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URINARY TRACT INFECTION BACTERIA AND THEIR DRUG RESISTANCE

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Abstract. Urinary tract infections (UTIs) are among the most common bacterial infections, and the increasing antimicrobial resistance of uropathogens progressively limits the options for empirical therapy. The aim of this study was to determine the spectrum of bacteria causing UTIs in outpatients of City Clinical Hospital No. 1 (Tashkent, Uzbekistan) and to evaluate their susceptibility to commonly used antibiotics. Sixty urine samples from patients with clinically diagnosed UTI and significant bacteriuria ($\geq 10^5$ CFU/mL) were cultured, and the isolates were identified by MALDI-TOF mass spectrometry. The susceptibility of the predominant isolates to ten antibiotics belonging to seven classes was determined by the disc diffusion method. Women accounted for 56.7% of the patients, and the mean age was 38.82 ± 15.3 years. Gram-positive and Gram-negative bacteria represented 52% and 48% of the isolates, respectively. *Escherichia coli* (43%) was the most frequent uropathogen, followed by *Staphylococcus haemolyticus* (35%) and *Staphylococcus aureus* (10%). None of the tested isolates produced an inhibition zone with oxacillin or ampicillin, and most were resistant to piperacillin and co-trimoxazole, whereas tetracycline produced inhibition zones against all isolates. The *S. aureus*, *Enterococcus faecalis* and *Klebsiella pneumoniae* isolates showed no inhibition by agents of three or more antimicrobial classes, consistent with a multidrug-resistant phenotype. These results suggest that resistance to commonly used antibiotics is frequent among uropathogens in this clinical setting and support the use of urine culture and susceptibility testing before treatment.

Keywords: urinary tract infection, uropathogens, antimicrobial resistance, multidrug resistance, *Escherichia coli*, *Staphylococcus haemolyticus*, disc diffusion method.

Annotatsiya. Siydik yo‘llari infeksiyalari (SYI) eng ko‘p uchraydigan bakterial infeksiyalardan biri bo‘lib, uropatogenlarning antimikrob vositalarga chidamliligining ortib borishi empirik davolash imkoniyatlarini tobora cheklamoqda. Tadqiqotning maqsadi Toshkent shahar 1-sonli klinik shifoxonasi (O‘zbekiston) ambulator bemorlarida SYI qo‘zg‘atuvchi bakteriyalar tarkibini aniqlash va ularning keng qo‘llaniladigan antibiotiklarga sezgirlikni baholashdan iborat. Klinik jihatdan SYI tashxisi qo‘yilgan va sezilarli bakteriuriyasi ($\geq 10^5$ KHB/ml) bo‘lgan bemorlardan olingan 60 ta siydik namunasi ekildi, ajratilgan izolyatlar MALDI-TOF mass-spektrometriya usulida identifikatsiya qilindi. Asosiy izolyatlarning yetti sinfga mansub o‘nta antibiotikka sezgirlik disk-diffuziya usulida aniqlandi. Bemorlarning 56,7 %ini ayollar tashkil etdi, o‘rtacha yosh $38,82 \pm 15,3$ yilni tashkil qildi. Izolyatlarning 52 %i grammusbat va 48 %i grammanfiy bo‘lgan bakteriyalar aniqlandi. Eng ko‘p uchragan uropatogen *Escherichia coli* (43 %) bo‘lib, undan keyin *Staphylococcus haemolyticus* (35 %) va *Staphylococcus aureus* (10 %) qayd etildi. Tekshirilgan izolyatlarning hech biri oksatsillin va ampitsillin atrofida o‘shishni to‘xtatish zonasini hosil qilmadi, ularning aksariyati piperatsillin va ko-trimoksazolga chidamli bo‘ldi, tetratsiklin esa barcha izolyatlarga nisbatan o‘shishni to‘xtatish zonasini hosil qildi. *S. aureus*, *Enterococcus faecalis* va *Klebsiella pneumoniae* izolyatlari uch va undan ortiq antimikrob sinf vakillariga nisbatan zona hosil qilmadi, bu ko‘p dori vositalariga chidamli fenotipga mos keladi. Olingan natijalar ushbu klinikada uropatogenlar orasida keng qo‘llaniladigan antibiotiklarga chidamlilik tez-tez uchrashini ko‘rsatadi va davolashdan oldin siydik ekmasi hamda antibiotiklarga sezgirlikni aniqlash maqsadga muvofiqligini tasdiqlaydi.

