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A Study of Antimicrobial Substances in Urine in Patients Attending the Outpatient Department in a Tertiary Care Hospital

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ABSTRACT

Infections of the genitourinary tract are a common cause of morbidity in both healthy and immunocompromised individuals. Urine analysis, microscopy, culture and antibiotic susceptibility testing are clinical microbiology procedures that aid in the diagnosis and elucidation of UTI etiology. The presence of antibiotics in urine samples presented for culture might further complicate the identification of UTIs, especially when they are easily accessed without a prescription. This condition may impair bacterial pathogen recovery and precise colony count, resulting in false-negative findings and diagnostic problems, especially in symptomatic patients. Antibiotics can temporarily reduce bacterial counts per milliliter of urine, resulting in a transient remission of clinical symptoms, which can obscure treatment success in chronic or recurrent asymptomatic infections. Therefore, we aimed to study the association between data collection from patients pertaining to antibiotic history and the presence of AMS in urine samples, to construe the effect of antibiotics in diagnosis of UTIs and correlate the presence of AMS in urine with culture outcome also to study antibiotic susceptibility pattern of bacteria isolated from such urinary tract infections. During a year and a half study period, a prospective study was carried out with three hundred cases of patients who visited the genitourinary OPD of the tertiary care setting and were instructed to get a microbiological investigation of urine culture and sensitivity. Patients who were first-time OPD visitors with signs and symptoms of UTI were included. In addition to obtaining a thorough history from each patient and filling out a proforma, these patients were asked whether they had recently or presently consumed any antimicrobial agents. After collection of midstream urine samples from the patients, these samples were subsequently subjected to standard microscopy, a microbiology test for the presence of AMS, culture identification and antibiotic susceptibility in accordance with CLSI criteria. Majority 29.1% (87) of the cases observed were from the age group 21-30 years with female predominance observed among the study population. The signs and symptoms observed were abdominal pain 73% (219), increased frequency 61% (183), fever with chills 37.3% (122), followed by dysuria 30.6% (92) and 4% (12) cases of blood in urine. Out of the 300 cases observed, 23% (69) cases had AMS in the samples. 29.0% (20) of AMS cases had Obstructive pathology followed by Diabetes Mellitus 5.8% (4) and 01.4% (1) case of pregnancy was observed under comorbidities. 40.8% (29) of cases who were on Antibiotic had AMS which was significantly higher ($p = 0.001$) as compared to the 17.5% (40) of cases who were not on Antibiotics but had AMS. It was observed that, 27.6% (35) of the cases having adequate history on requisition forms had AMS which was higher than the 19.7% (34) cases who did not have adequate history on requisition forms. Amongst the 20 uropathogens obtained among AMS patients, the most common organism was *E. coli* 40% and 20% CONS (coagulase negative staphylococcus) while *Enterococcus* species, Pseudomonas aeruginosa, *Acinetobacter* species, Proteus Mirabilis were found to be 5%. Presence of antimicrobial substances in urine has a great impact on the interpretation of urine culture reports as the antimicrobial substances tend to lower the microbial counts leading to false negative reports and undiagnosed UTI. While this can be prevented by obtaining adequate patient history including the intake of antibiotics, in most cases, the patient fails to provide the information as they are either not well informed about their medicinal intake or due to the easy availability of antibiotics over-the-counter the patient fails to report the same which leads to false negative reports of the urine sample hence a delay in diagnosis and treatment.

