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ETIOLOGY AND DRUG SENSITIVITY PATTRENS OF URINARY TRACT PATHOGENS ISOLATED FROM DISTRICT MANSHERA AND USE OF BACTERIOPHAGE THERAPY

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Abstract

Among the most often acquired bacterial illnesses in hospitals and the community are urinary tract infections. While cystitis (bladder infection) is infection of the lower urinary tract, pyelonephritis kidney infection is infection of the upper urinary tract. The cystitis symptoms include pain with urination and frequent urine. Apart from the cystitis symptoms, pyelonephritis also causes fever and flank pain. In female than in male urinary tract infections are more common. About 10% of women have a urinary tract infection every year; more than half have one in their lifetime; 2 to 8% of children under the age of 10 years have one as well. In frequent urinary tract infections, gram negative bacteria

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are the primary cause. Urinary tract infections also result from less often gram positive bacteria.The urinary tract infection gets complicated due to emergence of antibiotic resistance bacteria. The present study was aimed to isolate and identify the commonly encountered pathogens causing UTI in district Mansehra. Furthermore, antibiotic resistance pattern was determined and previously isolated and characterized bacteriophages were also applied to test their in vitro efficacy against the resistant bacterial strains. The samples were collected from patients of different age and sex at King Abdullah hospital, Mansehra medical complex, and Regional health center Shankyari Mansehra inside sterilized containers. Biochemical tests and Gram staining helped confirm the infections. Five distinct uropathogens were recovered from 920 samples using the Kirby-Bauer method on MHA media 740 samples were positive for E. coli, 55 for Pseudomonas aeruginosa, 84 for Staphylococcus aureus, and 41 for E. faecalis. Urinary tract infections were common in women as opposed to men. UTIs were more common in ages greater than 20 years (32.60%), ages less than 60 years (53.36%), and ages greater than 60 years (4.56%). Our research reveals that E. coli is the primary causal agent. It is an MDR pathogen so I can apply bacteriophage treatment against it.

INTRODUCTION

A urinary tract infection is a type of illness that specifically targets the urinary tract [1]. Pyelonephritis is the Upper Urinary System illness, specifically the kidneys, whereas cystitis refers to the infection of the lower urinary tract, specifically the bladder.[2]The signs of cystitis infection include urinary urgency with an empty bladder, dysuria, and frequent urination.[1] Pyelonephritis is characterised by the presence of fever and flank discomfort, together with the symptoms of cystitis.[3]The signs of cystitis infection include urinary urgency with an empty bladder, dysuria, and frequent urination.[4] Pyelonephritis is characterised by the presence of fever and flank discomfort, together with the symptoms of cystitis.[4]Urinary tract infection is more prevalent in females than males.[5]Approximately 10% of females encounter a urinary tract infection (UTI) each year, while over 50% will experience at least one UTI in their lifetime. Among children under the age of 10,

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the prevalence of UTIs ranges from 2 to 8%.[6] Gram-negative bacteria are the primary etiological agents responsible for frequent urinary tract infections.Occasionally, gram-positive bacteria, such as *Staphylococcus saprophyticus*, can also lead to urinary tract infections (UTIs). In younger females, this particular bacteria is responsible for 5 to 15% of UTI cases.The primary aetiology of urinary tract infection is Escherichia coli, which accounts for around 80 to 85% of urinary tract infections acquired in the community.The pathogenic strain of *E. coli*, known as UPEC, is responsible for over 90% of urinary tract infections.[7] Healthcare-associated urinary tract infections, primarily caused by urinary catheterization, involve a diverse range of bacteria, including *E. coli* (27%), *Pseudomonas* (11%), *Klebsiella* (11%), *enterococcus* (7%), and *Candida albicans* (9%). [8] In young children, fever may manifest as the sole symptom of a urinary tract infection, whereas in the elderly, symptoms such as weariness and alterations in mental well-being may indicate a UTI.[9]The management of urinary tract infections is determined by factors such as the patient's age, gender, causative agent, underlying medical condition, and whether the illness is cystitis or pyelonephritis.[10] In the case of an uncomplicated urinary tract infection, a shorter course of antibiotics such nitrofurantoin or sulfamethoxazole is required, however in the case of a complex urinary tract infection, a longer course of antibiotics is required. [11] Bacteriophage treatment is the use of bacteriophages that can infect and eliminate certain harmful bacteria when they come into contact with them. Bacteriophages attach to bacteria (host species), insert their DNA, use the host cell's machinery to make more of themselves, and then break down the bacteria, releasing virion progeny and starting the cycle over again. So, bacteriophages are different from other antibiotics because they can increase their numbers when they come into contact with certain types of bacteria. Bacteriophages are reasonably affordable compared to the last generation of antibiotics, and medical professionals can give the treatment using a catheter for intravesical instillation numerous times a day. In addition, bacteriophage therapy for UTIs will only be given locally and not as a systemic treatment. We looked into how bacteriophage therapy affects E. coli and bacteria such as K. pneumoniae strains that were found in the urine of people with urinary tract infections. This was because urinary tract infections

