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MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE PATTERN OF DIFFUSELY ADHERENT *ESCHERICHIA COLI* (DAEC) STRAINS ISOLATED FROM THE CHILDREN AND ADULTS WITH DIARRHOEAL SYMPTOMS – A STUDY FROM TERTIARY REFERRAL HOSPITAL, PUDUCHERRY, INDIA

Microbiology

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ABSTRACT

Background: Diarrhoea is a major global threat in developing countries especially in children below the age of five and the commonest bacterial etiologic pathogen of infectious diarrhoea is diarrhoeagenic E. coli (DEC). Among all the types of DEC, knowledge about the role of DAEC in diarrhoeal disease and their antimicrobial resistance (AMR) profile is limited in our center.

Aim: To detect the DAEC pathotypes from diarrhoeal stool specimen of children and adult and to study their toxin and antimicrobial resistance (AMR) genes profile (27 genes belongs to nine classes of antibiotics).

Methodology: Overall, 220 (120 children and 100 adults) stool samples from diarrhoeal cases were collected and inoculated on MacConkey agar. E.coli was confirmed using standard biochemical profiling. Convention polymerase chain reaction (PCR) was employed to detect virulence, toxin, AMR and integrons genes.

Results: Out of 220 patients, 8 were positive for DAEC infection. No *hlyA*, *hlyB*, *cfn1*, *cfn2*, and *ast* toxin genes detected among these strains. AMR genes detected in DAEC isolates were *sulI* 75% (6), *qnrB* 62.5% (5), *sulII* 62.5% (5), *qnrS* 50% (4), *tetA* 37.5% (3), *tetD* 37.5% (3), *blaSHV* 37.5% (3), *blaTEM* 37.5% (3), *tetC* 25% (2) *tetB* 25% (2) *catI* 25% (2) *aadB* 12.5% (1) *blaCTX* 12.5% (1). 3 DAEC strains harboured *int1* gene 37.5% (3) (class 1 integrons)

Conclusion: DAEC was found to be a predominant cause of diarrhoea in children and adults. These strains harboured various classes of AMR genes. Existence of class 1 integrons genes in these strains emphasize the significance of horizontal gene transfer (HGT).

KEYWORDS

Diarrhoeagenic Escherichia coli (DEC), diffusely adherent Escherichia coli (DAEC), antimicrobial resistance (AMR) genes, diarrhoeal, class 1 integrons.

INTRODUCTION

Diarrhea is a major global threat, the impact of diseases is high in developing countries like India especially in children below the age of five.[1, 2] *Escherichia coli* (bacterial pathogen) is the commonest etiological agent of infectious diarrhoea next to Rotavirus among the childhood diarrhoea.[1] Six pathotypes of diarrhoeagenic *E. coli* are classified into Enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETEC) , and diffusely adherent *E. coli* (DAEC) on the basis of cell culture pattern, pathogenesis and virulence factors.[3] Among this, DAEC association with the diarrhoeal disease remains a contentious issue.[3] Still, DAEC with diarrhoea has been reported across many studies worldwide in humans belonging to various age groups, significantly in children (<5 years of age).[4,5,6,7] DAEC was detected in travellers' diarrhoea cases and immunocompromised individuals like HIV patients with diarrhoeal diseases.[8, 9] Hybrid DAEC like DAEC/EAEC (presence of virulence gene more than one DEC pathotypes) have been reported across the globe with increased virulence provoking severe diarrhoeal episodes.[6] High drug resistance has been reported among these strains in few studies.[10] Amelioration of drug resistance in DAEC also made situation worst especially in treating immunocompromised patients and infants with diarrhoea. Hence, this study was conducted to characterize DAEC pathotypes and their antimicrobial resistance (AMR) genes profiling.

MATERIALS AND METHODS

Ethical statement

This study (Project No. JIP/IEC/2015/15/743) was endorsed by the Institute Ethics Committee (Human studies), JIPMER, Puducherry, India.

