Article published online: 2023-08-24

Prophage and Plasmid-Mediated Beta-Lactamases in Multidrug-Resistant Extraintestinal *Escherichia coli*

Juliet Roshini Mohan Raj[1] Akshatha Dinesh[2] Rajeshwari Vittal[3] Anusha Rohit[4]

[1] Division of Infectious Diseases, Nitte University Center for Science Education and Research, Mangaluru, Karnataka, India
[2] Nitte University Center for Science Education and Research, Mangaluru, Karnataka, India
[3] Division of Environmental Health and Toxicology, Nitte University Center for Science Education and Research, Mangaluru, Karnataka, India
[4] Department of Microbiology, Madras Medical Mission, Chennai, Tamil Nadu, India

Address for correspondence Juliet Roshini Mohan Raj, PhD, Division of Infectious Diseases, Nitte University Center for Science Education and Research, Paneer Campus, Kotekar-Beeri Road, Derelakatte, Mangaluru 575018, Karnataka, India (e-mail: julietm@nitte.edu.in).

J Health Allied Sci[NU] 2024;14:420–425.

Abstract

Objectives Antibiotic resistance can arise as a mutation to adapt to stress or be mediated by horizontal gene transfer. This study aimed at identifying the resistance determinants present in the mobile genetic elements of prophages and plasmids within multidrug-resistant (MDR) extraintestinal *Escherichia coli*.

Materials and Methods Thirty-five anonymized MDR *E. coli* isolates of nonintestinal infections were confirmed for their antimicrobial resistance to six categories of antimicrobials by the disk diffusion test. Genes coding for beta-lactamases and carbapenemases in bacterial genome, plasmid, and prophage fractions were separately determined by polymerase chain reaction. Transducing ability of prophages carrying resistance genes was determined.

Results Twenty-six isolates were positive for the gene $bla_{\text{CTX-M}}$, nine for bla_{TEM}, one each for bla_{KPC} and bla_{VIM}, thirteen for bla_{NDM}, and seven for bla_{OXA}. A majority of these isolates carried these determinants in plasmids and prophage fractions. Twenty-one percent of the prophage fractions (4 of 19) were able to successfully transfer resistance to sensitive isolates.

Conclusion This study indicates $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{NDM} genes that are reported most frequently in MDR isolates are more frequent in the plasmid and prophage fractions thus supporting for increased mobility.

Keywords
- beta-lactamase
- carbapenemases
- mobile genetic elements
- MDR *E. coli*

Introduction

Extraintestinal infections like urinary tract infections (UTIs) and bacteremia, caused by *Escherichia coli*, have surfaced in recent decades and this organism is now a dreaded pathogen. The impact of virulence is enhanced by the concomitant occurrence of antibiotic resistance. *E. coli*, the most prevalent pathogen causing UTI, has developed resistance to many antibiotics including the last line beta-lactams and carbapenems.[1] In India, as of 2019, more than 83% of invasive *E. coli* isolates exhibit resistance to cephalosporin and 41% are resistant to carbapenems.[2] An approximate 10% increase in resistance was observed in a 2-year reporting period.[2] Plasmids have gained attention as prime mediators of antimicrobial resistance (AMR) not only in clinical isolates but in the environment as well.[3,4] Bacterial genomes are impregnated with prophages that are speculated to be important mediators

article published online
August 24, 2023

DOI https://doi.org/10.1055/s-0043-1772707.
ISSN 2582-4287.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

of AMR in species that do not generally support transformation and conjugation.[5] Prophages may act as reservoirs for AMR genes. The occurrence of resistance genes within prophage elements of multidrug-resistant isolates and the contribution of these prophages to the transfer and spread of AMR is known. However, not well quantified.[6] The presence of beta-lactamases and carbapenemases in the genome, plasmid, and prophage fractions of extraintestinal *E. coli* was detected in this study. The ability and rate of transfer of prophages induced from the drug resistant bacteria to transform sensitive isolates to multidrug resistant ones were determined. Status on resistance patterns, genes that code for AMR in multidrug-resistant (MDR) organisms, and understanding mechanisms of transfer aid in developing strategies to combat and control the spread of AMR effectively.

Materials and Methods

The study was initiated after obtaining institutional ethics committee and biosafety committee approval. All experiments were conducted in triplicates maintaining the necessary controls.

