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Bacteriological Profile and Antibiotic Susceptibility Profile of Emerging Gram-positive Uropathogens in Tertiary Care Centre, Visakhapatnam, India.

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ABSTRACT: Amongst the common infections, Urinary tract infection (UTI) including both community and hospital acquired infections is seen to be responsible for high morbidity and mortality across all genders and age groups. The aim of the present study is to isolate and identify the pathogenic Gram-positive emerging uropathogens which are responsible for UTI in both male and female subjects and to identify the antibiotic susceptibility profile of isolated microorganisms. Urine samples were collected from both Inpatients (IP) and Outpatients (OP) at GIMSR Hospital, Visakhapatnam using clean-catch method. The specimens are examined to isolate the uropathogens by using standard microbiological procedures like Microscopy, Culture methods and Biochemical methods. Antimicrobial susceptibility of isolates is tested for all bacterial Gram-positive uropathogens by the disk diffusion according to Clinical Laboratory Standards Institute (CLSI) guidelines. Out of 624 samples, 266 samples yielded positive cultures and the rest had no growth. 266 culture positive cases yielded 270 isolates and among them 97 isolates were Gram positive bacteria. Majority of the Gram-positive bacteria isolated were Staphylococcus aureus followed by Coagulase negative Staphylococcus (CONS) and Enterococcus species. Majority of Gram-positive cocci showed resistance to penicillin followed by resistance to norfloxacin. Majority of the Gram positive cocci were sensitive to linezolid with Staphylococcus aureus sensitivity of 39 (71%), CONS 19 (86.4%) and Enterococcus species 15 (75%). They were moderately sensitive to vancomycin with Staphylococcus aureus sensitivity of 41 (74.6%), CONS 18 (81.9%) and Enterococcus species 10 (50%).The prevalence of new emerging pathogens causing urinary tract infections (UTIs) especially Gram-positive bacteria can be studied along with their antibiotic profile in this part of the country. There is an urgent need to enforce infection control measures and antimicrobial stewardship program.

KEYWORDS: Urinary tract infection, Gram-positive uropathogens, Microbiological techniques, Clinical Laboratory Standards Institute, Antimicrobial susceptibility

INTRODUCTION

One of the most commonly encountered bacterial infection is Urinary tract infection (UTI) encountered by the clinicians in developing countries and come across under both community and hospital(nosocomial) settings. It is one of the challenging problem to the clinicians because of multidrug resistant pathogens which increases the morbidity and mortality rates.The major symptoms noticed in the UTI are burning sensation during micturition and sensation to urinate frequently, an urgency to urinate all the time in the absence of vaginal discharge and significant abdominal pain [1]. These symptoms may vary from very mild to severe [2] and in otherwise healthy women may last an average of about six days[3]. Symptoms of an upper urinary tract infection or pyelonephritis, such as flank pain, rise in temperature, or nausea and vomiting along with the classic symptoms of a lower urinary tract infection, bloody urine or visible pus in the urine are also seen[2,4].

By examination of urine, if it has significant proportion of bacteria and the person is symptomless, the condition is known as asymptomatic bacteriuria [2]. Microbiologically it is defined as greater than or equal to 10^5 colony forming unit per milliliter of the organism in two consecutive urine specimens[5]. The frequency of asymptomatic

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bacteriuria vary from different populations. The bacteria which is isolated from the urine of patients with asymptomatic bacteriuria usually originate as colonizing flora of the gut, vagina or periurethralarea[1].

In women the UTI is more common specifically seen between the ages of 16 and 35 years, with around 10% of women getting an infection yearly and around 60% having an infection at some point in their lifetime[1,4]. Women are more prone to UTIs than men because of the urethra, which is much shorter and closer to the anus[6]. As the estrogen levels decreases with menopause, her risk of contracting urinary tract infections increases due to the loss of protective vaginal flora[6]. Regional studies help to gain the knowledge about the type of pathogens responsible for urinary tract infections and their antibiotic resistance patterns may help the clinician to choose the correct empirical treatment.

Among the different genera of microorganisms which cause UTI, the most common pathogens are *Enterobacteriaceae* members which are Gram-negative bacilli (GNB) like *Pseudomonas aeruginosa, Candida albicans, Escherichia coli, Acinetobacter baumannii, Proteus species, Clostridium difficile, Citrobacter species* etc. During the past decade, Gram positive cocci isolates have exhibited a remarkable ability to rapidly develop antibiotic resistance and it is of great concern. Recently, some studies have reported Gram-positive pathogens (GPC) *Staphylococcus saprophyticus, Enterococcus faecalis* and *Streptococcusagalactiae,Aerococcus,Corynebacterium,Actinobaculum* and *Gardnerella* are emerging[7].The supportive treatment for urinary tract infections is antibiotics. Gram positive bacteria showed highest antibiotic resistance to methicillin, ampicillin, amoxycillin, amikacin, ciprofloxacin, imipenem etc.

