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Prevalence and antibiotic resistance patterns of uropathogenic bacteria isolated from urinary tract infected patients in northwestern Algeria

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Abstract

Urinary tract infections (UTIs) are the most common community-acquired and nosocomial infection, they represent high health concern due to the emergence of antibiotic-resistant uropathogens. This study aimed to investigate the prevalence of uropathogenic microorganisms isolated from UTIs in Tiaret, Algeria and to determine their antibiotic resistance patterns.

A total of 132 urine samples were analyzed, 62.11% among them were positive to UTI. Women were most susceptible with a sex ratio F/M of 1.5. We identified gender, age, diabetes and hospitalization as risk factors of UTIs. *Escherichia coli* (52.43%) was the most predominant uropathogen followed by *Enterococcus* spp. (25.6%), *Klebsiella* spp. (6.50%), *Staphylococcus aureus* (6.09%), *Proteus* spp. (3.65%), *Serratia marcescens* (2.43%), *Pseudomonas* spp (2.05%) and *Staphylococcus* spp. (1.25%).

Our results showed that 90% of the isolated strains of *E.coli* were resistant to amoxicillin, 80% to metronidazole and 75% to erythromycin. In *Enterococcus* spp. the resistance to amoxicillin, metronidazole and erythromycin was higher than 80%, a high rate of antibiotic resistance was also observed toward amoxicillin and metronidazole for the rest of strains. Gentamicin and amoxicillin – clavulanic acid were the most effective antibiotics against all the tested uropathogens.

This study highlight the high antibiotics resistance levels of uropathogenic microorganisms isolated from UTI, this is due to the inappropriate use of the antibiotic therapy which have to be managed in a rational manner.

Keywords: Urinary tract infection, antibiotic-resistance, uropathogens, prevalence.

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections worldwide; it is describe as an inflammatory infection caused by the colonization of uropathogens into the urinary tract (Odoki et al., 2019). It is estimated that 150 million UTIs occur every year worldwide, resulting in more than 6 billion US dollar in direct healthcare costs (Arslan et al., 2005). In Algeria, UTIs evoke a major healthcare concern with a heavy financial cost estimated of 340 euro to 4000 euro for a single infection (Graba 2010).

The most commonly encountered microorganisms causing UTIs are Gram negative bacteria represented by *Escherichia coli*, *Citrobacter spp.*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Klebsiella spp.* (Prakash et al., 2013). Gram positive bacteria such as *Staphylococcus saprophyticus* are also very frequently found on UTIs.

Uropathogens can produce toxins, siderophore and adhesins while colonizing the urinary tract, they can be transmitted by food, water, person to person contact and instrumentation. The symptoms are usually represented by a painful urination (dysuria), blooded urine (hematuria), pain in the back (backache), etc. (Khan et al., 2020). UTIs can be non-complicated when it leads to pyelonephritis end cystitis, or complicated when it is associated with anatomical (renal damage), functional, or metabolic abnormalities of the urinary tract (Lee et al., 2007). Moreover, catheter-associated UTIs have higher risk of mortality (Li et al., 2019). Generally, the overall incidence of UTIs requires often a rapid intervention that leads on prescription of empirical antibiotics treatments without referring to the antibiotic resistance patterns of the uropathogenic agent, evoking an alarming situation of drug resistance bacteria (Ait-Mimoune et al., 2022). Recently, multidrug resistance of bacteria is becoming an emerging and major public health problem due to the massive misuse of antibiotics resulting in failure of treatment or to complexities associated with chronic infection (Khan et al., 2020). Since 2019, the antibioresistance rates are booming very fast, as a large proportion of population infected with SARS-COV-2 were initially treated with antibiotics.

To date, there is unfortunately a great lack of data that outlines the factors associated with urinary tract infections and the antibiotics resistance patterns in Algeria. In this context, these study objectives were to assess the local prevalence of UTIs in Tiaret, Algeria, to determine as well the etiology and the antibiotics resistance profiles of bacteria isolated from UTIs. It is the first investigation of UTIs situation done in the area of study.

Methods

Data collection

The study was conducted at microbiology laboratories of two hospitals and a clinic in Tiaret, Algeria, over a period from January to April 2023.