This cross-sectional study was conducted at JIPMER hospital (a tertiary care referral multispecialty hospital cum research institute) located in Pondicherry, India. About 220 continuous (non-duplicative) diarrhoeal stool samples received from children (n=120) below the age of five years and adults (n=100) over 18-year-old during the time frame from July 2015 to June 2016 were enrolled in this study. The specimens were inoculated on to MacConkey agar, HiMedia, India. Three to five distinct *E. coli* alike colonies were picked and subjected to conventional standard biochemical profiling. Phenotypically confirmed strains were preserved in Luria-Bertani (LB) broth supplemented with 50% glycerol at −80 °C till further use. QIAamp DNA Mini Kit, Qiagen (Germany), was exerted to extract the DNA of *E.coli* isolates and used for the DAEC virulence gene detection using conventional polymerase chain reaction (PCR). DAEC positive strains were subjected to EAEC virulence gene (to detect hybrid DAEC/EAEC strains as mentioned elsewhere[6]), toxin and AMR genes using conventional PCR performed in Eppendorf Mastercycler Nexus thermal cycler (Eppendorf, Germany). The details of virulence, toxin, and AMR genes along with PCR conditions followed as described in supplementary tables (Tables A1, A2 and A3). 1% agarose (Sigma-Aldrich, USA) was used for the Gel electrophoresis stained with ethidium bromide to measure amplicons size. Gel Doc XR System (Bio-Rad, USA) was used to visualize the separated PCR products.

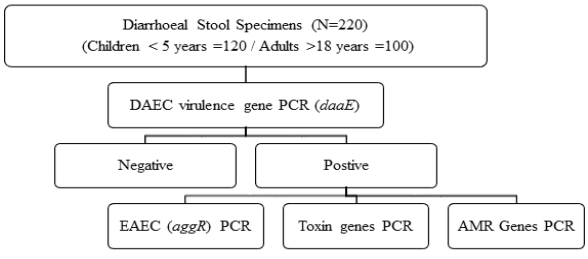
Statistical analysis

Percentages (%) was used to express all categorical variables. Likelihood of AMR genes existing in DAEC pathotypes isolated from children and adults was calculated using odds ratio (OR) and 95% confidence intervals (CIs) using Chi-square or Fisher's exact tests. A p-value of ≤ 0.05 was considered significant. OpenEpi Version 3.01 and Epidata Analysis V2.2.3.187 was used to perform Statistical analysis.

Sequence analysis

Using NCBI BLAST, sequenced data were analyzed for the highly significant matches. Sequencing service was done at Eurofins Scientific, Bengaluru, India.

Workflow of the study design



RESULTS

Totally, 220 diarrhoeal cases (120 children from the age group of 0 and 60 months and 100 adults more than 18 years of age) was collected from July 2015 to June 2016. Of which 63% (n=76) was male and 37% (n=44) was female among children and 57% (n=57) was male and 43% (n=43) was female among adults.

Distribution of DAEC in children and adults

An aggregate of 3.69% (n=8/220; p value= <0.05) of children and adult were positive for DAEC (Table 1). The clinical symptoms of DAEC infected children and adults were diarrhoea 75% (n=6) and acute gastroenteritis 25% (n=2) respectively. Likelihood of finding DAEC in children less than 12 month of age (odds ratio = 3.892; 95% CI 0.4727, 98.85) is 3.8 times more than children in 13 to 60 month. Similarly, the likelihood of finding DAEC in children below 5 years is 1.3 time (odds ratio = 1.387; 95% CI 0.3128, 7.2) high than adults (<18 years). No *hlyA, hlyB, cfn1, cfn2, stx1, stx2* and *ast* toxin genes detected in any of the DAEC isolates. No hybrid DAEC/EAEC strains detected.

