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Bacteriological and Mycological Profile of Urinary Tract Infections among Diabetic Patients at a Tertiary Care Centre

Dr. Bhagyashree. S. Patil[1], Dr. Anjana gopi[2], Dr. Swati Jain[3], Dr. Faiza samreen[4], Dr. Madhulatha C. K.[5]

[1](MD Microbiology) Tutor, Department of Microbiology Kempegowda Institute of Medical Science, Banashankari, Bangalore-560070

[2](MD Microbiology) Professor and Head, Department of Microbiology Kempegowda Institute of Medical Science, Banashankari, Bangalore-560070

[3](MD Microbiology) Senior Resident, Department of Microbiology Kempegowda Institute of Medical Sciences and SUM Hospital, Bhubaneshwar -751003

[4](MD Microbiology) Assistant Professor, Department of Microbiology Kempegowda Institute of Medical Science, Banashankari, Bangalore-560070

[5](MD Microbiology) Assistant Professor, Department of Microbiology Kempegowda Institute of Medical Science, Banashankari, Bangalore-560070

Email id- *[1]bhagyashreedr@gmail.com, [2]dranjanagopi1989@gmail.com, [3]drswati_jain@ymail.com, [4]faizasamreen6@gmail.com [5]madhulathaharish@gmail.com*

Abstract:

Introduction *- Urinary tract infections (UTIs) are among the most common bacterial infections. Patients with diabetes often have increased antimicrobial resistance and complications. The incidence of Methicillin resistance Staphylococcus aureus (MRSA), extended spectrum beta lactamases (ESBL) and Metallo-beta lactamases (MBL) producing organisms have been steadily increasing over the past few years resulting in limited therapeutic options.* ***Material and Methods*** *- It is a prospective study conducted at a tertiary care centre for a period of 18 months from December 2014- June 2016. A total of 200 urine samples of clinically suspected UTI among diabetic patients were collected aseptically. The isolates were identified by conventional methods along with HiCrome UTI agar and Antibiotic susceptibility testing (AST) was performed using Kirby Bauer disc diffusion method according to CLSI guidelines. Staphylococcus aureus (S. aureus) isolated was further studied for MRSA using Cefoxitin disc diffusion test. ESBL and MBL producers among gram negative isolates were detected by combined disc diffusion test as per CLSI guidelines. The Candida isolates were speciated by the germ tube test and chromogenic media. Antifungal susceptibility testing by Kirby Bauer disc diffusion method to fluconazole, voriconazole and amphotericin B was performed.* ***Results*** *- Of 200 samples, 100 (50%) yielded growth. Escherichia coli (E.coli) (32.43%) being the most common isolate followed by Klebsiella pneumoniae (K.pneumoniae) (18.91%). MRSA was observed in 50% (three out of six) S. aureus isolates. ESBL production was observed in 47.36% of all gram negative isolates and MBL production was observed in 50% of all nil-fermenting gram negative isolates. Out of 22 Candida isolates, 20 (90.9 %) were susceptible to amphotericin-B followed by voriconazole 19 (86.3%).* ***Conclusion*** *- Most of the isolates were multidrug resistant making available therapeutic choices limited.So antibiotic surveillance, infection control practices and an effective antibiotic policy are required to address this problem of increasing resistance.*

Keywords *- UTI, MRSA, ESBL, MBL.*

Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections that lead patients to seek medical care.[1] During the course of lifetime with diabetes, UTIs would be ranked among the top ten concurrent or complicating illnesses by most experts and patients.[2]

Patients with diabetes often have increased complications of UTI such as emphysematous cystitis, pyelonephritis and fungal infections (particularly due to candida species). There is greater likelihood of UTI associated with antimicrobial resistance or atypical uropathogens and risk of upper urinary tract involvement is increased. Hence, choice of antibiotic therapy should depend on local susceptibility patterns of the infecting organism.[3] Methicillin Resistant *Staphyloccocus aureus* (MRSA), Extended Spectrum Beta Lactamases (ESBL) and Metallo Beta Lactamases (MBL) producing

organisms have been steadily increasing over the past few years resulting in limited therapeutic options.[3,4,5]

Hence, this study was conducted to identify the organisms causing urinary tract infections among diabetic patients and to know the prevalence of MRSA, ESBL and MBL producing organisms and their susceptibility pattern, which in turn helps to reduce morbidity and duration of stay in the hospital.