132 midstream urine samples from patients presenting symptoms of urinary tract infection were collected in sterile urine containers. The data collection was made possible through questionnaires of every patient giving urine sample, and from previous data collected by the laboratories of the clinic and the two hospitals where this study was conducted.

Bacterial isolation and identification

Urine samples were inoculated on 3 different media: CHROMagar, Chapman and Hektoen followed by incubation of the agar plates aerobically at 37°C for 24h.

The cultured plates giving at least 10^5 colonies forming unit (UFC) per milliliter were considered positive for urinary tract infection. Morphological characters of colonies and bacterial cells were inspected by standard methods.

Gram staining was performed for all isolated strains. The identification of the isolates was based in the first time on the discriminatory growth of colonies on CHROMagar medium, and in the second time, by using Analytical Profile Index (API 20 E and API 20NE).

Antibiotics susceptibility determination

Antibacterial susceptibility testing was performed according to the recommendations of the CLSI guidelines (Wayne, 2010).

The uropathogenic isolated strains were tested for their susceptibility to antibiotics usually used in the study area to treat UTIs. For this, an antibiogram was performed on Muller-Hinton agar plates using discs diffusion method, the test discs included: Gentamicine (GEM, 10 UI), Amoxicilline-Clavulanate: Augmentin (AUG, 20/10 µg), Erythromocin (E, 15UI), Metronidazole (MET, 50µg) and Amoxicilline (AML, 25 µg). *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls.

Results

Epidemiological and clinical study

In this study, a total of 132 urine samples from patients suspected with UTIs were analyzed. The results showed that 62.11% of samples were positive to UTI, among them, 59.75 % were female patients against a rate of 40.24% presented by male patients. Moreover, the incidence of the UTI was higher in patients aged more than 50 years (33%) with feminine prevalence (62%) , followed by adults with age bracket between 16 to 30 years (30.48%). children (≤ 15 years) were the least affected with a percentage of 8.53% (Table 1).

Referring to the clinical cases of patients, our study demonstrates that 49.12% of positive samples were from outpatients with no history of disease, when 19.35% were diabetic outpatients. Furthermore, 25.08% of positive UTIs were rated in hospitalized patients, with an average of hospital stay ranged from 8 to 11 days, which highlight a nosocomial infection. Among them, 54.35 % and 39.12% were, respectively, indoor patient with urinary catheter and prostate disease. We found also that 6.15% of samples were from outpatients previously diagnosed with UTI with a failure of empirical antibiotherapy.

Table 1. Clinical characteristics of patients with UTI and risk factors (n=82).

	Number (n=82)	Percent (%)
Gender		
Female	49	59.75
Male	33	40.24
Age groups		
0-14	8	8.53
15-30	24	29.48
30 - 50	23	28.04
> 50	27	34
Clinical cases		
Out patients	40	49.12
Diabetes	15	19.35
Hospitalized patients	22	25.08
Catheters	11	54.35
Prostate diseases	8	39.12

Others	6	6.53
Previous declined antibiotherapy	5	6.15

Prevalence of uropathogens isolated from UTIs

In this work, isolation and identification of uropathogenics from urine samples were performed in order to determine the prevalence of causative agents of UTI.

Our results showed a predominance of *Escherichia coli* (52.43%) with higher frequency in females (51.16%) than in male (48.83%), followed by *Enterobacter spp.* (25.6%), *Klebsiella spp.* (6.50%), *Staphylococcus aureus* (6.09%), *Proteus spp.* (3.65%), *Serratia marcescens* (2.43%) and *Pseudomonas spp* (2.05%), negative-coagulase *Staphylococcus spp.* (1.25%). A significant prevalence of Gram negative bacteria were noted in our study as 90.2% compared with a rate of 9.8% of Gram positive bacteria. The prevalence of UTI causative bacteria according to gender are presented in Figure 1.

