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ORIGINAL ARTICLE

Significant bacteriuria among requested repeat urine samples and its clinical correlation

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ABSTRACT

Background and Objectives: Urinary tract infections (UTI) are the most common bacterial infections in both outpatient and inpatient department received for routine bacterial culture and sensitivity. We looked for significant bacteriuria in requested repeat urine sample after primary urine culture yielded significant growth (>10^5 CFU/ml) of ≥3 types of colonies. Also studied, different isolates grown with their sensitivity pattern and contamination rates of urine samples from different departments.

Materials and Methods: In routine, primary urine cultures yielding ≥3 types of colonies on Cystine Lactose Electrolyte Deficient (C.L.E.D) were requested for repeat samples, collected with aseptic precautions after proper instructions. Data was analyzed for the Microbiological profile and its clinical correlation.

Results: Among 617 received requested urine samples, 292 (47.3%) yielded significant bacteriuria. Clinical details were available for 252 cases out of which 100 (39.7%) showed asymptomatic bacteriuria, 87 (34.5%) complicated UTI and 65 (25.7%) uncomplicated UTI. Null hypothesis was rejected as 292 (47.3%) of the received repeat samples showed significant bacteriuria and 325 (53%) showed normal flora/no growth i.e. there is a 50% chance of getting either a positive culture or normal flora/no growth in repeat urine samples after the primary urine culture showed ≥3 types of colonies. It indicates the importance of requesting repeat urine samples for an accurate urine culture report. Male patients were significantly associated with significant bacteriuria and complicated UTI (p= 0.001). *Escherichia coli* (n=112, 28%) was the most common followed by *Klebsiella* species (n=66, 16.4%) and *Enterococcus* species (n=69, 17.2%). 183 (45.6%) isolates were Multi-Drug Resistant (MDR) Gram Negative Bacilli (GNBs), *Escherichia coli* (50.3%) being most common. Vancomycin Resistant *Enterococcus* (VRE) (n=8, 2.0%) was also isolated.

Conclusion: Our study justifies the rationale for asking a repeat urine samples which helps in providing an appropriate microbiological report with antibiotic sensitivity pattern, hence preventing unwanted reporting of commensals/contaminants facilitating evidence based therapy.

Keywords: Repeat urine samples; Significant bacteriuria; Multidrug resistant; Urinary tract infection

INTRODUCTION

Urinary tract infections (UTI) are believed to be one of the most common bacterial infection affecting 150 million people worldwide and consist of infection, invasion and inflammation of urethra (urethritis), bladder (cystitis), ureters, kidneys (acute and chronic pyelonephritis) and adjacent structures such as perinephric fascia, epididymis and prostrate (1-3). Despite the fact that UTIs are a huge problem for patients and a burden for the Microbiology laboratory, still there is no actual count of the cases suf-

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fering from UTIs as it is not a notifiable disease. It has been implicated in causing morbidity in children <2 years of age (both in community and hospital setting), males above 50 years of age and females of all ages (4, 5). Clinically UTI has been classified as uncomplicated and complicated. Uncomplicated UTI is when the patient is otherwise normal and does not have functional (neurological) and structural abnormality in genitourinary tract (GUT). They are further classified into lower UTIs (cystitis) and upper UTIs (pyelonephritis) (4). Complicated UTIs are defined as UTI with a comorbid condition such as a risk factor for UTI with a structural abnormality like stricture urethra, neurological defect in GUT or a foreign body such as renal calculus and indwelling catheters (2-5). Increased morbidity and mortality has been associated with Catheter associated UTIs (CAUTIs) with risk factors such as prolonged catheterization, female gender, old age and Type 2 Diabetes Mellitus (1, 4). The diagnosis of uncomplicated UTI is dependent on clinical symptoms confirmed by a positive culture (2). Asymptomatic bacteriuria is defined as a positive urine culture for significant growth (>10^5 CFU/ml) of a pathogen in absence of clinical symptom. It puts pregnant patients at high risk of developing clinical pyelonephritis and symptomatic UTI, though it has a small role in preventing prematurity (1, 4). Detection of asymptomatic bacteriuria especially during childhood defines the population at risk of developing symptomatic infection later in life. Studies suggest that 50% girls with significant bacteriuria at school-age develop symptomatic bacteriuria when they become sexually active. Other high risk group patients are diabetics in whom the treatment for asymptomatic bacteriuria needs to be individualized (1).

