Molecular markers of resistance in coagulase-negative staphylococci implicated in catheter-related bloodstream infections

Wilhelmina Strasheim[1,*], Marleen M. Kock[1,2], Andries W. Dreyer[2] and Marthie M. Ehlers[1,2]

[1]Department of Medical Microbiology, Faculty of Health Science, University of Pretoria, Private Bag x323, Arcardia Pretoria, 0007, Gauteng, South Africa
[2]Department of Medical Microbiology, Faculty of Health Science, University of Pretoria, National Health Laboratory Services, Tshwane Academic division (NHLS/TAD), Gauteng, South Africa
[*]Corresponding author: Miss W Strasheim wilkestrasheim@gmail.com

Coagulase-negative staphylococci (CoNS) have emerged as important opportunistic pathogens in healthcare facilities, specifically in patients with invasive medical devices. Diagnosis of intravascular catheter-related infections caused by CoNS is daunting, since the clinical significance of CoNS isolated in the diagnostic laboratory is obscured. Intravascular catheter-related infections are also difficult to treat, since CoNS in healthcare facilities often display multidrug resistance.

Keywords coagulase-negative staphylococci; intravascular catheter-related infections; antimicrobial-resistance

1. Introduction

Intravascular catheters play an irreplaceable part in critical care medicine [1]. The usage of intravascular catheters does, however, pose a significant risk to patients for developing a catheter-related infection [1, 2]. An intravascular catheter-related infection is a costly healthcare-associated infection, with a poor prognosis, leading to significant morbidity and mortality [3, 4].

The leading cause of intravascular catheter-related infections is coagulase-negative staphylococci (CoNS) [5, 6]. Coagulase-negative staphylococci had long been thought to be clinically insignificant when isolated in the diagnostic laboratory, since these bacteria form part of the normal skin microflora and can easily contaminate clinical specimens [7, 8]. Extreme difficulty is experienced in separating CoNS contaminants from infecting CoNS strains and no molecular markers currently exist to predict the bacteria's clinical relevance [9-11]. A number of diagnostic strategies have been developed (roll-plate method, sonification, differential time to positivity, paired quantitative blood cultures) to diagnose intravascular catheter-related infections and to evaluate the clinical significance of CoNS [12].

Treatment of intravascular catheter-related infections caused by CoNS is complicated. Coagulase-negative staphylococci colonise catheters through biofilm formation and biofilms are intrinsically resistant to antimicrobials [7, 13]. In addition, CoNS can become resistant to a number of antimicrobials through various resistance mechanisms leading to therapeutic failure. The purpose of this mini-review is to provide background knowledge on CoNS from a microbiological viewpoint, to discuss CoNS' association with intravascular catheter-related infections and to summarise the antimicrobial-resistance mechanisms described in CoNS.

2. Historical background on intravascular access and its association with staphylococci

In 1929, when Werner Forssmann first pierced his own left antecubital vein and inserted a central ureteric catheter, centrally into his right atrium, he would not have been able to believe that the presence of his breakthrough invention will be associated with the leading cause of healthcare-associated infections in the next century [5, 14]. Even though Forssmann's invention led to his dismissal as German surgeon, his work received widespread attention, which led to the refinement of his technique and ultimately resulted in the mass production of intravascular catheters in the 1950's [14].

Staphylococci were already discovered in 1878 by Louis Pasteur and Robert Koch, followed by more in depth studies by Ogston and Rosenbach [15, 16]. Ogston derived the *Staphylococcus* genus name, while Rosenbach was the first to culture staphylococci [16]. The culturing of staphylococci allowed Rosenbach to differentiate staphylococcal species on the basis of colonial pigmentation [16]. Rosenbach noted that the more pathogenic staphylococci produced golden colonies, whereas the less pathogenic staphylococci formed white colonies and named the bacteria, *Staphylococcus aureus* and *S. albus* (now *S. epidermidis*), respectively [16]. Later studies during 1953 showed that coagulase production provides a better correlation with pathogenicity [17]. The grouping of staphylococci as pathogenic (coagulase-positive) and non-pathogenic (coagulase-negative) led to CoNS not receiving much attention [18].

The initial reports regarding the pathogenicity of CoNS in septicaemia studies occurred about the same time (late 1950s) when intravascular catheters were established as a vital component in medical care [14, 18, 19]. Yet, it was only during the late 1970s that the association between CoNS infection and the presence of an intravascular catheter were suspected [18, 19].

3. Intravascular catheters and associated infections

Intravascular catheters are used for the care of critically ill patients requiring additional vascular access for the administration of fluids, antimicrobial therapy, total parental nutrition and renal replacement therapy [1]. In order to accurately define an intravascular catheter, the following aspects should be mentioned: i) the type of vessel it occupies (peripheral venous vs. central venous vs. arterial), ii) it's intended life span *in situ* (temporarily short-term vs. permanent long-term), iii) the insertion site (subclavian, femoral, internal jugular, peripheral and peripherally inserted central catheter), iv) the pathway from insertion site to the type of vessel it occupies (tunnelled vs. non-tunnelled), v) the physical length of the catheter line (long vs. short) and vi) any special characteristic of the catheter (e.g. the presence/absence of a cuff, impregnated with antimicrobials/antiseptics/heparin, the number of lumens) [20, 21].

Terminology (catheter-related bloodstream-infections (CRBSI) vs. central-line associated bloodstream-infections (CLABSI)) used to describe intravascular catheter-related infections are confusing and it is important to distinguish between a CLABSI and a CRBSI [21]. A CLABSI is a surveillance term, used by the Centres of Disease Control and Prevention's National Healthcare Safety Network [21, 22]. Central-line associated bloodstream-infections can be defined as a primary bloodstream infection (BSI) in patients that had a central-line within a 48 hour period, before the development of the BSI and when the BSI is not related to an infection at another site [21]. A CRBSI is a clinical term used during patient diagnosis and treatment, which requires specific laboratory testing (Section 7) in order to identify the catheter as the source of the BSI [21]. There are three types of CRBSI involving: i) catheter colonisation, ii) CRBSI and iii) exit site infections [1, 23]. Each type of infection has different microbiological criteria used in diagnosis, which will be discussed later in this review (Section 7) [23, 24].

Catheter colonisation can be defined as a positive catheter distal tip culture, which generate a significant number of bacteria, according to the culture method used [20, 24, 25]. Catheter colonisation occurs via six recognized routes: i) extraluminal arising from the catheter insertion site (the source associated with short-term catheters), ii) intraluminal via the catheter hub or the catheter tubing connection (the source associated with long-term catheters), iii) haematogenous seeding from another area of infection, iv) infusate contamination, v) impactation of the catheter's distal tip with skin microflora at the time of insertion and vi) direct contact with contaminated fluids, hands of patients or during catheter manipulations [20, 21, 25-27]. The majority of CRBSIs are associated with both extraluminal and intraluminal colonisation [27]. An exit site infection can be described as a localised infection of the skin and soft tissue, around the catheter insertion (exit) site [25]. Erythema, purulence, induration and tenderness are often present [20, 24, 25]. The true definition of a CRBSI involves a systemic infection ranging in severity (from minimal to life-threatening) and diagnosis requires the isolation of the same microorganism from a catheter tip culture and from the blood of a patient, with clinical signs of sepsis and without another apparent source of infection [20, 24, 25].

