

1 Resistance pattern of Nitrofurantoin of Uropathogens in 2 different age groups at *Dr. Lal Path Labs*, National Reference 3 Laboratory, Rohini, Delhi.

4 Abstract

5 **Aims and objectives:** This study was undertaken to highlight the resistance pattern of Nitrofurantoin
6 among 5162 UTIs causing isolates at Microbiology Department of *Dr. Lal Path Labs* between April to
7 June 2019.

8 **Materials and Methods:** This retrospective study was performed in Department of Microbiology at *Dr.*
9 *Lal Path Labs*, Delhi during period April to June 2019. Standard loopful midstream urine samples
10 collected in a sterile container were inoculated on UTI Chromagar and incubated overnight at 37°C
11 and demonstrating significance colony count of $\geq 10^5$ CFU/ml. Common Enterobacteriaceae group of
12 isolates and Gram positive isolates identified by MALDI TOF-MS (Bruker, Daltonics) were included in
13 this study. To determine Nitrofurantoin resistance by VITEK-AST (Biomérieux) system on 280/P628
14 antibiotic susceptibility testing cards respectively as per as CLSI M100-S-29. *Proteus spp.*,
15 *Pseudomonas spp.*, *Acinetobacter spp.*, of complicated UTI isolates were excluded from this study.

16 **Results:** Of the 29485 urine samples tested during April to June 2019, 5162 (17.5%) were culture
17 positive. Out of the 5162 positive isolates 2856 (55.3%) were isolated from female patients and 2306
18 (44.7%) from male patients. The most common bacterial isolates were members of
19 Enterobacteriaceae 4728 (91.5%) and Gram positive were 434 (8.5%) . The most predominant age
20 group infected with uropathogens were elderly adults ≥ 50 years of age constituted 58.1% of the UTIs
21 culture positive cases. *Klebsiella pneumoniae* demonstrated highest resistance of 92.3% whereas
22 *Staphylococcus aureus* demonstrated the least resistance of 8.3%.

23 **Discussion and conclusion:** The alarming substantial resistance to Nitrofurantoin in decreasing
24 order has been noticed in Enterobacteriaceae i.e., *Klebsiella pneumoniae*(92.3%) , *Enterobacter*
25 *spp.*(58.2%), *Citrobacter spp* (42.6%) and Gram positive *Enterococcus spp.* (45.6%) which is across
26 all age groups. Most susceptible to Nitrofurantoin were *Escherichia coli* (69.9%) in Gram negative and
27 *Staphylococcus aureus* (91.7%) in Gram positive. This finding emphasis the need of robust restriction
28 of Nitrofurantoin antibiotic policy and usage to uncomplicated UTIs caused by *Escherichia coli* and
29 *Staphylococcus aureus*.

30 **Keywords:** Nitrofurantoin, Uropathogens, UTIs (urinary tract infection), Enterobacteriaceae, Gram
31 positive.

32 **Introduction:** Urinary UTIs, is one of the most frequent infection in mankind, and are still among the
33 most common bacterial infections in the world. It is estimated to affect 150 million people each year
34 world wide. ¹ Nitrofurantoin is a broad spectrum, cheap and best bactericidal antibiotic which is used
35 for treating uncomplicated UTIs and nosocomial lower UTIs, that is an antibiotic for affects both Gram
36 -ve and Gram+ ve bacteria including *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter*,
37 *Enterobacter*, *Enterococcus*, *Staphylococcus aureus*, ESBL producing strains, also active against
38 VRE and VSE ^{1,2}. Nitrofurantoin is active against most common uropathogens but most *Proteus*
39 *species*, *Serratia marcescens* and *Pseudomonas aeruginosa* are naturally resistant. ²

40 Incidence, prevalence and antibiogram of adults and pediatric UTIs differ from country to country and
41 within same country between different geographical areas and also in different age groups. ¹⁵ The
42 alarming rise of resistant to Nitrofurantoin is a matter of concern about the use of Nitrofurantoin
43 limitations in Indian scenario.