However, increasing antibiotic resistance is a cause of concern about the future of treating those with complicated and recurrent UTI[8,9]. Among Gram positive uropathogens the rate of resistance is high and frequency of resistance to antibiotics and drugs is directly linked to consumption of antibiotics[10]. It is thus, very important to carry out antibiotic sensitivity testing prior to the treatment of UTI to ensure the efficacy of the antibiotics prescribed[11]. The overprescription and misuse of antibiotics because of economic and social pressures may contribute to the increasing resistance [12].

With this background, the present study was undertaken to identify the Gram positive isolates causing UTI among patients attending a tertiary care centre and to study their antibiotic susceptibility pattern.

MATERIALS AND METHODS:

Study setting: The present study was conducted in the Department of Microbiology, GITAM Institute of Medical Sciences and Research (GIMSR), Visakhapatnam, Andhra Pradesh from a period of June 2019-July 2019.It is a hospital based descriptive study involving a data containing 624 patients having UTI. The urine samples were collected from both outpatient(OP) and inpatient(IP) departments of GIMSR Hospital suffering with UTI reported from various clinical departments

Method of collection of data:

After obtaining a written informed consent from patients satisfying the inclusion criteria, a detailed history was recorded, a complete clinical examination was done and relevant investigations performed. The investigator also asked help of the Staff nurses, Juniorresidents and Interns for the conduct of the study. They were given adequate training regarding methods of collecting urine sample for investigation. The investigator visited the Emergency room, Medicine ward, Surgery ward, Obstetrics and Gynaecology ward, Orthopaedics ward and Paediatric wards daily during the study periods and screened patients with UTIs. All the patients screened by the investigator during the study period who were eligible were taken as the denominator for the study. The criteria for selecting a patient was any patient admitted at GIMSR who fulfillthe criteria for urinary tract infection(UTIs).

Materials required:

Specimen type and collection method:

Urine samples were taken from patients showing the signs and symptoms of UTI. Specimen for urine culture was obtained carefully to prevent contamination by periurethral flora. A clean-catch midstream urine specimen was collected in a wide, sterile universal container. Contamination by periurethral and prepucial organisms was reduced by washing the genitalia with soap and water. In infants, urine samples were preferably obtained by urethral catheterization. The specimen was mostly plated within one hour of collection. A urine culture was repeated in case any contamination was suspected, *e.g.*, mixed growth of two or more pathogens, or growth of organisms that usually constitute the periurethral flora (like *lactobacilli* in healthy girls and *Enterococci* in infants and toddlers). The culture was also repeated in situations where UTI was strongly suspected but colony counts were equivocal.

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Processing of sample:

The samples were processed according to the standard Microbiological methods which include:

Macroscopic examination, Colour of the specimen.

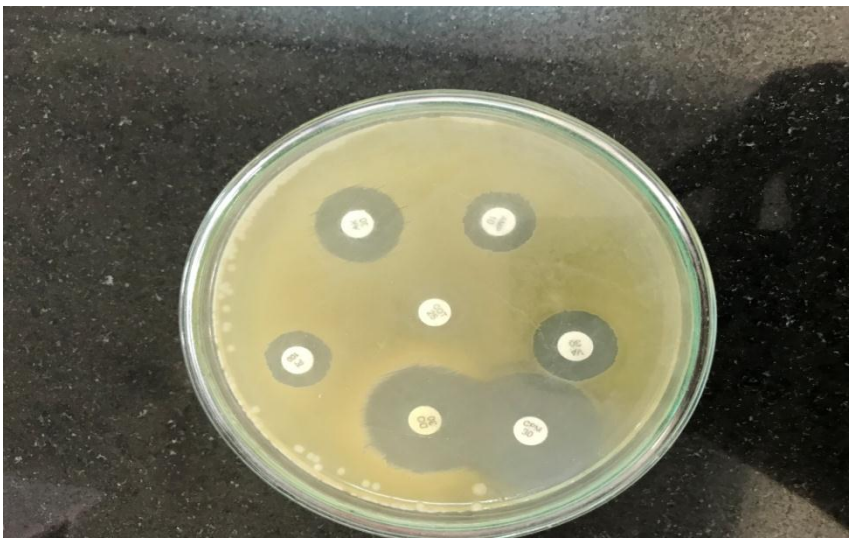
- Appearance of the specimen (turbid or clear) was noted.
- Microscopic examination through Wet mount examination to observe pus cells, red blood cells, yeast cells, casts was done.
- Culturing of the specimen: Before inoculation, the urine sample was mixed properly. An inoculating loop of SWG 28 was used. It was inserted vertically into the urine sample to withdraw 0.004ml of urine. The urine was inoculated in the medium like 5% Sheep blood agar, Mac Conkey agar and Cysteine Lactose Electrolyte Deficient (CLED) agar which is mainly used to isolate urinary pathogens were used because it gives consistent result and allows the growth of both gram positive and gram negative pathogens. After inoculation, it was incubated at 37^{o}C overnight.
- Biochemical confirmation of pathogens was done by using tests like catalase test, coagulase test, mannitol fermentation, gelatin liquefaction, phosphatase enzyme production etc. for Staphylococci. Bacitracin and optochin sensitivity test, catalase test etc. for Streptococci. Bile esculin hydrolysis test, PYR test for *Enterococci* species.

