Species Distribution, Virulence factors and Antibiotic Sensitivity Pattern of Coagulase Negative Staphylococci Isolated from the Clinical Specimens in a Tertiary Care Centre.

Mr Nagaraju Vanaparti[1], Dr Kalpana Sadawarte[2]
[1]Assistant professor R.V.M IMS & RC, Laxmakkapally, Siddipet, Telangana,
[2]Professor and Head, Microbiology, People's University, Bhanpur, Bhopal, Madhya Pradesh
Corresponding Author: Mr Nagaraju Vanaparti, [1]Assistant professor R.V.M IMS & RC, Laxmakkapally, Siddipet, Telangana. **Email:** vsnr369@gmail.com

ABSTRACT

Coagulase-Negative Staphylococci (CoNS) are generally less virulent but have emerged as significant pathogens in healthcare settings, particularly in immunocompromised patients or those with indwelling medical devices. Coagulase-Negative Staphylococci (CoNS) strains are increasingly resistant to various antibiotic classes, including macrolides, aminoglycosides, and fluoroquinolones, along with beta-lactam group, posing challenges in clinical treatment. the current study aims to investigate the occurrence, species distribution, antibiogram, virulence factors, of Coagulase-Negative Staphylococci (CoNS) isolates obtained from clinical specimens in a tertiary care centre. Coagulase-Negative Staphylococci (CoNS) were isolated and identified by standard Tests Catalase Test, tube coagulase, acid formation from D-trehalose, alkaline phosphatase Voges-proskauer test, β-glucosidase, ornithine decarboxylase, β-galactosidase, urease, oxidase, anaerobic growth, β-glucoronidase, acid from mannitol, PYR test from clinical specimens. Antibiotic sensitivity was done by Kirby Bauers Disk Diffusion method. Detection of virulence factors slime layer production and biofilm formation were done by Congo red and tissue culture methods. A total of 522 Coagulase-Negative Staphylococci (CoNS) were isolated. Majority of the isolates (>90%) produced both the virulence factors and showed antimicrobial resistance. Diagnosis, speciation and antimicrobial susceptibility study are required for formation of effective antibacterial policy for the early treatment of CoNS infections.

Key words: Coagulase-Negative Staphylococci (CoNS), slime layer, Biofilm Production, Antimicrobial resistance.

INTRODUCTION

Coagulase-Negative Staphylococci (CoNS) are a group of Gram-positive bacteria, primarily characterized by their inability to produce the enzyme coagulase. Coagulase-Negative Staphylococci (CoNS) are generally less virulent but have emerged as significant pathogens in healthcare settings, particularly in immunocompromised patients or those with indwelling medical devices. As of right now, the CoNS group contains roughly
40 species of Gram-positive cocci. The newest member of the CoNS group is Staphylococcus microti. But very few are thought to be the source of infection in people [1]. The CoNS pathogenicity is ascribed to a variety of virulence features, including the production of exoenzymes and toxins, as well as biofilm formation. Exoenzymes such as alkaline

phosphatase, lipase, lipoprotein lipase, protease, elastase, esterase, urease, and DNase play essential roles in pathogenesis of CoNS infection [2]. CoNS strains are increasingly resistant to various antibiotic classes, including macrolides, aminoglycosides, and fluoroquinolones, along with beta-lactam group, posing challenges in clinical treatment. In view of this the current study aims to investigate the occurrence, species distribution, antibiogram, virulence factors, of CoNS isolates obtained from clinical specimens in a tertiary care centre [3].

MATERIALS AND METHODS

A prospective cross-sectional study conducted at Department of Microbiology, Peoples Medical College Bhopal for the isolation, species distribution, detection of virulence factors and antibiotic sensitivity test for the Coagulase-Negative Staphylococci (CoNS) isolated from various clinical samples Urine, Blood, Pus, Vaginal swabs, Pleural fluid, CSF, Miscellaneous samples received at the laboratory. A total of 522, CoNS isolated from various clinical samples were subjected to the study. The identification of the genus and species differentiation were done with standard tests by Gram-staining technique, catalase test, coagulase test, anaerobic mannitol fermentation, susceptibility to bacitracin, phosphatase test and novobiocin disk test [12]. Antibiotic sensitivity test is done by Kirby Bauer's disk diffusion test using penicillin (10 U), oxacillin (1 µg) ciprofloxacin (5 µg), gentamicin (10 µg), erythromycin (15 µg), chloramphenicol (30 µg), vancomycin (30 µg) according to CLSI guidelines. Antibiotics disks and media were obtained from HI Media Private Ltd, Mumbai, India. Detection of virulence factors like slime layer production and biofilm formation[24] was done by Congo-red agar method and tissue culture plate method respectively.

