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Genotypic identification and antibiotic susceptibility of coagulase negative staphylococci isolates from urine samples of patients in southwest Nigeria

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Abstract:

Background: Urinary tract infection (UTI) largely affects women and is mostly caused by bacteria, among which coagulase negative staphylococci has received little attention. The objectives of this study are to isolate and genotypically identify coagulase negative staphylococci (CoNS) from urine samples of adult females attending selected healthcare facilities from three States (Oyo, Osun and Ogun) in southwest Nigeria, and to determine their susceptibility to selected antibiotics.
Methodology: A case-control study of 471 adult female participants with urinary symptoms in 205 (case) and no urinary symptoms in 266 (control), was conducted over a period of 10 months. Mid-stream voided urine samples were collected from the participants. The samples were cultured on mannitol salt and blood agar plates, and bacteria growths were presumptively identified for CoNS and other bacterial isolates using routine biochemical tests. The CoNS isolates were confirmed by conventional PCR amplification with *Staphylococcus saprophyticus* specific primers and 16S rRNA sequencing with phylogenetic analysis using MegaX software 18 version 10.0.5. Antibiotic susceptibility testing was performed on genotypically identified CoNS isolates using VITEK 2 system. Chi square and Fisher Exact tests were used to compare the prevalence of bacterial UTIs across the States and between symptomatic and asymptomatic participants at α=0.05.
Results: Of the urine samples of 471 participants examined, 178 (37.8%) had bacterial UTIs on cultures, 66 of 117 (56.4%) were from Oyo State, 98 of 241 (40.7%) from Osun State, and 14 of 113 (14.1%) from Ogun State (x^2=49.113, p<0.0001). A total of 34 (7.2%) CoNS isolates were phenotypically identified, with 29 (14.1%) from 205 symptomatic and 5 (1.9%) from 266 asymptomatic participants (OR=8.601, 95% CI=3.266-22.653, p<0.0001). *Staphylococcus saprophyticus* PCR amplified 19 of the 34 CoNS isolates while 16S rRNA sequencing identified 5 CoNS species among the 19 isolates, with *S. saprophyticus* (n=10, 52.6%) *S. lentus* (n=5, 2.8%), *S. xylosus* (n=2, 1.1%), *S. sciuri* (n=1, 0.6%), and *S. rodentium* (n=1, 0.6%). All the CoNS isolates were resistant to daptomycin, sulfamethoxazole-trimethoprim and oxacillin indicating they were multi-drug resistant (MDR) but were all sensitive to nitrofurantoin.
Conclusion: The high resistance rates to commonly used antibiotics such as the β-lactams, sulfamethoxazole-trimethoprim and daptomycin from our study underscore the need for routine genotypic surveillance and antibiotic stewardship in the management of UTIs. Our findings emphasize the clinical relevance of CoNS in UTIs and the necessity for targeted diagnostics and treatment strategies.

Keywords: *Staphylococcus saprophyticus*, Urinary tract infection, multi-drug resistance

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Identification génotypique et sensibilité aux antibiotiques d'isolats de staphylocoques à coagulase négative issus d'échantillons d'urine de patients du sud-ouest du Nigéria

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Résumé:

