# In vitro activity of Gentamicin against Uropathogens under Diabetic conditions

Korochikar Sujata, Korochikar Premkumar

**Abstract -** The diabetic individuals are more susceptible to urinary tract infections due to presence of glucose in the urine. Gentamicin is an antibiotic that treats gram negative microorganisms, most of that cause UTIs. The aim of this study was to determine the in vitro effectiveness of Gentamicin against uropathogens in presence of glucose. Uropathogens were isolated from the urine samples obtained prior to antibiotic therapy from the diabetic patients with glycosuria and urinary tract infection by culturing on Mac-Conkey's agar. The colony characteristics, Gram nature and biochemical characteristics of the isolates were studied and identified as Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli & Klebsiella preumonae respectively. Minimum Inhibitory Concentration of commercially available Gentamicin injection vial containing 40mg/ml (i.e. 40000µg/ml) was determined by using microbroth dilution method. The MIC of, Klebsiella pneumonae was 0.4µg/ml, Staphylococcus aureus was 0.04µg/ml, Escherichia coli and Pseudomonas aeruginosa was 4µg/ml respectively. Gentamicin antibiotic assay was carried using specific MIC against different glucose concentration like 100mg/dl, 250mg/dl, 500mg/dl, 1000mg/dl and 2000mg/dl, and growth was observed after 3, 6, 12, 24 and 48 hours of incubation at 370C. Growth was detected for Escherichia coli at 2000mg/dl glucose after 3hrs, Klebsiella pneumonae at l00mg/dl glucose after 24hrs, at 250mg/dl, 500mg/dl, l000 mg/dl glucose after 6 hours and at 2000mg/dl glucose after 3hrs of incubation, Staphylococcus aureus at 250mg/dl, 500mg/dl glucose after 24hrs and at l000mg/dl, 2000mg/dl glucose after 3hours of incubation. Pseudomonas aeruginosa showed growth at 500mg/dl glucose after 48 hours, l000mg/dl after 24hrs and at 2000mg/dl glucose after 3hrs of incubation. Throughout experiment control uropathogens showed no growth. The efficiency of Gentamicin against uropathogens is reduced with respect to increasing glucose concentration. Gentamicin may be used in individuals with mild to moderate glycosuria however in severe diabetic individuals the drug can be used by increasing concentration.

**Key words:** Urinary tract infection (UTI), Minimum inhibitory concentration (MIC), Uropathogens, Gentamicin, Diabetes, Glycosuria, Sensitivity

## 1 Introduction

Diabetes is a condition with impaired glucose metabolism leading to increased blood glucose concentrations beyond the normal levels. This condition is caused due to impaired insulin secretion (insulin dependent) or ineffectiveness of hormone insulin (Non insulin dependent) secreted by pancreas. The excessive glucose beyond the threshold level is excreted through urine. This condition is called as glycosuria. Due to presence of glucose in the urine, which is nutritive to the microorganisms, diabetic individuals are more susceptible to urinary tract infections as compared to non diabetic subjects. The factors like impaired neutrophil function, Glucose in urine supporting bacterial growth and increased adherence of uropathogens to epithelial cells of urinary tract have been suggested as possible causes [3].

A urinary tract infection (UTI) is an infection caused by pathogenic organisms (for example, bacteria, fungi, or parasites) in any of the structures that comprise the urinary tract. More specific terms that localize the urinary tract infection to the major structural segment involved are urethritis (urethral infection), cystitis (bladder infection), ureter infection, and pyelonephritis (kidney infection). Other structures that eventually connect to or share close anatomic proximity to the urinary tract (for example, prostate, epididymis, and vagina) are sometimes included in the discussion of UTIs. UTIs are more common in women than men. Although some infections go unnoticed, UTIs can cause problems that range from dysuria (pain and/or burning when urinating) to organ damage [1]. The bacteria associated with urinary tract infections include *E. coli, K. pneumonae, S. aureus, P. aeruginosa, S. saprophyticus, P. mirabilis, E. fecalis*. [3]

