

A Case Study on Antibiotic Resistance against Urinary Tract Infectious Cultures

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ABSTRACT

Back ground selection of empire antibiotics for urinary tract infections (UTI) has become more challenging because of the increasing rates of multi drug resistant infectious cultures. This respective study objective was to determine antibiotic resistance patterns, risk factors and appropriate empiric antibiotic selection for multi-drug resistant Urinary tract infectious cultures. Urinary tract infections are common among the female populations. Adult patients seen in emergency department with gram negative and gram positive infections , which are resistant to at least 5 different categories of antibiotics. This study concluded the antimicrobial activity of antibiotics against 6 different infectious and normal urine samples. The female sample shows more resistance towards antibiotics than the male samples. Among them the samples of diabetic patients show more resistance towards combined and mono therapy. In case of mono therapy Nitrofurantoin show more growth inhibition zone than Norfloxacin. As the concentration of drug increases the variation in growth inhibition is not varied much. We found 25% drug resistance against normal and 85% against abnormal samples due to different risk factors such as mutations, drug resistant plasmids and different physical pressures etc. So we concluded that the UTI therapy must be changed to protect the patients from fatal conditions.

Keywords: Antibiotics, drug resistance, gram positive, gram negative, infectious cultures, urine samples

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I. INTRODUCTION

A Urinary tract infection (UTI) is an infection involving the kidneys, ureters, bladder, or urethra. These are the structures that urine passes through before being eliminated from the body. Simple infections occur in healthy urinary tracts and do not spread to other parts of the body. They usually go away readily with treatment. Complicated infections are caused by anatomic abnormalities, spread to other parts of the body, are worsened by underlying medical conditions, or are resistant to many antibiotics, they are more difficult to cure. If predisposing factors are not identified and removed, UTI can lead to more consequences, in particular kidney damage and renal failure. These infections are much more common in girls and women than in boys and men younger than 50 years of age. The reason for this is not well

understood, but anatomic differences between the genders (a shorter urethra in women) might be partially responsible [1]. About 40% of women and 12% of men have a urinary tract infection at some time in their life. The major symptoms of UTI are frequency in urination, painful burning sensation, Discomfort or pressure in the lower abdomen. Pain in the pelvic area or back, urine often has a strong smell, looks cloudy, or contains blood and Occasionally, fever develops. Escherichia coli, Klebsiella spp., and Proteus spp. are the uropathogens with the highest prevalence among patients with UTIs. However, the antibiotic susceptibility patterns of Enterobacteriaceae have been constantly changing due to the continuous development of new resistance mechanisms, like the production of extended-spectrum beta-lactamases or

carbapenemases by bacteria and the spread of genes on mobile elements (2). Among patients with hospital-acquired UTIs, 50% of the isolates were susceptible to amoxicillin-clavulanic acid, 77% to ceftazidime, 78% to ciprofloxacin, 50% to nalidixic acid, 48% to gentamicin, 41% to trimethoprim-sulfamethoxazole, 50% to nitrofurantoin, and 30% to tetracycline [3]. The association between antibiotic use and antimicrobial resistance has been convincingly demonstrated [4-6]. On the individual patient level, this is a clinical problem in patients with urinary tract infections (UTI), in particular in women with recurrent UTI (rUTI). The (recurrent) empirical antimicrobial treatment in these women exerts significant resistance pressure on the uropathogens [7]. This pressure also affects the faecal flora, which serves as a resistance reservoir for potential uropathogens [8,9]. However, rates differed widely from one study to another. For example [10,11] investigated UTIs in young women who were outpatients and found resistance rates of 0 to 0.2%, whereas others investigators found that resistance rates for *E. coli* strains isolated from urine were as high as 20.6% and that 20% of strains from hospitalized patients were ciprofloxacin resistant [12,13]. In addition, investigators have found that 29% of strains from nursing home patients were norfloxacin resistant [14]. Antibiotic resistance of *Escherichia coli* from community-acquired urinary tract infections in relation to demographic and clinical data [15].

II. MATERIALS AND METHODS

2.1. Chemicals:

All the chemicals used in the present study were of pure quality grade. The culture medium constituents were of pure quality grade and analytical grade. The optical density values were measured by ELICO UV-Visible Spectrophotometer.

