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Original Research Article

Nitrofurantoin as an option in the treatment of Extended Spectrum Beta-Lactamase Producing *Escherichia Coli* and *Klebsiella Pneumoniae* in Uncomplicated UTI

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Abstract
Extended Spectrum Beta- Lactamase (ESBL) producing organisms causing urinary tract infections (ESBL-UTI) are increasing in incidence and pose a major burden to health care. Although 95-100% ESBL organisms are considered sensitive to meropenem, rapid emergence of carbapenem resistance has been documented in many countries. The choice of treatment for carbapenamase producing organisms is very limited. The present study is aimed to evaluate the antibiotic susceptibility patterns in urinary tract infections caused by ESBL producing E.coli and Klebsiella species.
A total of 576 Urine samples from out-patients are taken and data regarding previous hospitalization and antibiotic administration is collected. E.coli and Klebsiella pneumoniae are major isolates in this prospective study. The study also involves the effect of demographic variables on ESBL producers and sensitivity to antibiotics. Maximum ESBL isolates were reported from K.pneumoniae (93%) and majority of the isolates were from female urine samples. ESBL producing E.coli also showed maximum sensitivity to colistin (95%) followed by nitrofurantoin (88.4%) and ESBL producing K.pneumoniae showed maximum sensitivity to colistin (93.4%) followed by nitrofurantoin (58.9%).From the present study it was concluded that, Nitrofurantoin can be incorporated as a key antibiotic for Antimicrobial stewardship programme for empirical therapy.
Keywords: *UTI, ESBL, Nitrofurantoin, Colistin.*

Introduction

Globally there are an estimated 150 million UTIs each year leading to more than 6 billion dollars in direct healthcare costs[1]. In UTI, pathogenic organisms are identified in the urine, urethra, bladder, kidney or prostate. In majority of the cases, the microbial growth greater than 10^5 organisms/mL by examining the midstream "clean catch" urine sample specify infection. Management of acute uncomplicated UTI

(cystitis) is generally straightforward, with a predictable distribution of uropathogens isolated[2].

First-line treatment of acute uncomplicated UTI has traditionally involved a 3-day regimen of trimethoprim-sulfamethoxazole (TMP-SMX) or TMP alone for patients with sulfa allergies. Alternative first-line agents include the fluoroquinolones, nitrofurantoin, and fosfomycin. Factors to be considered in the selection of appropriate antimicrobial therapy include pharmacokinetics, spectrum of activity of the antimicrobial agent, resistance prevalence for the community, potential for adverse effects, and duration of therapy[3]. Ideal antimicrobial agents for UTI management have primary excretion routes through the urinary tract to achieve high urinary drug levels. In addition, there are special considerations in the management of UTI among selected populations, including postmenopausal and pregnant women, and for women with frequent recurrent UTIs[4].

Mortality is higher following bacteremia with strains resistant to antimicrobials than with strains sensitive to antimicrobials although this is most likely due to inappropriate empiric antimicrobial therapy rather than an association with increased virulence of the E. coli strain.[1]. Extended Spectrum Beta- Lactamase producing organisms causing urinary tract infections (ESBL-UTI) are increasing in incidence and pose a major burden to health care[5]. ESBL production was first reported in *K. pneumoniae* strain, which was capable of hydrolysing oxyamino-cephalosporins (Knothe, 1983). Today, acquired resistance to beta-lactams is mainly mediated by the extended spectrum beta-lactams (ESBLs), Amp C-type cephalosporinases, Carbapenemases[6,7,8].

The first described ESBLs evolved through random point mutations in isolates with broad-spectrum beta-lactamases, i.e. TEM-1, TEM-2, TEM-13, SHV-1 and SVH-11, which were already widespread in clinical settings when extended-spectrum beta-lactams were introduced. While ESBL producing Klebsiella species seem to account for most nosocomial outbreaks, ESBL-producing E. coli has been isolated from both hospitalized and non-hospitalized patients [5]. Although 95-100% ESBL organisms are still considered sensitive to meropenem, rapid emergence of carbapenem resistance has been documented in many countries[5]. The choice of treatment for carbapenamase producing organisms is very limited. In vitro susceptibility to colistin, tigecycline and aminoglycosides is mostly preserved, but the impact of these antibiotics in vivo is still uncertain and the mortality rates remain high, despite treatment according to the results of susceptibility testing[9].