RESULTS

Species. Distribution of Coagulase-Negative Staphylococci (CoNS). A total of 9species of Coagulase-Negative Staphylococci (CoNS) were isolated 522 isolates from different. Clinical specimens. Staphylococcus epidermidis, Staphylococcus haemolyticus and Staphylococcus saprophyticus were the most common species isolated from various clinical specimens representing 219 (41.9%), 151 (28.9%) and 73 (13.9%) of total. species of CoNS respectively (Table 1).

Table: 1. Various Species. of Coagulase Negative Staphylococci Isolated from Different Clinical Specimens.

CoNS isolate	N (%)
S. epidermidis	219 (41.9)
S. haemolyticus	151 (28.9)
S. saprophyticus	73 (13.9)
Staphylococcus hominis	36 (6.9)
S. lugdunensis	22 (4.2)
Staphylococcus capitis	07 (1.3)
S. auricularis	06 (1.2)

Staphylococcus warneri	04 (0.8)
S. schleiferi	04 (0.8)
Total	522

Virulence factors: Out of 522 Coagulase-Negative Staphylococci (CoNS) isolates from various clinical specimens, a total of 468 (89.7%) showed slime layer production by Congo-red agar-based test whereas 54 (10.7%) showed no slime layer production. Biofilm production by MTP method was shown by 471 (90.2%) (Table 2).

Slime layer production and biofilm formation among Coagulase Negative Staphylococci (CoNS) isolates.

Table: 2. Slime layer production and biofilm formation among Coagulase Negative Staphylococci (CoNS) isolates.

Total isolates	Slime layer production		Biofilm production	
	Yes	No	Yes	No
522	468 (89.7%)	54 (10.7%)	471 (90.2)	51 (9.8)

Species. wise slime layer & biofilm production. in Coagulase Negative Staphylococci (CoNS) isolates.

Slime layer production was significantly high in CoNS. isolates like S. epidermidis. S. haemolyticus, S. saprophyticus, S. hominis *and* S. lugdunensis. Whereas out of a total of 522 CoNS isolates from various clinical specimens, 471 (90.2%) showed biofilm formation by MTP method (Table 3).

Table 3. Species. wise slime layer & biofilm production. in Coagulase Negative Staphylococci (CoNS) isolates.

CoNS Isoilate	Total isolate	Slime layer production		Chi Square Test, *P* value	Biofilm production		Chi Square Test, *P* value
		Yes (%)	**No (%)**		**Yes (%)**	**No (%)**	
S. epidermidis	**219**	215 (98.2)	O4 (1.8)	<0.00001**	215 (98.2)	04 (1.8)	<0.00001**
S. haemolyticus	**151**	142 (94.1)	09 (5.9)	<0.00001**	149 (98.7)	02 (1.3)	<0.00001**
S. saprophyticus	**73**	61 (83.6)	12 (16.4)	<0.00001**	63 (86.3)	10 (13.7)	<0.05*
S. hominis	**36**	29 (80.6)	07 (19.4)	<0.0001**	23 (61.2)	13 (38.8)	>0.05
S. lugdunensis	**22**	16 (72.7)	06 (27.3)	<0.05*	15 (63.6)	07 (36.4)	>0.05
S. capitis	**07**	02 (28.6)	05 (71.4)	>0.05	03 (28.6)	04 (71.4)	>0.05
S. auricularis	**06**	01 (16.7)	05 (83.4)	>0.05	01 (16.7)	05 (83.3)	>0.05
S. warneri	**04**	01 (25)	03 (75)	>0.05	01 (25)	03 (75)	>0.05
S schleiferi	**04**	01 (25)	03 (75)	>0.05	01 (25)	03 (75)	>0.05
Total	**522**	**468 (89.7)**	**54 (10.3)**		**471 (90.2%)**	**51(9.8%)**	

* Statistically highly significant, †statistically significant

Antimicrobial susceptibility profile of Coagulase Negative Staphylococci (CoNS) isolates by disc diffusion method.