Antibiotic susceptibility testing: (according to CLSI guidelines)

Disc diffusion method:

AST was performed according to CLSI guidelines using Mueller-Hinton agar (MHA) plates using the concentration of antibiotics per discs. The plates were incubated at 37°C for 16-18h hrs. The antibiotic discs used in this study wereAmpicillin(10µg), Tetracycline(30µg), Cotrimoxazole(25µg), Ciprofloxacin(5µg), Nitrofurantoin(300µg), Gentamicin(30µg), Vancomycin(30µg), Linezolid(30µg), Norfloxacin(10µg).The inhibition zone was measured according to CLSI guidelines (CLSI Catalogue, 2016) (Figure 1).

Figure 1: Figure depicts antibiotic sensitivity test for various antibiotics



RESULTS:

Of the total 624 urine specimens collected and processed during the study period, 266 (42.63%) samples yielded positive cultures and the rest had no growth. 266 culture positive cases yielded 280 isolates. Out of them, 160 (57.14%) were Gram negative bacteria, 97 (34.64%) were Gram positive bacteria and 23 (8.21%) were Candida species. Majority of the Gram positive bacteria were isolated from females i.e. 70(72.16%). Majority i.e.35 (36.08%) were from patients in the age group of 21-30 years followed by 18 (18.55%) in 61-70 years as shown in Table 1. It was found that, 42 (43.3%) of Gram positive isolates were from outpatient department whereas 55 (56.7%) were from inpatient department as shown in Table 1.

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Table 1: Different variables associated with Gram positive bacteria (n = 97)

Variables		Frequency	Percentage (%)
Age	0-10	2	2.06
	11-20	11	11.34
	21 - 30	35	36.08
	31 - 40	15	15.46
	41 - 50	5	5.15
	51 - 60	9	9.27
	61-70	18	18.55
	71-80	2	2.06
Sex	Female	70	72.16
	Male	27	27.84
OP/IP	OP	42	43.3
	IP	55	56.7

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

Table 2depicts the frequency of distribution of Gram positive bacteria from patients of various departments. Majority i.e. 49 (50.51%) were from the department of General Medicine followed by 37 (38.14%) from Obstetrics and Gynaecology Department, 4 (4.12%) from General Surgery, 5 (5.15%) from Paediatrics and only 2 (2.06%) from Orthopaedics Department.

Table 2: Distribution of Gram positive bacteria according to various departments (n=97)

Department	Frequency	Percentage (%)
General Medicine	49	50.51
OBG	37	38.14
General Surgery	4	4.12
ORTHO	2	2.06
PAED	5	5.15
Total	97	100.0

Table 3shows the distribution of various Gram positive bacteria isolated from urine samples. *Staphylococcus aureus* 55 (56.70%) is seen as the most common organism isolated followed by Coagulase negative Staphylococcus (CONS) 22(22.68%) and *Enterococcus* species 20 (20.61%) (Figure 2).