Bacterial Cultures

MDR-*E. coli* cultures ($n = 35$) previously isolated from blood and urine were anonymized using secondary identifiers and revived from −80°C glycerol stocks to be included in this study. Environmental isolates of *E. coli* ($n = 5$) were also included. Standard quality control strain *E. coli* ATCC 25922 was maintained as control. Cultures were maintained on nutrient agar. The isolates were confirmed for antibiotic resistance by the disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines (2019).[7] Antibiotics include cefpodoxime (CAZ 30 µg), ceftazidime (CTX 30 µg), meropenem (MRP 10 µg), imipenem (IMP 10 µg), chloramphenicol (C 30 µg), co-trimethoxazole (COT 25 µg), ciprofloxacin (CIP 5 µg), and tetracycline (TE 30 µg).

Screening Isolates for Beta-Lactamase Genes

One milliliter of overnight culture was pelleted at 4,000g for 10 minutes and pellet suspended in 100 µL 10mM tris-ethylenediaminetetraacetic acid (EDTA) buffer. The suspension was heated at 98°C for 10 minutes and flash cooled on ice. The resultant solution was used as DNA source. The presence of beta-lactamase (*bla*) genes $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}, bla_{KPC}, bla_{VIM}, and bla_{OXA} was detected by polymerase chain reaction (PCR) using specific primers at optimized cycling conditions in a thermocycler (Nexus GX2, Eppendorf; ►**Supplementary Table S1**). PCR products were resolved by horizontal electrophoresis in a 2% agarose gel incorporated with SYBR safe dye. The gel was visualized and analyzed using a gel documentation system (GelDoc, BioRad).

Detection of Beta-Lactamase Genes in Plasmid

Plasmid was extracted using QIAprep Miniprep kit (Qiagen, United States)[8] and quantified using a nanodrop spectrophotometer (Implen, United States). Presence of plasmid-mediated *bla* genes was determined using the plasmid as template for PCR.

Detection of Beta-Lactamase Genes in Prophages

Prophages were chemically induced using different reagents. Isolates were grown in 5 mL nutrient broth at 37°C, 180 revolutions per minute (rpm) till $OD_{600} \sim 0.6$. The cultures were separately treated with 1% sodium dodecyl sulphate, mitomycin C (1 µg/mL), and nalidixic acid (12.5 µg/mL) and incubation continued for 5 hours. The flasks were observed for visual clearance. The lysate was clarified by centrifugation at 4,000 g for 10 minutes followed by filtration through 0.2 µm filters. The lysate was subjected to ultracentrifugation (Optima XPN-100, Beckman Coulter, United States) at 23,000 g for 3 hours. The pellet was suspended in 1 mL sodium chloride magnesium sulphate and gelatin (SM) buffer to obtain pure concentrated phage fractions. The concentrated phage fraction was treated with 10 units/mL DNase for 37°C for 1 hour to remove any residual bacterial DNA. Phage DNA was extracted by treatment with TENS buffer (50 mMTris-100 mM EDTA: 0.3% sodium dodecyl sulphate (SDS): 100mM NaCl) followed by phenol-chloroform extraction.[9] Nucleic acid was quantified by spectrophotometry. The presence of *bla* genes in the prophage was detected by PCR using the phage DNA as template.

Transducing Ability of the Prophages

The ability of prophage elements to induce AMR by transduction was determined as follows. The activity of the phages in the clarified mitomycin C-induced lysates of the MDR-*E. coli* was determined by spot assay wherein 5 µL of lysate was spotted on a lawn of environmental *E. coli* (as these were susceptible to all antibiotics tested).[10] For lysates that showed activity on the environmental *E. coli* isolates, prophage induction in 50 mL volume was performed using mitomycin C (1 µg/mL). Mitomycin C-induced lysates of the 9 MDR isolates that were positive for the AMR genes in prophage fraction and showed lytic activity on the environmental isolates (►**Supplementary Table S2**) were selected for transduction studies. The test contained 1 mL of the sensitive *E. coli* culture and 100 µL of prophage lysate from MDR isolate, while culture and lysate were separately maintained as controls. The experimental tubes were incubated at 37°C overnight. Cultures were centrifuged at 4,000g for 20 minutes and the supernatant discarded. The cell pellet washed thrice in 1 mL aliquots of sterile saline and suspended in Luria Bertani (LB) broth. The tubes were further incubated at 37°C 180 rpm for 1 hour. One hundred µL from each tube were spread on LB agar and LB_{AMP} agar (LB agar incorporated with 100 µg/mL ampicillin) and incubated at 37°C overnight. Colonies that developed on LB_{AMP} agar were tested for antimicrobial susceptibility using all the antibiotics mentioned earlier and the presence of *bla* genes tested by PCR. Colonies positive for *bla* genes were considered as true transformants obtained after transduction.