If UTI is suspected, a midstream urine sample is usually obtained in a sterile cup.. The urine sample is then sent for urinalysis and microbial culture and sensitivity tests. A positive urinalysis is usually detection of about two to five leukocytes, about 15 bacteria per high-power microscopic field, positive for bacteria as > 1,000 bacteria cultured per milliliter of urine. A definitive test is usually considered to be isolation and identification of the infecting pathogen at a level of about 100,000 bacteria per cc of urine with genus of pathogen identified and antibiotic sensitivity determined by lab studies. [1]

Antibiotics are the primary measures used to treat UTI. simple or complicated. Different antibiotics are used for cystitis and pyelonephritis. The common antibiotics used in the treatment of UTI include trimethoprim, nitrofurantoin, cephalosporins, penicillins, fluoroquinolones, and fosfomycin. The doctor may prescribe another antibiotic after receiving the results of the culture of the urine. Gentamicin is an antibiotic that treats gram negative microorganisms, such as the majority of bacteria that cause UTIs. Gentamicin simply kills the bacteria in the bladder and is not absorbed into the bloodstream. It also does not increase resistance of bacteria since it works locally and not

The choice of antibiotic is influenced by its effectiveness, side effects, resistance levels, costs and whether the UTI is

i) *Korochikar Sujata - Microbiologist, B.K.L Walawalkar Hospital and Diagnostic center, Dervan*
ii) *Korochikar Premkumar -Head of the Department, P.G. Diploma in Medical Laboratory Technology at SVJCT's College of Advanced Studies, Dervan*

systemically. [11]

Gentamicin is an aminocyclitol containing aminoglycoside antibiotic complex, produced by Micromonospora species, echinospora and purpurea. Gram negative organisms responsive to Gentamicin are both indole positive and indole negative *Proteus, Pseudomonas, E.coli, Aerobacter, Klebsiella, Salmonella* and *Shigella*. It is also effective against *Mycobacterium tuberculosis*. In adults, it is usually given in daily doses of 240–360 mg [4].

Commercially available Gentamicin is a mixture of Gentamicin sulphate C1, C2 and C3. Gentamicin Sulfate is the sulfate salt, or a mixture of such salts, of the antibiotic substance [12]. The three compounds Gentamicin sulphate C1, C2 and C3 have similar antimicrobial activities to one another. It has a potency equivalent to not less than 590µg of Gentamicin per mg, calculated on the dried basis. The antibacterial activity of this antibiotic is markedly increased by alkaline pH of urine [9]. The presence of glucose in the urine causes slight increase in urine pH. The changes in urine pH may interfere with the activity of drugs used for treatment of Urinary Tract Infections as well as the glucose provides nutritive medium and adds to the survival of the uropathogens in urine [5].

**2 Aims and Objectives**

i) Isolation of uropathogens from urine samples collected from the diabetic patients suffering with UTI.
ii) Determination of minimum inhibitory concentration of Gentamicin by broth dilution method.
iii) Gentamicin sensitivity assay in varying glucose concentrations.
iv) Comparison of growth of uropathogens at varying glucose concentrations.

**3 Materials and Methods**

To isolate uropathogens, total 10 diabetic patients with age group 40 to 60 years with mild to severe glycosuria and showing symptoms of urinary tract infections were screened for significant bacterial growth. The random midstream urine samples were collected aseptically prior to antibiotic therapy and transported to the laboratory immediately within one hour.

0.01ml of each urine sample was inoculated on sterile MacConkey agar plates using standard loop technique and Incubated at $37^0$c for 24 hours. Colony count was performed and organisms were identified using conventional method [3],[8],[9],[10],[11]. Microbroth dilution method was employed to determine minimum inhibitory concentration (MIC) of confirmed clinical isolates against the drug Gentamicin (Abbott, 40 mg/ml) using Nutrient broth. Four sets of seven tubes were prepared. In each tube 3ml sterile nutrient broth & 1ml Gentamicin solution of varying dilutions (4000µg/ml, 400µg/ml, 40µg/ml, 4µg/ml, 0.4µg/ml, 0.04µg/ml, & 0.004µg/ml) and 0.1ml of inoculum equivalent to 0.5 McFarland unit at 540 nm of respective isolate was added. Gentamicin sensitivity assay against the isolates was performed with varying glucose concentrations (Himedia glucose 100, 250, 500, 1000 and 2000mg/dl) to determine the effect of glucose on the gentamicin activity after 3 hrs, 6 hrs, 12 hrs, 24 hrs and 48 hrs of incubation at 37oc. 4 sets of 3ml sterile nutrient broth with varying glucose concentration was prepared. 0.1 ml of inoculum of uropathogens was added and after specific duration of incubation optical density of each tube was measured at 540 nm on colorimeter. The set of 4 tubes with 3ml nutrient broth containing 0.1ml inoculum and without glucose was used as control.

