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Prevalence and Phenotypic Characterization of Uropathogens in Pregnant Women from Al-Bayda, Libya

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Abstract

Background: Urinary tract infections (UTIs) during gestation represent a critical clinical concern associated with adverse maternal and fetal outcomes. Accurate local epidemiological data are paramount to optimizing empirical antimicrobial regimens. **Objectives:** This study aimed to isolate, phenotypically characterize, and evaluate the age-stratified distribution of bacterial uropathogens among pregnant inpatients at Al-Bayda Medical Center, Libya.

Methods: A cross-sectional analysis of 132 midstream urine specimens was conducted. Pathogens were isolated using differential media (MacConkey, Blood, CLED, and Mannitol Salt Agar) and systematically verified via standardized morphological and biochemical matrices. Statistical evaluations were performed using the Chi-square test ($\chi^2 = 6.41$, $P = 0.0113$) analysis to assess age-related variance.

Results: Significant bacteriuria was detected in 93 out of 132 samples (70.45%). Gram-positive cocci exhibited a slight predominance, accounting for 50.5% (n=47) of the positive cultures, compared to Gram-negative bacilli at 49.4% (n=46).

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Staphylococcus aureus was identified as the primary uropathogen (29.03%), followed by *Escherichia coli* (20.43%), *Staphylococcus epidermidis* (17.20%), and *Klebsiella* spp. (15.05%). Age-stratified categorization demonstrated a significantly higher infection rate within the older cohort (31–43 years: 84.4%) relative to the younger cohort (17–30 years: 63.2%), a correlation that was confirmed to be statistically significant using the Chi-square test (χ^2 = 6.41, P = 0.0113). Furthermore, *S. aureus* demonstrated a profound predominance (n=18) in the younger cohort, whereas the older group exhibited a synchronized distribution of *E. coli* and *S. aureus*.

Conclusion: The high prevalence of gestational UTIs, coupled with the unusual emergence of staphylococcal species over classic enterobacteria and a statistically verifiable escalation of risk in older pregnant individuals, emphasizes the necessity of implementing mandatory routine screening profiles within prenatal diagnostic protocols in eastern Libya.

Keywords: Urinary tract infections; Pregnancy; Uropathogens; Staphylococcus aureus; Age-stratified distribution; Al-Bayda; Libya.

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Prevalence and Phenotypic Characterization of Uropathogens in Pregnant Women from Al-Bayda, Libya

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انتشار وخصائص النمط الظاهري لمسببات الأمراض البولية لدى النساء الحوامل في البيضاء، ليبيا

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الملخص

تُعد التهابات المسالك البولية (UTIs) أثناء الحمل من المشكلات السريرية المهمة نظرًا لارتباطها بمضاعفات سلبية على صحة الأم والجنين. وتُعد البيانات الوبائية المحلية الموثوقة ضرورية لتوجيه العلاج التجريبي بالمضادات الحيوية وتحسين الرعاية الصحية للأمهات، وهدفت هذه الدراسة إلى عزل وتحديد وتقييم التوزيع العمري لمسببات التهابات المسالك البولية البكتيرية بين النساء الحوامل المترددات على مركز البيضاء الطبي، ليبيا.

كما أُجريت دراسة مقطعية على 132 عينة بول من منتصف مجرى البول جُمعت من نساء حوامل. تم زرع العزلات البكتيرية على أوساط ماكونكي آغار، وآغار الدم، وآغار كليد، وآغار ملح المانيتول، ثم جرى التعرف عليها باستخدام الاختبارات المورفولوجية والكيميائية الحيوية القياسية. وأُجري التحليل الإحصائي باستخدام اختبار كاي تربيع (Chi-square)لتقييم الفروق المرتبطة بالعمر في معدلات الإصابة.

