

Molecular Study of the Spread of Antibiotic Resistance Genes in the *E. coli* with PCR

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Abstract: This examination was completed to distinguish the dispersion of antibiotic safe qualities in multi-antibiotic safe microscopic organisms isolated. Basic strategies were taken after in this to separate and describe the antibiotic safe microorganisms by the regular phenotypic, morphological, biochemical and sub-atomic characters. The 60 multidrug safe microscopic organisms isolates were haphazardly decided for disconnecting the antibiotic resistance qualities. Around 47% of antibiotic safe tried microscopic organisms were isolated from pee tests and 53% from stool. The examination additionally meant to break down antibiotic resistance rates against ordinarily utilized antibiotics among bacterial populace of pee and feces tests. These bacterial isolates were recognized and sorted into eight species. The isolates showed resistance in diminishing request for clindamycin (83%), penicillin G (69.6%), rifampin (64.7%), cefotaxime (53.6%), cefaclor (51.7%), ceftriaxone (47.2%), nitrofurantoin (44.2%) and norfloxacin (39.7%). Most extreme resistance to extended spectrum β -lactam antibiotics happened in 11.3% of isolates and the creation of expanded range β -lactamase was accomplished by 3.5% of isolates. Numerous resistances to at least three antimicrobial operators were recorded. PCR technique was utilized to disconnect the antibiotic resistance qualities for breaking down the sub-atomic characterization of these isolates. It depended on CTX-M1, CTX-M2 and *mecA* qualities which were utilized for quick task of microbes into genera and species. The outcomes show that all isolates harbor at least one of antibiotic resistance qualities and that the PCR system is a quick, useful and fitting strategy for deciding the nearness of antibiotic-resistance genes.

Key words: Antibiotic resistant bacteria, *Escherichia coli*, multidrug resistant bacteria, PCR technique, β -lactamase, penicillin

INTRODUCTION

There is overall a deep concern about the appearance and ascent of bacterial resistance to generally utilized antibiotics. In such manner, programs for checking resistance have been executed in numerous nations to protect the health of people (Morosini *et al.*, 2006). These projects more often than not screen marker microscopic organisms, for example, *Escherichia coli*. *E. coli* is ordinarily found in human and creature intestinal tracts and because of faecal tainting or pollution amid sustenance creature butcher is frequently found in soil, water and nourishments (El-Jakee *et al.*, 2011).

Various *E. coli* strains are perceived as essential pathogens of Colibacillosis in poultry and some of them can cause extreme human sicknesses, for example, haemorrhagic colitis and haemolytic uremic disorder (Fallah *et al.*, 2013). The treatment of sicknesses caused by this bacterium frequently requires antimicrobial treatment. The choice to utilize antimicrobial treatment relies upon the weakness of the microorganism and the pharmacokinetics of the medication for accomplishing the coveted helpful focus at the site of disease and along these lines clinical viability (Nourizadeh *et al.*, 2013). Be

that as it may, veterinary professionals have a restricted selection of antimicrobials for use in the poultry business because of antimicrobial resistance issues and human wellbeing concerns. In addition, the rehased and inadmissible utilization of antibiotics has prompted an expanding rate of antimicrobial resistance (Rohlf, 2000). Antibiotic utilization chooses for resistance in pathogenic microscopic organisms as well as in the endogenous vegetation of uncovered people or populaces. Thusly, the antibiotic choice weight for resistance in microbes in poultry is high and subsequently, their fecal greenery contains a generally high extent of safe microscopic organisms (National Committee for Clinical Laboratory Standards, 1998).

Antibiotic resistant microscopic organisms are an expanding danger to general health, as featured by an ongoing appraisal that in the US Methicillin-Resistant *Staphylococcus Aureus* (MRSA) may add to a bigger number of passings than HIV (Galani *et al.*, 2008). Methicillin-safe strains of *S. aureus* were at first reported in the 1960 and have been related with higher death rates than their medication touchy partners. The pervasiveness of multi-antibiotic safe microscopic organisms in ongoing decades makes an enthusiasm to scan for new option and successful antibiotics. The antibiotic safe microscopic

organisms are distinguished that are not murdered by usually utilized antibiotics. At the point when microscopic organisms are presented to similar antibiotics again and again, the microbes can change and are never again influenced by the medication because of changing of bacterial layer, emission of chemicals by target living beings, adjustment of site receptors as well as because of hereditary reasons (Quinn *et al.*, 2002).

