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Exploring staphylococcus in urinary tract infections: A systematic review and meta-analysis on the epidemiology, antibiotic resistance and biofilm formation

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ABSTRACT

This study aimed to determine the epidemiology, biofilm formation and antibiotic resistance of staphylococci collected worldwide in the context of UTIs.

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Forty studies from 23 countries were selected for quantitative review. Electronic databases (PubMed, Scopus, Google Scholar, and Web of Sciences) were searched for articles published between 2010 and 2024 on the epidemiology, biofilm formation, and antibiotic resistance of uropathogenic staphylococci. Strict inclusion and exclusion standards were applied during the review of the articles.

Forty articles were included in this systematic review. The prevalence of uropathogenic staphylococci varies from country to country, with the pooled prevalence of *S. aureus* and coagulase-negative staphylococci (CoNS) being 8.71 % (95 %CI: 6.145–11.69) and 13.17 % (95 %CI: 8.08–19.27) respectively. Among CoNS isolates, *S. epidermidis* was the most common with 19.3 % (95 %CI: 5.88–38.05). The prevalence of methicillin-resistant *S. aureus* isolates increased significantly from 23 % in 2010–2015 to 47 % in 2021–2024 ($p = 0.03$). *S. haemolyticus* is the most antibiotic-resistant species in CoNS, with 45 % of isolates resistant to methicillin, 33 % to gentamicin, and 29 % to tetracycline.

Eighty-eight *S. aureus* strains were biofilm producers, including 35 % moderate biofilm producers and 48 % strong biofilm producers. The combined frequencies of *ica*A, *clf*A and *fnbp*A were 100, 99, and 89 %, respectively.

The development of antibiotic resistance and biofilm formation by staphylococci involved in UTIs explains the need for periodic regional surveillance of these infections, which poses a serious public health problem.

1. Introduction

Urinary tract infections (UTIs) are the second most frequent microbial infections in hospitals and communities after respiratory tract infections, accounting for 18–25 % of all infections [1]. An estimated 150–250 million cases of UTIs occur annually worldwide [2], leading to substantial economic costs for treatment and high morbidity rates [2]. The treatment and expenses associated with UTIs, including hospitalization and medical costs, account for more than 6 billion dollars in healthcare costs [3]. Various Gram-negative and Gram-positive bacteria are involved in the etiology of UTIs [2]. The predominant gram-positive bacteria are *Staphylococcus* spp., including *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) [4].

S. aureus is a human pathogen that causes UTIs in approximately 1–3 % of individuals; this variation in prevalence may be due to the detection methods used in the laboratory and the geographical regions where the samples were taken. In addition, this concern is heightened in approximately 30–50 % of healthy adults who are frequent carriers of

Data availabilityThere are no original data associated with this review manuscript and the relevant information provided has been cited appropriately.

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S. aureus in their nasal passages, as they have an increased risk of contracting UTIs. This microorganism, which originates from the skin, has the potential to invade and infect any tissue in the body, potentially leading to severe life-threatening illnesses.

Untreated *S. aureus* UTIs can lead to serious and potentially fatal conditions. More data are now available on the mechanisms by which *S. aureus* induces UTIs, particularly on the role and mode of action of polypeptides, toxins, and adhesion factors involved in its pathogenicity [5,6].

Species-level identification of CoNS isolated from UTIs, including *S. epidermidis, S. hominis, S. haemolyticus* and *S. warneri*, is uncommon, which makes it difficult to determine their role in UTIs [7]. *S. saprophyticus* is the second most common cause of tract infections in sexually active young women aged 15–30 years. Other CoNS are mainly isolated from catheterized and immunocompromised patients [2]. Some clinicians consider CoNS to be saprophytes, and they are rarely considered the cause of disease. Nevertheless, an increasing number of studies have indicated that CoNS can lead to UTIs in a wide array of patients with or without urinary tract instrumentation. Furthermore, when these bacteria are detected in urine samples, it is necessary to test them for etiological agents [8,9].

The pathogenesis of staphylococci in the urinary tract is complex and associated with virulence factors, including bacterial adhesion, biofilm formation in renal cells and antibiotic resistance. The global public health crisis caused by MDR *Staphylococcus* remains an ongoing concern. Methicillin-resistant staphylococci and vancomycin-resistant staphylococci, which are critical pathogens, pose a significant threat to global health due to their potential to cause widespread mortality in the absence of effective containment and treatment options [10].

Biofilms play a significant role in causing infections in humans, with over 80 % of all infections being attributed to them. Additionally, these biofilms contribute to increased resistance to antibiotic treatment and the host immune system. The formation of the biofilm matrix facilitates the interaction between bacteria and the horizontal transfer of genetic material [11].

In recent years, numerous studies have evaluated the prevalence and characteristics of antibiotic resistance and biofilm formation in staphylococci isolated from UTIs patients. However, there is a limited understanding of the global scope of these issues and the factors that contribute to their development. To address this knowledge gap, it is essential to collect and organize the available information to gain insight into trends in antibiotic resistance and biofilm formation. This knowledge can then be used to identify urgent situations that require strategic action to ensure the responsible use of antimicrobials.

This study aimed to provide a comprehensive summary of scientific data that examined the epidemiology of staphylococcal UTIs and the genetic and phenotypic characteristics of biofilm formation and antibiotic resistance over the period 2010–2024.

2. Methods

This systematic review followed the guidelines established in Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [12]. The review process involved five distinct stages: development of the search strategy, selection of studies, identification of the outcome variable, extraction of relevant information and statistical analyses.

2.1. Literature search methodology

The scientific research papers were collected from multiple databases, including MEDLINE-PubMed, Scopus, Google Scholar and Web of Sciences, with publication dates ranging from 2010 to 2024. This collection was achieved by using a combination of keywords, such as "Staphylococci UTIs," "*S. aureus*," "CoNS," "antibiotic resistance," "UTIs," and "biofilm formation." The search syntax for these keywords is available in the Supplementary Data (Supplementary Table 1). Articles were screened based on their abstracts and titles to determine their relevance and eligibility according to the inclusion and exclusion criteria.

2.2. Study selection

First, eligible articles were screened by three independent researchers. All titles and abstracts were reviewed; irrelevant or duplicate articles were excluded and the remaining articles were assessed for inclusion.

The scientific articles included in this meta-analysis were selected based on the following criteria: original articles published in peer-reviewed English journals and reporting the epidemiology of staphylococci UTIs based on different years, areas, isolate species, antimicrobial resistance testing, biofilm formation and genetic characterization. When one article showed results from different countries, sample times, methodologies, or different years of sampling, each condition was considered an independent study.

We excluded review articles, articles without original data, duplicate reports or overlapping articles, meta-analyses and/or systematic reviews, conference abstracts or articles without clear full-text information on staphylococci UTIs and articles without a peer review system (such as a thesis or editorial notes).

2.3. Data extraction

The three researchers independently extracted information from the selected articles using Microsoft® Excel (Microsoft Corporation, Redmond, WA, USA). The collected data included the country and continent of origin, sampling and publication year of Staphylococci species, antibiotic susceptibility testing methodology, number of staphylococci UTIs isolated and analyzed and a guide for determining the susceptibility of bacteria to antibiotics, antibiotic resistance, biofilm formation ability, adhesion and biofilm related genes.

