

BACTERIAL ETIOLOGY AND THEIR ANTIBIOGRAMS IN COMMUNITY ACQUIRED URINARY TRACT INFECTIONS WITH SPECIAL CONSIDERATION ON STERILE PYURIA

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ABSTRACT

Urinary tract infections are caused by heterogenous etiological agents and high proportions of samples are without significant growth. Antibiotic resistance issue constitutes a major public health problem.

A retrospective study was conducted from a period of January, 2021 to May 2023 in which a total of 419 mid urine samples were collected. The ages were ranged from 4 months to more than 65 years. Adults were 348 (83.1%) and children were 71 (16.9%). Females were 358 (85.5%) and Males were 61 (14.5%).

From 419 samples 193 (46.3%) were normal (pus cells < 5/HPF), while 225 (53.7%) were abnormal (Pus cells >5/HPF). Among 225 abnormal samples, 100 (44.5%) showed no growth, while 125 (54.5%) yielded significant growth. Among males 21/123 (17%) of the samples showed growth compared to 102/123 (83%) in females. Among the isolated pathogens, *E. coli* was the predominant (71%) followed by *Staphylococcus aureus* (14%), *Streptococcus spp.* (6%), *Proteus spp.* (5%), *Pseudomonas aeruginosa* (3%) and *Klebsiella pneumoniae* (1%). High percentages of total UTIs and *E. coli* were found among age group 20-30 years. The highest percentage of resistant was against doxycycline (87.61%) followed by trimethoprim (86.66%), cephalothin (86.06%), ceftriaxone (60.97%), amoxycylav (57.02%), ciprofloxacin (41.22%) while the lowest resistant was against Amikacin (25.2%) followed by Gentamicin (34.14%), nitrofurantoin (37.39%).

KEYWORDS: Urinary Tract Infection, Bacteria, Antibiogram

1. INTRODUCTION

Urinary tract infections (UTIs) are the most frequently reported bacterial infection in the community after respiratory tract infections (Thass *et al.*, 2019). UTIs are heterogeneous with regard to their etiology, clinical manifestations, and disease course, which range from simple (e.g., urethritis and cystitis) to severe (e.g., pyelonephritis, bacteremia, and septic shock) (Behzadi *et al.*, 2005). Furthermore, the pathogenic microorganisms of UTI are various, with significant changes by years and differences by countries or regions (Tandogdu *et al.*, 2016). About 150 million people worldwide are affected by UTIs every year, spending about 6 billion US dollars (Djordjevic *et al.*, 2016). Globally, 404.61 million cases, 236,790 deaths, and 520,200 Daily-Disability life Adjusted (DALYs) were estimated in 2019 (Yang *et al.*, 2022). UTI occurs in all age in both genders but more common in females. About 50% of all females will experience at least an episode of UTI during their lifetime (Al-Badr *et al.*, 2013). UTIs can be nosocomial or community acquired which are more common but with unknown mechanisms of occurrence and

spreading (Djordjevic *et al.*, 2016). UTI may be occurred either symptomatic or asymptomatic and the symptoms are either acute or chronic and may be uncomplicated or complicated. The clinical manifestation of urinary tract infections depends on; causative agent, severity of the bacterial infection and the ability to immune response (Olowe *et al.*, 2015). UTIs are caused by heterogenous etiological agents but bacteria remain the major causative agents which are responsible for more than 95% of the infection (Bonadio *et al.*, 2001). Both gram negative and gram-positive bacteria are associated with UTIs. *E. coli* is the most frequent causative agent of UTIs. Other organisms reported include members of the family *Enterobacteriaceae* (i.e., *Klebsiella*, *Proteus*, *Citrobacter* and *Enterobacter spp.*), *Enterococcus species*, *Pseudomonas species*, *streptococci* and *staphylococci* (Mahato *et al.*, 2018). Antibiotic resistance in the treatment of UTI and other bacterial infections constitute a major public health problem especially in the developing countries. Irrational and indiscriminate use of antibiotics as well as fake and substandard drugs, including antibiotics is common in these countries (Arjunan *et al.*, 2010).

In view of these and attendant tendency for changes in bacteriological profile, it is worthwhile that the degree of susceptibility and resistance of these uropathogens to various antibiotics be known to clinicians for effective treatment of infections they cause and to avoid antibiotic misuse. However, in view of the increasing bacterial resistance, regular monitoring of resistance patterns is necessary to improve guidelines for empirical antibiotic therapy. The aims of the present study were to detect bacterial causes of UTI among children and adult men and women as well as to determine their antibiograms. Moreover, to find out the proportion of pyuria without growth and to give reasonable justification.