**4 Result**

Out of total 10 random urine samples from diabetic cases with glycosuria analyzed, 8 cases (80%) showed significant bacteriuria. Predominant isolate was *Escherichia coli* (62.5%) followed by *Klebsiella pneumonae, Staphylococcus aureus* and *Pseudomonas aeruginosa* (12.5%) each.

The MIC of, *Klebsiella pneumonae* was 0.4µg/ml, *Staphylococcus aureus* was 0.04µg/ml, *Escherichia coli* and Pseudomonas aeruginosa was 4µg/ml respectively.

*Escherichia coli* showed sensitivity against Gentamicin at l00mg/dl, 250mg/dl, 500mg/dl, l000mg/dl of glucose level, but increasing growth is observed at glucose level 2000mg/dl with increase in duration of incubation. *Klebsiella pneumonae* showed growth after 24hrs of incubation at l00mg/dl of glucose level, and after 6 hours of incubation at 250mg/dl, 500mg/dl, l000mg/dl and 2000mg/dl of glucose level. *Staphylococcus aureus* is found to be sensitive to Gentamicin at l00mg/dl of glucose concentration and showed growth after 24hrs of incubation at 250mg/dl and 500mg/dl of glucose concentration. Growth was observed after 3 hours of incubation at l000mg/dl and at 2000mg/dl of glucose concentration. *Pseudomonas aeruginosa* was found to be sensitive to Gentamicin at l00mg/dl and 250mg/dl of glucose concentration. Growth was observed at 500mg/dl after 48 hours of incubation and at l000mg/dl of glucose after 24hrs of incubation and at 2000mg/dl of glucose concentration after 3hrs of incubation. Throughout experiment control showed no growth of respective uropathogens

**Table 1: Gentamicin sensitivity assay at varying concentrations of glucose and duration of incubation.**

| Sr. | Uropathogens | MIC of Gentamicin (µg/ml) | Hours of Incubation at 37°C | OD at 540 nm | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Glucose concentrations (mg/dl) | | | | | |
| | | | | 100 | 250 | 500 | 1000 | 2000 | Control |
| 1 | *Escherichia coli* | 4 | 3 | 0 | 0 | 0 | 0 | 0.03 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 6 | 0 | 0 | 0 | 0 | 0.03 | 0 |
| | | | 12 | 0 | 0 | 0 | 0 | 0.03 | 0 |
| | | | 24 | 0 | 0 | 0 | 0 | 0.08 | 0 |
| | | | 48 | 0 | 0 | 0 | 0 | 0.08 | 0 |
| 2 | *Klebsiella pneumonae* | 0.4 | 3 | 0 | 0 | 0 | 0 | 0.02 | 0 |
| | | | 6 | 0 | 0.05 | 0.06 | 0.06 | 0.06 | 0 |
| | | | 12 | 0 | 0.07 | 0.09 | 0.1 | 0.1 | 0 |
| | | | 24 | 0.05 | 0.09 | 0.1 | 0.13 | 0.13 | 0 |
| | | | 48 | 0.05 | 0.09 | 0.1 | 0.13 | 0.13 | 0 |
| 3 | *Staphylococcus aureus* | 0.04 | 3 | 0 | 0 | 0 | 0.02 | 0.03 | 0 |
| | | | 6 | 0 | 0 | 0 | 0.03 | 0.06 | 0 |
| | | | 12 | 0 | 0 | 0 | 0.04 | 0.08 | 0 |
| | | | 24 | 0 | 0.06 | 0.06 | 0.1 | 0.16 | 0 |
| | | | 48 | 0 | 0.06 | 0.06 | 0.1 | 0.16 | 0 |
| 4 | *Pseudomonas aeruginosa* | 4 | 3 | 0 | 0 | 0 | 0 | 0.03 | 0 |
| | | | 6 | 0 | 0 | 0 | 0 | 0.06 | 0 |
| | | | 12 | 0 | 0 | 0 | 0 | 0.06 | 0 |
| | | | 24 | 0 | 0 | 0 | 0.05 | 0.09 | 0 |
| | | | 48 | 0 | 0 | 0.02 | 0.05 | 0.09 | 0 |