2.4. Statistical analysis

Statistical analysis was conducted using the Stata version 14.0 software (Stata Corp., College Station, TX, USA). The data were pooled using the fixed-effects model and the random-effects model. Cochran's Q test and I^2 statistics were used to quantify and assess the presence of heterogeneity between the studies. Publication bias was assessed using Egger's test. All statistical interpretations were reported on a 95 % confidence interval (CI) basis and statistical significance was set at $p < 0.05$.

3. Results

3.1. Characteristics of the included studies

According to the literature search, a total of 2093 records were found in the initial search from three databases: 863 articles from PubMed, 872 articles from Scopus and 358 articles from Web of Science. Subsequently, 480 articles were removed due to duplication, while 879 articles were excluded during the evaluation of their titles and summaries due to their irrelevance. After carefully reviewing all documents and applying the specified exclusion and inclusion criteria, 694 articles were removed from the remaining 734 articles. The present systematic review included 40 geographically diverse articles that met all required criteria (Fig. 1). The majority of studies in this meta-analysis originated in Africa (45 %) and Asia (45 %), with Europe contributing a lower proportion (7.5 %).

All articles included in this meta-analysis reported the prevalence of uropathogenic staphylococci, 37 articles investigated antibiotic resistance, and five studies mentioned its ability to form biofilms (Fig. 2).

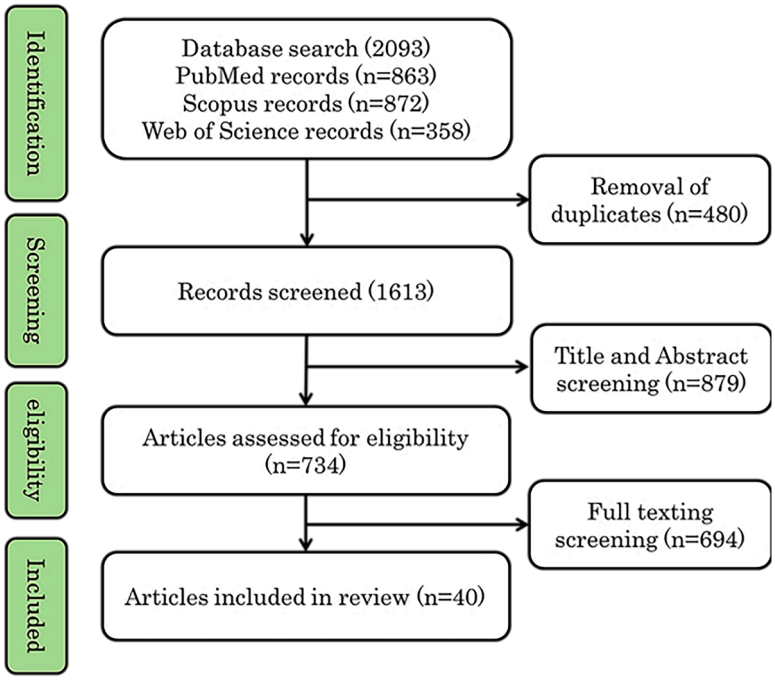

Fig. 1. Flow diagram of the study selection process.

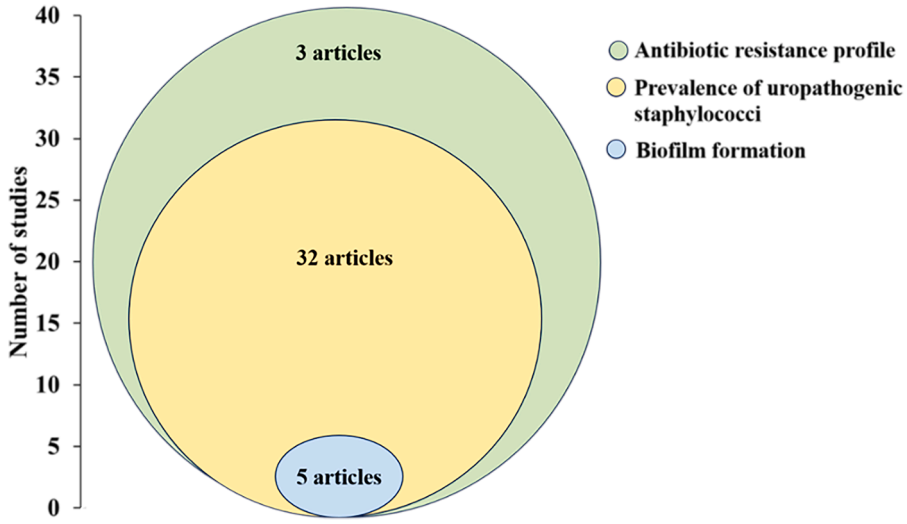

Fig. 2. Distribution of articles included in the systematic review according to published topics concerning uropathogenic staphylococci.

Fig. 3 shows that the number of articles reporting the prevalence of uropathogenic staphylococci increased from 7 in 2010–2015, to 11 in 2016–2020 and reached 22 in 2021–2024. Similarly, the number of newly published articles on antibiotic resistance has increased each year: 7 articles were published between 2010 and 2015, 8 articles from 2016 to 2020, and 22 articles from 2021 to 2024. In recent years, few studies have been published on biofilm formation.

3.2. Prevalence of uropathogenic staphylococci

Most articles included in this meta-analysis used biochemical methods for bacterial identification (82.5 %). Only 5 % of the articles used the VITEK 2® COMPACT 15 system (Biomerieux, France) and 7.5 % used biochemical tests followed by PCR confirmation. Whole-genome sequencing was used as a reference for bacterial identification in 2.5 % of articles.

UTIs caused by *Staphylococcus* spp. have been reported in various countries, including Ethiopia (10 %), Iran (10 %), Saudi Arabia (10 %), Pakistan (7.5 %), India (5 %), Morocco (5 %), Turkey (5 %) and Italy (2.5 %).

3.2.1. Staphylococcus aureus

In recent years, the prevalence of *S. aureus* UTIs (the frequency of *S. aureus* among pathogens found in urine samples from patients with suspected UTIs) has varied across countries. Studies conducted between 2010 and 2024 showed that the prevalence of *S. aureus* isolates ranged from 0.4 % to 35.27 % in the countries included in this review. Specifically, 13.85 % of the isolates were reported in Morocco, whereas 17.96 % were identified in India. Additionally, 22.58 % of the isolates were reported from Cameroon, 20 % from Sudan, and 0.5 % from Italy. Notably, Selim et al. reported higher frequencies of *S. aureus* UTIs in Saudi Arabia (35.27 %) and Nigeria (28 %) (Fig. 4).