2.2 Collection of Samples:

In our study we collected some infected and normal urine samples of from different microbiology laboratories of nearby our college. We have visited some microbiology laboratories at NRI medical college and A to Z laboratories in vijayawada to gather brief details about UTI infectious cultures (Fig: 1)

and their characterization techniques. For the exact diagnosis they were allowing the cultures to grow on the Blood agar medium (Figure 3), Mac-Conkey agar medium. They inoculated the infected samples on the Blood agar medium, Mac-Conkey agar medium and subjected for incubation for 24 hours. The grown cultures were isolated for characterisation and differentiation of the cultures by staining and motility techniques. In the present study we have been used 65 yrs normal female sample, 35 yrs diabetic male sample, 45 yrs abnormal female sample, 13 yrs normal male sample, 28 yrs abnormal male sample, 28 yrs abnormal female sample.

2.3. Isolation of UTI infectious cultures from the collected samples:

At first all the infectious cultures were isolated by standard plate method. The isolated cultures were subjected to different straining techniques such as simple staining, Gram staining (Figure 2 A & B), Acid fast staining, Spore staining to know the exact characteristics of the cultures. And the cultures are subjected to motility test by hanging drop method to know its motility characteristics. On this study the following infectious cultures were identified – *Escherichia Coli* (Gm-ve, rod shaped), *Staphylococcus saprophyticus* (Gram-positive, globular and resembles clusters of grapes), *Enterococci* (Gm-ve, spore forming, spherical cultures), *Klebsiella species* (Gram-ve, rod-shaped, anaerobic), *Ureaplasma urealyticum* (smallest free-living organisms lack of cell wall) in the collected samples. After the isolation of each and every pathogenic culture the pure cultures were again isolated by using a respective selective or enrichment medias such as *Blood gar* medium, *Mac-Conkey* agar medium, *Mueller-Hinton* agar medium, *Simmons citrate agar* medium for the isolation of the above cultures.

2.4. Selective media for the isolation of UTI infectious cultures

2.4.1) Blood agar media – The components were Nutrient agar (37gms), Blood (50ml) and disilled water up to 100ml.

2.4. 2) Mac-Conkey agar media – Peptone (3gms), Pancreatic digest of gelatin (17gms), Lactose monohydrate (10gms), Bile salta(1.50gms), Crystal violet

(0.001gms), Nacl(5gms), neutral red (0.3gms), and gar(13.5gms) and dist.water up to 1000ml.

2.4.3) Muller-Hinton agar media – Beef extract (30gms), Casein (50gms), Starch (1.50gms), agar (17gms) and distilled water up to 1000ml at Ph.7.3.

2.4.4) Simmons citrate agar media – Magnesium sulphate (0.20gms), Ammonium dihydrogen phosphate (1.00gms), Dipotassium phosphate (1.00gms), Sodiumcitrate (2.00gms), Bromothymol blue (0.08gms), Agar (15gms), and distilled water up to 1000ml at PH.6.8.

The infectious cultures were isolated by simple streaking shown in the (figure 3).

The above isolated pure cultures were subjected to the **antibiotic sensitivity test** by the standard methods:

A. Disc diffusion method / Cup plate method / Cylinder plate method [16].

B. Turbidimetric method [17].

In Paper disc method the Standard concentrations of 20µg, 40µg, 60µg, 80 µg and 100µg of different antibiotics such as Nitrofurantoin, Norfloxacin, cotrimoxazole, Piperacillin+ Tazobactam, Ticarcillin+ Clavulonic acid solution is prepared and the antibiotic sensitivity of all the infectious samples such as 65yrs normal, 35yrs diabetic samples, 45yrs abnormal samples, 13yrs normal male, 28 yrs male, female abnormal samples were known by studying its growth inhibition zone diameter in the respective solidified selective medias, shown in the (figure 4).

In turbidimetric method the Standard concentrations of 20µg, 40µg, 60µg, 80 µg and 100µg of different antibiotics such as Nitrofurantoin, Norfloxacin, cotrimoxazole, Piperacillin+ Tazobactam, Ticarcillin+ Clavulonic acid solution is prepared and the antibiotic sensitivity of all the infectious samples such as 65yrs normal, 35yrs

diabetic samples, 45yrs abnormal samples, 13yrs normal male, 28 yrs male, female abnormal samples were known by measuring its absorbance by observing its turbidity, shown in the (Figure 5).