Antibiotic resistance can come about likewise from vast genomic changes, for example, the obtaining of whole plasmids or portable components encoding resistance factors (CLSI, 2013). To contemplate the antibiotic resistance capacity, the key advances are the separation and purging of the antibiotic safe strains. Distinguishing proof of the disengaged antibiotic safe life form utilizing atomic procedures, for example, 16 SrRNA, Random Amplification of Polymorphic DNA (RAPD) and plasmid profile causing irresistible procedures is generally fundamental for viable antimicrobial and strong therapy (Sambrook *et al.*, 1989). Starting treatment might be empiric in view of the microbiologic the study of disease transmission of the contamination and the patient indications. Be that as it may, the recognizable proof of the irresistible living being guides the doctor in treatment of the infection on the grounds that the important information on the causal pathogen including its phenotypic and biochemical characters encourage the decision of appropriate and powerful antibiotic (Duran *et al.*, 2012). The point of the present examination was recognized and distinguished of antibiotic resistance genes in multi-antibiotic safe microscopic organisms separated from hospitalized patients in the KSA amid the 2013-2014 years. Furthermore, the present investigation was focused to evaluate the hereditary assorted variety among the diverse bacterial species by atomic (RAPD-PCR) markers (Schlegelova *et al.*, 2008).

MATERIALS AND METHODS

Bacterial isolation: Eighty-one *K. pneumoniae* were isolated from the patients (44 guys and 37 females). Tests were isolated from various clinical examples including pee, 49 (60.5%), blood societies, 16 (19.7%) injuries, 5 (6.2%) sputum, 4 (5%), intra-stomach, 3 (3.7%) and others 4 (5%). The clinic ward dispersion was as per the following: pediatric ward, 24 (29.6%) outpatient, 18 (22.2%) emergency unit, (12.3%) careful unit, 6 (7.4%) contamination unit, 2 (2.5%) and others, 21 (26%).

Assurance of antibiotic resistance and KPC enzyme detection: Antibiogram exhibited that the resistance rates of the separates were according to the accompanying:

Table 1: Antibiotic resistance patterns

Resistance pattern number	Number of isolates (%)	Antibiotics
A1	11(13.6)	OFX, NOR, CIP, IMI, K, GM, AK, TOB, HLS
A2	19(23.4)	OFX, NOR, CIP, IMI, GM, AK, TOB, K
A3	29(35.8)	OFX, NOR, CIP, IMI, K, GM
A4	4(4.9)	CIP, OFX, NOR, IMI, AK, CO
A5	12(14.8)	AK, GM, TOB, K, HLS
A6	25(30.9)	CIP, OFX, NOR, IMI, AK
A7	33(40.7)	CIP, OFX, NOR, IMI
A8	30(37)	CIP, OFX, NOR, AK
A9	52(64.2)	CIP, OFX, NOR
A10	11(13.6)	TR, TIC, C
A11	25(30.9)	IMI, AK

OFX: Ofloxacin; NOR: Norfloxacin; CIP: Ciprofloxacin; IMI: Imipenem; GM: Gentamicin; AK: Amikacin; TOB: Tobramycin; HLS: Streptomycin; K: Kanamycin; CO: Colistin; TR: Trimethoprim; TIC: Ticarcillin; C: Chloramphenicol

Table 2: Antibiotic resistance examples and nearness of armA quality

Isolate name	Resistance pattern	armA
1	OFX, NOR, CIP, IMI, K, GM, AK, TOB, HLS	+
2	OFX, NOR, CIP, IMI, K, GM, AK, TOB	+
3	OFX, NOR, CIP, IMI, K, GM, AK, TOB	-
4	OFX, NOR, CIP, IMI, K, GM	-
5	OFX, NOR, CIP, IMI, K, GM	+
6	OFX, NOR, CIP, IMI, K, GM	+