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are very common, antibiotic resistance is on the rise around the world, and there is renewed interest in using bacteriophage therapy in the Western world.

MATERIALS AND METHODS

A research project was planned in September 2024, and many sites were selected. Using conventional microbiological techniques including King Abdullah hospital, Mansehra medical complex, and Regional health center Shankyari Mansehra, the 920 urine samples were gathered from various patients from district Manshera for isolation of urinary tract pathogens. Different cultures medium were utilized for the development of uropathogens. All the samples were cultured on Cysteine Lactose Electrolyte Deficient media (CLED). It is a valuable and growth inducing medium which is used for the identification and differentiation of microorganisms that cause urinary tract infections. It contains cysteine and lactose and is electrolyte deficient which prevents the swarming of proteus species and Macconkey agar.

The plates were incubated for 18 to 24 hours at 34°C under aerobic conditions. After 18 to 24 hours of incubation, colonies were counted and processed for identification e.g., morphology and different biochemical tests.

The isolated pathogens were then tested against commonly used antibiotics for sensitivity patterns using Kirby Bauer method. 3-5 isolated colonies were added to the normal saline to make bacterial suspension. Lawn was prepared on Muller Hinton media (nutrient media) on a sterilized cotton swab and then 4 antibiotics disk were applied on each plate and incubated for about 18-20 hours. After incubation for 18-20 hours the zones of inhibition were measured according to the clinical and laboratory standard institute (CLSI).

For antimicrobial susceptibility testing (AST) the discs used were Amoxicillin (5mg), Ampicillin (10mg), Gentamicin (30µg), Erythromycin (30µg), Tobramycin (30mg), Cefataxime (5 µg), Ciprofloxacin (5 µg), Vancomycin (10µg).

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Results

From 920 clinical samples in all, four distinct pathogenic bacterial strains 80.43% *E. coli*, 9.13% *Staphylococcus aureus*, 5.97% *pseudomonas aeruginosa*, and 4.45% *E. faecalis* were identified. **Table 1and figure 2** shows that female rather than male urinary tract infections were more common. **Table 2 and figure 2** shows a higher frequency of urinary tract infections between the ages of 21–40 and 41–60.

Table1. Frequency distribution of isolated uropathogens.

Uropathogen	Male	Female	Total
E. coli	246	494	740
Pseudomonas aeruginosa	10	45	55
Staphylococcus aureus	40	44	84
E.faecalis	14	27	41
Total	310	610	920

Table2. Frequency distribution of Uropathogen in different age groups.

Age group (years)	*E. Coli*	*P.aeruginosa*	*S.aureus*	*E.faecalis*	Total
1-20	65	00	08	05	78
21-40	228	19	23	30	300
41-60	418	26	43	04	491
61-80	22	08	10	02	42
80>	07	02	0	00	09
					920

