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Effectiveness of Amikacin, Gentamicin and Tobramycin antibiotics against uropathogenic MDR *Escherichia coli*

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ABSTRACT

The emergence of multidrug-resistant (MDR) *Escherichia coli* poses a major global health concern, particularly in urinary tract infections (UTIs). The present study focuses on the isolation of *E. coli* strains from urine samples, their antimicrobial susceptibility profile, and molecular identification of MDR isolates using 16S rRNA gene sequencing. Urine samples were collected from patients with suspected UTIs and cultured on selective media for the isolation of *E. coli*. The isolates were subjected to antibiotic susceptibility testing against various classes of antibiotics using the disc diffusion method. MDR *E. coli* strains showing resistance to multiple antibiotics were further identified and confirmed by 16S rRNA gene sequencing. The Minimum inhibitory concentration (MIC) assays were performed to determine the efficacy of different concentrations of selected antibiotics. In the present investigation the *E. coli* isolates were isolated on MacConkey's agar plate. The E. coli isolate SUC-1 showed resistance to antibiotics from class β-lactam, cephalosporin, sulphonamide, quinolones, macrolides, glycopeptides, polymyxin and synthetic antibiotics oxazolidinones. The MDR isolate SUC-1 was identified as *Escherichia coli* it was deposited in GenBank under Accession No. PP854585. Among the tested antibiotics, aminoglycoside class drugs amikacin, gentamicin, and tobramycin showed significant inhibitory activity against MDR *E. coli* isolates, indicating their continued effectiveness. The findings suggest that aminoglycosides remain potent therapeutic options for treatment of MDR urinary infections.

INTRODUCTION

The amikacin, gentamicin and tobramycin belong to class of aminoglycosides antibiotics [(1)]. The target site of these antibiotics on 30S bacterial ribosome leading to inhibition of protein synthesis [(2)]. The amikacin and gentamicin are effective against infections caused by *Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa, Nocardia spp.,* Klebsiella *spp., Salmonella spp., Shigella spp., Proteus spp., Serratia spp., Enterobacter spp.* and other gram-negative bacteria. These amikacin and gentamicin are also choice of antibiotics for treating infection caused by bacterial pathogen which are resistant to other group of antibiotics [(3,4,5)]. The development of antibiotic resistance by gram negative bacterial pathogen against these antibiotics reported to be moderate whereas certain gram-negative bacterial pathogen has amikacin resistance due to acetylation by the aminoglycoside 6′-N-acetyltransferase type Ib, an enzyme coded by a gene found in integrons, transposons, plasmids, and

chromosomes [6]. The pathogenic E. coli strains are responsible for causing diarrhoea, dysentery, gastroenteritis, pneumoniae, meningitis, bladder infection and urinary tract infection [7]. The untreated UTI and due to non-effective of antibiotics the UTI infection causes serious consequences to bladder, kidney and also increase risk of premature birth if infection during pregnancy [8]. Therefore, proper and effective treatment with antibiotics is crucial to prevent complications. The most commonly recommended antibiotics to treat infection caused by pathogenic E. coli strains are antibiotics from class β-lactam, cephalosporin, sulphonamide, nitrofurantoin, quinolones, macrolides, glycopeptides, polymyxin and synthetic antibiotics oxazolidinones. However, infection caused by certain strains of E. coli not control even by these classes of antibiotics [9, 10]. The member of class aminoglycoside viz. amikacin and gentamicin will be effective against both extended spectrum β-lactamase producing E. coli and non- extended spectrum β-lactamase pathogenic strain of E. coli [11].

Therefore, in the present investigation the *E. coli* strain will be isolated from urine samples of UTI patients. The isolated *E. coli* strain will be evaluated for antibiotic resistance and sensitivity against different class of antibiotics and the minimum inhibitory concentration of antibiotics of effective antibiotics against MDR *E. coli* strain will be determined.