Materials and Methods

If you The present study was carried out in the Department of Microbiology- Kempegowda Institute Of Medical Sciences Hospital and Research centre, Bangalore over a period of 18 months from December 2014 to June 2016. A total of 200 early morning mid-stream clean catch urine samples from suspected UTI cases with Diabetes Mellitus (DM) were collected under aseptic precautions into sterile, wide mouthed, screw capped containers. Specimens were subjected to gram stain to look for pus cells and organisms. By standard loop method, known volume of urine was inoculated into Blood Agar, MacConkey Agar along with the chromogenic agar (HiCrome UTI agar). Plates were incubated at 37°C for 24-48 hours and checked for significant bacteriuria by estimating the colony count by Kass concept. The isolates were identified by colony morphology and standard biochemical tests. Antibiotic susceptibility testing of the organism was done by Kirby Bauer disc diffusion method using standard CLSI guidelines.[6]

S. aureus when isolated was studied for methicillin resistance (MRSA) using cefoxitin (30mcg) disc diffusion test.[7] Gram negative isolates resistant to cephalosporins were studied for ESBL production using ceftazidime (30mcg) plus ceftazidime/clavulanic acid (30/10mcg) and cefotaxime (30mcg) plus cefotaxime/clavulanic acid (30/10mcg) disc diffusion test.[8] Nil fermenting gram negative isolate resistant to carbapenems were studied for MBL production using imipenem (10mcg) plus imipenem-EDTA (10-750mcg) combined disc test.[9]

When gram positive budding yeast like cells were visualized on gram stain and based on the morphology on culture plates, candida was speciated using chrome agar and antifungal susceptibility done by disc diffusion method using voriconazole (1mcg), fluconazole (25mcg) and amphotericin B (10mcg).[10]

Results

Of 200 diabetic patients with suspected UTI, 104 (52%) were male patients and 96 (48%) were female patients. Maximum number were in the age group of 61-70 years (31%) followed by 51-60 years (29%). (**Figure-1**).

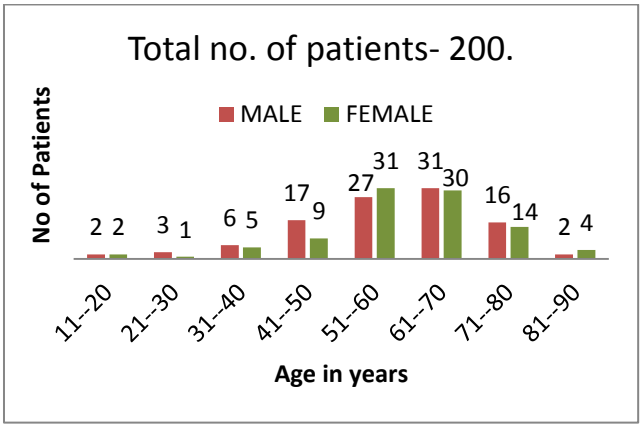


Figure 1: Graphic representation of age and gender wise distribution

Gram stain performed from 200 urine samples was viewed for pus cells and organisms. The positive gram stain (pus cells with organisms) and negative gram stain (few/ no pus cells without organism) was correlated with growth on culture plate. The difference between positive and negative gram staining for prediction of subsequent culture results was statistically significant ($p<0.01$) by Fisher exact test. (Table-1)

Table 1: Direct microscopy and culture positivity

GRAM STAIN	CULTURE POSITIVE	CULTURE NEGATIVE	TOTAL
Positive	**83**	**12**	**95**
Negative	**17**	**88**	**105**
Total	**100**	**100**	**200**

Of 200 suspected UTI patients, 100 samples were culture positive. 89 (89%) samples yielded a single organism and 11 (11%) samples yielded two organisms in each (there were 111 isolates altogether).*E.coli* (32.9%) was the most common organism isolated followed by *K. pneumoniae*. (**Figure-2**)