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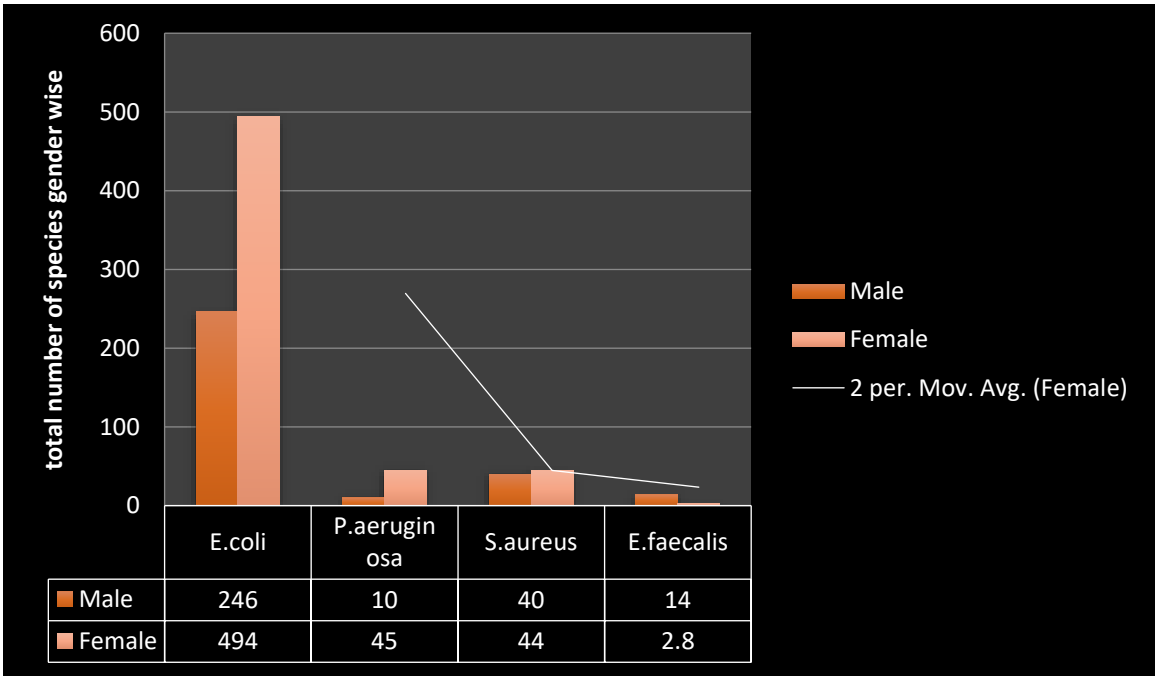

Figure1. Show the comparative analysis of UTI between female and male. Female have high number of positive cases then Male Trend line show the Avg. (female).

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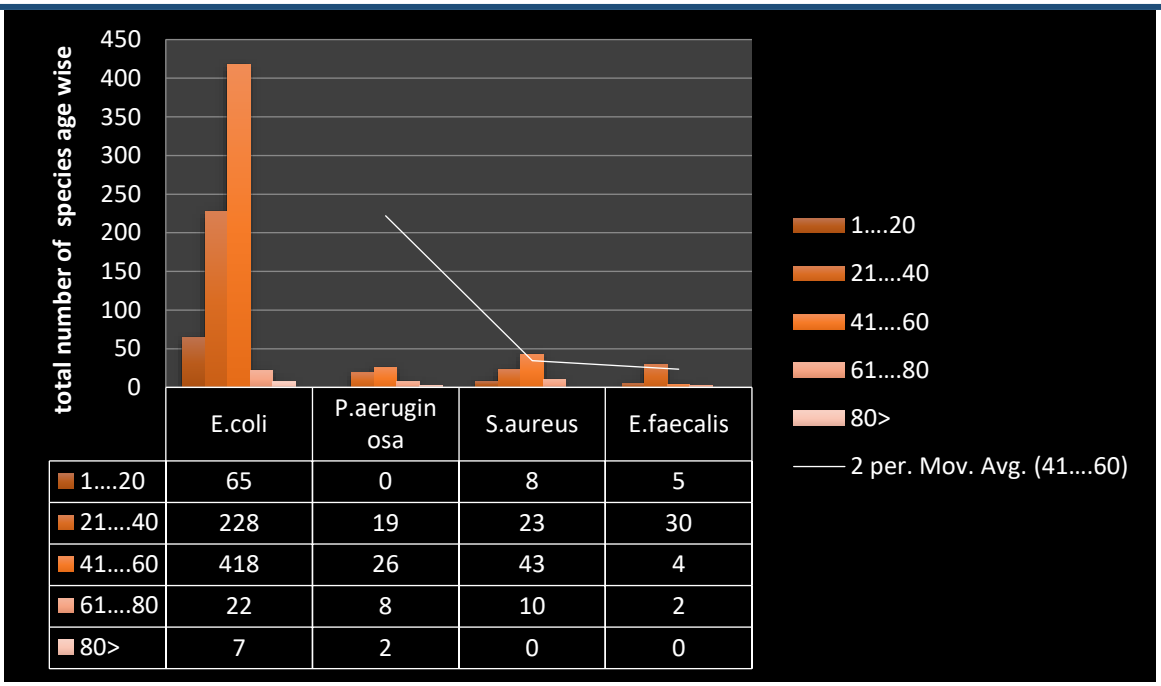