MATERIALS AND METHODS

Collection of clinical samples

The urine samples were collected from patients suffering from UTI from Government Hospital and Medical College, Vishnupuri, Nanded in a sterile sample collecting bottle, closed tightly and stored in the refrigerator at 4°C until processed [12].

Isolation of *E. coli* from urine sample

The 1ml of urine samples of patients were added in 9ml sterile saline and serially diluted up to 10^{-4} to 10^{-5}. From these dilutions 0.1ml of sample suspensions were spreaded on pre-sterilized MacConkey's agar plates, EMB plates and Endo agar plates respectively, incubated for 24 hours at 37°C. The well isolated colonies were selected for its cultural and biochemical characterization including IMViC test for identification of *E. coli* [13].

Antibiotic sensitivity/resistance profiling of E. coli isolates

All the clinical *E. coli* isolates were evaluated using HiMedia™ discs to assess antibiotic sensitivity/resistance against various classes of antibiotics. The active culture of all the *E. coli* isolates were spreaded on respective Muller Hinton agar plates and incubated for 10 minutes at room

temperature. After incubation, aseptically HiMedia™ antibiotic Icosa Pseudo-1 disc, Icosa G-I Plus disc and Icosa G-1 Minus disc were transferred to the surface of media plate aseptically and all the plates were incubated for 24 hours at 37°C. After incubation, the inhibitory zone was measured and resistance was determined as per the Clinical Laboratory Standard Institute's standards [14].

Molecular Identification of MDR isolates

The most resistant isolate SUC-1 was selected for molecular identification using 16S rRNA sequencing. To amplify the bacterial 16S rRNA gene, the universal primers 16S27F and 16S1492R were used [15].

Determination of Minimum Inhibitory Concentration (MIC) of Antibiotics

HiComb MIC™ Strips of the antimicrobial agents viz. Amikacin, Amoxyclav, Ampicillin, Azithromycin, Aztreonam, Cefalexin, Ceftriaxone, Chloramphenicol, Ciprofloxacin, Co-Trimoxazole, Erythromycin, Gentamicin, Nalidixic acid, Oxacillin, Piperacillin/Tazobactam, Streptomycin, Tobramycin and Vancomycin were used for determination of MIC values by an agar dilution technique on Mueller-Hinton agar according to the CLSI recommendations [14].

OBSERVATIONS AND RESULTS

Isolation of *E. coli* strains

A total of 10 *E. coli* strains were isolated from urine samples on MacConkey's agar plate as shown in Figure.1.

Fig.1 Growth of pure *E. coli* isolates on MacConkey's agar plate

Antibiotic Resistance/Sensitivity profiling of *E. coli* isolates against different Class of Antibiotics

The *E. coli* isolate SUC-1 showed resistance to antibiotics from class β-lactam, cephalosporin, sulphonamide, quinolones, aminoglycosides, macrolides, glycopeptides, polymyxin and synthetic antibiotics oxazolidinones as shown in Table 1-5 and Figure.2.

Table.1 Resistance/Sensitivity profiling of *E. coli* isolate against β-lactam Antibiotics

Antibiotics	Conc. (In mcg)	SUC-1

	Zone of inhibition in mm	
Oxacillin (OX)	1 mcg	R
Methicillin (MET)	5 mcg	R
Penicillin (P)	10 unit	R
Ampicillin (AMP)	10 mcg	R
Amoxyclav (AMC)	30 mcg	R
Augmentin (AMC)	30 mcg	R
Ticarcillin/Clavulanic acid (TCC)	75/10mcg	R
Aziocillin (AZ)	75mcg	R
Ticarcillin (TI)	75mcg	R
Meziocillin (MZ)	75mcg	R
Carbenicillin (CB)	100mcg	R
Piperacillin (PI)	100mcg	R
Piperacillin/Tazobactam (PIT)	100/10mcg	R

* R = Resistance

Table. 2 Resistance/Sensitivity profiling of *E. coli* isolate against Cephalosporin, Carbapenem, Monobactam & Sulphonamide class of Antibiotic

