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Coagulase-Negative Staphylococci in Hospital settings: Distribution, antibiotic resistance and biofilm formation

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Abstract

Coagulase-Negative staphylococci (CoNS) are increasingly recognized as important opportunistic pathogens in health- care settings. These organisms are commonly found on human skin and mucous membranes but can cause serious infections, particularly in immunocompromised patients and those with implanted medical devices. The aim of this study is to investigate the prevalence and distribution of CoNS species in various hospital environment sites and assess their potential role as reservoirs for health-care associated infections. This study was conducted in Algerian hospital over 6 months. 200 environmental samples were collected from different sites (beds, door, tables, serum stands, etc.). isolates were identified API Staph kits and Vitek 2 t automated system. Antibiotic susceptibility was determined by disk diffusion method. A total of 78 *Staphylococcus* strains (52 CoNS strains) were recovered from 200 samples. the most prevalent species identified were *Staphylococcus haemolyticus* (n=15), *Staphylococcus hominis* (n=10) and *Staphylococcus Epidermidis* (n=7). Higher concentrations were found on frequently touched surfaces. High levels of antibiotic resistance were observed, particularly against Penicillin (96%). Notably, all strains demonstrated the ability to form biofilms. Our findings highlight the ubiquitous presence of CoNS in hospital environment. The high prevalence of antibiotic-resistant strains underscores the importance of effective infection control measures and judicious use of antibiotics to mitigate the risk of healthcare-associated infections caused by these opportunistic pathogens.

Key words: Coagulase-Negative Staphylococci, Hospital environment, Antibiotic-resistance, Biofilm

Introduction

Healthcare- associated infections (HAIs) remain a significant global health challenge, with environmental surfaces playing a crucial role in pathogen transmission (**Alamer et al., 2022; CDC, 2024**). Among the diverse microbial communities found in the hospital environments, Coagulase-Negative Staphylococci (CoNS) have

emerged as important opportunistic pathogens, particularly in vulnerable patient populations **(Heilmann et al., 2019; Becker et al., 2020;**). Once considered mere contaminants, CoNS are now recognized as leading causes of nosocomial bloodstream infections and device-associated infections, accounting for a substantial proportion of HAIs worldwide (**Heilmann et al., 2019**). The clinical significance of CoNS has grown considerably in recent years, paralleling advances in medical interventions and the increasing use of implantable medical devices. These microorganisms, which are part of the normal human skin microbiota, can become formidable pathogens when they breach the body's natural defenses or colonize medical implants (**Michel et al., 2021**). The most clinically relevant species, including *Staphylococcus epidermidis*, *S. haemolyticus* and *S. hominis*, have demonstrated remarkable adaptability to the hospital environment and the ability to form robust biofilm on both biotic and abiotic surfaces (**Otto, 2008; Franca et al.,2021**). The clinical management of CoNS infections is further complicated by the increasing prevalence of multidrug-resistant strains (**Deyno et al.,2018**). The selective pressure exerted by widespread antibiotic use in healthcare settings has led to emergence of CoNS isolates resistant to multiple classes of antibiotics. This trend not only limits therapeutic options but also raises concerns about the potential for these organisms to serve as reservoirs genes that could be transferred to more virulent pathogens (**May et al.,2014; Asante et al.,2021**). Hospital surfaces serve as potential reservoirs of CoNS, facilitating their persistence and transmission within healthcare settings (**Cruz-Lopez et al., 2021**). The ability of these microorganisms to survive on dry surfaces for extended periods, coupled with their propensity for biofilm formation, makes them particularly challenging to eradicate from the hospital environment (**Becker et al.,2014**). High-touch surfaces such as bed rails, door handles, and medical equipment have been identified as hotspot for CoNS contamination, potentially serving as fomites for patient -to-patient transmission (**Dresch et al.,2018).** Despite their clinical significance, comprehensive data on the prevalence, species distribution, and antimicrobial resistance profiles of CoNS contaminating hospital surfaces remain limited in many healthcare settings specially in Algeria. This knowledge gap hampers the development of targeted infection control strategies and may contribute to the underestimation of the role these organisms play in HAIs. The present study aims to address this gap by investigating the prevalence of CoNS on hospital environmental surfaces, identifying their species diversity, and evaluating their antimicrobial susceptibility patterns.