2. MATERIALS AND METHODS

2.1. Sample collection

A retrospective study was conducted from a period of 1st January, 2021 to end of May 2023 in which a total of 419 mid urine samples were collected from outpatients who referred by physicians for culture and sensitivity to the private medical laboratory at Nawroz medical settlement and hospital in Zakho independent administration-Kurdistan Region-Iraq. The ages were ranged from 4 months to more than 65 years. Adults were 348 (83.1%) and children were 71 (16.9%). Females were 358 (85.5%) and Males were 61 (14.5%). Patients who received antibiotics for at least 3 days were excluded. All samples were collected in sterile containers and cultured immediately and their general urine examination was performed to determine presence of pus cells. Samples with pus cells more than 5 per high power field considered abnormal, while those with less than 5 pus cells per high power field considered as normal urine sample.

2.2. Culture

Samples were immediately cultured on MacConkey and blood agars (Neogen, UK), incubated at 37°C for 24 hrs, then growth with colony forming units (CFU) more than 10^5 from adults and 10^3 from children considered as a significant bacteriuria and subjected to antimicrobial drug susceptibility test, while samples with few mixed growths or with CFU $<10^5$ for adults or $<10^3$ for

children were considered contamination or non-significant growth.

2.3. Antimicrobial susceptibility test

The antimicrobial susceptibility test was done on Mueller-Hinton agar (Himedia, India) using the Kirby-Bauer disc diffusion method and interpreted according to Clinical Laboratory Standard Institute (CLSI) guidelines. Nine different antimicrobial drugs were used and all were from Bioanalyse-Turkey: Doxycycline- Do 10µg, Trimethoprim-TMP 10, Amoxicillin/Clavulanic acid -AMC 30µg, Nitrofurantoin-F 100 µg, Ciprofloxacin-CIP 10 µg, Ceftriaxone - CRO 10 µg, Amikacin- AK 10 µg, Gentamicin- CN 10 µg, Cephalothin- KF10 µg.

2.4. Statistical analysis

Graph Pad Prism 8.1 software and Excel were used for statistical analysis. The Chi-square test and Two-way ANOVA have been performed to compare the significant differences between data, and $p < 0.05$ considered as a significant difference.

3. RESULTS

A total of 419 midstream urine samples were collected aseptically from outpatients who referred to culture and sensitivity with ages ranging from 4 months to more than 65 years. Males were 61 (14.5%) while females were 358 (85.5%). Out of 419 samples 123 (29.4%) showed growth and 296 (70.6%) without growth. From 419 samples 193 (46.3%) were normal (pus cells <5 /HPF), while 225 (53.7%) were abnormal (Pus cells >5 /HPF) as shown in figure 1. Among 225 abnormal samples, 100 (44.5%) showed no growth, while 125 (54.5%) yielded significant growth as shown in figure 2. No significant differences were found between abnormal urine with and without significant growth. Among males 21/ 123 (17%) of the samples showed growth compared to 102/123 (83%) in females as shown in figure 3. A significant association was detected between sex and growth. Among the isolated pathogens, *E. coli* was the predominant (71%) followed by *Staphylococcus aureus* (14%), *Streptococcus spp.* (6%), *Proteus spp.* (5%), *Pseudomonas aeruginosa* (3%) and *Klebsiella pneumoniae* (1%) as shown in figure 4.