Characterization of the Beta-Lactamase Genes in Mobile Genetic Elements

DNA sequencing of the gene $bla_{\text{CTX-M}}$ from isolates that were positive for this gene in both plasmid and phage DNA fractions was undertaken to determine the presence of the $bla_{\text{CTX-M}}$ variant present. Sequencing was performed at Eurofins Genomics India Pvt Ltd, Bengaluru, India. Sequences

were subject to NCBI BLAST tool and multiple sequence alignment in Multalin (http://multalin.toulouse.inra.fr/multalin/) to determine the similarity and gene relatedness.

Results

Bacterial Isolates

Thirty-five *E. coli* of clinical origin and five of environmental origin were included in this study. All 35 clinical isolates were resistant to CTX, 34 (97%) to MRP, CAZ, and CIP, 30 (86%) to COT, 27 (77%) to IPM, 24 (69%) to TET, and 4 (11%) to C. All 35 isolates were resistant to at least three classes of antimicrobials conferring them as MDR. The environmental isolates were sensitive to all antibiotics tested. The resistance pattern observed is shown in ►**Table 1**.

Beta-Lactamase Genes in Mobile Genetic Elements

The thirty-five MDR *E. coli* were screened for genes coding for beta-lactamases by PCR. Presence of resistance determinants in the isolates as tested from crude DNA is shown in ►**Table 1**. The number of plasmid DNA and induced phage fraction that carried the *bla* genes is shown in ►**Table 2**. Genes encoding multiple resistance determinants were detected in several isolates. Concomitant resistance to carbapenems IMP and MRP in the absence of the carbapenemases tested was observed in isolates U5663 and P5943. The χ^2 test of association compared presence in plasmid and phage for individual resistance determinants. For the resistance determinants $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{NDM}, the concomitant presence in plasmid and prophage fractions was found to be associated (χ^2 (1) = 3.313, $p < 0.05$; χ^2 (1) = 0.1042, $p < 0.05$; and $\chi^2(1) = 0.0925$, $p < 0.05$, respectively).

Transducing Ability of the Prophages

Prophages were easily induced on all chemical treatments used. Nineteen prophage lysates were screened for active phages by spot assay on the anitbiotics susceptible environmental isolates. Nine lysates showed lytic activity (►**Supplementary Table S2**). Transduction experiments were carried out using lysate-isolate combinations as per ►**Supplementary Table S2**. Transformants that grew on LB_{AMP} plates were screened for resistance genes that were present in the respective phage lysates. To supplement the genotype information, antimicrobial susceptibility test was performed. Transformants of transduction from four lysates showed phenotypic resistance to antimicrobials identical to their respective parent cultures U2368, U5663, B5773, and U2642 (►**Table 3**).

Characterization of the Beta-Lactamase Genes in Mobile Genetic Elements

The $bla_{\text{CTX-M}}$ amplicon of six isolates B5773, U6040, U2368, U5133, U5663, and IRU1123, which were positive for the gene in both plasmid and phage DNA, was sequenced. Alignment of our sequences revealed that all the sequences were identical to $bla_{\text{CTX-M 15}}$. The sequences have been deposited in Genbank (accession numbers MN692192 to MN692197).

Discussion

E. coli has gained the attention of the healthcare sector over the past five decades as a pathogen with increased resistance to antimicrobial agents. Reports of extraintestinal *E. coli* infections are increasing and the prevalence of extended spectrum beta-lactamases (ESBLs) and carbapenemases among them are alarming. Among the beta-lactamases, $bla_{\text{CTX-M}}$ has superseded the variants bla_{TEM} and bla_{SHV} to become a global dominant menace. To date, more than 175 variants of the $bla_{\text{CTX-M}}$ gene have been reported with $bla_{\text{CTX-M-15}}$ being highly prevalent among clinical isolates of *E. coli*. The carbapenemases bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, and $bla_{\text{OXA-48}}$ type are regarded as the most effective in carbapenem hydrolysis and geographical spread.[11] Modified human activities, poor access to good sanitary facilities, and poor hygiene support the spread of CTX-M and NDM genes.[12] The nature of these resistance genes and an understanding of triggers for transfer would aid in complementing antimicrobial stewardship practices by developing intervention strategies to combat the spread of the AMR pandemic.