Contexte: Les infections urinaires (IU) touchent majoritairement les femmes et sont principalement d'origine bactérienne, parmi lesquelles les staphylocoques à coagulase négative ont reçu peu d'attention. Les objectifs de cette étude sont d'isoler et d'identifier génotypiquement les staphylocoques à coagulase négative (SCN) à partir d'échantillons d'urine de femmes adultes fréquentant des établissements de santé sélectionnés dans trois États (Oyo, Osun et Ogun) du sud-ouest du Nigéria, et de déterminer leur sensibilité à certains antibiotiques.
Méthodologie: Une étude cas-témoins a été menée sur une période de 10 mois auprès de 471 femmes adultes, dont 205 présentaient des symptômes urinaires (cas) et 266 n'en présentaient aucun (témoin). Des échantillons d'urine mictionnelle ont été prélevés chez les participantes. Les échantillons ont été cultivés sur des plaques de gélose au sel de mannitol et au sang, et les croissances bactériennes ont été identifiées de manière présomptive pour le CoNS et d'autres isolats bactériens à l'aide de tests biochimiques de routine. Les isolats de CoNS ont été confirmés par amplification PCR conventionnelle avec des amorces spécifiques de *Staphylococcus saprophyticus* et séquençage de l'ARNr 16S avec analyse phylogénétique à l'aide du logiciel MegaX 18 version 10.0.5. Des tests de sensibilité aux antibiotiques ont été réalisés sur les isolats de CoNS identifiés génotypiquement à l'aide du système VITEK 2. Les tests du chi carré et de Fisher Exact ont été utilisés pour comparer la prévalence des infections urinaires bactériennes à travers les États et entre les participants symptomatiques et asymptomatiques à α=0,05.
Résultats: Sur les échantillons d'urine de 471 participants examinés, 178 (37,8%) présentaient des infections urinaires bactériennes sur les cultures, 66 sur 117 (56,4%) provenaient de l'État d'Oyo, 98 sur 241 (40,7%) de l'État d'Osun et 14 sur 113 (14,1%) de l'État d'Ogun (x^2=49,113, p<0,0001). Au total, 34 (7,2%) isolats de CoNS ont été identifiés phénotypiquement, avec 29 (14,1%) provenant de 205 participants symptomatiques et 5 (1,9%) de 266 participants asymptomatiques (OR=8,601, IC à 95%=3,266-22,653, p<0,0001). Français La PCR de *Staphylococcus saprophyticus* a amplifié 19 des 34 isolats de CoNS tandis que le séquençage de l'ARNr 16S a identifié 5 espèces de CoNS parmi les 19 isolats, avec *S. saprophyticus* (n=10, 52,6%), *S. lentus* (n=5, 2,8%), *S. xylosus* (n=2, 1,1%), *S. sciuri* (n=1, 0,6%) et *S. rodentium* (n=1, 0,6%). Tous les isolats de CoNS étaient résistants à la daptomycine, au sulfaméthoxazole-triméthoprime et à l'oxacilline, indiquant qu'ils étaient multirésistants aux médicaments (MDR), mais étaient tous sensibles à la nitrofurantoïne.
Conclusion: Les taux élevés de résistance aux antibiotiques couramment utilisés, tels que les bêta-lactamines, le sulfaméthoxazole-triméthoprime et la daptomycine, observés dans notre étude, soulignent la nécessité d'une surveillance génotypique systématique et d'une gestion responsable des antibiotiques dans la prise en charge des infections urinaires. Nos résultats soulignent la pertinence clinique de la neurosynthèse des antibiotiques dans les infections urinaires et la nécessité de diagnostics et de stratégies thérapeutiques ciblés.

Mots-clés: *Staphylococcus saprophyticus*, infection urinaire, multirésistance aux médicaments

Introduction:

Urinary tract infection (UTI) is the most common type of bacterial infections usually reported in the healthcare facilities (1). It is an infection of the urinary system which consists of the bladder, ureter, kidney and urethra with significant symptoms which include increase rate of urination, enhanced desire to empty the bladder and painful urination, thereby adding to the hospital bills of the affected individual, most especially if it involves multi-drug-resistant (MDR) isolates (1,2). Urinary tract infection is confirmed by the presence of 10^5 microorganisms or of a single strain of bacterium per milliliter in two consecutive mid-stream samples of urine (3).

Among the bacteria that colonize urinary system, *Staphylococcus saprophyticus* is the second most common bacterium in UTIs, mostly common in females due to menarche, menopause and sexual intercourse (4,5). *Staphylococcus saprophyticus* is a commensal Gram-positive, coagulase negative staphylococcus (CoNS) and therefore has the potential to exist among contaminating bacteria, which often leads to lower UTI in young women as a community-acquired UTI (6). Colonization of the gut and genital areas has been linked to this kind of UTI (7).

Antimicrobial resistance (AMR) is an inevitable outcome of evolutionary adaptation of microbes which has emerged as epidemic crisis in clinical medicine worldwide (8,9). Bacterial resistance to antimicrobial drugs is one of the most serious menaces to global public health (10,11). Human and improper uses of antimicrobial drugs have driven the intense rapid and prevalent outburst of resistance in both pathogenic and commensal organisms (12). The challenge of AMR knows no limitation, drug-resistant microbes of all kinds can move among humans and animals, and from countries to countries (13).

The looming threat of incurable staphylococcal infections most especially coagulase negative staphylococci is at the latest corkscrew in an international public health night-

mare, increasing bacterial resistance to many antibiotics that once cured (14). When *Staphylococcus* species are present and multiply in generally sterile sites such as the urinary tract, they cause harm to the host. However, *Staphylococcus* species are usually harmless on the skin, gastrointestinal tract, and periurethral area.

The dominance of *S. saprophyticus* infections had been in reality since the inception of mankind because the organism has the ability to adhere to urothelial cells and produce urease. Colonization is a very common phenomenon even in an environment thought to be nearly sterile (15). *Staphylococcus saprophyticus* has been accorded little attention as an important causative agent of UTIs. This study was therefore carried out to determine the prevalence of *S. saprophyticus* in UTI and antibiotic susceptibility of the isolates to selected antibiotics.