The range of causative organisms associated with CRBSI are: i) CoNS, predominantly *S. epidermidis*, followed by *S. haemolyticus*, ii) *S. aureus*, iii) methicillin-resistant *S. aureus* (MRSA), iv) *Candida* species (spp.), v) *Enterococcus* spp., vi) *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*) and vii) *Pseudomonas aeruginosa* [5, 21, 24, 25, 28]. In the remainder of this mini-review only CoNS, as causative organisms of intravascular catheter-related infections, are discussed.

4. Coagulase-negative staphylococci from a microbiological viewpoint

4.1. Classification of human-associated staphylococci

Among the universal tree of life, based on ribosomal RNA gene sequencing, staphylococci branches from the *Bacteria* domain [29]. In the *Bacteria* domain, as shown in Table 1, the *Staphylococcus* genus can further be classified as *Eubacteria*, with a low G+C content (33%) and is closely related to the *Enterococcus*; *Streptococcus; Lactobacillus* and the *Listeria* genera [15].

Table 1 The *Linnaeus* classification of staphylococci [15].

Taxonomic order of life	Order in which human-associated staphylococci group
Domain	*Bacteria*
Kingdom	*Eubacteria / Gram-positive bacteria*
Phylum	*Firmicutes*
Class	*Bacilli*
Order	*Bacillales*
Family	*Staphylococcaceae*
Genus	*Staphylococcus*
Species	*Epidermidis*
Binomial nomenclature	*Staphylococcus epidermidis*

Staphylococci can further be classified, based on DNA-DNA hybridisation studies, according to coagulase production and novobiocin susceptibility, as shown in Table 2 [15].

Table 2 The classification of staphylococci according to coagulase production and novobiocin susceptibility based on DNA-DNA hybridisation studies [15, 30, 31].

	CoNS	**CoPS***
Novobiocin susceptible species	*S. epidermidis*; *S. capitis*; *S. caprae*; *S. haemolyticus*; *S. hominis*; *S. lugdunensis*; *S. saccharolyticus*; *S. warneri*	*S. aureus*; *S. intermedius*
Novobiocin resistant species	*S. saprophyticus*; *S. sciuri*	-

*coagulase-positive staphylococci

Coagulase-negative staphylococci (e.g. *S. epidermidis*, *S. capitis*, *S. caprae*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, *S. warneri*) are distinguished from coagulase-positive staphylococci (e.g. *S. aureus, S. intermedius*) by not producing the coagulase enzyme, whereas novobiocin-susceptible staphylococci are mostly associated with human disease, except *Staphylococcus* subspecies (subsp.) *novobiosepticus* and. *S. saprophyticus* [7, 15, 32].

4.2. General characteristics of staphylococci

Staphylococci are all catalase-positive, non-motile, non-sporulated, facultative anaerobic Gram-positive cocci, occurring either singly, in pairs, in tetrads or in bunches [15, 30, 32, 33]. *Staphylococcus* species can be differentiated among each other based on i) cell wall composition, ii) haemolytic activity, iii) carbohydrate utilisation patterns and iv) anaerobic growth patterns in thioglycolate medium [34,35]. Staphylococcal species have a specific niche preference on the human skin as shown in Figure 1 [15, 19, 30, 36].

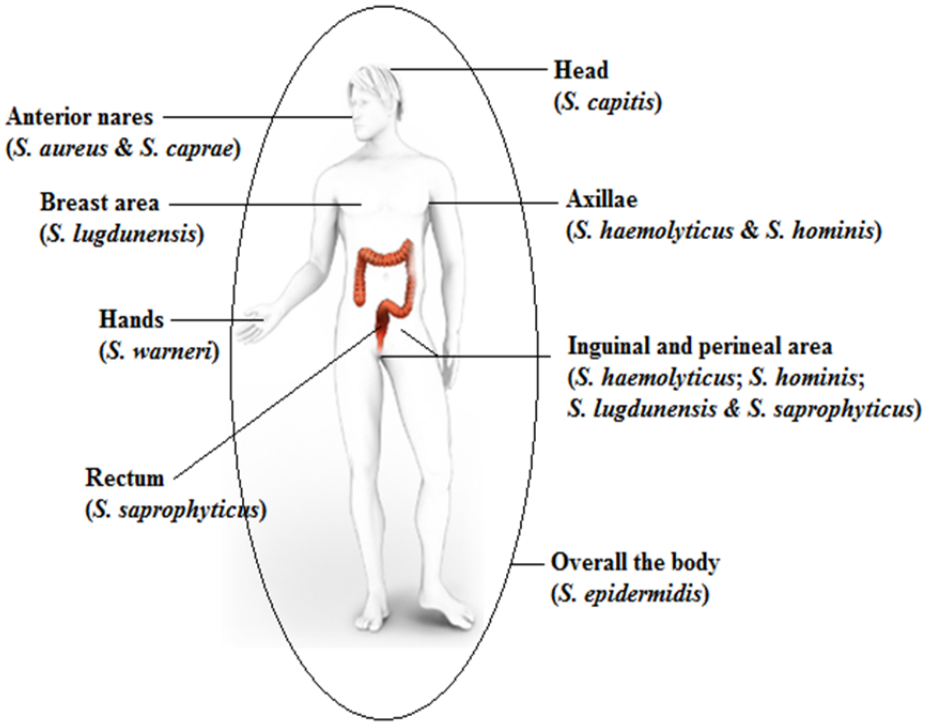

Fig. 1 Colonisation of pre-defined sites on the human body by staphylococci [19, 33, 37-39].

Staphylococcus epidermidis occurs in densities of 10^3 cells.cm^{-2} to 10^4 cells.cm^{-2} and is the most widely distributed staphylococcal species (90%) on the human skin. [30, 33]. *Staphylococcus capitis* populations are mainly concentrated on the human head and *S. warneri* can be recovered from human hands [19, 39]. *Staphylococcus haemolyticus* and *S. hominis* are most commonly recovered from the axillae, inguinal and perineal areas [19]. *Staphylococcus saprophyticus* can be isolated from the perineal area, specifically the rectum [19, 40]. *Staphylococcus aureus* and *S. caprae* are frequently isolated from the anterior nares [38]. It is important to distinguish between *S. aureus* and *S. caprae*, since *S. caprae* can mimic MRSA [38]. *Staphylococcus lugdunensis*, similar to *S. aureus*, has a predefined niche preference for the inguinal and breast areas [31, 37, 41].

4.3. Staphylococci as opportunistic pathogens

Coagulase-negative staphylococci are opportunistic pathogens and will only cause disease if the external barrier of the skin or mucous membranes have been breached [9, 28, 30]. Patients most at risk are the ones with intravascular catheters, as well as the immunocompromised, which include: i) premature neonates, ii) HIV-positive patients, iii) patients with malignant diseases, iv) post-chemotherapeutic or post-transplant patients and v) intravenous drug abusers [7, 9, 19, 42].

Infections caused by CoNS other than intravascular catheter-related infections include: i) bacteraemia, ii) vascular graft infections, iii) central venous shunt infections, iv) native valve and prosthetic valve endocarditis, v) cardiac pacemaker infections, vi) prosthetic joint infections, vii) surgical-site infections, viii) endophthalmitis (after surgery or trauma), ix) infections in neonates associated with umbilical catheters, x) osteomyelitis, xi) healthcare-associated urinary tract infections and xii) peritonitis and peritoneal-dialysis related infections [19, 28, 43, 44]. *Staphylococcus saprophyticus* is the only exception and is associated with community-acquired urinary tract infections in young, sexually-active women [19, 28].