Table 3: Distribution of different Gram positive bacteria isolated from urine samples (n=97)

Organisms	Number	Percentage
Enterococcus species	20	20.61
Staphylococcus saprophyticus	09	9.28
Other CONS (Coagulase negative Staphylococcus)	13	13.4
Staphylococcus aureus	55	56.70

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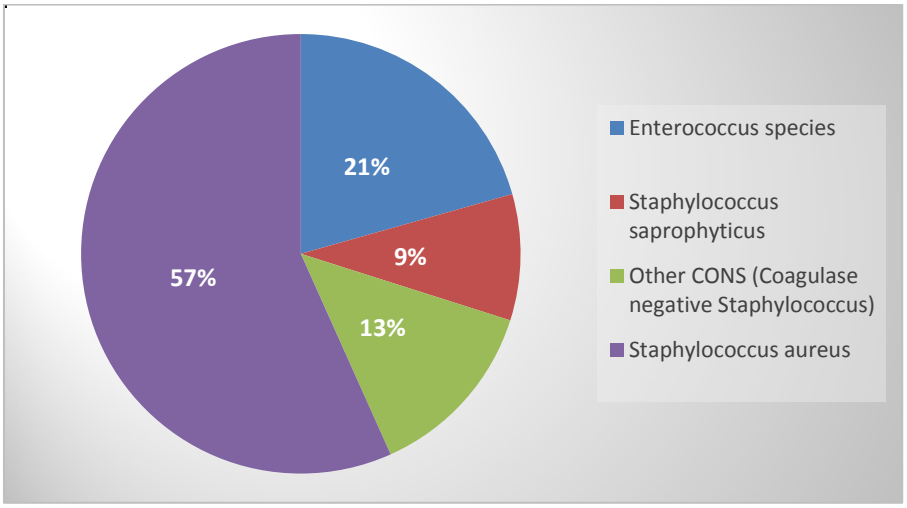
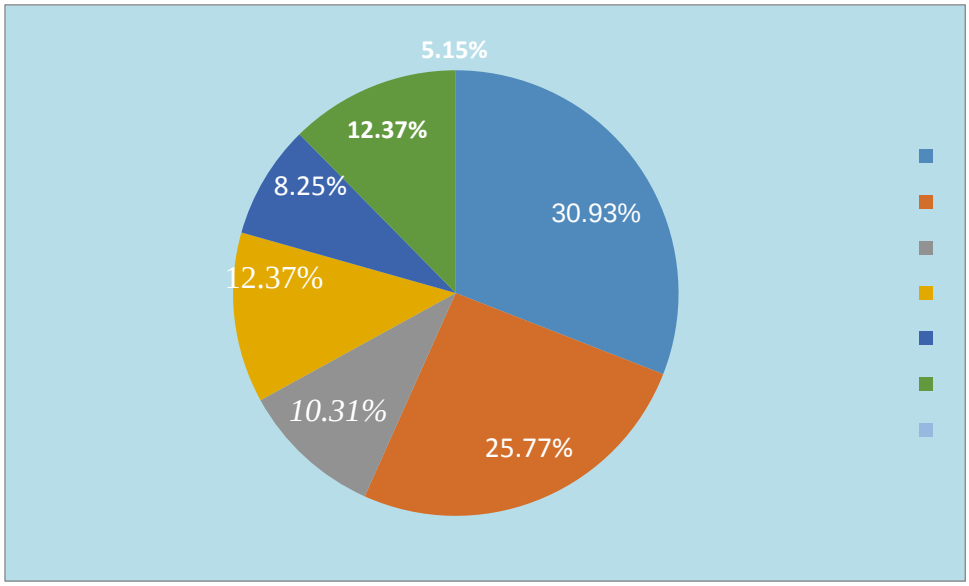
Figure 2: Distribution of different Gram positive bacteria isolated from urine samples



Among the 97 isolates of Gram positive cocci, Methicillin sensitive *Staphylococcus aureus* (MSSA) were 30 (30.93%), Methicillin resistant *Staphylococcus aureus* (MRSA) were 25 (25.77%), Methicillin sensitive CONS were 10(10.31%) and Methicillin resistant CONS were 12 (12.37%). *Enterococcus faecalis*accounted for 8 (8.25%), other *Enterococcus* species 12 (12.37%) as depicted in Fig. 3

Fig. 3: Distribution of various Gram positive isolates from urine samples



Majority i.e. 96 (98.97%) of Gram positive cocci showed resistance to penicillin followed by resistance to norfloxacin in 94 (96.91%) as shown in **Table 4.**

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Table 4: Antimicrobial susceptibility profile of Gram positive isolates (n = 97).