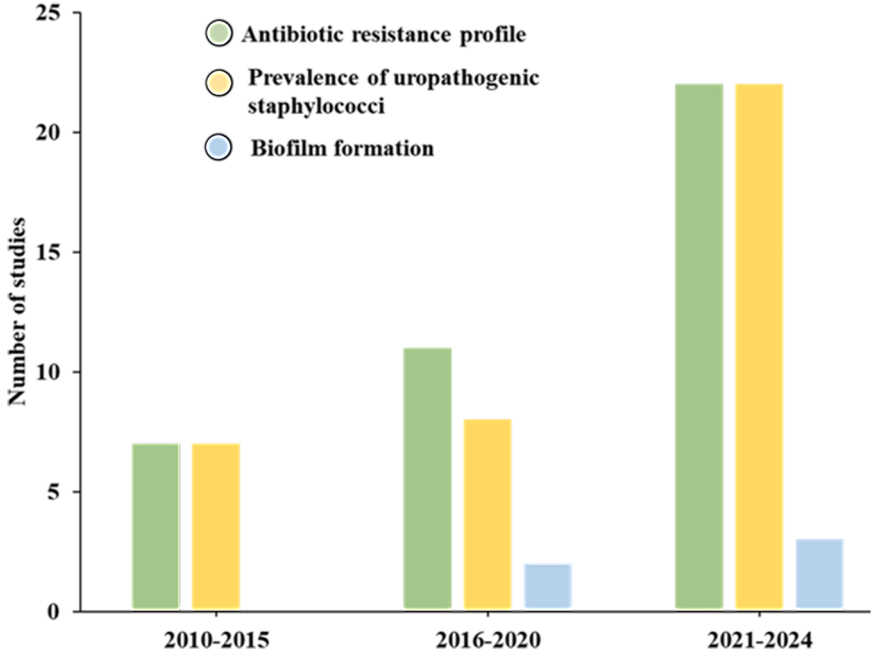

Fig. 3. Number of articles by year of publication.

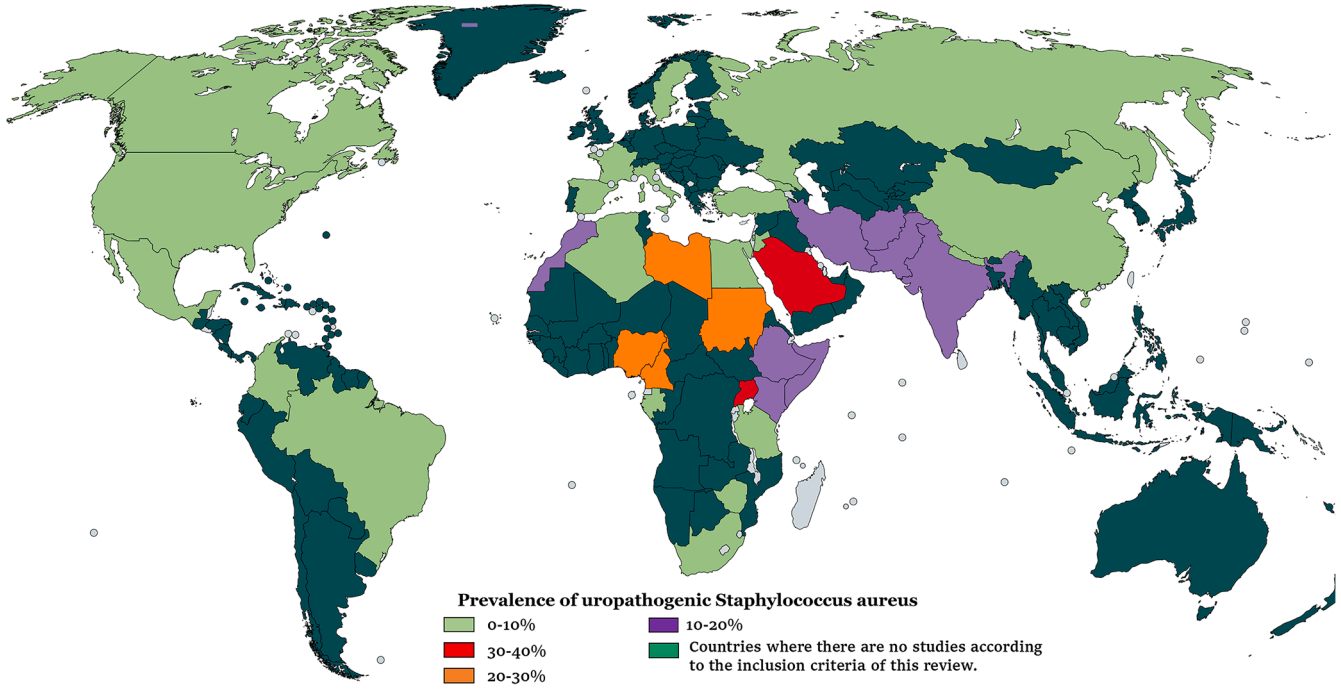

Fig. 4. Epidemiology of Staphylococcus aureus isolated from urinary tract infection.

The combined prevalence of *S. aureus* in different countries included in this review was reported by 8.71 % (95 % CI: 6.145–11.69) and heterogeneity was observed ($Q = 886.6$, $I^2 = 96.95$) (Fig. 5).

3.3. Coagulase negative staphylococci (CoNS)

CoNS are frequently associated with UTIs. Studies conducted between 2010 and 2024 showed that the prevalence of CoNS isolates (the frequency of CoNS among pathogens found in urine samples from patients with suspected UTIs) in the countries included in this review ranged from 2.7 to 24.2 %. Specifically, 24.2 % of the isolates were reported in Ethiopia, while 21.7 % were identified in Jordan, and 16.1 % in Zimbabwe (Fig. 6). The combined prevalence of CoNS in different countries included in this review was 13.17 % (95 % CI: 8.08–19.27), and heterogeneity was observed ($Q = 147.8$ and $I^2 = 92.56$) (Fig. 7). Among the CoNS isolates, *S. epidermidis* was the most common isolate, with 19.3 (95 % CI: 5.89–38.06), followed by *S. haemolyticus* (6.28 %; 95 % CI: 2.59–11.44) and *S. saprophyticus* (11.71; 95 % CI: 0.00–41.186).