5. Biofilm formation as molecular marker of resistance in CoNS

The ability of CoNS to result in an intravascular catheter-related infection is ascribed to biofilm formation on a catheter [7, 30, 42]. In CoNS, specifically *S. epidermidis*, biofilm formation is encoded by the *ica*ADBC operon [7]. It is advantageous for CoNS to reside in a biofilm. These bacteria are protected from the external environment, allowing microbial communication in the form of horizontal gene transfer and have enhanced virulence due to immune evasion [45, 46].

Biofilm formation is associated with a non-specific mechanism of antimicrobial resistance [36]. This can be explained by the physiological different states between planktonic cells and cells residing in a biofilm [9, 36, 47]. Antimicrobials target fast-growing cells, whereas cells residing in a biofilm have decreased transcription and translation rates, which result in a non-aggressive, but persistent, protected mode of growth, that is less sensitive to antimicrobials [9, 42]. Coagulase-negative staphylococci embedded in a biofilm are 100 to 1000 times more resistant to antimicrobials compared to these bacteria's cultured counterpart, which complicate treatment [48].

6. Antimicrobial-resistance genes in coagulase-negative staphylococci

Treatment of intravascular catheter-related infections caused by CoNS is in itself a clinical challenge [49]. Treatment involves the administration of oral or intravenous antimicrobials, antibiotic lock technique or the removal and re-insertion of the catheter [50]. According to the pathogen specific treatment recommendations of the Infectious Diseases Society of America, CRBSI caused by CoNS should be treated with antimicrobials for 5 days to 7 days if the catheter is removed and for 10 days to 14 days, if the catheter is retained, in combination with antibiotic lock therapy [51]. However, a CRBSI caused by *S. lugdunensis* should be treated according to the recommendations of *S. aureus*, which involve antimicrobial therapy for 4 weeks to 6 weeks [51]. Vancomycin forms the basis of empirical antimicrobial therapy of CRBSI caused by CoNS [50]. However, the efficiency of vancomycin monotherapy shows conflicting results and treatment should rather be a combination of vancomycin and other antimicrobials, with antibiofilm activity, such as rifampicin, gentamicin or clindamycin [50].

In order to effectively treat a CRBSI caused by CoNS, it is important to have a basic understanding of the antimicrobial-resistance mechanisms in CoNS, since these bacteria are resistant to multiple antimicrobials, which limits treatment options [52]. Antimicrobial-resistance mechanisms in CoNS will be explained in reference to the antimicrobial mode of action, which include: i) inhibition of cell wall synthesis, ii) inhibition of protein synthesis and iii) inhibition of nucleic acid synthesis [43].

6.1. Resistance against cell wall synthesis inhibitors

6.1.1. Resistance against β-lactams

Coagulase-negative staphylococci lack an outer membrane and β-lactams can diffuse straight through the cell wall and bind to penicillin-binding proteins (PBPs) [43]. Penicillin-binding proteins are transpeptidases, involved in the cross-linking of peptidoglycan, the main constituent of the Gram-positive bacteria cell wall [52]. A β-lactam antimicrobial prevents the cross-linking of peptidoglycan, which will result in a damaged cell wall and ultimately lead to cell lysis [43, 52].

The two main mechanisms of β-lactam-resistance in CoNS are i) degradation of β-lactams, specifically penicillin, by the production of an penicillinase enzyme, which is encoded by the *blaZ* gene and ii) the production of a modified PBP, called PBP2a, which is encoded by the *mec*A gene harboured on a mobile genetic element, called the Staphylococcal Chromosomal Cassette *mec* (SCC*mec*) [52, 53]. Methicillin-resistance among CoNS is of great concern, since CoNS

are more likely to be resistant to other antimicrobials and all other β-lactams, when harbouring the *mec*A gene [52, 54, 55]. Methicillin-resistance is widespread among healthcare-associated CoNS, with the exception of *S. lugdunensis* [7, 9]. Methicillin-resistance in *S. lugdunensis* is rare and the first-time detection of the *mec*A gene in *S. lugdunensis* were in 2003 [56]. A new mechanism of methicillin-resistance in *S. lugdunensis* was recently described by Kotsakis and colleagues (2011), which involve a mutational alternation of PBP1A/1B [57]. The alternation prevents the binding of β-lactams to the active site of the PBP1A/1B [57].

6.1.2. Resistance against glycopeptides

Glycopeptides are recommended as an empirical antimicrobial treatment option for CoNS infections and are used as a last resort, due to increased resistance to methicillin among CoNS [58, 59]. This makes the emergence of glycopeptide-resistance among CoNS of great concern [58]. Glycopeptides inhibit peptidoglycan synthesis by binding to precursor molecules, making these unavailable as substrates for transglycosylases and transpeptidase, two enzymes which are involved in cross-linking of peptidoglycan [59]. Vancomycin and teicoplanin are the two clinically used glycopeptides [59].

Although glycopeptide-resistance has first been described in CoNS, glycopeptide-resistance among enterococci received more attention, since it was less common among staphylococci [58]. The CoNS species mainly associated with glycopeptide-resistance are *S. haemolyticus* and *S. epidermidis*, but it has also been described in *S. warneri* and *S. capitis* [60]. Glycopeptide-resistance in CoNS applies nearly completely to teicoplanin, in contrast to *S. aureus* [61]. The mode of teicoplanin-resistance is not yet fully understood, but CoNS resistant to teicoplanin tend to show cell wall thickening and cellular aggregates [60, 61].

6.2. Resistance against inhibitors of protein synthesis

6.2.1. Aminoglycoside-resistance

Aminoglycosides, such as gentamicin and tobramycin, are increasingly being used in both treatment (alone or in combination with β-lactams or glycopeptides for life-threating staphylococcal infections, such as endocarditis) and prevention (incorporated in prosthetic materials in the form of coatings, cements or beads, for local antimicrobial delivery) of staphylococcal infections [62, 63]. Aminoglycosides prevents protein synthesis, by binding to the 30S ribosomal subunit, subsequently preventing attachment of mRNA [43, 62]. This interferes with the reading of the mRNA sequence, leading to the insertion of the wrong amino-acid or the inability to link amino-acids in the growing peptide chain [43, 63].

The main mechanism of aminoglycoside-resistance in CoNS is aminoglycoside modifying enzymes (AME) [62, 64]. Aminoglycoside modifying enzymes impairs the inhibitory protein-synthesis function and can co-exist among another [53, 62, 64]. The three most common AME in staphylococci are summarised in Table 3.

Table 3 The main AME prevalent in staphylococci and the mobile genetic elements and the phenotypic resistance characteristics associated with it [62-64].