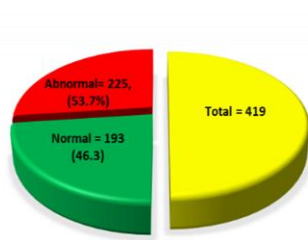


Fig.(1):- Total number with normal and abnormal

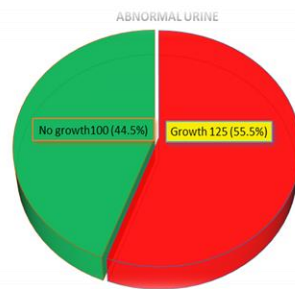


Fig. (2):- Results of abnormal urine

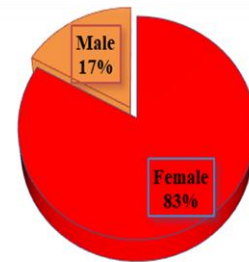


Fig.(3):- Results of growth according to the gender

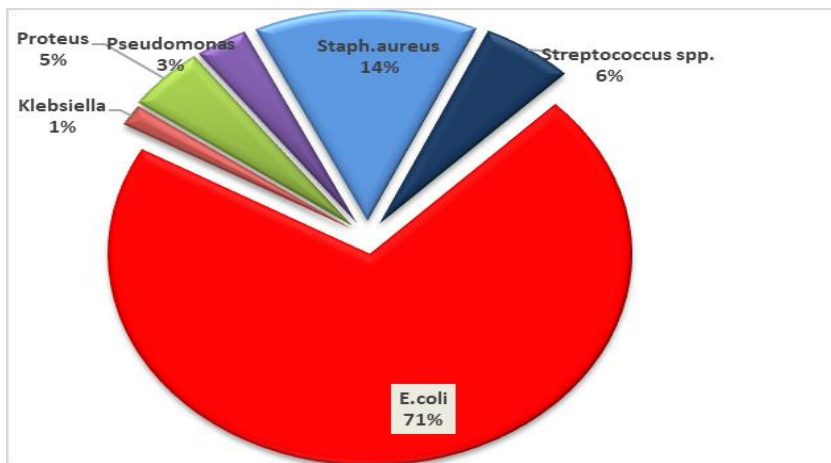


Fig.(4):- Frequency of isolated pathogens

According to the age groups, high percentages of total UTIs and *E. coli* were found among age group 20-30 years then declined in age groups 30-40 and 40-50 and raised in age group 50-60 years

as shown in figure 5. No such results were found with other detected causes of UTI with age groups.

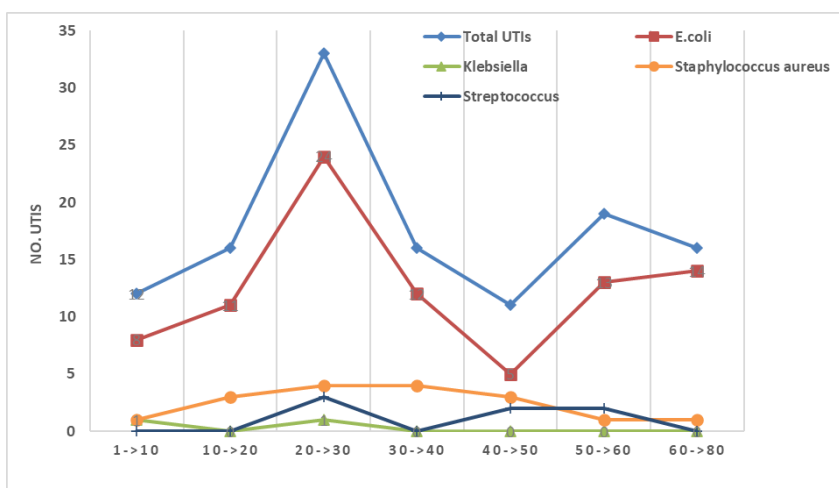


Fig.(5):- Distribution of total UTIs and causative agents with age groups

The antibiogram of the isolated causative agents against nine antibacterial drugs is shown in

figure 6. The highest percentage of resistant was against doxycycline (87.61%) followed by

trimethoprim (86.66%), cephalothin (86.06%), ceftriaxone (60.97%), amoxicillin/clavulanic acid (57.02%), ciprofloxacin (41.22%) while the lowest resistant was against Amikacin (25.2%)

followed by Gentamicin (34.14%), nitrofurantoin (37.39%). No single antimicrobial drug showed sensitivity to all isolated pathogens.