Materials and method:

Study setting and design:

This was a laboratory-based case-control study carried out in selected healthcare facilities from three States (Oyo, Ogun and Osun) in southwest region of Nigeria. The facilities from Oyo State were University Health Service (UHS) and Oyo State Hospital Management Board (OYSHMB); from Ogun State, Sacred Heart Hospital (SHH) and Federal Medical Centre (FMC) Abeokuta, and from Osun State, Osun State University Teaching Hospital and Osun State Hospital Management Board (OSHMB) Osogbo.

Study participants, sample size and method of sampling:

The sample size of 471 participants (205 with urinary symptoms as case and 266 without urinary symptoms as control) was determined using the appropriate sample size formula. Participants were individuals attending the outpatient departments of each selected hospital in the States and were randomly selected over a period of 12 months, with 117 from Oyo, 113 from Ogun and 241 from Osun State. Participants who have received antibiotics in the 30 days preceding recruitment and those who declined to give consent were excluded.

Ethical approval and informed consent:

Ethical approval was obtained from the Ethical Committees of the three selected hospitals in Oyo, Osun and Ogun States approval numbers AD16/479/634, OSHREC/PRS/569T/173 and HPRS/381/341 respectively. Informed consent was obtained from each participant, and the rights of each participant to remain anonymous and decline study participation were upheld.

Urine sample collection:

Clean catch specimen of voided midstream urine was collected from each participant into a sterile urine sample bottle and transported to the Pharmaceutical Microbiology laboratory, University of Ibadan, within one hour of collection for microbiological analysis.

Urine culture, isolation and identification of coagulase negative staphylococci:

The urine sample of each participant was carefully mixed and about 1µL inoculated by streaking onto mannitol salt and blood agar plates using a sterile wire loop and incubated aerobically at 37^{o}C overnight. The culture plates were observed for growth of staphylococcal-like colonies and identified by Gram staining and biochemical tests including catalase, coagulase, and novobiocin disc tests (17). Urinary tract infection caused by CoNS was confirmed when there was ≥ 10^5 colony-forming units of a single CoNS per milliliter (CFU/ml) of urine.

Antibiotic susceptibility testing of CoNS isolates using VITEK 2 system:

Inoculum of the overnight culture of each CoNS isolate was prepared in 0.85% saline and the turbidity adjusted to 0.5 McFarland turbidity standard using VITEK 2 densitometer. The inoculum was then transferred to a second tube containing 3.0 ml of saline. The bacterial suspension tubes containing susceptibility card cassette were loaded into the Vitek 2 compact processor, having loaded the cassette information into the workstation computer.

The AST cards containing benzyl-penicillin, clindamycin, erythromycin, ciprofloxacin, gentamicin, levofloxacin, daptomycin, nitrofurantoin, oxacillin, rifampin, teicoplanin, tetracycline, tigecycline, trimethoprim-sulfamethoxazole, linezolid and vancomycin, was automatically loaded, sealed, and introduced into VITEK 2 reader-incubator module (incubation temperature, 35°C), where it was subjected to kinetic absorbance spectra every 15 min using a vacuum device.

The results were analyzed using the AST-GPC database, and the final results were calculated automatically. All of the used cards were instantly discarded. The MIC for each antimicrobial agent was determined and used to classify each CoNS isolate as susceptible, intermediate or resistant in line with Clinical Laboratory and Standards Institute (CLSI) guidelines (18).

Extraction of DNA:

Bacterial DNA was extracted using Quick DNA^{TM} Bacterial Miniprep Kit. About 50-100µg of bacterial cells was re-suspended in 200 µl of isotonic buffer (PBS) in a Zymo Research (ZR) Bashing-bead^{TM}. The bacterial

DNA was extracted with Zymo Research (ZR) Bashing-bead™ following manufacturer's instruction.

Polymerase chain reaction (PCR) amplification of *S. saprophyticus* specific gene:

Molecular identification of *S. saprophyticus* isolate was carried out following the method described by Morot-Bizot et al., (19), using *S. saprophyticus* primers; F-5'-TCAAAA AGTTTTCTAAAAAATTTAC-3' (annealing position 169 to 193) and R-5'-ACGGGCGTCCAC AAAATCAATAGG A-3' (annealing position 355 to 379) with the amplicon size of 221bp alongside the positive control. The following PCR conditions were used; 5 min at 95°C, 40 cycles of 30 sec at 95°C, 60 sec at 55°C, 30 sec at 72°C and a final extension of 3 min at 72°C.

Agarose gel electrophoresis of PCR amplicons:

Electrophoresis of amplicon DNA was carried out in 1.5% agarose in 1xTBE buffer on horizontal gel apparatus. Ten microliters of amplicon DNA and 2 µL of loading buffer were mixed well and loaded into the gel containing 10 µL ethidium bromide (1 µg/mL in water) and placed in Tris-borate buffer according to the method of (20)**.** The electrophoresis was conducted for 60-90 min at constant voltage 75v. The gel was examined on transilluminator at wavelength 312 nm. Photography was carried out using a Polaroid Camera SP 34. The positive control was *S. saprophyticus* subsp*. bovis* DSM 18669 and nuclease free water was used as the negative control.