Aminoglycoside modifying enzyme (AME)	**Gene which encodes AME**	**Mobile genetic element associated with AME**	**Antimicrobials to which this AME confers resistance to**
Bifunctional enzyme with aminoglycoside-6'-N-acetyltransferase and 2''-O-phosphotransferase activity	*acc*-(6')-*Ie-aph*(2'')	Tn*4100* in *S. aureus* *Tn4031* in *S. epidermidis*	Gentamicin; Tobramycin; Kanamycin Dibekacin; Netilmicin; Amikacin;
Aminoglycoside 3'-phosphotransferease	*aph*(3')-*IIIa*	Tn*3854* Tn*5404*	Neomycin; Kanamycin
Aminoglycoside 4'-adenyltransferase	*ant*(4')-*Ia*	pSK41	Neomycin; Kanamycin; Tobramycin; Amikacin

The most prevalent AME is *acc*-(6')-*Ie-aph*(2''), followed by *aph*(3'')-*IIIa* and *ant*(4')-*Ia* [53, 64]. Aminoglycoside modifying enzymes are all harboured on mobile genetic elements, such as plasmids and transposons, which can facilitate the rapid spread through horizontal gene transfer of aminoglycoside resistance determinants among closely-related species [53, 63, 64].

6.2.2. Resistance against macrolides, lincosamides and streptogramins

Macrolides, lincosamides and streptogramins (MLS) antimicrobials are grouped together, based on a similar mode of action, even though these antimicrobials are chemically different [65]. Inhibition of protein synthesis by MLS antimicrobials are achieved through binding to the 23S rRNA of the 50S ribosomal subunit [43, 65]. The number of

atoms forming the lactone ring of macrolides are used to classify this group of antimicrobials as either 14-membered (e.g. erythromycin), 15-membered (e.g. azithromycin) or 16-membered (e.g. spiramycin) [65]. Clinically used lincosamides include lincomycin and clindamycin, whereas streptogramins are a mixture of streptogramin A (e.g. dalfopristin) and streptogramin B (e.g. quinuprisitin) that act synergistically [65]. Macrolides, lincosamides and streptogramins are regarded as alternatives for the treatment of methicillin-resistant staphylococcal infections, which has led to MLS antimicrobials being prescribed more often [66, 67]. However, the increased usage of MLS antimicrobials has led to an increase in resistance [67, 68].

The three major mechanisms of MLS resistance among CoNS include: i) ribosomal target modification through methyltransferases encoded by the *erm* genes [65, 67], ii) active efflux pump encoded by the *msr* or the *vga* genes, [65, 67, 69] and iii) enzymatic antimicrobial inactivation encoded by either the *mph*C, *lnu*A, *vgb* or the *vat* genes [65-67, 69]. The predominant mechanisms of resistance to MLS antimicrobials are, however, the *erm* (target site modification) and the *msr* (active efflux pump) genes, while the other mechanisms are encountered infrequently in CoNS [69].

In order to prevent treatment failure, a clear understanding of the MLS_B resistance phenotype is necessary. A MLS_B resistance phenotype is the result of a conformational change in the ribosome, due to the methylation of an adenine at position 2058 in the V domain of the 23S rRNA, caused by the methyltransferase encoded by the *erm* (either A, B or C) genes [62, 68, 69]. The expression of the MLS_B resistance phenotype can either be inducible ($iMLS_B$) or constitutive ($cMLS_B$) [65, 67, 68]. Coagulase-negative staphylococci, with an $iMLS_B$ resistance phenotype are resistant to 14-membered and 15-memberd macrolides, but susceptible to lincosamides, streptogramin B and 16-membered macrolides, whereas CoNS with a $cMLS_B$ resitance phenotype, are resistant to all MLS antimicrobials [67]. Although, $iMLS_B$ CoNS are *in vitro* resistant to erythromycin and *in vitro* sensitive to clindamycin, prescribing clindamycin may lead to treatment failure [68]. The presence of the *msr* genes, which encodes an active efflux pump, only confers resistance to macrolides and streptogramins, but not to lincosamides in CoNS [68]. This makes clindamycin, as a treatment choice effective [68]. It is important to distinguish the two resistance mechanisms from another, to prevent unnecessary avoidance of clindamycin [68].

6.2.3. Resistance against the oxazolidinone, linezolid

Linezolid is the first member of the new synthetic oxazolidinone antimicrobial class [69-71]. Linezolid is currently used for the treatment of patients with serious methicillin-resistant staphylococcal infections, such as osteomyelitis or pneumonia [70, 72, 73]. Linezolid binds to the peptidyl-transferase centre, preventing the formation of the tRNA-ribosome-fMet-mRNA-complex, thus inhibiting protein synthesis [71, 74, 75].

Linezolid-resistance among CoNS is a rare phenomenon [71, 73]. However, linezolid-resistance has been detected in *S. epidermidis*, *S. haemolyticus*, *S. hominis* and *S. capitis* in North America (United States, Mexico), Europe (Greece, Spain, France, Germany, Italy, Ireland), South America (Brazil) and Asia (Japan, Korea, China) [71-74, 76]. Three mechanisms of linezolid-resistance have been reported in CoNS [73]. The most common mechanism of linezolid-resistance is a mutation in the central loop domain V of the 23S rRNA, called G2576T [73, 74, 76]. This mutation is called G2576T, since it occurs at position 2576 of the 23S rRNA due to a change of a guanine (G) to a thymine/uracil (T) substitution [76]. Another common mechanism associated with linezolid-resistance is the presence of the plasmid-encoded *cfr* gene [73]. The *cfr* gene encodes a methyl-transferase, which is responsible for the post-transcriptional methylation of an adenosine at position 2503 in the 23S rRNA [72, 76]. The *cfr* gene also provide resistance to streptogramin A, lincosamides, chloramphenicol and pleuromutilins, but not to erythromycin and have the ability to spread among closely-related species [72, 73]. A rare mechanism of linezolid-resistance in CoNS is mutations in the L3 and L4 ribosomal proteins of the peptide translocation centre, which are close to the binding site of linezolid [72, 73].

Linezolid-resistance among CoNS is frightening, since these strains are rarely considered as clinically significant pathogens and may not be detected by infection control practices [73, 76]. The presence of the *cfr* gene is alarming, since it can lead to the wide dissemination of linezolid-resistance, among closely-related species, through horizontal gene transfer, in hospital settings [73]. The usage of linezolid should be strictly regulated in order to maintain its clinical value [76].

6.3. Resistance against nucleic acid synthesis inhibitors

6.3.1. Resistance against the rifamycin, rifampicin

The most important antimicrobial in the rifamycin family is rifampicin [43]. Rifampicin is used for the treatment of tuberculosis and leprosy, as well as in combination with other antimicrobials for the treatment of intravascular catheter-related infections caused by CoNS [43, 48, 77]. Rifampicin is effective in the treatment of biofilm-associated intravascular catheter-related infections, since rifampicin can penetrate the biofilm and has a low bactericidal concentration, which is active against CoNS in the stationary growth phase [77, 78]. Rifampicin prevents bacterial DNA replication, by inhibiting the bacterial DNA-dependent RNA polymerase enzyme, which is encoded by the *rpo*B gene [43, 48, 77]. Rifampicin-resistance can emerge rapidly at a frequency of 10^{-8}, due to single point mutations at any location in the *rpo*B gene [48, 77, 78].

As already mentioned, rifampicin is used in the treatment of tuberculosis [43]. This can possibly lead to CoNS residing in the normal skin microflora, being preliminary exposed to rifampicin, giving rise to rifampicin-resistant mutants in the tuberculosis patient's CoNS population [78]. In the somewhat rare, but likely event of catheterisation of the tuberculosis patient, *rpo*B CoNS mutants already exist, which can possibly lead to the ineffectiveness of rifampicin combination therapy in intravascular catheter-related infections of tuberculosis patients, making rifampicin-resistance among CoNS alarming.