Antimicrobial resistance among the isolates were done by Kirby Bauers disk diffusion method. Among fluoroquinolone class of antibacterial agents, levofloxacin resistance was observed in 50.9% isolates, ciprofloxacin resistance was noted in 35.8% of CoNS isolates whereas 50.4% of isolates were found to moxifloxacin resistant. Therefore, among fluoroquinolones, CoNS were more susceptible to ciprofloxacin. When considering the beta-lactam agents, resistance to penicillin, erythromycin and clindamycin was observed in 25.1, 30.1 and 48.1% CoNS isolates respectively. A total 48.7% of CoNS isolates were resistant to cefoxitin. Therefore 48.7% were methicillin resistant by disc diffusion test. When tested against vancomycin, the glycopeptide class of antibacterial agent, a total of 54 (10.3%) CoNS isolates were resistant. Among aminoglycosides, gentamicin resistance was observed in 67.8% of isolates. Resistance to tigecycline was observed in 9.4% of CoNS isolates. Among various antibacterial agents, CoNS isolates were less resistant to linezolid (5.9%) and trimethoprim/sulfamethoxazole (7.3%). Table 4.

Table 4. Antimicrobial susceptibility profile of Coagulase Negative Staphylococci (CoNS) isolates by disc diffusion method.

Antimicrobial agent	Susceptible (%)	Resistant (%)
Penicillin	391 (74.9)	131 (25.1)
Erythromycin	318 (60.9)	204 (30.1)
Cefoxitin	268 (51.3)	254 (48.7)
Clindamycin	271 (51.9)	251 (48.1)
Levofloxacin	256 (49.1)	266 (50.9)
Moxifloxacin	259 (49.6)	263 (50.4)
Trimethoprim/sulfamethoxazole	484 (92.7)	38 (7.3)
Gentamicin	168 (32.2)	354 (67.8)
Vancomycin	468 (89.6)	54 (10.3)
Linezolid	491 (94.1)	31 (5.9)
Ciprofloxacin	335 (64.2)	187 (35.8)
Tigecycline	473 (90.6)	49 (9.4)

DISCUSSION

The present study reveals the species distribution, production of virulence factors like slime layer production and biofilm formation and antibiotic susceptibility of various Coagulase-Negative Staphylococci (CoNS) isolates. In most human infections, the rank order of the frequencies of the ten predominant species of CoNS is similar to the rank order of the cutaneous population density of each species. Therefore, the most common Coagulase-Negative Staphylococci (CoNS) pathogen, S. epidermidis, typically produces the largest coagulase negative staphylococcus populations of human epidermis [7]. S. epidermidis and other CoNS

have been a significant contributor to nosocomial infections in recent years. Immunocompromised individuals, such as premature babies, leukaemia patients, and those suffering from other cancerous illnesses, are typically infected by these germs. Previous researchers have indicated that among Coagulase-Negative Staphylococci (CoNS) species, S. epidermidis predominates [4].

Recent research indicates that the incidence was between 60 and 90 %. Because S. epidermidis is so common on human skin, it has been found to cause >80% of CoNS bacteraemias that occur after implant surgery. Coagulase-Negative Staphylococci (CoNS)− caused bacteraemia seldom results in death if treated slowly, yet frank sepsis syndrome can happen, particularly in patients with impaired immune systems [5].

This oversimplified explanation of Coagulase-Negative Staphylococci (CoNS) opportunism, however, falls short of explaining the emergence of these organisms as important pathogens in some infections that were previously and infrequently attributed to them, as well as the propensity of some species, like S. saprophyticus, to colonize and infect particular body sites, like the urogenital tract [6-7]. In the current study, the isolation rate of S. saprophyticus was 16.2%. A uropathogen called S. saprophyticus is linked, globally, to 10-20% of urinary tract infections (UTI) in young, sexually active women [8]. There has been evidence of potential side effects, including acute pyelonephritis, urethritis, and endocarditis, particularly in immunocompromised people. The human gastrointestinal tract, cervix, urethra, vagina, perinaeum, and rectum are frequently colonized by S. saprophyticus [9].