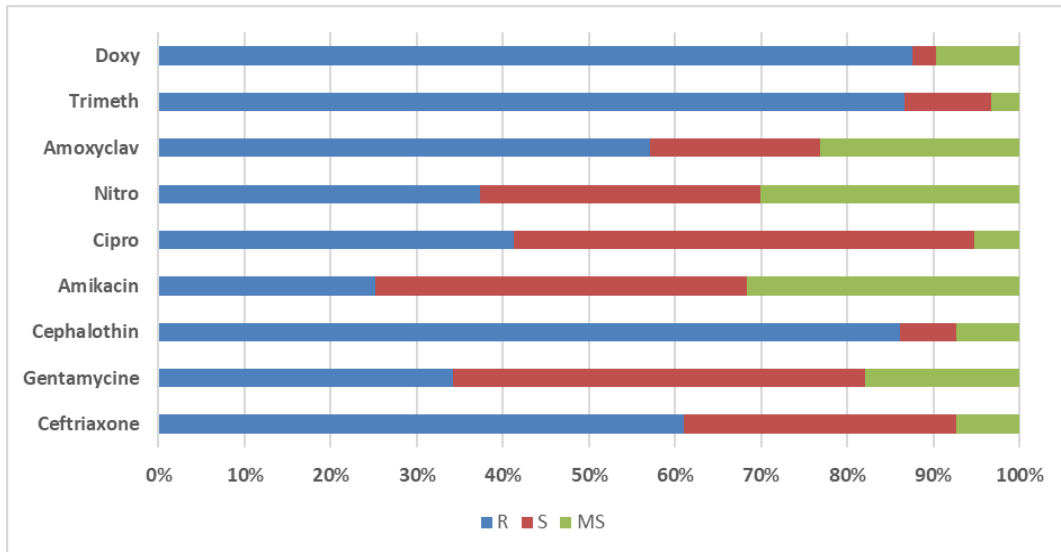


Fig.(6):- Antibiogram of overall isolated pathogens against antimicrobial drugs

The antibiogram profiles of 87 isolates of *E. coli* are shown in figure 7. The highest percentage of resistant was 93.1% for cephalothin followed by 83.9% for both doxycycline and trimethoprim, 66.6% for ceftriaxone and 65.5% for

amoxicillin/clavulanic acid, while the lowest resistant was 17.24% for amikacin followed by nitrofurantoin 24.1%, gentamicin 26.4% and ciprofloxacin 37.9%.

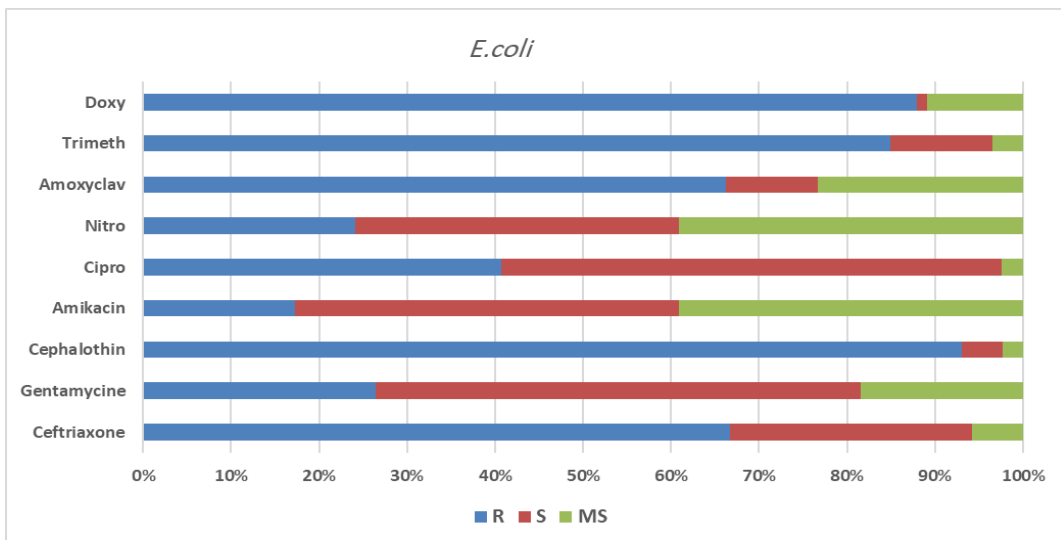


Fig.(7):- Antibiogram of *E. coli* isolates

The antibiogram of *Proteus* spp. isolates is shown in figure 8. The highest resistant was 100% for doxycycline followed by 83.33% for both trimethoprim and nitrofurantoin, 66.66% for

cephalothin and 50% for gentamicin, ceftriaxone and ciprofloxacin, while the lowest resistant was 20 % for amoxicillin/clavulanic acid and 33.3% for amikacin.