The frequency of AMR genes in DAEC isolates

More than one class of AMR genes detected in all DAEC isolates. Majority of DAEC isolates harbored sulphonamides (*sul1* & *sul2*) and Quinolone (*qnrB* & *qnrS*) resistance genes followed by β lactams, tetracycline, chloramphenicol, trimethoprim, aminoglycoside. No *mcr-1, ermA, tetE, tetY, qnrA, qnrC, qepA, aac(6')-Ib, sulIII, aac(3)-IV* resistance genes detected among these isolates (Table 2). 2 DAEC isolates harbored integrons genes of class 1. While none harbored *int2* and *int3* genes (Table 2).

DISCUSSION

Our study results clearly show that DAEC has emerged as one of the potential etiological agent of diarrhoea in all the age groups, especially in children less than five years of age. Studies from India have reported 0.5% (n=2/203) and 0% (n=0/120) of DAEC respectively,[11,12] whereas in our study we detected 3.6% (n=8/220) of DAEC. Worldwide many similar studies reported 8.18% (n=9), 5.1% (n=34), 4.6% (N=43), 4.4% (41), 0.3% (n=3), 13% (n=21), 5% (N=4), of DAEC from China, Mexico, Peru, Japan, United States, Iran, and Mozambique respectively.[4, 6, 7, 13, 14, 15, 16] Within Brazil, various study have reported 12.8% (n=42), 0.4% (n=1), 0.7% (n=3) and 0% (n=0) of DAEC. These reports suggest that the prevalence rate of DAEC varies from nil to a prominent level.[6,17, 18,19]

Generally, DAEC was most commonly associated with diarrhoea in children than adults which are similar to our study findings and this was in concurrence with an earlier study report.[14] Contradictorily, DAEC was most commonly isolated from the adult diarrhoeal patients when compared with children in few studies too.[20, 21] These studies clearly depict that the DAEC emerged as a potential etiological agent of diarrhoea in all age groups.

Study conducted by Garcia C et al and Gassama-Sow et al in HIV positive and negative patients with diarrhoea found 10.1% (n=7/69) and 0% (n=0/40), 32.9% (n=52/158) and 28.0% (n=34/121) were infected with DAEC respectively, which clearly state that DAEC is a typical opportunistic diarrhoeal pathogen in immunocompromised patients.[9,22] In our study, we did not include the immunocompromised risk group with diarrhoea.

Vargas et al, Schultsz et al, and Meraz et al reported that 9.41%, 1.2% and 11% (comprise of European tourist, developed travellers' diarrhoea when they visited India (Kolkata (n=4) and Goa (n=3)) of the patient were infected with DAEC.[8,23,24] A study conducted by Smith et al in Ecuador has documented DAEC associated with travellers' diarrhoea among the local domestic travelers, stating that DAEC is most commonly associated with both international and domestic travellers' diarrhoea.[25]

Li D et al have reported 44.4% (n=9) and 0% (n=0) of DAEC strains positive for *hlyA* and *cfn1* toxin genes respectively.[4] Whereas no toxin genes were detected among DAEC strains in this study.

Flexibility in acquiring new genes (virulence, AMR, etc.,) due to horizontal gene transfer (HGT) leads to the emergence of heterogenicity among the DEC, leading to hybrid DEC strains which are becoming common and is responsible for the considerable amount of outbreaks across the globe. One such emerging Hybrid combination is DAEC/EAEC and it has been reported from diarrhoeal stool specimen of children by Patzi-Vargas from Mexico, and Sumbana et al from Maputo, Mozambique, 0.8% and 1% respectively.[6, 16] On the upper hand, DAEC/EAEC hybrid strains have been isolated from bacteremia cases of children less than five years old.[26] In this study, we did not detect any such hybrid strains.

