N'Guessan et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (1): 32 - 38 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jan 2026; Vol.27 No.1
AJCEM/2451. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Phenotypic identification and species characterization of clinically significant coagulase-negative staphylococci isolates at Bouaké University Hospital, Côte d'Ivoire

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Abstract:

Background: Coagulase-negative staphylococci (CoNS) are now recognized as important agents of nosocomial infections, particularly in immunocompromised patients in the healthcare settings, due to their ability to form biofilms and their high resistance to methicillin. The aim of this study is to identify and characterize different species of CoNS isolated at the Bacteriology-Virology Laboratory of the Bouaké University Hospital, Côte d'Ivoire
Methodology: This was a laboratory-based study conducted at the Bacteriology-Virology Laboratory of the Bouaké University Hospital (CHUB) over a two-month period (November to December 2021). Coagulase negative staphylococci isolated from biological samples of hospitalized patients in the routine laboratory were first re-identified by Gram staining and conventional biochemical tests (catalase, coagulase) and further characterized into species using the API® Staph gallery (BioMérieux, France). Antibiotic susceptibility of the isolates was determined by the Kirby-Bauer disc diffusion method.
Results: A total of 100 CoNS were isolated from samples received from various departments of the CHUB during the period of the study. The main CoNS species identified among these were *Staphylococcus xylosus* (n=30), *Staphylococcus haemolyticus* (n=20), *Staphylococcus epidermidis* (n=18), *Staphylococcus lentus* (n=12), *Staphylococcus saprophyticus (*n=7), *Staphylococcus capitis* (n=4), and *Staphylococcus chromogenes (*n=3). The proportion of CoNS isolates resistant to penicillin was 91.0%, tetracycline 72.0%, cotrimoxazole 67.0%, cefoxitin 65.0%, kanamycin 61.0%, tobramycin 54.0%, levofloxacin 53.0%, and gentamicin 50.0%. The most frequent species, *S. haemolyticus* (100%) and *S. xylosus* (60.0%) were resistant to methicillin. Kanamycin, tobramycin and gentamicin (KTG) aminoglycoside resistance phenotypes were seen in 95.0% of S. *haemolyticus* and 86.0% of *S. saprophyticus* isolates. The constitutive MLS_B resistance phenotype was seen in 13.0% of *S. xylosus* and 11.0% of S. *epidermidis.*
Conclusion: A variety of CoNS species were isolated in this study, with *S. xylosus* and *S. haemolyticus* being the most frequently identified species. Methicillin resistance rate was 100.0% for *S haemolyticus* isolates which are likely involved in serious, potentially fatal nosocomial infections.

Keywords: Coagulase-negative staphylococci, phenotype, resistance, Côte d'Ivoire

Received Sept 11, 2025; Revised Sep 30, 2025; Accepted Oct 3, 2025

Identification phénotypique et caractérisation des espèces d'isolats de staphylocoques à coagulase négative cliniquement significatifs au CHU de Bouaké, Côte d'Ivoire

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Résumé:

Contexte: Les staphylocoques à coagulase négative (SCN) sont désormais reconnus comme d'importants agents