The objective of this study was to evaluate antibiotic susceptibility patterns in urinary tract infections caused by ESBL producing *E.coli* and *Klebsiella* species.

Materials and Methods

A total of 576Urine samples from out-patients were taken and data regarding previous hospitalization and antibiotic administration was collected. *E.coli* and *Klebsiella pneumonia* were major isolates. The antibiogram of other isolates belonging to Enterobacteriaceae family which are less than 30 in number are not considered in the analysis of this study. The study was conducted between June 2018 to December 2018. Demographic variables of the patient were recorded include age and sex. As the study included samples submitted to the laboratory, approval for institutional ethics board was waived.

Microbiological examination of urine samples:

All the 576 urine samples were examined for the presence of pathogenic microorganisms by using standard microbiological techniques. Urine samples were inoculated into CLED agar, The morphological examination of the microorganisms was performed through Gram's staining reaction. Basing on Gram's stain, shape and arrangement of organisms were noted. The culture of pathogens enables colonies of pure growth to be isolated for identification and Biochemical tests include carbohydrate fermentation reactions, Indole test,

Methyl red test, Voges-proskauer test, Citrate utilization, Urease test, Oxidase test, Catalase test, Nitrate reduction test, Triple sugar iron agar test etc. are used for culture conformation tests.

Antibiotic susceptibility testing

Disk diffusion method: Disk diffusion refers to the diffusion of an antimicrobial agent of a specified concentration from disks or strips, into the solid culture medium that has been seeded with the selected inoculum isolated in a pure culture. Disk diffusion is based on the determination of an inhibition zone proportional to the bacterial susceptibility to the antimicrobial present in the disk. The diffusion of the antimicrobial agent into the seeded culture media results in a gradient of the antimicrobial. AST was performed according to CLSI guidelines using Muller-Hinton agar (MHA) plates using the concentration of antibiotics per discs, recommended by the WHO experts committee on biological standardization. The plates were incubated at 37°C for 16-18h hrs. The antibiotic discs used in this study were listed in the Tables. The inhibition zone was measured according to CLSI guidelines (CLSI Catalogue, 2016)[10]. Phenotypic testing of ESBL production was done by using demonstration of synergy between clavulanic acid and broad-spectrum cephalosporins according to CLSI guidelines.

ESBL confirmation: ESBL production was tested with the CLSI confirmatory test using CAZ (30 µg) disc alone and in combination with CA (10 µg). At least 3cm distance was maintained between the disks. The test was considered positive when an increase in the growth-inhibitory zone around the CAZ disk with CA was 5 mm or greater of the diameter around the disk containing CAZ alone. The plates were incubated at 37°C for 18h [7].

Confirmation of resistance of Nitrofurantoin detected by vitek -2: Antimicrobial susceptibility testing was performed using VITEK 2, Gram Negative Susceptibility Test Card AST-N209 (bio Me´rieux), which includes amoxicillin-clavulanic acid, ampicillin, aztreonam, cefotaxime, cefoxitin, cefpirome, ceftazidime, cefuroxime, ciprofloxacin, gentamicin, mecillinam, meropenem, nalidixic acid, nitrofurantoin, piperacillin-tazobactam, tobramycin, trimethoprim and trimethoprim-sulfamethoxazole.

Detection of colistin resistant organisms by E-test initially a suspension of each test bacterial isolate in Mueller-Hinton broth was prepared and adjusted to 0.5 McFarland standards. The suspensions were now swabbed onto MHA plates. When once the surface of the agar was completely dry, a colistin E-strips (concentration ranging from 0.06 to 1,024 µg/ml were applied to each plate separately and incubated at 35°C for 16-20h. The results were noted as MIC where inhibition of growth intersected the E-strip. A ≥4 µg/ml for colistin concentration was used as the breakpoint to select as resistant isolates [11].