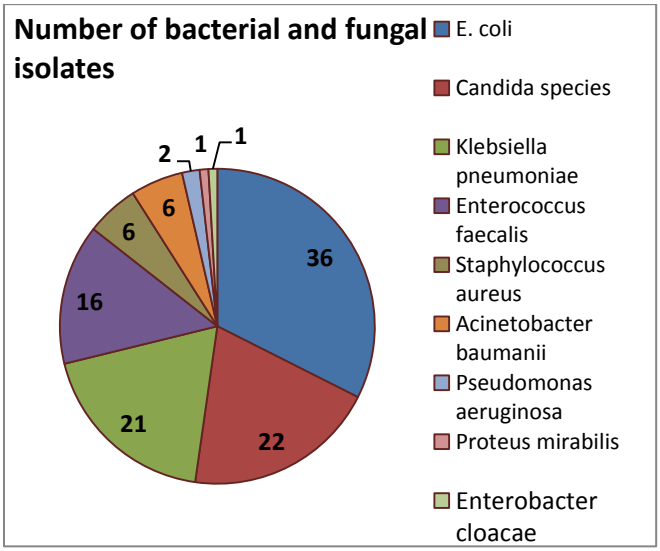


Figure 2: Graphic representaion of culture positive cases according to spectrum of bacterial and fungal isolates (n=111)

Out of 111 isolates, 22 isolates belonged to gram positive organisms, 59 gram negative organisms, eight nil fermenting gram negative organisms and 22 fungal isolates (Candida).

Among 22 gram positive isolates, *Enterococcus faecalis (E. faecalis)* 16 (73%) was the most common organism isolated followed by *S. aureus* six (27%).

Hicrome UTI agar and Blood agar supported the growth of all 111 (100%) isolates whereas MacConkey agar (MA) did not support the growth of three *Enterococcus* isolates, hence yielded 108 (97.29%) bacterial growth. The rate of presumptive identification of the bacterial isolates on HiCrome UTI agar was high due to their distinctive colour. (**Table-2**)

Table 2: Colony morphology of different UTI isolates on HiChrome agar

ORGANISM	COLONY MORPHOLOGY
Enterococcus faecalis	blue, small
Staphylococcus aureus	golden yellow.
Escherichia coli	pink to purple.
Klebsiella pneumonia	blue, mucoid.
Pseudomonas spp	colourless (greenish pigment may be observed)
Acinetobacter spp	cream colour
Proteus mirabilis	light brown
Enterobacter spp	blue to blue green

Out of six *S. aureus* strains, three (50%) were MRSA by cefoxitin disc diffusion test.

The gram positive organisms were most susceptible to vancomycin and linezolid (95.45%). (**Figure-3**).

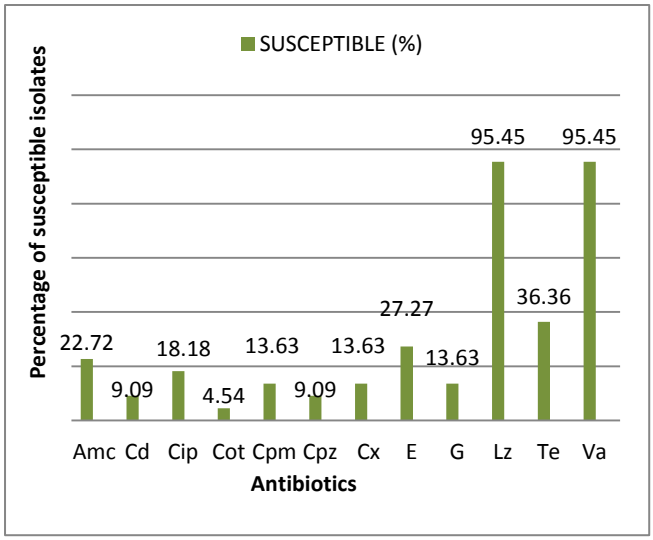


Figure 3: Graphic representation of antibiotic susceptibility pattern of gram positive isolates

Amc - amoxyclav, Cpm- cefepime, Cpz- cefoperazone, Cip- ciprofoxacin, Cd- clindamycin, Cot- Cotrimoxazole, E- erythromycin, G- gentamycin, Lz- linezolid, Cx- Cefoxitin, Te- tetracycline, Va- vancomycin.

