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Original Article

Microbiology Section

Susceptibility Profile and Clinical Response of Fosfomycin and Other Antibiotics against Multidrug Resistant Gram Negative Urinary Isolates: A Cross-sectional Study

CHINMOY SAHU[1], SWETA SINGH[2], SANGRAM SINGH PATEL[3], NIDHI YADUVANSHI[4], SANJAY SINGH[5], UJJALA GHOSHAL[6]

ABSTRACT

Introduction: Irrational use of antibiotics to treat Urinary Tract Infections (UTI) has led to the development of Multidrug Resistant (MDR) bacteria in both community as well as the hospital settings. Fosfomycin has emerged as a novel therapeutic option to treat these UTI patients along with empirically used routine antibiotics.

Aim: To assess the sensitivity, molecular resistance mechanisms and clinical response of fosfomycin along with other urinary antibiotics like nitrofurantoin, colistin, and imipenem.

Materials and Methods: It was a cross-sectional observational study conducted from July 2018 to June 2019 in SGPGIMS, Lucknow, India. Stream urine samples of 24,782 patients were collected with clinical suspicion of UTI. The antibiotics were tested by disc diffusion and Minimum Inhibitory Concentration (MIC) methods. Genotypic analysis was done for testing resistance mechanisms in fosfomycin resistant isolates. Statistical tests were performed using Statistical Package for the Social Sciences (SPSS) software for Windows version 14.0.

Results: Out of the 24,782 urine samples, 2,776 (11.2%) showed significant growth of pathogens with 334 drug resistant isolates among them. Gram negative bacilli 1846 (66.50%) was the most predominantly isolated pathogen in the cultures. Among the 334 drug resistant specimens, Escherichia coli {124 (37.13%)} were maximum in number. Total 79.6% (266/334) of the isolates were sensitive to fosfomycin including 88.7% (110/124) of *E. coli*, and 91.3% (105/115) of *K. pneumoniae* isolates. Colistin showed sensitivity in 87.1% (108/124) of the *E. coli* isolates; followed by Imipenem in 49.2% (61/124) and nitrofurantoin in 37.1% (46/124) of the isolates. Fos A genes were found to be the most prevalent in Fosfomycin resistant. About 41% of the patients showed favourable outcome and were cured with initiation of treatment as per sensitivity pattern.

Conclusion: Fosfomycin has emerged as a safer option in MDR urinary isolates as compared to other urinary antibiotics including colistin. The drug needs to be more widely studied for its possible pharmacokinetics and dynamics as well as it's possible implications in healthcare settings and patient management.

Keywords: Colistin, Disc diffusion test genotype analysis, Minimum inhibitory concentration method

INTRODUCTION

The Urinary Tract Infections (UTI), both complicated as well as uncomplicated are on a rise in today's scenario. Irrational use of antibiotics is leading to UTI by MDR bacteria in both community as well as the hospital settings [1,2]. Empirical antibiotics used commonly for treating UTI consist of nitrofurantoin, fluroquinolones, aminoglycosides; whereas carbapenems and colistin are used as salvage therapy for otherwise untreatable gram negative infections, most notably MDR and Extensively Drug Resistant (XDR) strains [3].

Fosfomycin, originally named phosphonomycin, was discovered in Spain in 1969 [4]. Synergistic action may be seen with beta-lactam antibiotics, aminoglycosides, etc., [5-8]. It is also effective against MDR pathogens like Extended Spectrum Beta Lactamase (ESBL) producing *Enterobacteriaceae*, *Klebsiella Pneumoniae* Carbapenemase (KPC) producing bacteria and Vancomycin Resistant *Enterococci* (VRE). Currently, it has been approved by Food and Drug Administration (FDA) for use in uncomplicated UTI infections [8].

Bacterial resistance to fosfomycin is exerted by different mechanisms-genetic mutation in phosphoenol pyruvate synthase (murA) and/or chromosomally encoded transport systems GlpT and UhpT; or by a fosfomycin modifying enzyme that brings structural changes in fosfomycin with no antibacterial activity [9,10], e.g., fosfomycin (FosA), L-cysteine-fosfomycin (FosB), ATP-fosfomycin (FosC), and water-fosfomycin (FosX) adducts. These plasmid mediated resistance genes can be of significant concern in heathcare settings because it can give rise to a clone of fosfomycin resistant bacterial isolates [11].

