# Gram positive uropathogens and their antibiogram: Data analysis at a tertiary care hospital in Karnataka

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## Abstract

**Introduction:** Urinary tract infections (UTI) are one of the most common infections encountered in clinical settings, being amenable to easy diagnosis with established laboratory methods. However, in recent years, antibiotic resistance has become a major public health problem worldwide owing to the extensive and inappropriate use of antimicrobial agents.

**Objectives:**

1. To determine the various Gram positive isolates causing urinary tract infections.
2. To determine the antibiotic susceptibility pattern of Gram positive cocci.

**Material and Methods:** A cross sectional study was conducted at a private tertiary care hospital in Shivamogga district of Karnataka, using secondary data of Gram positive bacteria isolated from urine samples maintained in the Microbiology laboratory registers for a period of 1 year from January 2016 to December 2016. Standard protocols were followed for bacteria isolation, identification and to assess their antibiotic susceptibility. Statistical analysis was done using MS office Excel 2010.

**Results:** Of the total 1254 urine specimens, 466 (37.16%) revealed positive cultures. Out of the 512 isolates from positive cultures, 196 (38.28%) were Gram positive bacteria. The predominant isolates i.e. 86 (43.87%) belonged to Enterococcus species. All the isolates were susceptible to Linezolid and Vancomycin. Majority i.e. 184 (93.87%) were sensitive to nitrofurantoin followed by gentamicin 146 (74.48%) and majority showed resistance to ampicillin i.e.126 (64.28%).

**Conclusion:** The rise in the resistance among uropathogens alerts us against indiscriminate use of antibiotics, indicating the need to establish antibiotic policies along with stringent measures to ensure effectiveness of the same.

**Keywords**: Antibiogram, Gram positive isolates, Urinary tract infection.

## Introduction

Currently, Urinary tract infections (UTI) represent one of the most commonly encountered morbidities in health care settings with an estimated 150 million cases per annum worldwide. They are caused by invasion of the urinary tract by pathogenic microorganisms.[1] The clinical manifestations depend on the part of the urinary tract affected, the causative organisms, the severity of the infection and the patient's ability to mount an immune response.[2] Females are more prone to UTI than males due to anatomical and physiological reasons.[1,3,4] They occur most frequently between the ages of 16 and 35 years, with 10% of women getting an infection at some point in their lives. Recurrences are common, with nearly half of people getting a second infection within a year.[5]

Gram negative bacteria like *Escherichia coli*, Proteus species, Klebsiella species, *Pseudomonas aeruginosa*, Acinetobacter, Serratia and *Morganella morgagni* are isolated from 75-95% cases of uncomplicated UTI which is most common in young, sexually active, non pregnant, premenopausal women.[6] The remaining cases are associated with a variety of organisms, including the Gram positive bacteria like Enterococcus, Staphylococcus especially coagulase negative staphylococci, *Streptococcus agalactiae* and other less frequently isolated organisms.[7] However, Gram positive bacteria are found more often as etiologic agents in vulnerable groups such as pregnant women and the elderly. Complicated UTI is defined as cystitis or pyelonephritis that occurs in individuals with predisposing anatomic, metabolic or functional risk factors making their treatment difficult.[7] and these populations are at greater risk of Gram positive and polymicrobial UTI.[8,9]

Current management of UTIs is usually empirical, without the use of a urine culture or susceptibility testing to guide therapy. During the past decade, Gram positive cocci isolates exhibited a remarkable ability to rapidly develop antibiotic resistance and it is of great concern.[5] Further, the Infectious Disease Society of America also recommends that physicians must keep on updating information on local susceptibility pattern of organisms causing urinary tract infections to monitor changes in their susceptibility which is a prerequisite for any hospital infection control program.[1,3] With this background, the present study was undertaken to identify the Gram positive isolates causing UTI among patients attending a tertiary care hospital and to study their antibiotic susceptibility pattern.

## Materials and Methods

A cross sectional study was conducted in the Microbiology department of a private tertiary care

hospital in Shivamogga district of Karnataka. Prior permission for the study was obtained from concerned authorities. Secondary data maintained in the laboratory registers of Microbiology department of Gram positive bacteria isolated from urine samples of patients presented with UTI for a period of 1 year from January 2016 to December 2016 were collected for the study. The following information were noted – Name, Age, Sex, Case history, Organism isolated and their antibiotic susceptibility pattern.