Among 59 gram negative isolates, *E.coli* 36 (61%) was the most common organism isolated followed by *K. pneumoniae* 21 (35.59%). The gram negative organisms were most susceptibility to nitrofurantoin (74.57%), amikacin (74.57%) and gentamycin (74.57%). (**Figure-4**) *E. coli* was found to be more ESBL producer when compared to *K. pneumoniae* (20 out of 36 (55.5%) isolates of *E. coli* and seven out of 21 (33.3%) isolates of *K. pneumoniae* were ESBL producers). (**Picture-1**)

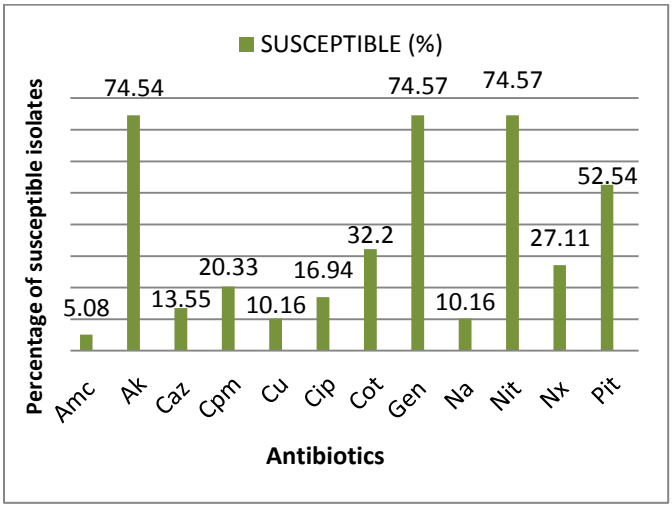
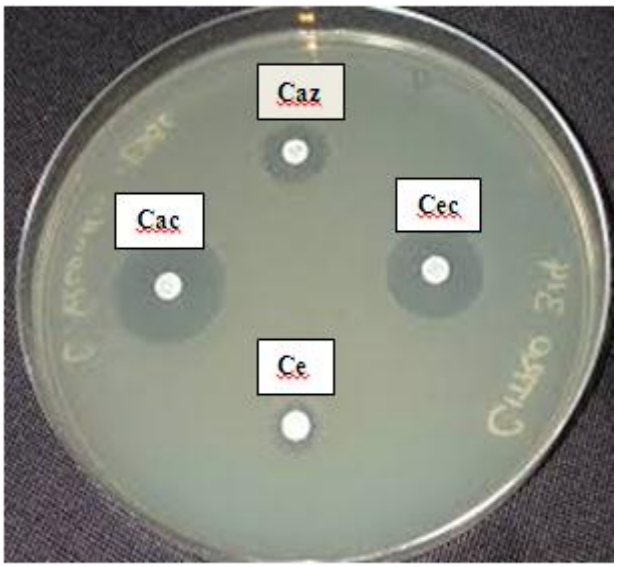


Figure 4: Graphic representation of antibiotic susceptibility pattern of gram negative isolates