Kalit so‘zlar: siydik yo‘llari infeksiyasi, uropatogenlar, antimikrob chidamlilik, ko‘p dori vositalariga chidamlilik, *Escherichia coli*, *Staphylococcus haemolyticus*, disk-diffuziya usuli.

Аннотация. Инфекции мочевыводящих путей (ИМП) относятся к наиболее распространённым бактериальным инфекциям, а растущая антимикробная резистентность уropatогенов всё больше ограничивает возможности эмпирической терапии. Целью исследования было определение спектра возбудителей ИМП у амбулаторных пациентов Городской клинической больницы № 1 (Ташкент, Узбекистан) и оценка их чувствительности к широко применяемым антибиотикам. Проведён посев 60 образцов мочи пациентов с клинически установленным диагнозом ИМП и значимой бактериурией ($\geq 10^5$ КОЕ/мл); выделенные изоляты идентифицированы методом MALDI-TOF масс-спектрометрии. Чувствительность преобладающих изолятов к десяти антибиотикам семи классов определяли диско-диффузионным методом. Женщины составили 56,7 % пациентов, средний возраст – $38,82 \pm 15,3$ года. Грамположительные и грамотрицательные бактерии составили 52 % и 48 % изолятов соответственно. Наиболее частым уropatогеном была *Escherichia coli* (43 %), за ней следовали *Staphylococcus haemolyticus* (35 %) и *Staphylococcus aureus* (10 %). Ни один из исследованных изолятов не образовывал зону задержки роста вокруг дисков с оксациллином и ампициллином, большинство были устойчивы к пиперациллину и ко-тримоксазолу, тогда как тетрациклин образовывал зоны задержки роста у всех изолятов. Изоляты *S. aureus*, *Enterococcus faecalis* и *Klebsiella pneumoniae*

не образовывали зон задержки роста к препаратам трёх и более классов, что соответствует фенотипу множественной лекарственной устойчивости. Полученные результаты указывают на частую устойчивость уропатогенов к широко применяемым антибиотикам в данном лечебном учреждении и подтверждают целесообразность посева мочи и определения чувствительности к антибиотикам до начала лечения.

Ключевые слова: инфекция мочевыводящих путей, уропатогены, антимикробная резистентность, множественная лекарственная устойчивость, *Escherichia coli*, *Staphylococcus haemolyticus*, диско-диффузионный метод.

INTRODUCTION. Urinary tract infection (UTI) is one of the most common bacterial infections, second only to respiratory tract infections. Global estimates indicate that more than 400 million UTI cases occur each year, and the disease burden has continued to grow over the last three decades [1]. UTIs are more frequent in women, children and the elderly; approximately one in two women and one in twenty men experience at least one episode during their lifetime. According to the affected part of the urinary system, UTIs are classified as urethritis, cystitis and pyelonephritis [2]. Most infections are caused by bacteria of intestinal origin that ascend from the periurethral region to the bladder and, in complicated cases, to the kidneys [3].

The principal causative agents of UTIs are *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus* spp. and *Staphylococcus* spp., including *S. saprophyticus* and other coagulase-negative staphylococci [3]. Because antibiotics are usually prescribed empirically, often before the results of urine culture are available or without culture at all, uropathogens are continuously exposed to selective pressure. As a result, resistance to β -lactams, fluoroquinolones and sulfonamides has become widespread, and recurrent infections respond progressively worse to repeated courses of antibiotics.