**Statistical analysis**

Analysis was done using MS Office Excel 2010.

**Bacterial isolation and identification**

Cases of clinically suspected urinary tract infection were routinely sent for microbiological analysis. These samples were processed in the laboratory for direct microscopy and aerobic culture and sensitivity as per the standard protocol. Routine microscopic examination of urine samples were done to look for pus cells, red blood cells and epithelial cells. Semi quantitative urine culture was done using a calibrated loop. A loopful (0.001 ml) of well mixed un-centrifuged urine was inoculated onto the surface of MacConkey agar, blood agar media and Cysteine lactose electrolyte deficient agar (CLED). All plates were then incubated at 37°C aerobically for 24 hours.

The plates were examined macroscopically for bacterial growth. Unlike Gram negative bacilli that needs more than 100 colonies corresponding to $10^5$ cfu/ml to be significant, Gram positive cocci, irrespective of the colony count were considered significant.[10] The Gram positive bacterial isolates were identified using standard bacteriological tests.[11] Colonial appearance and morphological characters of isolated bacteria were noted and isolated colonies were subjected to preliminary tests like Gram staining and catalase test. These preliminary tests were followed by biochemical reactions for identification of the isolated organism.

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing was performed on Mueller Hinton agar as per CLSI guidelines[12] and was tested for the following antibiotics - Ampicillin (10µg), Tetracycline (30µg), Ciprofloxacin (5µg), Norfloxacin (10µg), Nitrofurantoin (300µg), Gentamycin (10µg), High level Gentamycin (120µg), Linezolid (30µg),Vancomycin (30µg).

## Results

Of the total 1254 urine specimens collected and processed during the study period, 466 (37.16%) samples yielded positive cultures and the rest had no growth.

466 culture positive cases yielded 512 isolates. Out of them, 306 (59.76%) were Gram negative bacteria, 196 (38.28%) were Gram positive bacteria and 10 (1.95%) were non albicans Candida.

Majority of the Gram positive bacteria were isolated from females i.e. 166 (84.69%). Majority i.e. 80 (40.81%) were from patients in the age group of 21-30 years followed by 24 (12.24%) in 31-40 years as shown in **Table 1**.

136 (69.38%) of Gram positive isolates were from outpatient department whereas 60 (30.61%) were from inpatient department as shown in **Table 1**.

**Table 1: Different variables associated with Gram positive bacteria (n = 196)**

| **Variables** | | **Gram positive isolates** | |
|---|---|---|---|
| | | **No.** | **%** |
| **Gender** | Male | 30 | 15.30 |
| | Female | 166 | 84.69 |
| **Age (years)** | 0-10 | 14 | 7.14 |
| | 11-20 | 22 | 12.79 |
| | 21-30 | 80 | 40.81 |
| | 31-40 | 24 | 12.24 |
| | 41-50 | 18 | 9.18 |
| | 51-60 | 22 | 11.22 |
| | 61-70 | 14 | 7.14 |
| ***OP/IP** | OP | 136 | 69.38 |
| | IP | 60 | 30.61 |

*OP- Out patient department
*IP- In patient department

**Table 2** depicts the frequency of distribution of Gram positive bacteria from patients of various departments. Majority i.e. 132 (67.34%) were from the department of Obstetrics and Gynaecology followed by 28 (14.28%) from General Surgery, 16 (5.81%) from General Medicine, 14 (7.14%) from pediatrics and only 4 (2.32%) from orthopedics.

**Table 2: Distribution of Gram positive bacteria according to various departments (n=196)**

| Department | No. | % |
|---|---|---|
| Medicine | 16 | 5.81 |
| OBG | 132 | 67.34 |
| Surgery | 28 | 14.28 |
| Orthopedics | 4 | 2.32 |
| Pediatrics | 14 | 7.14 |