Antibiotics	**Conc. (In mcg)**	**SUC-1**
	Zone of inhibition in mm	
Cefoperazone (CPZ)	75mcg	R
Cefpodoxime (CPD)	10 mcg	R
Ceftriazone (CTR)	30mcg	R
Cephalothin (CEP)	30 mcg	R
Ceftriaxone (CTR)	30 mcg	R
Imipenem (IPM)	10mcg	R
Co-Trimoxazole (COT)	25 mcg	R
Aztreonam (AT)	30mcg	R

* R = Resistance

Table.3 Resistance/Sensitivity profiling of *E. coli* isolate against Quinolones class of Antibiotic

Antibiotics	Conc. (In mcg)	SUC-1
	Zone of inhibition in mm	
Ciprofloxacin (CIP)	5mcg	20
Levofloxacin (LE)	5mcg	27
Ofloxacin (OF)	5mcg	25
Gatifloxacin (GAT)	5mcg	26
Moxifloxacin (MO)	5 mcg	R
Sparfloxacin (SPX)	5 mcg	23
Norfloxacin (NX)	10mcg	19
Nalidixic Acid (NA)	30 mcg	R

* R = Resistance

Table.4 Resistance/Sensitivity profiling of *E. coli* isolate against Aminoglycosides and Macrolides class of Antibiotic

Antibiotics	Conc. (In mcg)	SUC-1
	Zone of inhibition in mm	
Amikacin (AK)	10mcg	24
Gentamicin (GEN)	10mcg	21
Tobramycin (TOB)	10mcg	20
Streptomycin (S)	25 mcg	R
Kanamycin (K)	30 mcg	25
Netillin (NET)	30mcg	22
Erythromycin (E)	15 mcg	R
Azithromycin (AZM)	15 mcg	R
Clanthromycin (CLR)	15 mcg	R

* R = Resistance

Table.5 Resistance/Sensitivity profiling of *E. coli* isolate against Glycopeptide, Tetracycline, Chloramphenicol, Polymyxin, Oxazolidinones, Lincomycin and Aminocoumarin class of Antibiotic

Antibiotics	Conc. (In mcg)	SUC-1
	Zone of inhibition in mm	
Vancomycin (VA)	30 mcg	R
Teicoplanin (TEI)	10 mcg	R
Tetracycline (TE)	30 mcg	R
Chloramphenicol (C)	30 mcg	R
Colistin (CL)	10 mcg	R
Linezolid (LZ)	30 mcg	R
Clindamycin (CD)	2 mcg	R
Novobiocin (NV)	5 mcg	R

* R = Resistance

Figure.2 Antibiotic resistance/sensitivity profiling of *E. coli* SUC-1 isolate against Icosa Pseudo-1-disc antibiotics

Identification of MDR isolates

On the basis of 16S rRNA gene sequence analysis the MDR isolate SUC-1 was identified as *Escherichia coli* and designated as *Escherichia coli* strain SUC-1 and it was deposited in GenBank under Accession No. PP854585 and phylogenetic tree indicating the taxonomic position of

Escherichia coli strain SUC-1 showed in Figure.3.