Reports from healthcare settings differ in the resistance patterns and the occurrence of resistance determinants. Beta-lactamases and carbapenemases appear in multiple combinations, thereby conferring resistance to virtually all β-lactam antibiotics.[3,12,13] In our study, the $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{NDM} were the most common *bla* genes encountered. The phenotypic resistance to carbapenems in isolates U5663 and P5943 but absence of resistance genes tested in this study highlights the increasing adaptability of the ARDs and the presence of other resistance mechanisms that were not included in this study.

ESBLs $bla_{\text{CTX-M}}$, bla_{TEM} and the carbapenemases bla_{KPC}, bla_{VIM}, bla_{NDM}, and bla_{OXA} are often reported as plasmid mediated. Phages are important vehicles for horizontal gene exchange between and within different bacterial species and are important agents for antimicrobial gene transfer.[14–16] In our study, $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{NDM} occurred frequently as plasmids and in the prophage fractions. Procedural limitations of genomic DNA contamination in the plasmid and prophage DNA preparations are a concern while interpreting these results. However, since the results mentioned herein are the consensus results of three biological replicates, we consider the percentage of carriage of the *bla* genes in plasmids and phages to accurately represent the samples. Phage-plasmids are extra-chromosomal elements that act both as plasmids and as phages. Phage-like plasmids carry the resistance gene KPC-2.[17] Many genes encoding resistance determinants are found in specific phage-plasmids and these phage-plasmids contribute widely to the dissemination of AMR.[18] We speculate that the detection of the AMR genes in both plasmids and phages may be due to the presence of phage-plasmids. Genomic data of these MDR *E. coli* isolates would clarify if these elements are indeed phage-plasmids.

From a total of 19 phage lysates that carried resistance genes, 9 were selected for the transduction study and of these, 4 were capable of successfully transferring resistance via transduction as screened on LB_{AMP}. The use of ampicillin

Table 1 Multidrug resistance and the beta-lactamases observed in extra-intestinal *E. coli* isolates

Isolates	Class of antimicrobial						Beta-lactamase gene present						
	CEP	CARB	TET	FLO	C	FPI	CTX-M	TEM	SHV	KPC	NDM	VIM	OXA
U6451	R	R	S	R	S	S		+			+		
B5308	R	R	S	R	S	S	+	+					
B5773	R	R	S	R	S	S	+						
U6106	R	R	S	S	S	R					+		
U5546	R	R	S	R	S	R							+
IRU2553	R	R	S	R	S	R							
B6127	R	R	S	R	S	R		+			+		
U2368	R	R	S	R	S	R	+	+					
U5265	R	R	S	R	S	R							
U3659	R	R	R	R	S	S	+						+
U5801	R	R	R	R	S	S	+				+		+
U2592	S	R	R	R	S	R							
IRU1123	R	R	R	R	S	R	+					+	
U5663	R	R	R	R	S	R	+						
B3263	R	R	R	R	S	R	+				+		+
U5849	R	R	R	R	S	R	+						
U6320	R	R	R	R	S	R				+			
IRU1070	R	R	R	R	S	R							
U5620	R	R	R	R	S	R	+				+		
U5133	R	R	R	R	S	R	+				+		
U5861	R	R	R	R	S	R							
U6487	R	R	R	R	S	R	+	+			+		
U5389	R	R	R	R	S	R	+						+
U6040	R	R	R	R	S	R	+	+			+		
U3842	R	R	R	R	S	R	+						+
IRU1729	R	R	R	R	S	R	+	+					
U3731	R	R	R	S	S	R	+	+			+		
U5926	R	R	S	R	S	R	+						
U5187	R	R	R	R	R	R	+						
U2642	R	R	R	R	R	R							
U6306	R	R	R	R	S	R	+				+		
U3542	R	R	R	R	S	R	+						+
U5716	R	R	R	R	S	R	+				+		
U3239	R	R	R	R	R	R							
P5943	R	R	R	R	R	R	+						