تم الكشف عن بيلة جرثومية ذات دلالة إحصائية في 93 من أصل 132 عينة (70.45%). وشكلت المكورات إيجابية الغرام 50.5% (47 = n) من المزارع الإيجابية، في حين مثلت العصيات سلبية الغرام 49.5% (46 = n). وكانت المكورات العنقودية الذهبية (Staphylococcus aureus) أكثر الممرضات البولية شيوعًا بنسبة 29.03%، تلتها الإشريكية القولونية (Escherichia coli) بنسبة 20.43%، ثم المكورات العنقودية البشروية (Staphylococcus epidermidis) بنسبة 17.20%، والكليبسيلا (Klebsiella spp.) بنسبة 15.05%. وأظهر التحليل حسب الفئات العمرية ارتفاعًا ملحوظًا في معدل الإصابة بين النساء الحوامل الأكبر سنًا (31–43 سنة؛

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84.4%) مقارنة بالنساء الأصغر سنًا (17–30 سنة؛ 63.2%). وقد ثبتت دلالة هذه العلاقة إحصائيًا ($\chi^2 = 6.41$ ، $P = 0.0113$). بالإضافة إلى ذلك، كانت المكورات العنقودية الذهبية هي الأكثر انتشارًا في الفئة العمرية الأصغر (n = 18) ، بينما ظهرت الإشريكية القولونية والمكورات العنقودية الذهبية بمعدلات متساوية تقريبًا بين المشاركات الأكبر سنًا.

يشير الانتشار المرتفع لالتهابات المسالك البولية بين النساء الحوامل، إلى جانب غلبة أنواع المكورات العنقودية على البكتيريا المعوية التي تُعد تقليديًا المسبب الأكثر شيوعًا، وكذلك الزيادة الملحوظة في خطر الإصابة بين الحوامل الأكبر سنًا، إلى أهمية إدراج الفحص الروتيني والتشخيص المبكر ضمن برامج الرعاية السابقة للولادة في شرق ليبيا.

الكلمات الرئيسية: التهابات المسالك البولية؛ الحمل؛ مسببات الأمراض البولية؛ المكورات العنقودية الذهبية(Staphylococcus aureus) ؛ التوزيع العمري؛ البيضاء؛ ليبيا.

1. Introduction

Urinary tract infections (UTIs) constitute one of the most pervasive bacterial complications encountered during pregnancy, imposing a substantial burden on healthcare systems globally (Mancuso *et al.,* 2023). Gestation triggers complex physiological, anatomical, and hormonal alterations; specifically, elevated progesterone levels induce ureteral dilation, reduce detrusor muscle tone, and promote vesicoureteral reflux (Sobel, 2016). Concurrently, the mechanical compression of the bladder by the gravid uterus creates a state of persistent urinary stasis. This environment, enhanced by gestational glucosuria and aminoaciduria, provides an optimal substrate for the rapid proliferation of both facultative and obligate uropathogens.

The clinical consequences of unmanaged UTIs during pregnancy range from asymptomatic bacteriuria to acute cystitis and life-threatening pyelonephritis are profoundly linked to severe obstetric risks. These include maternal anemia, preeclampsia, premature rupture of membranes (PROM), low birth weight (LBW), and

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spontaneous miscarriage or preterm labor (Delzell & Lefevre, 2000).

While Gram-negative bacilli, predominantly *Escherichia coli*, are globally documented as the primary etiological agents of UTIs, recent epidemiological updates reveal shifting microbiological profiles characterized by a rising incidence of Gram-positive organisms and evolving antimicrobial resistance strains (Gajdács *et al.,* 2020). Because geographic and socio-environmental factors actively shape the local microbial landscape, continuous localized surveillance is essential. This investigation provides a rigorous phenotypic, biochemical, and statistical assessment of uropathogens isolated at Al-Bayda Medical Center, analyzing their distribution patterns across distinct maternal age groups to guide regional empirical interventions.

2. Materials and Methods

2.1. Ethical Approval and Study Timeline

The study protocol was formally reviewed and officially approved by the Al-Jabal Akhdar Branch Committee for Bioethics (JCB), under the Libyan National Committee for Biosafety and Bioethics (NBC), Libya (Reference Number: NBC: 004, H. 25. 14). The official approval was granted during the committee's meeting number (8) held on Tuesday, April 22, 2025. Following ethical clearance, clinical specimen collection was prospectively carried out over a designated period spanning from December 2, 2023, to March 20, 2024. The research was conducted in strict accordance with national and international bioethical standards. Additionally, prior to specimen enrollment, informed oral/written consent was obtained from all participating pregnant women, and the confidentiality of all personal and clinical data was rigorously maintained.