d'infections nosocomiales, en particulier chez les patients immunodéprimés en milieu hospitalier, en raison de leur capacité à former des biofilms et de leur forte résistance à la méthicilline. L'objectif de cette étude est d'identifier et de caractériser différentes espèces de SCN isolées au laboratoire de bactériologie-virologie du CHU de Bouaké, en Côte d'Ivoire.
Méthodologie: Il s'agit d'une étude en laboratoire menée au laboratoire de bactériologie-virologie du CHU de Bouaké (CHUB) sur une période de deux mois (novembre à décembre 2021). Français Les staphylocoques à coagulase négative isolés d'échantillons biologiques de patients hospitalisés au laboratoire de routine ont d'abord été ré-identifiés par coloration de Gram et tests biochimiques conventionnels (catalase, coagulase) puis caractérisés en espèces en utilisant la galerie API® Staph (Bio Mérieux, France). La sensibilité aux antibiotiques des isolats a été déterminée par la méthode de diffusion sur disque de Kirby-Bauer.
Résultats: Au total, 100 CoNS ont été isolés d'échantillons reçus de différents services du CHUB pendant la période de l'étude. Les principales espèces de CoNS identifiées parmi celles-ci étaient *Staphylococcus xylosus* (n=30), *Staphylococcus haemolyticus* (n=20), *Staphylococcus epidermidis* (n=18), *Staphylococcus lentus* (n=12), *Staphylococcus saprophyticus* (n=7), *Staphylococcus capitis* (n=4) et *Staphylococcus chromogenes* (n= 3). Français La proportion d'isolats de CoNS résistants à la pénicilline était de 91,0%, à la tétracycline de 72,0%, au cotrimoxazole de 67,0%, à la céfoxitine de 65,0%, à la kanamycine de 61,0%, à la tobramycine de 54,0%, à la lévofloxacine de 53,0% et à la gentamicine de 50,0%. Les espèces les plus fréquentes, *S. haemolyticus* (100.0%) et *S. xylosus* (60,0%), étaient résistantes à la méthicilline. Des phénotypes de résistance à l'aminoside kanamycine, tobramycine et gentamicine (KTG) ont été observés chez 95,0% des isolats de *S. haemolyticus* et 86,0% des isolats de *S. saprophyticus.* Le phénotype de résistance constitutif au MLS_B a été observé chez 13,0% de *S. xylosus* et 11,0% de *S. epidermidis*.
Conclusion: Diverses espèces de CoNS ont été isolées dans cette étude, *S. xylosus* et *S. haemolyticus* étant les plus fréquemment identifiées. Le taux de résistance à la méthicilline était de 100,0% pour les isolats de *S. haemolyticus,* probablement impliqués dans des infections nosocomiales graves, potentiellement mortelles.
Mots-clés: Staphylocoques à coagulase négative, phénotype, résistance, Côte d'Ivoire

Introduction:

Coagulase negative staphylococci (CoNS) are commensals of the skin and mucous membranes and have long been described as contaminants when routinely isolated in clinical bacteriology laboratories (1,2). Over the past few decades, their incidence has significantly increased, and CoNS have emerged as a cause of nosocomial infections (3). The distinct populations in which CoNS infections play a predominant role are premature newborns, patients with implanted medical devices, immunocompromised patients, and those with other comorbidities (4). CoNS form biofilms that allows bacteria to adhere to medical devices and, as a result, protects them from antibiotics (5). Infections caused by CoNS are numerous and can be very serious, and CoNS strains isolated from clinical samples are increasingly resistant to antibiotics, particularly methicillin (6).

The prevalence of methicillin resistance in CoNS strains isolated in hospitals is estimated at 75% in several countries around the world (7). New species have been described in recent years, while clinical information on most of these species is still scarce (8). Differentiating between clinically pathogenic strains and strains considered to be contaminants is one of the major challenges facing clinical biology laboratories (7). Identifying CoNS species is still difficult for most clinical laboratories (9) and are detected by conventional methods available in the laboratory. These methods include the use of Chapman agar for the isolation and enumeration of staphylococci, which allows the differentiation of species that ferment mannitol from those that do not ferment mannitol, and the coagulase test or a rapid slide agglutination test for the simultaneous detection of the affinity of fibrinogen, protein A, and capsular polysaccharides, which are the key markers of the bacterium.

Due to the emergence of infections caused by potentially pathogenic and antibiotic-resistant CoNS, these clinical strains should be properly identified and characterized. In our practice, any CoNS isolate was considered a contaminant and the result was reported as negative. The aim of our study is to provide information that will contribute to the identification and better understanding of CoNS species isolated from biological samples at the Bouaké University Hospital, Côte d'Ivoire.

Materials and method:

Study setting and design:

This was a laboratory-based study of CoNS isolated from clinical specimens of hospitalized patients and stored in the laboratory of the Bouake University Hospital (CHUB) during a 2-month period (November to December 2021). These strains were stored in heart-brain broth (HBB) containing glycerol at -80°C in the laboratory.