Some of the methods routinely used for urine sample collection which might have contamination are (1):

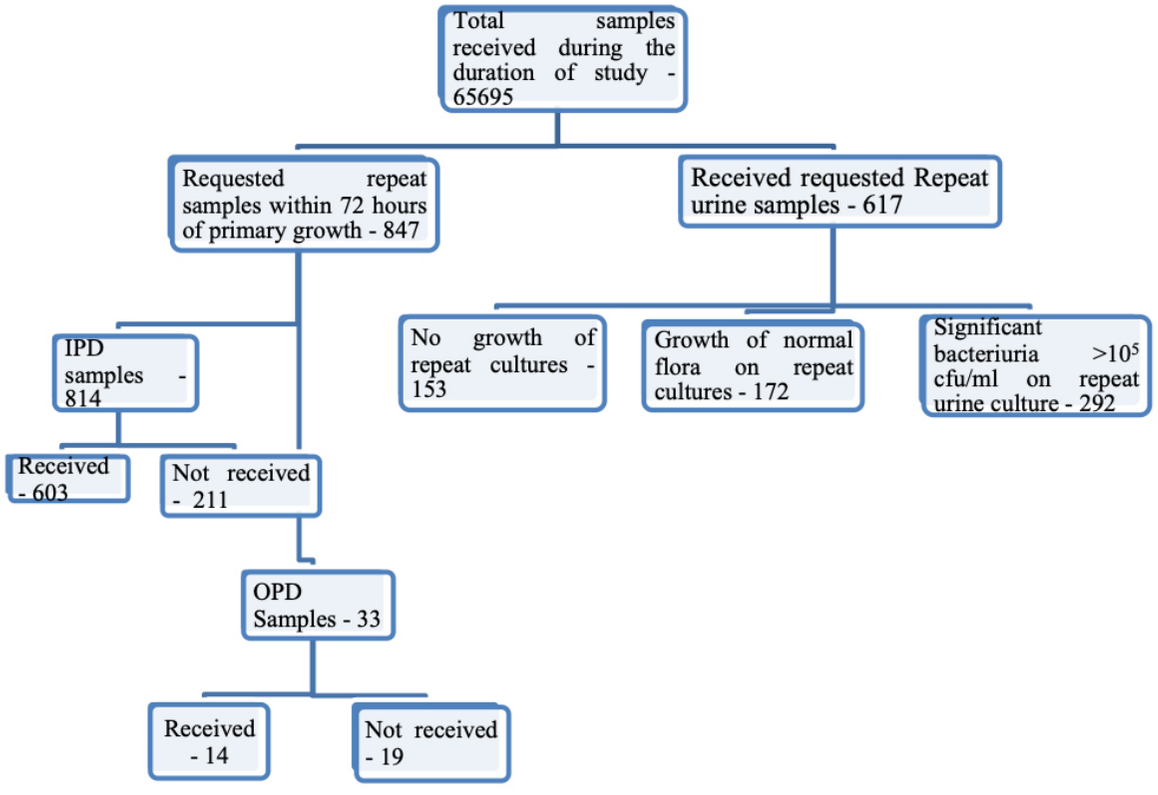
1. Urine sample collected without appropriate and clear instructions
2. Random samples delivered without instructions
3. Samples from long term urethral catheter bag or uro-condom without aseptic precautions

Guidelines suggest a compulsory urine culture for cases of upper or complicated UTI as the diagnosis is difficult and aetiology is quite different with increased frequency of complications and antimicrobial resistance (6). Sometimes, clinical microbiologists face a situation in which significant growth of ≥3 types of colonies on the primary culture plates are yielded. Reporting in those cases might lead to misinterpretation of commensal flora or contaminants as urinary pathogens resulting in misuse of antibiotics, leading to emergence of drug resistance. On the other hand, non-reporting may deprive the patient from getting an appropriate treatment. This was the rationale to conduct our study for requesting repeat samples after proper collection to isolate and differentiate significant pathogens from commensal flora/ contaminants.

MATERIALS AND METHODS

We performed a retrospective descriptive study for a duration of two and a half years from 1st January 2016 to 31st July 2018 in a multispeciality tertiary care hospital catering 2000 beds. Institutional Ethics Committee clearance (IEC Code no.: 233/2018) was obtained. It was done to look for prevalence of significant bacteriuria and microbiological profile of the isolates obtained with a clinical correlation in samples where primary cultures grew ≥3 types of colonies. Requested Repeat Urine Sample was defined as a properly collected repeat urine sample which was specifically requested when there was significant growth (>10^5 CFU/ml) of ≥3 types of colonies in culture from the primary sample. Multidrug Resistant (MDR) strain was defined as a strain resistant to >3 classes of antibiotics (7, 8) (Fig. 1).