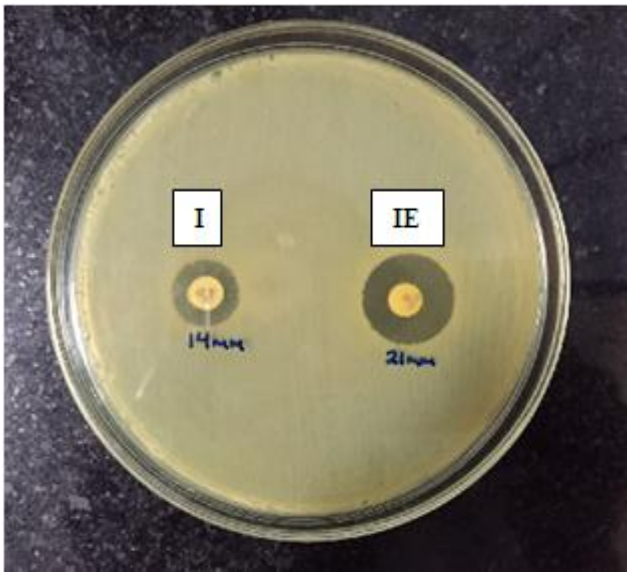
Amc- amoxyclav, Ak- amikacin, Caz- ceftazidime, Cpm- cefepime, Cu- cefuroxime, Cip- ciprofloxacin, Cot- cotrimoxazole, Gen- gentamycin, Na- nalidixic acid, Nit- nitrofurantoin, Nx- nalidixic acid, Pit- piperacillin tazobactam.



Picture 1: ESBL producer confirmed by combined disc diffusion test

Among eight nil fermenting gram negative organisms, *Acinetobacter baumanii* six (75%) was most common organism followed by *Pseudomonas aeruginosa* two (25%).

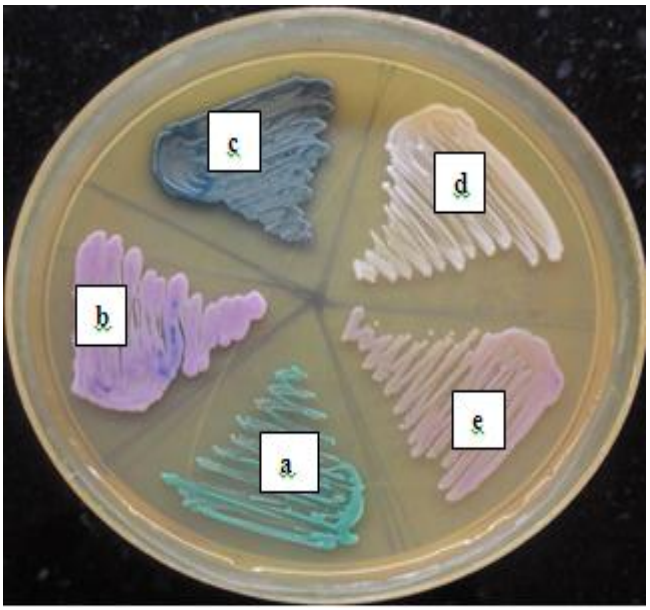
The nil fermenting gram negative organisms were most susceptibile to levofloxacin(62.5%), and amikacin (62.5%). MBL production was seen in two out of six (33.33%) isolates of *A. baumanii* and two out of two (100%) isolates of *P. aeruginosa*. (**Picture-2**)



Picture 2: Imipenem plus imipenem-EDTA combined disc test showing MBL production.

Among 22 fungal isolates (Candida), the predominant candida species isolated was *Candida albicans* nine(40.90) followed by *Candida tropicalis* eight(36.3%), *Candida glabrata* three(13.6%), *Candida parapsilosis* one(4.5%) and *Candida krusei* one(4.5%). (**Picture-3**)

Out of 22 isolates, 20 were susceptibile to amphotericin B (90.9%), 19 to voriconazole (86.3%) and 16 to fluconazole (72.7%).



Picture 3: Candida species on chrome agar (a) *C. albicans* (b) *C. glabrata* (c) *C. tropicalis* (d) *C. parapsilosis*(e) *C. krusei*.

Discussion

UTIs are common among diabetic patients and more severe and carry worst outcomes. They are also more often caused by drug resistant pathogens including MRSA, ESBL and MBL producing organisms. Correct identification of these organisms in due time is necessary not only for optimal patient management but also for immediate institution of appropriate infection control measures to prevent the spread of these organisms. This study was a small step towards the same.