ofloxacin 65 ciprofloxacin 68.7%, norfloxacin 66.3%, gentamicin 66.3%, amikacin 51.8%, tobramycin 56.7%, kanamycin 79.5%, ticarcillin 82%, streptomycin 16.9%, cefotaxim 85.5%, ceftazidime 78.3%, azetreonam 79.5%, imipenem 45.8%, trimethoprim 74.7%, chloramphenicol 21.7% and colistin 16.9%. One colistin-safe isolate had a MIC of 8 µg/mL and the other all had MIC estimations of 4 µg/mL. All the colistin-safe segregates except for one of them had a place with one mending office. Five colistin-safe *K. pneumoniae* showed resistance to imipenem of which one was certain for KPC age. The antibiotic resistance case of the segregates is showed up in Table 1. The most bewildering rate of the resistance had a place with the A9 plan (64.2%) which saw to be impenetrable to three flouroquinolons. Also, minimal rate of resistance had a place with the A4 plan (4.9%) which was impenetrable to ciprofloxacin, ofloxacin, norfloxacin, imipenem, amikacin and colistin. MDR is generally chosen as resistance to no <3 classes of antibiotics. In the present examination, 66 out of 81 secludes were impenetrable to no <3 classes of antibiotics in this way, 81.5% of the disconnects were seen to be MDR. In like manner, the changed Hodge test exhibited that 6 disconnects (7.4%) were sure for KPC creation with 3 resistance plans (Table 2).

Molecular detection: The PCR demonstrated that 54 isolates (66.7%) of the isolates had armA quality. Aminoglycoside resistance designs in armA positive isolates (Table 3).

Table 3: Aminoglycoside resistance in *armA* positive isolates

Aminoglycosides	Resistance number (%)	<i>armA</i> +Number (%)
Kanamycin	66(79.5)	46(69.7)
Tobramycin	66(79.5)	32(68)
Gentamicin	55(66.3)	38(69)
Amikacin	43(51.8)	32(74.4)
Streptomycin	14(16.9)	12(87.7)

RAPD-PCR: RAPD investigation was performed by Moschetti *et al.*, Hill and Wachsmuth (1996) utilizing distinctive eight operon preliminaries. The RAPD-PCR intensification responses were performed in C1000TM Thermo Cycler Bio-Rad, utilizing the accompanying PCR program: 1 cycle at 94°C, 4 min, 35 extra cycles comprising of 94°C for 45 sec, 36°C for 60 sec and 72°C for 45 sec. Opened up DNA items were investigated by electrophoresis in 1.5% agarose gel keep running in TBE cushion. The gels were recolored with ethidium bromide (5 µg-mL⁻¹). Quality Ruler TM 100 pb. DNA Ladder (Fermentas) was utilized as a standard marker. DNA was pictured by UV light and was shot by a Bio-Rad Gel Doc 2000 gadget.

Antibiotic sensitivity test: Around eight sorts of antibiotics, Ceftriaxone (CRO), Norfloxacin (NOR), Clindamycin (DA), Penicillin G (P), Cefotaxime (CTX), Nitrofurantoin (F), Cefaclor (CEC) and Rifampin (Ra) were utilized for circle dispersion bioassay as indicated by Ehinmidu. Clinical bacterial separates suspensions were spread by sterile glass poles on the surface of supplement agar media. At that point antibiotic circles (Bioanalyse) were set onto the surface of the immunized supplement agar plates. The plates were then brooded at 28°C for 48 h and afterward hindrance zones were watched.

Biochemical characteristics of antibiotic resistant bacteria: The social, morphological and biochemical criteria of the 60 antibiotic resistant microorganisms secludes were utilized as ordered criteria. Gram recolor was the key advance and was completed by Hassanien (2004). Additionally, cell morphology was recorded utilizing oil inundation lense, motility test, catalase test and oxidase test as per cappuccino and sherman.

Genomic DNA extraction: The cell pellets from all disengages were utilized to extricate genomic DNA utilizing (Jena Bioscience, Germany) extraction unit following the producer's directions.