Ethical considerations:

Ethical approval was obtained from the Institutional Review Board of the Medical Scientific Directorate of Bouaké University Hospital. The study used samples from the laboratory's collection of isolates, therefore, there were no risks of physical harm to patients. Nevertheless, the data were anonymized to protect patient confidentiality.

Re-identification of CoNS isolates:

The clinical isolates were revived from a bacterial suspension in heart-brain broth,

then seeded on blood-enriched agar to purify them. The strains were then cultured on Chapman agar medium and identified on the basis of their cultural characteristics on Chapman agar medium and biochemical characteristics (coagulase test). Morphological characteristics were verified by Gram staining and catalase test, as well as purity.

Latex agglutination for the simultaneous detection of the affinity factor for fibrinogen, protein A, and capsular polysaccharides of *Staphylococcus aureus,* and detection of free staphylocoagulase on human citrated plasma were performed to differentiate *S. aureus* from CoNS species. The different species of CoNS were confirmed using the API Staph gallery, which was done following preparation of a homogeneous bacterial suspension equivalent to 0.5 McFarland by emulsifying an 18-24-hour colony in an ampoule of API Staph medium.

The microtubes were inoculated with the bacterial suspension to obtain colored turns according to the manufacturer's instructions. These reactions were read using the reading chart, and identification was obtained using the API Staph analytical catalog.

Antibiotic susceptibility testing of CoNS isolates:

Antibiotic susceptibility test (AST) of the CoNS isolates was performed by the Kirby Bauer disc diffusion method on Mueller Hinton agar medium (10) as recommended by the WHO. The AST was performed using young colonies incubated for 18 to 24 hours on Chapman agar to prepare inoculum suspension that was standardized to 0.5 McFarland turbidity standards.

Antibiotic discs were applied to Mueller Hinton (MH) agar medium that has been seeded with the standardized inoculum prepared from the pure young CoNS colonies. The antibiotic discs include penicillin G (P, 10IU), cefoxitin (CX, 30µg), vancomycin (VA, 30µg), kanamycin (K, 5µg), gentamicin (CN, 10µg), tobramycin (TOB., 10µg), fosfomycin (FF, 200 µg), ciprofloxacin (CIP, 5µg), norfloxacin (NOR, 10µg), erythromycin (E, 15µg), clindamycin (DA, 2µg), pristinamycin (PT, 15µg), fusidic acid (FA,10µg), and rifampicin (RA, 5µg).

After 18 to 24 hours of incubation in an aerobic incubator at 37°C, the zone diameters of inhibition of the different antibiotics were measured and compared to the reference diameters of the 2021 European Committee on Antimicrobial Susceptibility Testing (EUCAST) to determine sensitivity or resistance of each isolate to the selected antibiotics.

Statistical analysis:

Data entry was done on Microsoft Word version 2019 and Excel version 2019, and the analysis was performed using EPI INFO 7 software. The results were expressed as averages for quantitative variables and as percentages for qualitative variables.

Results:

Source of biological samples for CoNS isolates:

A total of 100 CoNS were isolated from clinical samples of hospitalized patients including 82.0% from blood, 10.0% from pus, and 3.0% from invasive medical devices (Fig 1). The CoNS isolates are mostly of hospital origin (87.0%), mainly from pediatrics (30.0%), internal medicine (17.0%), and intensive care (9.0%), compared to 13.0% that are of community origin (Fig 2).

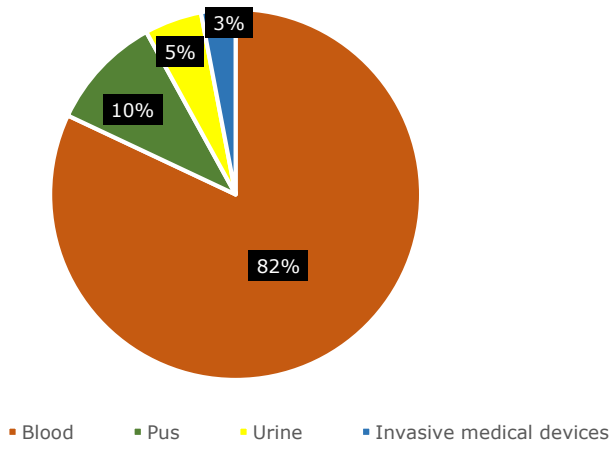

Fig 1: Distribution of coagulase negative staphylococci isolates by sample sources