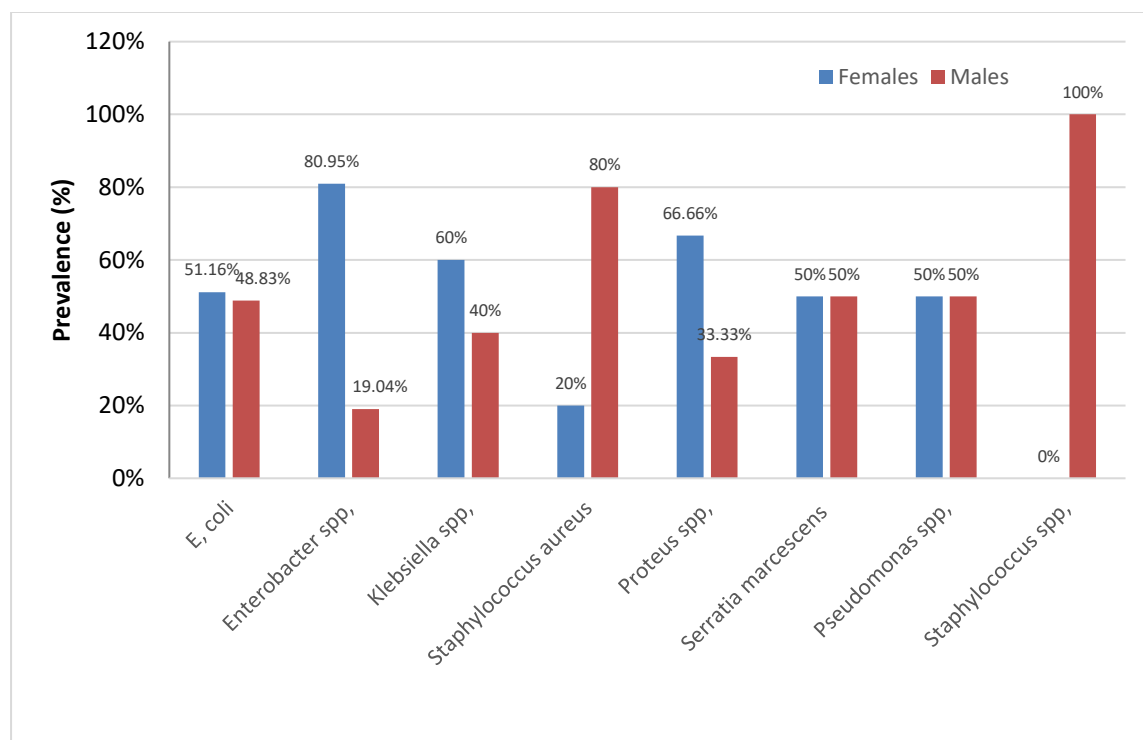


Figure 1. Prevalence and frequency of uropathogenics among positive UTI patients according to gender.

Antibiotic resistance patterns of the isolated uropathogens

Antibioresistance study was performed on uropathogens isolated by us while the other resistance data were collected from previous analyses carried out in two hospital laboratories and clinic laboratory during the year 2023.

A total of 215 uropathogenic *E.coli* isolated from UTIs were tested to antibiotic susceptibility. Our results showed a high rate of resistance (90%) to Amoxicillin, to Metronidazole (80%) and to Erythromycin (75%). However, Gentamycin was the only effective antibiotic against 81.40% of *E.coli* isolates.

Serratia marcescens was completely resistant to Metronidazole, Erythromycin and Amoxicillin (100%), and completely susceptible to Gentamycin and Amoxicillin/clavulanic acid (Augmentin). Among the 105 isolates of *Enterobacter spp.*, we observed a significant rate of resistance to Amoxicillin, Metronidazole and Erythromycin ($\geq 80\%$), while Gentamycin and Amoxicillin/Clavulanic acid (Augmentin) were very active against 80% and 70% respectively of the isolates.

Similarly, we observed that *S.aureus* isolates were only sensitive to Gentamycin (100%) and Amoxicillin/Clavulanic acid (Augmentin; 80%). They showed a high rate of resistance to Amoxicillin (100%), Metronidazole (100%) and Erythromycin (80%). Resistance rates of *E.coli*, *Enterobacter spp.* and *S. aureus* are shown in Figure 2.