	E.coli	P.aerugin osa	S.aureus	E.faecalis
1....20	65	0	8	5
21....40	228	19	23	30
41....60	418	26	43	4
61....80	22	8	10	2
80>	7	2	0	0

Figure2. Show the comparative analysis of UTI between different ages. Most positive cases are ages greater than 20 years (32.60%) and less ages than 60 years old (53.36%).

Among the investigated antibiotics, ampicillin, amoxicillin, tobramycin, ciprofloxacin, and cefotaxime displayed the best resistance for Gram-negative bacteria. Erythromycin, ciprofloxacin, and gentamicin exhibited the Gram-positive bacterial greatest resistance. The most often used isolate, *E. coli*, offered great resistance to ciprofloxacin (95.2%), gentamicin (80.4%), ampicillin (99.0%), and amoxicillin (98.5%), With great resistance to erythromycin (99.2%), ciprofloxacin (98.5%), gentamicin (79.1%), and vancomycin(88.2), *Staphylococcus aureus*, the second most identified pathogen, revealed Third most isolated organism, *Pseudomonas aeruginosa*, demonstrated great resistance to gentamicin (99.2%), cefotaxime (98.5%), tobramycin (96.4%), and ciprofloxacin (95.3%). Fourth most isolated organism *Enterococcus faecalis* exhibited strong resistance to

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ciprofloxacin (99.2%), amoxicillin (98.5%), ampicillin (99.2%), and gentamicin (98.0%). As **Table 3 and Table 4** both illustrate. **Figure 3, 4 and 5.**

Table3. Antibiotic resistance pattern of gram negative urinary tract pathogen.

Antibiotic	P.aeruginosa(R)%	E.coli(R)%
Ampicillin	~~	99.0
Amoxicillin	~~	98.5
Tobramycin	96.4	~~
Ciprofloxacin	95.3	95.2
Cefataxime	98.5	~~
Gentamicin	99.2	80.2

Table4. Antibiotic resistance pattern of gram positive urinary tract pathogen.

Antibiotic	S.aeurus(R)%	E.faecalis(R)%
Ciprofloxacin	98.5	99.2
Amoxicillin	~~	98.5
Ampicillin	~~	99.2
Gentamicin	79.1	98.0
Erythromycin	99.2	~~
Vancomycin	88.2	~~

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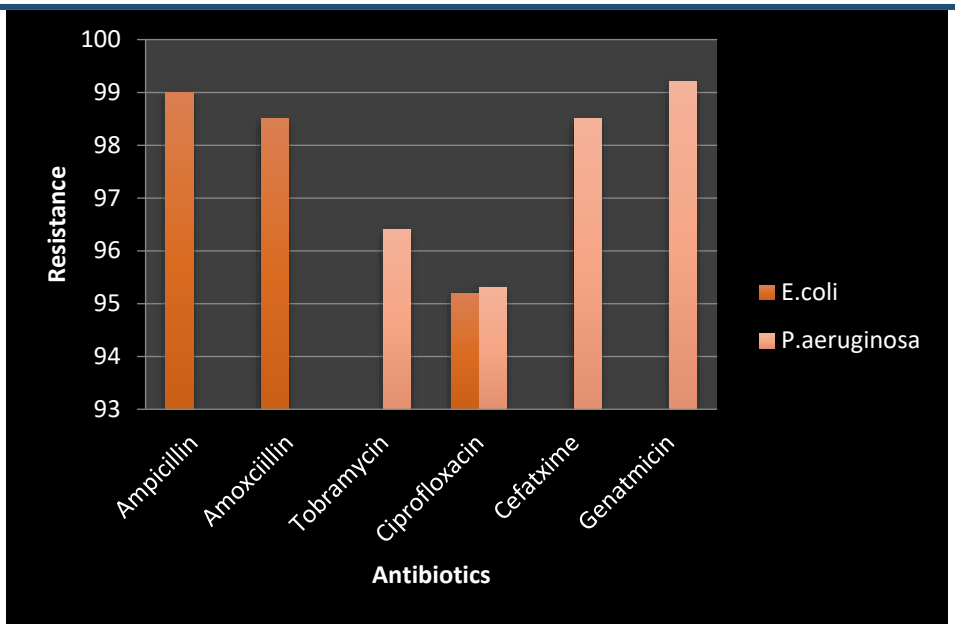