Results

Out of 576 urine samples examined for the presence of pathogenic microorganisms, 395 samples were reported to be sterile and 181 samples are culture positive (Figure 1). The organisms were identified based on the morphology, culture and biochemical characteristics (Figure 2) as *E.coli* (42%), *Klebsiella pneumonia* (24%) and other isolates (34%). The other isolates are Gram-positive microorganisms and those belonging to Enterobacteriaceae family other than *E.coli* & *K*lebsiella species and are <30 in number. Table 1and Figure 3 showed that the highest percentage of ESBL production was reported from *K. pneumoniae* 40 (93%) out of 43 isolates. The study also found that out of 109 urinary isolates, maximum ESBL producers were obtained from urine samples of females and less from i.e. urine samples of males (Figure 4). Among the age wise distribution of ESBL producers, maximum ESBL *E.coli* were reported in the age group of 21-30 years patient urine samples (Figure 5).Table 2 showed that *E.coli* have maximum sensitivity to the antibiotic colistin (96%) followed by nitrofurantoin (89.4%) whereas *K.pneumoniae* showed maximum sensitivity to colistin (94%)

followed by nitrofurantoin (61.9%). ESBL producing *E.coli* also showed maximum sensitivity tocolistin (95%) followed by nitrofurantoin (88.4%) and ESBL producing *K.pneumoniae* showed maximum sensitivity to colistin (93.4%) followed by nitrofurantoin (58.9%) (Table 3)

Figure 1: Distribution of total urine samples based on culture report

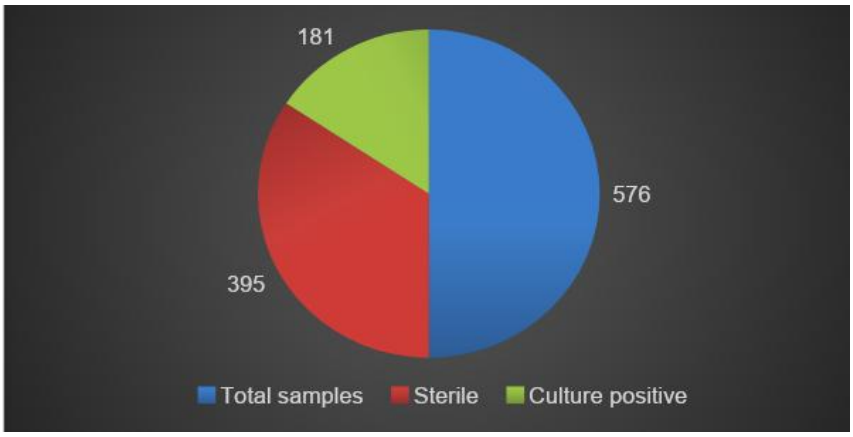

Figure 2: Distribution of pathogenic microorganisms isolated from various infections

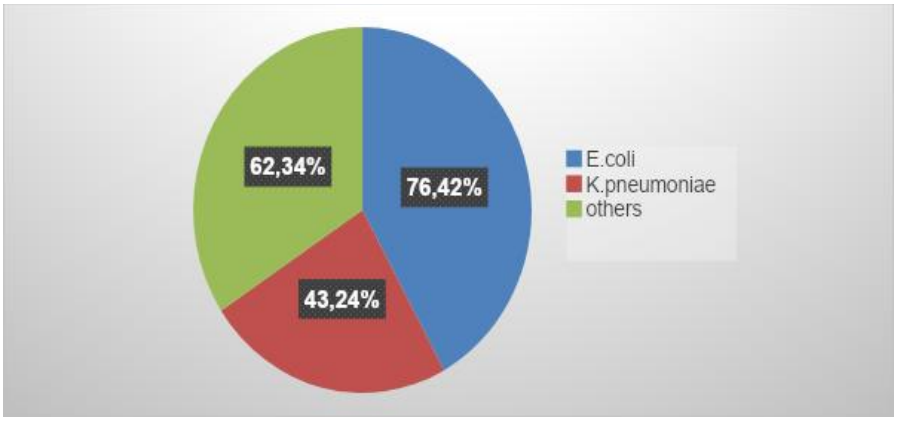

Table 1: Distribution of Extended spectrum Beta-Lactamase producing urinary microbial isolates

Urine microbial isolates	Total number of microbial isolates (n=119)	ESBL producers (n=109)	Percentage
E.coli	76	69	90.7%
Klebsiella pneumoniae	43	40	93%

Figure 3: Distribution of Extended spectrum Beta-Lactamase producing urinary microbial isolates