Detection of antibiotic genes: PCR amplification for acknowledgment the three antibiotic genes CTX-M1, CTX-M2 and *mecA* was finished. PCR amplification of CTX-M1 was finished using the going with preparations: 5'-GGT TAA AAA ATC ACT GCG TC-3' (forward) and

5'-TTG GTG ACG ATT TTA GCC GC-3' (alter) with an amplicon size of 860 bp. Foundations used for CTX-M2 were: 5' ATG ACT CAG AGC ATT CG-3' (forward) and 5'-TGG GTT ACG ATT TTC GCC GC-3' (pivot) with an amplicon size of 890 bp. Fundamentals used for *mecA* were: 5'-TCCAGATTACAACCTTCACCAG3' (forward) and 5'-CAATTCATATCTTG TACCG-3' (reverse) with an amplicon size of 162 bp. PCR mixes (25 µL) contained 1 µL of DNA design, 12.5 µL pro mix (Promega) and 1 pM of each fundamental and 9.5 µL sanitized refined water. PCR amplifications were performed in C1000TM Thermo Cycler Bio-Rad using the going with venture for blaIMP: beginning denaturation at 95°C for 5 min, trailed by 30 cycles of 30 sec at 94°C, 30 sec of treating at 54°C and 1 min of extension at 72°C with a last growth of 7 min at 72°C. For CTXM1 and CTX-M2, amplification was finished with a basic denaturation at 95°C for 5 min, trailed by 30 cycles of 1 min at 95°C, 1 min of fortifying at 60°C, and 1 min of development at 72°C with a last expansion of 10 min at 72°C.

For *mecA*, amplification was passed on using CTX-M1 program with toughening at 58°C for 45 sec. PCR things were continued running on 1.5% agarose gels, recolored with ethidium bromide and imagined by UV illumination and were caught by a Bio-Rad Gel Doc 2000 device. Data examination in order to choose the innate relationship among pondered microorganisms, RAPD data were scored for proximity (1) or nonattendance (0) of the gatherings using gene tools programming from Syngene. A fundamental organizing coefficient was surveyed by strategies for the Jaccard's coefficient to build up a closeness framework. Bundle examination and dendrogram were made in light of the Unweighted Typical Match Amass Technique (UPGMA) using the NTSYS-PC statistical package.

RESULTS AND DISCUSSION

Antibiotic frailty testing: Antimicrobial frailty testing was performed by the Bioanalyse circle dispersal procedure using supplement agar plates, according to the Clinical and Laboratory Standards Institute rules. The antimicrobial masters attempted and their looking at obsessions were according to the accompanying: nitrofurantoin (300 mg/plate), cefaclor (30 mg/circle), rifamin (5 mg/circle), cefotaxime (30 mg/circle), clindamycin (2 mg/circle), penicillin G (10 U/plate), norfloxacin (10 mg/plate), ceftriaxone (30 mg/circle). In the wake of bring forth the vaccinated plates energetically at 28°C for 48 h, the powerlessness of the 60 isolates to each antimicrobial pro was recognized using Combination Disk Diffusion Test (CDDT) and the results were deciphered according

to criteria gave by CLSI. All the 60 isolates were impenetrable to no <1 antimicrobial administrator. For example, the CDDT showed that among the 60 isolates strain number W-55 was resistance to seven of eight antimicrobial administrators while separate UR-17 was sensitive to each attempted antibiotic.

The resistance case of the each one of the 60 isolates to the eight antimicrobial administrators attempted. About 48 of the isolates were seen to be the most broadly perceived finding to the resistance to the clindamycin (83%), trailed by 42 bacterial strains were seen to be resistance to penicillin G (69.6%). Cefotaxime resistance was found in around 36 bacterial strains (53.6%). The rate estimations of other antimicrobial master like cefaclor, ceftriaxone, nitrofurantoin and norfloxacin were 51.7, 47.2, 44.2 and 39.7%, exclusively. Starting late CTX-M synthetic concoctions as the most surely understood extended territory β -lactamase have been represented.

Unmistakable sorts of this protein have been perceived and reported. Thusly, in this examination, we cleared the regularity of this kind of β -lactamase protein. This impetus gets from a hospetal bacterium and shows higher activity against cefotaxime than ceftazidime. PCR examination for CTX-M and mecA genes. The PCR amplification aftereffects of mecA, CTX-M1 and CTX-M2 genes making in *Staphylococcus aureus* and *E. coli* restrict.