2.2. Specimen Collection and Participant Selection (Inclusion/Exclusion Criteria)

A total of 132 urine specimens were prospectively collected from pregnant inpatients presenting at Al-Bayda Medical Center. To ensure a rigorously defined study population, specific inclusion and exclusion criteria were strictly applied:

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Inclusion Criteria: Eligible participants included pregnant women of any gestational age and parity who presented with or without clinical symptoms of urinary tract infections (UTIs) during the designated study timeline.

Exclusion Criteria: Patients were systematically excluded if they had received any systemic antimicrobial therapy within the 14 days (2 weeks) prior to specimen collection, as prior antibiotic exposure could lead to false-negative cultures or alter the natural uropathogenic profile. Additionally, patients with a known history of structural or functional renal abnormalities, permanent urinary catheterization, or those unable to provide a reliable midstream clean-catch specimen were excluded from the study.

To minimize contamination from the periurethral and vaginal microbiota, samples were acquired utilizing the standard midstream clean-catch technique under the direct supervision of trained nursing staff (Alzaher *et al.,* 2023). A volume of 5–10 ml of midstream urine was captured in sterile, screw-capped, wide-mouth containers (Cheesbrough, 2006). In accordance with standard diagnostic protocols, first-morning voided specimens were prioritized due to the physiological concentration of bacterial counts overnight (Forbes *et al.* , 2018).

2.3. Microscopic Analysis and Primary Cultivation

All clinical samples were transferred to the laboratory and processed within one hour of collection. Aliquots were subjected to centrifugation, and the resulting sediment was examined via high-power field (HPF) light microscopy. The presence of ≥8–10 leukocytes/HPF was classified as indicative of significant pyuria (Simerville *et al.,* 2005). Concurrently, the presence of erythrocytes, epithelial cells, casts, crystals, and yeast forms was documented.

For primary isolation, uncentrifuged, well-mixed urine specimens were inoculated onto standard differential media, including Blood Agar, MacConkey Agar, and Cystine Lactose Electrolyte-Deficient (CLED) agar. Inoculated media plates were incubated aerobically at 37°C and evaluated sequentially at 24 and 48 hours to assess significant colony-forming units (CFUs) (Forbes *et al.,* 2018).

2.4. Morphological and Gram Characterization

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Following macro-colonial development, representative discrete colonies were subjected to standard Gram staining protocols to differentiate isolates into Gram-positive cocci or Gram-negative bacilli (Baron *et al.,* 1994). Cellular arrangements and morphology were confirmed under oil immersion light microscopy.

2.5. Biochemical Identification Matrix

Definitive species-level verification was performed using classical microbiological phenotypic assays (Koneman *et al.,* 2017). Pure colonies were isolated, suspended in nutrient broth, and inoculated into specific substrate media:

- **Catalase and Coagulase Assays:** The catalase test (3% H_2O_2) differentiated *Staphylococcus* spp. from *Streptococcus* spp. Slide and tube coagulase tests utilized rabbit plasma to separate *Staphylococcus aureus* from coagulase-negative staphylococci (CoNS) (Baron *et al.,* 1994).
- **Novobiocin and Optochin Susceptibility Testing:** Performed on Mueller-Hinton and Blood agar, respectively. A zone of inhibition 12 mm defined *Staphylococcus saprophyticus* resistance, whereas zones >16 mm confirmed *Staphylococcus epidermidis*. Optochin disks (5µg) yielding inhibition zones 14 mm were diagnostic for *Streptococcus pneumoniae* (Murray *et al.,* 2020).
- **Enteric Gram-Negative Bacilli Differentiation:**
- **Triple Sugar Iron (TSI) Test:** Evaluated to determine carbohydrate fermentation patterns (lactose, sucrose, glucose), gas evolution, and hydrogen sulfide (H_2S) precipitation.
- **IMViC Profiles and Urease Test:** Standard assays for indole production (Kovac's reagent), Simmons citrate utilization, and Christensen's urea agar slants were executed to complete the diagnostic matrix (Forbes *et al*., 2018).