III. RESULTS AND DISCUSSIONS

3.1. Effect of antibiotics on infectious samples by paper-disc method:

After 24 hrs incubation period, the effect of different antibiotics such as Nitrofurantoin, Norfloxacin, Cotrimoxazole, Piperacillin+ Tazobactam, Ticarcillin+ Clavulonic acid on different infectious samples were observed and it was measured in terms of growth inhibition zones diameters for different samples at five different concentrations such as 20,40, 60, 80 and 100 micrograms. The results were tabulated in table 4.1.A, 4.2.A, 4.3.A, 4.4A and in 4.5.A. and in the graph 5.1A, 5.2A, 5.3A, 5.4A and in 5.5A. On our observation there is no drastic change in the therapeutic efficiency of antibiotics on different infectious and normal samples due to drug resistance of infectious cultures either because of physical factors such as mutations, drug resistance plasmids, gene transfer, social pressures, inappropriate drug use, hospital use and inadequate diagnostics etc.

3.2. Effect of antibiotics on infectious samples by Turbid-metric method:

After 12hrs incubation period the antibiotic sensitivity of different antibiotics were measured in terms of absorbance on each testing and normal samples. The O. D. values were measured at 540 nm in U.V. spectrophotometer for five different concentrations. The results were tabulated in table.4.1.B, 4.2.B, 4.3.B, 4.4.B and 4.5.B and in the graph 5.1B, 5.2B, 5.3B, 5.4B and in 5.5B. On our observation there is no drastic change in the therapeutic efficiency of antibiotics on different infectious and normal samples because of various factors as mentioned earlier.



Figure 1: UTI Infected Samples from NRI Laboratories at Mangalagiri

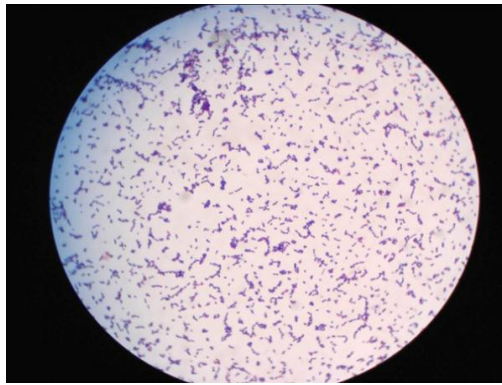


Figure 2A: GM+ VE Strains Of Female Diabetic Urine Sample

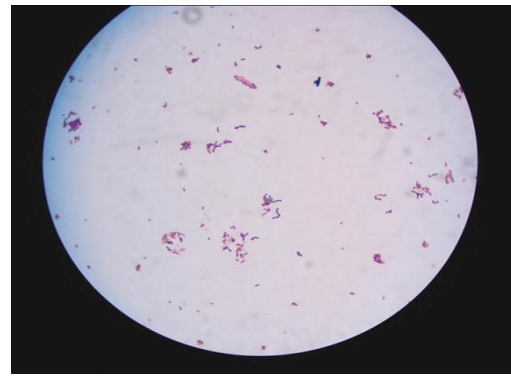


Figure 2B: GM-VE Strains Of Female Normal Sample

Figure 2: Isolated Cultures of Infected Samples on GM- Staining



Figure 3: Isolation by Simple Streaking

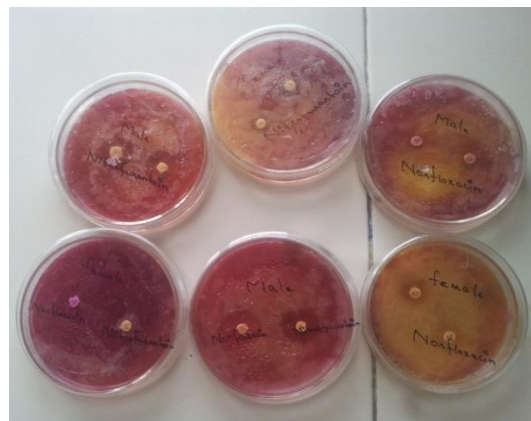


Figure 4: Antibiotic Sensitivity by Paper Disc Method



Figure 5: Antibiotic Sensitivity by Turbidimetric Method

IV. TABLES

4. A. Effect of antibiotics on infectious samples by paper-disc method:

4.1A. Effect of Nitrofurantoin on UTI Infected Person:

S. No.	Drug concentration (µg/ml)	65 yrs normal Female	35 yrs diabetic male	45 yrs abnormal female	13 yrs normal male	28 yrs abnormal male	28 yrs abnormal female
1	20	1.5	1.8	1.5	1.5	1.3	1.6
2	40	1.7	1.9	1.8	1.8	1.5	1.9
3	60	1.9	2.0	1.9	1.9	1.6	2.0
4	80	2.0	2.2	2.0	2.0	1.8	2.2
5	100	2.2	2.4	2.3	2.1	2.0	2.4

4.2A. Effect of Norfloxacin on UTI Infected Person:

S. No.	Drug concentration (µg/ml)	65 yrs normal Female	35 yrs diabetic male	45 yrs abnormal female	13 yrs normal male	28 yrs abnormal male	28 yrs abnormal female
1	20	1.3	1.1	1.3	1.3	1.2	1.1
2	40	1.5	1.5	1.5	1.5	1.4	1.5
3	60	1.8	1.6	1.8	1.6	1.9	1.6
4	80	1.9	1.8	1.9	1.8	2.0	1.8
5	100	2.0	1.9	2.0	2.2	2.2	1.9

4.3A. Effect of Co-Trimoxazole on UTI Infected Person:

S. No.	Drug concentration (µg/ml)	65 yrs normal Female	35 yrs diabetic male	45 yrs abnormal female	13 yrs normal male	28 yrs abnormal male	28 yrs abnormal female
1	20	1.3	1.2	1.3	1.1	1.5	1.3
2	40	1.5	1.4	1.5	1.3	1.8	1.5
3	60	1.7	1.5	1.7	1.4	1.9	1.8
4	80	1.9	1.7	1.9	1.8	2.0	2.0
5	100	2.0	2.0	2.1	2.0	2.2	2.2

4.4A. Effect of Piperacillin+ Tazobactam on UTI Infected Person

S. No.	Drug concentration (µg/ml)	65 yrs normal Female	35 yrs diabetic male	45 yrs abnormal female	13 yrs normal male	28 yrs abnormal male	28 yrs abnormal female
1	20	1.5	1.4	1.5	1.1	1.5	1.3
2	40	1.8	1.6	1.8	1.3	1.7	1.5
3	60	1.9	2.0	1.9	1.6	1.9	1.9
4	80	2.0	2.2	2.0	1.8	2.0	2.0
5	100	2.5	2.4	2.2	2.0	2.2	2.2

4.5A. Effect of Ticarcillin+ Clavulonic Acid on UTI Infected Person

S. No.	Drug concentration ($\mu\text{g/ml}$)	65 yrs normal Female	35 yrs diabetic male	45 yrs abnormal female	13 yrs normal male	28 yrs abnormal male	28 yrs abnormal female
1	20	1.1	1.2	1.1	1.4	1.1	1.2
2	40	1.4	1.6	1.4	1.6	1.4	1.4
3	60	1.6	1.8	1.5	1.8	1.6	1.8
4	80	1.8	1.9	1.7	1.9	1.8	2.0
5	100	1.9	2.0	1.9	2.1	1.9	2.4

4. B. Effect of Antibiotics on Infectious Samples by Turbidi-Metric Method**4.1B. Effect of Nitrofurantoin on UTI Infected Persons:**

S. No.	Drug concentration ($\mu\text{g/ml}$)	65 yrs normal Female	35 yrs diabetic male	45 yrs abnormal female	13 yrs normal male	28 yrs abnormal male	28 yrs abnormal female
1	20	0.87	0.86	0.89	0.85	0.85	0.82
2	40	0.84	0.81	0.82	0.82	0.78	0.79
3	60	0.79	0.79	0.79	0.79	0.75	0.76
4	80	0.73	0.76	0.75	0.77	0.71	0.71
5	100	0.71	0.73	0.70	0.75	0.69	0.67