In our study, all the DAEC strains harbored more than one class of AMR genes. Very few studies have analyzed the AMR pattern in DAEC strains worldwide; one such study was conducted by Li et al wherein, they reported all DAEC strains isolated (n=9) were highly resistant to most classes of drug and all the strains reported resistance for both sulphonamide and tetracycline by the in-vitro method.[4] Similar to this, another study conducted by Spano et al in DAEC isolates have reported a high burden of resistance for ciprofloxacin (57.1% (n=24)) and cefotaxime (52.3% (n=22)).[5] Resistance towards sulphonamides, quinolones, β-lactam and tetracycline groups of the drug was high among these strains in these studies and these reports were concordant to other study reports elsewhere.[10,21]

The increasing pattern of drug resistance among the pathogenic bacteria is majorly due to attaining of extra AMR genes by mobile genetic elements through HGT because of antibiotic selective pressure. Among these, plasmid-mediated resistance plays a crucial role than other modes of resistance mechanisms. In our study most of the strains harboured sulphonamide resistance genes followed by quinolone resistance genes, it could be due to the fact that sulphonamide and trimethoprim combination was the most common choice of drug for the treatment of a wide range of infections including diarrhoea for many decades earlier which could have led to the development of resistance among these strains gradually and emergence and rapid spread of plasmid-mediated resistance genes (*sul1, sul2,* and *dhfr1*) among the enteric pathogens especially by Inc plasmid especially in *IncF* plasmids through class 1 integrons.[27, 28] In addition to this, *IncA/C* plasmids (140- to 160-kb) harbouring multiple resistance genes at the same time, in clinical and environmental strains are emerging and have become a real threat to the current scenario.[29] Most of DAEC strains in our study also showed a similar profile of resistance genes. Recent studies have reported *sul3* resistance gene also emerging, but in this study, we could not find any isolates harbouring this resistance gene.[27]

Next, to sulphonamide resistance genes, most of the DAEC strains harboured quinolones resistance genes. This could be due to the usage of fluoroquinolone for the treatment of diarrhoeal disease (expect diarrhoea due to STEC) especially in travellers' diarrhoea and other types of infection since the 1960s. *qnrB* gene has been reported to be the most common than other *qnr* genes, similarly in our study also *qnrB* was most commonly detected followed by *qnrS*, while no *qnrA* and *qnrC* genes were detected. A study conducted by Herrera-León et al documented that all the DEC strains isolated from travelers patient harboured either *qnrB* and *qnrS* resistance genes.[30] High prevalence of *qnrB* and *qnrS* could be due to the rapid dissemination of plasmid through mobile genetic elements which carries *qnrB* and *qnrS* genes like *ISCR, Orf1005, IS26, ISEcp1, IS2, ISEcl2,* and *mic* among the pathogens.[31]

blaCTX is the most common β-lactam resistance genes among the Enterobacteriaceae, but in our study blaSHV was most common among the DAEC strains followed by *blaCTX* and *blaTEM*. A recent study from our center to study other DEC pathotypes other than DAEC found *blaCTX* gene high in number followed by *blaSHV*.[32] These β lactams resistance genes are also carried on the *IncF* plasmids.[30] Very recently *blaCTX* and *qnrS* was detected on the same new *IncF* plasmid isolated from fecal commensal *E.coli* of pregnant women.[33]

In this study, a considerable amount of DAEC strains were tetracycline resistant. Though it is not used in the treatment of diarrhoea, tetracycline resistance is common among the clinical strains and non-clinical strains due the extensive practice of using tetracycline in animal husbandries, aquaculture, poultry etc., as prophylactic and therapeutic agent in feeds and fertilizers for many decades, leading to circulation of these tetracycline resistance genes in clinical and nonclinical bacterial strains due to high selective pressure of these antibiotic through HGT.[34] In this study class 1 integrons, genes were detected in two DAEC isolates, class 1 integrons play a major role in spreading of multiple resistance genes of various classes at the same time in these strains.[27] Extraintestinal infections due to hybrid DEC strains like DAEC/EAEC with multiple drug resistant have affected the therapeutic strategies in treating patients.