16S rRNA sequencing and phylogenetic analysis of *S. saprophyticus* isolates:

About 50 ng DNA templates of 20 selected CoNS isolates amplified with *S. saprophyticus* primers were further amplified with the V5 region of 16S rRNA which spanned the site of the primer (27F 5'-AGAGTTTGATCCT GGCTCAG-3'; 1492R 5'-GGTTACCTTGTTACGA CTT-3'). The following conditions were used for PCR: 95°C for 10 minutes, then 30 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 45 sec, followed by a 10 min extension at 72°C. The PCR products were purified using the Big-Dye Terminator Purification x kit (Applied Biosystems) and sequenced using 1 µL of forward and reverse 16s rRNA primer set (Thermo Fisher Scientific, Waltham, MA) in a 3130 Genetic Analyzer (Applied Bio-systems).

To identify *Staphylococcus* species, the partial sequences of 16S rRNA were compared to sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/index.html). Sequences were aligned using Mega X software (version 10.0.5), which was also utilized to establish phylogenetic relationships between several *Staphylococcus* species and build phylogenetic trees (21).

Statistical analysis of data:

The prevalence of UTI due to CoNS and other bacterial isolates was determined among the case and control participants and compared across the facilities in the three States using Chi square and Fisher Exact tests. Statistical significance was determined at *p*-value < 0.05.

Results:

Distribution of the participants by States and healthcare facilities:

Of 471 recruited participants, 205 (43.5%) had urinary symptoms (case) while 266 (56.5%) were asymptomatic (control); 117 (24.8%) were from Oyo, 113 (24.0%) from Ogun and 241 (56.2%) were from Osun State. The distribution of participants by facilities from each State is shown in Table 1. With regard to age group distribution, Fig 1 shows the age group 19-30 years as the most represented (n=192, 40.8%) followed by age group 31-40 years (n=125, 26.5%).

Prevalence of bacterial urinary tract infections in the study participants:

Table 2 shows the prevalence of UTIs according to the States. Of the 471 participants, 178 had bacterial UTI by cultures, giving a prevalence of 37.8% in the study. Sixty-six of 117 (56.4%) from Oyo State, 98 of 241 (40.7%) from Osun State, and 14 of 113 (14.1%) from Ogun State had bacterial UTI (x^2=49.113, p<0.0001). Compared to the overall prevalence of 37.8%, the prevalence of UTI in Oyo (56.4%) is significantly higher (OR =2.796, p<0.0001) and that of Ogun (12.4%) is significantly lower (OR=0.1673, p<0.0001), but the prevalence of UTI in Osun is not significantly different (OR=1.286, p=0.2166) from the overall prevalence in the study. Participants in age group 19-30 years had the highest prevalence of UTIs (26.0%, n=50).

Table 1: Frequency distribution of urinary symptomatic and asymptomatic study participants across the healthcare facilities in the three States, southwest Nigeria

State	Facilities	Participants			
		Symptomatic	Asymptomatic	Total (%)	
Oyo	OYHMB	9	41	50	**117 (24.8)**
	UHS	67	0	67	
Ogun	FMC	27	38	55	**113 (24.0)**
	SHH	28	20	48	
Osun	OSHMB	26	105	131	**241 (56.2)**
	OSUTH	48	62	110	
Total (%)		**205 (43.5)**	**266 (56.5)**	**471 (100.0)**	

OYHMB = Oyo State Hospital Management Board; UHS = University Health Service; FMC = Federal Medical Center; SHH = Sacred Heart Hospital; OSHMB = Osun State Health Management Board; OSUTH = Osun State University Teaching Hospital