The second most frequent Coagulase-Negative Staphylococci (CoNS) isolate in our investigation was S. haemolyticus. The literature indicates that S. haemolyticus is the second leading species among CoNS that cause illnesses linked to healthcare. In addition to causing blood infections and sepsis, it is frequently isolated from eye infections. Urinary tract infections, peritonitis, and otitis media are also linked to S. haemolyticus. It is important to remember that S. haemolyticus is a species that is known to pick up resistance genes quickly. As a result, the majority of this species' strains are resistant to the antimicrobial drugs now on the market [10-11].

In the current investigation, the isolation rate of S. lugdunensis was 4.2%. It has been observed that S. lugdunensis is an opportunistic pathogen. Few cases of S.lugdunensis infection in neonates have been documented. Nonetheless, research on adult patients has demonstrated the importance of this species as a pathogen. The majority of individuals with positive Coagulase-Negative Staphylococci (CoNS) growth possessed indwelling equipment, such as intravascular lines, and prosthetic limbs. Patients admitted to the medical, surgical, and intensive care units showed higher rates of this. According to some theories, the Coagulase-Negative Staphylococci (CoNS) blood isolates were probably pollutants rather than infections [12].

It is not possible to distinguish clinically significant pathogenic Coagulase-Negative Staphylococci (CoNS) from a nonpathogenic commensal Coagulase-Negative Staphylococci (CoNS) on the basis of biochemical reactions or antimicrobial susceptibilities. Hence isolation of Coagulase-Negative Staphylococci (CoNS) from a clinical specimen often results in a diagnostic dilemma.Among the specific virulence factors that permit differentiation of nonpathogenic CoNS from pathogenic species slime production is very important. Coagulase-Negative Staphylococci (CoNS) forms a complex multi-layer of cells covered with

polysaccharide slime and form biofilm, which allows Coagulase-Negative Staphylococci (CoNS) to persist on the foreign body and become less susceptible to antibiotics.

Biofilm provide survival advantages to the organism by making the cells less accessible to the defence system of the host and also by impairing the action of antibiotics. The ability to form biofilm is the most important virulence factor of CoNS which facilitate its adherence and colonization on artificial materials.

Biofilm formation is a four-step process involving attachment, accumulation, maturation, and detachment. The initial attachment is mediated through various cell wall anchored proteins like Bhp, AtlE, and Fbe as well as intercellular adhesion [13].

The biological factors known to affect slime production include species of CoNS and phase variation of CoNS isolates which represent a virulence factor i.e., slime formation or polymer adherence that contributing to bacterial survival and growth under changing environmental conditions. The technical factors that influence the slime production in vitro are the type of medium, the atmosphere of incubation and the nature of the solid surface [14-15].

In the present study adherence of Coagulase-Negative Staphylococci (CoNS) to different smooth surface were evaluated by Congo red method and microtitre plate method (MTP). A total of 468 (89.7%) showed slime layer production by Congo-red agar-based test whereas 54 (10.7%) showed no slime layer production whereas out of a total of 522. CoNS isolates from various clinical specimens, 471 (90.2%) showed biofilm formation by MTP Method.

The inference of result is subjective in the case of Congo red method and objective in the case of spectrophotometric method. Biological and technical factors might have contributed to the observed results of these tests, as the Congo red was performed in a glass plate and the spectrophotometric method in a microlitre plate. Congo red being simple method can be used for screening of slime layer/biofilm formation in CoNS whereas MTP can be used for confirmation.

In this study, biofilm formation was seen in all Coagulase-Negative Staphylococci (CoNS) spp. isolated from various clinical specimens. Strong biofilm formation was seen predominantly in S. epidermidis. Similar observation was noted by Mulder and Degener [16].

Antimicrobial susceptibility profile of CoNS: Antimicrobial resistance (AMR) in bacteria is an increasing public health issue jeopardizing many achievements of modern medicine. Accordingly, monitoring the resistance situation in major human pathogens like Enterobactereals, Nil-fermenters or Staphylococcus aureus is in the focus of surveillance programs.

Far less attention however is paid to commensal, low pathogenic and environmental microorganisms, which may carry AMR genes as well. In this context, commensal and environmental bacteria are considered to play a role as putative AMR gene reservoirs that may fuel the resistance gene pool of more pathogenic bacteria through horizontal gene transfer (HGT).