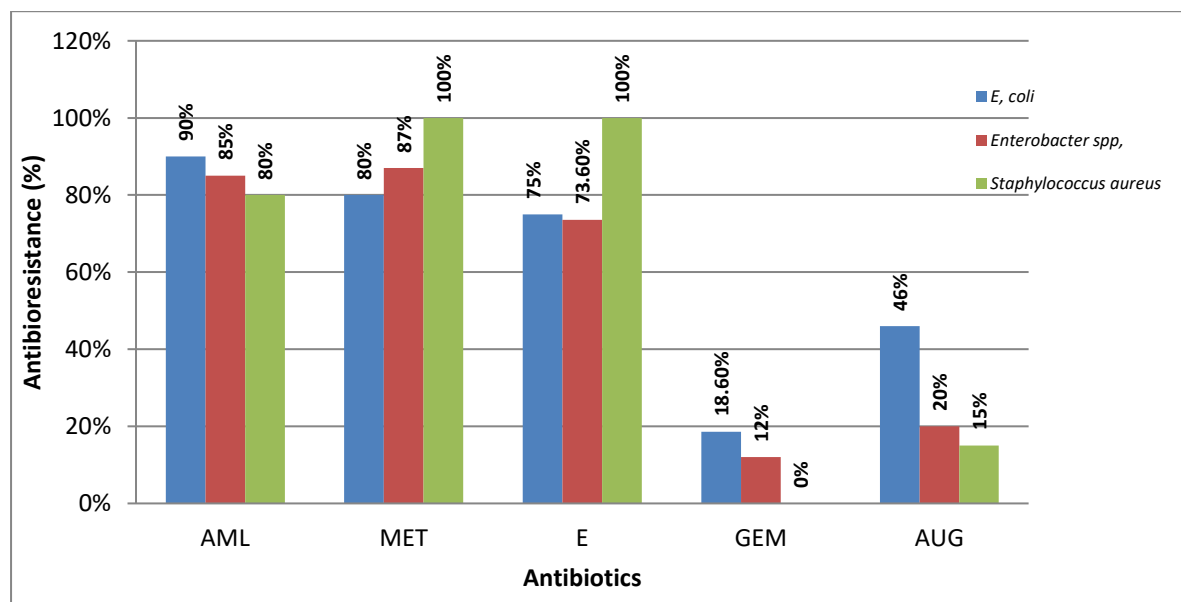


Figure 2. Antibiotic resistance rates of isolated uropathogenic strains from UTIs. AML: Amoxicillin, MET: Metronidazole, E: Erythromecine, GEM: Gentamycine and AUG: Augmentin. Results are expressed in terms of the percentage of resistance.

Discussion

In the present work we aimed to give a data of the urinary tract infections prevalence in Northwestern Algeria (Tiaret), to determine the etiology of infection and to analyze the antibiotic resistance patterns among the uropathogens.

Prevalence studies are effective tools to give data and to monitor the epidemiological situation in a region, from them; efficient solutions can be proposed and applied to control infections.

This study was performed on 132 urine samples of patients from 3 hospitals, results show that 62.11% of samples have given a growth positive urine cultures indicating UTI. This rate of infection was higher in comparison with previous studies in other regions in Algeria, 35.5% in Tizi-Ouzou (Ait-Mimoune et al., 2022), 13.15% in Algies (Aouf et al., 2018), 45.52% in Bechar (Benyagoub et al., 2021). Similarly, dramatic high rates of UTI were recorded in other countries, 73% in Bangladesh (Islam et al., 2022), 53.82% in Meerut City in india (Prakash et al., 2013), 40% in spain (Rodríguez-Baño et al., 2008). Recently, Zhu et al. (2021) reported that the incident cases of UTIs increased by 60.40% in 2019 compared with 1990 in a study that was carried out in 203 countries. The burden of UTIs was mainly concentrated in developing countries, particularly, in south Asia and tropical Latin America.

As expected, our results revealed that women were more affected by UTI (59.75 %) compared to men patients (40.24%) with a sex ratio 1.5 (F/M), this was the same ratio value reported by Mohammed et al. (2016) in Libya. Furthermore, our findings are similar to those reported in previous studies in Algeria (Aouf et al., 2018; Benyagoub et al., 2021; Ait-Mimoun et al., 2022). In fact, 10 to 35 % of women have an episode of urinary tract infection at least once in their lifetime (Zhu et al., 2021). A study from the USA reported by Kurtaran et al. (2010) estimated that one-third of women are affected by UTIs before 24 years, 10.8% of women aged over 18 years reported that they experienced UTIs at least once in the previous 12 months.

