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Expulsion of plasmid-mediated antibiotic resistance genes in *E. coli* by ethidium bromide and acridine orange treatment

Muhammad Salman Rasool[1], Muhammad Akbar Hassan[2], Kauser Siddiqui[3], Faisal Mazhar[1] Akhtar Hussain Shar[4], Khalid Hussain Rind[4], Nadir Ali Rind[4], Fasih Uddin Ansari[5] and Shahana Urooj Kazmi[6]

[1]Department of Microbiology, Shaheed Benazir Bhutto University Shaheed Benazirabad, Nawabshah, Pakistan
[2]Department of Pathology, Baqai Medical University, Karachi, Pakistan
[3]Pharmaceutical Research Center, PCSIR Laboratories, Karachi, Pakistan
[4]Department of Molecular Biology and Genetics Shaheed Benazir Bhutto University Shaheed Benazirabad, Nawabshah, Pakistan
[5]Department of Microbiology, University of Karachi, Karachi, Pakistan
[6]Department of Microbiology, University of Karachi, Women University of Sawabi, Khyber Pakhtunkhwa, Pakistan

Abstract: Plasmid borne antibiotics resistance is the global threat to healthcare facilities. Such antibiotics resistance is inherited stably within the same bacterial generations and transmitted horizontally to other species of bacteria. The elimination of such resistance plasmid is of great importance to contain dispersal of antibiotics resistance. *E. coli* strains were identified, screened for the presence of antibiotics resistance by disc diffusion method, and cured by sub-lethal concentrations of Ethidium bromide and Acridine orange. After curing, again antibiotic resistance was determined. Before and after curing, plasmids were extracted by column spin Kit and subjected to 1% agarose gel electrophoresis and antibiotic resistance genes were identified by PCR. The Ethidium bromide was more effective than Acridine orange in eliminating antibiotics resistance and resistance genes bearing plasmids (4, 5, 6, 8, 9, 10 and >10kb). The most frequently eliminated antibiotic resistance was against Imipenem and Meropenem followed by Cefoperazone-sulbactam, Amikacin and cephalosporins in sequence. The loss of antibiotic resistance was associated with the elimination of plasmid-borne antibiotic resistance genes; *bla*-TEM, *bla*-SHV, *bla*-CTX-M, *qnr*A, *qnr*B, *qnr*C and *qnr*D. Some *E. coli* strains did not show the removal of antibiotics resistance and plasmids, suggesting the presence of resistance genes on main chromosome and or non-curable plasmids.

Keywords: Plasmid curing, antibiotic resistance plasmids, *bla*-TEM, *bla*-SHV, *bla*-CTX-M, *qnr*A, *qnr*B, *qnr*C and *qnr*D

INTRODUCTION

The plasmid mediated resistance is on rise throughout the world and is reducing treatment choices and About 5 million fatalities are caused by drug resistant infections per year (Fursova *et al*., 2022). Further, such antibiotic resistance markers can move not only to descendants but also to across the bacterial species (Aslam *et al*., 2018). To stop dispersal of plasmid mediated antibiotic resistance, it is necessary to examine the anti-plasmid efficiency of various compounds on drug resistant bacteria. Elimination of antibiotic resistance plasmids (curing) is a way to diminish the global load of antimicrobial resistance (AMR). Plasmid curing can also be applied to dismiss plasmid containing antimicrobial resistance genes (ARGs) from bacteria in wastewater before their discharge in environment (Buckner *et al*., 2018). This strategy could be useful for the removal of drug resistance from hospital sewage. Animal and human waste is employed to fertilize agriculture soil which can incorporate high levels of ARGs (Meek *et al.*, 2015).