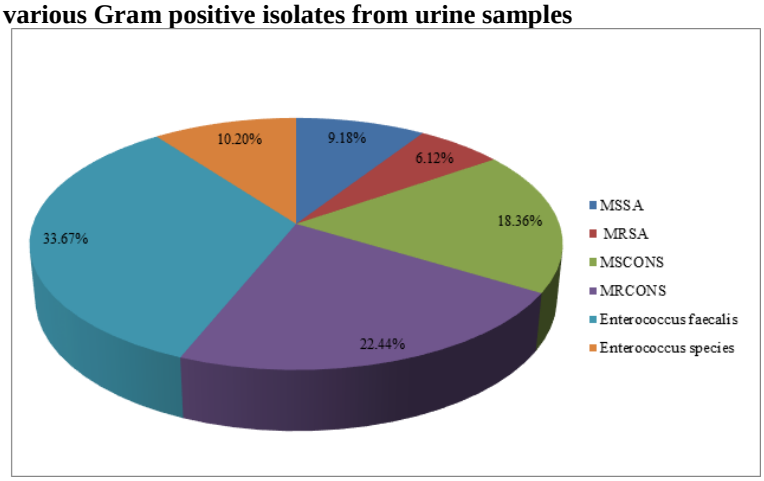
**Table 3** shows the distribution of various Gram positive bacteria isolated from urine samples. Enterococcus 86 (43.87%) were the most common organisms isolated followed by Coagulase negative Staphylococcus (CONS) 80 (40.81%) and *Staphylococcus aureus* 30 (15.3%).

**Table 3: Distribution of different Gram positive bacteria isolated from urine samples (n=196)**

| Organisms | No. | % |
|---|---|---|
| Enterococcus species | 86 | 43.87 |
| CONS (Coagulase negative Staphylococcus) | 80 | 40.81 |
| Staphylococcus aureus | 30 | 15.3 |

Among the 196 Gram positive cocci, Methicillin sensitive *Staphylococcus aureus* (MSSA) were 18 (9.18%), Methicillin resistant *Staphylococcus aureus* (MRSA) were 12 (6.12%), Methicillin sensitive CONS were 36 (18.36%) and Methicillin resistant CONS were 44 (22.44%). *Enterococcus faecalis* accounted for 66 (33.67%) and other Enterococcus species 20 (10.2%) as depicted in **Fig. 1**

**Fig. 1: Distribution of various Gram positive isolates from urine samples**




Majority i.e. 126 (64.28%) of Gram positive cocci showed resistance to penicillin followed by resistance to norfloxacin in 100 (51.02%) as shown in **Table 4.**

**Table 4: Antimicrobial susceptibility pattern of Gram positive isolates (n = 196).**

| Antibiotics | Susceptible | | Resistant | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Ampicillin | 70 | 35.71 | 126 | 64.28 |
| Tetracycline | 134 | 68.36 | 62 | 31.63 |
| Cotrimoxazole | 42 | 21.42 | 72 | 36.73 |
| Ciprofloxacin | 120 | 61.22 | 76 | 36.2 |
| Nitrofurantoin | 184 | 93.87 | 12 | 6.12 |
| Gentamicin* | 146 | 74.48 | 50 | 26.12 |
| Vancamycin | 196 | 100 | 0 | 0 |
| Linezolid | 196 | 100 | 0 | 0 |
| Norfloxacin | 96 | 48.97 | 100 | 51.02 |

*High level gentamicin in case of Enterococcus species

All the Gram positive cocci were sensitive to Vancomycin and Linezolid. Majority of the Gram positive cocci were sensitive to nitrofurantoin with *Staphylococcus aureus* sensitivity of 28 (93.33%), CONS 78 (97.5%) and Enterococcus species 78 (90.69%). They were moderately sensitive to gentamicin (high level gentamicin in case of Enterococcus species) with *Staphylococcus aureus* sensitivity of 22 (73.33%), CONS 62 (77.5%) and Enterococcus species 62 (72.09%) as shown in **Table 5.**