2.6. Statistical Analysis

Statistical analysis was performed using SPSS software (Statistical Package for the Social Sciences, version 26.0). Categorical variables, including isolation rates and uropathogen distribution across the two distinct maternal age cohorts, were expressed as

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frequencies and percentages. The Chi-square test or Fisher's exact test was employed to determine the statistical significance of differences between the evaluated groups. A P-value of < 0.05 was considered statistically significant for all epidemiological comparisons.

3. Results

3.1. General Prevalence and Age-Stratified Growth Dynamics

Out of the 132 analyzed specimens, 93 (70.45%) demonstrated significant bacterial growth, while 39 (29.55%) exhibited no growth. Gram-positive isolates slightly predominated, constituting 50.5% (n=47) of the positive cultures, whereas Gram-negative isolates accounted for 49.4% (n=46).

Stratification by maternal age revealed a notable increase in infection rates with advanced age. In the younger cohort (17–30 years, n=87), positive bacterial growth was verified in 55 samples (63.2%), with 32 samples (36.8%) showing no growth as shown in figure 1.

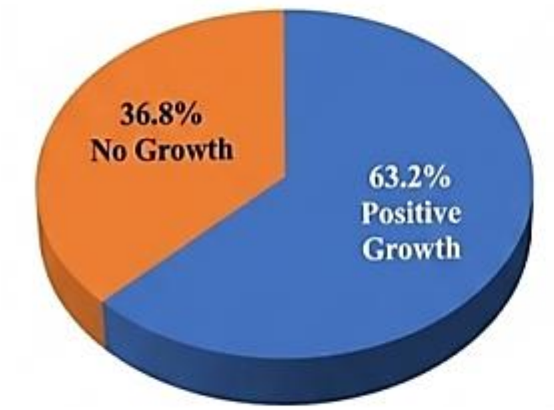

Figure 1: Percentage distribution of significant bacteriuria (Positive Growth) and no significant growth (No Growth) in urine specimens from the younger

Conversely, in the older cohort (31–43 years, n=45), positive bacterial growth was identified in 38 samples (84.4%), with only 7 samples (15.6%) remaining culture-negative as shown in figure 2. Chi-square analysis demonstrated that the higher prevalence of

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UTIs within the older maternal group was statistically significant using the Chi-square test ($\chi^2 = 6.41$, $P = 0.0113$), rejecting the null hypothesis of equal distribution.

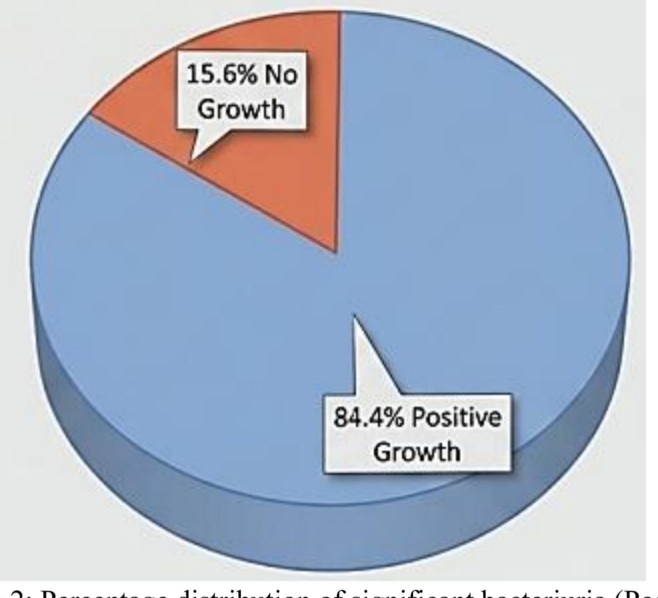

Figure 2: Percentage distribution of significant bacteriuria (Positive Growth) and no significant growth (No Growth) in urine specimens from the older

3.2. Colonial Characteristics on Differential Media

The recovered uropathogens demonstrated consistent and highly reproducible phenotypic properties across both evaluated cohorts:

- **MacConkey Agar (MAC):** *E. coli* and *Klebsiella* spp. exhibited rapid lactose fermentation, yielding smooth, vibrant pink colonies; *Klebsiella* colonies displayed a characteristic hyper-mucoid consistency. Non-lactose-fermenting *Pseudomonas* spp. generated pale, translucent colonies, with a subset producing a distinct green pyoverdin pigment and an aromatic, grape-like odor. *Proteus mirabilis* produced flat, non-lactose-fermenting colonies. *Stenotrophomonas maltophilia* and *Acinetobacter baumannii* uniformly yielded

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milky-white colonies. Gram-positive isolates were successfully inhibited on this medium.

- **Blood Agar:** Strong \beta-hemolytic clearing zones were recorded around *S. aureus* and *Streptococcus* spp. colonies. *Proteus mirabilis* demonstrated its characteristic multi-concentric swarming film. *E. coli* and *Klebsiella* spp. appeared as large, white, non-hemolytic colonies. *Pseudomonas* isolates demonstrated variable profiles, ranging from non-hemolytic colonies to zones of active hemolysis.
- **CLED Agar:** Lactose-fermenting *E. coli* and *Klebsiella* spp. manifested as bold yellow colonies. Conversely, *Pseudomonas* spp. formed white-to-green colonies indicative of pyoverdin secretion and absence of lactose fermentation. *Proteus mirabilis* presented as pale, non-swarming colonies. *Staphylococcus* spp. presented variable white-to-yellowish colony hues.
- **Mannitol Salt Agar (MSA):** Selectively differentiated staphylococci; *S. aureus* demonstrated robust mannitol fermentation resulting in distinctive golden-yellow colonies, while *S. epidermidis* grew as pink colonies, confirming an inability to ferment mannitol.

3.3. Pathogen Distribution Profiles

The definitive epidemiological distribution of the 93 isolated uropathogens across both maternal age groups is compiled in Table 1 and figure 1.

Table 1: Distribution of Bacterial Uropathogens Isolated from Total Urine Samples Across Maternal Age Groups

Pathogen Species	Gram Status	Age Group: 17–30 (n=55)	Age Group: 31–43 (n=38)	Total Isolates (N=93)	Prevalence Percentage (%)
Staphylococcus aureus	Gram-Positive	18	9	27	29.03%
Escherichia coli	Gram-Negative	10	9	19	20.43%

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Pathogen Species	Gram Status	Age Group: 17–30 (n=55)	Age Group: 31–43 (n=38)	Total Isolates (N=93)	Prevalence Percentage (%)
Staphylococcus epidermidis	Gram-Positive	9	7	16	17.20%
***Klebsiella* spp.**	Gram-Negative	9	5	14	15.05%
Pseudomonas spp.	Gram-Negative	5	4	9	9.68%
***Streptococcus* spp.**	Gram-Positive	2	2	4	4.30%
Acinetobacter baumannii	Gram-Negative	1	1	2	2.15%
Proteus mirabilis	Gram-Negative	1	0	1	1.08%
Stenotrophomonas maltophilia	Gram-Negative	0	1	1	1.08%
Total Positive Isolates	—	**55**	**38**	**93**	**100.00%**