6.3.2. Bypass of the folic acid synthesis pathway, conferring resistance to co-trimoxazole

Co-trimoxazole is a combination of trimethoprim and the sulphonamide, sulfamethoxazole, which acts synergistically, to inhibit the bacterial folic acid synthesis pathway, which in turn disrupts nucleic acid synthesis [32, 43]. Trimethoprim and sulfamethoxazole have similar modes of actions [79]. Sulfamethoxazole is a structural analogue of *para*-aminobenzoic acid (PABA) (involved in the initial stages of folic acid synthesis) and competes with it for dihydropteroic acid synthetase [32]. Trimethoprim inhibits dihydrofolate reductase, an enzyme responsible for the reduced form of folic acid, which can act as a co-enzyme in the synthesis of purines and thymidine [32].

Although not the main clinical use, co-trimoxazole can be used for the treatment of intravascular catheter-related infections, with a good clinical outcome in infections caused by methicillin-resistant staphylococci [79]. However, resistance to co-trimoxazole can arise due to the overproduction of PABA or due to the acquisition of trimethoprim-resistant dihydrofolate reductases, which are encoded by either the *dfr*A (previously known as *dfr*S1), *dfr*D, *dfr*G or *dfr*K genes [43, 80].

Co-trimoxazole is nowadays used as prophylaxis against opportunistic infections in HIV-positive patients. The CoNS population of a HIV-positive patient are continuously exposed to co-trimoxazole, leading to the possibility of developing of co-trimoxazole resistance. In the case of catheterisation of the HIV-positive patient, co-trimoxazole resistant CoNS mutants could already exist, which could render co-trimoxazole treatment of an intravascular catheter-related infection fruitless.

7. Diagnosis of intravascular catheter-related infections caused by coagulase-negative staphylococci

The diagnosis of an intravascular catheter-related infection is a major challenge, since difficulty is experienced in differentiating a CoNS contaminant from an invasive CoNS strain [1, 10, 49]. Numerous methods have been developed for the diagnosis of intravascular catheter-related infections to ensure that the organism, such as CoNS, is not a contaminant and these techniques either involve the removal of the catheter (catheter segment culture), or the saving of the catheter (blood cultures) [12, 51].

Paired quantitative blood cultures (simultaneous blood cultures drawn through the catheter and percutaneously, where both cultures are positive with the catheter yielding 5-fold higher growth than the percutaneous culture) are the most accurate diagnostic test, based on sensitivity and specificity, but these tests are labour intensive and costly [12, 81]. A simple and cheap approach for the diagnosis of intravascular catheter-related infection involves the isolation of the same organism from a single blood culture and catheter, by semi-quantitative analysis (Five centimetres of the catheter are removed and rolled four times across a blood agar plate and incubated) [12] yielding ≥15 colony forming unit (cfu) for the catheter tip [22, 81]. This approach can be problematic in ICU patients, since these patients may have multiple short-term intravascular catheters, with any one being the cause of the infection [22].

In order to further evaluate the clinical significance of CoNS isolated from catheter or blood cultures, the identification of CoNS is important. Coagulase-negative staphylococci are routinely identified in diagnostic laboratories through automated systems, such as VITEK® 2 (bioMérieux, Marcy l'Etoile, France) [82]. The VITEK® 2 Gram-positive card system (bioMérieux, Marcy l'Etoile, France) identifies CoNS based on metabolic reactions, through colorimetric changes [82, 83]. However, problems are experienced with phenotypic identification of CoNS and should not always be considered accurate [84]. The problems experienced with the identification of CoNS include: i) imprecise results due to phenotypic variation [85], ii) the lack of unique biochemical markers to differentiate between CoNS species [86], iii) the dependence on the expression of metabolic activities and/or morphological features [87] and vi) polyclonality of CoNS growing on catheter tips [88, 89].

The Matrix-Assisted Laser Desorption/Ionization time of flight Mass Spectrometry (MALDI-tof) is a highly sensitive and rapid method, used for microbial species-level identification [90]. This method is based on the ionization of co-crystallised sample material by short laser pulses [90]. A study by Loonen and colleagues, which compared five CoNS identification methods [VITEK® 2 (bioMérieux), ID 32 Staph strip (bioMérieux) 16S rRNA sequencing, *tuf* gene sequencing and MALDI-tof MS (Bruker Daltonics)] found that MALDI-tof (Bruker Daltonics) or VITEK® 2 (bioMérieux) combined with partial *tuf* gene sequencing is the method of choice for the identification of CoNS [91].

8. Prevention of CRBSI through central-line bundles

Specific prevention strategies of intravascular catheter-related infections require 100% adherence to the central-line bundle [23]. The five components of the central-line bundle are i) hand hygiene, ii) maximum sterile barrier precautions, iii) chlorhexidine skin preparation, iv) optimal catheter insertion site and v) review of the necessity of the catheter, to be removed without delay, if found unnecessary [22, 23]. A strict hand washing protocol, followed by the wearing of sterile gloves is required before a catheter is touched for whatever reason, since the source of infection is contamination of the catheter hub [23]. The insertion of a catheter requires maximum sterile barrier precautions to be followed [23]. This includes wearing a head cover, a mask, a gown and sterile gloves by the healthcare worker and a body drape for the patient [23]. Lines inserted in an emergency setting, without maximum sterile barrier precautions, should be removed and replaced under maximum sterile barrier precautions, as soon as practical [23]. The skin should be prepared before catheter placement, by using two percent (2%) chlorhexidine or five percent (5%) iodine with 70% alcohol in patients with allergies [22, 23]. The skin should be allowed to air dry before the skin is punctured [23]. Catheters inserted into the subclavian vein have lower infection rates than femoral catheters [22, 23]. Infection rates increase after five to seven days of catheterisation and therefore it is crucial to remove catheters, if no longer required [22, 23].

9. Conclusion

Coagulase-negative staphylococci are important emerging opportunistic pathogens in the clinical setting. Not only is the diagnosis of an intravascular catheter-related infection caused by CoNS complicated, the treatment of a CoNS infections is not straightforward, due to biofilm formation and the numerous antimicrobial-resistance genes prevalent in CoNS. In order to prevent the spread of multidrug-resistant CoNS in the clinical setting vigilant surveillance and infection control policies need to be adapted.

Acknowledgements The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF.