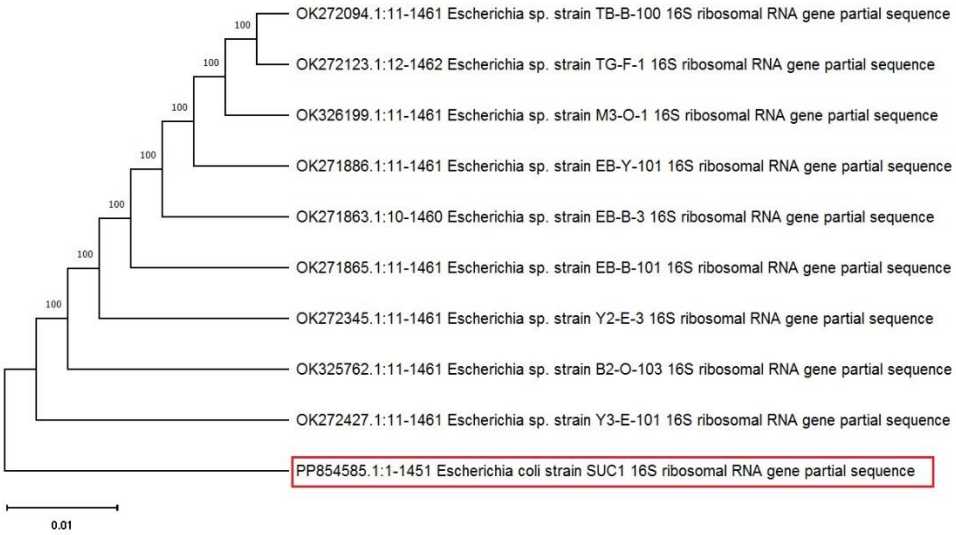

Figure. 3

Molecular Phylogenetic analysis of *Escherichia coli* strain SUC-1

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of antibiotics from class β-lactam, cephalosporin macrolide, aminoglycoside, sulphonamide, glycopeptide and quinolones against MDR *E. coli* SUC-1 were determined in accordance with CLSI guidelines as shown in Table 6, Figure 4, and Figure 5.

Table.6 The MIC of antibiotics against MDR *E. coli* strain SUC-1

Sr. No.	Antibiotics	Standard range in mcg as per CLSI guidelines	*E. coli* (PP854585)
		Concentration in mcg	
1.	Amikacin (AK)	0.5-4	4
2.	Amoxyclav (Amoxicillin/Clavulanic acid)	2-8	240
3.	Ampicillin (AMP)	2-8	256
4.	Azithromycin (AZM)	≤16	128
5.	Aztreonam (AT)	0.06-0.25	240
6.	Cefalexin (CN)	0.1-5	240
7.	Ceftriaxone (CTR)	0.03-0.12	240
8.	Chloramphenicol (C)	2-8	30
9.	Ciprofloxacin (CIP)	0.004-0.016	30
10.	Co-Trimoxazole (COT)	≤ 0.5	240
11.	Erythromycin (E)	1-4	240
12.	Gentamicin (GEN)	≥ 5	5
13.	Nalidixic acid (NA)	1-4	240
14.	Oxacillin (OX)	8-32	256
15.	Piperacillin/Tazobactam (PIT)	1-4	30
16.	Streptomycin (S)	2-8	30
17.	Tobramycin (TOB)	0.25-1	8
18.	Vancomycin (VA)	1-4	240

Figure 4. The MIC of antibiotics against MDR *E. coli* strain SUC-1

Figure 5. The MIC of antibiotics against MDR *E. coli* strain SUC-1

DISCUSSION

Uropathogenic *Escherichia coli* (UPEC) is the most common cause of urinary tract infection and urine samples are crucial for diagnosis and monitoring the infection. The *E. coli* were isolated on MacConkey's agar plate from urine sample used for experimental work. Identification of MDR *E. coli* is important because it signifies a significant public health threat as these strains are resistant to multiple antibiotics making infection harder to treat and potentially leading to increase morbidity and mortality. The MDR *E. coli* strains were identified using molecular PCR techniques as *Escherichia coli* and designated as *Escherichia coli strain* SUC1 and it was deposited in GenBank under Accession No. PP854585. A bacterial strain, designated BzDS03 was characterized by using 16S ribosomal RNA gene and 16S-23S rRNA internal transcribed spacer region sequences. Phylogenetic analysis showed that 16S rRNA sequence of the isolate formed a monophyletic clade with genera Escherichia. The closest phylogenetic relative was *Escherichia coli* with 99% 16S rRNA gene sequence similarity. The result of Ribosomal database project's classifier tool revealed that the strain BzDS03 belongs to genera Escherichia.16S rRNA sequence of isolate was deposited in GenBank with accession number FJ961336 [16].