Furthermore, our results indicate that the incidence of UTIs increase markedly in women over 50 years of age, especially in postmenopausal period. Medina and Edgardo Castillo-Pino

(2019) have indicated that the prevalence of UTI increases with age, and in women aged over 65 approximately double the rate (20%) seen in the female population (11%) overall. The higher risk of UTIs occurrence in women is due to anatomical factors, in one hand, female urinary system have a short urethra measuring 5 cm in length and in the second hand, the proximity between the anus and the external urethral opening facilitates bacterial access to the bladder.

Beside gender and age, other risk factors are associated with UTIs; our results rated 19.35% of positive samples from diabetic patients. Actually, UTIs occur more commonly in patients with diabetes, and have more often a higher risk to develop subsequent complications and failure of antimicrobial treatments (Stapleton, 2002).

Moreover, our findings demonstrated that 25.08 % of positive samples were from hospitalized patients, the average of hospital stay before infection diagnosis was 8 to 11 days. These results are very similar to those reported by Amazian et al. (2010) from a multicentre study they carried in 27 hospitals in Algeria, Italy, Egypt, Morocco and Tunisia to evaluate the prevalence of nosocomial infections. They found that UTIs were the most common reaching a rate of 25.9% of all nosocomial infections contracted in the studied hospitals. This high prevalence can be explained by the patient length stay in hospitals leading to be exposed to multiple types of microbes, placement of invasive devices, previous heavy antibiotic therapy and admission in intensive care units (Nedjai et al., 2012).

Escherichia coli is the most common causative agent of UTIs around the world; it is responsible for about 64.5-82% of community acquired UTIs and about 50% of hospital acquired infections (Huang et al., 2014).

Our findings line with several other studies reporting that the most predominant causative uropathogen in UTIs was *E. coli* (Prakash et al., 2013; Aouf et al., 2018; Benyagoub et al., 2021; Ait-Mimoun et al., 2022), also, we found that the prevalence of *E. coli* isolates in Tiaret (52.43%) is higher than that reported by Ayad et al. (2016) in three hospitals of Oran, Tlemcen, and Sidi Bel Abbes (28%) situated in Northwestern Algeria (same geographical area of Tiaret).

The virulence of *E. coli* is related to its ability to adhere to epithelial cells using their fimbriae that interact with glycoprotein and glycolipid receptors on uroepithelium, same structures will prevent the bacterium from being flushed by urine (Lee et al., 2007). Biofilm formation also will make the bacterium more resistant to the immune system and to antibiotics.

We found that *Enterococcus spp.* was the most prevalent cause of UTIs after *E. coli* (25.6%), the bacterium share some characteristics with the *E. coli* and they are classified into the *Enterobacteriaceae* family, the identification of *Enterobacter* species can be very challenging due to the high identical phenotypical characteristics between species. Jain et al. (2021) have reported that *E. cloacae* was the most commonly isolated species (50%) from patients urine samples in their study, followed by *E. aerogenes* and *E. asburiae*.

Moreover, other Gram negative urophthogens were found causative agents in our study, *Klebsiella spp* (6.50%), *Proteus spp* (3.65%), *Serratia marcescens* (2.43%), and *Pseudomonas spp* (2.05%). Previous studies (Kurtaran et al., 2010; Nedjai et al., 2012; Odoki et al., 2019; Khan et al., 2020; Islam et al., 2022) have reported the implication of these bacteria in UTIs etiology but with different rates. It has been found that those bacteria were highly associated with nosocomial infections and with urinary infections due to catheter.

Antibiotic resistance is a global emergency which is receiving much political and international attention. The chaotic and the overuse of antibiotics are leading to the occurrence of more than 20,000 resistance genes listed in a current data-base (Chinmayee and Dalia, 2023). Hence, all researches, findings and data around the world indicate that the pre-antibiotic era is yet to come.