Our results showed that the three genes were recognized in some isolates. Six isolates of *Staphylococcus aureus* saw to be passed on mecA quality, that in a couple of edges. While, CTX-M1 quality was found in three *E. coli* isolates and CTX-M2 in five *E. coli* isolates. Strikingly, segregate number 40 passed on both CTX-M1 and CTX-M2 genes Surprisingly, when PCR was done to disconnect CTX-M2 quality, isolates numbers 40, 44 and 58 indicated distinctive band estimate in harboring the CTX-M2 quality (around 580 bp) than isolates numbers 32 and 34 which have the genuine size of the CTX-M2 quality (890 bp). It might be conceivable that these isolates harbor same quality with various atomic weight. This could be because of that the isolates numbers 40, 44 and 58 are unexpected write in comparison

to isolates numbers 23 and 34. In this way in the closest future, we will endeavor to recognize these isolates utilizing 16 sec rRNA and furthermore, we will attempt to distinguish the grouping of CTX-M2 quality in isolates numbers 40, 44 and 58. The quality associated with the creation of this protein was set on mobile components called ISEcp1 which can exist in various districts of the genome of the bacterium. *E. coli* is the most prevailing pathogen conveying CTX-M protein quality. The strains which convey this quality are frequently isolated in bacteremia or gastroenteritis contaminations.

Consequences of antibacterial affectability:

Consequences of antibiotic affectability test on 19 isolates of *S. aureus* recuperated from crude drain, meat, and their items, 14 isolates showed resistance against penicillin (73.6%), 11 isolates were safe against antibiotic medication (57.8%), while 5 isolates displayed resistance to erythromycin (26.3%) and 8 isolates were impervious to kanamycin (42.1%) (Table 4).

RAPD-PCR analysis: The PCR-based RAPD fingerprinting strategy, using subjective oligonucleotides is especially, an intense apparatus for hereditary examinations and it is valuable as a screening genotyping technique (Quinn *et al.*, 2002). RAPD can create different unique mark designs with boundless number of preliminaries. In this examination, 8 RAPD groundworks were utilized for assessing of hereditary decent variety of antibiotic resistance microbes. RAPD responses were performed in copy and all amplification items were observed to be reproducible (CLSI., 2013). The RAPD-PCR comes about utilizing groundwork (OPA-10) has demonstrated a sum of 20 groups in these 60 utilized antibiotic resistance microbes extended from 250 bp-1900 bp. Three regular groups were seen in all isolates which displayed around 15% monomorphism while the other 17 pieces have indicated 85% polymorphism.

This groundwork distinguished an extraordinary piece, more than 1.5 kbp, particular to detach No. 45 (Data not appeared). If there should arise an occurrence of OPA-03 preliminary, an aggregate of sixteen sections

Table 4: Results of antibiotic sensitivity tests

Antibacterial agent	Milk and milk products (total n = 9)			Meat and meat products (total n = 10)		
	Sensitive n (%)	Intermediate n (%)	Resistant n (%)	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Penicillin group						
Penicillin	2 (22.2)	0 (-)	7 (77.7)	3 (30)	0 (-)	7 (70)
Tetracycline group						
Tetracycline	2 (22.2)	0 (-)	7 (77.7)	3 (30)	3 (30)	4 (40)
Aminoglycoside group						
Kanamycin	2 (22.2)	2 (22.2)	5 (55.5)	4 (40)	3 (30)	3 (30)
Macrolide group						
Erythromycin	6 (66.6)	1 (11)	2 (22.2)	5 (50)	2 (20)	3 (30)