Our null hypothesis suggested that there should be no change in growth results on requesting a repeat urine sample. Sample size for the study was calculated based on a pilot study which was conducted in the same laboratory but on a different set of patients among 100 random samples during same period. This methodology was adopted due to lack of evidence in literature pertaining to any kind study on requested repeat urine samples and to calculate the prevalence of repeat samples yielding significant bacteriuria. Sample size, n=816 for requested repeat urine samples was calculated with prevalence (32%) (from the pilot study) to have 95% Confidence Interval (C.I.) and 10% variability. As per this data we requested 847 samples in our hospital but received 617 (response rate of 73%) samples only. However, the available literature shows that the prevalence of urine culture contamination can vary from 32% to 40% (9, 10). Considering the variability in the prevalence of



IPD – Inpatient Department; OPD – Outpatient Department

Fig. 1. Flow diagram of requested repeat urine samples received during the study period

repeat sample, our study had adequate sample size for 32% prevalence with precision of 12% and 95% C.I. Clinical data was captured from case sheets of the patients from Medical Records Department (MRD) by principal investigator. Patient's confidentiality was maintained by coding each patient separately. As per International Clinical Practice Guidelines (IDSA) (11), Catheter Associated – Urinary Tract Infections (CA-UTI) in patients with indwelling urethral, indwelling suprapubic, or intermittent catheterization was defined by the presence of symptoms or signs compatible with UTI with no other identified source of infection along with $\geq 10^3$ colony forming units CFU/mL of ≥1 bacterial species in a single catheter urine specimen or in a midstream voided urine specimen from a patient whose urethral, suprapubic, or condom catheter has been removed within the previous 48 hours. The method of collection in adults and paediatric age group was according to the protocol followed by treating clinician i.e. either a Midstream Clean Catch Urine or urine from catheter line or suprapubic collection. Received samples were collected, processed and cultures were read according to standard protocol (Table 1).

Inclusion criteria. All requested repeat urine samples received during the study period within 72 hours. Typically a span of 24 hours already existed before requesting for a repeat urine sample and antimicrobial therapy would have little effect on the outcomes of prognosis with added duration especially in the patients of uncomplicated cystitis (6) and also it has been stated that if there is no improvement in the patient on antimicrobial therapy then a repeat urine culture should be requested within 72 hours.

Exclusion criteria. Any sample yielding heavy growth of ≥3 types both in primary and repeat urine sample was not included in the study. Any sample which could not be collected due to discharge or death of the patient was not included in the study.

Statistical analysis. Statistical analysis was done on the master excel sheet by SPSS software version

Table 1. Methodology of Urine sample processing and culture interpretation (12, 13).

Method	Description
Sample Processing	Appropriate Standard precautions were followed while handling samples in the laboratory. Gram Stain was made for urine sample and was looked for pus cells, host cells, bacteria and other organisms to correlate with the growth on culture plate. Each plate with appropriate sample number was inoculated with a 2 mm calibrated loop delivering 0.005 ml of urine sample.
Media Used for Inoculation	5% Sheep Blood Agar (5% SBA) (HiMedia, India) and Cystine Lactose Electrolyte Deficient Agar (CLED) (HiMedia, India)
Inoculation of media	After sterilization on a flame loop is inserted into the urine container vertically, and inoculated onto the culture medium. Both 5% SBA and CLED were inoculated with one loop full each using modified Mayo technique
Culture Reading	Each loop delivers 0.005 ml; 0.005 × 200 = 1ml Therefore, total no of colonies × 200 = Colony forming units per ml done from CLED media and interpretation will be done as per the Standard protocols. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method following the latest CLSI guidelines (14, 15). For all Meropenem resistant organisms, Colistin was tested by automated Vitek 2 system and interpretation done as per CLSI guidelines (2016-2018).

23. Descriptive statistical analysis was performed using mean and standard deviation for continuous variables. Categorical variable was reported using numbers and percentages. The proportion of repeat urine samples were reported. p value <0.05 was considered as significant on performing the Chi square test. Student paired t test was performed to calculate the mean age of the cohorts studied.

RESULTS

During the study duration a total of 65,695 urine samples for aerobic culture and sensitivity were received, out of which 847 showed growth of ≥3 types of colonies and were requested for repeat urine samples with written instructions for proper sample collection. Among 847 requested samples male patient samples were 328 (38.7%) and female were 519 (61.3%) i.e. M:F ratio was 0.63. Fig. 2 gives demographic details of the patients which were included in our study.