3.4. Antibiotic resistance profile

According to the results of this meta-analysis, 82 % of the authors

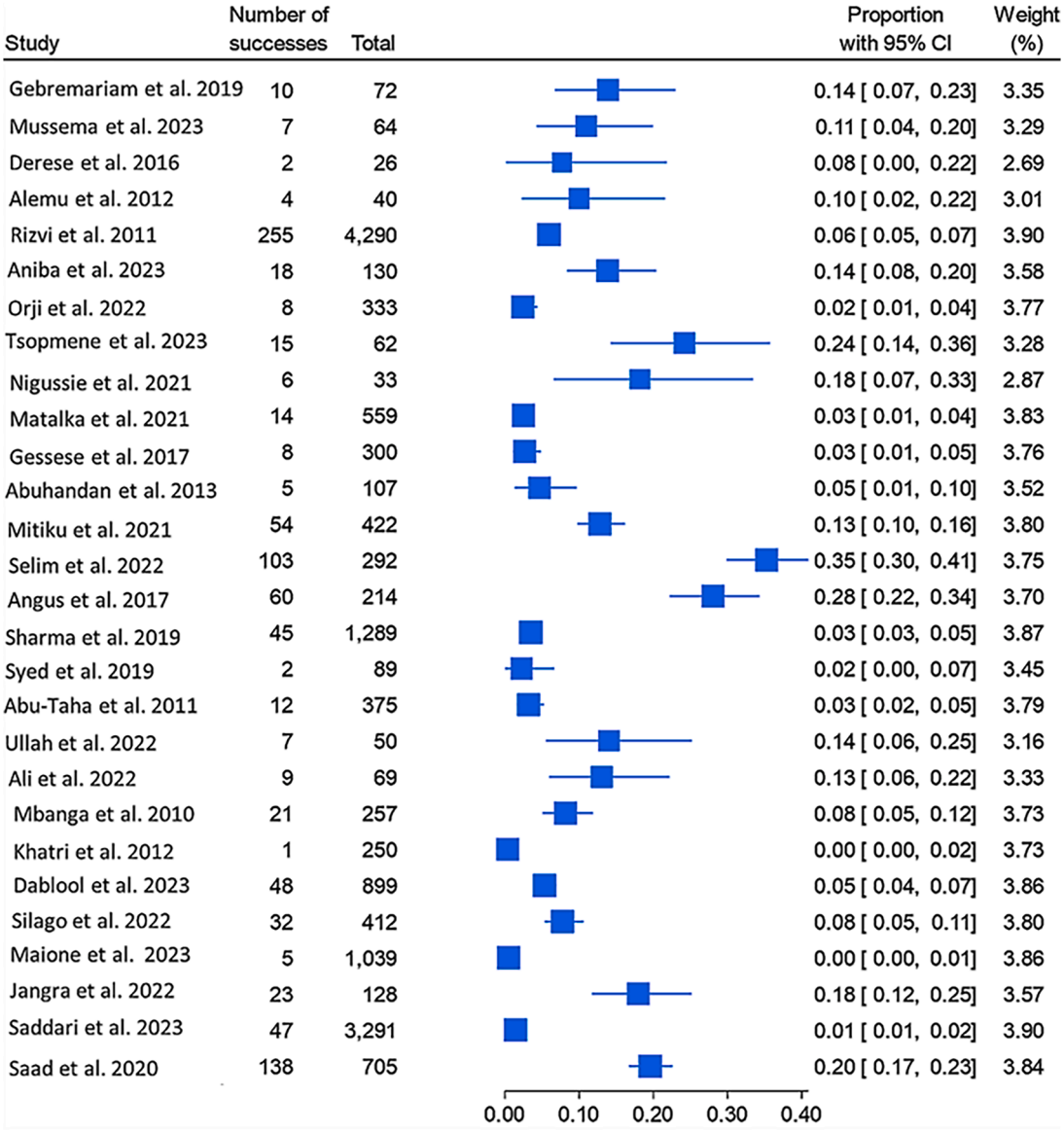

Fig. 5. Forest plot of the meta-analysis of the prevalence of S. aureus isolated from UTIs patients.

used the Clinical and Laboratory Standards Institute guidelines for antimicrobial testing, whereas 18 % referred to the guidelines of the Antibiogram Committee of the French Society. This section aims to qualitatively summarize the data available worldwide on the antibiotic resistance patterns of bacteria isolated from UTIs.

3.5. *Antibiotic resistance profile of S. aureus isolates*

S. aureus isolates from urine showed the highest prevalence of resistance to the four antibiotics, clindamycin with pooled estimates of 80 % (95 % CI: 73–83), trimethoprim-sulfamethoxazole with pooled estimates of 0.73 (95 % CI:0.65–0.79) and tetracycline with pooled estimates of 0.65 (95 % CI: 0.58–0.73). On the contrary, these isolates showed the lowest prevalence of resistance to fosfomycin with pooled estimates of 0.12 (95 % CI:0.00–0.42) and vancomycin with pooled estimates of 0.01 (95 % CI: 0.00–0.03) (Table 1).

The overall pooled prevalence of methicillin-resistant *S. aureus* (MRSA) in different countries included in this review was 45 % (95 % CI: 41 %–49 %) (Table 1).

To assess the trends in the prevalence of MRSA strains in recent years, we conducted a subgroup analysis based on the study year. Three distinct periods—2010–2015, 2016–2020, and 2021-2024—are designated. The prevalence of MRSA isolates demonstrated a remarkable increase, rising from 23 % (95 % CI: 10–39) during the period of 2010–2015 to 46 % (95 % CI: 24–69) in 2016–2020, and finally settling at 47 % (95 % CI: 40–54) in the 2021–2024 period. The prevalence of MRSA was significantly associated with the study year ($p = 0.03$) (Table 2).

The prevalence of MRSA differed between the geographical regions in the subgroup analysis (Table 3). MRSA prevalence was 36 % (95 % CI: 23–50) of MRSA strains in seven Asian studies and 44 % (95 % CI: 32–57) of strains in 13 African studies. In addition, there was a significant association between the prevalence of MRSA and the study location country ($p < 0.001$) (Table 2).

S. aureus isolates from Sudan, Tanzania, Saudi Arabia, and South Africa showed a higher prevalence of methicillin resistance than other countries studied. In Sudan, pooled MRSA prevalence estimates were 76 % (95 % CI: 69–83), wihile pooled MRSA prevalence estimates in Tanzania were 53 % (95 % CI: 0.36–0.70). In addition, 58 % (95 % CI: 0.43–0.72) of MRSA cases were reported in Saudi Arabia and 50 % (95 % CI: 15–85) in South Africa (Table 3).

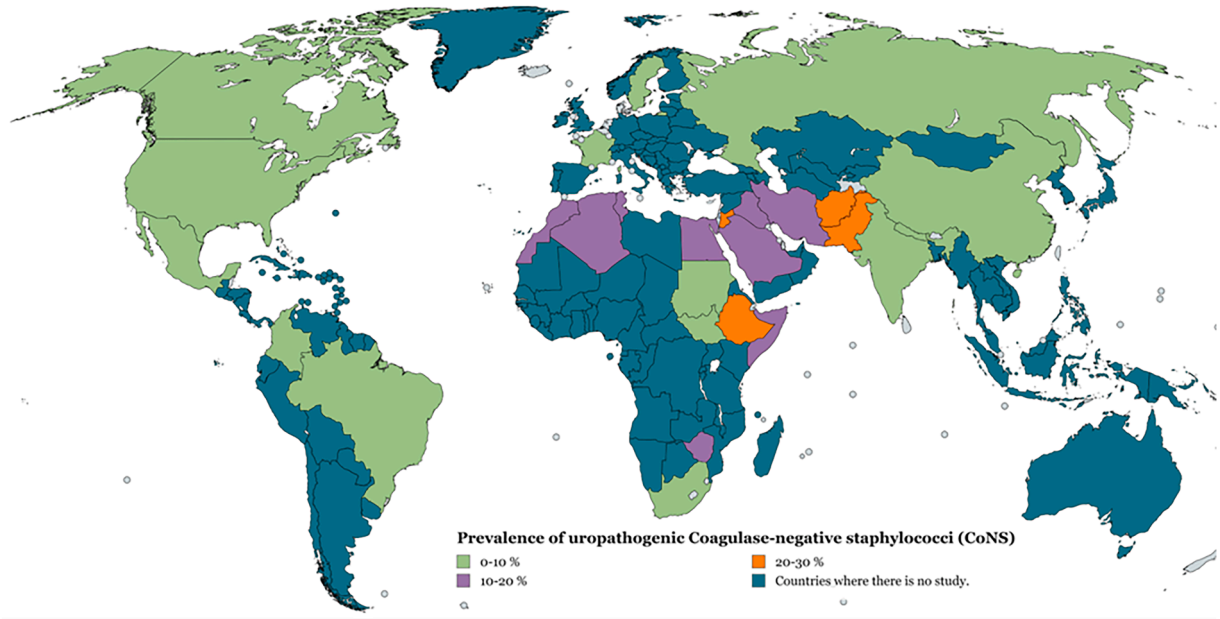

Fig. 6. Epidemiology of Coagulase-negative staphylococci (CoNS) isolated from urinary tract infection.