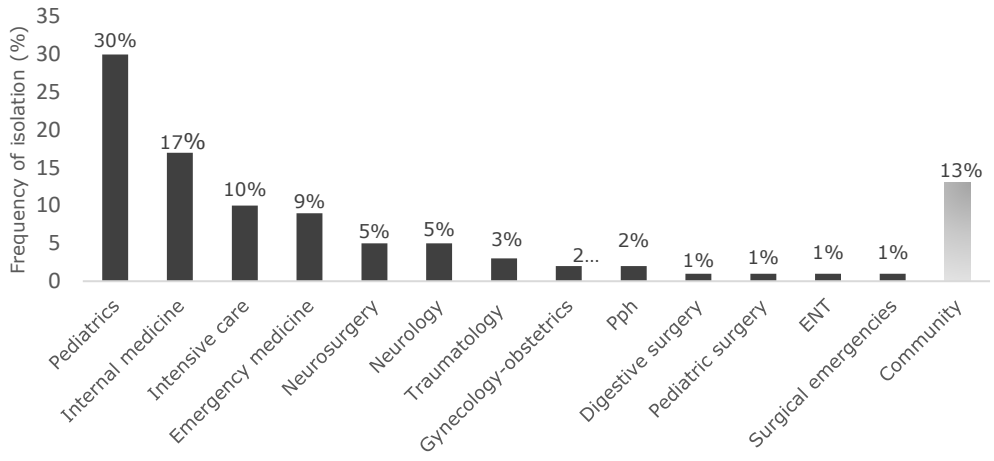

Fig 2: Frequency distribution of coagulase negative staphylococci isolates in hospital and community settings

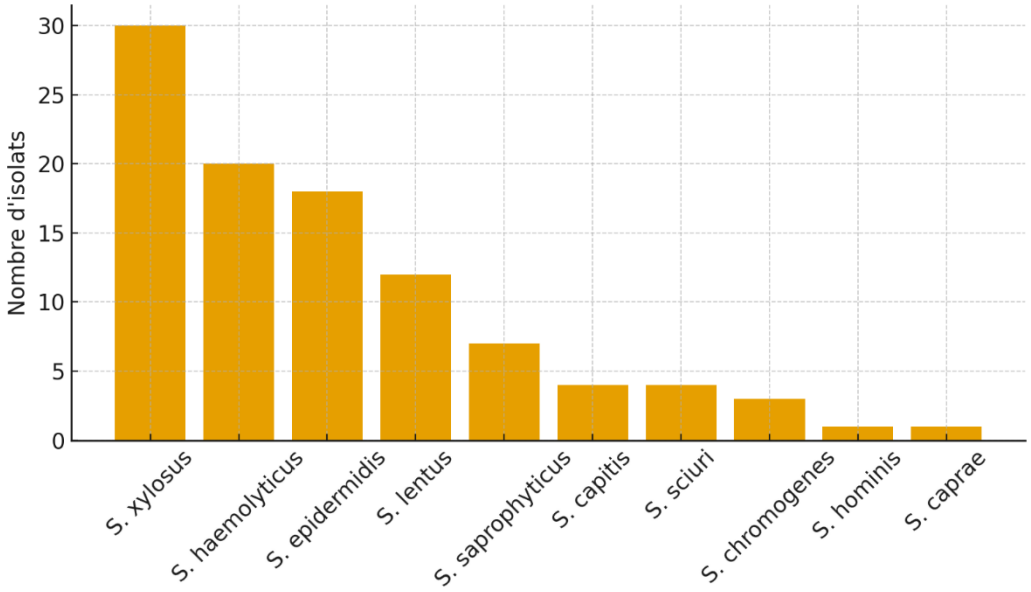

Fig 3: Frequency of coagulase negative staphylococci species identified

Frequency of CoNS species:

Ten CoNS species were identified (Fig 3). The main species were *S. xylosus* (30.0%), *S. haemolyticus* (20.0%), *S. epidermidis* (18.0%), and *S. lentus* (12.0%).

