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

Abstract

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

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

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

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

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

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

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

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

Methods: This retrospective study was perfomed in Department of microbiology at *Dr Lal Path Labs*, Delhi for a period April to June 2019. A total of 29,485 midstream urine samples were submitted to microbiology department of *Dr Lal Path Labs* for processing. According to the standard microbiological techniques with standard (10µl) loopful urine was inoculated on UTI Chrom agar and incubated overnight at 37˚C under aerobic conditions. Based on Cfu/ml, the cultures were classified as negative, insignificant, Significant and contamination as per standard recommendations. Significant growth was determined as $>10^{5}$˚colony forming units CFU/ml of midstream urine,$>10^{2}$ CFU/ml of a catheter specimen and any no. of colonies from a suprapubic sample. More than two types of bacteria on culture were excluded from this study.

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

CLSI M100-S-29[9] interpretive criteria for Enterobacteriaceae and Gram positive were utilized for Nitrofurantoin. For Enterobacteriaceae, *Enterococcus, Staphylococcus aureus* breakpoints were ≤32µg/ml (susceptible), 64µg/ml (intermediate) and ≥128 µg/ml (resistant). *Proteus spp., Pseudomonas spp., Acinetobacter spp.*, of complicated UTI isolates were excluded from this study.

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

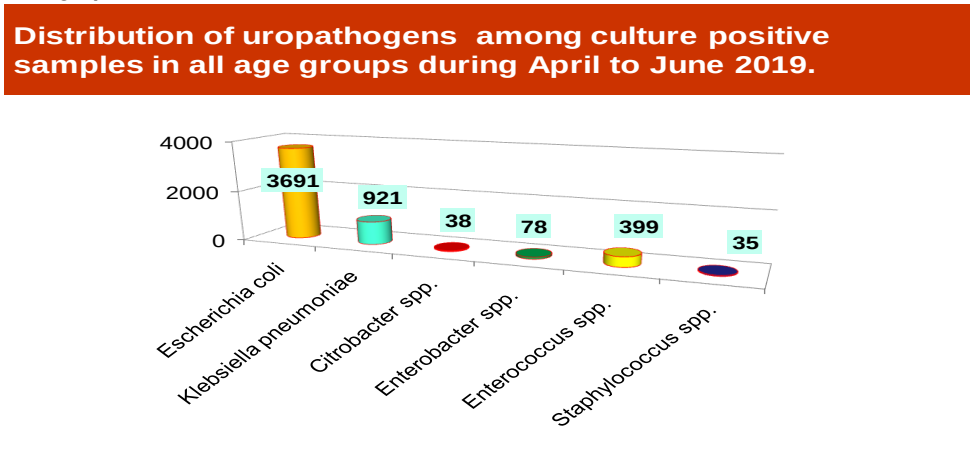
statistical analysis program was used.

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

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

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



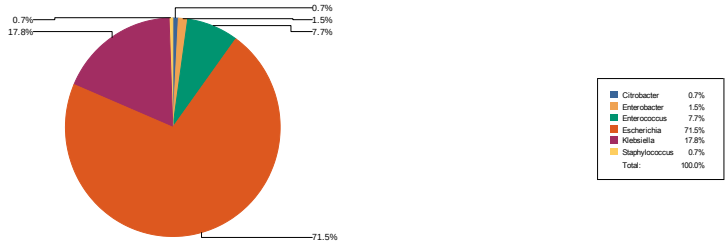
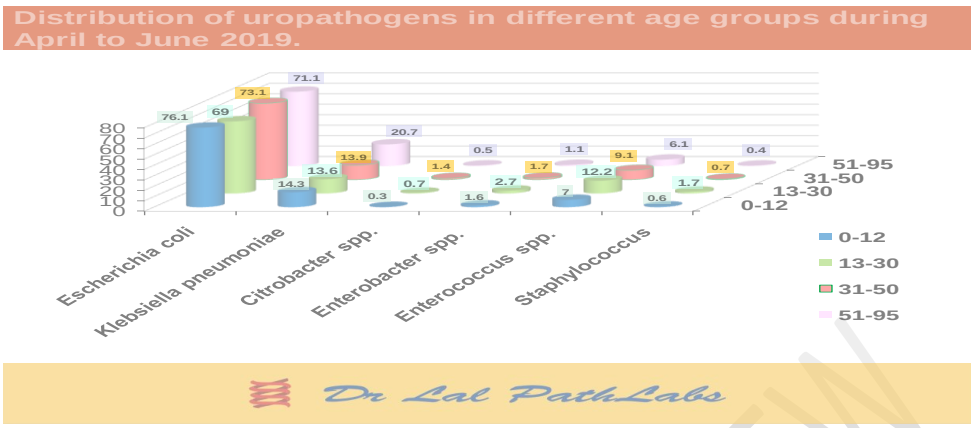


Figure 2: Age specific distribution of uropathogens during April to June 2019.



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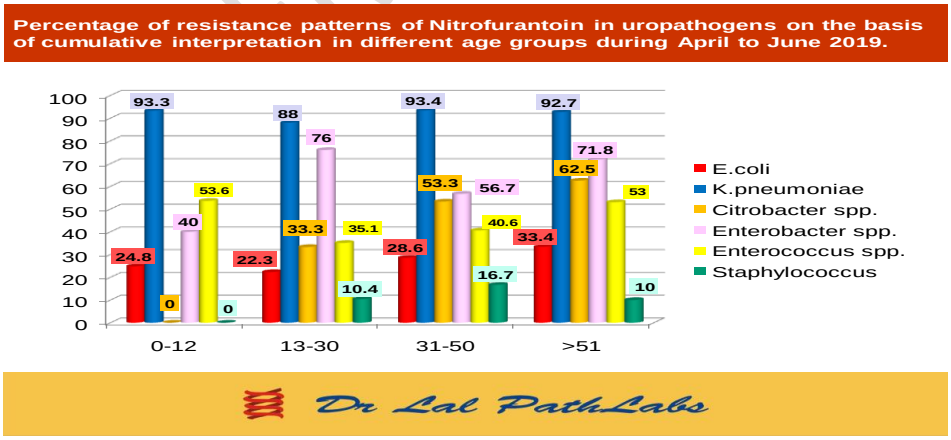
98 **Table 2: Percentage of Cumulative interpretation of Nitrofurantoin resistance in all age**
99 **groups.**

Escherichia.coli	*Klebsiella pneumoniae*	*Citrobacter spp.*	*Enterobacter spp.*	*Enterococcus spp.*	*Staphylococcus aureus.*
30.1%	**92.3%**	**42.6%**	**58.2%**	**45.6%**	**8.3%**

100

101 The cumulatative interpretation of Nitrofurantoin drug in different age groups shown dramastically
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 >=51year 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.**



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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 (Biomerieux, India)which causes complicated UTIs such as *Klebsiella pneumoniae* , *Enterobacter* and

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

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

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

-- -Not tested

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

Comparing the age groups most commonly affected by UTIs with different uropathogens in our study was the elderly group aged >=50 age and least affected (0-12) years of age which is similar to other studies.[12, 15]

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

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

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

Comment [u1]: Review this paragraph what is the meaning? Explain

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

On concordance to the finding of various other previous studies which documented among the gram positive organisms *Enterococcus spp.* (45.6%) showed very high level of resistance to nitrofurantoin [16] This finding was in contrast with previously perfomed studies in which *Enterococcus species* were reported sensitive to Nitrofurantoin.[12,14,15]

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

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

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

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

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