44 It was observed that increasing of MIC of Nitrofurantoin in pediatric and adults UTIs has not been
45 reported from this part of our country that is northern India, Delhi. In this study, the objective was to
46 investigate the Cumulative MIC of Nitrofurantoin for different types of uropathogens. On the basis of
47 our findings Nitrofurantoin should no longer be recommended for initial empirical therapies for
48 *Klebsiella pneumoniae*, *Enterobacter*, *Enterococcus*, noscomial *E. Coli*. Hence this study was
49 undertaken to determine the cumulative interpretation and MIC of Nitrofurantoin of pediatric and
50 adults UTIs.

51 **Methods:** This retrospective study was performed in Department of microbiology at *Dr Lal Path Labs*,
52 Delhi for a period April to June 2019. A total of 29,485 midstream urine samples were submitted to
53 microbiology department of *Dr Lal Path Labs* for processing. According to the standard
54 microbiological techniques with standard (10µl) loopful urine was inoculated on UTI Chrom agar and
55 incubated overnight at 37°C under aerobic conditions. Based on Cfu/ml, the cultures were classified
56 as negative, insignificant, Significant and contamination as per standard recommendations.
57 Significant growth was determined as >10⁵ colony forming units CFU/ml of midstream urine, >10²
58 CFU/ml of a catheter specimen and any no. of colonies from a suprapubic sample. More than two
59 types of bacteria on culture were excluded from this study.

60 Common Enterobacteriaceae group of isolates *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter*,
61 *Enterobacter*, and Gram positive isolates of *Enterococcus*, *Staphylococcus aureus* identified by
62 MALDI TOF-MS (Bruker, Daltonics) were included in this study. VITEK-2 (Biomerieux) system was
63 employed for the antibiotic susceptibility testing of isolates from the pure culture of isolated colonies of
64 the uropathogens on UTI Chrom agar, the Gram negative and Gram positive bacteria were inoculated
65 on to N280/P628 cards respectively.

66 CLSI M100-S-29⁹ interpretive criteria for Enterobacteriaceae and Gram positive were utilized for
67 Nitrofurantoin. For Enterobacteriaceae, *Enterococcus*, *Staphylococcus aureus* breakpoints were
68 ≤32µg/ml (susceptible), 64µg/ml (intermediate) and ≥128 µg/ml (resistant). *Proteus spp.*,
69 *Pseudomonas spp.*, *Acinetobacter spp.*, of complicated UTI isolates were excluded from this study.

70 **Statistical analysis:** For the evaluation of the study data Myla (bioMerieux, India Pvt.Ltd).

71 statistical analysis program was used.

72 **Results:** We assessed the activity of Nitrofurantoin against 5162 (17.5%) that comprises
73 Enterobacteriaceae and Gram positive UTIs uropathogens that were collected during the study
74 period, they consisted of *Escherichia coli* (71.5%), followed by *Klebsiella pneumoniae*(17.8%),
75 *Citrobacter spp.*(0.7%), *Enterobacter spp.*(1.5%), *Enterococcus spp.* (7.7%), *Staphylococcus aureus*
76 (0.7%) (**Figure1**). Out of the 5162 positive isolates 2856 (55.3%) were isolated from female patients
77 and 2306 (44.7%) from male patients. 6.1% of isolates of them belonging to the age group of 0-12
78 years and rest were 93.9% of 13-95 years. In our study the most predominant age group infected with
79 uropathogens were elderly adults ≥50 years (58.1%) followed by adults (20%), young adults (15.8%)
80 and children (6.1%) (**Table: 1**). The prevalence of isolates among Enterobacteriaceae and Gram
81 positive were stratified by age group, the most frequently identified bacteria in pediatric age group
82 were *Escherichia coli* (76.1%) (**Figure2**). Antibiotic resistance to Nitrofurantoin was elevated across
83 all age groups for Enterobacteriaceae and Gram positive bacterial species, but it was especially high
84 among isolates of *Klebsiella pneumoniae* (92.3%), *Enterobacter* (58.2%) and *Enterococcus* (45.6%).
85 Conversely *Escherichia coli* (30.1%) and *Staphylococcus aureus*(8.3%) resistance to Nitrofurantoin
86 were low (**Table: 2**). The prevalence of resistance increased by age group for several uropathogens
87 for example, *Citrobacter*, *Staphylococcus aureus* resistant to Nitrofurantoin were relatively low among
88 isolates from pediatric age group(**Figure3**).