Nosocomial Coagulase-Negative Staphylococci (CoNS) are particularly notorious for readily acquiring numerous resistance traits, resulting in (multidrug-) resistance toward many commonly used antimicrobials. In addition, some species (e.g., S. epidermidis) are capable of forming biofilms on indwelling medical devices, making Coagulase-Negative Staphylococci (CoNS) infections sometimes extremely difficult to treat. In the present study, Coagulase-

Negative Staphylococci (CoNS) demonstrated resistance to various classes of antimicrobials including fluoroquinolone and beta-lactam agents [17-22].

Methicillin resistance was observed in 48.7% of Coagulase-Negative Staphylococci (CoNS) isolates. Methicillin resistance by cefoxitin disc diffusion method was noted in 5 species of CoNS including S. epidermidis (69.8%), S. haemolyticus (57.6%), S. saprophyticus (10.9%), S. hominis (5.5%) and S. lugdunensis (9.1%).

The detection of methicillin resistance (MR) in Coagulase-Negative Staphylococci (CoNS) can be critically important for isolates from normally sterile sites. However, detection of MR CoNS is problematic and less reliable than the detection of MR Staphylococcus aureus (MRSA) [23].

CONCLUSION

In present study S. epidermidis, S. haemolyticus and S. saprophyticus were the most common species of Coagulase-Negative Staphylococci (CoNS) isolated from various clinical specimens. A total of 468 (89.7%) showed slime layer production by Congo-red agar-based test whereas 54 (10.7%) showed no slime layer production. Slime layer production was significantly high in CoNS isolates from clinical specimens like urine, blood, pleural fluid and CSF. The resistance to all classes of antibacterial agents was significantly high in S. epidermidis. Coagulase-Negative Staphylococci (CoNS) isolates producing virulence factors like slime layer and biofilms showed maximum resistance to all classes of antibacterials. Consequently, there is a high risk of impaired treatment and dissemination of infection. and, increasing the morbidity and mortality of the admitted patients. Frequent monitoring of species distribution, biofilm development and antibiotic resistance profiles in Coagulase-Negative Staphylococci (CoNS) isolates is recommended from patient's samples. Our understanding of these factors might help us design an effective antibacterial policy for the early treatment of infection.

Conflict of interest: **Nil**
Financial support: **Nil.**

REFERENCES

1. Koneman E, Allen S, Janda W, Schreckenberger P. The gram-positive cocci Part I: Staphylococci and related organism in colour Atlas and Textbook of Diagnostic Microbiology. 5th ed. Lippincott; 1997.
2. Ieven M, Jansens H, Ursi D, Verhoeven J. Rapid detection of methicillin-resistance in CONS. by commercially available Fluorescence Test. J Clin Microbiol 1995; 33: 2183-2185.
3. Davenport DS, Massanari RM, Pfaller MA, Bale MJ, Streed SA, Hierholzer WJ. Usefulness of a test for slime production as a marker for clinically significant infections with coagulase-negative staphylococci. J Infect Dis. 1986;153(2):332-9. doi: 10.1093/infdis/153.2.332, PMID 2935582.
4. Poyart C, Quesne G, Boumaila C, Trieu-Cuot P. Rapid and accurate species-level identification of coagulase-negative staphylococci by using the sodA gene as a target. J

Clin Microbiol. 2001 Dec 1;39(12):4296-301. doi: 10.1128/JCM.39.12.4296-4301.2001, PMID 11724835.