A total number of 617 (72.8%) requested samples were received within 72 hours from the time of request and were included as per the inclusion criteria of our study. On performing T-test no difference was found between the mean age of two cohorts (no growth/normal flora and significant bacteriuria) which were studied in primary analysis. It is a significant finding, as we can generalise our findings onto the samples which were not received but showed significant growth of ≥3 types of colonies on primary culture plates.

Out of the received samples, 292 (47.3%) showed significant bacteriuria (culture positive) on repeat cultures whereas the remaining 325 (52.7%) showed either growth of normal flora or no growth in culture as the final result. The percentage of significant bacteriuria in repeat urine sample, was found maximum in Pediatric Wards (around 67% repeat samples showed significant bacteriuria) followed by Urology, Surgery (General Surgery, Neurosurgery, Pediatric Surgery), Medicine [General Medicine, Physical Medicine and Rehabilitation (PMR), Psychiatry], Nephrology and Obstetrics and Gynecology (Fig. 3).

Clinical details were collected for 252 (86.3%) patients, out of which 127 (50.4%) were males and 125 (49.6%) were females. Among them, growth of only one type of bacteria causing significant bacteriuria was observed in 181 (71.8%) patients whereas the remaining 71 (28.2%) showed growth of >1 type of microorganisms with both the isolates showing significant growth (>10^5 CFU/ml). Among these 71 patients, complicated UTI were 29 (40.8%) and uncomplicated UTI were 42 (59.2%). Among 252 patients, 100 (39.6%) patients were diagnosed with asymptomatic bacteriuria, 87 (34.5%) were complicated UTI and remaining 65 (25.7%) had uncomplicated UTI (Fig. 4).

Among 127 male patients, 66 (52%) were diagnosed with complicated UTI, whereas 68 (54.4%) females had other diagnosis with asymptomatic bacteriuria. Association of different types of UTI with gender was

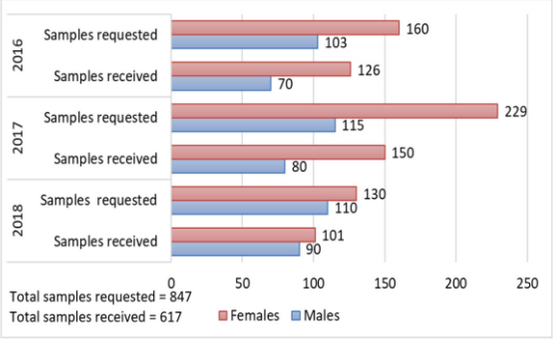


Fig. 2. Demographic data representative of male and female urine samples

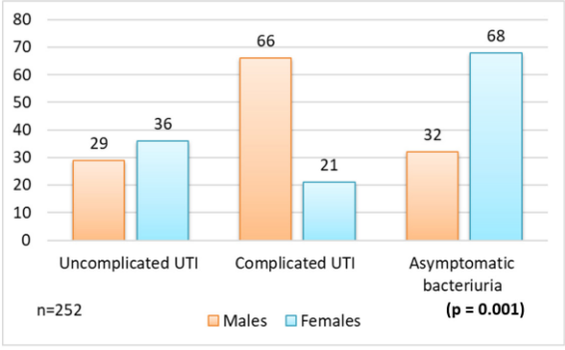


Fig. 4. Patients with significant bacteriuria and their clinical diagnosis

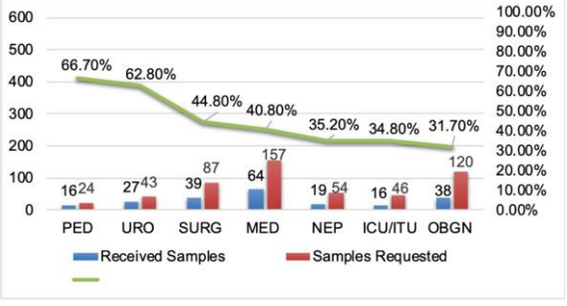


Fig. 3. Significant bacteriuria in requested repeat urine samples from different wards; PED – Pediatrics Wards; URO-Urology Wards; SURG – Surgery Wards (General Surgery, Neurosurgery, Pediatric Surgery); Medicine Wards [General Medicine, Physical Medicine and Rehabilitation (PMR), Psychiatry]; NEP – Nephrology Wards; OBGN - Obstetrics and Gynecology Wards