Methods

Samples collection

This is a prospective study conducted from March to August 2023. It involves two hundred Samples collected from various sites identified as the most highly contaminated areas (the most likely to be touched: tables, doors, beds, serum stands, scopes etc.) within an Algerian hospital's environment using sterile swabs.

Samples identification

Samples were pre incubated on Brain Heart Infusion Broth (BHIB) for 24hours at 37°C. Positive swabs showing turbidity were then cultured on Mannitol Salt Agar medium (MSA) for 24 to 48 hours at 37°C (**Salo et al.2000**). Purification was performed on UriSelect chromogenic agar. Macroscopic and microscopic observation of colonies were performed after that, species were identified using rapid orientation tests such as Gram staining, catalase and coagulase (**Brown et al.,2005**). Further identification was carried out using API STAPH Kit (Biomérieux), with confirmation provided by VITEK automated system.

Antibiotic susceptibility testing

Antibiotic susceptibility detecting was determined using the disk diffusion method on Muller -Hinton solid medium (MH) following the Clinical and Laboratory Standards Institute (CLSI) recommendations (**CLSI, 2023**). The antibiotics tested were: Penicillin G (P) (10 µg), Cefoxitin (CX) (30 µg), Gentamycin (CN) (10 µg), Amikacin (AK) (30 µg), Erythromycin (E) (15 µg), Clindamycin (CD) (2 µg), Ciprofloxacin (CIP) (5 µg), Levofloxacin (LE) (5 µg), Trimethoprim+ Sulfamethoxazole (SXT) (1.25/23.75 µg), Rifampicin (RIF) (5 µg), Tetracycline (TE) (30 µg), Chloramphenicol (C) (30 µg), Fusidic acid (FA) (10 µg), Quinupristin-dalfoprostin (PR) (15µG).

Complementary tests

According to the CLSI, 2023 two complementary tests were performed. The first was the inducible Clindamycin resistance test, using clindamycin and erythromycin disks placed 12mm apart on Muller-Hinton agar medium. The second was the oxacillin resistance test, also known as the gene mecA screening test, which was conducted using CX on the same medium.

Biofilm formation screening test

The ability of all isolated strains to form biofilms was tested using the Congo Red Agar method (CRA). The experiment was performed in triplicate and repeated three times (**Mathur et al.,2006; Freeman et al.,1989**).

Results

Out of 200 samples collected, 78 *staphylococcus* strains were isolated. Gram staining, catalase and coagulase tests, as well as identification using API and the Vitek 2 automated system, enabled differentiation between coagulase-positive and coagulase-negative staphylococci. Analysis of the resultant data, as presented in Table 1, revealed a notable predominance of coagulase-negative *Staphylococcus* species (n=52) (66.66%) within the sample population (**Table 1**).

Table 1. Prevalence of *staphylococcus* species

	Species	n	%	
Coagulase Positive Staphylococci (CoPS)	*S.aureus*	26	33.33	
Coagulase Negative Staphylococci (CoNS)	*S.hominis*	10	19.23	66.66 %
	S.auricularis	4	7.69	
	S.cohnii	3	5.76	
	S.lentus	2	3.84	
	S.xylosus	6	11.54	
	S.chromogenes	2	3.84	
	S.saprophyticus	3	5.76	
	S.epidemidis	7	13.46	
	S. haemolyticus	15	28.85	

Prevalence and distribution of coagulase negative staphylococci species by sites

Beds are the most significant hot spot, with the highest diversity and abundance of *staphylococcus* species. *S. haemolyticus* is particularly prevalent here (8 occurrence). Tables are the second most significant hot spot, with a similar diversity to beds but slightly lower counts overall. *S. haemolyticus* is the most widely distributed species, found in high numbers: on beds and tables, and present on serum stands trolleys and windows. *S. hominis* is also widely distributed, found on beds, tables, serum stands, trolleys and windows. Some species show sites specificity:

- *S.epidermidis* is mainly found on doors , serum stands , and trolleys.
- *S.saprophyticus* is only found on beds and tables.
- *S.lentus*, *S.chromogenes* and *S.cohnii* are primarily found on beds and tables.