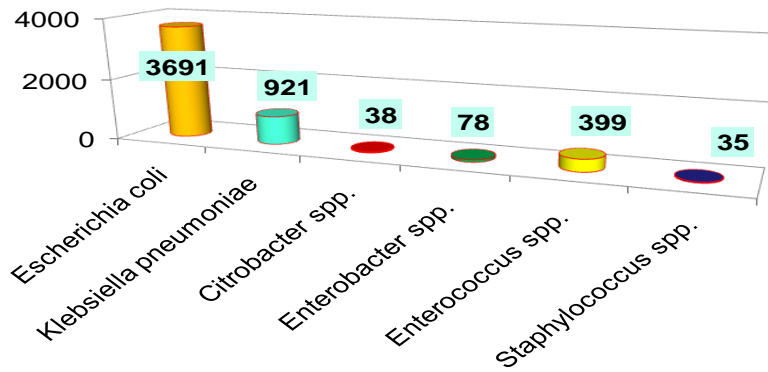
89 **Table 1: Distribution of uropathogens in different age groups.**

Age Groups	Total number of Uropathogens	% of Uropathogens
0-12	314	6.1
13-30	814	15.8
31-50	1035	20
>51-95	2999	58.1

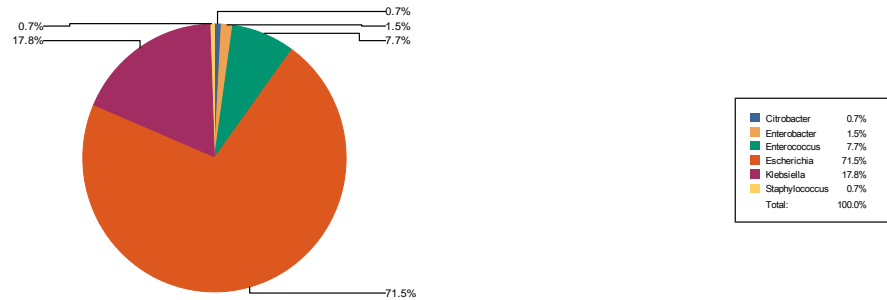
90

91 **Figure 1: Distribution of uropathogens among culture positive samples in all age groups**
 92 **during April to June 2019.**

Distribution of uropathogens among culture positive samples in all age groups during April to June 2019.



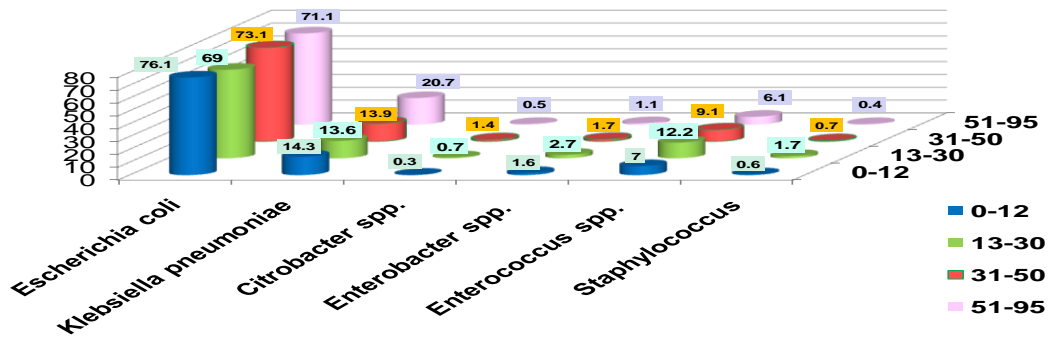
93



94

95 **Figure 2: Age specific distribution of uropathogens during April to June 2019.**

Distribution of uropathogens in different age groups during April to June 2019.