Abbreviations: +:gene present; C, chloramphenicol; CARB, carbapenem; CEP, cephalosporin; E. coli, *Escherichia coli*; FLO, fluoroquinolone; FPI, folate pathway inhibitor TET, tetracycline.

selects for resistance to third-generation cephalosporins, and vice-versa and hence can be used for screening transformants.[19] Since the hosts were sensitive to all antibiotics, the use of ampicillin could screen for cephalosporin and carbapenem resistance. The transformants obtained after transduction were resistant to several other antibiotics as determined by the disk diffusion method and included the use of 8 antibiotics. The genes tested for the transduction study include only the $bla_{\text{CTX-M}}$ and bla_{TEM}. However, it is possible that genes encoding resistance to other classes of antibiotics but not screened for in this study were present as a single cassette, and thus horizontally transferred by the phage. Hence, the antibiogram could be established for different classes of antibiotics but genotype only for two genes.

Table 2 Distribution of antibiotic resistance genes in various genetic fractions

Genes	No. of isolates			
	Crude DNA	Plasmid only	Phage only	Plasmid and phage
CTX-M	26	7	10	9
TEM	9	4	0	5
SHV	0	0	0	0
KPC	1	1	0	0
NDM	13	5	1	7
VIM	1	0	0	1
OXA	7	5	0	2

Table 3 Phenotype and genotype of transformants obtained from transduction

Antibiotic sensitive host	Characteristics of prophage source	Characteristics post-transduction
EC153	U5663: TE^R,CIP^R,COT^R,CTX^R,CAZ^R,IPM^RMRP^R Genotype: bla_{CTX-M}	COT^R,CTX^R,CAZ^R,IPM^RMRP^R Genotype: bla_{CTX-M}
EC69	U2642: TE^R, CIP^R,COT^R,CTX^R,CAZ^R,IPM^RMRP^R Genotype: bla_{CTX-M}	CTX^R,CAZ^R,IPM^RMRP^R Genotype: bla_{CTX-M}
EC69	B5773 CIP^R,CTX^R,CAZ^R,IPM^RMRP^R Genotype: bla_{CTX-M}	CTX^R,CAZ^R,IPM^RMRP^R Genotype: bla_{CTX-M}
EC69	U2368 CIP^R,COT^R,CTX^R,CAZ^R,IPM^RMRP^R Genotype: bla_{CTX-M},bla_{TEM}	COT^R,CTX^R,CAZ^R,IPM^RMRP^R Genotype: bla_{CTX-M},bla_{TEM}

The bla_{CTX-M} of isolates positive for the gene in both plasmid and phage DNA were identical to $bla_{CTX-M-15}$ as determined by sequencing. This concomitant presence in multiple mobile elements in the same isolate reinforces and provides an insight for $bla_{CTX-M-15}$ being highly prevalent among clinical isolates of *E. coli* and probably as phage-plasmids.[18]

The limited number of isolates included in the study and the few resistance genes tested have narrowed the spectrum of this study, but provide insights into the probability of the role of many more AMR genes as phages. As this study focused on prophage mediated AMR dissemination, the ability of the plasmids to transfer the AMR genes by conjugation was not determined.

Shedding of infectious organisms from diseased personnel into the environment is unavoidable in cases where quarantine is not possible. Cleaning products that contain certain antimicrobial compounds are speculated to accelerate the development and spread of AMR.[20] Many components of cleaning products like detergents act as plasmid and prophage inducers. This study reiterates that genes like bla_{CTX-M}, bla_{TEM}, and bla_{NDM} that occur frequently in both the mobile genetic elements, plasmids and prophages, easily get induced on exposure to cleaning agents and thus disseminate faster than other genes. However, these findings do not hinder phage therapy as phage therapy specifically used lytic phages only.

Approvals Statement

The study was approved by the Institutional Research Advisory Committee via order INST/RAC/2017-18/9 and the Institutional Ethics Committee via sanction order INST.EC/2017-18/001 dated 22.01.2018.

Conflict of Interest

None declared.