Fosfomycin has the potential to replace other parenteral antibiotics for the treatment of both complicated and uncomplicated UTI. Fosfomycin can be a good oral alternative to colistin and carbapenems in hospital acquired UTI especially for *E. coli*. It's activity is good against other *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Hence, this study was planned to assess the sensitivity, Minimum Inhibitory Concentration (MIC) ranges, molecular resistance mechanisms, clinical response for fosfomycin and other urinary antibiotics like nitrofurantoin, colistin, imipenem in UTI isolates of gram negative bacteria.

MATERIALS AND METHODS

This study was a cross-sectional observational study and undertaken for a period of one year from July 2018 to June 2019 in SGPGIMS, Lucknow, India. Institutional Ethics Committee (IEC) permission was taken before the study (IEC Code: 2018-56-IMP-103). Informed consent was obtained from all the patients and their legal guardians (in case of minors) regarding the publication of images and clinical information in the journal.

Inclusion criteria: Patients presenting with symptoms of UTI (fever, burning micturition, frequency, urgency) were included in the study. Both inpatients and outpatients were included.

Exclusion criteria: Rest all other patients not presenting with the symptoms of UTI and having other diagnosis were excluded from the study.

Mid-stream urine specimens were collected in wide mouthed universal plastic containers. For catheterised patients, urine was collected from port site after proper disinfection. The samples were processed and cultured as per standard protocol [11].

Study Procedure

1. **Sample selection:** A total of 24,782 urine samples were collected with clinical suspicion of UTI. Among these, 334 drug resistant cases were further studied for their clinical outcome and involved resistance mechanisms.
2. **Culture:** Cultures that yielded significant bacterial growth of atleast 104 colony forming units/mL were included for further sensitivity testing and follow-up in the study [12].
3. **Identification:**

a. Conventional method using biochemical tests: The bacterial isolates were first identified using routine staining and biochemical tests as per existing laboratory protocols [11,12].

b. Automated methods: The identity of bacteria was later confirmed by Vitek 2 system (Biomerieux, France), an automated identification and susceptibility testing system [13].

4. **Resistance detection method:**

a. Disc diffusion test: Antibiotic susceptibility testing of all the isolates were done by Kirby-Bauer's disk diffusion method on Muller Hinton agar and interpreted based on Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. Interpretations of zone diameters were as follows: Fosfomycin ≥16 mm as sensitive, 13-15 mm as intermediate and ≤12 mm as resistant; Nitrofurantoin: ≥17 mm sensitive, 15-16 mm intermediate, ≤14 mm as resistant; Imipenem: ≥23 mm sensitive, 20-22 mm intermediate, ≤19 mm as resistant (Oxoid Ltd, Basingstoke, Hampshire, England).

b. Minimum Inhibitory Concentration (MIC): Fosfomycin MIC were determined initially by E-test [15] and finally confirmed by agar dilution method in cation adjusted Mueller-Hinton medium supplemented with 25 mg/L of G-6-P (glucose-6-phosphate; Sigma Chemical Co., India) [13]. Interpretative criteria according to CLSI guidelines are as follows: ≤64 µg/mL as sensitive, 128 µg/mL as intermediate, ≥256 µg/mL as resistant [14,16]. Colistin MIC was detected by microbroth dilution method. The MICs of colistin were determined by the broth microdilution method with cation adjusted Muller-Hinton broth (Oxoid, Code: CM0405, UK) according to Clinical Laboratory Standard Institute (CLSI) guidelines [14,17]. Colistin sulfate (Sigma-Aldrich, St. Louis, MO, USA) was tested over a range of dilutions (0.06-32 µg/mL), and (0.25-256 µg/mL), respectively. One hundred microliters of freshly prepared colistin were added to 96-well U bottom microplates. Bacterial suspensions prepared from non selective culture media, were inoculated in microplates and incubated for 24 hour at 37°C in ambient air [14]. The MIC breakpoints for colistin according to CLSI guidelines was ≤2 ug/mL as sensitive/intermediate and ≥4 ug/mL as resistant.

c. Genotypic method: All fosfomycin resistant strains according to CLSI guidelines were characterised genotypically for plasmid mediated resistance gene FosA and FosC by Polymerase Chain Reaction (PCR) using the primers as described in earlier studies [14,18,19]. Sequence analysis was performed with a dye primer and a dye terminator cycle sequencing kit (Applied Biosystems) and with a 310 gene analyser (ABI Prism). The primers used were as shown in [Table/Fig-1].