The curing mode of action of Acridine orange (AO) and Ethidium bromide (EthBr) is to intercalate with plasmid DNA and to stop its replication (Letchumanan *et al*., 2015). In *Enterobacter aerogenes*, *Salmonella* spp and *E. coli*, EthBr effectively removes AMR makers located on plasmids (Bouanchaud and Chabbert, 1971; Poppe and Gyles, 1988; Pulcrano *et al*., 2016). These agents are being applied in curing experiments and typically associated with the removal of the entire plasmid (Salisbury *et al*., 1972; Costa *et al*., 2014). Various studies showed, *E. coli*, *Vibrio parahaemolyticus*, *Lactobacillus plantarum*, *S. aureus*, *Bacteroides fragilis* and *B. thetaiotamicron* were cured by acridine orange (Jetten and Vogels 1973; Rotimi *et al*., 1981; Keyhani *et al*., 2005; Zaman *et al*., 2010; Adeyemo and Onilude 2015; Letchumanan *et al*., 2015;). Ethidium bromide eliminates plasmids from *S. aureus*, *E. coli*, *Bacillus cereus*, *Enterobacter aerogenes* (bearing *bla*-TEM, *bla*-KPC and pKpQIL plasmids), and Salmonella (Borah and Yadav, 2015; Pulcrano *et al*., 2016). These curing agents are still beneficial in vitro studies to cure plasmids (Coleri *et al*., 2004; Mesas, 2004; Chin *et al*., 2005; Zaman *et al*., 2010; Adeyemo and Onilude, 2015; Pulcrano *et al*., 2016; Buckner *et al*., 2018). The present investigation was

**Corresponding author:* e-mail: salman.rasool@sbbusba.edu.pk, sangimsr@yahoo.com

aimed to investigate the efficiency of EthBr and AO to knock out plasmid-based antibiotic resistance genes.

MATERIALS AND METHODS

Collection of E. coli cultures

E. coli isolates of urine and blood samples were collected from various hospitals and diagnostic laboratories of Karachi and were re-confirmed by biochemical identification (indole, urease, triple sugar iron agar, and citrate utilization tests) (Oxoid). Isolated colony of *E. coli* was picked with sterile wire-loop of needle and inoculated on all agar medium by streak-stab method and placed in incubator (Binder) for 24hrs at 37ºC.

Curing

Sub-minimal inhibitory concentrations (Sub-MICs) of Ethidium bromide (EthBr) (from stock of 10mg/mL, Sigma-Aldrich) and Acridine orange (AO) (from stock of 10mg/mL, Sigma-Aldrich) were used as curing concentrations. Sub-minimum inhibitory concentrations range from 125µg/ml to 1000µg/ml (125µg/ml, 250µg/ml, 500µg/ml and 1000µg/ml), were utilized for both curing agents (Brousseau *et al*., 1999; Zaman *et al*., 2010; Hassan *et al*., 2020).

Antibiotic resistance profiling

Antibiotic resistance profile before and after curing was checked by disc diffusion method by adopting the protocols of clinical laboratory standard institute (Wayne, 2014).

PCR amplification of plasmid mediated resistance genes

Plasmids were extracted from *E. coli* strains before and after curing by Plasmid purification by EZ-10 spin column kit (K0502). Presence of plasmids was confirmed by 1% agarose gel electrophoresis. These plasmid preps were used for the detection of antibiotic resistance genes by conventional PCR. The following primers were used; TEM-F 5'-CATTTCCGTGTCGCCCTTATTC-3', TEM-R 5'-CGTTCATCCATAGTTGCCTGAC-3' (Dallenne *et al*., 2010), SHV-F 5'-TATCTCCCTGTTAGCCACC-3',SHV-R5'-GATTTGCTGATTTCGCTCGG-3'CTX-M-F5'-SCSATGTGCAGYACCAGTAA-3',CTX-M-R5'-CCGCRATATGRTTGGTGGTG-3',QnrA-F5'- AGAGG ATTTCTCACGCCAGG-3'(Cattoir *et al*., 2007), QnrA-R 5'-GCCATACCTACGGCGATACC-3'(Robicsek *et al*., 2006),QnrB-F5'-GATCGTGAAAGCCAGAAAGG-3', QnrB-R 5'- ATGAGCAACGATGCCTGGTA-3'(Kim *et al*., 2009), QnrC-F 5'-GGGTTGTACATTTATTGAATC G-3',QnrC-R 5'-CACCTACCCATTTATTTTCA-3' (Pribul *et al*., 2016), QnrD-F 5'-CGAGATCAATTTACG GGGAATA-3'and QnrD-R 5'-AACAAGCTGAAGCGC CTG-3') (Cavaco *et al*., 2009). These primers were specified for the detection of cephalosporins resistance genes (*bla*-TEM, *bla*-SHV, *bla* CTX-M) and quinolones resistance genes (*qnr*A, *qnr*B, *qnr*C and *qnr*D).