Figure3. Comparative analysis of Antibiotics profiling Gram negative pathogens

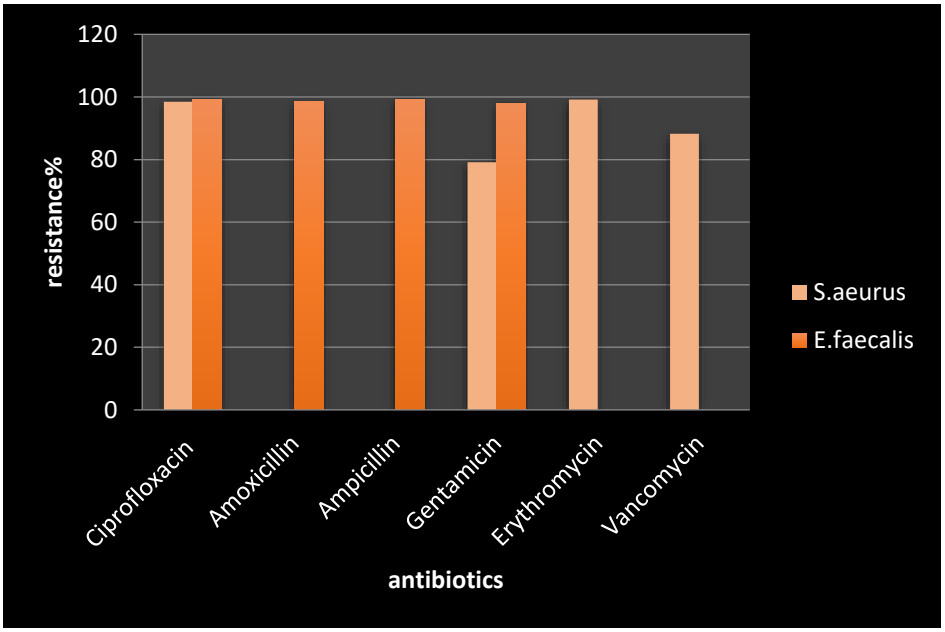

Figure4. Comparative analysis of Antibiotic profiling gram positive pathogens

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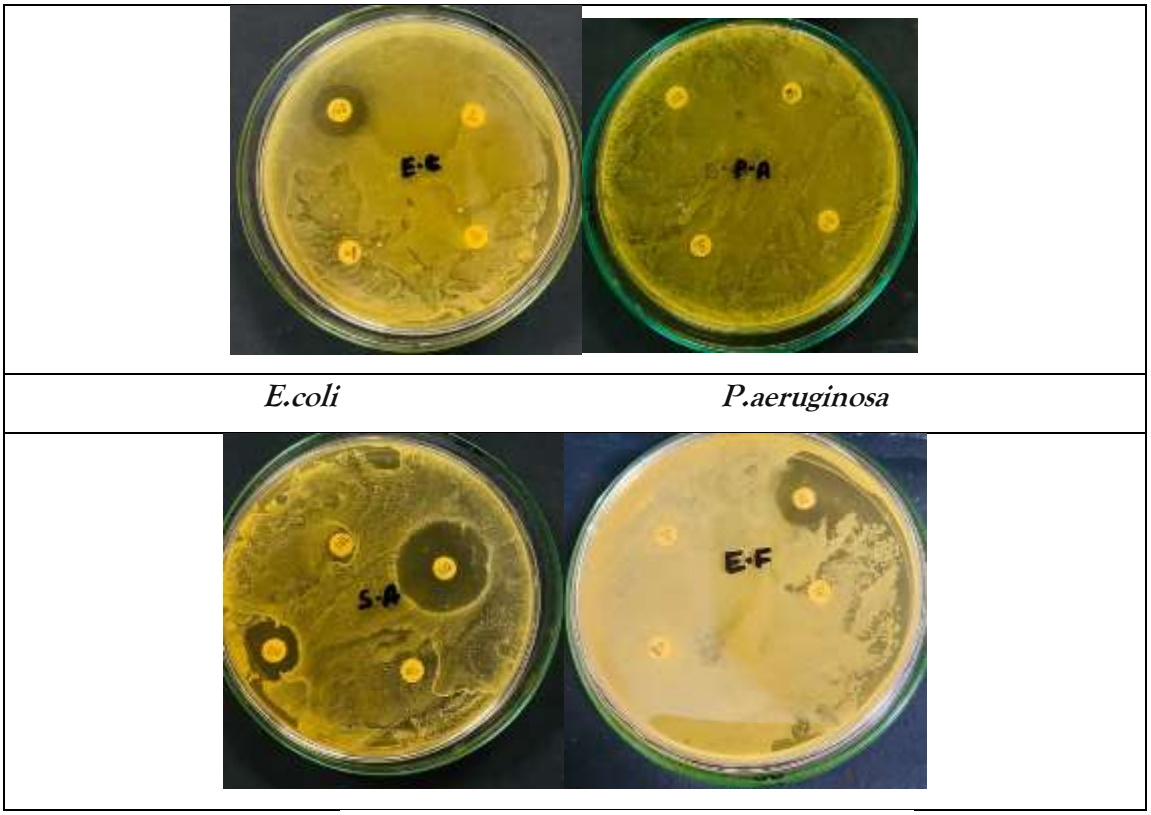