96

97

98 **Table 2: Percentage of Cumulative interpretation of Nitrofurantoin resistance in all age**
 99 **groups.**

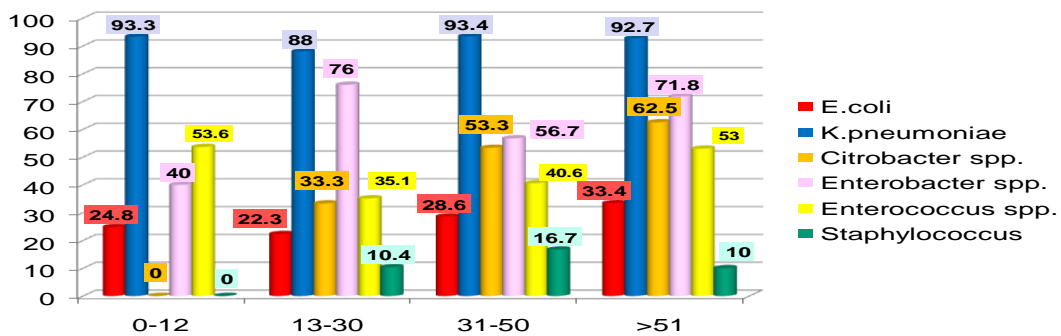
<i>Escherichia.coli</i>	<i>Klebsiella pneumoniae</i>	<i>Citrobacter spp.</i>	<i>Enterobacter spp.</i>	<i>Enterococcus spp.</i>	<i>Staphylococcus aureus.</i>
30.1%	92.3%	42.6%	58.2%	45.6%	8.3%

100

101 The cumulative interpretation of Nitrofurantoin drug in different age groups shown dramatically
 102 changes we found that resistance of Nitrofurantoin were not related to age groups all uropathogens
 103 consistently increasing in all age groups. Only *Staphylococcus aureus* shown sensitive against
 104 Nitrofurantoin and most predominant age group were >=51 year of age (Figure: 3).

105 **Figure 3: Percentage of Cumulative interpretation of Nitrofurantoin resistance patterns of age**
 106 **specific distribution of uropathogens during April to June 2019.**

Percentage of resistance patterns of Nitrofurantoin in uropathogens on the basis of cumulative interpretation in different age groups during April to June 2019.



107

108 And this study describes for the first time cumulative MIC interpretation of Nitrofurantoin resistance
 109 patterns among Enterobacteriaceae and Gram positive isolates with help of Myla statistical analysis
 110 (Biomérieux, India) which causes complicated UTIs such as *Klebsiella pneumoniae*, *Enterobacter* and

111 *Enterococcus* is increasing in community acquired UTIs in Delhi. Total 3691(71.5%) *Escherichia coli*
 112 isolates tested against Nitrofurantoin, 45% of *Escherichia coli* isolates was having MIC \leq 16 μ g/ml g
 113 and 70% of isolates tested was having MIC \leq 32 μ g/ml (**Table 3**). Out of 921(17.8%) tested isolates
 114 of *Klebsiella pneumoniae* only 3% isolates having MIC \leq 16 μ g/ml and 8% of isolates was having MIC
 115 \leq 32 μ g/ml. Nitrofurantoin activity (MIC_{50/90} 128/512) against *Klebsiella pneumoniae* demonstrated that
 116 50 % of isolate were within 128 μ g/ml MIC and 90% isolates were within 512 μ g/ml, *Klebsiella*
 117 *pneumoniae* were recorded high resistance rate (92.3%) in this study (**Table 3**). Second highest
 118 resistance recorded to Nitrofurantoin in *Enterobacter spp.* (58.2%) in Delhi. The distribution of
 119 Nitrofurantoin MIC values against resistant patterns of other uropathogens followed in (**Table 3**).

