and insertion of vascular catheters, as well as the rational use of antimicrobials and attention to protocols of catheters use, is of great importance to prevent CRBSI.

The hypothesis that a RNAIII-inhibiting peptide (RIP) can be used to cover catheters in order to prevent biofilm formation and consequent infections related to these devices is a future perspective to help prevent CRBSI. New studies about CRBSI prevention are needed to elucidate still unanswered issues, to help taking decisions based on the existing controversies and to support the implementation of new technologies and their application in the clinical practice, which will be certainly reflected in the quality of treatment to patients with central venous catheters.

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### References