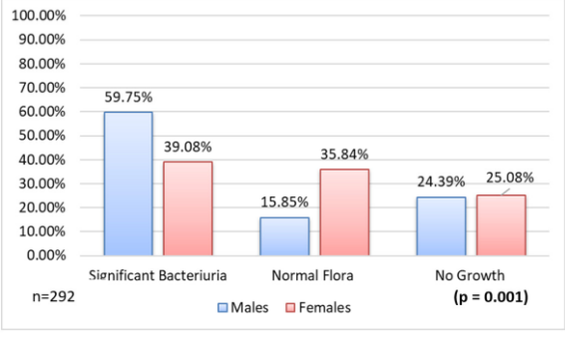


Fig. 5. Microbiological profile of requested repeat urine samples among males and females with diagnosis.

found to be statistically significant (p = 0.001) (Fig. 4).

On performing Pearson Chi-Square test association of significant bacteriuria in male patients among received repeat samples was found to be statistically significant (p = 0.001) as compared to the female patients (Fig. 5).

The most common systemic manifestation among the patients with complicated UTI was hypertension (HTN) 27 (10.71%) and type 2 Diabetes Mellitus (T2DM) 26 (10.3%). Patients with HTN also had associated renal dysfunction with anatomical and functional anomalies included stricture urethra 2 (7.4%), Benign Prostrate Hypertrophy (BPH) 3 (11.1%), neurogenic bladder 2 (7.4%), Chronic Kidney Disease 12 (44.4%), carcinoma of cervix and prostrate (1, 3) which cause both structural and functional abnormality.

Midstream Clean Catch urine (MSCCU) method of collection was used in 133 (52.8%) of the patients and the rest 119 (47.2%) samples were either sent after collection from a catheter or a urinary condom (Fig. 6). On performing Pearson Chi-Square test we observed uncomplicated and complicated UTI were found to be significantly associated with samples collected from Foley's Catheter or Urinary Condoms (p = 0.003) (Fig. 6). Growth of one kind of pathogen resulting in UTI was found to be strongly associated with MSSCU as a method of collection, whereas growth of more than one kind of pathogen was associated with collection of urine from a Foley's catheter or Urinary Condom (p = 0.0253).

Among 292 repeat samples which showed significant bacteriuria, a total of 401 isolates were identified (Fig. 7). Among those 401 isolates, 300 (74.8%) were Gram negative bacteria, 87 (21.7%) were Gram positive cocci and 14 (3.5%) grew *Candida* spp. *Escherichia coli* was the most common bacteria isolated with 112 (27.9%) isolates. The second most common isolate was *Enterococcus* species accounting for

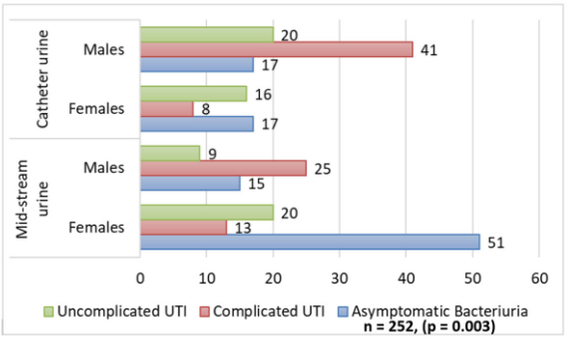


Fig. 6. Schematic representation of different methods of requested repeat urine sample collections.

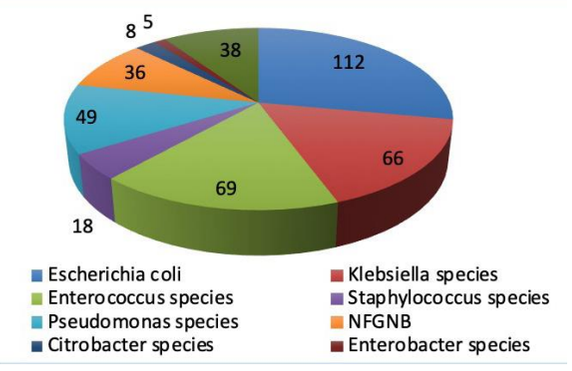


Fig. 7. Spectrum of clinically important pathogens isolated from requested repeat urine sample.