PCR mix of 25µl was prepared by using master mix 12.5µl, forward and reverse primers 1.5µl each, plasmid DNA 2µl and distilled water 7.5µl. For the detection of *bla*-TEM, *bla*-SHV and *bla* CTX-M, PCR reaction mix was denatured at 94°C for 5 min, then further proceeded for thirty cycles of denaturation for 1min at 94°C, annealing for 1 min at 55°C, elongation for 1 min at 72°C, in last amplified for 10 min at 72°C and cooled at 4°C (Jena *et al*., 2017). For the confirmation of *qnr*A, *qnr*B, *qnr*C and *qnr*D, PCR mix was denatured initially at 95°C for 15 min, further proceeded for thirty rounds of 1 min at 95°C, for 1 min at 55°C or (1 min at 56°C for only *qnr*D), and 5minutes at 72°C and 1 round of ultimate extension at 72°C. PCR products (~10µl) were subjected to 2% agarose gel electrophoresis containing ethidium bromide (0.5µg/ml). 1 kb DNA ladder (Fermentas, USA) was used to calibrate the product band sizes. PCR products of 800bps, 795bps, 544bps, 516bps, 476bps, 307bps and 582bps were considered positive for *bla*-TEM, *bla*-SHV, *bla* CTX-M, *qnr*A, *qnr*B, *qnr*C and *qnr*D, respectively (El-Badawy *et al*., 2017).

STATISTICAL ANALYSIS

Variables were expressed in line diagrams and analyzed by using Microsoft Excel 2010.

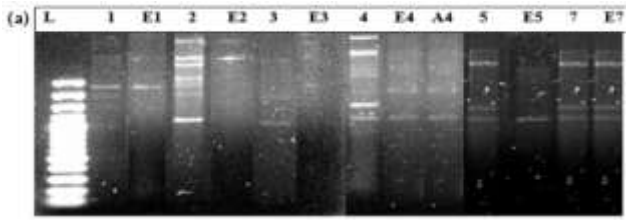
RESULTS

Antibiotic resistance, respective plasmids and plasmid-mediated resistance genes were more significantly removed after the treatment with Ethidium bromide (EthBr) than Acridine orange (AO) (table 1). *E. coli* No. 7 did not lose any plasmids and resistance genes and antibiotic resistance after treatment with EthBr and AO. *E. coli* No. 1, 2, 3, 5, 10 and 12 did not manifest any change in plasmid profile, resistance genes and antibiotic resistance after treatment with AO.

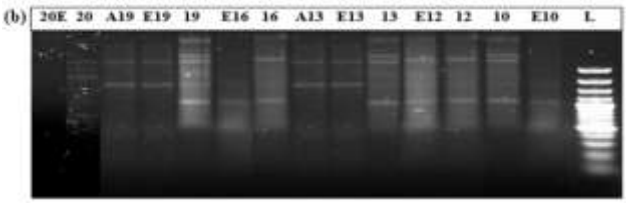
Loss of plasmids, plasmid borne genes and drug resistance after treatment with EthBr

Plasmids of variable sizes (4, 5, 6, 8, 9, 10, >10kbp) noticed in *E. coli* strains. *E. coli* No.1 dropped two plasmids i.e., 8kb and >10kb plasmids and *bla*-TEM gene, and respective three resistance markers Cefoperazone/ sulbactam, Imipenem and Meropenem. *E. coli* No. 2 lost five plasmids i.e., 5kb, 8kb, 9kb, 10kb and one >10kb plasmids and two relevant resistance determinants Imipenem and Meropenem, but did not lose *bla*-TEM gene after treatment with EthBr. *E. coli* No. 3 gave up all three plasmids i.e., 4kb, 8kb and >10kb and *bla*-TEM, *bla* CTX-M-ve and *qnr*D genes and resistance to Cefoperazone/ sulbactam, Imipenem and Meropenem. *E. coli* No. 4 deprived of 6kb, 10kb and two >10kb and *bla*-TEM, *bla*-SHV, *qnr* C and *qnr* D genes and resistance to Cefoperazone/ sulbactam, Imipenem and Meropenem. *E. coli* No. 5 dropped three plasmids i.e., 6kb and two >10kb