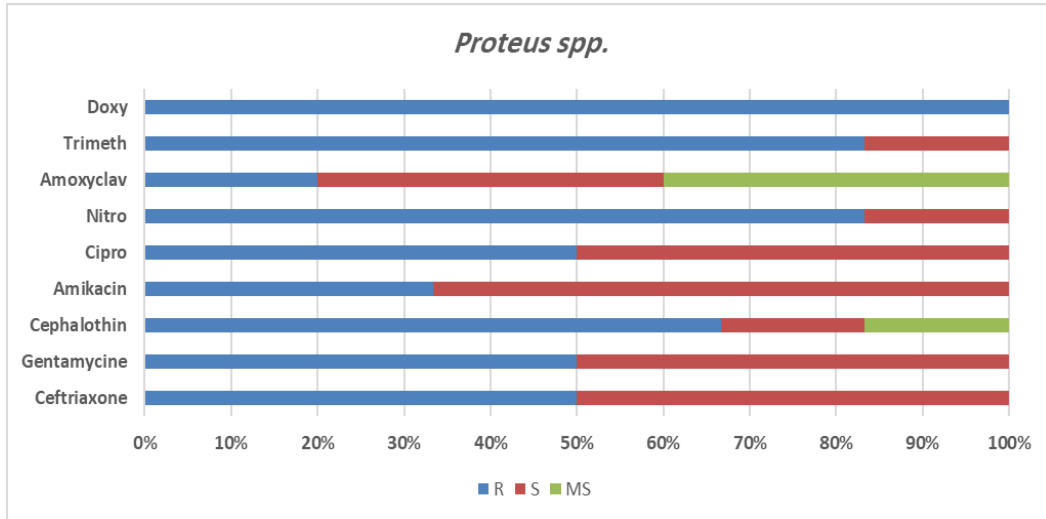


Fig.(8):- Antibiogram of *Proteus* spp.

The antibiogram of *Pseudomonas aeruginosa* isolates is shown in figure 9. The highest resistant was 75% for doxycycline, trimethoprim, amoxicillin/clavulanic acid, gentamicin and

ceftriaxone followed by 66.66% for cephalothin, 50% for nitrofurantoin, while the lowest resistant was 25% for both amikacin and ciprofloxacin.

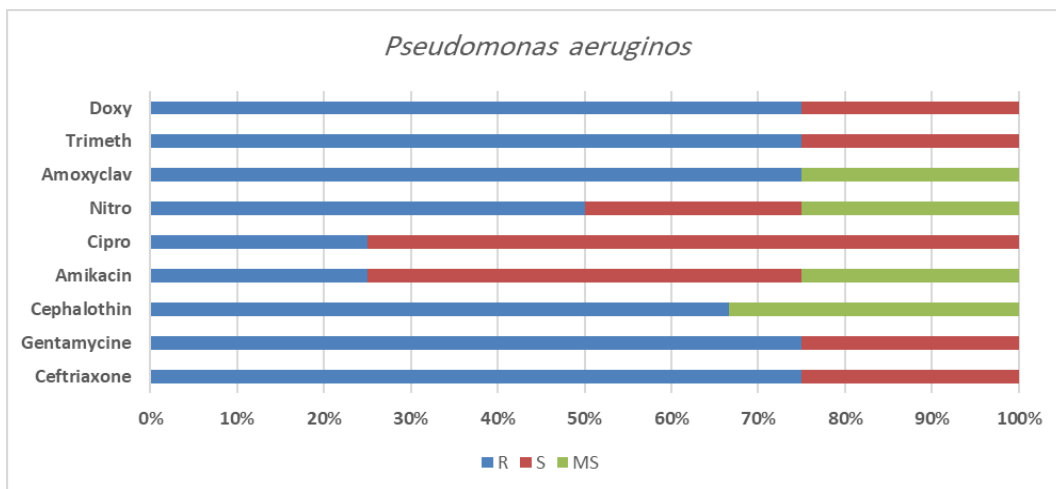


Fig.(9):- Antibiogram of *Pseudomonas*

The antibiogram of *Staphylococcus aureus* isolates is shown in figure 10. The highest resistant was 94.1% for trimethoprim followed by doxycycline 93.75% ,70.58% for both nitrofurantoin and cephalothin, 58.8% for

gentamicin, 52.94% for both amikacin and ceftriaxone and 50% for ciprofloxacin, while the lowest resistant was 35.29% against Amoxicillin/clavulanic acid.