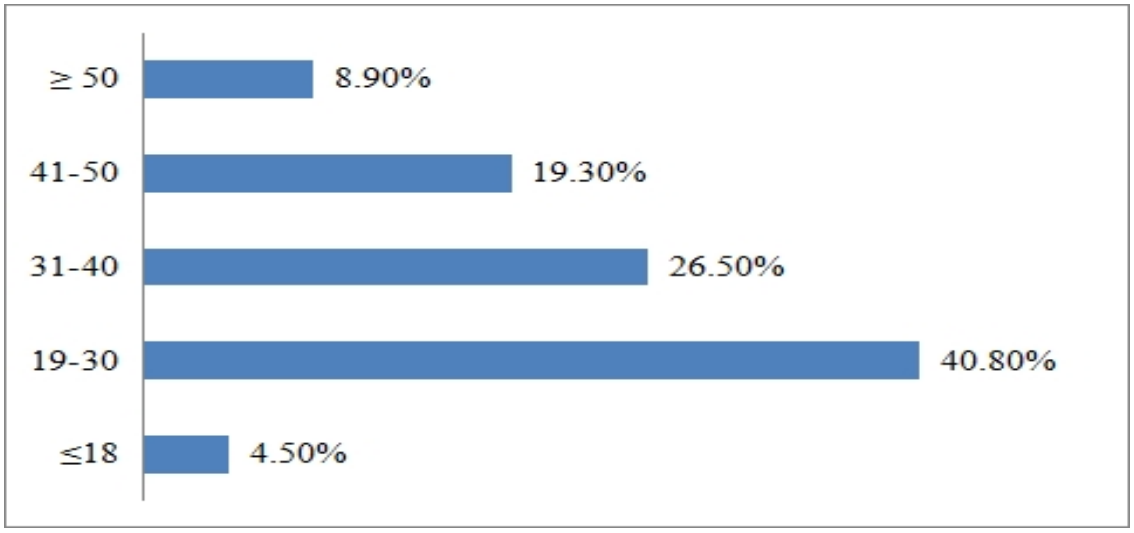


Fig 1: Age distribution of the study participants

Table 2: Prevalence of bacterial urinary tract infection among study participants across the three States, southwest Nigeria

State	Participants		OR (95% CI)	*p* value	x^2	*p* value
	UTI (%)	No UTI (%)				
Oyo (n=117)	66 (56.4)	51 (43.6)	2.796 (1.821-4.293)	<0.0001*	49.113	<0.0001*
Ogun (n=113)	14 (12.4)	99 (87.6)	0.1673 (0.0921-0.304)	<0.0001*		
Osun (n= 241)	98 (40.7)	143 (59.3)	1.286 (0.8842-1.867)	0.2166		
Total (n=471)	**178 (37.8)**	**293 (62.2)**				

UTI = urinary tract infection; x^2 = Chi square; OR= Odd ratio; CI= Confidence interval * = statistically significant

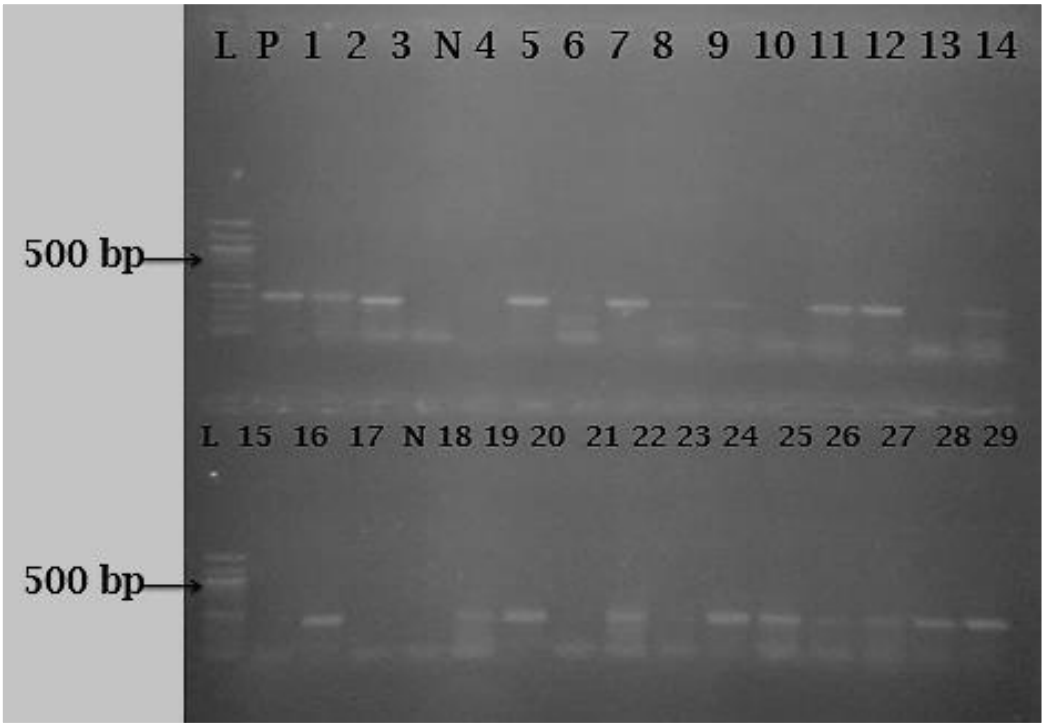
Table 3: Prevalence of urinary tract infections caused by genotypically identified coagulase negative staphylococci across the three States in southwest Nigeria