and *bla*-TEM, *bla* CTX-M, *qnr*D genes and resistance to Amikacin, Ceftazidime, Cefepime, Cefoperazone/ sulbactam, Imipenem and Meropenem. *E. coli* No. 10 gave up four plasmids of >10kb and*bla*-TEM, *bla*-SHV, *qnr*D genes and resistance to Cefoperazone/ sulbactam and Imipenem. *E. coli* No. 12 deprived of only one plasmid i.e., 8kb and *bla*-SHV, *qnr*D genes and resistance to Amikacin. *E. coli* No. 13 lost four plasmids i.e., 5kb and three >10kb and*bla*-TEM, *bla* CTX-M, *bla*-SHV, *qnr*A, *qnr*B genes and resistance to Cefaclor, Ceftriaxone, Cefotaxime, Ceftazidime, Cefepime, Ciprofloxacin, Cefoperazone/ sulbactam, Imipenem and Meropenem. *E. coli* No. 16 deprived of three plasmids i.e., 8kb and two >10kb and *bla*-TEM, *bla*-SHV, *qnr*D genes and resistance to Cefoperazone/ sulbactam, Imipenem and Meropenem. *E. coli* No. 19 lost four plasmids i.e., 5kb and three of >10kband resistance to Amikacin, Imipenem and Meropenem, but did not lose any resistance genes. *E. coli* No. 20 gave up two plasmids 9kb and >10kb and *bla*-TEM gene and resistance to Amikacin and Cefoperazone/ sulbactam (table. 1, fig. 1a and b).



Keys: L= 1Kb Ladder, 1=*E. coli* No.1, E1=EthBr treated *E. coli*No. 1, 2=*E. coli*No. 2, E2=EthBr treated *E. coli*No. 2, 3= *E. coli* No. 3, E3=EthBr treated *E. coli* No 3, 4=*E. coli* No. 4, E4=EthBr treated *E. coli* No. 4, A4=AO treated *E. coli* No. 4, 5=*E. coli* No. 5, E5=EthBr treated *E. coli* No. 5, 7=E. coli No. 7, E7=EthBr treated *E. coli* No. 7



Keys: L=1 Kb ladder, E10=EthBr treated *E. coli* No. 10, 10=*E. coli* No. 10, 12=*E. coli* No. 12, E12=EthBr treated *E. coli* No. 12, E12=EthBr treated *E. coli* No. 12, 13=*E. coli* No. 13, E13=EthBr treated *E. coli* No. 13, A13=AO treated *E. coli* No. 13, 16=*E. coli* No. 16, E16= EthBr treated *E. coli* No. 16, 19=*E. coli* No. 19, E19=EthBr treated *E. coli* No. 19, A19=AO treated *E. coli* No. 19, 20=*E. coli* No. 20, 20E=EthBr treated *E. coli* No. 20

Fig. 1: Gel elctrophoratic representation of plasmids bands before and after treatment with Ethidium bromide and Acridine orange.

Loss of plasmids, plasmid born genes and drug resistance after treatment with AO

E. coli No. 4 lost 6kb, 10kb and two >10kb and *bla*-TEM gene only and resistance to Cefoperazone/ sulbactam, Imipenem and Meropenem. *E. coli* No. 16 gave up two plasmids i.e., of >10kb and resistance to Imipenem and Meropenem but did not lose resistance genes. The plasmid, plasmid born resistance genes and antibiotic resistance loss patternsin *E. coli* No.13, 19 and 20 were same as of after treatment with EthBr (table.1, fig. 1a, b).

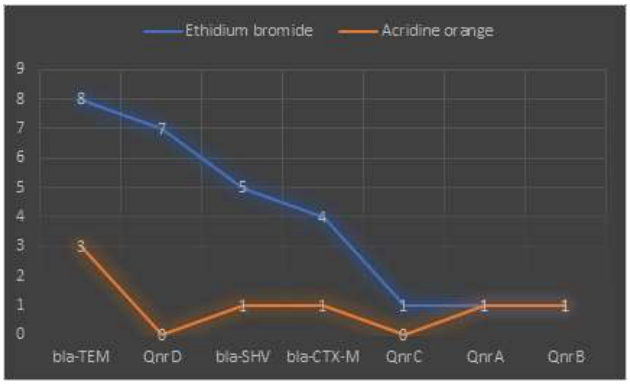


Fig. 2: Comparison of resistance genes removal after treating with Acridine orange and Ethidium bromide treatment

Comparison of Ethidium bromide and acridine orange

Ethidium bromide was noticed more effective than Acridine orange in eliminating resistance genes. Ethidium bromide removed *bla*-TEM, *qnrD*, *bla*-SHV, *bla*-CTX-M, *qnrC*, *qnrA* and *qnrB* in 8, 7, 5, 4, 1, 1 and 1 *E. coli* strains, respectively. But, Acridine orange removed resistance genes *bla*-TEM, *bla*-SHV, *bla*-CTX-M, *qnrA* and *qnrB* in 3, 1, 1, 1 and 1 *E. coli* strains, respectively (fig. 2).