69 (17.20%) of total isolates followed by *Klebsiella* species 66 (16.45%), Non-Fermenting Gram Negative Bacilli (NFGNB) 36 (8.97%) and *Pseudomonas* species 49 (12.21%). Others were *Proteus* species 10 (2.49%), *Morganella* species 1, *Providencia* species 1, *Citrobacter* species 8 (1.99%), *Enterobacter species* 5 (1.24%), *Serratia marcescens* 2 (0.5%) and *Aeromonas* (1 isolate). In Gram positive cocci, *Staphylococcus* species 18 (4.48%) was the second most common isolate followed by Group B -hemolytic streptococcus (3 isolates) with pure growth in the repeat cultures. All the 14 yeast isolates were identified as *Candida* species other than *Candida albicans.*

Antibiotic sensitivity was performed using Kirby Bauer Disc Diffusion test and was interpreted using latest CLSI standards (14, 15). Out of 300 GNB isolates, 183 were found to be MDR-GNB (Table 2), among which *E. coli* (50.3%) was the commonest MDR-GNB followed by *Klebsiella* species (25.7%). Among 183 MDR strains, 115 were found to be carbapenem (meropenem) resistant. Colistin sensitivity was performed as per the CLSI guidelines (2016-2018) for 88 (76.5%) isolates out of all meropenem resistant GNBs. On performing colistin sensitivity, 14 (16%) isolates were found to be resistant to colistin. Among them,4 isolates were *Klebsiella* species, 4 were NFGNB, 3 were *P. aeruginosa*, 1 isolate was *Enterobacter* species and remaining 2 were *Proteus* spp. and *Morganella* spp. which are intrinsically resistant to colistin. 8 VRE were also isolated (Table 3). No growth of MRSA was observed. All 3 isolates of -Beta Hemolytic Streptococci (BHS) (GBS) were sensitive to penicillin.

DISCUSSION

Most of the work in a Clinical Microbiology Laboratory can be denoted to routine urine cultures (16). Therefore accurate handling of pre-analytic phase of urine culture such as collection, preservation, and storage of urine specimens is an important factor in generation of reliable and accurate culture reports (1, 6). As urine samples for culture and sensitivity are majority of the specimens received, it was felt that there was a need to study and formulate a protocol for requesting repeat urine samples to access significant bacteriuria for primary cultures which gave heavy growth (>10^5 CFU/ml) of ≥3 types of colonies in a period of 24 hours (6).

In urine cultures which had significant growth of ≥3 types of colonies written and oral instructions were given to the patients for collection of a repeat sample as suggested in a study conducted by College of American Pathologists, that, there is statistically significant decrease in urine contamination on instructing the patients about proper sample collection technique (10). After primary analysis we found that 48% of the received samples had significant bacteriuria and rest had either growth of normal flora or no growth as final result which emphasizes on importance of proper sample collection technique.

According to Burd et al. routine urine cultures should not be done for uncomplicated cystitis as those cases have predictable microbiology, low morbidity and in many cases reliable clinical diagnosis leading to start of an empirical therapy (6). They also reported that mixed growth with colony count more than 10^4/10^5 CFU/ml warrants a repeat urine culture especially when growth of a single pathogen is suspected. For children suffering from critical illness, thresholds of ≥5 × 10^4 CFU/ml in catheterized patients and ≥10^5

Table 2. Antibiotic Resistance of all the Gram negative bacteria isolated from requested repeat Urine samples (n=263)

Organism AST† panel – GNB (mcg)	*E. coli* n = 112	*Pseudomonas* spp. n = 49	*Klebsiella* spp. n = 66	NFGNB n = 36
Netilmicin	29.50%	26.50%	46.90%	44.40%
Gentamicin (10)	43.80%	34.70%	53%	41.70%
Amikacin (30)	42.90%	32.70%	53%	38.90%
Nitrofurantoin (300)	24.10%	-	91%	72.20%
Cefotaxime (30)	87.50%	-	69.70%	58.30%
Ceftazidime (30)	83.90%	30.60%	68.10%	52.80%
Norfloxacin (10)	82.14%	-	63.60%	44.40%
Ciprofloxacin (5)	79.50%	44.90%	68.10%	38.90%
Meropenem (10)	40.20%	34.70%	48.50%	41.70%
Piperacillin-Tazobactum (100/10)	58%	36.70%	60.60%	41.70%
Colistin (MIC)	0%	6%	6%	11.10%

Table 3. Antibiotic Resistance of all the Gram positive bacteria isolated from requested repeat urine samples (n=84)

Organism AST† panel – GPC (mcg)	*Enterococcus* species n = 69	*Staphylococcus* species n = 15
Ampicillin (25)	26%	-
Ciprofloxacin (5)	82.60%	73.30%
Gentamicin (10)	44.90%	33.33%
Nitrofurantoin (300)	18.84%	-
Penicillin (10)	33.33%	86.70%
Teicoplanin (30)	10.20%	-
Vancomycin (30)	11.60%	-
Trimethoprim-Sulfamethoxazole (23.75/1.25)	-	40%
Amikacin (30)	-	60%
Cefoxitin (30)	Erythromycin (15)	53.33%
Erythromycin (15)		73.33%

CFU/ml of not more than 2 types of colonies in uncatheterized patients maybe appropriate (6).