**Table 5: Antimicrobial susceptibility pattern of various Gram positive bacteria (n = 196).**

| Antibiotics | Staphylococcus aureus (n=30) | | CONS (n=80) | | Enterococcus species (n=86) | |
|---|---|---|---|---|---|---|
| | S | R | S | R | S | R |
| | No. % | No. % | No. % | No. % | No. % | No. % |
| Ampicillin | 12 (40) | 18 (60) | 6 (7.5) | 74 (92.5%) | 52 (60.46) | 34 (39.53) |
| Tetracycline | 18 (45) | 12 (40) | 66 (82.5) | 14 (17.5) | 50 (58.13) | 36 (41.86) |
| Cotrimoxazole | 8 (26.66) | 22 (73.33) | 14 (17.5) | 66 (82.5) | 20 (23.25%) | 66 (76.74%) |
| Ciprofloxacin | 14 (46.66) | 16 (53.33) | 60 (75) | 20 (25) | 46 (53.48) | 40 (46.51) |
| Nitrofurantoin | 28 (93.33) | 2 (6.66) | 78 (97.5) | 2 (2.5) | 78 (90.69) | 8 (9.30) |
| Gentamicin* | 22 (73.33) | 8 (26.66) | 62 (77.5) | 18 (22.5) | 62 (72.09) | 24 (27.9) |
| Norfloxacin | 8 (26.66) | 22 (73.33) | 44 (55) | 36 (45) | 44 (51.16) | 42 (48.83) |
| Vancamycin | 30 (100) | 0 (0) | 80 (100) | 0 (0) | 86 (100) | 0 (0) |
| Linezolid | 30 (100) | 0 (0) | 80 (100) | 0 (0) | 86 (100) | 0 (0) |

*High level gentamicin in case of Enterococcus species

## Discussion

The present study shows the pattern of UTIs prevalent in different age and sex groups, their causative organisms and the antimicrobial susceptibility pattern of Gram positive bacteria isolated. Out of the total 1254 urine samples processed, only 464 (37%) showed growth which is comparable with other studies done in different study settings and time periods.[8,13-16] However, the proportion of positive cultures was higher in a couple of studies[3,15] while few other studies reported low isolation rates.[1,4,17,18] Further, 196 (38.28%) Gram positive bacteria were isolated in our study similar to the findings of other studies[15,18] while lower rates were reported by other studies.[4,14,17]

The proportion of females was more in patients presenting with symptoms of UTI i.e.166 (84.69%) which correlates well with the findings of other studies.[3-5,17-20] Females are more prone to UTIs than men because of short urethra and its close proximity to the anus, which could be the reason for present study findings. On the contrary a study by Bajpai T et. al. reported higher prevalence of UTI in males.[14] Majority i.e. 80 (40.81%) of the patients were in the age group of 21-40 years. Similar trend was reported by Kumar S. et. al.[4]

In our study, Enterococcus species was the commonest isolated Gram positive cocci 86 (43.87%) followed by CONS 80 (40.81%). This result is in agreement with previous studies.[3,14,21-23] On the contrary some studies have isolated CONS[4,15] and Staphylococcus aureus[17,18] as the predominant uropathogens in their studies. This may be due to geographical differences in study settings, differences in practices related to hygiene and differences in healthcare practices. Personal hygiene plays an important role in reducing the incidence of UTI as pathogens causing UTI are usual commensals in the perianal and vaginal regions.

All the Gram positive cocci were sensitive to Vancomycin and linezolid in the present study. This was in accordance with other studies.[4,17,18,23,24] Majority of the Gram positive bacteria i.e. 93.88% were found to be sensitive to nitrofurantoin, similar to various studies done previously.[17,20,23-26] and in contrast to some other studies which have observed a higher degree of resistance to nitrofurantoin.[1,26] Owing to its narrow spectrum of activity, limited number of indications, limited tissue distribution and limited contact with bacteria outside the urinary tract, Nitrofurantion has the highest activity among commonly used oral antibiotics for treatment of UTI.[1] On the other hand, the least effective drugs against Gram positive bacteria were ampicillin and norfloxacin with 64.28% and 51.02% of resistant strains respectively. Ampicillin resistance may be determined by the organisms due to the production of enzymes such as betalactamase.[18,23,24,28-30]

## Conclusion

Knowledge of the uropathogens and their antimicrobial susceptibility pattern in an area is essential for providing effective therapy and control of UTI. Antibiotic sensitivity pattern may vary even from place to place and from time to time, making periodical evaluation of antibiotic sensitivity a prerequisite for any hospital infection control program. The rise in the resistance pattern alerts us against indiscriminate usage of antibiotics and indicates the need to establish

antibiotic policies along with stringent measures to ensure effectiveness of the same.

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