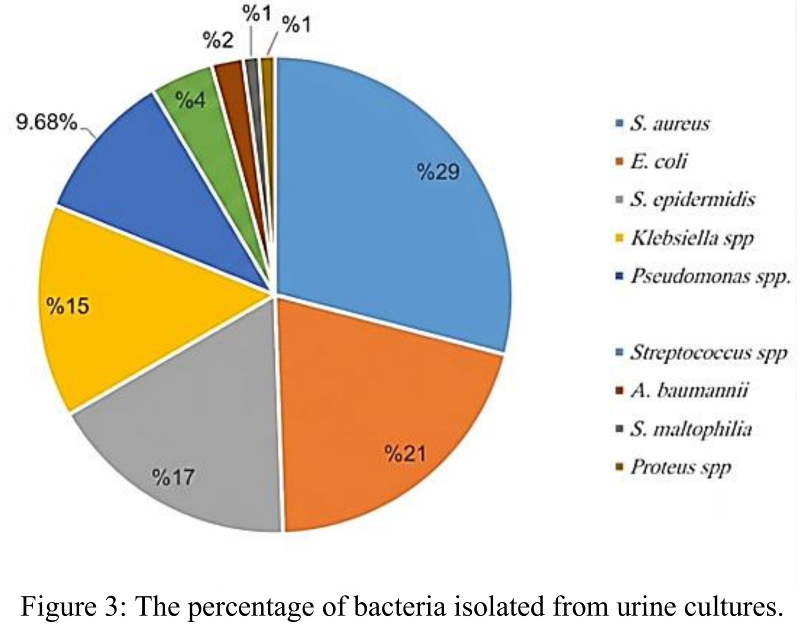

Figure 3: The percentage of bacteria isolated from urine cultures.

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3.4. Biochemical Differentiation Matrix

The systematic verification of the isolates was confirmed via specialized biochemical testing groups:

- **Gram-Positive Verification:** All staphylococci were confirmed as catalase-positive, effectively differentiating them from catalase-negative *Streptococcus* spp. Positive coagulase reactions uniquely separated *S. aureus* from CoNS. *S. epidermidis* isolates were confirmed by demonstrating uniform sensitivity to novobiocin.
- **Gram-Negative Enteric Profiles:** *E. coli* exhibited an A/A (acid/acid) reaction on TSI with gas production, and was uniquely indole-positive and citrate-negative. *Klebsiella* spp. demonstrated positive citrate utilization and negative indole reactions. *Proteus mirabilis* was rapidly identified by strong urease production and H2S generation on TSI. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* yielded K/K (alkaline/alkaline) reactions on TSI, confirming their status as non-fermenters.

4. Discussion

The calculated UTI prevalence rate of 70.45% observed among pregnant inpatients at Al-Bayda Medical Center indicates a high localized rate of urinary tract colonization within eastern Libya. This frequency significantly exceeds rates documented in North India (17.0%) (Chooramani *et al.,* 2020) and Saudi Arabia (23.4%) (Almutawif & Eid, 2023). However, it aligns with findings reported from the District of Anuppur in India (65.5%) (Sharma *et al.,* 2020) and data from the Ethiopian Public Health Institute (60%) (Seifu & Gebissa, 2018), while remaining below the exceptional infection rate reported at Shashemene Referral Hospital in Ethiopia (90.1%). These variations emphasize how distinct diagnostic thresholds, socio-environmental standards, and local clinical screening protocols can shape recorded UTI rates (Mohammed *et al.,* 2016).

A compelling finding of this study is the slight predominance of Gram-positive organisms (50.5%) over Gram-negative pathogens (49.4%). This represents a departure from the classic paradigm identifying Gram-negative enterobacteria as the overwhelming

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cause of UTIs. *Staphylococcus aureus* emerged as the primary uropathogen, accounting for 29.03% of all positive cultures. This matches epidemiological findings from Kenya, which reported an *S. aureus* prevalence of 29.7% in gestational asymptomatic bacteriuria (Ayoyi *et al.,* 2017). It also aligns with regional observations within Libya; for instance, a study at Al-Wahda Hospital reported *S. aureus* as a prominent Gram-positive uropathogen at 14.3% (Abd Alqawi, 2023). This trend is further supported by historical data from Nigeria, where staphylococcal infection rates (22.8%) frequently exceeded those of *E. coli* and *Klebsiella* spp. (Akortha & Ibadin, 2008).

The high prevalence of *S. aureus* may be linked to physiological changes during pregnancy that facilitate the colonization of skin and perineal flora into the urinary tract. Concurrently, *Staphylococcus epidermidis* was isolated at a rate of 17.20%. While historically dismissed as a skin contaminant, modern clinical microbiology recognizes its role as an opportunistic uropathogen. This is supported by data from Morocco, where coagulase-negative staphylococci (CoNS) accounted for 16.84% of urinary isolates (Aniba *et al.,* 2023), as well as data from Ethiopia (Eshetie *et al.,* 2018).