Distribution of CoNS isolates by associated pathologies:

Table 1 shows that a total of 45 CoNS were isolated from patients with infectious syndrome, with 16 *S. xylosus* (35.5%), 7 *S. epidermidis* (15.5%) and 6 *S. lentus* (13.3%) the main species isolated. In addition, *S. haemolyticus* was frequently isolated in patients with infectious syndrome, respiratory distress syndrome in premature babies, and neuro-encephalitic syndrome.

Antibiotic resistance profiles of CoNS isolates:

Of the 100 CoNS isolates tested, 91.0% were resistant to penicillin G, and 65% to cefoxitin, implying a high proportion of methicillin-resistant CoNS (MR-CoNS) isolates (Table 2). Cross-resistance to aminoglycosides of the kanamycin, tobramycin and gentamicin (KTG) phenotype was found in 95.0% of S*. haemolyticus* and 86.0% of *S. saprophyticus*. The constitutive resistance phenotype to MLS_B was found in 13.0% of S*. xylosus* and 11.0% of S*. epidermidis*.

Table 1: Distribution of coagulase negative staphylococci isolates by associated pathologies

Species	**Infectious syndrome (%) (n=45)**	**Suppuration (%) (n=3)**	**Burning sensation during urination (%) (n=1)**	**Neuro-encephalitic syndrome (%) (n=11)**	**Respiratory distress syndrome (%) (n=13)**
Staphylococcus xylosus	16 (35.5)	3 (75.0)	0	1 (9.1)	3 (23.1)
Staphylococcus saprophyticus	3 (6.6)	0	0	2 (18.2)	0
Staphylococcus epidermidis	7 (15.5)	0	1 (100.0)	1 (9.1)	4 (30.8)
Staphylococcus lentus	6 (13.3)	0	0	3 (27.3)	1 (7.7)
Staphylococcus hominis	1 (2.2)	0	0	0	0
Staphylococcus haemolyticus	5 (11.1)	0	0	2 (18.2)	4 (30.8)
Staphylococcus capitis	3 (6.6)	0	0	1 (9.1)	0
Staphylococcus chromogenes	2 (4.4)	0	0	1 (9.1)	0
Staphylococcus sciuri	2 (4.4)	1 (35.5)	0	0	1 (7.7)
Staphylococcus caprae	0	0)	0	0	0

Table 2: Antibiotic resistance of coagulase negative staphylococci isolates to selected antibiotics

Antibiotics	***S. xylosus*** (%) (**n=30**)	***S. lentus*** (%) (**n=12**)	***S. haemolyticus*** (%) (**n=20**)	**Total coagulase-negative staphylococci** (%) (**n=100**)
Penicillin G	29 (97.0)	10 (83.3)	20 (100.0)	91 (91.0)
Cefoxitin	17 (57.0)	5 (41.7)	20 (100.0)	65 (65.0)
Kanamycin	12 (40.0)	8 (66.7)	20 (100.0)	61 (61.0)
Gentamicin	9 (30.0)	6 (50.0)	20 (100.0)	50 (50.0)
Tobramycin	10 (33.3)	7 (58.3)	19 (95.0)	54 (54.0)
Chloramphenicol	5 (16.7)	1 (8.3)	1 (5.5)	12 (12.0)
Erythromycin	11 (36.7)	5 (41.7)	11 (55.5)	43 (43.0)
Clindamycin	4 (13.3)	4 (33.3)	4 (20.0)	16 (16.0)
Linezolid	8 (26.7)	5 (41.7)	3 (15.0)	16 (16.0)
Ciprofloxacin	9 (30.0)	8 (66.7)	17 (85.5)	48 (48.0)
Levofloxacin	11 (36.7)	7 (58.3)	18 (90.0)	53 (53.0)
Tetracycline	22 (73.3)	6 (50.0)	15 (75.0)	72 (72.0)
Cotrimoxazole	17 (57.0)	7 (58.3)	18 (90.0)	67 (67.0)
Vancomycin	3 (10.0)	1 (8.3)	2 (10.0)	12 (12.0)
Fosfomycin	11 (36.7)	2 (16.7)	8 (40.0)	27 (27.0)
Rifampicin	11 (36.7)	4 (33.3)	3 (15.0)	24 (24.0)
Fusidic acid	17 (57.0)	8 (66.7)	7 (35.0)	48 (48.0)
Pristinamycin	2 (6.7)	1 (8.3)	22 (10.0)	7 (7.0)
Minocycline	6 (20.7)	6 (50.0)	9 (45.0)	36 (36.0)