CoNS	**States**			**Total (n=471)**	**x^2**	***p* value**
	Oyo (n=117)	**Ogun (n=113)**	**Osun (n= 241)**			
S. saprophyticus	7 (5.9)	3 (2.7)	0	10 (2.1)		
S. lentus	3 (2.6)	1 (0.9)	1 (0.4)	5 (1.1)		
S. xylosus	0	2 (1.8)	0	2 (0.4)		
S. sciuri	0	1 (0.9)	0	1 (0.2)		
S. rodentium	1 (0.9)	0	0	1 (0.2)		
Total	**11 (9.4)**	**7 (6.2)**	**1 (0.4)**	**19 (4.0)**	**18.224**	**<0.0001***

Prevalence of coagulase negative staphylococci UTI in the study:

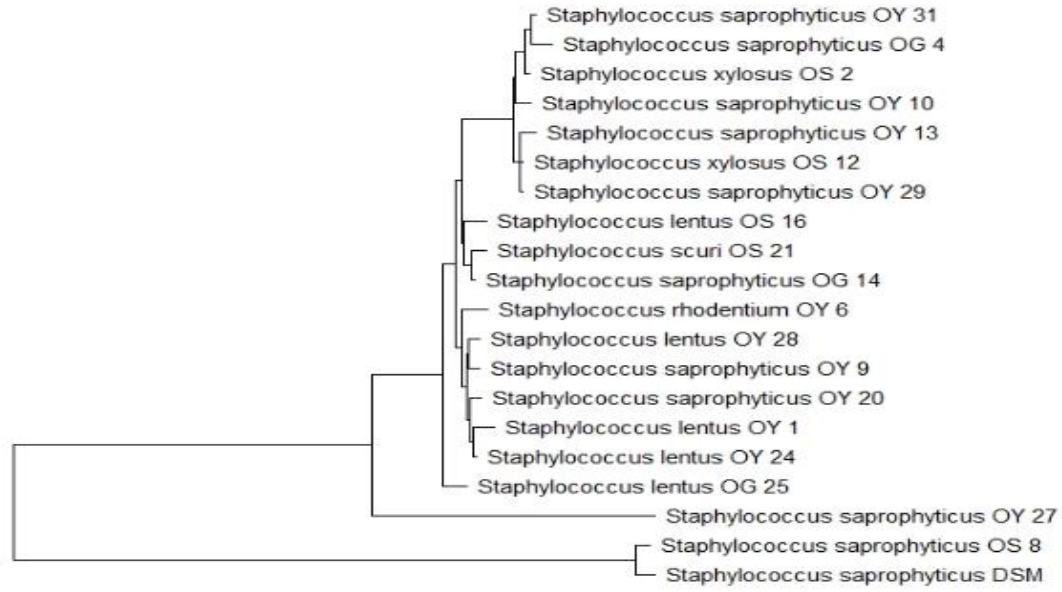
Coagulase negative staphylococci were phenotypically identified in urine samples of 34 (7.2%) of the 471 participants, 29 (14.1%) of which were from 205 symptomatic (case) and 5 (1.9%) from 266 asymptomatic (control) participants (OR=8.601, 95% CI=3.266-22.653, p<0.0001). In term of frequency of isolation, CoNS constituted 19.1% (n=34) of the 178 bacterial pathogens isolated from the urine samples of the participants, 19 (10.7%) of which were genotypically confirmed to be *S. saprophyticus* by PCR and 10 (5.6%) by 16S rRNA sequencing.

Table 3 shows the prevalence of UTI caused by confirmed CoNS by PCR and 16S rRNA sequencing in the study participants. Of the 471 participants, CoNS was identified in 19 patients' samples, giving a prevalence of 4.0%, with 9.4% (n=11) in Oyo, 6.2% (n=7) in Ogun and 0.4% (n=1) in Osun State (x^2=18.224, p<0.0001). Fig 2 shows the *S. saprophyticus* amplified products using the *S. saprophyticus* specific primer. Fig 3 represents the phylogenetic tree of the relatedness of the CoNS isolates.



Lane 1,2,5,7,8,9,11,12,14,16,18,19,21,22,24,25,26,27,28 and 29 at 380bp; Lane L, 1, 4 and N are ladder, positive control and negative control respectively

Fig 2: Agarose gel electrophoresis of amplified PCR amplicons of *Staphylococcus saprophyticus* isolates



OY = Oyo State; OS = Osun State; OG = Ogun State; DSM = Standard *Staphylococcus saprophyticus*
The 19 positive amplicons with *S. saprophyticus* specific-primer further sequenced with 16S rRNA and aligned with the sequences available in GenBank. The strains were established as belonging to the *Staphylococcus* Genus (similarity =95%). Some of the 19 staphylococci were identical to each other. The 19 distinct strains were grouped into 5 *Staphylococcus* species, with the principal species being *S. saprophyticus* of 10 strains, followed by 5 *S. lentus*, 2 *S xylosus,* and 1 each of *S. sciuri*, *S. rodentium*. A control strain, *S. saprophyticus bovis*, gave 98% similarity with *S. saprophyticus* in the GenBank.