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Key Words
Antimicrobial substance, Urinary Tract Infection, Patient history, Diagnostic error, Urine culture

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INTRODUCTION

Genitourinary tract infections are a frequent cause of morbidity in both healthy and immunocompromised individuals. Various types of genitourinary tract infections include Genital Tract Infections (GTI) including urethritis, cervicitis, epididymitis, genital ulcerative diseases, endometritis and pelvic inflammatory disease and Urinary Tract Infections (UTI) including cystitis, pyelonephritis and prostatitis[1]. UTIs are one of the most common types of human infections, with an estimated 150 million to 250 million people globally developing them each year[2]. 8.6 million medical visits and 1.6 billion dollars' worth of projected expenses are incurred annually due to UTIs. Uropathogens from fecal flora, primarily Escherichia coli, usually cause UTI by ascending the urethra and infecting the bladder[3]. Complete voiding, which eliminates 9 bacteria from the bladder, urinary acidification from concentrated urea and other 10 excreted organic acids, vesicoureteral valves, which prevent bacterial reflux to the upper urinary tract, leukocyte phagocystosis and IgA production are all normal defense mechanisms against UTIs[4-6].

Urine analysis, urine microscopy, urine culture and antibiotic susceptibility testing are clinical microbiology procedures that help diagnose and elucidate the etiology of UTIs. Urine must be collected properly to prevent specimen contamination and transported expeditiously to the lab for processing to prevent bacterial overgrowth.

Therapeutic failures may stem from improper urine cultures or incorrect data interpretation. The presence of antibiotics in urine samples submitted for culture can complicate the diagnosis of UTIs further, particularly when they are easily acquired over the counter, without a prescription[7-10]. This situation may jeopardize the recovery of bacterial pathogens and their precise colony count, leading to false-negative findings and diagnostic challenges, particularly in symptomatic patients. Antibiotics can temporarily lower bacterial counts per milliliter of urine, providing a brief remission of clinical symptoms, which can obfuscate the effectiveness of treatment in chronic or recurrent asymptomatic infections[11]. This investigation was carried out to emphasize the association between data collection from patients and the effect of unreported antibiotic use prior to urine culture in the diagnosis of UTIs.

Aims and objectives:

- To study the association between data collection from patients pertaining to antibiotic history and the presence of AMS in urine samples
- To construe the effect of antibiotics in diagnosis of UTIs
- To correlate presence of AMS in urine with culture outcome
- To study antibiotic susceptibility pattern of bacteria isolated from such urinary tract infections

MATERIAL AND METHODS

A prospective study was conducted including 300 cases of patients visiting the genitourinary OPD of the tertiary care setting during the 1 year and 6 month study period who were advised microbiological investigation of urine culture and sensitivity. Patients with signs and symptoms of UTI visiting the OPD for the first time were included. These patients were enquired about current or recent ingestion of antimicrobial agents as well as a detailed history was obtained and a proforma was filled for each patient. All the patients were provided with sterile wide mouth containers and were given detailed instructions regarding sterile aseptic precautions to be followed before and during the sample collection.

Midstream urine samples collected from the patients were further subjected to routine microscopy, microbiology assay for the presence of AMS, culture identification and antibiotic susceptibility as per CLSI guidelines.

RESULTS

Majority 29.1% (87) of the cases observed were from the age group 21-30 years followed by individuals 20.1% (61) cases under <u><</u>10 years. With 57% (171) and 43% (129) female to male ratio, female predominance was observed (Table 1).

Among the cases observed, 73% (219) had abdominal pain, 61% (183) of the AMS cases had increased frequency. About 37.3% (122) had fever with chills, followed by dysuria 30.6% (92) and 4% (12) cases of blood in urine.

Out of the 300 cases observed, 23% (69) cases had AMS in the samples (Table 2).

About 29.0% (20) of AMS cases had Obstructive pathology followed by Diabetes Mellitus 5.8% (4) and 01.4% (1) case of pregnancy was observed (Table 3).

About 40.8% (29) of cases who were on Antibiotic had AMS which was significantly higher (p = 0.001) as

Table 1: Distribution of patients according to their age groups

Age (Years)	No. of cases (N = 300)	Percentage
≤10	61	20.1
11-20	22	7.4
21-30	87	29.1
31-40	47	15.7
41-50	34	11.4
51-60	21	7.0
> 60	28	9.4
Mean age	30.59	

Table 2: Distribution of signs and symptoms among the study cases

Signs and symptoms	No. of cases (N = 300)	Percentage
Abdominal pain	219	73.0
Increased frequency	183	61.0
Fever with chills	122	37.3
Dysuria	092	30.6
Blood in urine	012	04.0

compared to the 17.5% (40) of cases who were not on Antibiotics but had AMS (Table 4).