CONCLUSION

DAEC is one of the potential etiological agent of diarrhoea in both children and adults. DAEC strains were high antibiotic resistant. Class 1 integrons play a predominant role in spreading of AMR genes in these strains through HGT due to antibiotic selective pressure which strongly recommends the use of antibiotics needs to be regulated under proper guidelines. Well build surveillance network is essential to study the frequency and the role of DAEC strains in diarrhoeal disease.

Limitation of this study

We did not look for any plasmids which could have provided more information regarding the HGT.

Financial support and sponsorship

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Conflicts of interest

There are no conflicts of interest.

Table 1. Distribution of DAEC in children and adults (age and gender wise distribution)

*Median (Non- Normally Distribution), ** IQR - interquartile range, †SD: Standard Deviation, ‡ p-value = <0.05

Total of diarrhoeal stool samples (n=220)		Children (<5 of age) = 120						Adults (>18 of age) = 100				
		Age in months expressed in median* (IQR)** – 12 (5.25-24)						Age in years expressed in mean [±SD†] - 44.74 [±15.24]				
		0–12	13–24	25–36	37–48	49–60	Total	18–24	25–36	37–48	49–85	Total
DAEC Positive (n=8/220)‡		4	0	0	1	0	5‡	0	0	2	1	3‡
Gender	Male	3	0	0	1	0	4	0	0	2	1	3
	Female	1	0	0	0	0	1	0	0	0	0	0
Clinical Diagnosis	Diarrhoea	3	0	0	1	0	0	0	0	1	1	0
	Acute -Gastroenteritis	1	0	0	0	0	0	0	0	1	0	0
Toxin genes	*hlyA*	0	0	0	0	0	0	0	0	0	0	0
	hlyB	0	0	0	0	0	0	0	0	0	0	0
	cfn1	0	0	0	0	0	0	0	0	0	0	0
	cfn2	0	0	0	0	0	0	0	0	0	0	0
	stx1	0	0	0	0	0	0	0	0	0	0	0
	stx2	0	0	0	0	0	0	0	0	0	0	0
	ast	0	0	0	0	0	0	0	0	0	0	0

Table 2. The associations between AMR and Integron genes among DAEC strains isolated from children and adults (n = 8)
OR- odds ratio

AMR genes	DAEC in children % (n=5)	DAEC in adults % (n=3)	Total (n=8)	Associations of genes (OR, 95% confidence interval)
qnrA	0	0 (0)	0 (0)	-
qnrB	80 (4)	33.3 (1)	62.5 (5)	8.0 (0.31 - 206.37)
qnrS	80 (4)	0 (0)	50 (4)	0.2 (.03 - 1.15)
qnrC	0 (0)	0 (0)	0 (0)	-
qepA	0 (0)	0 (0)	0 (0)	-
aac(6')-Ib	0 (0)	0 (0)	0 (0)	-
sulI	80 (4)	66.6 (2)	75 (6)	2.0 (0.07 - 51.59)
sulII	60 (3)	66.6 (2)	62.5 (5)	0.7 (0.03 - 14.97)
sulIII	0 (0)	0 (0)	0 (0)	-
dhfrI	40 (2)	0 (0)	0 (0)	0.6 (0.29 - 1.22)
aac(3)-IV	0 (0)	0 (0)	0 (0)	-
aadB	20 (1)	0 (0)	12.5 (1)	0.8 (0.51 - 1.24)
tetA	40 (2)	33.3 (1)	37.5 (3)	1.3 (0.06 - 26.61)
tetY	0 (0)	0 (0)	0 (0)	-
tetD	40 (2)	33.3 (1)	37.5 (3)	1.3 (0.06 - 26.61)
tetE	0 (0)	0 (0)	0 (0)	-
tetC	40 (2)	0 (0)	25 (2)	0.6 (0.29 - 1.22)
tetB	0 (0)	66.6 (2)	25 (2)	3.0 (0.60 - 14.86)
catI	20 (1)	33.3 (1)	25 (2)	0.5 (0.01 - 12.89)
blaCTX	20 (1)	0 (0)	12.5 (1)	0.8 (0.51 - 1.24)
blaSHV	60 (3)	0 (0)	37.5 (3)	0.4 (0.13 - 1.17)
blaTEM	0 (0)	100 (3)	37.5 (3)	-
ermA	0 (0)	0 (0)	0 (0)	-
mcr1	0 (0)	0 (0)	0 (0)	-
int1	40 (2)	33.3 (1)	37.5 (3)	1.3 (0.06 - 26.61)
int2	0 (0)	0 (0)	0 (0)	-
int3	0 (0)	0 (0)	0 (0)	-