References

[1] Goede MR, Coppersmith CM. Catheter-related bloodstream-infections. *Surgical Clinic of Northern America*. 2009;89:463-474.
[2] Leonidou L, Gogo CA. Catheter-related bloodstream infections: Catheter management according to pathogen. *International Journal of Antimicrobial Agents*. 2010;36:S26–S32.
[3] Tacconeli E, Smith G, Hieke K, Lafuma A, Bastide P. Review – Epidermiology, medical outcomes and costs of catheter-related bloodstream infections in intensive care units of four European countries: Literature and registry-based estimates. *Journal of Hospital Infection.* 2009;72:97-103.
[4] Michalopoulus A, Falagas ME, Karatza DC, Alexandropoulou P, Papadakis E, Gregorakos L, Chalevelaks G, Pappas G. Epidemiologic, clinical characteristics, and risk factors for adverse outcome in multiresistant gram-negative primary bacteremia of critically ill patients. *American Journal of Infection Control*. 2011;39:396-400.
[5] Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK. Antimicrobial-resistant pathogens associated with healthcare-associated infections: Annual summary of data reported to the National Healthcare Safety Network at the Centres for Disease Control and Prevention, 2006-2007. *Infection Control and Hospital Epidemiology*. 2008;29:996-1011.
[6] Sievert DM, Ricks P, Edwards JR, Schneinder A, Patel J, Srinivasan A, Kallen, Limbago B, Fridkin S for the National Healthcare and Safety Network (NHSN) Team and Participating NHSN Facilities. Antimicrobial-resistant pathogens associated with healthcare-associated infections: Summary of data reported to the National Healthcare Safety Network at the Centres for Disease Control and Prevention, 2009–2010. *Infection Control and Hospital Epidemiology.* 2013;34:1-14.
[7] Otto M. *Staphylococcus epidermidis* – The "accidental" pathogen. *Nature Reviews Microbiology.* 2009;8:555-567.
[8] Piette A, Verschraegen G. Role of coagulase-negative staphylococci in human disease. *Veterinary Microbiology*. 2009;134:45-54.
[9] Schoenfelder SMK, Lange C, Eckart M, Hennig S, Kozytska S, Ziebuhr W. Success through diversity – How *Staphylococcus epidermidis* establishes as a nosocomial pathogen. *International Journal of Medical Microbiology*. 2010;300:380-386.
[10] Rahkonen M, Luttinen S, Koskela M, Hautala T. True bacteraemia caused by coagulase-negative staphylococci are difficult to distinguish from blood culture contaminants. *European Journal of Clinical Microbiology and Infectious Diseases*. 2012;3:1-6.
[11] Otto M. Molecular basis of *Staphylococcus epidermidis* infection. *Seminars in Immunopathology.* 2012;34:201-204.
[12] Abad CL, Safdar N. Catheter-related bloodstream-infections. *The Lancet - Infectious Disease Special Edition.* 2011;1:84-98.
[13] Revidiwala S, Rajdev BM, Mulla S. Characterization of bacterial etiologic agents of biofilm formation in medical devices in critical care setup. *Critical Care Research and Practice*. 2012;12:1-6.
[14] Beheshti MV. A concise history of central venous access. *Techniques in Vascular and Interventional Radiology*. 2011;14:184-185.
[15] Götz F, Bannerman T & Schleifer KH. *The Prokaryotes – A Handbook on the Biology of Bacteria, Volume 3: Archaea, Bacteria: Firmicutes, Actinomycetes.* 3rd ed. New York, USA: Springer Science and Business Media; 2006.

[16] Crossley KB, Jefferson KK, Archer G, Fowler VG Jr. *Staphylococci in human disease.* 2nd ed. New York, USA: Blackwell Publishing; 2009.
[17] Katz DS. Coagulase Test Protocol. The Microbe Library – American Society of Microbiology. Available at: http://www.microbelibrary.org/library/laboratory%20test/3243-coagulase-test. Accessed February, 2012.
[18] Pfaller MA, Herwaldt LA. Laboratory, clinical and epidemiological aspects of coagulase negative staphylococci. *Clinical Microbiology Reviews.* 1988;1:281-299.
[19] Widerström M, Wiström J, Sjöstedt A, Monsen T. Coagulase-negative staphylococci: Update on the molecular epidemiology and clinical presentation, with a focus on *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. *European Journal of Clinical Microbiology and Infectious Diseases*. 2012;31:7-20.
[20] Kelly L, Buchan E, Brown A, Criggie L. Care and maintenance of central venous catheter devices – Vascular Access Service, NHS Greater Glasgow and Clyde. Available at: http://www.beatson.scot.nhs.uk/content/mediaassets/doc/CVAD%20guidelines%20September%2008%20final.pdf. Accessed April 2012.
[21] O'Grady NP, Alexander M, Burns LA, Dellinger EP, Garland J, Heard SO, Lipsett PA, Masur H, Mermel LA, Pearson ML, Raad II, Randolph A, Rupp ME, Sanjay S, Healthcare Infection Control Practices Advisory Committee. Guidelines for the Prevention of Intravascular Catheter-Related Infections. Available at: http://www.cdc.gov/hicpac/BSI/BSI-guidelines-2011.html. Accessed April 2012.
[22] Edgeworth J. Intravascular catheter infections – Review. *Journal of Hospital Infection.* 2009;73:323-330.
[23] Meek ME. Diagnosis and treatment of central venous access-associated infections. *Techniques in Vascular and Interventional Radiology*. 2011;14:212-216.
[24] Von Eiff C, Jansen B, Kohnen W, Becker K. Infections associated with medical devices - Review article. *Drugs.* 2005;65:179-214.
[25] Hamilton H, Bodenham A. *Central venous catheters*. West Sussex, UK: Wiley-Blackwell Publishing; 2009.
[26] Costa SF, Miceli MH, Anaisssie EJ. Mucosa or skin as source of coagulase-negative staphylococcal bacteraemia? *The Lancet Infectious Diseases*. 2004;4:278-286.
[27] Mermel LA. What is the predominant source of intravascular catheter infections? *Clinical Infectious Diseases*. 2011;52:211-212.
[28] Heikens E, Fleer A, Paauw A, Florijn A, Fluit AC. Comparison of genotypic and phenotypic methods for species-level identification of clinical isolates of coagulase-negative staphylococci. *Journal of Clinical Microbiology*. 2005;43:2286-2290.
[29] Madigan MT, Martinko JM, Dunlap PV, Clark DP. *Brock Biology of Microorganisms* . 12th ed. San Francisco, USA: Pearson Benjamin Cummings; 2009.
[30] Longauerova A. Coagulase-negative staphylococci and their participation in pathogenesis in human infections. *Bratislava Medical Journal*. 2006;107:448-452.
[31] Frank KL, Del Pozo JL, Patel R. From clinical microbiology to infection pathogenesis: How daring to be different works for *Staphylococcus lugdunensis*. *Clinical Microbiology Reviews*. 2008;21:111-133.
[32] Ryan KJ, Ray CG. *Sherris Medical Microbiology*. 5th ed. USA: McGraw Hill; 2010.
[33] Goering RV, Dockrell HM, Zuckermann M, Wakelin D, Roitt IM, Mims C, Chiodini PL. *Mims' Medical Microbiology*. 4th ed. Philadelphia, USA: Mosbt-Year Book Europe, Elsevier Ltd; 2008.
[34] Kloos WE, Schleifer KH. Isolation and characterization of staphylococci from human skin - Descriptions of four new species: *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus hominis* and *Staphylococcus simulans*. *International Journal of Systematic Bacteriology*. 1975;25:62-79.
[35] Schleifer KH, Kloos WE. Isolation and characterisation of staphylococci from human skin – Amended descriptions of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* and descriptions of three new species: *Staphylococcus cohnii*, *Staphylococcus haemolyticus* and *Staphylococcus xylosus*. *International Journal of Systematic Bacteriology* 1975;25:50-61.
[36] Otto M. Staphylococcal biofilms. *Current Topics in Microbiology and Immunology*. 2008;322:207-228.
[37] Mateo M, Maestre JR, Aguilar L, Cafini F, Puente P, Sánchez P, Alou L, Giménez MJ, Prieto J. Genotypic versus phenotypic characterisation, with respect to susceptibility and identification, of 17 clinical isolates of *Staphylococcus lugdunensis*. *Journal of Antimicrobial Chemotherapy.* 2005;56:287-291.
[38] Ross TL, Fuss EP, Harrington SM, Cai M, Perl TM, Merz WG. Methicillin-resistant *Staphylococcus caprae* in a neonatal intensive care unit. *Journal of Clinical Microbiology.* 2005;43:363-367.
[39] Cimiotti JP, Haas JP, Della-Latta P, Wu F, Saiman L, Larson EL. Prevalence and clinical relevance of *Staphylococcus warneri* in a neonatal intensive care unit. *Infection Control and Hospital Epidemiology*. 2007;28:326-330.
[40] Raz R, Colodner R & Kunin CM. Who are you – *Staphylococcus saprophyticus*? *Clinical Infectious Diseases.* 2005;40:896-898.
[41] Tan TY, Ng SY, He J. Microbiological characteristics, presumptive identification and antimicrobial susceptibilities of *Staphylococcus lugdunensis*. *Journal of Clinical Microbiology*. 2008;46:2393-2395.
[42] McCann MT, Gilmore BF, Gorman SP. *Staphylococcus epidermidis* device-related infections: Pathogenesis and clinical management. *Journal of Pharmacy and Pharmacology.* 2008:60:1551-1571.
[43] Török E, Moran E, Cooke F. *Oxford Handbook of Infectious Diseases and Microbiology*. New York, USA: Oxford University Press; 2009.
[44] Sani NAM, Sapri HF, Noordin A, Neoh H, Hussin S. Species identification of coagulase-negative staphylococci (CoNS) isolates in University Kebangsaan Malaysia Medical Centre (UKMMC). *Asia-Pacific Journal of Molecular Medicine* 2011;1:1-5.
[45] Madsen JS, Burmølle M, Hansen LH, Sørensen SJ. The interconnection between biofilm formation and horizontal gene transfer. *FEMS Immunology and Medical Microbiology.* 2012;65:183-195.
[46] Percival SL, Emanuel C, Cutting KF, Williams DW. Microbiology of the skin and the role of biofilms in infection. *International Wound Journal*. 2012;9:14-32.