It was observed that, 27.6% (35) of the cases having adequate history on requisition forms had AMS which was higher than the 19.7% (34) cases who did not have adequate history on requisition forms. The difference noted was statistically non-significant (Table 5).

Among of the 300 cases 87.7% of the cases had anogenital wash and 84.3% collected midstream urine samples (Table 6).

Table 3: Distribution of comorbidities among AMS cases

Co-morbidity	No. of AMS cases (N = 69)	Percentage
Obstructive pathology	20	29.0
Diabetes Mellitus	04	05.8
Pregnancy	01	01.4

Table 4: Association between antibiotic history and study population

		No. of cases			
		AMS		Non AMS	
Antibiotic	No. of cases	No.	%	No.	%
Yes	071	029*	40.8	042	59.2
No	229	040	17.5	189	82.5

Table 5: Association between adequate history on requisition forms and study population

		No. of cases			
		AMS		Non AMS	
Adequate information on requisition forms	No. of Cases	No.	%	No.	%
Yes	127	035	27.6	092	72.4
No	173	034	19.7	139	80.3

Table 6: Distribution of other variables

Status	No. of cases (N = 300)	Percentage
Midstream urine	253	84.3
Anogenital wash	263	87.7

Amongst the total 20 uropathogens obtained among AMS patients the most common organism is *E. coli* 40% followed by 20% CONS (coagulase negative staphylococcus). *Enterococcus* species, Pseudomonas aeruginosa, *Acinetobacter* species, Proteus Mirabilis were found to be 5% (Table 7).

Gram positive and negative growth were found to be 40 and 60% respectively.

The aforementioned isolates were tested for antimicrobial susceptibility in accordance with CLSI guidelines 2017 for urine and the results revealed that *E. coli* was isolated from urine culture in (40%) patients.

E. coli isolates were sensitive to Colistin (100%) and Tigecycline (100%) before Meropenem (66%) and Piperacilin+ Tazobactam. Imapenem (8.33% of Klebsiella group isolates) was the drug of choice, followed by Meropenem (77.77%) and Levofloxacin (72.22%).

Isolates from the Pseudomonas group were responsive to Imipenem (85.71%), followed by Meropenem (78.57%) and Levofloxacin (64.28%). Imipenem and Meropenem were the most effective antimicrobials against Proteus group isolates (91.66% and 91.66%, respectively), followed by Levofloxacin (83.33%).

DISCUSSION

Urinary tract infection (UTI) is one of the most frequent bacterial infection that general practitioners encounter[12]. UTIs are the most common outpatient infections, with a lifetime incidence of 50-60% in an adult women[13].