Table A1. PCR Primers used for the detection of virulence genes of DAEC and EAEC in this study

S.No	DEC Pathotypes	Genes	Primers (53)	Product size (bp)	Reference
1.	DAEC	*daaE (Dr fimbrial adhesin - F1845 fimbriae)*	F:GAACGTTGGTTAATGTGGGGTAA R:TATTCACCGGTCGGTTATCAGT	542	Pérez C, Gómez-Duarte O, Arias M. Diarrheagenic Escherichia coli in Children from Costa Rica. Am J Trop Med Hyg.2010; 83 (2):292-297.
2.	EAEC	*aggR (transcriptional regulator)*	F: GTATACACAAAAGAAGGAAGC R: ACAGAATCGTCAGCATCAGC	254	

DEC- Diarrheagenic *E. coli;* **DAEC-** Diffusely Adherent E. Coli; **EAEC-** Enteroaggregative *E. coli;* **bp-** base pairs.

Table A2. PCR Primers used for the detection of toxin genes

S.No	Toxin Genes	Primers(53)	Product size (bp)	Reference
1.	*hlyA* (Hemolysin toxin)	F: GTATTCGGCACAGCAGAGAAA R: TTAATGCTG CAGCTGTGTC	323	Al-Shammari FJH. Detection of hemolysin genes producing Enterohemorrhagic Escherichia coli isolated from sheep by Multiplex PCR technique. Int J Adv Res. 2014; 2(6): 614-616
2.	*hlyB* (Hemolysin toxin)	F: TTACTGGCGCTACCGATCTC R: CGAATAACCGGTGCAACAAT	575	

3.	*astA* (Heat-stable enterotoxin)	F: TGCCATCAACACAGTATATCCG R: ACGGCTTTGTAGTCCTTCCAT	102	Bonkoungou IJ, Lienemann T, Martikainen O, Dembelé R, Sanou I, Traoré AS et al. Diarrhoeagenic Escherichia coli detected by 16-plex PCR in children with and without diarrhoea in Burkina Faso. Clin Microbiol Infect. 2012 Sep;18(9):901-6. doi: 10.1111/j.1469-0691.2011.03675.x. Epub 2011 Oct 10.
4.	*CFN1* (Cytotoxic Necrotizing Factors-1)	F: GAACTTATTAAGGATAGT R: CATTATTTATAACGCTG	543	Orden JA, Ruiz-Santa-Quiteria JA, Cid D, García S, de la Fuente R. Prevalence and characteristics of necrotoxigenic Escherichia coli (NTEC) strains isolated from diarrhoeic dairy calves. Vet Microbiol. 1999;66(4):265-273.
5.	*CFN2* (Cytotoxic Necrotizing Factors-2)	F: AATCTAATTAAAGAGAAC R: CATGCTTTGTATATCTA	543	

Table A3. PCR primers used for the detection of antimicrobial resistance and integrons genes