Fig 3: Phylogenetic tree of 16S rRNA sequences of coagulase negative staphylococci isolates

Table 4: Antibiotic resistance profiles of genotypically identified coagulase-negative staphylococci isolates from urine samples

Antibiotic	**S. saprophyticus** (%) **(n=10)**	**S. lentus** (%) **(n=5)**	**S. xylosus** (%) **(n=2)**	**S. rodentium** (%) **(n=1)**	**S. sciuri** (%) **(n=1)**	**Total CoNS (%) (n=19)**
Gentamicin	3 (30.0)	3 (60.0)	0	1 (100.0)	0	7 (36.8)
Ciprofloxacin	0	1 (20.0)	0	0	0	1 (5.3)
Levofloxacin	1 (10.0)	1 (20.0)	0	0	0	2 (10.5)
Erythromycin	2 (20.0)	2 (40.0)	0	0	0	4 (21.1)
Clindamycin	9 (90.0)	3 (60.0)	1 (50.0)	1 (100.0)	1 (100.0)	15 (78.9)
Linezolid	1 (10.0)	1 (20.0)	0	1 (100.0)	0	3 (15.8)
Daptomycin	10 (100.0)	3 (60.0)	2 (100.0)	1 (100.0)	1 (100.0)	17 (89.5)
Teicoplanin	1 (10.0)	1 (20.0)	0	0	0	2 (10.5)
Vancomycin	2 (20.0)	1 (20.0)	0	0	0	3 (15.8)
Tetracycline	2 (20.0)	2 (40.0)	0	0	0	4 (21.1)
Tigecycline	0	1 (20.0)	0	0	0	1 (5.3)
Nitrofurantoin	0	0	0	0	0	0
Rifampicin	4 (40.0)	3 (60.0)	0	1 (100.0)	0	8 (42.1)
Penicillin	5 (50.0)	2 (40.0)	0	1 (100.0)	1 (100.0)	9 (47.4)
Oxacillin	10 (100.0)	4 (80.0)	2 (100.0)	1 (100.0)	1 (100.0)	18 (94.7)
Trimethoprim-sulfamethoxazole	10 (100.0)	4 (80.0)	2 (100.0)	1 (100.0)	1 (100.0)	18 (94.7)

Antibiotic resistance profiles of the coagulase negative staphylococci:

The CoNS isolates exhibited high level of resistance to oxacillin (94.7%), sulfamethoxazole-trimethoprim (94.7%), clindamycin (78.9%) and daptomycin (89.5%). All *S. saprophyticus, S. xylosus, S. rodentium* and *S. sciuri* isolates were 100% resistant to daptomycin, sulfamethoxazole-trimethoprim and oxacillin. *S. xylosus, S. rodentium* and *S. sciuri* were also 100% resistant to daptomycin and oxacillin. All *S. rodentium* and *S. sciuri* were

resistant to clindamycin and *S. rodentium* was resistant to gentamicin and linezolid. All *S. saprophyticus, S. xylosus, S. rodentium, S. sciuri* isolates and 3 (60%) of 5 *S. lentus* were multidrug resistant, with resistance to three or more different classes of antibiotics. However, all CoNS isolates were susceptible to nitrofurantoin. All *S. xylosus, S. rodentium* and *S. sciuri* were susceptible to ciprofloxacin and levofloxacin. All *S. rodentium* and *S. sciuri* were sensitive to erythromycin and all *S. xylosus* and *S. sciuri* were susceptible to gentamicin, erythromycin and linezolid (Table 4).

Discussion:

Females of reproductive age are more susceptible to *S. saprophyticus* due to rectal, vaginal and urethral colonization by this organism (22). Genito-urinary colonization by *S. saprophyticus* is also a common feature in female gender as well as changes in the hormonal influences during menstruations, with *Candida* infection which affect the vaginal flora (23). Of the bacterial UTI pathogen isolated in 178 participants in this study, 34 (19.1%) were phenotypically identified as CoNS, 10 (5.6%) of which were genotypically confirmed to be *S. saprophyticus* as the cause of UTI among the selected female participants. *S. saprophyticus* is a prime uropathogen in young women of reproductive age especially below the age of 30 years (13).