DISCUSSION

The role of plasmids has been appreciated in antibiotic resistance. Most of the ESBLs in *E. coli* are located on plasmids and 88% of the uropathogenic *E. coli* bears antibiotic resistance plasmids (Li *et al*., 2019). It is also noticed in present study twelve, nine and seven *E. coli* strain carried *bla*-TEM, *bla*-SHV and *bla*-CTX-M genes and having plasmid bands (Paterson and Bonomo,2005; Bush, 2010; Ali *et al*., 2014). Many studies revealed the efficacy of Acridine orange for the removal of antibiotic resistance but in present study, EthBr was found more prominent than AO in eliminating plasmids borne resistance genes. As it could remove plasmid mediated resistance in 11 out of 12 *E. coli* strains, while AO could displace plasmid borne resistance in 5 out of 12. However, the pattern of antibiotic resistance and plasmid removal by each agent was similar. According to Otokunefor and colleagues, plasmid curing by acridine orange caused the decline in resistance to many antibiotics (7-8) in *E. coli*. Comparatively, in current investigation, loss of 1 to 4 antibiotics in many *E. coli* strains and 8 antibiotics in one *E. coli* strain related to the loss of plasmids after treating with EthBr. In another

Table 1: Resistance patterns and comparison of plasmid profiles of *E. coli* before and after treatment with Ethidium bromide and Acridine orange