120

121 **Table 3: Percentage of Cumulative MIC interpretation and antimicrobial activity of**
 122 **Nitrofurantoin against uropathogens from all age groups during April to June 2019.**

Uropathogens	MIC (μ g/ml)/ cumulative%									
	16	32	64	128	256	512	MIC ₅₀	MIC ₉₀	%S	%R
<i>Escherichia coli</i>	45	70	90	96	99	100	32	128	69.9	30.1
<i>Klebsiella pneumoniae</i>	3	8	32	58	78	100	128	512	7.7	92.3
<i>Citrobacter spp.</i>	13	57	76	89	95	100	64	256	57.4	42.6
<i>Enterobacter spp.</i>	8	42	76	100	--	--	64	128	41.8	58.2
<i>Enterococcus spp.</i>	28	54	63	82	93	100	64	256	54.4	45.6
<i>Staphylococcus aureus</i>	50	92	90	90	100	---	\leq 16	64	91.7	8.3

123 -- -Not tested

124

125 **Discussion:** Many countries as well as different part of India reported Nitrofurantoin as first line of
 126 antibiotic for treatment and prophylaxis of acute lower UTIs and noscomial infection of UTIs. ^{1,5,7,12,14}
 127 Our study describes the distribution and antibiotic resistance of Nitrofurantoin based on Cumulative
 128 interpretation and MIC across all age groups.

129 Comparing the age groups most commonly affected by UTIs with different uropathogens in our study
 130 was the elderly group aged \geq 50 age and least affected (0-12) years of age which is similar to other
 131 studies. ^{12, 15}

132 This study highlighted potential and the limitation of this agent in the era of antibiotic resistance
 133 especially in Delhi, India. *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter*, *Enterobacter* and
 134 *Enterococcus* isolates are reported to be the most common organisms causing UTIs in not only
 135 noscomial infections but community acquired infections.

136 In accordance with the several global and national reports our study revealed *Escherichia coli* (71.5%)
 137 as the most predominantly isolated uropathogen associated with UTIs in all age groups. ^{1-8, 10, 14}
 138 Throughout the entire study, 30.1% of *Escherichia coli* isolates showed resistant against
 139 Nitrofurantoin. Similar finding were also reported by several authors. ^{12, 14, 15}

140 The present study of cumulative MIC of Nitrofurantoin resistance have reported high level of
 141 resistance on *Klebsiella pneumoniae* (92.3%), which is in agreement with the findings of few studies
 142 from India and Taiwan quoted high resistance against Nitrofurantoin ($>$ 75%) in *Klebsiella*
 143 *pneumoniae*. ^{13,17, 18} Interesting thing is that the world seem Nitrofurantoin is sensitive in world. ^{1-10, 14,15}

144 To best of our knowledge 58.2% and 42.6% isolates of *Enterobacter*, *Citrobacter*, respectively that
145 causes complicated UTIs having resistant to Nitrofurantoin. This finding was in contrast with
146 previously performed studies in which *Citrobacter spp.* were reported sensitive to
147 Nitrofurantoin.^{12,14,15} Among the Gram negative organisms isolated in our study *Citrobacter spp.*
148 (42.6%)and *Enterobacter spp.* (58.2%) had a high level of resistance to Nitrofurantoin, this is in
149 consistence with findings of other studies.^{11,13}