Among received samples female urine samples were significantly higher with a M:F ratio of 0.63 and is consistent with findings of Harshkumar B et al. (17) and Iregbu et al. (18). It also stands firm with our previous knowledge that there is more chance of contamination in urine samples collected from female patients as compared to the male patients (1, 18). This can be explained by the fact that female urethra (3-7 cm) is much smaller as compared to male urethra (10-12 cm) and lies near perianal region leading to increased chances of contamination in a urine sample, if not collected properly (1, 3).

After primary analysis, our null hypothesis was rejected i.e. there is almost a 50% chance of getting a growth of normal flora/no growth or significant bacteriuria on repeating the urine culture where primary urine samples are contaminated resulting in heavy growth of ≥3 types of colonies in primary culture plates. Approximately, equal number of samples were obtained by MSCC Urine Technique (52.7%) and catheter line or uro-condom. It adds more value to rejection of null hypothesis as conventionally, it is thought that ≥3 types of colonies are seen in the samples which are obtained by catheter lines or uro-condoms.

This is a big leap towards preventing antibiotic resistance and treatment in patients who suffer from complicated UTI or debilitating chronic illnesses e.g. quadriplegia, end stage renal disease, as repeat cultures give a better picture of microbiological profile with resistance patterns for antibiotics used to treat UTIs. It is also further supported by study done by Eileen M Burd et al. (6) that if cultures of samples from indwelling catheter yield >3 types of bacterial colonies on culture, they should not be worked upon, especially if catheters are in place for long periods because they are readily colonized by organisms which are not actually present in bladder. Moreover, this fact is further supported by published literature (1). Our finding corroborates it as we found out that only 47.3% (292) of the received requested urine samples showed significant bacteriuria and rest of the samples either showed no growth in culture or normal flora.

Percentage significant bacteriuria was observed maximum in paediatric patients which is an important finding with respect to the fact that collec-

tion of urine sample is difficult in these patients. It is also a vulnerable group of patients for contracting UTIs (males>females). Our study emphasizes on the importance of evidence-based therapy with proper administration of antibiotics supporting antibiotic stewardship, especially in these patients so as to prevent development of resistance to first line drugs.

Second most common patient group with significant bacteriuria on repeat cultures belonged to Urology wards. They mostly consisted of patients suffering from complicated UTIs with prolonged indwelling catheter. Uncomplicated and complicated UTI cases were also found to be significantly associated with sample collection from Foley's catheter line and uro-condom (p=0.003). Growth in samples from Foley's catheter line and uro-condom was also significantly associated with >1 type of pathogen on repeat cultures especially in patients with neurogenic bladder (p=0.03) which is line with the fact that patients with catheter have a risk of developing polymicrobial and nosocomial UTI (3). Approximately 72% of the patients showed growth of single kind of pathogen but the remaining 28% who showed growth of >1 type of pathogen, were the ones suffering from chronic debilitating disease like Anaemia, CKD, End stage Renal Disease, Carcinoma of either prostrate or cervix, structural and functional abnormalities like neurogenic bladder, stricture urethra or had multiple chronic co-morbidities such as Type 2 Diabetes Mellitus, Ischemic Heart Disease, Renal parenchyma damage due to hypertension, B/L Hydronephrosis and paraplegia (3).

When these findings are coupled together it is important that antibiotic sensitivity, as accurate as possible, should be reported because there are more chances of growing a >1 type of pathogen on repeat urine cultures. Patients who suffer from >1 pathogen, though, are not statistically significant still accurate reporting of antibiotic sensitivity is important in this high risk group to prevent infection developing due to MDR organisms. In our study, 29% of the patients had complicated UTI due to renal abnormality.

In male patients statistically significant association of significant bacteriuria and diagnosis of complicated UTI was observed. It can be explained by the fact that most of the samples received were from Urology and Neurology wards in which male patients were on long-term indwelling catheter due to neurogenic bladder, paraplegia, stricture urethra or TURP which are predisposing factors for developing UTI as stated in already published literature (3, 5, 19). It also leads us to a conclusion that there is a definite need to request a repeat urine sample in male patients with complicated UTI, as there is a high likelihood of them resulting in significant growth of a pathogen. It is also true for female samples which were significantly associated with growth of normal flora or diagnosis of asymptomatic UTI.