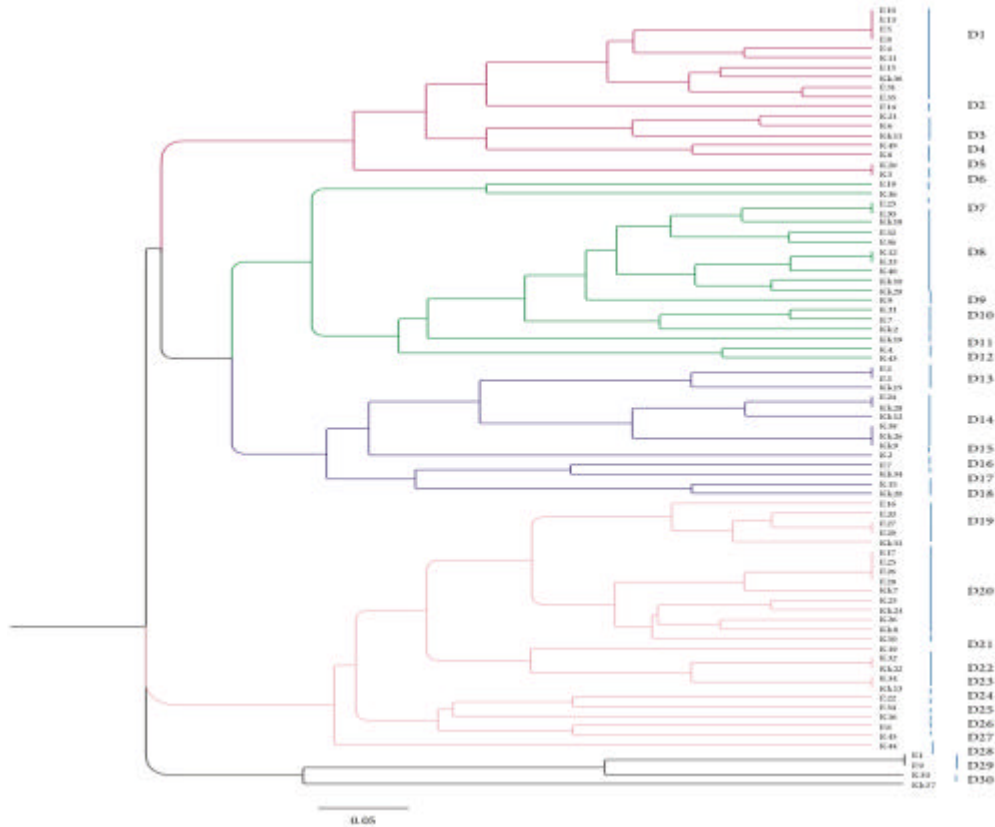


Fig. 1: Cluster analysis of *Klebsiella pneumoniae* based on RAPD typing

have indicated 100% polymorphism among the 60 isolates. The sub-atomic size of the amplicon items ran from 200 bp-1950 bp. Likewise, this preliminary perceived distinctive novel pieces at 250 bp particular to seclude No. 13. As indicated by hereditary similitude and intraspecies separation, the 60 isolates were gathered into four distinct bunches (A-D) with around 86% hereditary closeness.

Most isolates were assembled in bunches A and B. 25 isolates, for the most part isolated from pee tests were gathered in group A. Then again, 22 isolates, generally, isolated from feces tests, were assembled in group B. 11 of 60 isolates, quick amongst pee and feces tests were ascribed into third real bunch C. Strangely, bunch D represented just two one of a kind isolates. RAPDs were ended up being helpful as hereditary markers in antibiotic resistance microbes fingerprinting. Albeit real groups from RAPD responses are exceptionally reproducible, minor groups can hard to rehash because of arbitrary preparing nature of this PCR response and potential puzzling impacts related with co-movement with different markers (Sambrook *et al.*, 1989). The utilization of various groundwork sets in RAPD

investigation can be utilized as a rapid technique for starter biotyping of multidrug safe strains (Duran *et al.*, 2012) (Fig. 1).

In a past report utilizing distinctive Operon groundworks, the biased intensity of RAPD and its capacity to portray strains was illustrated. Operon groundworks likewise have been utilized as a part of a few past examinations and showed to effectively segregate epidemiologically related isolates (Schlegelova *et al.*, 2008).