5. Longauerova A. Coagulase negative staphylococci and their participation in pathogenesis of human infections. Bratisl Lek Listy. 2006 Jan 1;107(11-12):448-52. PMID 17425165.
6. Crass BA, Bergdoll MS. Involvement of coagulase-negative staphylococci in toxic shock syndrome. J Clin Microbiol. 1986 Jan;23(1):43-5. doi: 10.1128/jcm.23.1.43-45.1986, PMID 3700606.
7. Michalik M, Samet A, Podbielska-Kubera A, Savini V, Międzobrodzki J, Kosecka-Strojek M.Coagulase-negative staphylococci (CoNS) as a significant etiological factor of laryngological infections: A review. Annals of clinical microbiology and antimicrobials. 2020; 19:1-0.
8. Biavasco F, Vignaroli C, Varaldo PE. Glycopeptide resistance in coagulase-negative staphylococci. Eur J Clin Microbiol Infect Dis. 2000; 19:403-17.
9. HALL SL. Coagulase-negative staphylococcal infections in neonates. Pediatr Infect Dis J. 1991 Jan 1;10(1):57-67. doi: 10.1097/00006454-199101000-00012, PMID 2003057.
10. 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. J Clin Microbiol. 2005 May;43(5):2286-90. doi: 10.1128/JCM.43.5.2286-2290.2005, PMID 15872257.
11. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM et al. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol. 1985 Dec;22(6):996-1006. doi: 10.1128/jcm.22.6.996-1006.1985, PMID 3905855.
12. Suzuki EI, Hiramatsu KE, Yokota TA. Survey of methicillin-resistant clinical strains of coagulase-negative staphylococci for mecA gene distribution. Antimicrob Agents Chemother. 1992 Feb;36(2):429-34. doi: 10.1128/AAC.36.2.429, PMID 1605606.
13. Tornero E, García-Oltra E, García-Ramiro S, Martínez-Pastor JC, Bosch J, Climent C et al. Prosthetic joint infections due to Staphylococcus aureus and coagulase-negative staphylococci. Int J Artif Organs. 2012; 35(10):884-92. doi: 10.5301/ijao.5000148, PMID 23138701. Yeat:2003, page no: 384-404.
14. Cunha MD, Rugolo LM, Lopes CA. Study of virulence factors in coagulase-negative staphylococci isolated from newborns. Mem Inst Oswaldo Cruz. 2006;101(6):661-8. doi: 10.1590/s0074-02762006000600014, PMID 17072480.\
15. John JF, Harvin AM. History and evolution of antibiotic resistance in coagulase-negative staphylococci: susceptibility profiles of new anti-staphylococcal agents. Ther Clin Risk Manag. 2007 Dec 30;3(6):1143-52. PMID 18516271.
16. Bannerman T, 2003. Staphylococcus. *Micrococcus* and other catalase-positive cocci that grow aerobically. In: Murray P, Baron E, Jorgerson J, Pfaller M, Yolken R, editors. Manual of clinical microbiology. Washington: ASM Press.
17. Azimi T, Mirzadeh M, Sabour S, Nasser A, Fallah F, Pourmand MR. Coagulase-negative staphylococci (CoNS) meningitis: a narrative review of the literature from 2000 to 2020.

New Microbes New Infect. 2020; 37:100755. doi: 10.1016/j.nmni.2020.100755, PMID 33014383.

18. Schreckenberger PC, Ilendo E, Ristow KL. Incidence of constitutive and inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci in a community and a tertiary care hospital. J Clin Microbiol. 2004 Jun;42(6):2777-9. doi: 10.1128/JCM.42.6.2777-2779.2004, PMID 15184468.
19. Peters G, Locci R, Pulverer G. Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters. J Infect Dis. 1982 Oct 1;146(4):479-82. doi: 10.1093/infdis/146.4.479, PMID 7119478.
20. Tenover FC, Jones RN, Swenson JM, Zimmer B, McAllister S, Jorgensen JH. Methods for improved detection of oxacillin resistance in coagulase-negative staphylococci: results of a multicenter study. J Clin Microbiol. 1999 Dec 1;37(12):4051-8. doi: 10.1128/JCM.37.12.4051-4058.1999, PMID 10565931.
21. Jean-Baptiste N, Benjamin DK, Cohen-Wolkowiez M, Fowler VG, Laughon M, Clark RH et al. Coagulase-negative staphylococcal infections in the neonatal intensive care unit. Infect Control Hosp Epidemiol. 2011 Jul;32(7):679-86. doi: 10.1086/660361, PMID 21666399.
22. Barbier F, Ruppé E, Hernandez D, Lebeaux D, Francois P, Felix B et al. Methicillin-resistant coagulase-negative staphylococci in the community: high homology of SCCmec IVa between Staphylococcus epidermidis and major clones of methicillin-resistant Staphylococcus aureus. J Infect Dis. 2010 Jul 15;202(2):270-81. doi: 10.1086/653483, PMID 20550456.
23. Ieven M, Verhoeven J, Pattyn SR, Goossens H. Rapid and economical method for species identification of clinically significant coagulase-negative staphylococci. J Clin Microbiol. 1995 May;33(5):1060-3. doi: 10.1128/jcm.33.5.1060-1063.1995, PMID 7615705.