Doors, aerosol, scopes and windows shows very low diversity with only one species found at each of these sites. Trolleys shows moderate diversity, with four different species present (**Tables 2a and 2b**).

Table 2a. Distribution of environmental coagulase negative staphylococci (CoNS) strains by sites

Sites	n	%
Beds	20	38.46
Tables	15	28.85
Serum stands	7	13.46
Scopes	1	1.92
Doors	2	3.85
Aerosol	1	1.92
Trolleys	5	9.61
Windows	1	1.92

Table 2b. Coagulase-negative staphylococci species Hot Spot Analysis

Site	*S.auricularis*	*S.chromogenes*	*S.cohnii*	*S.epidermidis*	*S.haemolyticus*	*S.hominis*	*S.lentus*	*S.saprophyticus*	*S.xylosus*
Beds	2	1	1	0	8	3	1	1	3
Tables	2	1	1	0	3	3	1	2	2
Serum stands	0	0	1	2	2	2	0	0	0
Doors	0	0	0	2	0	0	0	0	0
Aerosol	0	0	0	1	0	0	0	0	0
Scopes	0	0	0	0	0	1	0	0	0
Trolleys	0	0	0	2	1	1	0	0	1
Windows	0	0	0	0	1	0	0	0	0

Antimicrobial susceptibility testing results

Antimicrobial susceptibility testing was conducted to assess the efficacy of several antibiotics against the bacterial isolates collected during the study. The results demonstrated a varied susceptibility profile among the isolates. According to Table 3, a highest to moderate high resistance were observed with Penicillin (96.15%), Cefoxitin (62.23%), Amikacin (73.08%) and, Gentamycin (59.62%). However, Rifampicin, Fusidic acid, Clindamycin, Levofloxacin, Pristinamycin and Trimethoprim+ Sulfamethoxazole showed a moderate resistance. In conjunction with this, Table 4 categorizes the isolates into distinct resistotype groups based on their resistance patterns (**Tables 3and 4**).

Table 3. Resistance profile of Coagulase-negative staphylococci strains

Antibiotics	n	%
Penicillin G (P) (10 µg)	50	96.15
Cefoxitin (CX) (30 µg)	36	62.23
Gentamycin (CN) (10 µg)	31	59.62
Amikacin (AK) (30 µg)	38	73.08
Erythromycin (E) (15 µg)	45	86.54
Clindamycin (CD) (2 µg)	26	50
Ciprofloxacin (CIP) (5 µg)	35	67.31

Levofloxacin (LE) (5 µg)	28	53.85
Trimethoprim+ Sulfamethoxazole (SXT) (1.25/23.75 µg)	25	48.08
Rifampicin (RIF) (5 µg)	33	63.46
Tetracycline (TE) (30 µg)	39	75
Chloramphenicol (C) (30 µg)	4	7.69
Fusidic acid (FA) (10 µg)	30	57.69
Quinupristin-dalfoprostin (PR) (15µG)	27	51.52

Table 4. Resistotype distribution of coagulase-negative staphylococci strains

Resistotype	Antimicrobial resistance profile	Isolates n (%)
I	P/CX/CN/AK/E/CD/CIP/LE/SXT/RIF/TE/C/FA/PR	11 (21.2%)
II	P/CX/CN/AK/E/CD/CIP/LE/RIF/TE/FA	8 (15.4%)
III	P/CX/CN/AK/E/CIP/LE/TE/FA	5 (9.6%)
IV	P/E/CIP/LE/SXT/RIF/TE/FA	4 (7.7%)
V	P/CX/AK/E/CD/TE/FA	3 (5.8%)
VI	P/CN/AK/E/CD/CIP/LE/FA/PR	3 (5.8%)
VII	P/CX/CN/E/CD/RIF/TE	2 (3.8%)
VIII	P/E/CD/CIP/LE/SXT/FA/PR	2 (3.8%)
IX	P/CN/AK/E/CIP/LE/SXT/RIF/TE	2 (3.8%)
X	Other unique patterns	12 (23.1%)