In this study, the largest number of participants with urinary complaints recruited into the study were in the age group 21-30 years (40.8%) and lowest were in the age group ≤ 20 years (4.5%). Females in the age group 21-30 years are more prone to UTI and therefore likely to visit healthcare facility with urinary symptoms. This agrees with other researchers who found that young adult women are more susceptible to *S. saprophyticus* colonization and subsequent UTI. Oladeinde et al., (24) reported highest prevalence of UTI in the age group 21-30 years. Onyemelukwe and Nwokocha (10) in Enugu Nigeria also reported 6.3% incidence of *S. saprophyticus* in female urine samples. Farina et al., (3) reported its predominance in sexually active young women. Ekwealor et al., (25) also reported *S. saprophyticus* as a cause of UTIs in sexually active women. The high bacterial colonization of urethral of women within the reproductive age might be due to increased sexual activity in this age group and association of UTI with poor genital hygiene practices (26).

In our study, *S. saprophyticus* were genotypically confirmed in 10.7% of patients whose urine samples were positive for bacterial pathogens. Jhora et al., (27) reported 7.0% frequency of *S. saprophyticus* in UTI. Jombo et al., (28) reported 7.7% incidence from Calabar, Nigeria, Nasiru et al., (29) reported 6.8% incidence from Abuja Nigeria, and Jemikalajah (30) reported 11.1% in Delta State Nigeria, which agree with the rate reported in the present study. However, Vemulapalli and Rao (31) reported a higher frequency of 20.5% for *S. saprophyticus* in India and Alao et al., (32) reported extremely high frequency of 88% for *S. saprophyticus* in southwestern Nigeria, which contrast the finding of our current study. Other researchers from Nigeria have also reported *S. saprophyticus* in urine samples of symptomatic patients (10,28,29).

Stepanovic et al., (33) isolated 5 *S. sciuri* in urine samples of asymptomatic patients. Despite being predominantly associated with animals, members of the *S. sciuri* group have been known to colonize humans (34), and associated with significant infections, most frequently wound infections (35,36). The 16S rRNA sequencing in our study identified 5 species among the 19 *S. saprophyticus* identified by PCR, which included 10 *S. saprophyticus*, 5 *S. lentus*, *S.* 2 *xylosus*, 1 *S. rodentium* and 1 *S. sciuri* from the urine samples of symptomatic patients. We are not unaware that even with well-designed primers, high genomic similarity between *S. saprophyticus* and closely related CoNS species can result in false positives, particularly if the primers target conserved genes such as 16S rRNA, where sequence identity is ≥ 97%. *Staphylococcus lentus*, *S. xylosus*, and other CoNS share significant sequence identity with *S. saprophyticus* in the amplified region. This suggests the need to target more variable or unique genetic markers.

Antimicrobial resistance in CoNS is becoming more common and severe around the globe (37,38). Both human and animal health experts are facing difficulties due to the spread of antibiotic-resistant staphylococci. CoNS in this study exhibited multidrug resistance to the selected antimicrobials tested with high level of resistance to oxacillin (94.7%), sulfamethoxazole-trimethoprim (94.7%), clindamycin (78.9%) and daptomycin (89.5%). Production of β-lactamase was the initial resistance mechanism of staphylococci to β-lactam antibiotics through horizontal transfer of antimicrobial resistance plasmid genes (39). But resistance to oxacillin is mediated by chromosomal *mecA* or *mecC* genes that encodes abnormal penicillin binding proteins (PBPs) with low affinity of binding to β-lactam antibiotics (40).

The relative sensitivity of *S. saprophyticus* (80.0%) to erythromycin in this study agrees with a recent study by Fatholahzadeh et al., (41) who reported 80% susceptibility of *S. saprophyticus* to the same antibiotic. Susceptibility to levofloxacin and nitrofurantoin in this study was 100% in contrast to the study

by Sarzynski et al., (42) who reported 55.6% and 44.4% sensitivity of *S. saprophyticus* to levofloxacin and nitrofurantoin respectively. Agvald-Ohman et al., (43) reported that erythromycin and gentamicin resistance in clinical staphylococci was 48% and 54% respectively, higher than the rates obtained in our study for erythromycin (21.1%) and gentamicin (26.3 %). According to Antoinette et al., (44) clinical isolates in general have increasing levels of antibiotic resistance as a result of recurrent antibiotic exposure.

Conclusion:

Coagulase negative staphylococci, particularly *S. saprophyticus*, are important bacterial pathogen involved in UTIs and should not be discounted as commensals or contaminants. Molecular identification, antibiotic susceptibility, and resistance gene detection are necessary for accurate assessment of clinical significance of CoNS involved in UTI.

Contributions of authors:

OBO participated in the research design and benchwork; COI prepared and revised the manuscript and analysed the data; OBF was involved in manuscript-proofing and data analysis; OEA participated in the research design and supervision. All the authors read and approved the final manuscript**.**

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