[47] Bridier A, Briandet R, Thomas V, Dubois-Brissonnet D. Resistance of bacterial biofilms to disinfectants: A review. *Biofouling*. 2011;27:1017-1032.
[48] Forrest GN, Tamura K. Rifampin combination therapy for nonmycobacterial infections. *Clinical Microbiology Reviews*. 2010;23:14-34.
[49] Elzi L, Babouee1 B, Vögeli N, Laffer R, Dangel M, Frei R, Battegay M, Widmer AF. How to discriminate contamination from bloodstream infection due to coagulase-negative staphylococci: A prospective study with 654 patients. *Clinical Microbiology and Infection*. 2012;18:355-362.
[50] Esposito S, Purrello SM, Novelli A, Tripodi F, Pascale R, unal S, Milkovich G. Central venous catheter-related biofilm infections. An up-to-date focus on methicillin-resistant *Staphylococcus aureus*. *Journal of Global Antimicrobial Resistance.* 2013; Article in press. Available at: http://dx.doi.org/10.1016/j.jgar.2013.03.002.
[51] Mermel LA, Allom M, Bouza E, Craven DE, Flynn P, O'Grady NP, Raad II, Rijnders BJA, Sherertz RJ, Warren DK. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infections: 2009 Update by the Infectious Diseases Society of America. *Clinical Infectious Diseases*. 2009;49:1-45.
[52] Zapun A, Contreras-Martel C, Vernet T. Review article – Penicillin-binding proteins and β-lactam resistance. *FEMS Microbiology Reviews*. 2008;32:361–385.
[53] Duran N, Burcin O, Duran GG, Onlen Y, Deir C. Antibiotic resistance genes & susceptibility patterns in staphylococci. *Indian Journal of Medical Research*. 2012;135:389-396.
[54] Koksal F, Yasar H, Samasti M. Antibiotic resistance patterns of coagulase-negative staphylococcus strains isolated from blood cultures of septicemic patients in Turkey. *Microbiological Research*. 2009;164:404-410.
[55] Haas W, Pillar CM, Torres M, Morris TW, Sahm DF. Monitoring antibiotic-resistance in ocular microorganisms: Results from the antibiotic-resistance monitoring in ocular microorganisms (ARMOR) 2009 surveillance study. *American Journal of Ophthalmology*. 2011;52:567-574.
[56] Starlander G, Wire M, Melus A. First documented case of a *Staphylococcus lugdunensis* strain carrying the *mec*A gene in Northern Europe*. Infection Ecology and Epidemiology*. 2011;1:8410 - DOI:10.3402/iee.v1i0.8410.
[57] Kotsakis SD, Tzouvelekis LS, Zerva L, Liakopoulus A, Petinaki E. *Staphylococcus lugdunensis* strain with a modified PBP1A/1B expressing resistance to β-lactams. *European Journal Clinical Microbiology and Infectious Diseases*. 2012;31:169-172.
[58] Natoli, S, Fontana C, Favaro M, Bergamini A, Testore GP, Minelli S, Bossa MC, Casapulla M, Broglio G, Beltrame A, Cudillo L, Cerretti R, Leonardis F. Characterisation of coagulase-negative staphylococcal isolates from blood with reduced susceptibility to glycopeptides and therapeutic options. *BMC Infectious Disease*. 2009;9:83-91
[59] Périchon B, Courvalin P. Glycopeptide resistance. In: Doughterty TJ, Pucci MJ (eds.) *Antimicrobial Discovery and Development*. New York: Springer Science + Business; 2012:515-542.
[60] Sujatha S, Praharaj I. Glycopeptide resistance in Gram-positive cocci: A review. *Interdisciplinary Perspectives on Infectious Diseases*. 2012.
[61] Cremniter J, Slassi A, Quincampoix JC, Sivadon-Tardy V, Bauer T, Porcher R, Lortat-Jacob A, Piripou P, Judet T, Herrmann JL, Gaillard JL, Rottman M. Decreased susceptibility to teicoplanin and vancomycin in coagulase-negative staphylococci isolated from orthopaedic-device-associated infections. *Journal of Clinical Microbiology.* 2010;48:1428-1431.
[62] Campoccia D, Montanaro L, Pirini V, Ravaioli S, Arciolo CR. Prevalence of genes for aminoglycoside-modifying enzymes in *Staphylococcus epidermidis* isolates from orthopaedic postsurgical and implant-related infections. *Journal of Biomedical Materials Research.* 2009;88A:654-663.
[63] Liakopoulus A, Foka A, Vourli S, Zerva L, Tsiapara F, Protonatoriou E, Dailiana Z, Economou M, Papoutsidou E, Koutsia-Carouzou C, Anastassiou ED, Diza E, Zintzaras E, Spiliopoulou I, Petinaki E. Aminoglycoside-resistant staphylococci in Greece: prevalence and resistance mechanisms. *European Journal of Microbiology and Infectious Disease*. 2011;30:701-705.
[64] Emaneini M, Taherikalani M, Eslampour MA, Sedaghat H, Aligholi M, Jabalameli F, Shahsavan S, Sotoudeh. Phenotypic and genotypic evaluation of aminoglycoside resistance in clinical isolates of staphylococci in Tehran, Iran. *Microbial Drug Resistance*. 2009;15:129-132.
[65] Le Bouter A, Leclercq R, Cattoir V. Molecular basis of resistance to macrolides, lincosamides and streptogramins in *Staphylococcus saprophyticus* clinical isolates*. International Journal of Antimicrobial Agents*. 2011;37:118-123.
[66] Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. *Journal of Medical Microbiology*. 2007;56:342-345.
[67] Cetin ES, Gunes H, Kaya S, Aridogan BC, Demirci M. Distribution of genes encoding resistance to macrolides, lincosamides and streptogramins among clinical staphylococcal isolates in a Turkish University Hospital. *Journal of Microbiology, Immunology and Infections*. 2010;43:524-529.
[68] Juyal D, Shamanth AS, Pal S, Sharma MK, Prakask R, Sharma N. The prevalence of inducible clindamycin resistance among staphylococci in a tertiary care hospital – A study from Garhwal Hills of Uttarakhand, India. *Journal of Clinical and Diagnostic Research*. 2013;7:61-65.
[69] Mendes RE, Deshpande LM, Farrell DJ, Spanu T, Fadda G, Jones RN. Assesment of linezolid-resistance mechanisms among *Staphylococcus epidermidis* causing bacteraemia in Rome, Italy. *Journal of Antimicrobial Chemotherapy*. 2010;65:2329-2335.
[70] Stefani S, Bongiorno D, Mongelli G, Campanile F. Linezolid resistance in staphylococci. *Pharmaceuticals*. 2010;3:1988-2006.
[71] Wong A, Reddy SP, Smyth DS, Aguero-Rosenfeld ME, Sakoulas G, Robinson DA. Polyphyletic emergence of linezolid-resistant staphylococci in the United States. *Antimicrobial Agents and Chemotheraphy.* 2010;54:742-748.
[72] Bongiorno D, Campanile F, Mongelli G, Baldi MT, Provenzani R, Reali S, Russo CL, Santagati M, Stefani S. DNA methylase modifications and other linezolid resistance mutations in coagulase-negative staphylococci in Italy. *Journal of Antimicrobial Chemotherapy.* 2010;65:2336-2340.
[73] Gu B, Kelesidis T, Tsiodras S, Hindler J, Humphries RM. The emerging problem of linezolid-resistant *Staphylococcus*. *Journal of Antimicrobial Chemotheraphy.* 2013;68:4-11.