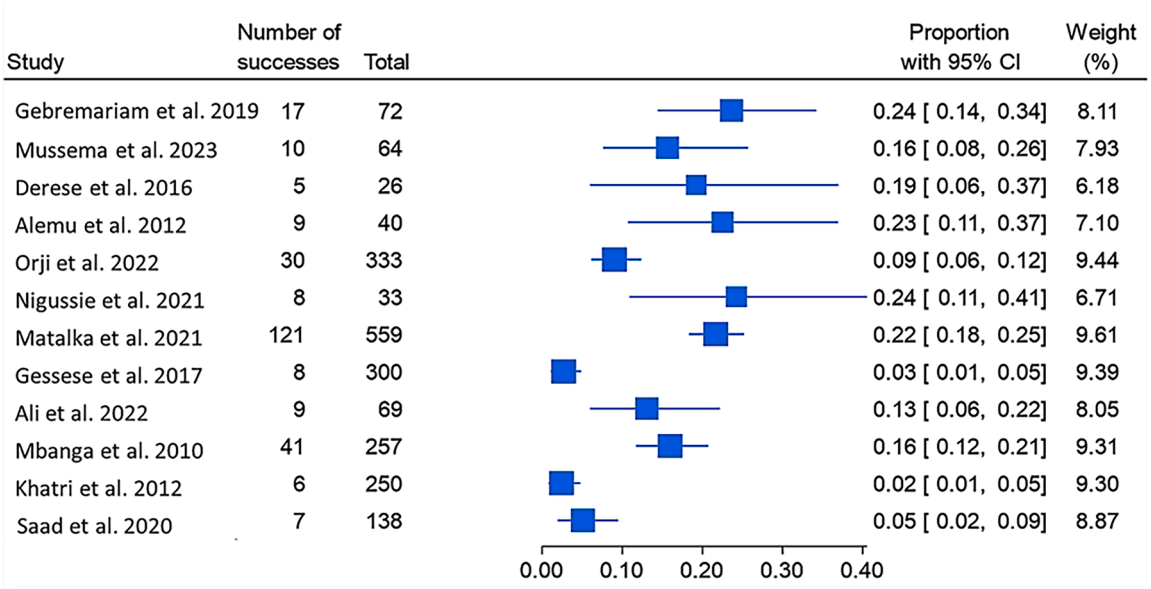

Fig. 7. Forest plot of the meta-analysis of the prevalence of CoNS isolated from UTIs patients.

3.6. Antibiotic resistance profile of CoNS isolates

S. haemolyticus isolates exhibited the highest prevalence of resistance to methicillin, with pooled estimates of 0.45 (95 % CI: 0.06–0.88), and gentamicin, with pooled estimates of 0.33 (95 % CI: 0.21–0.46). Furthermore, *S. saprophyticus* isolates had a higher pooled estimate of ampicillin resistance, at 0.81 (95 % CI: 0.30–1.00). It is worth noting that *S. epidermidis* had the highest rates of antibiotic resistance for ampicillin (72 %; 95 % CI: 23–100), tetracycline (55 %; 95 % CI: 6–98 %) and erythromycin (0.5 %)

3.7. Biofilm formation

For biofilm formation, all authors utilized the microtiter plate method and the Congo red agar plate method to evaluate the ability of uropathogenic staphylococci to form biofilm. This section aims to qualitatively summarize the data available worldwide on biofilm formation by staphylococci isolated from UTIs. The pooled rates of biofilm formation in *S. aureus* strains were obtained at 88 % (95 % CI: 66–100). Also, 35 %, 48 %, and 12 % of isolates were able to produce strong, moderate, and weak biofilms, respectively (Table 3). The combined frequencies of *ica*A, *ica*D, *clf*A, and *fnbp*A genes were 100 % (95 % CI: 98–100), 49 % (95 % CI: 29–68), 99 % (95 % CI: 92–100), and 89 % (95 % CI: 69–100), respectively. The frequencies of other biofilm-related genes have been demonstrated in Table 3.

The combined biofilm formation in *S. saprophyticus* was reported to be 88 % (95 % CI: 39–100). Also, 28 % (95 % CI: 13–45), 33 % (95 % CI: 20–47), and 39 % (95 % CI: 24–54) of *S. saprophyticus* isolates were strong, moderate, and weak biofilm producers, respectively.

Table 1
Rate of antibiotic resistance in uropathogenic staphylococci.

	Antibiotic	Pooled estimate prevalence of isolates resistant to the antibiotics	Confidence interval (95 % CI)	Heterogeneity index (*I*2, %)
S. aureus	Ampicillin	0.84	0.75–0.91	65.95
	Methicillin	0.45	0.41–0.49	81.76
	Gentamicin	0.3	0.26–0.34	94.57
	Tetracycline	0.65	0.58–0.73	0
	Fosfomycin	0.12	0.00–0.42	27.06
	Erythromycin	0.61	0.56–0.65	91.57
	SXT	0.73	0.65–0.79	74.49
	Ciprofloxacin	0.43	0.38–0.48	86.83
	Clindamycin	0.80	0.73–0.83	87.54
	Vancomycin	0.01	0.00–0.03	96.55
S. haemolyticus	Ampicillin	0.45	0.06–0.88	78.74
	Methicillin	0.45	0.06–0.88	78.74
	Gentamicin	0.33	0.21–0.46	0
	Tetracycline	0.29	0.00–0.96	91.96
	Erythromycin	0.17	0.00–0.62	78.78
	SXT	0.02	0.00–0.32	74.03
	Ciprofloxacin	0.17	0.00–0.62	78.78
S. saprophyticus	Ampicillin	0.81	0.30–1.00	95.05
	Methicillin	0.12	0.00–0.34	79.84
	Gentamicin	0.04	0.00–0.21	70.94
	Tetracycline	0.03	0.00–0.13	55.09
	Erythromycin	0.26	0.04–0.57	87.03
	SXT	0.10	0.00–0.34	73.21
	Ciprofloxacin	0.05	0.00–0.24	80.43
	Clindamycin	0.43	0.15–0.72	0
S. epidermidis	Ampicillin	0.72	0.23–1.00	96.68
	Methicillin	0.47	0.26–0.68	75.82
	Gentamicin	0.35	0.02–0.79	95.92
	Tetracycline	0.55	0.06–0.98	96.62
	Erythromycin	0.50	0.06–0.94	96.85
	Ciprofloxacin	0.22	0.00–0.60	93.35
	Vancomycin	0.09	0.01–0.20	68.94

Trimethoprim-sulfamethoxazole (SXT)

4. Discussion

Uropathogenic staphylococci are a major global threat, causing serious infections in both healthcare facilities and the community, despite the relatively low frequency of urinary tract infections. It is essential to treat these infections because, if left untreated, they can have serious health consequences. Moreover, the growing resistance of these organisms to antibiotics and their tendency to form biofilms has become a major health issue.

Based on the data available to date, we conducted the first large-scale systematic review and meta-analysis of available data on the epidemiology of uropathogenic staphylococci. The primary objective of this study was to determine the overall prevalence of staphylococci isolated from UTIs by combining data from various studies and to evaluate the distribution patterns of different staphylococcal species, as well as the emergence of antibiotic resistance and biofilm formation.