References

1 Tacconelli E, Carrara E, Savoldi A, et al; WHO Pathogens Priority List Working Group. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 2018;18(03):318–327
2 The Center for Disease Dynamics. Economics & Policy. Resistance Map Antibiotic Resistance. 2021. https://resistancemap.cddep.org/AntibioticResistance.php
3 Smyth C, Leigh RJ, Delaney S, Murphy RA, Walsh F. Shooting hoops: globetrotting plasmids spreading more than just antimicrobial resistance genes across One Health. Microb Genom 2022;8(08):mgen000858
4 Rozwandowicz M, Brouwer MSM, Fischer J, et al. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. J Antimicrob Chemother 2018;73(05):1121–1137
5 Brabban AD, Hite E, Callaway TR. Evolution of foodborne pathogens via temperate bacteriophage-mediated gene transfer. Foodborne Pathog Dis 2005;2(04):287–303

6 Kondo K, Kawano M, Sugai M. Distribution of antimicrobial resistance and virulence genes within the prophage-associated regions in nosocomial pathogens. MSphere 2021;6(04):e0045221
7 CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 29th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute 2019
8 Su MT, Venkatesh TV, Bodmer R. Large- and small-scale preparation of bacteriophage λ lysate and DNA. Biotechniques 1998;25 (01):44–46
9 Woods WH, Egan JB. Prophage induction of noninducible coliphage 186. J Virol 1974;14(06):1349–1356
10 Castanheira M, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC Antimicrob Resist 2021;3(03):dlab092
11 Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother 2017;72(08):2145–2155
12 Jaggi N, Chatterjee N, Singh V, et al. Carbapenem resistance in *Escherichia coli* and *Klebsiella pneumoniae* among Indian and international patients in North India. Acta Microbiol Immunol Hung 2019;66(03):367–376
13 Suzuki Y, Sato T, Fukushima Y, et al. Contribution of β-lactamase and efflux pump overproduction to tazobactam-piperacillin resistance in clinical isolates of Escherichia coli. Int J Antimicrob Agents 2020;55(04):105919
14 Balcazar JL. Bacteriophages as vehicles for antibiotic resistance genes in the environment. PLoS Pathog 2014;10(07):e1004219
15 Muniesa M, García A, Miró E, et al. Bacteriophages and diffusion of β-lactamase genes. Emerg Infect Dis 2004;10(06):1134–1137
16 Mohan Raj JR, Vittal R, Huilgol P, Bhat U, Karunasagar I. T4-like Escherichia coli phages from the environment carry bla_{CTX-M}. Lett Appl Microbiol 2018;67(01):9–14
17 Galetti R, Andrade LN, Varani AM, Darini ALC. A phage-like plasmid carrying blaKPC-2 gene in carbapenem-resistant Pseudomonas aeruginosa. Front Microbiol 2019;10:572
18 Pfeifer E, Bonnin RA, Rocha EPC. Phage-Plasmids spread antibiotic resistance genes through infection and lysogenic conversion. MBio 2022;13(05):e0185122
19 Quinn JP, Miyashiro D, Sahm D, Flamm R, Bush K. Novel plasmid-mediated β-lactamase (TEM-10) conferring selective resistance to ceftazidime and aztreonam in clinical isolates of Klebsiella pneumoniae. Antimicrob Agents Chemother 1989;33(09):1451–1456
20 McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999;12(01):147–179
21 Batchelor M, Hopkins K, Threlfall EJ, et al. bla(CTX-M) genes in clinical *Salmonella* isolates recovered from humans in England and Wales from 1992 to 2003. Antimicrob Agents Chemother 2005;49(04):1319–1322
22 Mahrouki S, Ben-Achour N, Chouchani C, Ben-Moussa M, Belhadj O. Identification of plasmid-encoded extended spectrum beta-lactamases produced by a clinical strain of Proteus mirabilis. Pathol Biol (Paris) 2009;57(04):e55–e59
23 Babini GS, Livermore DM. Antimicrobial resistance amongst *Klebsiella* spp. collected from intensive care units in Southern and Western Europe in 1997–1998. J Antimicrob Chemother 2000;45(02):183–189
24 Yigit H, Queenan AM, Anderson GJ, et al. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumonia*. Antimicrob Agents Chemother 2001;45(04):1151–1161
25 Shenoy KA, Jyothi EK, Ravikumar R. Phenotypic identification & molecular detection of bla (ndm-1) gene in multidrug resistant Gram-negative bacilli in a tertiary care centre. Indian J Med Res 2014;139(04):625–631
26 Fallah F, Borhan RS, Hashemi A. Detection of bla(IMP) and bla (VIM) metallo-β-lactamases genes among Pseudomonas aeruginosa strains. Int J Burns Trauma 2013;3(02):122–124
27 Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70:119–125