Amplified gene	Primer	Sequence	Amplicon size (bp)
Fos A	FAF	5'-ATCTGTGGGTCTGCCTGTCGT-3'	271
	FAR	5'-ATGCCCGCATAGGGCTTCT-3'	
Fos C	FCF	5'-TGGAGGCTACTTGGATTTG-3'	217
	FCR	5'-AGGCTACCGCTATGGATTT-3'	

[Table/Fig-1]: Genotypic primers used in the study.

5. **Patients' follow-up:** Culture, sensitivity and other study parameters like co-morbidities/risk factors were kept in computer database along with patient's profile. The culture follow-up was done upto six months (July to December 2019) for any repeat culture, new isolates, change in antibiotic sensitivity pattern and other study parameters.

STATISTICAL ANALYSIS

Statistical tests were performed using Statistical Package for the Social Science (SPSS) software for Windows version 14.0 (SPSS, Inc., Chicago, IL, USA). Categorical data was described using numbers and percentages. Geometric MIC was calculated by Graph Pad Prism Software and one-way Analysis of Variance (ANOVA) with two-sided Bonferroni multiple comparison test was used for calculation of significance. The p-values <0.05 were taken as statistically significant.

RESULTS

A total of 24,782 urine samples with clinical suspicion of UTI were received in the laboratory. Of these; 2,776 (11.2%) showed significant growth of pathogens and the rest were either sterile or contaminated with more than three different types of bacterial growth. The predominant pathogen in these cultures were gram negative bacilli in 66.50% (1846/2776) cases and rest cultures comprised of gram positive isolates like *Staphyloccoccus* and Enterococcus group. Among these isolates; 18.1% (334/1846) were drug resistant; which were MDR, XDR or PDR (according to standard definitions). Majority of these isolates were from males (56.2%) and the maximum age group was of adolescents with mean age of 39 years. These 334 isolates was further studied for their co-morbidities, present diagnosis, sensitivity pattern and follow-up or outcomes. Among, these drug resistant isolates catheterisation was identified as the most common risk factor (47%); followed by renal calculi and diabetes mellitus. Urological surgical procedures, chronic kidney and liver diseases were also identified as important risk factors in these drug resistant cases [Table/Fig-2]. Among the 194 (58%) MDR isolates, were of mixed infections since mixed infections are common in UTIs and rest were single isolates.

S. No.	Co-morbidities/Diagnosis/Risk factors	n (%)
1.	Renal calculus	151 (45.2%)
2.	Chronic Kidney disease/Acute tubular necrosis	59 (17.7%)
3.	Chronic Liver disease/Alcoholism/Hepatitis	52 (15.6 %)
4.	Urological surgical procedures/Urethral strictures	107 (32.0 %)
5.	Catheterisation procedures	157 (47%)
6.	Fistula/Vesicovaginal Fistula (VVF)	32 (9.58 %)
7	Renal transplant recipients	40 (12%)
8.	GIT related surgeries	17 (5.1%)
9.	Diabetes mellitus	149 (44.6%)
10.	Gynaecological surgeries/procedures	22 (6.6%)

[Table/Fig-2]: Co-morbidities/Risk factors in the Cases (N=334).

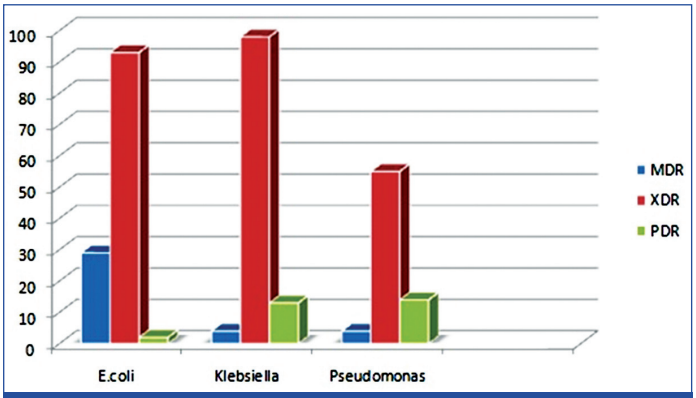
Of these 334 isolates, *Escherichia coli* 124 (37.1%) was the predominant one; followed by, *Klebsiella pneumoniae* 115 (34.4%), and *Pseudomonas aeruginosa* 73 (21.9%) [Table/Fig-3].