The overall pooled prevalence estimates of *S. aureus* in different countries (the weighted average of the prevalence rates reported in individual studies included in the review) were found to be 8.71 % (95 % CI: 6.145–11.69), with a high level of heterogeneity (I^2= 96.95 %, $p < 0.001$). It is estimated that 30–50 % of healthy people are intermittently or chronically colonized with S. aureus. Chronic nasal colonization is a risk factor for S. aureus infections [13].

The pathogenicity of *S. aureus* is due to a series of toxins ((Enterotoxin and Enterotoxin, Toxic Shock Syndrome Toxin (TSST), Pantone-Valentine leucocidin (PVL) and Staphylococcal leukotoxin (LukE/LukD)) and adhesion factors (Agglutination factor B, elastin-binding protein, fibrinogen-binding protein, collagen-binding protein and fibronectin-binding protein) [5,6]. In addition, some studies have highlighted the critical role of urease in the pathogenic mechanism of *S. aureus* in UTIs. *S. aureus* strongly expresses urease, an enzyme that hydrolyzes urea to produce ammonia, thereby increasing the pH of its environment. This alkaline environment suppresses the expression of the Accessory Gene Regulator (Agr) system in *S. aureus*. Suppression of the Agr system results in reduced virulence but increased capacity for biofilm formation and adhesion, which are crucial for the persistence and survival of S. aureus in the urinary tract during UTIs [14].

S. aureus showed the highest levels of resistance to clindamycin, trimethoprim-sulfamethoxazole and tetracycline antibiotics, indicating the need to restrict the use of these antibiotics. The lowest levels of resistance were observed against vancomycin and fosfomycin. As a result, the rational use of these antibiotics has led to the discovery of new antibacterial targets and active drugs.

MRSA has been isolated since the 1960s. Initially, these cases were confined to hospitals, but today they occur in the community [15]. Furthermore, our results indicate a higher prevalence of MRSA strains of 45 % (95 % CI: 41–49) and a remarkable increase from 23 % in the 2010–2015 period to 46 % in 2016–2020, finally settling at 47 % in the 2021–2024 period, which was significantly associated with the study year ($p = 0.03$). This high rate of MRSA shows that multidrug-resistant *S. aureus* is an alarming problem in most parts of the world, especially in Africa. This means that international treatment guidelines need to be changed right away to include highly effective new antimicrobials that

Table 2
Subgroup meta-analyses for MRSA, by stratification variable.

Category	Subcategory	Number of studies	Pooled estimate	Confidence interval (95 % CI)	Qs	*p-values*
Year	2010-2015	3	0.23	0.10–0.39	7.19	0.03
	2016-2020	7	0.46	0.24–0.69		
	2021-2024	10	0.47	0.40–0.54		
Continent	Africa	13	0.44	0.32–0.57	0.72	0.40
	Asia	7	0.36	0.23–0.50		
Country	Ethiopia	6	0.36	0.19–0.54	85.81	<0.0001
	Cameroun	1	0.40	0.16–0.66		
	India	1	0.34	0.28–0.40		
	Iran	1	0.20	0.00–0.68		
	Jordan	1	0.43	0.18–0.70		
	Mauritania	1	0.52	0.32–0.72		
	Morocco	1	0.44	0.22–0.68		
	Pakistan	1	0.43	0.08–0.81		
	Saudi Arabia	2	0.58	0.43–0.72		
	Somaliland	1	0.11	0.00–0.42		
	Sudan	1	0.76	0.69–0.83		
	Tanzania	1	0.53	0.36–0.70		
	Turkey	1	0.20	0.11–0.32		
	South Africa	1	0.50	0.15–0.85		

Table 3
Prevalence of biofilm formation and expression of biofilm-related genes in S. aureus UTIs.

Biofilm formation	Pooled estimate prevalence of isolates resistant to the antibiotics	Confidence interval (95 % CI)	Heterogeneity index (*I*2, %)	Cochran *Q* *p*-value	Egger's *p*-value
Overall effect (Biofilm)	0.88	0.66–1.00	90.61	<0.0001	<0.0001
Biofilm types					
Strong	0.35	0.16–0.57	82.07	<0.0001	<0.0001
Moderate	0.48	0.29–0.69	79.07	<0.0001	<0.0001
Weak	0.12	0.03–0.23	62.14	0.04	<0.0001
The expression of biofilm-related genes					
icaA	1.00	0.98–1.00	0	0.94	<0.0001
icaD	0.49	0.29–0.68	65.16	0.09	<0.0001
*clf*A	0.99	0.92–1.00	64.41	0.07	<0.0001
*fnbp*A	0.89	0.69–1.00	85.08	<0.0001	<0.0001
bap	0.40	0.00–1.00	98.44	<0.0001	0.21

target MRSA. Simultaneously, it is imperative to implement comprehensive antimicrobial stewardship strategies accompanied by robust systemic surveillance. In addition, to curb MRSA transmission, it is essential to prioritize infection control measures, such as contact precautions, meticulous screening, proper sterilization of healthcare equipment and ensuring an aseptic environment.

Biofilm formation increases antibiotic resistance and leads to the use of higher concentrations of antibiotics in the treatment of infections caused by *S. aureus* isolates. Overall, the current study showed that the biofilm formation rate of *S. aureus* was 88 %. In addition, 35 %, 48 %, and 12 % of *S. aureus* isolates were strong, moderate, and weak biofilm producers, respectively. The eradication of infections caused by biofilm-producing *S. aureus* isolates through antibiotics is very troublesome because, in UTIs, bacterial biofilms spread on the bladder, causing recurrent UTIs, prostatitis, chronic cystitis, and protection against antimicrobial treatment. In the present meta-analysis, the genotypic evaluation of biofilm production in *S. aureus* isolates showed that the combined frequencies of the *ica*A, *ica*D, *clf*A, and *fnbp*A genes were 100 %, 49 %, 99 %, and 89, respectively. According to the results of this meta-analysis and other studies, *S. aureus* strains possessing IcaADBC clusters, *clf*B, *fnb*B, *clf*A, and *fnb*A are likely to produce biofilms [16]. Biofilm-associated proteins (Bap) are crucial for *S. aureus* adhesion and biofilm formation [17].

Biofilm production is another important virulence factor for *S. aureus* and is an important strategy adopted by bacteria to colonize and infect the urinary tract and abiotic surfaces. Biofilm formation contributes to antibiotic resistance through mechanisms such as genetic resistance elements, protective barriers against antibiotics, and host immune responses [18].

CoNS isolates constitute the main part of the human normal microbiota population. Patients who are immunocompromised, pregnant, or have inserted foreign objects or medical devices into their bodies are typically classified as high-risk patients who are more susceptible to developing UTIs [19]. Among CoNS isolates, *S. epidermidis* (19.3 %), *S. saprophyticus* (11.71 %), and *S. haemolyticus* (6.281 %) account for the majority of UTIs cases.

S. epidermidis UTIs are related to medical implant infections as well as the ability to form biofilms and colonize various surfaces, which can contribute to antimicrobial resistance. Therefore, according to the results obtained, most of the antibiotics used in the included studies, such as ampicillin, tetracycline, and erythromycin, were ineffective against the majority of *S. epidermidis* isolates. However, vancomycin and ciprofloxacin are the most effective treatment options for these isolates. To ensure the optimal use of these two molecules, it is essential to control and monitor the prescription of antibiotics belonging to the quinolone and glycopeptide families, and infection prevention remains the best available strategy [20].