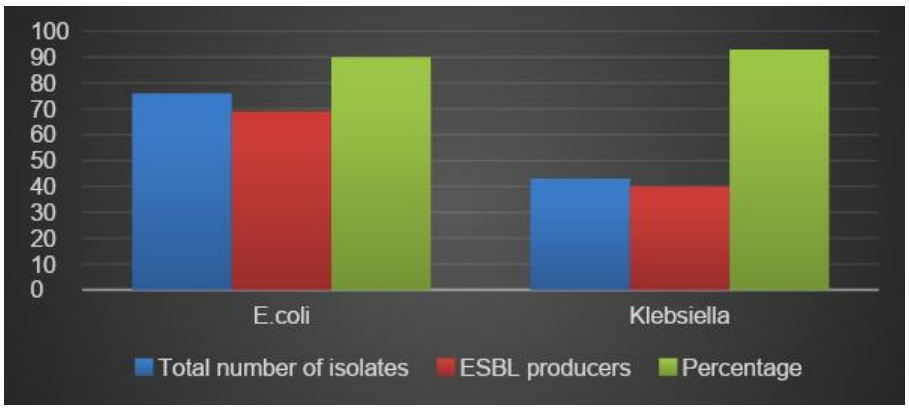

Figure 4: Gender wise distribution of ESBL producing *E.coli* and *Klebsiella pneumoniae*

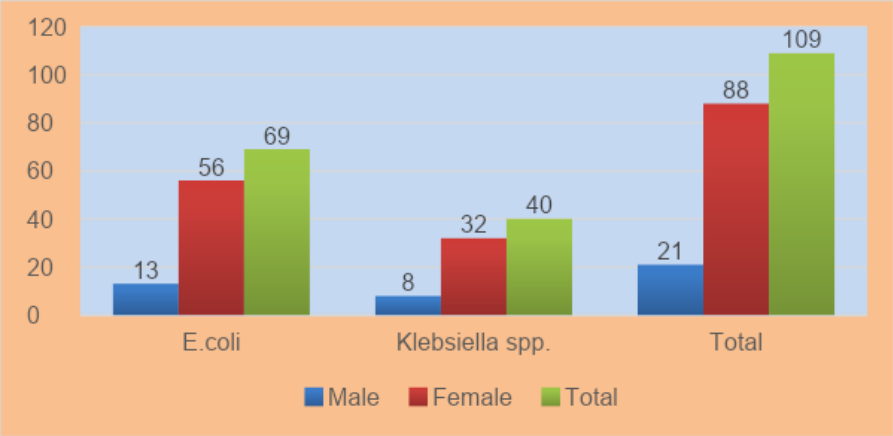

Figure 5: Age wise distribution of ESBL producing *E.coli* and *Klebsiella pneumoniae*

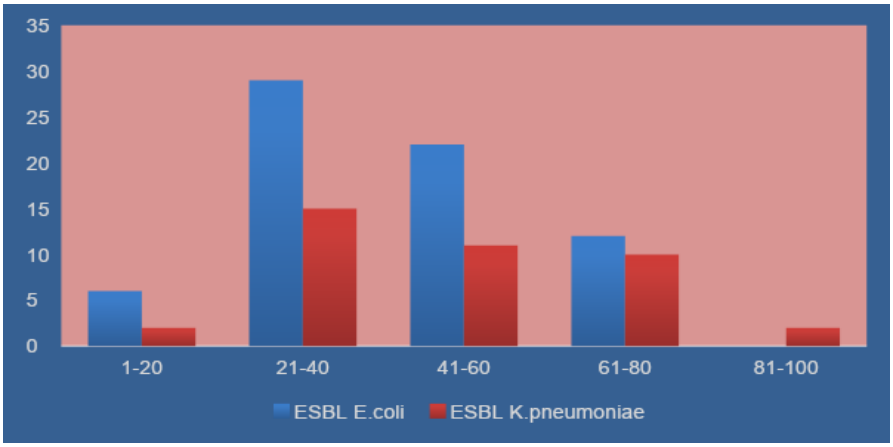

Table 2: Antibiotic sensitivity pattern of urinary microbial isolates

Antibiotic sensitivity (%)	***E.coli*** **n= 76 (64%)**	***K. pneumoniae*** **total n=43 (36%)**
Ceftazidime	9.2	6.9
Ceftazidime / clavulinic acid	9.2	6.9
Impenem	67	39.5
Meropenem	51.7	57
Nitrofurantoin	89.4	61.9
Ciprofloxacin	20.5	29
Gentamycin	35	51.2
Amikacin	55.4	54.7
Cotrimoxazole	30.5	23.6
Colistin	96	94