Table 7: Anti-microbial susceptibility among patients with AMS in urine

	Organisms							
Antimicrobial agent	*E. coli* (n = 1) (%)	*Enterococcus* (n = 1) (%)	Group D *Streptococcus* (n = 1) (%)	*Acinetobacter* (n = 1) (%)	*Pseudomonas* (n = 1) (%)	*Klebsiella* (%)	*Streptococcus* (%)	Proteus (%)
Amoxycillin-Clavulanic acid	37.5	NT	NT	100	NT	NT	NT	0
Cotrimoxazole	37.5	NT	NT	100	0	NT	NT	0
Nitrofurantoin	37.5	100	0	100	0	0	0	0
Cefazolin	12.5	NT	NT	100	NT	NT	NT	0
Amikacin	37.5	NT	NT	0	100	100	NT	100
Gentamycin	37.5	NT	NT	100	0	NT	NT	0
Ceftriaxone	12.5	NT	NT	NT	NT	NT	NT	100
Ceftazidime	NT	NT	NT	NT	NT	0	NT	NT
Ampicillin-Sulbactum	50	NT	NT	100	NT	NT	NT	NT
Imepenem	33	NT	NT	NT	100	100	NT	100
Piperacillin-Tazobactum	66	NT	NT	NT	100	100	NT	100
Meropenem	66	NT	NT	NT	100	100	NT	100
Colistin	100	NT	NT	NT	100	NT	NT	NT
Tetracycline	NT	NT	NT	100	0	NT	NT	0
Tigecycline	NT	NT	NT	NT	0	NT	NT	NT
Ampicillin	NT	100	100	NT	NT	NT	100	NT
Linezolid	NT	100	100	NT	NT	NT	100	NT
Vancomycin	NT	100	100	NT	NT	NT	100	NT
Penicillin	NT	0	0	NT	NT	NT	0	NT
Ciprofloxacin	NT	NT	NT	100	0	NT	NT	100
Norfloxacin	37.5	NT	NT	NT	NT	NT	NT	NT
Nalidixic acid	37.5	NT	NT	0	NT	NT	NT	NT
Pipericillin				100	100	NT	NT	NT

Among the cases recorded in our study, most of the affected population belonged to the 21-30 years age group. Shilpi *et al*.[14] reported that the highest prevalence was observed in the 21-40 year old reproductive age range, which was consistent with our findings. They had also linked UTI to malnutrition, poor hygiene and low socioeconomic level, all of which are prevalent in rural areas.

It was observed in the study that the prevalence of UTI was higher among females (57%) than in males (43%). Similarly, Antibiotic[15] in their study, found that female patients had a higher prevalence of UTI than male patients did. Also, in a study by Chowdhury and Parial [16] 64.5% of the cases diagnosed with UTI were females. UTIs are more common in females because their urethras are shorter and closer to the rectum which makes it easier for bacteria to enter the urinary tract. Other factors such as sexual activity, changes in the vaginal flora, pregnancy can increase the risk of UTIs.

Abdominal discomfort was present in 73%, increased frequency was observed in 61% and fever with chills was present in 37.3% of the cases. The most prevalent symptom in a similar study by Kakde *et al*.[17] was frequency of micturition (65.26%), followed by dysuria (62.10%) and fever was present in 45.26 percent of cases, which is equivalent to this study. Bignell *et al*.[18] observed that fever (60.81%) and dysuria (48.64%) were the most frequent symptoms, which is similar to the findings by Chandrasekhar *et al*.[19] in which fever (60.81%) and dysuria (48.64%) were the most frequent symptoms reported.

Drugs or their metabolites can be detected in urine tests, which have previously been found to be helpful in antibiotic compliance studies and in determining how reliable a patient's medication history is.

In our study, 69 (23%) of a total of 300 cases had AMS in their urine. Whereas, 4.1% of new and rebook patients at a genitourinary department had antibiotic compounds in their urine. In urine samples obtained as cases of probable urinary tract infection, there was a noticeably greater prevalence of antibiotic compounds (18.7%), according to a study by Bignell *et al*.[18] However, a research by Chandrasekhar *et al*.[20] found a reasonably high level of AMS in urine (23.4%) given the fact that it was carried out on in-patients with a relatively high antibiotic consumption. The results of AMS in urine could also be impacted by the antimicrobial class used, the presence of inactive metabolites produced in urine, or the use of antibiotics to treat infections in other anatomical sites. Since the use of antibiotics reduces the sensitivity of the culture, these findings highlight the need of having appropriate information about antibiotic medication at the time a urine examination is requested.