In present study, the incidence of UTI was highest in the age group of 61-70 years and is more common in men (52%) compared to women (42%), which correlates with the study done by Venkatesh et al.[11] This may be due to physiological alteration or pathological conditions which is seen in geriatric age group and more common in men may be because of increased prevalence of diabetes in men compared to women comorbidities such as risk of age related prostate disorders increase with age.[11, 12]

There was slightly lower yield of *Enterococcus spp.* on MA, this limitation can be explained as MA is a selective medium for the members of family Enterobacteriaceae. But the use of HiCrome UTI Agar facilitates improved sensitivity of identification of some Gram positive cocci *(e.g., Enterococci)* in mixed cultures with Enterobacteriaceae. It also enables shorter time for reporting (time saving), reduces work load by limiting use of identification tests. By promoting rapid identification of the etiological agent(s) it provides clinicians with relevant information regarding their choice of empirical antimicrobial therapy for patients. Similar results were observed by Usha M G et al.[13]

In the present study, 111 isolates were obtained from 100 culture positive cases. Most common organism isolated in our study was *E. coli* (32.43%), followed by *K. pneumoniae* (18.91%). Predominance of *E. coli* in present study is consistent with study from Hamdan Z et al[14] and Vinita Rawat et al.[15]

This could be due to:

- High number of coliforms present in proximity to the perineum.
- Ability of the uropathogenic bacteria to adhere, grow and resist against host defences resulting in colonization and infection of the urinary tract.[16]

In the present study of 22 gram positive organisms isolated, 16 were *E. faecalis* and six were *S. aureus.* This correlated with Janifer et al.[17] where the *E. faecalis* were the major isolates among gram positive cocci.

Among six strains of *S. aureus*, three strains (50%) were MRSA. This correlates with a study by Rawat et al[15] who reported MRSA in 50% of *S. aureus* isolates.

Of 59 gram negative isolates, 27 (47.36%) isolates were ESBL producers which included 20 (55.5%) *E.coli* and seven (33.3%) of *K. pneumoniae*. This correlates with the study done by Kumar P A et al[18] showed 44.4% of gram negative bacilli were ESBL producers. With *E. coli (34.1%)* being the predominant ESBL producer followed by *K. pneumonia. (30.9%).*

Out of eight nil fermenters, two (100%) of *P. aeruginosa* and two (33.33%) of *A. baumanii* were found to be MBL producers. The percentage of MBL production in *P. aeruginosa* (100%) is higher compared to other studies because the number of isolates in our study were few that is two in number and both were isolated from patients who were chronically ill with prolonged indwelling catheter.

Out of 22 Candida isolates, *Candida albicans* was predominant isolate followed by *Candida tropicalis* and this correlates with the study by Gopi A et al.[19] Among fluconazole, voriconazole and amphotericin B, highest resistance was seen to fluconazole and the highest resistance was shown by *C. glabrata* and *C. krusei*. These findings correlated with the study by Gopi A et al.[19] Most of the isolates were susceptible to amphotericin B but since the drug is highly nephrotoxic, it is not the first choice of treatment. Hence, further studies are required to assess the susceptibility to echinocandins in Indian settings.

Conclusion

To conclude, the pathogenesis of UTI among patients with DM appears to be multifactorial, with diverse risk factors as bladder dysfunction, alteration in immune function and instrumentation of urinary tract playing roles. The most common uropathogen is E.coli and fungal infections are also frequent among patients with DM. Because patients with diabetes are considered to have complicated UTI, resistant uropathogens are a special concern which include MRSA, ESBL and MBL producing organisms. These organisms make available therapeutic choices limited. Hence, treatment should be guided by an antibiotic susceptibility report as multidrug resistance had made empirical therapy ineffective.

Among gram positive isolates, vancomycin and linezolid were most effective antibiotics and aminoglycosides and nitrofurantoin among gram negative and most of the candida species were susceptible to amphotericin B but the usage is limited because of the toxicity. Hence voriconazole is the next effective antifungal in present study.

Continuous antibiotic surveillance, infection control practices and an effective antibiotic policy are required to address the problem of MRSA, ESBL and MBL infections.

The drawbacks of this study were lack of genotypic confirmation and differentiation of MRSA, ESBL and MBL types that were prevalent in the study sample.

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