Table 3: Antibiotics sensitivity pattern of ESBL isolates

Antibiotic sensitivity (%)	**ESBL *E.coli*** **n=69 (90.7%)**	**ESBL *K.pneumoniae*** **n=40 (93%)**
Ceftazidime	0	0
Ceftazidime/clavulinic acid	0	0
Cefipime	14.8	6.25
Impenem	64.7	35
Meropenem	45	53
Nitrofurantoin	88.4	58.9
Ciprofloxacin	14.7	21.4
Gentamycin	30.43	47.2
Amikacin	50.7	51.2
Cotrimoxazole	27.6	20
Colistin	95	93.4

Discussion

Most of the pathogenic urinary microorganisms develop resistance to most of the commonly used antibiotics, because of acquisition of genes encoding ESBLs[12]. These ESBL producing organisms also might acquire resistance to other classes of antibiotics and thus become multidrug resistant and limits the treatment options. In general, carbapenems like imipenem, meropenem etc. are used as the drugs of choice for ESBL producers[13]. They are the last line of defence against many organisms that are resistant to other antimicrobial agents. However there is an increase in the world-wide production of beta-lactamase enzymes by the resistant microbial strains, thus hydrolyze all the β-lactam antibiotics along with carbapenems[14]. However colistin is one of the older drugs but now has become a popular choice of clinicians faced with few options in the treatment of MDR bacteria. In the present study the urinary microbial isolate *E.coli* showed maximum sensitivity to the antibiotic colistin (96%) followed by nitrofurantoin (89.4%) whereas *K.pneumoniae* showed maximum sensitivity to colistin (94%) followed by nitrofurantoin (61.9%). Because the hospital is a Tertiary care hospital the ESBL producers are more when compared to others. Most of the patients has the history of hospitalization and prior antibiotic therapy within 3 months of duration.The ESBL producing *E.coli* also showed maximum sensitivity tocolistin (95%) followed by nitrofurantoin (88.4%) and ESBL producing *K.pneumoniae*showed maximum sensitivity to colistin (93.4%) followed by nitrofurantoin (58.9%). Though the isolates showed maximum sensitivity to the antibiotic colistin but it should be given to a patient through parenteral administration, not used as monotherapy and MIC should be confirmed by broth microdilution method which is a cumbersome procedure, and Colistin can be used as a drug of last resort .The other antibiotic to which isolates showed optimum sensitivity was Nitrofurantoin which can be taken as orally as monotherapy comparatively low toxicity than that of colistin so clinicians can be considered nitofurantoin as a better choice of drug for un complicated UTI for the empirical therapy before the actual antibiotic susceptibility of the isolate. Emerging multidrug resistant isolates is one of the great concerns as they pose task to the clinicians in the treatment of many infectious diseases. The early identification of such drug resistant microorganisms may help in the prompt antimicrobial therapy while beginning and thus evade the development and dissemination of these multidrug resistance strains in the hospital as well as community. This study reveals Nitrofurantoin can be incorporated as a key antibiotic for Antimicrobial steward ship programme for empirical therapy. The study helps the clinicians in choosing the correct antimicrobial agent which contribute not only to better treatment but there judicious use will also help in preventing the emergence of drug resistant strains which are still sensitive.

Conclusion

Nitrofurantoin which can be taken as orally as monotherapy comparatively low toxicity than that of colistin so clinicians can be considered nitofurantoin as a better choice of drug for uncomplicated UTI for the empirical therapy before the actual antibiotic susceptibility of the pathogenic isolate. Nitrofurantoin can be incorporated as a key antibiotic for Antimicrobial steward ship programme for empirical therapy. The study helps the clinicians in choosing the correct antimicrobial agent which contribute not only to better treatment but there judicious use will also help in preventing the emergence of drug resistant strains which are still sensitive.

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