There are 9 distinct resistotypes that occur in more than one isolate, accounting for 76.9% of all isolates. The most common resistotype (I) shows resistance to all 14 antibiotics tested, occurring in 21.2% of isolates. The second most common resistotype (II) is similar to type I but lacks resistance to Trimethoprim+ Sulfamethoxazole (SXT), Chloramphenicol(C), and Quinupristin-dalfoprostin (PR), occurring in 15.4% of isolates. The remaining 23.1% of isolates have unique resistance patterns, grouped under "Other unique patterns". Most common resistances across the major resistotypes are to Penicillin (P), Erythromycin (E), and Fusidic Acid (FA). Chloramphenicol resistance is only present in the most resistant group (type I), which aligns with its low overall resistance rate.

This analysis provides a clearer picture of the predominant resistance patterns in our bacterial population. It shows that while there is significant variability in resistance profiles, there are also several common patterns that emerge across multiple isolates.

Complimentary tests results

In addition to the primary antimicrobial susceptibility testing, we conducted supplementary tests to evaluate inducible Clindamycin resistance and resistance to Oxacillin. These tests provide further insights into the resistance mechanisms of the bacterial isolates. Antibiotic resistance testing revealed significant levels of resistance among the 52 *Staphylococcus* strains isolated. Notably, 26 strains (50%) demonstrated inducible

Clindamycin resistance, while 36 strains (60.23%) showed resistance to Oxacillin. These findings suggest a high prevalence of antibiotic-resistant *Staphylococcus* in the studied environment, with more than half of the isolates potentially being Methicillin-Resistant *Staphylococcus* species (MRS), as indicated by Oxacillin resistance (**Table 5, Figure 1a and 1b**).

Antibiotic resistance	Positive strains	Percentage (%)	Negative strains	Percentage (%)
inducible clindamycin resistance	26	50	26	50
oxacillin resistance	36	60.23	16	39.77