In the present investigation, 81 *K. pneumoniae* were isolated from clinical examples. Antibiotic resistance designs and their connections among various clonal isolates were examined (Hill and Wachsmuth, 1996). Eleven antibiotic examples were discovered (A1-A11), demonstrating much decent variety in the resistance designs. In the present examination, 81.5% were MDR. The nearness of multidrug resistance network gained *K. pneumoniae* features the requirement for exact intending to control and counteract of the scattering of MDR strains (Hassanien, 2004). The greater part of the KPC-delivering isolates harbored armA quality and

were impervious to carbapenems, aminoglycosides and fluoroquinolones. As per our past investigation, Real Time PCR demonstrated an expanded articulation level of OqxAB and AcrAB efflux directs in fluoroquinolone-safe isolates in correlation with the delicate ones (information not appeared) (El-Jakee *et al.*, 2008). Thus, the part of efflux draw in making fluoroquinolone-safe strains could be recognized in these isolates. Likewise, the aminoglycoside resistance rates recommended 16S rRNA methylase action.

In the present investigation, around 70% of the aminoglycoside-safe strains conveyed the armA quality. In this way, a portion of these *K. pneumoniae* isolates have three highlights of resistance: KPC, efflux pumps and armA quality (El-Jakee *et al.*, 2013). So, they can be impervious to fluoroquinolones, cephalosporins, carbapenems and a range of aminoglycosides also. These strains can swing out to an imperative test for network and healing facility authorities by scattering among the patients in clinics and influencing the treatment to process more troublesome (Jamali *et al.*, 2015). Conjunction of the dynamic efflux pump, armA quality and KPC chemicals in *K. pneumoniae* can oppose against the blend treatment. This theory is additionally suggested who watched KPC generation and armA quality in clinical isolates of *K. pneumoniae* (Fooladi *et al.*, 2010).

Besides, around 17% of the isolates were impervious to colistin, among which everything except one segregate had MIC 4 µg/mL. In spite of the fact that colistin-safe isolates were identified with five unique groups, 42.9% of them had a place just with the fourth bunch from one healing facility, demonstrating a hereditarily particular coursing group (Song *et al.*, 2015). Colistin is considered as successful treatment against MDR and carbapenem-safe microorganisms, for example, *K. pneumoniae*, however, resistance to this specialist has started to rise. In this way, more examinations to decide the best treatment for contaminations caused by safe *K. pneumoniae* are required (Gwida and El-Gohary, 2013). In the present examination, genotyping investigation demonstrated distinctive hereditary examples among pathogenic *K. pneumoniae* isolates. In this manner, this apparatus has the capacity to recognize related and random isolates (Hosny *et al.*, 2011).

The connection between the antibiotic resistance examples and RAPD investigation exhibited that diverse hereditary examples had distinctive antibiotyping profiles (El-Sayed *et al.*, 2011). Additionally, KPC-creating *K. pneumoniae* were found to have a place with various bunches and the after effects of RAPD PCR embroiled that there is no relationship between's the hereditary examples and nearness of armA quality or KPC-delivering

K. pneumoniae that nonclonally spread of ESBL-creating *K. pneumoniae* strains with armA or rmtB intervenes aminoglycoside resistance (Fox *et al.*, 2017). It appears that in light of the fact that most resistance genes are conveyed by the portable hereditary components, they can without much of a stretch transmit among the microorganisms.

Of significance, the low number of the isolates was one of our constraints. Gathering more clinical isolates, utilizing all the more great separating composing strategies, for example, PFGE and investigation of armA and blakpc gene's demeanor level may enhance the nature of our outcomes in the accompanying examinations (Pesavento *et al.*, 2007).

CONCLUSION

The release of these treated effluents into the oceanic condition could conceivable increment antibiotic-resistance in *E. coli* along these lines heightening the spread of medication safe microorganisms in networks utilizing the streams and water bodies getting the treated emanating. The high predominance of colistin-resistance quality in *E. coli* recuperated from the last gushing represent a high hazard to the general population in this examination zone and this is the main give an account of colistin-resistance *E. coli* conveying the mcr-1 quality. Aminoglycosides have been considered as a sufficient remedial against both Gram-negative and gram-positive pathogens and furthermore, mix treatment with β-lactams and aminoglycosides is very much acknowledged for the treatment of the foundational diseases caused by *K. pneumoniae*, so, synchronous recognition of resistance making operators to Fluroquinolones, β-lactams and aminoglycosides in these strains is of clinical significance. Accentuation on the reasonable utilization of antibiotics, compelling contamination control measures and recognizable proof of antibiotic resistance systems by atomic techniques are important to lessen the occurrence of diseases caused by antibiotic-safe organisms.

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