Antimicrobial resistance (AMR) is now recognized as one of the leading threats to public health. In 2019, bacterial AMR was directly responsible for an estimated 1.27 million deaths worldwide [4], and several typical uropathogens, including third-generation cephalosporin-resistant Enterobacterales and methicillin-resistant *S. aureus*, are listed by the World Health Organization as priority pathogens [5]. In many healthcare settings, diagnosis and treatment of UTIs still proceed without culture and susceptibility testing [6], although the aetiology of UTIs and the susceptibility of uropathogens change over time and differ considerably between countries and regions [7–9]. For this reason, international guidelines recommend that empirical therapy be chosen according to local resistance data; for example, co-trimoxazole should be used empirically for uncomplicated cystitis only where local resistance of uropathogens is below 20% [10].

Data on the aetiological structure and resistance patterns of uropathogens in Uzbekistan remain limited. Therefore, the aim of the present study was to determine the spectrum of bacteria causing UTIs in outpatients of City Clinical Hospital No. 1 (Tashkent) and to evaluate the susceptibility of the predominant isolates to antibiotics commonly used in clinical practice.

MATERIALS AND METHODS

Study Design and Patients. This cross-sectional study included 60 urine samples collected in 2024 from febrile male and female patients with clinical signs of UTI who attended the outpatient departments of City Clinical Hospital No. 1 (Tashkent, Uzbekistan). The diagnosis was confirmed by significant bacteriuria, defined as the presence of a single uropathogen at $\geq 10^5$ CFU/mL in suprapubic aspirated or catheterized urine. Demographic and clinical data (sex, age, medical history and vital signs) were recorded using a structured questionnaire on the patient’s arrival.

Culture and Identification of Uropathogens. Urine samples were cultured on Mueller–Hinton agar (HiMedia, India) and incubated under aerobic conditions at 35–37 °C for 18–24 h. Colonies were subcultured to obtain pure cultures, and bacterial identification was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Antimicrobial Susceptibility Testing. The susceptibility of isolates of the six predominant species (*S. haemolyticus*, *E. faecalis*, *E. coli*, *S. aureus*, *K. pneumoniae* and *S. epidermidis*) was determined by the disc diffusion method on Mueller–Hinton agar [11]. Ten antibiotics belonging to seven classes and frequently used in clinical practice were tested (HiMedia, India): cefoperazone/sulbactam (30 µg), penicillin G (10 U), oxacillin (10 µg), piperacillin (30 µg), ampicillin (10 µg), rifampicin (5 µg), ciprofloxacin (5 µg), chloramphenicol (5 µg), tetracycline (30 µg) and co-trimoxazole (30 µg). After incubation at 37 °C for 18–24 h, the diameter of the growth inhibition zone was measured in millimetres. The complete absence of an inhibition zone was recorded as resistance (R). All tests were performed in triplicate. An isolate showing no inhibition by at least one agent in three or more antimicrobial classes was considered to have a multidrug-resistant (MDR) phenotype [12]. Part of the susceptibility data was previously reported in a study of the antagonistic activity of *Lactobacillus* strains against uropathogens [13].

Statistical Analysis. Descriptive statistics were used for data analysis. The prevalence of each uropathogen and of antimicrobial resistance was calculated as the proportion of positive results in the study sample. Data were analysed using SPSS software; Student’s *t*-test and cross-tabulation were used for comparisons, and $p < 0.05$ was considered statistically significant.

RESULTS

Characteristics of Patients. A total of 60 urine samples were collected from the outpatient departments of City Clinical Hospital No. 1 (Tashkent), and all of them (100%) showed significant bacteriuria. Of the 60 patients, 34 (56.7%) were women and 26 (43.3%) were men (Table 1). The mean age ($\pm$ SD) of the patients was 38.82 ± 15.3 years.

Table 1. Distribution of UTI cases by sex among outpatients of City Clinical Hospital No. 1, Tashkent (2024)

Sex	Number of patients (n)	Frequency (%)
Male	26	43.3
Female	34	56.7
Total	60	100.0

Distribution of Uropathogens. UTIs in the study population were caused by both Gram-positive and Gram-negative bacteria [14]. Gram-positive bacteria accounted for 52% and Gram-negative bacteria for 48% of all isolates (Table 2). *E. coli* was the most frequently isolated uropathogen (43%), followed by *S. haemolyticus* (35%) and *S. aureus* (10%). *S. epidermidis* accounted for 3% of isolates; *E. faecalis*, *E. faecium*, *K. pneumoniae* and *P. aeruginosa* for 2% each; and *P. mirabilis* for 1%. Overall, 56.2% of the isolates were recovered from men and 43.8% from women. *P. mirabilis* and *P. aeruginosa* were isolated only from male patients.