Among the Gram-negative pathogens, *Escherichia coli* (20.43%) and *Klebsiella* spp. (15.05%) remained the most common. The isolation rate for *E. coli* was comparable to reports from Somalia (19%) (Mohamed *et al.,* 2024), but lower than rates in Kenya (23.5%) (Simba et al., 2022) and Uganda (28.78%) (Johnson *et al.,* 2021). Our finding of a mixed infection involving both *Klebsiella* spp. and *E. coli* in a 23-year-old patient who experienced a miscarriage highlights the clinical severity of these infections. Polymicrobial Gram-negative infections can exacerbate uterine complications and increase the risk of adverse pregnancy outcomes (Delzell & Lefevre, 2000; Nicolle, 2008).

Age-stratified analysis revealed distinct clinical trends. The older pregnant cohort (31–43 years) showed a significantly higher infection rate (84.4%) compared to the younger cohort (63.2%). This statistically significant difference ($\chi^2 = 6.41$, $P = 0.0113$) strongly supports the hypothesis that maternal age and associated

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factors, such as multiparity, structural urinary modifications, and diminished immune responsiveness over time, actively exacerbate susceptibility to uropathogenic exploitation. The older group also showed an equal distribution between *E. coli* and *S. aureus*, and included rare pathogens like *Stenotrophomonas maltophilia*. This finding aligns with the association of *S. maltophilia* with opportunistic or healthcare-associated exposures in older populations (JoVE, 2023). Conversely, the younger cohort (17–30 years) showed a strong predominance of *S. aureus* (n=18), suggesting that age-specific behavior, physiological differences, or localized environmental exposures can influence infection patterns (Arab Center for Health Sciences Translation, 2023).

4.1. Clinical Implications and Resistance Phenotypes

The high prevalence of *Staphylococcus aureus* (29.03%) and *Escherichia coli* (20.43%) isolated in this obstetric cohort introduces significant challenges for empirical prenatal therapy. Globally, gestational antibiotic selection is restricted due to potential teratogenic risks, making localized monitoring of resistance profiles imperative (Mancuso et al., 2023). Although extensive standard disk diffusion assays (e.g., Kirby-Bauer method) are required to map out definitive multidrug-resistant (MDR) patterns in Al-Bayda city, the documented emergence of staphylococci necessitates caution. The potential risk of Methicillin-Resistant *Staphylococcus aureus* (MRSA) or Extended-Spectrum Beta-Lactamase (ESBL) producing *E. coli* strains in pregnant patients represents a severe threat that can exacerbate therapeutic failure, highlighting the critical need for constant susceptibility surveillance.

5. Limitations and Future Recommendations

While this study provides crucial, updated epidemiological data regarding gestational UTIs in Al-Bayda, certain limitations must be acknowledged. First, the identification of uropathogens was strictly reliant on classical phenotypic and biochemical assays. Molecular characterization methods, such as Polymerase Chain Reaction (PCR) and 16S rRNA sequencing for the precise tracking of resistant genes were not performed (e.g., *bla_CTX-M* or *mecA*), due

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to localized infrastructural constraints. Second, this investigation utilized a single-center cross-sectional design, which may not fully represent the broader longitudinal shifts in community-acquired asymptomatic bacteriuria across eastern Libya.

Future multi-center studies integrating automated identification systems (such as VITEK-2) and molecular genotypic assays are strongly recommended. Furthermore, routine institutional monitoring of antimicrobial susceptibility testing (AST) should be strictly maintained to establish updated, safe, and effective empirical prescribing guidelines for the pregnant population in the region.

6. Conclusion

This study demonstrates a high prevalence of urinary tract infections (70.45%) among pregnant women in Al-Bayda, Libya. It highlights a notable shift, with Gram-positive staphylococci (*S. aureus* and *S. epidermidis*) matching or exceeding the prevalence of classic Gram-negative enterobacteria (*E. coli* and *Klebsiella* spp.). The significantly higher infection rate in older pregnant women (84.4%, $P < 0.05$) emphasizes the need for targeted clinical screening. These findings suggest that routine urine culture testing should be mandatory during prenatal care at Al-Bayda Medical Center to ensure early diagnosis and improve maternal and neonatal health.

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