Tests for drug resistance revealed maximum isolates as Extensively Drug Resistant (XDR) in both *E. coli* (93%) and *Klebsiella pneumonia*

S. No.	Isolates	n (%)
1	*E. coli*	124 (37.1)
2	*Klebsiella*	115 (34.4)
3	*Pseudomonas*	73 (21.9)
4	*Acinetobacter*	9 (2.7)
5	*Providencia*	2 (0.6)
6	*Proteus spp.*	4 (1.2)
7	*Morganella*	1 (0.3)
8	*Chryseobacterium*	4 (1.2)
9	*Burkholderia*	2 (0.6)

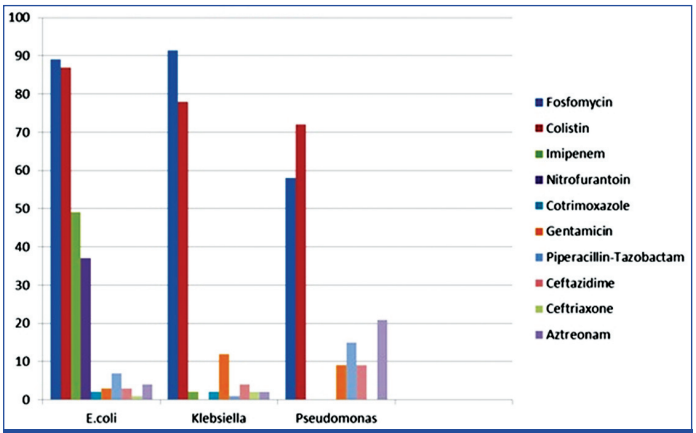
[Table/Fig-3]: Distribution of isolates in the cases (N=334).

(98%) subgroup. *Pseudomonas* comprised of 55% of the isolates as XDR and 14% as Pandrug Resistance (PDR). [Table/Fig-4] highlights the percentages of various isolates as XDR, MDR and PDR.



[Table/Fig-4]: Distribution of isolates according to resistance pattern (MDR, XDR, PDR). Bars representing the number of cases

Drug sensitivity testing of these 334 isolates, revealed 79.6% (266/334) of the isolates as sensitive to fosfomycin [Table/Fig-5]. An 88.7% (110/124) of *E. coli* and 91.3% (105/115) of *K. pneumoniae* isolates were sensitive to fosfomycin. Of the other urinary antibiotics which are commonly used for drug resistant UTI, colistin showed sensitivity of 87.1% (108/124) in the *E. coli* isolates; followed by Imipenem 49.2% (61/124) and nitrofurantoin 37.1% (46/124). On the other hand; 78.3% (90/115) of *K. pneumoniae* isolates were sensitive to colistin. On the other hand, colistin showed a higher sensitivity (72%) in *P. aeruginosa* isolates as compared to fosfomycin (58%). Comparison of the sensitivity patterns of various first line drugs in contrast with fosfomycin has been depicted for the isolates of *E. coli, K. pneumoniae* and *Pseudomonas* in [Table/Fig-5].



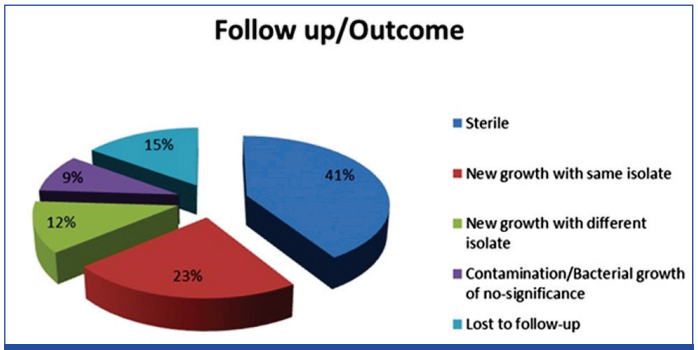
[Table/Fig-5]: Sensitivity pattern in *E. coli, Klebsiella*, and *Pseudomonas* to different drugs as compared to Fosfomycin.

Further, the MIC values of fosfomycin were also studied for the different isolates. The sensitive *E. coli* isolates 110/124 (88.7%) in the present study had lower MICs with range of 0.064-64 mg/L and *K. pneumoniae* had MIC range of 0.064-32 mg/L. On the other hand, sensitive *Pseudomonas* strains had MIC in the range of 2-64 mg/L. The MIC values for colistin resistant isolates ranged from 8-256 mg/L for Enterobacteriaceae group [Table/Fig-6].