Table 2

Distribution of bacteria causing UTI (% of clinical isolates)

Microorganism	Overall, %	Male, %	Female, %
Gram-positive bacteria	52.0	28.0	24.0
<i>Staphylococcus epidermidis</i>	3.0	1.5	1.5
<i>Staphylococcus haemolyticus</i>	35.0	20.0	15.0
<i>Staphylococcus aureus</i>	10.0	5.0	5.0
<i>Enterococcus faecalis</i>	2.0	0.5	1.5
<i>Enterococcus faecium</i>	2.0	1.0	1.0
Gram-negative bacteria	48.0	28.2	19.8
<i>Escherichia coli</i>	43.0	25.0	18.0
<i>Klebsiella pneumoniae</i>	2.0	0.2	1.8
<i>Proteus mirabilis</i>	1.0	1.0	0
<i>Pseudomonas aeruginosa</i>	2.0	2.0	0
Total	100.0	56.2	43.8

Antimicrobial Susceptibility of Uropathogens. The results of disc diffusion testing are presented in Table 3 and summarized in Figure 1. None of the six tested isolates produced an inhibition zone around oxacillin or ampicillin discs, and five of six were not inhibited by piperacillin. Co-trimoxazole produced no inhibition zone against *E. coli*, *K. pneumoniae*, *S. aureus* and *E. faecalis*, while only narrow zones (8–10 mm) were observed for *S. haemolyticus* and *S. epidermidis*. In contrast, tetracycline produced inhibition zones (10–17 mm) against all isolates, and rifampicin was active against all isolates except *S. aureus*.

Among Gram-negative bacteria, *E. coli* and *K. pneumoniae* were resistant to oxacillin, ampicillin, piperacillin and co-trimoxazole; *K. pneumoniae* was additionally resistant to ciprofloxacin, and only a narrow inhibition zone was observed for *E. coli* (8 mm). Among Gram-positive bacteria, all three staphylococcal species (*S. haemolyticus*, *S. aureus* and *S. epidermidis*) were resistant to cefoperazone/sulbactam as well as to oxacillin, ampicillin and piperacillin. *S. aureus* showed the broadest resistance, with no inhibition zone for 8 of the 10 antibiotics, including rifampicin, chloramphenicol and co-trimoxazole. *E. faecalis* was resistant to β -lactams (oxacillin and ampicillin), ciprofloxacin, chloramphenicol and co-trimoxazole. Thus, the *S. aureus* (five classes), *E. faecalis* (four classes) and *K. pneumoniae* (three classes) isolates showed an MDR phenotype.

Table 3

In vitro susceptibility of uropathogens to antibiotics (diameter of growth inhibition zone, mm)

No.	Antibiotic	Class	Disc content	S. haemolyticus	E. faecalis	E. coli	S. aureus	K. pneumoniae	S. epidermidis
1	Cefoperazone/sulbactam	I	30 µg	R	14	13	R	14	R
2	Penicillin G	II	10 U	18	22	23	R	22	R
3	Oxacillin	II	10 µg	R	R	R	R	R	R
4	Piperacillin	II	30 µg	R	11	R	R	R	R
5	Ampicillin	II	10 µg	R	R	R	R	R	R
6	Rifampicin	III	5 µg	12	25	16	R	17	12
7	Ciprofloxacin	IV	5 µg	16	R	8	16	R	10
8	Chloramphenicol	V	5 µg	6	R	10	R	10	25
9	Tetracycline	VI	30 µg	16	12	12	17	12	10
10	Co-trimoxazole	VII	30 µg	8	R	R	R	R	10

Note: R – resistant (no growth inhibition zone). Antibiotic classes: I – cephalosporin/β-lactamase inhibitor combination; II – penicillins (β-lactams); III – ansamycins (rifamycins); IV – fluoroquinolones; V – amphenicols; VI – tetracyclines; VII – sulfonamide/trimethoprim combination.