Table 5. Complementary tests results

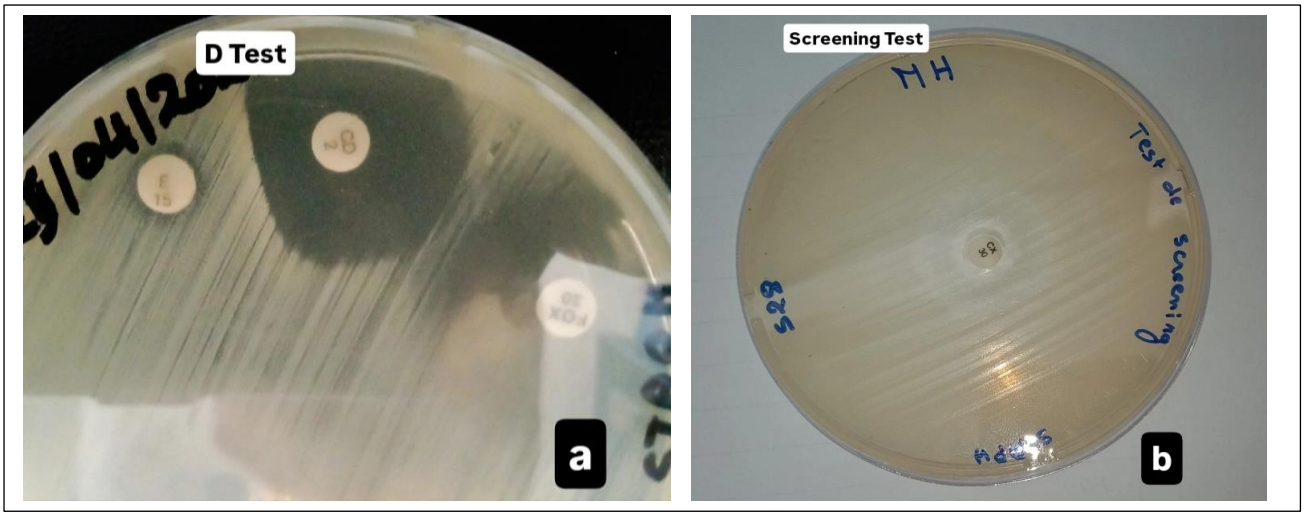

Figure 1. D-test (a) and Oxacillin resistance test (b) results

Biofilm formation screening test results

All isolated *Staphylococcus* strains (n=52) were evaluated for their biofilm-forming ability using the Congo Red Agar (CRA) method. This qualitative method allows for the visual detection of biofilm production based on the

color and texture of bacterial colonies grown on CRA plates. Our results indicated that 100% (52/52) of the tested strains demonstrated biofilm-forming capacity, as evidenced by black colonies with a dry crystalline consistency on CRA (**Figure 2**).

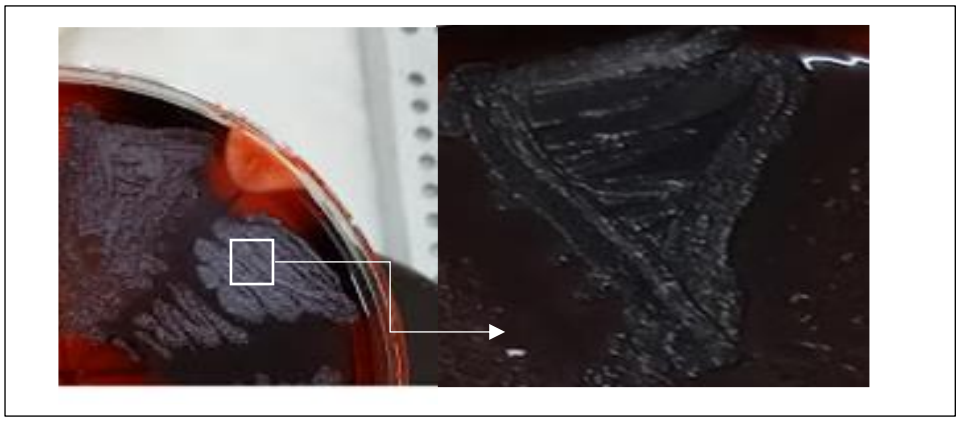

Figure 2. Black colonies with dry crystalline consistency on CRA indicating biofilm forming

Discussion

The prevalence and distribution of *Staphylococcus* species, particularly coagulase-negative staphylococci (CoNS), in hospital environments is a critical area of study due to their potential impact on healthcare-associated infections (HAIs). Our study, which examined 200 samples from various hospital sites, found that 39% (78/200) of samples were positive for *Staphylococcus* species, with more than half being CoNS.

The overall prevalence of *Staphylococcus* species in our study (39%) is comparable to findings from other hospital-based studies. For instance, **Chaoui et al. (2019)** reported a 39.5% (79/200) prevalence of *Staphylococcus* species in a Moroccan hospital. Our finding that CoNS comprised more than half of the positive samples aligns with the growing recognition of CoNS as important opportunistic pathogens in healthcare settings (**Becker and al.,2014**). These results corroborate the findings of **Shi et al. (2020),** where CoNS constituted 54.5% (222 strains) of the isolated species. Our study revealed a diverse distribution of CoNS in the hospital environment, with *Staphylococcus haemolyticus* being the most prevalent species, followed by *S. hominis* and *S. epidermidis*. This distribution aligns with recent trends observed in healthcare settings, where *S. haemolyticus* has emerged as a significant nosocomial pathogen due to its multidrug resistance and biofilm-forming capabilities (**Chi et al.,2022**). The prominence of *S. epidermidis* and *S. hominis* is consistent with their status as common skin commensals and their increasing recognition as opportunistic pathogens in healthcare-associated infections (**Becker and al.,2014**). The identification of less common species such as *S. auricularis*, *S. cohnii*, *S. lentus*, *S. xylosus*, *S. chromogenes*, and *S. saprophyticus* underscores the complex microbial ecology of hospital environments and the potential for these species to serve as reservoirs for antimicrobial resistance genes.