E. coli	Specimen	Resistance profile	Plasmid profile	PCR of plasmid preps.
1 Original	Urine	AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, IPM, MEM, TZP, PIP, F	>10kb, 9kb, 8kb	*bla*-TEM +ve
1 After curing with EthBr		AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, TZP, PIP, F	9kb	*bla*-TEM -ve
1 After curing with AO		No change/ as original	No change/ as original	No change/ as original
2 Original	Urine	AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, IPM, MEM, TZP, PIP, F	(3)>10kb, 10kb, 9kb,	*bla*-TEM +ve
2 After curing with EthBr		AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, TZP, PIP, F	8kb and 5kb (2)>10kb	*bla*-TEM +ve
2 After curing with AO		No change/as original	No change/ as original	No change/ as original
3 Original	Urine	AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, IPM, MEM, TZP, PIP	4kb, 8kb and >10kb	*bla*-TEM+ve, *bla* CTX-M+ve, *bla*-SHV+ve, *qnr*D+ve
3 After curing with EthBr		AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, TZP, PIP	No plasmid seen	*bla*-TEM-ve, *bla* CTX-M-ve, *bla*-SHV+ve, *qnr*D-ve
3 After curing with AO		No change/as original	No change/as original	No change/as original
4 Original	Blood	AMC, AK, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, IPM, MEM, TZP (Resistant to all antibiotics)	(2)>10, 10kb, 8kb, 6kb, 5kb	*bla*-TEM+ve, *bla* CTX-M+ve, *bla*-SHV+ve, *qnr*C +ve, *qnr*D+ve
4 After curing with EthBr		AMC, AK, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, TZP	5kb and 8kb	*bla*-TEM-ve, *bla* CTX-M+ve, *bla*-SHV-ve, *qnr*C-ve, *qnr*D-ve
4 After curing with AO		AMC, AK, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, TZP	5kb and 8kb	*bla*-TEM-ve, *bla* CTX-M+ve, *bla*-SHV+ve, *qnr*C +ve, *qnr*D+ve
5 Original	Urine	AMC, AK, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, IPM, MEM, TZP, PIP, F	(2)>10kb, 6kb and 5kb	*bla*-TEM+ve, *bla* CTX-M+ve, *qnr*D+ve
5 After curing with EthBr		AMC, CEC, CXM, CFM, CTX, CRO, CIP, TZP, PIP, F	5kb	*bla*-TEM-ve, *bla* CTX-M-ve, *qnr*D-ve
5 After curing with AO		No change/as original	No change/as original	No change/as original
7 Original	Urine	AMC, AK, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, IPM, MEM, TZP, PIP	(2)>10kb, 6kb and 5kb	*bla*-TEM+ve, *bla* CTX-M+ve, *bla*-SHV+ve
7 After curing with EthBr		No change/ as original	No change/ as original	No change/ as original
7 After curing with AO		No change/ as original	No change/ as original	No change/ as original
10 Original	blood	AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, IPM, TZP	(4)>10kb, 8kb and 5kb	*bla*-TEM+ve, *bla* CTX-M+ve, *bla*-SHV+ve, *qnr*D+ve
10 After curing with EthBr		AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, TZP	8kb and 5kb	*bla*-TEM-ve, *bla* CTX-M+ve, *bla*-SHV-ve, *qnr*D-ve
10 After curing with AO		No change/ as original	No change/ as original	No change/ as original
12 Original	Urine	AMC, AK, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, IPM, MEM, TZP, PIP, F	(2)>10kb, 8kb and 5kb	*bla*-TEM+ve, *bla*-SHV+ve, *qnr*D+ve
12 After curing with EthBr		AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, IPM, MEM,	(2)>10kb and 5kb	*bla*-TEM+ve, *bla*-SHV-ve, *qnr*D-ve
12 After curing with AO		TZP, PIP, F		
		No change/ as original	No change/ as original	No change/ as original
13 Original	Urine	AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, IPM, MEM, TZP, PIP	(4)>10kb,8kb and 5kb	*bla*-TEM+ve, *bla* CTX-M+ve, *bla*-SHV+ve, *qnr*A+ve, *qnr*B+ve
13 After curing with EthBr		AMC, CEC, CXM, CFM, TZP, PIP	(1)>10kb and 8kb	*bla*-TEM-ve, *bla* CTX-M-ve, *bla*-SHV-ve, *qnr*A-ve, *qnr*B-ve
13 After curing with AO		AMC, CEC, CXM, CFM, TZP, PIP	(1)>10kb and 8kb	*bla*-TEM-ve, *bla* CTX-M-ve, *bla*-SHV-ve, *qnr*A-ve, *qnr*B-ve
16 Original	Blood	AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, IPM, MEM, TZP	(2)>10kb, 8kb and 5kb	*bla*-TEM+ve, *bla* CTX-M+ve, *bla*-SHV+ve, *qnr*D+ve
16 After curing with EthBr		AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, TZP	5kb	*bla*-TEM-ve, *bla* CTX-M+ve, *bla*-SHV-ve, *qnr*D-ve
16 After curing with AO		AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, TZP	8kb and 5kb	*bla*-TEM-ve, *bla* CTX-M+ve, *bla*-SHV+ve, *qnr*D-ve
19 Original	Blood	AMC, AK, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, IPM, MEM, TZP (Resistant to all antibiotics)	(4)>10kb, 8kb and 5kb	*bla*-TEM+ve, *bla*-SHV+ve
19 After curing with EthBr		AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, TZP	(1)>10 and 8kb	*bla*-TEM+ve, *bla*-SHV+ve
19 After curing with AO		AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, TZP	(1)>10 and 8kb	*bla*-TEM+ve, *bla*-SHV+ve
20 Original	Blood	AMC, AK, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, SCF, TZP	>10kb, 9kb	*bla*-TEM+ve, *bla* CTX-M+ve, *bla*-SHV+ve, *qnr*D+ve
20 After curing with EthBr		AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, TZP	No plasmid seen	*bla*-TEM-ve, *bla* CTX-M+ve, *bla*-SHV+ve, *qnr*D+ve
20After curing with AO		AMC, CEC, CXM, CFM, CAZ, FEP, CTX, CRO, CIP, TZP	No plasmid seen	*bla*-TEM-ve, *bla* CTX-M+ve, *bla*-SHV+ve, *qnr*D+ve

study, the highest decrease of Ceftazidime resistance (40%) followed by Ofloxacin resistance (27%) was observed (Otokunefor *et al*., 2019). Zaman and co-researchers could remove plasmids of 2.5 MDa and <2 MDa by Acridine orange. Conversely, in present study, antibiotic resistance, respective plasmids and plasmid-mediated resistance genes were more predominantly knocked out after the treating with Ethidium bromide (EthBr) than Acridine orange (AO). Accordingly, plasmids of 4kb to >10kb were noticeably excluded. The removal of plasmids was concomitant with loss of antibiotic resistance and relevant genes (Zaman *et al*., 2010).