[74] Cai JC, Hu YY, Zhang R, Zhou HW, Chen GX. Linezolid-resistant clinical isolates of methicillin-resistant coagulase-negative staphylococci and *Enterococcus faecium* from China. *Journal of Medical Microbiology*. 2012;61:1568-1573.
[75] Long KS, Vester B. Resistance to linezolid caused by modification at its binding site on the ribosome. *Antimicrobial Agents and Chemotherapy*. 2012;56:603-613.
[76] Mazzariol A, Cascio GL, Kocsis E, Maccacaro L, Fontana R, Cornaglia G. Outbreak of linezolid-resistant *Staphylococcus haemolyticus* in an Italian intensive care unit. *European Journal of Clinical Microbiology and Infectious Diseases*. 2012;31:523-527.
[77] Hellmark B, Söderquist B, Unemo M. Stimulataneous species identification and detection of rifampicin resistance in staphylococci by sequencing the *rpo*B gene. *European Journal of Clinical Microbiology and Infectious Diseases*. 2009;28:183-190.
[78] Achermann Y, Eigenmann K, Ledergerber B, Derksen L, Rafeiner P, Clauss M, Nüesch R, Zellweger C, Vogt M, Zimmerli W. Factors associated with rifampin resistance in staphylococcal periprosthetic joint infections: A matched case-control study. *Infection*. 2013:41:431-437.
[79] Wendlandt S, Fessler AT, Monecke S, Ehrich R, Schwarz S, Kadlec K. The diversity of antimicrobial resistance genes among staphylococci of animal origin. *International Journal of Medical Microbiology*. 2013; Article in press. Available at: http://dx.doi.org/10.1016/j.ijmm.2012.02.006.
[80] Goldberg E, Bishara J. Contemporary unconventional clinical use of co-trimoxazole. *Clinical Microbiology and Infection*. 2011;18:8-17.
[81] Seifert H. The clinical importance of microbiological findings in the diagnosis and management of bloodstream infections. *Clinical Infectious Diseases*. 2009;48:238-245.
[82] Delmas J, Chacorna JP, Robin F, Giammarinaro P, Talon R, Bonnet R. Evaluation of the VITEK® 2 card system with a variety of *Staphylococcus* species. *Journal of Clinical Microbiology*. 2007;46:311-313.
[83] D'Azevedo PA, Siquiera I, Gugel J, Antunes ALS, Secchi C, Pasternak J, Martino MDV. Evaluation of the automated system for identification and antimicrobial susceptibility testing of Brazilian Gram-positive cocci strains. *The Brazilian Journal of Infectious Diseases*. 2009;13:107-110.
[84] Kim M, Heo SR, Choi SH, Kwon H, Park JS, Seong MW, Lee DH, Park Ku, Song J, Kim EC. Comparison of the MicroScan, VITEK 2, and Crystal GP with 16S rRNA sequencing and MicroSeq 500 v2.0 analysis for coagulase-negative staphylococci. *BMC Microbiology*. 2008;8:233-240.
[85] Hirotaki S, Sasaki T, Kuwahara-Arai K, Hiramatsu K. Rapid and accurate identification of human-associated staphylococci by use of a multiplex PCR. *Journal of Clinical Microbiology*. 2011;49:3627-3631.
[86] Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki A, Kwakami T, Fukata T, Hiramatsu K. Multiplex-PCR method for species identification of coagulase-positive staphylococci. *Journal of Clinical Microbiology*. 2010;48:765-769.
[87] Layer F, Ghebremedhin B, Moder KA, König W, König B. Comparative study using various methods for identification of *Staphylococcus* species in clinical specimens. *Journal of Clinical Microbiology*. 2006;44:2824-2830.
[88] Poyart C, Quesne G, Boumaila C, Trieu-Cuot P. Rapid and accurate species-level identification of coagulase-negative staphylococci using the *sod*A gene as a target. *Journal of Clinical Microbiology*. 2001;39:4296-4301.
[89] Aldea-Mansilla C, de Viedma DG, Cercenado E, Martin-Rabadán P, Marin M, Bouza E. Comparison of phenotypic and genotypic procedures for confirmation of coagulase-negative *Staphylococcus* catheter-related bloodstream-infections. *Journal of Clinical Microbiology*. 2006;44:3529-3532.
[90] Wieser A, Schneider L, Jung J, Schubert S. Mini review: MALDI-tof MS in microbiological diagnostics – identification of microorganisms and beyond. *Applied Microbiology and Biotechnology*. 2012;93:965-974.
[91] Loonen AJM, Jansx AR, Bergland JNB, Valkenburg M, Wolffs PFG, Van den Brule AJC. Comparative study using phenotypic, genotypic and proteomics methods for identification of coagulase-negative staphylococci. *Journal of Clinical Microbiology*. 2012;50:1437-1439.