86 bacterial strains were isolated by us from patient's urine samples confirmed to have UTI, the strains were then tested for their antibiotics susceptibility. Also, we collected data of resistance patterns from tests performed on pre-isolated strains by the laboratories of the 3 hospitals where our study was carried out.

E. coli were the most common isolated strain from UTIs, 215 isolates were tested for antibiotics susceptibility, 63% from them were multidrug-resistant, a strong resistance were found for Amoxicillin, Metronidazole and Erythromecine (90%, 80 % and 75% respectively). Our findings are in agreement with those, previously reported in Algeria, by Kara et al. (2024) who noted high resistance rates of *E. coli* isolates towards Amoxicillin (86.04%), same with the results reported by Yahiaoui et al. (2015) finding that 96.8% of *E. coli* isolates were Amoxicillin-resistant. Similarly, *E. coli* isolates showed resistance to Metronidazole (80%) and to Erythromycin (75%). Antibiotic resistance in *E. coli* has an alarming increase worldwide since the germ is in constant evolution to develop high mechanisms of resistivity to the most used antibiotics around the world (Chinmayee and Dalia, 2023). Same resistance patterns were observed in our study in isolates of *Enterobacter spp* and *Serratia marcescens*,

this can be explained by their belonging to the *Enterobacteriaceae* family, and consequently, they can be involved in related pathologies leading clinicians to adopt the same therapy regimens. Our findings support the fact that our isolates are Extended-spectrum beta lactamases (ESBL) producing organism. ESBLs have the property of inactivating Penicillins, narrow- and broad-spectrum Cephalosporins, and Aztreonam, Quinolones, Trimethoprim-Sulfamethoxazole, and Aminoglycosides (Uzunovic-Kamberovic et al., 2006). ESBLs are frequently encountered as causatives of UTIs and reported in several studies worldwide. In Algeria, Nedjai et al. (2012), Gharout-Sait et al. (2012) and Baba Ahmed Kazi-Tani et al. (2014), reported the high incidence of ESBL organisms in UTIs founding that resistance to β -lactams were dominated by the production of ESBL CTX-M1, CTX-M-3, CTX-M-15 (>88%).

Further, although *S. aureus* accounts for only 0.5-6% of urinary tract infections (Muder et al., 2006), but the bacterium expressed a strong resistance to Amoxicillin and Metronidazole, our findings are in accordance with those reported by Alshomrani et al. (2023). Our study highlights the excessive use of these antibiotics in Algeria, even more; they are all current medications available often without prescription. Another fact has to be mentioned, is that the most UTIs are treated empirically, with broad spectrum antibiotics in most cases without referring to antimicrobial susceptibility testing.

However, Gentamicin and Augmentin revealed to be the most effective antibiotics against all the isolates, our results agree with previous published data of Haller et al. (2004), Bischoff et al. (2018), Khan et al. (2020) and Bousbia et al. (2022). Our results suggest that Gentamicin and Augmentin are likely the better choices to use for treatment of UTIs caused by multiply resistant bacteria in the studied area; however, they must be carefully managed due to the risk of significant side effects such as kidney and inner ear damage.

Conclusion

This study highlights the prevalence, etiology and antibiotics susceptibility patterns of urinary tract infection investigated in Tiaret, Algeria. Our findings demonstrate that females are most affected by the UTIs, age is the major risk factor and that *E. coli* remains the most commonly isolated pathogen followed by other uropathogens (*Enterococcus ssp*, *Klebsiella ssp*, *Staphylococcus aureus*, *Proteus ssp*, *Serratia marcescens* and *Pseudomonas ssp*)

The study also underscores the antibiotic resistance of UTIs causative bacteria, which was alarming, especially for common first line antibiotics such as Amoxicillin, Metronidazole and Erythromecin.

The observed patterns of resistance need urgent strategies, including the rational use of antibiotics, enhancement of diagnostic techniques, establishment of monitoring programs for prevalence and emergence of multidrug resistant uropathogens.

This study could be a reference containing data of the current situation of UTIs which is largely missing in the studied area, to effectively manage the infections and to prevent the spread of resistant uropathogens in the community and in health care settings.

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