S. epidermidis has a limited range of virulence factors compared to *S. aureus*. Unlike S. aureus, it lacks a large number of immune evasion proteins and cytolytic toxins. However, it has can express a variety of cell wall-anchored surface proteins, including those that promote biofilm formation and bind to fibronectin, collagen, and fibrinogen [21].

Biofilm formation on the surfaces of host cells/tissues and indwelling medical devices is a major contributor to pathogenicity. *S. epidermidis* employs two primary mechanisms for biofilm formation: the secretion of extracellular adhesion polysaccharides and the accumulation of associated proteins, which enhance resistance to phagocytosis and may contribute to antimicrobial resistance [22,23].

S. saprophyticus is a bacterium that commonly causes UTIs in young females worldwide, with prevalence rates ranging from 10 to 20 %. The success of this bacterium as a UTI pathogen is due to its ability to survive in harsh and toxic environments, which can be attributed to its high resistance to heavy metals and its ability to detoxify uric acid and D-serine [24]. Additionally, *S. saprophyticus* has been linked to its capacity to adhere to and infect uroepithelial cells through the use of adhesins, surface proteins and biofilm production. The current study found that 88 % of *S. saprophyticus* isolates could form biofilms and 28 %, 33 %, and 39 % of these isolates were classified as strong, moderate, and weak biofilm producers, respectively.

S. haemolyticus is part of the skin microflora and is one of the most important CoNS species, responsible for 10–20 % of clinical CoNS infections [25]. The present study revealed that *S. haemolyticus* was the second most prevalent and important CoNS species among UTI isolates, accounting for approximately 6.28 % of the cases.

S. haemolyticus exhibits remarkable resistance to antibiotics compared to other CoNS [25]. In addition, *S. haemolyticus* infections particularly affect immunocompromised patients and neonates and mainly occur as bloodstream and device-associated infections [26]. Compared to *S. aureus, S. haemolyticus* has few typical virulence factors, such as biofilm formation, adhesion proteins, cell wall-anchored proteins, the hemolysis of red blood cells, the production of phenol-soluble modulins and frequent phenotypic rearrangements due to numerous insertion sequences [27,28].

According to the results of this meta-analysis, *S. haemolyticus* isolates exhibited the highest prevalence of resistance to methicillin, with pooled estimates of 45 %, gentamicin 33 % and tetracycline 29 %. The presence and expression of antibiotic resistance genes accounts for the high antibiotic resistance in *S.* haemolyticus. Moreover, the presence of these genes and their propagation in the environment represents a potential threat, as this bacterium can host resistance genes and transfer them to other species. Biofilm formation by *S. haemolyticus* is the most crucial virulence factor in CoNS. It is a complex process that affects adhesion, increases antibiotic resistance and evades host defense mechanisms [29].

Antibiotic therapy remains the treatment of choice, especially in the early stages of UTIs. The choice of antibiotic therapy is based on signs of severity, susceptibility of the strain to methicillin, management of foreign bodies and the medical devices with which these infections may be associated.

Antistaphylococcal penicillins, such as cloxacillin and oxacillin,

remain the first-line treatment for UTIs caused by methicillin-sensitive staphylococci. However, the poor digestive absorption of the oral forms often leads to a preference for prescribing other antibiotics that are active on methicillin-sensitive staphylococci and have better bioavailability. Methicillin-sensitive staphylococci are most often sensitive to fluoroquinolones and macrolides. In addition, clavulanic acid can restore the sensitivity of methicillin-sensitive staphylococci to penicillin (amoxicillin and clavulanic acid). Aminoglycosides should be reserved for UTIs that require intravenous treatment [30].

The mechanism of resistance to methicillin is responsible for the resistance to all β-lactam antibiotics. In the case of hospital methicillin-resistant staphylococci, resistance to macrolides, fluoroquinolones, fosfomycin, and aminoglycosides is frequently observed [31]. Generally, glycopeptides (vancomycin or teicoplanin), rifampicin, fusidic acid, and linezolid remain active against these strains. However, community methicillin-resistant staphylococci have a distinct resistance phenotype and often retain susceptibility to macrolides, fluoroquinolones, tobramycin and have intermediate susceptibility to fusidic acid [32].

5. Conclusion

According to the results of this study, *Staphylococcus* species are becoming increasingly prevalent in UTIs, displaying increased resistance to antibiotics and being characterized by increased biofilm formation. These trends require immediate attention. However, vancomycin was found to be the most effective antibiotic. Periodic surveillance studies on uropathogenic staphylococci and their antibiotic resistance are necessary to effectively combat methicillin-resistant isolates and reduce antibiotic resistance rates. When selecting empirical treatment or self-medication, the inappropriate use of antibiotics, for example, can prevent and hinder the reduction of multidrug-resistant bacterial infections.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

CRediT authorship contribution statement

Rafik Aniba: Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation. **Asmaa Dihmane:** Formal analysis, Data curation, Conceptualization. **Habiba Raqraq:** Methodology, Investigation, Conceptualization. **Amina Ressmi:** Methodology, Investigation. **Kaotar Nayme:** Visualization, Validation, Supervision. **Mohammed Timinouni:** Visualization, Validation, Supervision. **Abouddihaj Barguigua:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology.

Declaration of competing interest

No conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2024.116470.

References

[1] Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Micro 2015;13:269–84. https://doi.org/10.1038/nrmicro3432.

[2] Terlizzi ME, Gribaudo G, Maffei ME. UroPathogenic Escherichia coli (UPEC) infections: virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. Front Microbiol 2017;8:1566. https://doi.org/10.3389/fmicb.2017.01566.

[3] Gebremariam G, Legese H, Woldu Y, Araya T, Hagos K, GebreyesusWasihun A. Bacteriological profile, risk factors and antimicrobial susceptibility patterns of symptomatic urinary tract infection among students of Mekelle University, northern Ethiopia. BMC Infect Dis 2019;19:950. https://doi.org/10.1186/s12879-019-4610-2.

[4] Kline KA, Lewis AL. Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. Microbiol Spectr 2016;4. https://doi.org/10.1128/microbiolspec.UTI-0012-2012.

[5] Assouma FF, Sina H, Dossou AD, Socohou A, Hounsou MC, Avogbe PH, et al. Antibiotic resistance profiling of pathogenic staphylococcus species from urinary tract infection patients in Benin. Biomed Res Int 2023;2023:6364128. https://doi.org/10.1155/2023/6364128.

[6] Baba-Moussa L, Anani L, Scheftel JM, Couturier M, Riegel P, Haïkou N, et al. Virulence factors produced by strains of Staphylococcus aureus isolated from urinary tract infections. J Hosp Infect 2008;68:32–8. https://doi.org/10.1016/j.jhin.2007.10.010.

[7] Natsis NE, Cohen PR. Coagulase-negative staphylococcus skin and soft tissue infections. Am J Clin Dermatol 2018;19:671–7. https://doi.org/10.1007/s40257-018-0362-9.

[8] Kumari H, Chakraborti T, Singh M, Chakrawarti MK, Mukhopadhyay K. Prevalence and antibiogram of coagulase negative Staphylococci in bioaerosols from different indoors of a university in India. BMC Microbiol 2020;20:211. https://doi.org/10.1186/s12866-020-01875-8.