Asymptomatic bacteriuria was associated with growth of a single pathogen and to sample collection by MSCCU technique hence leading us to a conclusion that it is rational to request for a repeat urine sample in patients of asymptomatic bacteriuria because a significant number of them showed growth of one type of pathogen on repeat culture. Therefore, reporting of ≥3 types of pathogens can lead to unwanted reporting of antibiotics in such cases, adding onto already increasing antibiotic selection pressure resulting increased rate of infections caused by MDR uro-pathogens.

In 252 patients (for which clinical details were available) we found that 87 (34.5%) had diagnosis of complicated UTI among which 57 (65.5%) had growth of only one type of uro-pathogen, both these numbers are clinically significant. Hence there is a need for a better picture of significant bacteriuria with its antimicrobial profile. Type 2 Diabetes Mellitus has been implicated in malfunctioning of immune system making the patient vulnerable to infections and is also a known risk factor for Catheter Associated UTI (CAUTI) (3-5).

It is a known fact that asymptomatic bacteriuria in male and female patients with Type 2 Diabetes Mellitus has same microbiological profile as non-diabetics but patients who have functional anomalies are at risk of developing infection by MDR pathogens (20). Most of the patients in our study who had Type 2 Diabetes Mellitus as a comorbid condition were suffering from chronic or debilitating diseases with functional anomalies like Chronic Kidney Disease, carcinoma – prostrate, neurogenic bladder. This emphasizes the need for appropriate reporting to prevent further progression of these patients towards developing UTIs by carbapenem resistant strains of GNBs. HTN was clinically associated with portal HTN and renal parenchyma changes (5). Abnormalities in the renal anatomy can cause obstruction of urine leading to patient being prone to recurrent UTIs.

Out of the samples which showed significant bacteriuria, a total of 401 uro-pathogens were isolated. *Escherichia coli* was the most common isolate accounting for approximately 112 (28%) of the total number which is in line with the published literature (3, 17). It was closely followed by Gram positive cocci, *Enterococcus* species which accounted for 69 (17.2%) of the total isolates. The total number of Gram Negative Bacilli were more than Gram Positive Cocci (GPC). This is similar to the previous knowledge that GNBs are commoner cause of UTI (both complicated and uncomplicated). The importance of repeat cultures is also emphasised by isolation of β-Hemolytic streptococci, which is a known cause of UTI resulting in complications in pregnancy like stillbirth, pre-term birth etc. (21).

The results of antibiotic sensitivity were interpreted using CLSI standards each year. Among the 401 isolates, approximately 54% isolates were multi-drug resistant, out of which 183 were GNBs and 34 were GPCs. This corroborates with the previously published literature that there has been an increasing trend in the resistance in GNBs which cause UTIs. Among the MDR-GNB, 69 (37.7%) were sensitive to carbapenem. Among the carbapenem resistant MDR-GNB, *Escherichia coli* was the most common isolate followed by *Klebsiella* and NFGNB. Colistin resistance was also observed in 28.6% of the carbapenem resistant *Klebsiella* and NFGNB. These results indicate that there is an increase in multi-drug resistance in general and also an increasing trend towards carbapenem resistance in GNB, therefore an increase in colistin resistance due to antibiotic selection pressure.

Our study signifies the rationale for asking repeat sample which avoids reporting of commensals and treatment with appropriate antibiotics. Despite that it was a retrospective study due to which clinical details of all the patients could not be captured. Due to retrospective nature of the study and inclusion criteria of samples to be studied which were received in 72 hours we were not able to include many of the samples even if they were just a few hours delayed. However, in a prospective study if a strict protocol is followed for collection of samples based on the gender, clinical classification of UTI and comorbidity, more significant results might be achieved. While requesting the repeat urine samples the time limit of 72 hours has to be followed as the best results are shown within this time period.

CONCLUSION

In conclusion, our study helped in developing a protocol for requisition of a repeat urine sample and also broadened horizons of our knowledge towards the prevailing contamination rates in different wards of our hospital. It not just emphasizes on the importance of proper urine collection technique, but also gives a unique perspective about clinical profile of the patient and an elaborated microbiological profile of significant bacteriuria in the requested repeat urine samples and its importance, hence rationalizing evidence based medicine which is in concordance with policies of antibiotic stewardship.

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