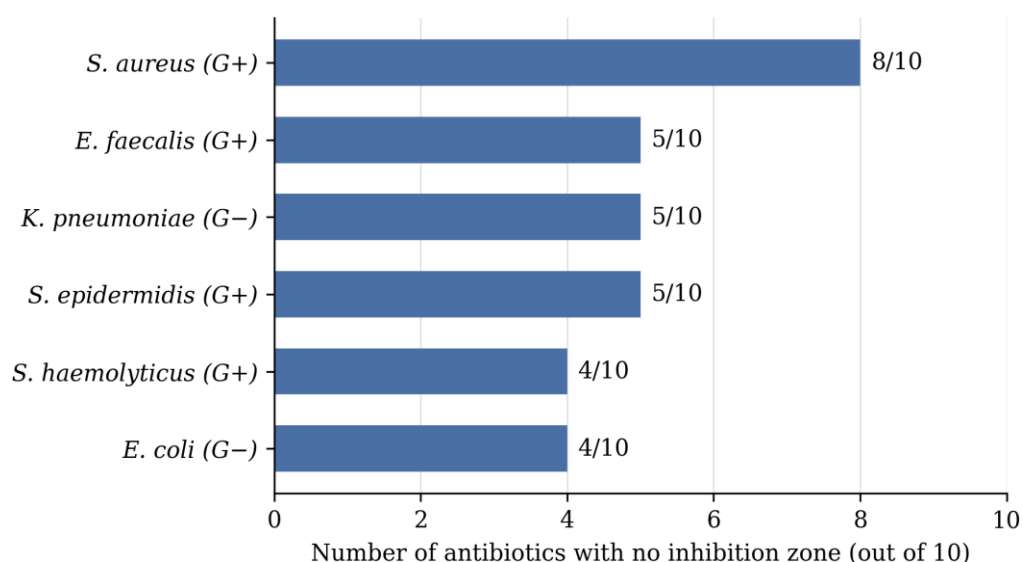


Figure 1. Number of antibiotics (out of 10) that produced no growth inhibition zone against each uropathogen isolate (G+ – Gram-positive; G– – Gram-negative).

DISCUSSION

The present study showed that *E. coli* remains the leading cause of UTIs among outpatients in Tashkent, which is consistent with data from other regions of the world [3,7,9]. At the same time, the proportion of Gram-positive bacteria (52%) was higher than is usually reported for community-acquired UTIs, mainly because of the high frequency of *S. haemolyticus* (35%). Coagulase-negative staphylococci are increasingly recognized as

opportunistic uropathogens, particularly in patients with predisposing conditions, and their high antibiotic resistance and ability to form biofilms contribute to their pathogenicity and make the infections difficult to treat [15,16]. Nevertheless, because coagulase-negative staphylococci are also common skin commensals, their clinical significance should always be interpreted together with the clinical picture and the colony count.

A key finding of this study is the uniform absence of inhibition zones around oxacillin and ampicillin discs. For Gram-negative bacteria, resistance to oxacillin and penicillin-type agents is largely intrinsic, and *K. pneumoniae* is naturally resistant to ampicillin [17]. However, the resistance of *E. coli* and *K. pneumoniae* to piperacillin and co-trimoxazole reflects acquired resistance and is clinically relevant. Since co-trimoxazole should be used for empirical therapy only where local resistance is below 20% [10], our results suggest that this drug should not be prescribed empirically for UTIs in this setting without susceptibility testing. The resistance of *K. pneumoniae* and *E. faecalis* to ciprofloxacin, and the narrow inhibition zone observed for *E. coli*, also argue against the empirical use of fluoroquinolones.

Among Gram-positive isolates, the absence of inhibition zones with oxacillin in all three staphylococcal species, together with resistance to cefoperazone/sulbactam, suggests methicillin resistance. Methicillin-resistant staphylococci are resistant to almost all β -lactam antibiotics, which considerably narrows the treatment options [5,15]. The *S. aureus* isolate was resistant to agents of five antimicrobial classes, including rifampicin, and the *E. faecalis* isolate to agents of four classes, confirming that MDR bacteria can pose considerable treatment challenges even in outpatients. Tetracycline retained activity against all isolates, although its clinical role in UTI treatment is limited.