[9] Phillip S, Mushi MF, Decano AG, Seni J, Mmbaga BT, Kumburu H, et al. Molecular characterizations of the coagulase-negative staphylococci species causing urinary tract infection in Tanzania: a laboratory-based cross-sectional study. Pathogens 2023;12:180. https://doi.org/10.3390/pathogens12020180.

[10] Belete MA, Gedefie A, Alemayehu E, Debash H, Mohammed O, Gebretsadik D, et al. The prevalence of vancomycin-resistant Staphylococcus aureus in Ethiopia: a systematic review and meta-analysis. Antimicrob Resist Infect Control 2023;12:86. https://doi.org/10.1186/s13756-023-01291-3.

[11] Aniba R, Dihmane A, Raqraq H, Ressmi A, Nayme K, Timinouni M, et al. Characterization of biofilm formation in uropathogenic Staphylococcus aureus and their association with antibiotic resistance. Microbe 2024;2:100029. https://doi.org/10.1016/j.microb.2023.100029.

[12] Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev 2015;4:1. https://doi.org/10.1186/2046-4053-4-1.

[13] Parlet CP, Brown MM, Horswill AR. Commensal staphylococci influence staphylococcus aureus skin colonization and disease. Trends Microbiol 2019;27:497–507. https://doi.org/10.1016/j.tim.2019.01.008.

[14] Xu K, Wang Y, Jian Y, Chen T, Liu Q, Wang H, et al. Staphylococcus aureus ST1 promotes persistent urinary tract infection by highly expressing the urease. Front Microbiol 2023;14:1101754. https://doi.org/10.3389/fmicb.2023.1101754.

[15] Harkins CP, Pichon B, Doumith M, Parkhill J, Westh H, Tomasz A, et al. Methicillin-resistant Staphylococcus aureus emerged long before the introduction of methicillin into clinical practice. Genome Biol 2017;18:130. https://doi.org/10.1186/s13059-017-1252-9.

[16] Navidinia M, Mohammadi A, Arjmand R, Dadashi M, Goudarzi M. Molecular typing, biofilm formation, and analysis of adhesion factors in *Staphylococcus aureus* strains isolated from urinary tract infections. Gene Rep 2021;22:101008. https://doi.org/10.1016/j.genrep.2020.101008.

[17] Pajohesh R, Tajbakhsh E, Momtaz H, Rahimi E. Relationship between biofilm formation and antibiotic resistance and adherence genes in *Staphylococcus aureus* strains isolated from raw cow milk in Shahrekord, Iran. Int J Microbiol 2022;2022:e6435774. https://doi.org/10.1155/2022/6435774.

[18] Hashemzadeh M, Dezfuli AAZ, Nashibi R, Jahangirimehr F, Akbarian ZA. Study of biofilm formation, structure and antibiotic resistance in Staphylococcus saprophyticus strains causing urinary tract infection in women in Ahvaz, Iran. New Microbes New Infect 2021;39:100831. https://doi.org/10.1016/j.nmni.2020.100831.

[19] 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. https://doi.org/10.1016/j.nmni.2020.100755.

[20] Skovdal SM, Jørgensen NP, Meyer RL. JMM profile: *Staphylococcus epidermidis*. J Med Microbiol 2022;71:001597. https://doi.org/10.1099/jmm.0.001597.

[21] Pauer H, Glatthardt T, Ferreira NV, Ferreira RBR, Antunes LCM. Chapter 12 - bioactive molecules of the human microbiome: skin, respiratory tract, intestine. J. Faintuch, S. Faintuch. Microbiome and metabolome in diagnosis, therapy, and other strategic applications. Academic Press; 2019. p. 115–25. https://doi.org/10.1016/B978-0-12-815249-2.00012-9.

[22] Foster TJ. Chapter 35 - Coagulase negative staphylococci. Y.-W. Tang, M.Y. Hindiyeh, D. Liu, A. Sails, P. Spearman, J.-R. Zhang. Molecular medical microbiology. 3rd Ed. Academic Press; 2024. p. 681–704. https://doi.org/10.1016/B978-0-12-818619-0.00016-2.

[23] Raafat D, Otto M, Reppschläger K, Iqbal J, Holtfreter S. Fighting staphylococcus aureus biofilms with monoclonal antibodies. Trends Microbiol 2019;27:303–22. https://doi.org/10.1016/j.tim.2018.12.009.

[24] Lawal OU, Barata M, Fraqueza MJ, Worning P, Bartels MD, Goncalves L, et al. Staphylococcus saprophyticus from clinical and environmental origins have distinct biofilm composition. Front Microbiol 2021;12:663768. https://doi.org/10.3389/fmicb.2021.663768.

[25] Eltwisy HO, Twisy HO, Hafez MH, Sayed IM, El-Mokhtar MA. Clinical infections, antibiotic resistance, and pathogenesis of staphylococcus haemolyticus. Microorganisms 2022;10:1130. https://doi.org/10.3390/microorganisms10061130.

[26] Kranjec C, Kristensen SS, Bartkiewicz KT, Brønner M, Cavanagh JP, Srikantam A, et al. A bacteriocin-based treatment option for Staphylococcus haemolyticus biofilms. Sci Rep 2021;11:13909. https://doi.org/10.1038/s41598-021-93158-z.
[27] Pain M, Hjerde E, Klingenberg C, Cavanagh JP. Comparative genomic analysis of staphylococcus haemolyticus reveals key to hospital adaptation and pathogenicity. Front Microbiol 2019;10. https://doi.org/10.3389/fmicb.2019.02096.
[28] Pereira PMA, Binatti VB, Sued BPR, Ramos JN, Peixoto RS, Simões C, et al. *Staphylococcus haemolyticus* disseminated among neonates with bacteremia in a neonatal intensive care unit in Rio de Janeiro, Brazil. Diagn Microbiol Infect Dis 2014;78:85–92. https://doi.org/10.1016/j.diagmicrobio.2013.06.026.
[29] Vanderhaeghen W, Piepers S, Leroy F, Van Coillie E, Haesebrouck F, De Vliegher S. Invited review: effect, persistence, and virulence of coagulase-negative *Staphylococcus* species associated with ruminant udder health. J Dairy Sci 2014;97:5275–93. https://doi.org/10.3168/jds.2013-7775.
[30] Lowe RA, Barber KE, Wagner JL, Bell-Harlan AM, Stover KR. Ceftriaxone for the treatment of methicillin-susceptible staphylococcus aureus bacteremia: a case series. J Pharmacol Pharmacother 2017;8:140–4. https://doi.org/10.4103/jpp.JPP_5_17.
[31] Brown NM, Goodman AL, Horner C, Jenkins A, Brown EM. Treatment of methicillin-resistant Staphylococcus aureus (MRSA): updated guidelines from the UK. JAC Antimicrob Resist 2021;3:dlaa114. https://doi.org/10.1093/jacamr/dlaa114.
[32] Kotsaki A, Tziolos N, Kontopoulou T, Koutelidakis IM, Symbardi S, Reed V, MacGowan A, et al. Oral minocycline plus rifampicin versus oral linezolid for complicated skin and skin structure infections caused by methicillin-resistant Staphylococcus aureus: the AIDA open label, randomized, controlled Phase 4 trial. eClinicalMedicine 2023;56. https://doi.org/10.1016/j.eclinm.2022.101790.