4.2 B. Effect of Norfloxacin on UTI Infected Person:

S. No.	Drug concentration ($\mu\text{g/ml}$)	65 yrs normal Female	35 yrs diabetic male	45 yrs abnormal female	13 yrs normal male	28 yrs abnormal male	28 yrs abnormal female
1	20	0.82	0.80	0.88	0.86	0.86	0.79
2	40	0.79	0.78	0.85	0.84	0.81	0.78
3	60	0.77	0.75	0.81	0.81	0.79	0.75
4	80	0.75	0.68	0.74	0.78	0.77	0.73
5	100	0.65	0.64	0.71	0.72	0.67	0.68

4.3 B. Effect of Cotrimoxazole on UTI Infected Person:

S. No.	Drug concentration ($\mu\text{g/ml}$)	65 yrs normal Female	35 yrs diabetic male	45 yrs abnormal female	13 yrs normal male	28 yrs abnormal male	28 yrs abnormal female
1	20	0.81	0.86	0.85	0.83	0.87	0.85
2	40	0.75	0.81	0.78	0.77	0.85	0.79
3	60	0.74	0.77	0.75	0.75	0.79	0.72
4	80	0.69	0.73	0.72	0.70	0.76	0.68
5	100	0.62	0.64	0.66	0.64	0.71	0.63

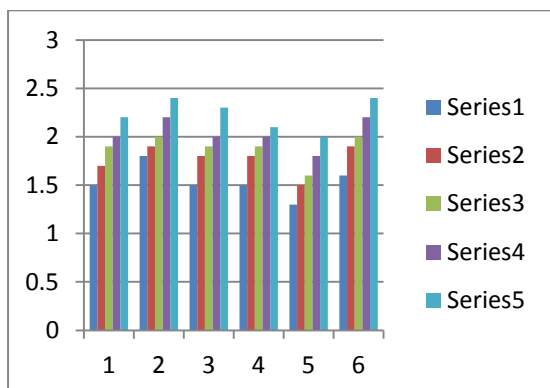
4.4 B. Effect of Piperacillin+ Tazobactam on UTI Infected Person

S. No.	Drug concentration ($\mu\text{g/ml}$)	65 yrs normal Female	35 yrs diabetic male	45 yrs abnormal female	13 yrs normal male	28 yrs abnormal male	28 yrs abnormal female
1	20	0.81	0.84	0.78	0.81	0.83	0.79
2	40	0.77	0.78	0.74	0.78	0.79	0.73
3	60	0.73	0.71	0.71	0.75	0.75	0.67
4	80	0.68	0.65	0.67	0.68	0.71	0.64
5	100	0.62	0.60	0.63	0.62	0.65	0.62

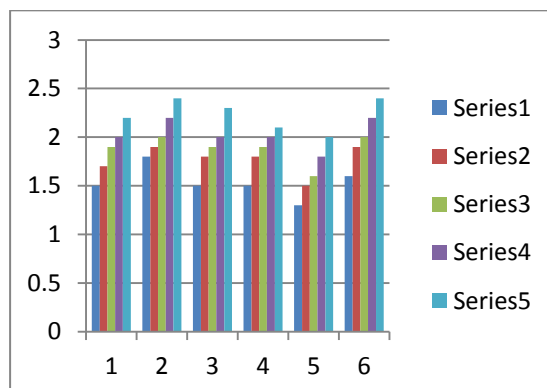
4.5 B. Effect of Ticarcillin+ Clavulonic Acid on UTI Infected Person

S. No.	Drug concentration (µg/ml)	65 yrs normal Female	35 yrs diabetic male	45 yrs abnormal female	13 yrs normal male	28 yrs abnormal male	28 yrs abnormal female
1	20	0.83	0.81	0.80	0.82	0.79	0.83
2	40	0.81	0.78	0.78	0.78	0.76	0.80
3	60	0.78	0.75	0.71	0.75	0.71	0.79
4	80	0.76	0.71	0.67	0.71	0.68	0.75
5	100	0.69	0.67	0.63	0.68	0.64	0.68