150 On concordance to the finding of various other previous studies which documented among the gram
151 positive organisms *Enterococcus spp.* (45.6%) showed very high level of resistance to nitrofurantoin¹⁶
152 This finding was in contrast with previously performed studies in which *Enterococcus species* were
153 reported sensitive to Nitrofurantoin.^{12,14,15}

154 Among the gram positive organisms isolated in our study *Staphylococcus aureus* had a very low level
155 of resistance (8.3%) to Nitrofurantoin used in this study this is similar with other studies.¹⁵

156 Study of all uropathogens indicate that resistance to Nitrofurantoin is on rise and treatment of UTIs is
157 becoming more difficult with time more over there are considerable regional and geographic
158 differences in the susceptibility pattern of uropathogens is required and choose the appropriate
159 empiric therapy of Nitrofurantoin for UTIs in children and adults.

160 To our knowledge, this is the first study that highlights MIC of Nitrofurantoin for *Escherichia coli*,
161 *Klebsiella pneumoniae*, *Citrobacter spp.*, *Enterobacter spp.*, *Enterococcus spp.* and *Staphylococcus*
162 *aureus* MIC₅₀ (concentration that inhibited 50% of isolates) was 32, 128, 64, 64, 16µg/ml and MIC₉₀
163 (concentration that inhibited 90% of isolates) was 128, 512, 256, 128, 256, 64 µg/ml respectively our
164 results clearly demonstrated that Nitrofurantoin remains available suitable option for community
165 acquired UTIs from *Escherichia coli* and *Staphylococcus aureus* in Delhi. This is in similar to other
166 studies.^{14, 19, 20}

167 **Conclusion:** To conclude that UTIs varies with age groups therefore, extensive evaluation among
168 interpretation by cumulative MIC of Nitrofurantoin increases with increasing age groups. Emergence
169 of increasing MIC of Nitrofurantoin to *Klebsiella pneumoniae*, *Enterobacter spp.*, *Enterococcus spp.*,
170 *Citrobacter spp.* has become the concern for policy makers and a urgent need of strict antibiotics
171 prescription policy in our country. Judicious selection of antibiotics as per organisms recommendation
172 by CLSI M-100. S-29 is the need of hour. Further Nitrofurantoin should be restricted to complicated
173 and non complicated UTI by *Klebsiella pneumoniae* only.

174 **References :**

- 175 1. Alqasim A, Jaffal AA, and Abdullah AA et al. Prevalence of Multidrug Resistance and
176 Extended-Spectrum β-Lactamase Carriage of Clinical Uropathogenic *Escherichia*
177 *coli* Isolates in Riyadh, Saudi Arabia. International Journal of Microbiology. (2018);
178 <https://doi.org/10.1155/2018/3026851>
- 179 2. Cunha BA, Schoch EP, Hage RJ et al., Mayo Clin Proc. 2011, 86(12):1243-1244.
- 180 3. Maria Jose Munoz-Davila. Review, Antibiotics. (2014); 3:39-48.
- 181 4. Gunduz S and Altun HU. Antibiotic resistance patterns of urinary tract pathogens in Turkish
182 children. Global Health Research and Policy (2018); 3:10.[https://doi.org/10.1186/s41256-](https://doi.org/10.1186/s41256-018-0063-1)
183 [018-0063-1](https://doi.org/10.1186/s41256-018-0063-1).
- 184 5. Keyhan H, Sedighi S, Mashayekhi B et al., Community Acquired Urinary Tract
185 Infections, Etiological Organisms and Antibiotics Susceptibility Patterns, Nephro-Urol
186 Mon.(2017); 9(5).e62146 doi: 10.5812/numonthly.62146