S. No.	Isolates	Fosfomycin (mg/L)
1.	*Escherichia coli*	0.064-64
2.	*Klebsiella pneumoniae*	0.064-32
3.	*Pseudomonas aeruginosa*	2-64

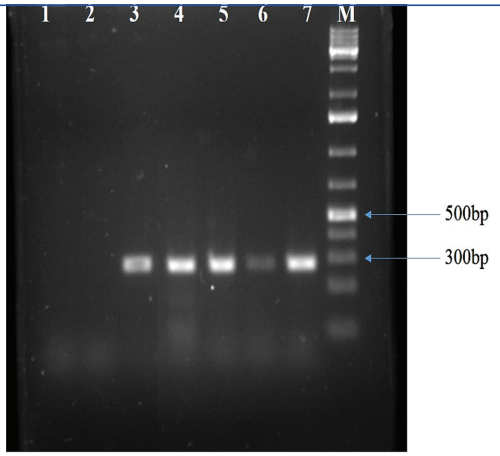
[Table/Fig-6]: MIC range Comparison of Fosfomycin in different sensitive isolates.

Finally, patients were studied for their outcomes after initiation of treatment and follow-up was planned for a period of three months. Of these 137 (41%) were reported as cured and cultures became sterile while; 50 (15%) of the patients were lost to follow-up. Detailed outcome of the cases is described in [Table/Fig-7].



[Table/Fig-7]: Outcomes on follow-up of the cases.

Genotype analysis for testing resistance mechanisms in the drug resistant isolates by PCR revealed 7 K. *pneumoniae* isolates as positive for Fos A4, 9 for Fos A5 and one for Fos C. Two *E. coli* isolates were positive for both Fos A4 and Fos A5 [Table/Fig-8,9].



Representative gel picture of FosA4 gene PCR
Lane 1-7 : Sample 1-7, Lane M: 1Kb+ Ladder

[Table/Fig-8]: Gel picture of Fos A4 gene PCR.

Isolates	No. of Fos A4 positive	No. of Fos A5 positive	No. of Fos C positive
Klebsiella pneumoniae	7	9	1
Escherichia coli	2	2	0

[Table/Fig-9]: Prevalence of Fos genes in different isolates.

DISCUSSION

Fosfomycin with its broad spectrum of action, oral dosing regimen and good bioavailability acts as a novel and suitable treatment option for drug resistant UTI isolates.

Current study showed *E. coli* and K. *pneumoniae* as the predominant isolates causing drug resistant UTI. This finding is in concordance with other similar studies by Demir T and Buyukguclu T (6.1%) [20] and Hirsch EB et al., (5.6%) [21]. Overall, fosfomycin showed much greater sensitivity of 79.6% as compared to other urinary drugs. Majority of the *E. coli* and *Klebsiella* isolates were sensitive

to fosfomycin with very low MIC values (0.064- 32 mg/l). In a similar study by Maraki S et al., fosfomycin emerged as the most active drug against a majority of drug resistant urinary isolates [22]. In another similar study by Rajenderan S et al., maximum UTI isolates of *E. coli* and *Klebsiella* were again found to be maximally sensitive to fosfomycin [23].

Of the 334 isolates, maximum isolates were reported as XDR in both *E. coli* and *K. pneumoniae*. All these isolates were mostly sensitive to fosfomycin. In a previous study; done by Gupta V et al., from Chandigarh, 52.6% of his isolates were drug resistant and all strains were susceptible to fosfomycin [24]. De Cueto M et al., also demonstrated the high in-vitro activity of drug resistant *E. coli* and *K. pneumoniae* strains on exposure to fosfomycin in his study [25].