S.No.	Antimicrobial class	Genes	Primers (53)	Product size (bp)	Reference
1.	Quinolone	*qnrA*	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	516	Natarajan M, Kumar D, Mandal J, Biswal N, Stephen S. A study of virulence and antimicrobial resistance pattern in diarrhoeagenic Escherichia coli isolated from diarrhoeal stool specimens from children and adults in a tertiary hospital, Puducherry, India. J Health Popul Nutr. 2018;37(1):17. doi:10.1186/s41043-018-0147-z.
		qnrB	F: GATCGTGAAAGCCAGAAAGG R: ATGAGCAACGATGCCTGGTA	476	
		qnrS	F: GCAAGTTCATTGAACAGGGT R: TCTAAACCGTCGAGTTCGGCG	428	
		qnrC	F: GGGTTGTACATTTATTGAATCG R: CACCTACCCATTTATTTTCA	307	
		qepA	F: AACTGCTTGAGCCCGTAGAT R: GTCTACGCCATGGACCTCAC	482	
		aac(6')-Ib	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGTTT	596	
2.	Sulphonamides	*sulI*	F: TTCGGCATTCTGAATCTCAC R: ATGATCTAACCCTCGGTCTC	822	
		sulII	F: CGGCATCGTCAACATAACC R: GTGTGCGGATGAAGTCAG	722	
		sulIII	F: GAGCAAGATTTTTGGAATCG R: CATCTGCAGCTAACCTAGGGCTTTGGA	880	
3.	Trimethoprim	*dhfrI*	F: AAGAATGGAGTTATCGGGAATG R: GGGTAAAAACTGGCCTAAAATTG	391	
4.	Aminoglycoside	*aac(3)-IV*	F: GTGTGCTGCTGGTCCACAGC R: AGTTGACCCAGGGCTGTCG	627	
		aadB	F: TCCAGAACCTTGACCGAAC R: GCAAGACCTCAACCTTTTCC	700	
5.	Tetracycline	*tetA*	F: GTGAAACCCAACATACCCC R: GAAGGCAAGCAGGATGTAG	888	
		tetY	F: ACCGCACTCATTGTTGTC R: TTCCAAGCAGCAACACAC	823	
		tetD	F: TGGGCAGATGGTCAGATAAG R: CAGCACACCCTGTAGTTTTC	827	
		tetE	F: TTAATGGCAACAGCCAGC R: TCCATACCCATCCATTCCAC	853	
		tetC	F: ACTTGGAGCCACTATCGAC R: CTACAATCCATGCCAACCC	881	
		tetB	F: CCTTATCATGCCAGTCTTGC R: ACTGCCGTTTTTTCGCC	774	
6.	Chloramphenicol	*catI*	F: AGTTGCTCAATGTACCTATAACC F: ACACTTTGCCCTTTATCGTC	547	
7.	β lactams	*blaCTX*	F: ATGTGCAGYACCAGTAARGT R: TGGGTRAARTARGTSACCAGA	1018	
		blaSHV	F: ATTTGTCGCTTCTTTACTCGC R: TTTATGGCGTTACCTTTGACC	1076	
		blaTEM	F: ATAAAATTCTTGAAGACGAAA R: GACAGTTACCAATGCTTAATC	544	
8.	Macrolide	*ermA*	F: TCTAAAAAGCATGTAAAAGAAA R: CGATACTTTTTGTAGTCCTTC	533	
9.	Colistin	*mcr1*	F:CGGTCAGTCCGTTTGTTC R: CTTGGTCGGTCTGTAGGG	309	
10.	class 1 integrons	*int1*	F: GGTCAAGGATCTGGATTTCG R: ACATGCGTGTAAATCATCGTC	436	
11.	class 2integrons	*int2*	F: CACGGATATGCGACAAAAAGG R: TGTAGCAAACGAGTGACGAAATG	788	
12.	class 3 integrons	*int3*	F: AGTGGGTGGCGAATGAGTG R: TGTTCTTGTATCGGCAGGTG	600	

bp – base pairs

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