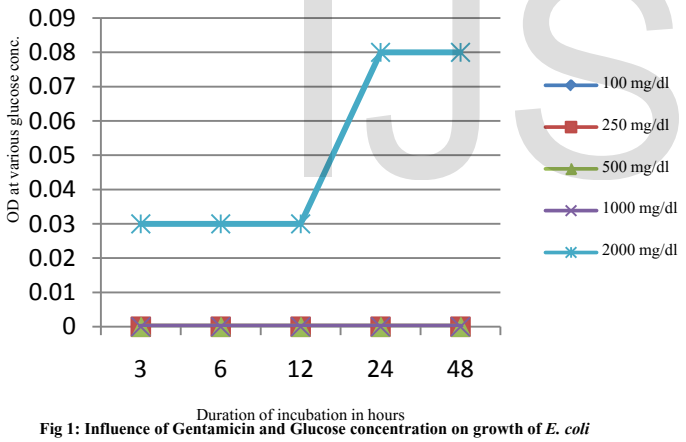


**Fig 1: Influence of Gentamicin and Glucose concentration on growth of *E. coli***

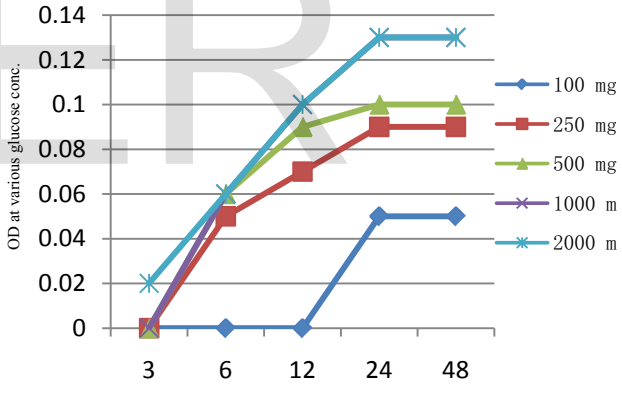


**Fig 2: Influence of Gentamicin and Glucose concentration on growth of *K. pneumonae***

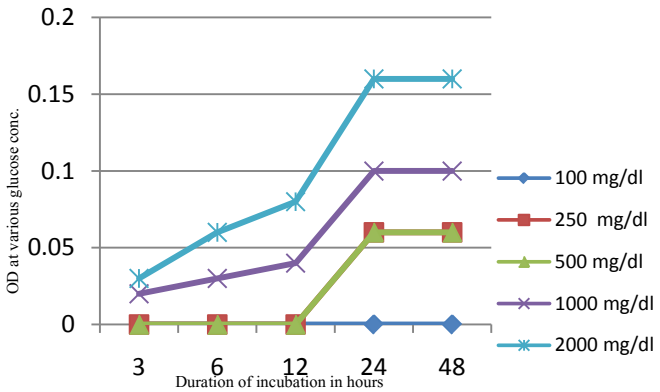


**Fig 3 Influence of Gentamicin and Glucose concentration on growth of *S. aureus***

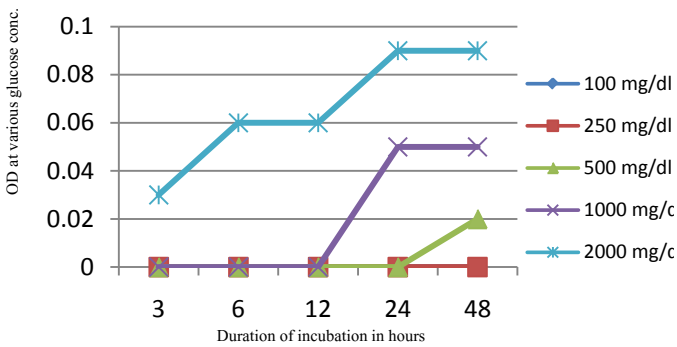


**Fig 4: Influence of Gentamicin and Glucose concentration on growth of *P. aeruginosa***

## 5 Discussion

The in vitro activity of gentamicin was determine to maximize the co-relation between susceptibility testing to the drug and the result of clinical therapy of urinary tract infection in diabetes. Generally glycosuria of 0.0-6.0 mg/dl is accepted as normal, l00mg/dl to 250mg/dl is as mild diabetes and 250mg/dl to l000mg/dl is as severe diabetes [1].