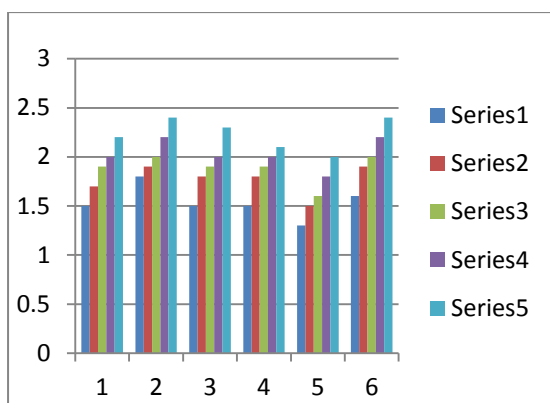
V. GRAPHS



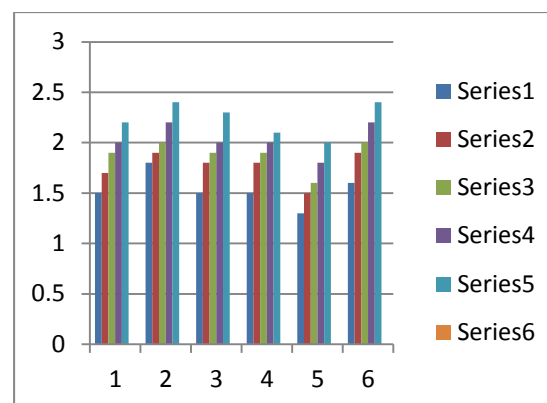
5.1A. Effect of Nitrofurantoin on Disc Diffusion Method



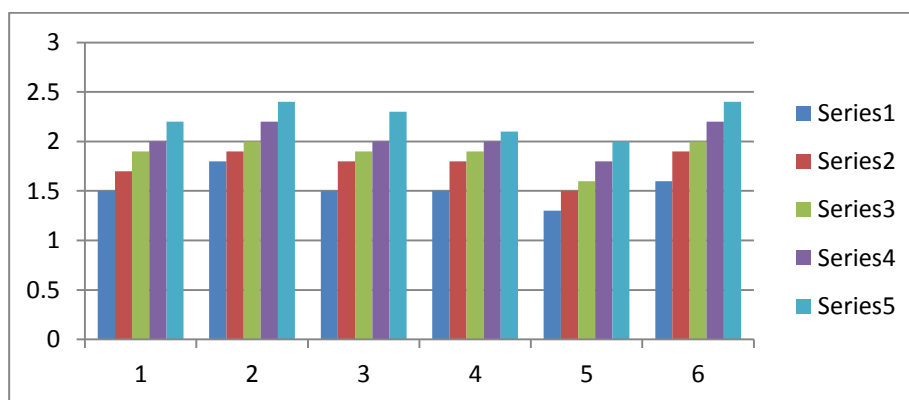
5.2A. Effect of Norfloxacin on Disc Diffusion Method



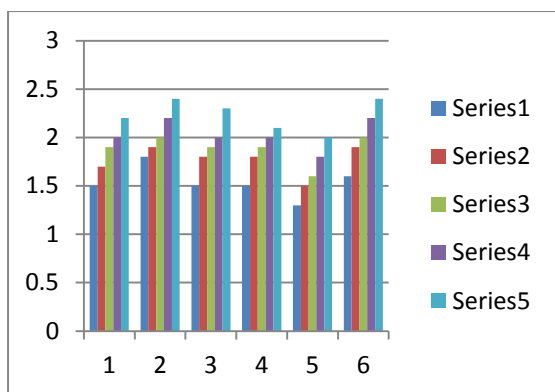
5.3A. Effect of Cotrimoxazole on Disc Diffusion Method



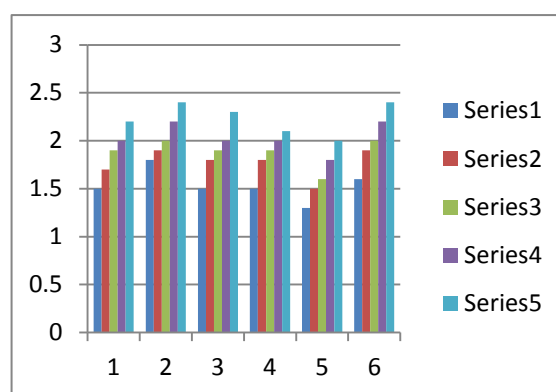
5.4 A. Effect of piperacillin+Tazobactam on Disc Diffusion Method