Klebsiella pneumoniae isolates of our study showed sensitivity of 91.3% to fosfomycin with MIC range of 0.064-32 mg/L. This finding is very much similar to the previous studies by Demir T and Buyukguclu T, and Perdigao Neto LV et al., who reported similar sensitivity patterns [20,26]. The *Pseudomonas aeruginosa* isolates in present study had sensitivity of 58% which was in contrast to the study by Perdigao Neto LV et al., in which almost all *P. aeruginosa* isolates were sensitive to fosfomycin [26]. This disconcordance in results may be due to the fact that CLSI has no clear MIC breakpoints for Pseudomonas isolates while; for *E. coli* and Enterococcus isolates, susceptibility to fosfomycin have been defined as an MIC ≤64 mg/L [16]. On the other hand; MIC ≤32mg/L of fosfomycin has been described as susceptible for urinary isolates by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for *Enterobacteriaceae* and *Pseudomonas* isolates [14]. This difference in interpretation of breakpoints by two established documents makes interpretation of results and comparison between different studies difficult. A 71% of the *Pseudomonas* isolates in present study had MIC values quite close to the breakpoint of 64 mg/L and there was perfect correlation between the results of disc diffusion and E-test.

Present study highlighted colistin as the second most effective drug against these drug resistant UTI isolates showing overall sensitivity of 87.1% (108/124) in the *E. coli* isolates and surprisingly showed greater sensitivity than fosfomycin in *Pseudomonas* isolates; 72.6% (53/73). However, Colistin is not a good option to treat UTI in all the cases due to many serious side-effects; Nephrotoxicity being the major one. Other therapeutic options like carbapenems, aminoglycosides, nitrofurantoin were much less effective as compared to fosfomycin in the current study.

During the follow-up period, 41% of the patients showed cure after initiation of treatment as per reported sensitivity pattern and subsequently; cultures were reported as sterile. Majority 73/137 (53%) of these patients showed improvement within 15 days; however 5.8% (8/137) of these showed remission within a time period of one to three months. A 23% (77/334) of the cases showed growth of the same isolate but with a different sensitivity pattern and 12% (40/334) cases revealed growth of a different isolate with a different sensitivity a pattern within a span of three months. The overall sensitivity pattern in these new isolates showed resistance to fosfomycin in 42.7% (50/117). A portion of the participants 15% (50/334) was also lost to follow-up. These cases could not be traced due to improper telephonic/contact details or only a single Outpatient Department (OPD) visit to our hospital.

Fos A genes were found to be most prevalent in the Fosfomycin resistant isolates of our study. This was in concordance with similar genotype studies done in other parts of the world. Ho PL et al., studied the low prevalence (<2%) of plasmid-mediated fos genes in clinical *E. coli* isolates of his study. Present study also reported maximum prevalence of resistance genes in *K. pneumoniae* and *E. coli* isolates [27,28].

Based on the study findings, Fosfomycin can be a good oral alternative to colistin and carbapenems in hospital acquired UTI especially for *E. coli*. The study here reported UTIs in 2776 cases of which approximately 39% were hospital acquired UTIs occurring most commonly due to urinary catheters in the patients. Current study also highlighted catheterisation procedures as the most important risk factor in 47% of the drug resistant cases and urinary catheters were seen in majority of the patients. Its activity is good against other *Enterobacteriaceae* and *Pseudomonas aeruginosa*. However, plasmid mediated fosA resistant genes can be problem especially in *Klebsiella pneumoniae* and *Enterobacter* spp isolates.

Limitation(s)

The study was planned for a time span of one year only, similar studies with a larger time frame will add more value to this work; also clinical trials will be of more help in this direction.

CONCLUSION(S)

Fosfomycin has the potential to replace other parenteral antibiotics for the treatment of both complicated and uncomplicated UTI. It's safety profile and the ease of oral dosage adds to its pros as a suitable treatment option for such cases. We strongly advocate the addition of this novel antibiotic in the routine sensitivity panel for drug resistant UTI cases. Hence, further studies and research work needs to be planned to explore this potential urinary antibiotic for its future use in hospital settings.

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PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Microbiology, SGPGI, Lucknow, Uttar Pradesh, India.
2. Senior Resident, Department of Microbiology, SGPGI, Lucknow, Uttar Pradesh, India.
3. Assistant Professor, Department of Microbiology, SGPGI, Lucknow, Uttar Pradesh, India.
4. Senior Resident, Department of Microbiology, SGPGI, Lucknow, Uttar Pradesh, India.
5. PhD Student, Department of Microbiology, SGPGI, Lucknow, Uttar Pradesh, India.
6. Professor and Head, Department of Microbiology, SGPGI, Lucknow, Uttar Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Sweta Singh,
Senior Resident, Department of Microbiology, SGPGI, Lucknow, Uttar Pradesh, India.
E-mail: swetasinghkarn@gmail.com

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