The high resistance levels observed in this study are most likely associated with the widespread empirical and often non-judicious use of antibiotics, including self-medication, which is common when culture and susceptibility testing are not performed [6,8].

This study has several limitations. It was conducted in a single centre with a relatively small number of samples, and susceptibility testing was performed on one isolate of each predominant species. Zone diameters were not interpreted using CLSI or EUCAST clinical breakpoints, minimum inhibitory concentrations were not determined, and the production of extended-spectrum β -lactamases and the presence of the *mecA* gene were not investigated. Larger multicentre studies using standardized interpretive criteria and molecular methods are needed to characterize the resistance mechanisms of uropathogens in Uzbekistan.

CONCLUSION

E. coli, *S. haemolyticus* and *S. aureus* were the predominant causative agents of UTIs among outpatients of City Clinical Hospital No. 1 in Tashkent. The tested uropathogens showed high resistance to penicillins (oxacillin, ampicillin and piperacillin) and co-trimoxazole, and the *S. aureus*, *E. faecalis* and *K. pneumoniae* isolates exhibited an MDR phenotype. These results suggest that resistance to commonly used antibiotics is frequent among uropathogens in this clinical setting. Whenever possible, the choice of antibiotic should be guided by urine culture and susceptibility testing, and larger studies are needed to assess the resistance situation across Tashkent.

REFERENCES

1. Yang X, Chen H, Zheng Y, Qu S, Wang H, Yi F. Disease burden and long-term trends of urinary tract infections: a worldwide report. *Frontiers in Public Health*. 2022;10:888205.
2. Bono MJ, Leslie SW, Reygaert WC. Uncomplicated urinary tract infections. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2024.
3. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology*. 2015;13(5):269–284.
4. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. 2022;399(10325):629–655.
5. World Health Organization. WHO Bacterial Priority Pathogens List, 2024. Geneva: World Health Organization; 2024.
6. Ahmed SS, Shariq A, Alsallloom AA, Babikir IH, Alhomoud BN. Uropathogens and their antimicrobial resistance patterns: relationship with urinary tract infections. *International Journal of Health Sciences (Qassim)*. 2019;13(2):48–55.
7. Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaeffer AJ, Stamm WE. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. *Clinical Infectious Diseases*. 1999;29(4):745–758.
8. Livermore DM, Pearson A. Antibiotic resistance: location, location, location. *Clinical Microbiology and Infection*. 2007;13(Suppl 2):7–16.
9. Magliano E, Grazioli V, Deflorio L, Leuci AI, Mattina R, Romano P, et al. Gender and age-dependent etiology of community-acquired urinary tract infections. *ScientificWorldJournal*. 2012;2012:349597.
10. European Association of Urology. EAU Guidelines on Urological Infections. Arnhem: EAU Guidelines Office; 2024.
11. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*. 1966;45(4):493–496.
12. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*. 2012;18(3):268–281.
13. Abdunabiev AM, Abduvohidova YO, Turayev AA, Urmonaliev ShU, Ermatova XY, Reyimbergenova ZA, et al. Antimicrobial activity of *Lactobacillus* strains against urinary tract infections. *Xorazm Ma'mun akademiyasi axborotnomasi*. 2024;(9/1):16–21.
14. Haghi-Ashtiani MT, Sadeghifard N, Abedini M, Soroush S, Taheri-Kalani M. Etiology and antibacterial resistance of bacterial urinary tract infections in children's Medical Center, Tehran, Iran. *Acta Medica Iranica*. 2007;45(2):153–157.
15. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. *Clinical Microbiology Reviews*. 2014;27(4):870–926.

16. Gaudio de Allori MC, Jure MA, Romero C, de Castillo MEC. Antimicrobial resistance and production of biofilms in clinical isolates of coagulase-negative *Staphylococcus* strains. *Biological and Pharmaceutical Bulletin*. 2006;29(8):1592–1596.

17. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 34th ed. CLSI supplement M100. Wayne (PA): CLSI; 2024.