The Uropathogenic *Escherichia coli* are resistant to various classes of antimicrobials with mechanism including the production of β-lactamase, mutation in gene coding for DNA gyrase and topoisomerase and acquisition of resistance genes. The isolated *E. coli* isolate SUC-1 showed resistance to 9 class of antibiotics. The growth of *E. coli* isolate SUC-1 was observed even at the vicinity of antibiotic disc having antibiotics from 9 class of antimicrobials. The growth of *E. coli* indicates inactivation or degradation of antibiotics by enzyme. These types of resistant *E. coli* will have hazardous impact on patients and patients infected by such strains will not be treated by antibiotics from this class. In the present investigation the *E. coli* isolate SUC-1 shown resistance to antibiotics from class β-lactam, cephalosporin, sulphonamide, quinolones, macrolides, glycopeptides, polymyxin and synthetic antibiotics oxazolidinones.

A total of 117 MDR *Escherichia coli* isolated from patient and surrounding hospital environment in Bangladesh. Out of 117 MDR *E. coli* 30 were highly drug resistant among the isolates, all were resistant to ampicillin, cefuroxime and cefotaxime [17]. They reported resistance

was due to ESBL. Similarly larger proportion of MDR *E. coli* were also shown resistance to antibiotics from class quinolone, monobactam, aminoglycoside, nitrofurantoin, sulphonamide and macrolide which are in accordance with previous studies [18]. The increased resistance may result from excessive use of these antibiotics frequently sold and used within the country [19]. In our study also MDR *E. coli* strain SUC-1 shown resistance to 31 antibiotics from 9 classes of antimicrobials. A total of 120 *E. coli* isolated from clinical specimen and determined antimicrobial susceptibility by using Kirby-Bauer disc diffusion method according to CLSI guidelines. The 42.5% *E. coli* were observed MDR and 50.9% of the MDR isolates have integrons [20]. The isolation of MDR *E. coli* from urine sample concludes that overuse and misuse of antibiotics in hospital setting leading to the selection and spread of resistance gene through horizontal gene transfer and use of mechanism like efflux pump, decrease membrane permeability, enzymatic inactivation and target site modifications.

The MIC for pathogenic *E. coli* varies depending on the antimicrobial agent, the specific strains and the method of MIC testing. The understanding of MIC is crucial for choosing effective antimicrobial treatment and monitoring antibiotic resistance pattern. The growth of MDR *E. coli* were inhibited by 4µg/ml, 5 µg/ml and 8µg/ml concentration of Amikacin, Gentamicin and Tobramycin respectively (Aminoglycosides class). The antibiotics Amikacin, Gentamicin and Tobramycin inhibits the growth of bacteria by blocking 30S ribosomal subunit. These antibiotics are broad spectrum and biocidal against aerobic and facultative anaerobes therefore it is prescribed for the treatment of infection caused by MDR *E. coli* strains. The MIC (90) value of gentamycin, tobramycin and amikacin as 32, 8 and 4 mg/L, respectively to clinical isolate *E. coli* and *Klebsiella pneumoniae* from New York city, an area where multidrug-resistant organisms are endemic. Whereas ACHN-490 a next generation aminoglycoside shown MIC (90) 1mg/L [21]. Also, the MIC value 64 µg/ml, 8 µg/ml, 256 µg/ml, 8 µg/ml and 32 µg/ml for Amikacin, Gentamicin, Kanamycin, Neomycin and Tobramycin from class aminoglycoside antibiotics against MDR *E. coli* [22].

It is concluded that antibiotics amikacin, gentamicin and tobramycin from aminoglycosides class have a broad spectrum of activity, rapid bactericidal action and favorable chemical and pharmacokinetics property make them suitable to treat infection caused by MDR *E. coli* and other strains.

CONCLUSION

The aminoglycoside antibiotics amikacin, gentamicin, and tobramycin have been reported as effective for treating infections caused by MDR *E. coli* strains.

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CONFLICT OF INTEREST

The author declare that there is no conflict of interest.

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