In the present study, 40.8% of the cases of AMS that were reported had a positive history of antibiotic use. In accordance with Schwarz *et al*.[21] In a primary care physician consultation, 48% of those who had an antimicrobial agent in their urine denied taking such a medication. The percentage of new and follow-up patients who denied using antibiotics was comparable. The 27.6% cases who had their history on requisition forms adequately filled had AMS in urine which was comparatively higher than the 19.7% of cases who had AMS but had not filled the requisition forms adequately. Although there haven't been many studies done to date, they do suggest a link between adequately filled out requisition forms and the presence of AMS in urine. According to a study by Khennavong *et al*.[22] patients' uncertainty about the medications they have taken, especially when prescriptions are not used and loose pills or capsules are given out in tiny unlabeled plastic bags, is reflected in the discrepancy between antibiotic activity in urine and patient recall of antibiotic use. Lack of eliciting antibiotic history and insufficient history writing by the doctor on the case record form may be attributed for inadequacy in the history.

The most effective screening test for asymptomatic bacteriuria is urine culture, which is the gold standard for diagnosing UTIs. Although catheterized specimens can occasionally be obtained, clean midstream urine capture is the most usual method of collecting urine samples. Before midstream urine collection, there is probably no difference in contamination between cleansing and not cleansing. Clean-catch midstream is just as reliable for diagnosing UTIs as catheterized urine, despite the limited evidence[23].

In our investigation, we found that *E. coli* and CONS (coagulase negative staphylococcus) were the two most prevalent uropathogens among the 20 uropathogens collected from AMS patients. All of the following were shown to be present in 5% of cases: *Enterococcus* species, *Pseudomonas aeruginosa*, *Acinetobacter* species and Proteus Mirabilis. According to Bignell *et al*.[18] the significant proportion of isolates were E. coli (48.6%), followed by *Klebsiella* spp. (17.6%), Citrobacter spp (10.8%), *Enterococcus* (9.4%), *Enterobacter* (5.4%), *Pseudomonas* spp. (4%) and coagulase-negative *Staphylococcus* (2.7%) and Proteus (1.3%). These findings are also supported by other studies conducted by Chowdhury *et al*.[16] and Galate *et al*.[24] Contrarily, a research by Hammoudi[25] found that the most common isolate was *Klebsiella* spp. (30.8%), followed by Staphylococcus aureus (27.2%), *E. coli* (22.2%), Enterobacter (13.6%), Proteus (3.1%) and *Pseudomonas* spp. (1.9%). Similarities and variances in the type and distribution of uropathogens may be brought about by many host and environmental factors, as well as by local socioeconomic norms, healthcare and educational policies and cleanliness practices.

The usual threshold for severe bacteriuria is 10^5 cfu mL^{-1}, but this standard has a high proportion of false negatives (94% sensitivity, 50-70% sensitivity)[26]. According to studies, between 30 and 50 percent of women with symptomatic UTIs have counts as low as 10^2 cfu mL^{-1} [27]. According to another study by Hooton *et al*.[6] an *E. coli* UTI had a 93% PPV at 10^2cfu/ml. Women who had already taken antibiotics as well as those infected with uropathogens such *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Serratia* and *Moraxella* may also see lower bacterial counts indicative of infection. Many laboratories, however, only provide results for numbers more than 10^4 cfu mL^{-1}, which may lead to the undertreatment of symptomatic women. Positive cultures are those in which a single uropathogen has grown significantly, while contaminated cultures are those in which the flora is mixed[5].

CONCLUSION

Presence of antimicrobial substances in urine has a great impact on the interpretation of urine culture reports as the antimicrobial substances tend to lower the microbial counts leading to false negative reports and undiagnosed UTI. While this can be prevented by obtaining adequate patient history including the intake of antibiotics, in most cases, the patient fails to provide the information as they are either not well informed about their medicinal intake or due to the easy availability of antibiotics over-the-counter the patient fails to report the same. Especially in symptomatic patients, this scenario may compromise the recovery of bacterial pathogens and their precise colony count, resulting in false-negative findings causing diagnostic difficulties and hence delay in treatment. This problem might possible be tackled by healthcare providers by creating awareness regarding self-medication and providing information to the patient about the medicines prescribed.

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