Other studies of plasmid curing had shown major decline in resistance to cephalosporins like Ceftazidime (Rains *et al*., 1995; Orhue *et al*., 2017; Alkali *et al*., 2018). However, in current investigation, 9, 7, 5 and 1 *E. coli* strains lost resistance to (Imipenem, Meropenem), (various Cephalosporins, Cefoperzone-sulbactam, Ceftazidime, Cefepime, Cefotaxime, Ceftriaxone), (Amikacin) and (Ciprofloxacin), respectively after cured with EthBr. The removal of such antibiotic resistance was due to the elimination of plasmids bearing carbapenamases, *bla*-TEM, *bla*-CTX, *bla*-SHV, aminoglycoside resistance genes and *qnr*A, *qnr*B, *qnr*C and *qnr*D genes. Moreover, such concomitant removal of multiple resistance genes revealed the presence of resistance genes on the same plasmids (Do-Carmo *et al*., 2008; Maina *et al*., 2013; El-Bouamri *et al*., 2015; Jacoby *et al*., 2015; Mbim *et al*., 2016; Chen *et al*., 2019). Interestingly in another study, it is noticeable that ESBL genes (*bla*-CTX-M, *bla*-SHV, *bla*-TEM) responsible for cephalosporin resistance were lost along with quinolones resistance genes (*qnrA*, *qnrB*, *qnrC* and *qnrD*)(Liu *et al*., 2013; Jacoby *et al*., 2015). MDR-plasmids have been found by researchers to harbor antibiotic resistance genes (*bla*-IMP, *bla*-CTX-M, *bla*-KPC, *bla*-SHV etc.) (Mendes *et al*., 2007; Espedido *et al*., 2008; Miró *et al*., 2010; Zhao *et al*., 2010; Jeong *et al*., 2011; Albrechtova *et al*., 2012; Yang *et al*., 2012; Liu *et al*., 2013).

More than 10kb plasmids have been reported in *Pseudomonas aeruginosa* by Paul and co-researchers. These plasmids bear *bla*-NDM gene responsible for carbapenem resistance (Zhao *et al*., 2021). Accordingly, SDS was found most effective in curing such plasmids. Several plasmids and respective antibiotics resistance determinants were lost by *E. coli* isolates after curing in present study. The most frequent loss of imipenem and meropenem was observed after curing. This may be due to loss of plasmids bearing metallo-betalactamase genes (Paul *et al*., 2021). Study of Paul and colleagues is quite relevant with the findings of present investigation. But in present study EthBr and AO (with low efficiency) have been found to cure such plasmids (>10Kbp) bearing Carbapenem resistance genes indicated by the removal of Imipenem and Meropenem resistance in many *E. coli* strains (novel finding). After treatment with Ethidium bromide, only four *E. coli* strains showed *bla*-TEM, *bla*-CTX-M and *bla*-SHV positive PCR and one strain(*E. coli* No. 7) did not lose any plasmids and resistance genes, and antibiotic resistance after treatment with EthBr and AO. These findings indicate that in these *E. coli* strains the location of resistance marker is the main chromosome or high copy number or non-removable plasmids (Rasool *et al*., 2003; Carattoli, 2013; Miller *et al*., 2014; Zhang *et al*., 2014; Churchill and Romanus, 2019). Anusha *et al*. performed PCR after curing to find out resistance genes. They did not find any genes pertaining to antibiotic resistance after curing in Uropathogenic *E. coli* (Anusha *et al*., 2015). It became evident that all the UPEC isolates turned to ESBL negative. The after curing PCR results of present study is found consistent with the study of Anusha *et al*. and others (Shahid *et al*., 2003; Anusha *et al*., 2015).

CONCLUSION

The ethidium bromide was more efficient than acridine orange in removing antibiotic resistance genes bearing plasmids. The loss of plasmids after treating with these agents was consistent with the loss of multiple antibiotics resistance and the disappearing of *bla*-TEM, qnrD, *bla*-SHV, *bla*-CTX-M, *qnr*A, *qnr*B and *qnr*C in *E. coli* strains indicated by PCR. Plasmids of various size (4, 5, 6, 8, 9, 10 and>10kb) were removed effectively by curing agents. The most common resistance eliminated against imipenem and meropenem followed by Cefoperazone-sulbactam, Amikacin and other cephalosporins in sequence.

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