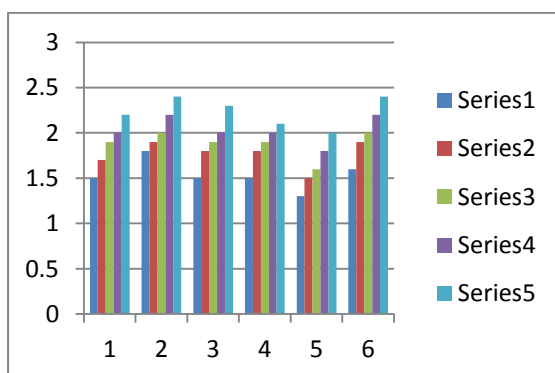
5.5A. Effect of Ticarcillin+ Clavulonic by Disc Diffusion Method



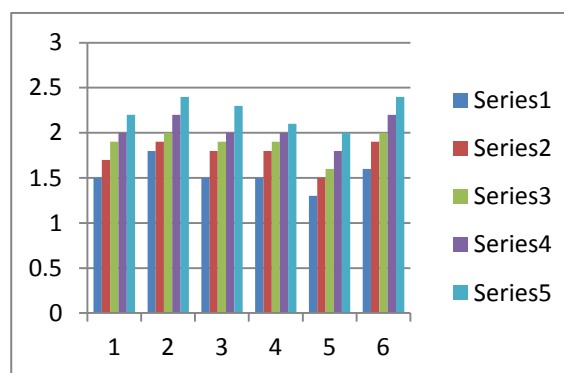
5.1B. Effect of Nitrofurantoin by Turbidimetric Method



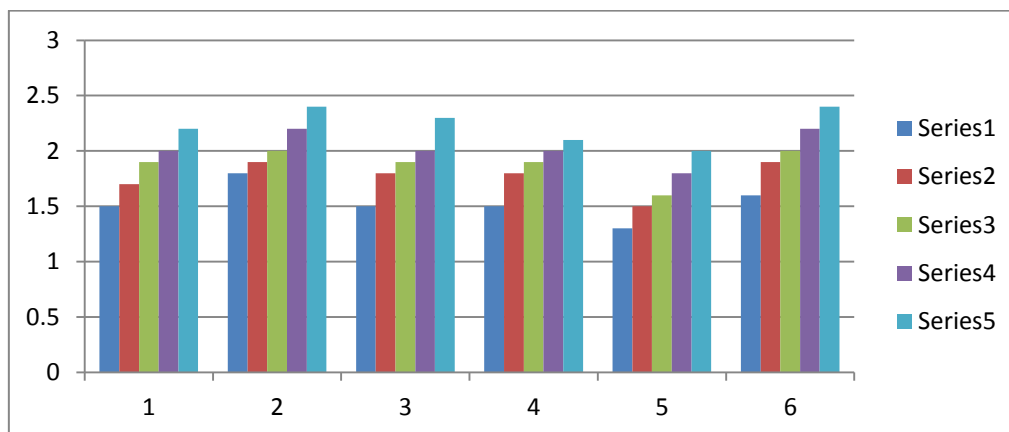
5.2A. Effect of Norfloxacin by Turbidimetric Method



5.3B. Effect of Cotrimoxazole by Turbidimetric Method



5.4B. Effect of Piperacillin+Tazobactam by Turbidimetric Method



5.5B. Effect of Ticarcillin+ Clavulonic Acid by Turbidimetric Method

CONCLUSION

- ❖ From the above study it was concluded that The female sample shows more resistance towards antibiotics than the male samples. Among them the samples of diabetic patients show more resistance towards combined and mono therapy.
- ❖ In case of mono therapy Nitrofurantoin show more growth inhibition zone than Norfloxacin.

- ❖ The concentration of 100µg/ml of individual drugs show nearer absorbance value to the standard absorbance value (blank).
- ❖ As the concentration of drug increases the variation in growth inhibition is not varied much.
- ❖ So, we concluded that the UTI therapy must be changed to protect the patients from fatal conditions.

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