Discussion:

In our study, the main source of biological specimens analysed for the identification of CoNS was from blood cultures of hospitalized or outpatient (community) patients, which agrees with the finding of Zaineb et al., (11) who reported in their study that blood cultures were the most common biological specimens in which CoNS were frequently isolated but Mea (12) reported in his study that the predominant biological specimen was pus while Modibo (13) reported that urine was the main biological specimens in his study. The CoNS isolates were predominantly from the pediatrics department. This predominance could be explained by the high demand for specimen analysis due to the high patient loads and sensitivity of the department as well as the communication that exists between bacteriology-virology and the pediatric department. Zaineb et al., (11) also reported that pediatrics was the department where the isolation rate of CoNS was high.

The identification of CoNS isolates in our study was performed using the API Staph gallery, a miniaturized system that allows the identification of numerous strains of the genera *Staphylococcus, Micrococcus*, and *Kocuria.* We identified 100 CoNS isolates and identify several species such as *S. capitis, S. hominis, S. xylosus,* S*. epidermidis, S. haemolyticus, S.*

saprophyticus, S. caprae, S. chromogenes, S. lentus, and *S. sciuri.* The main species identified was S. *xylosus* (30.0%), followed by S. *haemolyticus* (20%), *S. epidermidis* (18.0%), and *S*. *lentus* (12.0%). Our results differ from those of Nanoukon (14) in Benin in 2017 and Mea (12) in Côte d'Ivoire in 2019, which had a predominance of *S. haemolyticus* (44.0%) and S*. epidermidis* (23.0%), respectively. This difference in CoNS distribution could be due to variability in strain distribution depending on geographical location or sample size.

Staphylococcus xylosus, a commensal of human and animal skin and naturally present in food, is like other CoNS species apart from *S. epidermidis*, increasingly isolated in laboratories. This high frequency of strain isolation has led to renewed clinical interest. A study conducted by Émilie et al., (2) on *S. xylosus* showed their genomic diversity, with most strains being commensal and useful in food, while others could be potentially dan gerous. Tselenis-Kotsowillis et al., (15) were the first to report a case of pyelonephritis caused by S. *xylosus,* Tompkins et al., (16) reported *S. xylosus* infection in a cancer patient. In addition, S*. xylosus* has also been implicated in nosocomial infections, particularly in pneumonia and septicemia in newborns (5, 16,17,18).

Bacteriological results showed that 82.0% of CoNS species in our study were isolated in positive blood cultures. Our results are similar to those of Nanoukon in Benin (14) and Zaineb et al., (11) who reported CoNS isolates in their studies highest from blood cultures. However, the most frequently implicated bacterium responsible for contaminating blood cultures is CoNS, often making it difficult to interpret, as reported by some researchers (3,19). This situation may be the result of prior contamination of the blood culture bottle at the time of collection by healthcare personnel. Our study did not evaluate the risks of blood culture contamination during collection. Although this is a significant limitation of our study, we chose to prioritize the policy of compliance with hygiene, infection prevention, and control measures in place at the CHUB and clinical-biological communication in order to accept or rule out the diagnosis of bacteremia.

The CoNS isolates identified in our series showed variability in antibiotic resistance profiles. The highest resistance rates were observed for tetracycline (72.0%), cotrimoxazole (67.0%), cefoxitin (65.0%), kanamycin (61.0%), tobramycin (54.0%), levofloxacin (53.0%), and gentamicin (50.0%). The CoNS species identified were 91.0% resistant to penicillin. Our results are similar to those of Mea (12) in Ivory Coast, and Nanoukon (14) in Benin, who reported that 94% and 92% of isolated species were resistant to penicillin, respectively.