Antibiotics	Sensitivity		Resistance	
	Frequency	Percent	Frequency	Percent
AMP	1	1.03	96	98.97
TE	9	9.28	88	90.72
COT	38	39.18	59	60.82
CIP	5	5.15	92	94.85
NIT	46	47.42	51	52.58
G/GEN	12	12.37	85	87.63
VA	69	71.13	28	28.87
LZ	73	75.26	24	24.74
NOR	3	3.09	94	96.91
P≤0.05				

*High level gentamicin in case of *Enterococcus*speciesAMP=Ampicillin, TE=Tetracycline, COT=Cotrimoxazole, CIP=Ciprofloxacin, NIT=Nitrofurantoin, G/GEN= Gentamicin, VA=Vancomycin, LZ=Linezolid, NOR= Norfloxacin

Majority of the Gram positive cocci were sensitive to linezolid with *Staphylococcus aureus* sensitivity of 39 (71%), CONS 19 (86.4%) and *Enterococcus* species 15 (75%). They were moderately sensitive to vancomycin with *Staphylococcus aureus* sensitivity of 41 (74.6%), CONS 18 (81.9%) and *Enterococcus* species 10 (50%) as shown in Table 5.

Table 5: Antimicrobial susceptibility profile of various Gram positive bacteria (n = 97).

Antibiotics	CONS (n=22) (*Staphylococcus saprophyticus*& other CONS)		*Enterococcus* (n=20)		*Staphylococcus* (n=55)	
	Sensitivity	Resistance	Sensitivity	Resistance	Sensitivity	Resistance
AMP	0 (0)	22 (100)	1 (5)	19 (95)	0 (0)	55 (100)
TE	0 (0)	22 (100)	9 (45)	11 (55)	0 (0)	55 (100)
COT	18 (81.9)	4 (18.2)	2 (10)	18 (90)	18 (32.8)	37 (67.3)
CIP	3 (13.7)	19 (86.4)	1 (5)	19 (95)	1 (1.9)	54 (98.2)
NIT	12 (54.6)	10 (45.5)	17 (85)	3 (15)	17 (31)	38 (69.1)
G/GEN	1 (4.6)	21 (95.5)	1 (5)	19 (95)	10 (18.2)	45 (81.9)
VA	18 (81.9)	4 (18.2)	10 (50)	10 (50)	41 (74.6)	14 (25.5)
LZ	19 (86.4)	3 (13.7)	15 (75)	5 (25)	39 (71)	16 (29.1)
NOR	0 (0)	22 (100)	0 (0)	20 (100)	3 (5.45)	52(94.55)
P≤0.05						

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DISCUSSION: The present investigation was a short-term study intended to show the pattern of UTIs prevalent in different age and sex groups, their causative organisms and their antimicrobial susceptibility profile of Gram positive bacteria isolated. Out of the total 624 urine samples processed, only 266 (42.63%) showed growth which is comparable with other studies done in different study settings and time periods.[13] However, the proportion of positive cultures was higher in a couple of studies[38]while few other studies reported low isolation rates[14,15]. Further, 97(34.84%) Gram positive bacteria were isolated in our study similar to the findings of other studies[38] while lower rates were reported by other studies. [14]

The proportion of females was more in patients presenting with symptoms of UTI i.e.70 (72.16%) which correlates well with the findings of other studies [3-15]. 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 et al. reported higher prevalence of UTI in males[16]. Majority i.e. 35 (36.08%) of the patients were in the age group of 21-40 years. Similar trend was reported by Kumar etal.[16]

In our study, *Staphylococcus aureus* was the commonest isolated Gram positive cocci 55(56.70%) followed by CONS 22 (22.68%). This result were in agreement with the previous studies [16] On the contrary some studies have isolated CONS and *Enterococci*species[17, 18] as the predominant uropathogens in their studies.

Majority i.e. 96 (98.97%) of Gram positive cocci showed resistance to penicillin followed by resistance to norfloxacin in 94 (96.91%)similar to a few studies done previously[19] and in contrast to some other studies which have observed a higher degree of sensitivity to norfloxacin[17,18]. Majority of the Gram positive cocci were sensitive to linezolid with *Staphylococcus aureus* showing sensitivity of 39 (71%), CONS 19 (86.4%) and *Enterococcus* species 15(75%). They were moderately sensitive to vancomycin with *Staphylococcus aureus*sensitivity of 41 (74.6%), CONS 18 (81.9%) and *Enterococcus* species 10 (50%).

CONCLUSION:

The results of the present study has concluded that Gram positive cocci are the significant bacteria isolated in the UTI patients especially females in between the age group of 21-30 years. Among them *Staphylococcus aureus* is the most prevalent uropathogen showed maximum sensitivity to Linezolid followed by Vancomycin and completely resistant to Ampicillin. 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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