Figure5. show the result of isolated bacterial strain antibiotics profiling.

E. coli is the most often occurring isolated and primary causal agent found in our work. we thus can treat an MDR strain of coli with phage treatment. Using several isolated lytic phage samples against *E. coli* strains, F4, H1, A1, A2, M4, M2, and M1, This work presents a spot assay for phage treatment against E. coli results displayed in **figure 6.**

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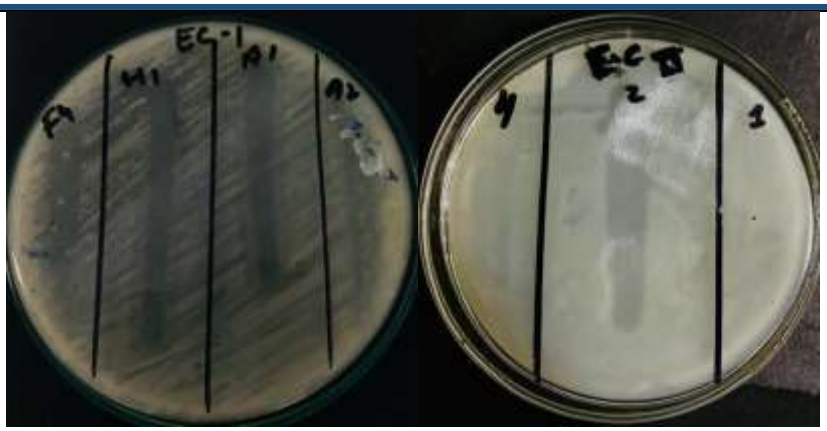

Figure6. Show the result of different isolated bacteriophages against different *E.coli* strains

Conclusion

Our analysis reveals that female urinary tract infections are more common than male ones. In both male and female urinary tract infections, female patients having positive UTI are 494 (53.69%) and Male positive UTI patients are 246 (26.73%), E. coli is the most common bacterium implicated (80.34%). While gram positive displays most resistance towards Erthromycin, Ciprofloxacin, Genatamicin, gram negative pathogens show highest resistance towards Ampicilin, Amoxcilin, Tobramycin, Cepataxime. . In our research study, phages and their derivatives were reported as an alternative strategy for the treatment of drug-resistant Urinary tract pathogen *E.coli*.

Discussion

Different species of bacteria include *E. coli, Klebsiella pneumonia, porteus, S. aureus, pseudomonas* and so forth cause urinary tract infections. Urinary tract infections can be simple and complex, upper and lower. The most often occurring bacteria in the urinary tract infection are gram negative Uropathogens. Men have less urinary tract infections than women. Comparatively to male (8.8%), UTIs were far more common among female (30.6%). Female also were 3.4 times more likely than male [12]. Our analysis reveals that 53.69% of female and 26.73% of male urinary tract infections

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were more common in female than male. Patients above 20 years and less 60 years have a greater urinary tract infection ratio—41.8%. This is in line with past studies where it was more in 33% of the same aged individuals. [13].

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