A similar study using Norfloxacin have revealed that the factors like altered pH and glycosuria play important role in lowering the activity of antibiotic against the uropathogens [3]. At higher glucose concentration and at alkaline pH Gentamicin activity was found to be reduced. Therefore it clearly reveals that the efficiency of Gentamicin is very high at normal (non diabetic) condition of urine glucose as compared to altered condition of urine glucose concentration. Growth of uropathogens is enhanced at higher glucose concentration like l000mg/dl, 2000mg/dl and within 3 hrs of incubation indicating decreased gentamicin sensitivity, whereas at low glucose concentration growth of uropathogens is delayed and observed after 6-8 hrs of incubation indicating sensitivity to gentamicin at low glucose concentrations. Bacterial growth observed after 12hrs of incubation has no clinical significance as patient is administered the second dose of Gentamicin.

These in vitro results may not exactly reflect the in vivo condition as the human urinary tract is the dynamic system in which fresh urine is constantly being formed. Furthermore the urine pH and osmolarity changes over time. For these results low urine pH as a result of bacterial metabolism properly does not play an important role as an inhibitor of further bacterial growth. In vitro and in clinical situation very high glucose concentration probably enhances bacterial growth. This may explain greater susceptibility of diabetics to urinary tract infection [3].

To conclude Gentamicin may be used to treat urinary tract infections in non- diabetic & diabetic individuals with mild to moderate glycosuria however in severe diabetic individuals the drug can be used by increasing concentration of Gentamicin.

## Bibliography

1. **Charles P. Davis (1998):** Urinary tract infection symptoms, causes, treatment and home remedies cited on http//:www.medicinenet.com
2. **Desai J.D & Anjana J. Desai (1980):** Methods in microbiology, microscopy & staining, Prashant Publishers, Vallabh Vidyangar. pp 88 -93.
3. **H. Anand kumar, A Dayananad, C. S. Vinodkumar and I. Kapur (2003)**: In vitro activity of Norfloxacin against uropathogens and drug efficiency in simulator bladder model under diabetic conditions, *Indian Journal of Medical microbiology*, 21 (1):37-42
4. **Himabindu, M Jetty, Annapurna. (2006):** Optimization of nutritional requirements of Gentamicin production by *Microspora echinospora*: Indian *Journal of Experimental Biology*, *Volume* - 44, pp 842 – 848
5. **Janine D. Cook, Kathy A. Strauss, Yale H. Caplan, Charles P. LoDico and Donna M. Bush (2007):** Urine pH: the Effects of Time and Temperature after Collection: Journal of Analytical Toxicology, Vol. 31, pp 486- 496
6. **Mackie and Macartney (2007):** Practical medical microbiology, 4th Ed. Churchill Livingstone, Elsveir, New Delhi, pp151 - 165
7. **Noel R. Krieg, John G Holt (1984):** Bergey's manual of systematic Bacteriology, volume - I, Williams & Wilkins, Baltimore, London. pp 140 – 173 & 409 – 516.
8. **Patel Rakesh J. (2008):** Experimental microbiology, volume – I, 5th Edition, Aditya, Ahmedabad, Gujrat. pp 110 -133
9. **Satoskar R. S, A. K. Kale, S. D. Bhandarkar (1970):** Pharmacology and Pharmacotherapeutics, 3rd Ed. Popular Prakashan and Popular press Bombay. pp 526 - 531
10. **Sneath, P.H.A. Mair. N.S & Sharpe N.E (1986)**: Bergey's manual of systematic Bacteriology volume – II, Williams & wilkins London. pp 1013 – 1018
11. **Susan Agrawal (2008):** cited on http://www.articles.complexchild.com/00013.html
12. **William W. Wright (2005):** Pharmaceutical analysis (PAT), cited on http://www.newdruginfo.com/pharmacopeia/usp28/v28230/usp28nf23s0_m34850.htm