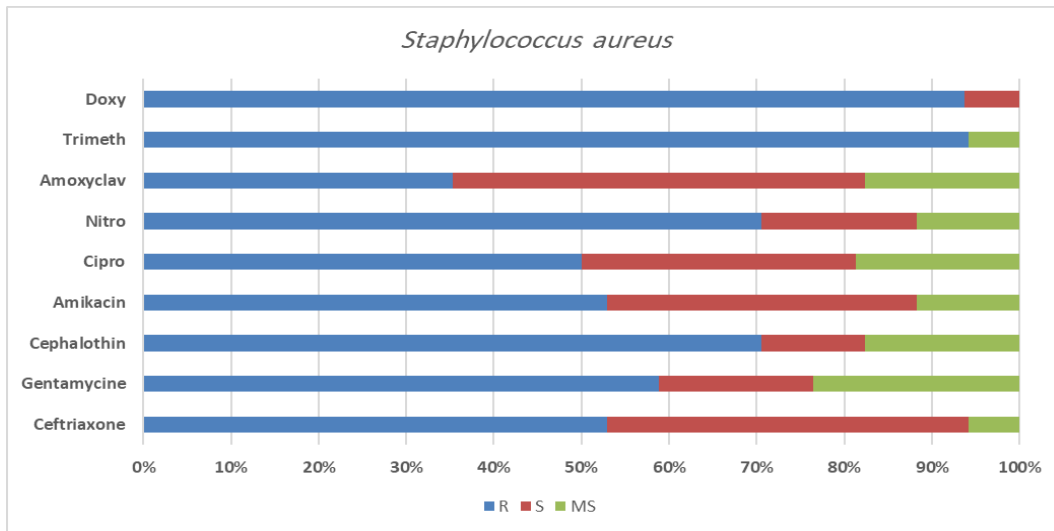


Fig.(10):- Antibiogram of *Pseudomonas aeruginosa*.

The antibiogram of *Streptococcus spp.* isolates is shown in figure 11. The highest resistant was 94.11% for trimethoprim followed by 93.75% for doxycycline, 70.58% for both nitrofurantoin and

cephalothin, 58.82% for gentamicin and 52.94% against both amikacin and ceftriaxone, while the lowest resistant was 35.29 % for amoxicillin/clavulanic acid.

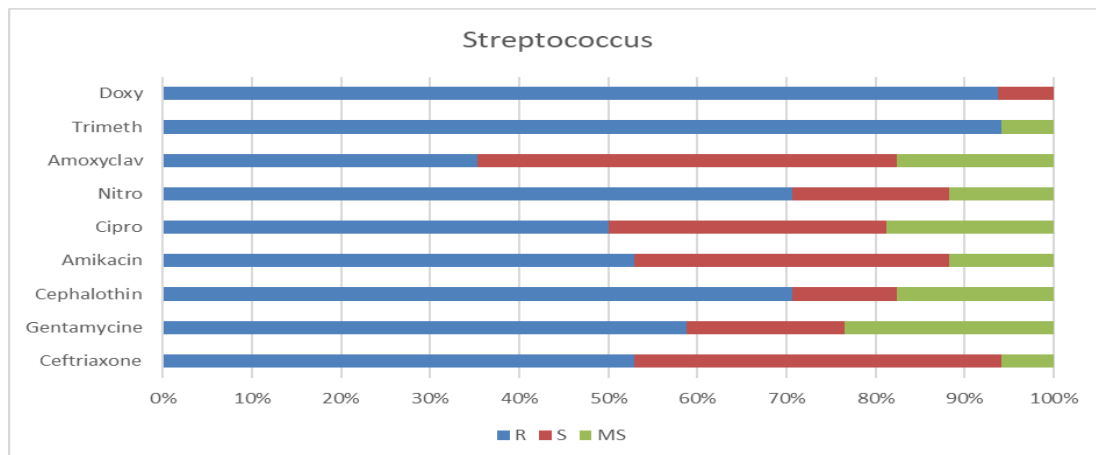


Fig.(11):- Antibiogram of *Streptococcus spp.*

1. DISCUSSION

The etiology and antimicrobial susceptibility properties of uropathogens have changed over time and place which will be continue in the future. Empirical antibiotic therapy is adapted by most physicians for treatment of UTI cases. However, the effectiveness of such treatment depends on the local antimicrobial resistance profiles. Most of the clinicians complaining from the results of laboratories in which a considerable percentage of samples showed no significant