The highest levels of contamination in our study were found on beds (n= 20), tables (n=15), serum stands (n=7), and trolleys (n=5), which are all high-touch surfaces in close proximity to patients. This finding aligns with several other studies investigating bacterial contamination in hospital environments. For instance, **Firesbhat et al. (2021)**, reported similar results where high touch surfaces were more contaminated. The high prevalence of multidrug-resistant *Staphylococcus* species observed in our study, particularly the near-universal resistance to penicillin (96%), aligns with recent findings in healthcare settings. **Xu et al. (2017)** reported similarly high rates of penicillin resistance among CoNS isolated from hospital surfaces in China. Our finding that over half of the strains exhibited both inducible clindamycin resistance and oxacillin resistance is particularly alarming. This rate is higher than that reported by **Marincola et al. (2021)** who found inducible clindamycin resistance in 7% of Staphylococcus isolates from individuals in Germany. The higher prevalence in our study might indicate a more severe problem with methicillin-resistant *Staphylococcus* (MRS) in our healthcare setting. However, its also mirror trends observed by **Asante et al. (2021)** who found high rate of MRS. The observation that all isolated Staphylococcus strains demonstrated biofilm-forming capabilities using the Congo Red Agar (CRA) method is a significant finding with important implications for hospital hygiene and infection control. This result underscores the widespread potential for biofilm formation among Staphylococcus species in healthcare environments.

Conclusion

In conclusion, this study reveals a significant presence of multi-drug resistant *staphylococcus* species on various hospital surfaces, highlighting a critical issue in healthcare-associated infections. Our investigation of 200 samples from different hospital surfaces yielded 78 *Staphylococcus* strains, with predominance of Coagulase-Negative Staphylococci (CoNs). Notably, *S. haemolyticus* emerged as the most common species followed by *S.hominis* and *S.epidemidis* underscoring the increasing relevance of CoNS in hospital environments.

The antibiotic resistance profile of these isolates present a concerning picture. nearly all strains (96.15%) demonstrated resistance to Penicillin, while high resistance rates were observed for several other antibiotics, including Erythromycin (86.54%), Cefoxitin (62%), Ciprofloxacin (67.81%), Amikacin (73.08%) and Tetracycline (75%). Moreover, over half of the strains exhibited inducible Clindamycin resistance and Oxacillin resistance suggesting a high prevalence of Methicillin Resistante *Staphylococcus*. these findings indicate a substantial challenge in treating potential infections caused by these environmental contaminants.

Perhaps most alarmingly, all isolated strains demonstrated biofilm forming capacity using the Congo Red Agar method. this universal biofilm formation ability, coupled with the high antibiotics resistance rates, suggest these strains possess enhanced survival capabilities in hospital environments and increased resilience against both antibiotic treatment and disinfections procedures.

These results underscore the critical need for robust infection control measures in health care settings. strength surface disinfection protocols, particularly focusing on frequently touched surfaces such as beds and tables, are crucial points. Furthermore, the high prevalence of antibiotic- resistance strains emphasize the importance of judicious antibiotic use to prevent further selection of resistante bacteria.

In light of these findings, further research into novel decontamination strategies and alternative antimicrobial agents is warranted. additionally regular surveillance of hospital surfaces for multi-drug resistant organisms should be considered as a part of comprehensive infection control programs. addressing this growing public health concern will require a multifaceted approach, combining improved hygiene practices, antibiotics stewardship, and innovative solution to combat biofilm formation and antibiotic resistance.

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