Methicillin resistance was detected in 65.0% of the isolates identified. In his 2019 study, Mea (12) reported that 40.0% of these isolates were methicillin resistant, with methicillin resistance increasingly observed in CoNS. The main species identified were also resistant to methicillin; S*. xylosus (*57.0%), *S. epidermidis* (72.0%), and *S. haemolyticus* (100%). In our study, *S. haemolyticus* was 100% resistant to penicillin, cefoxitin, kanamycin, gentamicin, and 95.0% resistant to tobramycin. Maria et al., (20) made the same observation in their 2019 study on the comparative analysis of *S. haemolyticus*, revealing the key to hospital adaptation and pathogenicity of *S. haemolyticus. S. haemolyticus* is the second most frequently encountered CoNS species in hospitals and has the highest level of antibiotic resistance.

Multidrug resistance, particularly to methicillin and aminoglycosides, among CoNS is frequently encountered in hospitals. In this study, *S epidermidis* strains showed variable resistance to the antibiotics tested, with 78.0% resistance to penicillin and 72.0% to cefoxitin (methicillin resistant). Our results are similar to that of Modibo (13) in Mali, who reported that S*. epidermidis* strains were 80% resistant to methicillin. *Streptococcus lentus* isolates showed 83.0% resistance to penicillin and 42.0% to cefoxitin (methicillin resistant). A single isolate of *S. caprae* was isolated, which was sensitive (100.0%) to almost all antibiotics tested except penicillin, fosfomycin, and minocycline.

The CoNS species identified were associated different disease syndromes in patients colonized or infected with these strains. These included 45 cases of infectious syndrome, 13 cases of respiratory distress, and 11 cases of neuro-encephalic syndrome. *Staphylococcus haemolyticus* was frequently isolated in one diabetic patient with neuroendocrine syndrome and two premature newborns with respiratory distress syndrome. The increased pathogenicity of CoNS could explain the numerous cases associated with these identified species in our study. Ricarda et al., (4) in their study on CoNS reported that the incidence of CoNS has steadily increased in recent years in parallel with advances in medicine, especially with regard to the use of foreign body devices.

Premature newborns, patients with implanted medical devices, immunocompromised patients, and those with other relevant comorbidities are distinct populations in which CoNS infections play a prominent role. Furthermore, Noshak et al., (21) reported that CoNS are one of the main nosocomial pathogens and can cause several infections such as catheter-related bloodstream infections, skin infections, urinary tract infections, peritonitis, and endocarditis.

Conclusion:

Coagulase-negative staphylococci are commensals of the skin and mucous membranes of humans and animals, and evidence of their contamination of biological samples analyzed in microbiology laboratories is widely reported. Although considered as contaminants in microbiology laboratories, they have become a major cause of infections in immunocompromised persons including premature infants. Specific CoNS species such as *S. xylosus, S. haemolyticus, S. epidermidis,* and *S. lentus,* sometimes of animal origin, are known to be involved in nosocomial infections due to their ability to form biofilms and their high resistance to several classes of antibiotics.

Our study results showed varying rates of resistance to commonly used antibiotics. The emergence of MR-CoNS strains and the numerous resistance profiles found in these bacteria make them potentially lethal. Correct identification of CoNS strains associated with potential clinical infections and genomic surveillance of virulence and resistance factors would be an excellent prospect for CoNS at the Bouake University Hospital Center.

Contributions of the authors:

NAM and AKC designed the study; NAM, MP, TA, TJON, OA and GKJ performed the technical and laboratory analysis of the biological samples collected; NAM and MP analyzed the data and wrote the manuscript; AKC, MP, TJON, GKJ and TA participated in the critical review of the manuscript. All authors read and approved the manuscript submitted for publication.

Source of funding:

This study was supported by the ANDEMIA project.

Conflict of interest:

Authors declare no conflict of interest.

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