growth in spite of pyuria which may make some laboratories to consider any non-significant growth as significant and perform antimicrobial sensitivity tests which eventually reflex adversely to the health of the patients and increase drug resistance. In the current study, 123 urine samples out of 419 (29.4%) yielded significant growth. This result agreed with the result of Dabobash *et al* (2017) from Libya who found 33.9% of urine samples gave significant growth, also with result of Adhikari *et al* (2019) from Nepal who found growth in 29.5%, Kavita *et al.* (2016) from India

found that 26.09% showed growth, Al-Gasha *et al* (2020) from Iraq found 26.68% yielded growth, Alkhateeb (2016) from Iraq found 31.9% showed growth. On the other hand, our results were lower than those found by Nahab *et al* (2022) in Iraq who found 86.0% of samples showed significant growth, Muhammed *et al* (2020) from Pakistan found 36.1% showed growth, Bhargava *et al* (2022) from India found 77.98% of urine samples showed significant growth, Osman (2019) in Erbil, Iraq found 80% showed growth, Oli *et al* (2017) from Nigeria found 89.1% yielded growth. This discrepancy in the results can be attributed to several factors such as study population whether samples from clinically suspected cases or from general individuals, methodology like manual versus automated machine, gender, ages, socio-economic status, residual drug effects and sampling process. In this study, among 225 urine samples with the number of pus cells more than 5 per HPF, 125 (54.5%) samples showed significant growth, while 100 samples (45.5%) without growth or with non-significant growth. These results were lower than those found by Oli *et al* (2017) from Nigeria who found that 89.1% urine samples from symptomatic women showed growth, while higher than by Muhammed *et al* (2020) from Pakistan who found that 36.1% of urine samples from clinically suspected UTI cases showed significant growth and from results of Saeed *et al* (2015) in Erbil, Iraq who found 43.4% urine samples from patients with signs and symptoms of UTI yielded growth. Explanation for these dissimilarities may be due to the study population, gender, age and methodology. In addition, high percentages of no growth in the presence of pyuria may be due to residual drug effect because there is no general consensus about duration of antibiotic abstinence and culture. Moreover, causes of sterile pyuria should be taken into consideration, because in routine culture, only rapid growing aerobic and facultative anaerobic bacterial causes were isolated, while slow growing, anaerobic bacteria and atypical bacteria like *Mycoplasma*, *Ureaplasma*, *Chlamydia* and *Mycobacterium tuberculosis* cannot be detected. Moreover, viruses and mechanical injury due to renal calculi are other causes of sterile pyuria. Although the spectrum of microorganisms isolated from subjects suffering from UTI is almost stable in which Gram-negative bacteria are more dominant than Gram positive bacteria and *E. coli* remains as the most

prevalent causative agent as found in the current study. However, certain recent studies carried out in Iraq, two from Duhok province (Ibrahim, 2019; Abduljabar and Hasan, 2023) and the other from Al-Diawaniya province (Al-Fatlawi and Jasim, 2022) found that Gram positive mainly *Staph.aureus* were the predominant uropathogens. These results are not reliable because other studies carried out in different Iraqi provinces had found that *E. coli* was the predominate uropathogen. In the current study, *Staph.aureus* was the second most commonly isolated uropathogen. This result was differed from the results of majority studies who found that *Klebsiella pneumoniae* was the second predominant pathogen (Kibret and Abera, 2014; Hussien *et al.*, 2018; Al-Gasha *et al.*, 2020; Muhammed *et al.*, 2020; Naqid *et al.*, 2020; Baloch *et al.*, 2022; Utami *et al.*, 2022; Ait-Mimoune *et al.*, 2022; Bhargava *et al.*, 2022), while similar to other who found *Staph.aureus* as a second most commonly isolated uropathogens (AlKhateeb, 2016; Oli *et al.*, 2017; Abdulrahman, 2018; Osman, 2019). Recently the role of *Staph.aureus* in causing UTI has been increased due to the increasing of the carrier stage among people in the area which is alarming signal. High percentages of total UTIs and *E. coli* infections were found in age group 20-30 year and 50-60 years. These findings are on line with those by Muhammed *et al* (2020), Al-Gasha *et al* (2020) who also found the majority of UTIs occurred in young aged patients 20-40 years as well as in older patients 50-60 years. The highest prevalence of UTIs in young aged patients could be due increased sexual activity, while in older aged women, the vaginal normal flora will decrease and allow Gram negative bacteria to grow as uropathogens (Muhammed *et al.*, 2020). The issue of antibiotic resistance is a major public health problem especially in the developing countries due to inappropriate and misuse of antibiotics. The antibiogram of the uropathogens changing over time, therefore periodic checking is necessary to provide update data for empirical therapy. High resistance rate of overall isolated uropathogens was detected against doxycycline (87.61%), trimethoprim (86.66%), cephalothin (86.06%), ceftriaxone (60.97%), amoxyclav (57.02%), ciprofloxacin (41.22%) while the lowest resistant was against amikacin (25.2%) followed by gentamicin (34.14%), nitrofurantoin (37.39%). No single antimicrobial drug showed