- 187 6. Huttner A, Verhaegh EM, Harbarth S, et al., Nitrofurantoin revisited: a systematic review
188 and meta analysis of controlled trials, J Antimicrob chemother **(2015)**; 70:2456-2464.
- 189 7. Ekwealor PA, Malachy CU, Ezeobi I, Amalukwe G, Ugwu BC, Okezie U, Stanley C,
190 Esimone C. Antimicrobial Evaluation of Bacterial Isolates from Urine Specimen of Patients
191 with Complaints of Urinary Tract Infections in Awka, Nigeria. J Int J Microbiol. **(2016)**;
192 2016.9740273.
- 193 8. Sanchez GV, Babiker A, Master RN, et al., Antibiotic Resistance among Urinary Isolates from
194 Female Outpatients in the United States in 2003 and 2012. Antimicrobial agents and
195 chemotherapy **(2016)**; DOI: 10.1128/AAC.02897-15.
- 196 9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk
197 susceptibility tests. Wayne Pa: Clinical and Laboratory Standards Institute: M100-S29;
198 **(2019)**.
- 199 10. Nahar A, Hasnat S, Akhter H et al .Evaluation of antimicrobial resistance pattern of
200 uropathogens in a tertiary care hospital in Dhaka city, Bangladesh. South East Asia Journal
201 of Public Health. **(2017)**; 7(2):12-18,
- 202 11. Patel HB, Soni ST, Bhagyalaxmi A and Patel NM. Causative agents of urinary tract
203 infections and their antimicrobial susceptibility patterns at a referral center in Western India:
204 An audit to help clinicians prevent antibiotic misuse. J Family Med Prim Care. Journal of
205 Family Medicine and Primary care. **(2019)**; 8: 1: 154-159
- 206 12. Patwardhan V, Kumar D, Goel V, Singh S. Changing prevalence and antibiotic drug
207 resistance pattern of pathogens seen in community-acquired pediatric urinary tract
208 infections at a tertiary care hospital of North India. **(2017)**; Journal of Laboratory
209 Physicians.9 (4):264-268.
- 210 13. Shailaja ST and Kumar MA. Antimicrobial Resistance among Uropathogenic Bacteria in
211 Rural Kerala, India. Int.J.Curr.Microbiol.App.Sci. **(2017)**; 6(9):2287-2296.doi:
212 <https://doi.org/10.20546/ijcmas.2017.609.2>.
- 213 14. Shakti L, Veeraraghavan B. Advantage and limitations of nitrofurantoin in multidrug
214 resistant Indian scenario. Indian J Med Microbiol. **(2015)**; 33(4):477-81.
- 215 15. Singh R M, Devi MU., Singh K L, et al., Evaluation of nitrofurantoin activity against the
216 urinary isolates in the current scenario of antimicrobial resistance. Ann Trop Med Public
217 Health. **(2015)**; 8(6): 280-285.
- 218 16. Benachinmardi K, Padmavathy M, Malini J, Navneeth BV. Microbiological profile and
219 antibiogram of uropathogen in pediatric age group. Int. J. Health. Allied Science. **(2015)**;
220 4:61-64.
- 221 17. Mishra MP, Debata NK, and Pathy RN. Surveillance of multidrug resistant uropathogenic
222 bacteria in hospitalized patients in India. Asian. Pac. J. Trop. Biomed. **(2013)**; 3(4):315-324.
- 223 18. Liu HY, Lin HC, Lin YC, Yu SH, Wu WH, Lee YJ. Antimicrobial susceptibilities of urinary
224 extended spectrum beta lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*
225 to fosfomycin and nitrofurantoin in a teaching hospital in Taiwan. J Microbiol. Immunol
226 Infect. **(2011)**; 44:364-8.

- 227 **19.** Komp Lindgren P, Klockars O, Malmberg C, Cars O. Pharmacodynamic studies of
228 nitrofurantoin against common uropathogens. *J. Antimicrob. Chemother.* **(2015)**; 70(4):1076-
229 82.
- 230 **20.** John Osei Sekyere. Genomic insights into nitrofurantoin resistance mechanism and
231 epidemiology in clinical enterobacteriaceae. *Future Sci OA.* **(2018)**; 4(5):FS0293.

UNDER PEER REVIEW