sensitivity to all isolated pathogens. These results clearly indicate to the prevalence of highly drug resistance bacteria in our area due to misuse of drugs and easily accessible from the pharmacy without physician prescription. In the current study 66.6% and 65.5% of *E.coli* isolates were resistance to ceftriaxone and amoxicillin/clavulanic acid respectively. Similar to the results of present study were recorded by Hussein *et al* (2018) in Duhok, Iraq who found that 75% and 65% of *E. coli* isolates were resistance to amoxicillin/clavulanic acid and ceftriaxone respectively. On the other hand, the lowest resistance was for amikacin (17.24%), Nitrofurantoin (24.1%) and gentamicin (26.4%). Similar to our results were also recorded by Kibret and Abera (2014) who found the lowest resistance was against nitrofurantoin and gentamicin as well as Osman (2019) found the lowest resistance was against amikacin and nitrofurantoin. The resistance pattern of *Proteus spp.* was different from that of *E. coli* which showed highest resistance (100%) to doxycycline followed by 83.33% for both trimethoprim and nitrofurantoin, 66.66% for cephalothin and 50% for gentamicin, ceftriaxone and ciprofloxacin, while the lowest resistant was 20 % for amoxicillin/ clavulanic acid and 33.3% for amikacin. Similar results were also found by Hussein *et al* (2018) in Duhok, Iraq who found the highest resistance was for gentamicin, nitrofurantoin and ceftriaxone, while the lowest resistance was against amikacin but disagreed with ciprofloxacin who found the lowest resistance 25% with ciprofloxacin in contrast to 50% in current study. The resistance pattern of *Pseudomonas aeruginosa* was 75% for doxycycline, trimethoprim, amoxicillin/clavulanic acid, gentamicin and ceftriaxone followed by 66.66% for cephalothin, 50% for nitrofurantoin, while the lowest resistant was 25% for both amikacin and ciprofloxacin. The results of current study showed increased in the resistance pattern of *Pseudomonas aeruginosa* when compared to the results recorded by Hussein *et al* (2018) in the same area.

The resistance profile of *Staphylococcus aureus* was 94.1% for trimethoprim, 93.75% for doxycycline, 70.58% for both nitrofurantoin and cephalothin, 58.8% for gentamicin, 52.94% for both amikacin and ceftriaxone and 50% for ciprofloxacin, while the lowest resistant was

35.29% against amoxicillin/clavulanic acid. These results were dissimilar to that by Hussein *et al* (2018) in the same area who found that no *Staphylococcus* isolates were resistant to amikacin, gentamicin, while 100% were resistance to amoxicillin/clavulanic acid and ciprofloxacin. The most reasonable explanation may be the low number of isolates or undetermined species of *Staphylococci* which differ in their antibiogram profiles or emergence of drug resistance strains.

The resistance pattern of *Streptococcus spp.* was 94.11% for trimethoprim followed by 93.75% for doxycycline, 70.58% for both nitrofurantoin and cephalothin, 58.82% for gentamicin and 52.94% against both amikacin and ceftriaxone, while the lowest resistant was 35.29 % for amoxicillin/clavulanic acid. Similar to our results were also found by Osman (2019) in Erbil, Iraq. Different species of *Streptococci* are causing UTIs with variable resistance profiles which demands more attention and studies.

4. CONCLUSION

High percentage rate of no growth was observed among urine samples with pyuria. High infection rate was found among females than males. High isolation rate was recorded among young females in reproductive age group as well as in older females. The most common causative agent was *E.coli* followed by *Staph.aureus*. Alarming resistance rates were observed toward commonly used antibiotics. Other bacterial causes of sterile pyuria should be investigated. Continuous surveillance of antimicrobial susceptibility pattern helps in initiating the empirical therapy of UTI. More studies are required to find out the minimum post antibiotic duration for isolation of uropathogens.

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