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Original Research Article

Genomewide sequencing reveals presence of multidrug resistant genes in *E. coli* isolated from chicken

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Abstract

This study was carried out to detect the distribution of antibiotic resistant genes in *Escherichia coli* isolates from chickens by whole genome sequencing. Isolation of *E. coli* from chicken confirm by biochemical test. The investigated genes included ampC, acrF, bacA, rarD, tehB, bcr, fsr, emrE were resistant to single antimicrobial agent and emrD, marR, marA, marB, mdtK, emrY, emrK, emrA, emrB were multiresistance. Multi-resistance which was defined as resistance to two or more tested agents. Whole genome sequencing and strategy to identify resistant genes reveals the presence of multidrug resistant genes for above tested antibiotics.
Key Words: Genomewide, *E. Coli.*

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INTRODUCTION

The use of antimicrobials in the veterinary medicines as well as in the commercial feed for cattle, pigs and poultry is very high. Due to the spatially congested environment and rapid breeding management, large breeding farms act as a pool of the antibiotic resistant microorganisms and resistance genes. *Escherichia coli* is commonly found in human and animal intestinal tracts. This microbe is usefully harmless, but it is also a medically important bacterium causing a number of significant infections. Recently, many strains of *Escherichia coli* have been found to be resistant to multiple, structurally unrelated antimicrobial classes (H. Momtaz *et. al.*, 2012) A number of E. coli strains are recognized as important pathogen of colibacillosis in poultry and some of them can cause severe human disease such as haemorrhagic colitis and haemolytic uremic syndrome (Riley *et.al* 1983; Chansiripornchai, 2009; Ferens and Hovde 2011). Various uses of antimicrobial agents in medicine, production of food animals, and crop protection are some of the reasons for increasing resistance to those agents (American Society of Microbiology, 2007). Today the development of antibiotic resistance and lack of discoveries of new antibiotics have created a serious public health concern. If bacteria come into contact with antibiotic but are not killed by antibiotic they may adapt their cell structure and/ or metabolism to make themselves resistant to that antibiotic. Once antibiotic resistance is acquired, they can share this information with other bacteria via vertical gene transfer. The veterinary practitioners have a limited choice of antibiotics for the treatment of animals, due to antimicrobial resistance issues and human health concerns. In view of this they use same antibiotics repeatedly, which leads to an increasing rate of antimicrobial resistance in bacteria (Mooljuntee *et al.* 2010). This resistance is not only limited to pathogenic bacteria but also spreads in the endogenous flora of exposed animals. There are several reports of the presence of antibiotic resistant bacteria in poultry and meat products. Researchers have reported high proportion of antibiotic resistant bacteria in the faecal flora of poultry (Piddock, 1996; Bogaard and Stobberingh 1999). Momtaz *et al* (2012) had carried out a study to detect the distribution of antibiotic-resistant genes in *Escherichia coli* isolates from slaughtered commercial chickens in

Iran. Similar studies have also been carried out in pigs during Metaphylactic Trimethoprim and Sulfamethoxazole treatment and in the Post-Exposure Period (Mazurek *et al* 2015). In some of the previous studies, transfer of antimicrobial-resistant bacteria from animal products to humans has been reported (Sanchez *et al*. 2002; Swartz 2002). In the last few years, many strains of *E.coli* have been reported to be resistant to multiple, structurally unrelated antimicrobial classes, like quinolones, cephalosporins, and aminoglycosides (Orden *et al*., 2001; Donaldson *et al*., 2006). Resistance among microorganisms can generally be detected either phenotypically or genotypically. The phenotypic approach is the usual method when testing bacteria for clinical purposes. However, in genotypic detection DNA based techniques are in use.

MATERIAL AND METHOD

Sample: Chicken sample were collected for microbial analysis from Nanded city. All the sample were collected aseptically, transported to the laboratory under chilled conditions and processed for microbiological analysis within 24 hrs of collection.

Enrichment: The samples were inoculated into 0.1 % peptone salt solution and incubate at 37 °*C* for 24hrs.

Plating: A loopful inoculum from 0.1 % peptone salt solution was streaked onto MacConkey's agar and plates were incubated at 37 °*C* for 24hrs and observed for pink colony on MacConkey's agar. The well separated pure colonies were subculture on EMB agar and plate incubated 37 °*C* for 24hrs and observed for the characteristic metallic sheen of *E. coli*. Pure colonies were picked up on nutrient slant as pure culture and subjected for biochemical test.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was performed by the Kirby- Bauer disc diffusion method using Mueller – Hinton agar (HiMedia Laboratories, Mumbai, India MV1084).

DNA extraction: E. coli were subcultured overnight in LB broth (Merck, Germany) and genomic DNA was extracted using a Genomic DNA purification kit (Fermentas, germany) according to the manufactures instructions.

Sequencing

1. Sample Prep. (Sample Preparation) For library construction, DNA is extracted from a sample. After performing quality control (QC), passed sample is proceeded with the library construction.
2. Library Construction: The sequencing library is prepared by random fragmentation of the DNA sample, followed by 5' and 3' adapter ligation. Alternatively, "tagmentation" combines the fragmentation and ligation reactions into a single step that greatly increases the efficiency of the library preparation process. Adapter-ligated fragments are then PCR amplified and gel purified.
3. Sequencing: For cluster generation, the library is loaded into a flow cell where fragments are captured on a lawn of surface-bound oligos complementary to the library adapters. Each fragment is then amplified into distinct, clonal clusters through bridge amplification. When cluster generation is complete, the templates are ready for sequencing. Illumina SBS technology utilizes a proprietary reversible terminator-based method that detects single bases as they are incorporated into DNA template strands. As all 4 reversible, terminator-bound dNTPs are present during each sequencing cycle, natural competition minimizes incorporation bias and greatly reduces raw error rates compared to other technologies. The result is highly accurate base-by-base sequencing that virtually eliminates sequence-context-specific errors, even within repetitive sequence regions and homopolymers.
4. Raw data: Sequencing data is converted into raw data for the analysis.

Preprocessing

1. Quality Control: After sequencing, analyze the quality control of the sequenced raw reads. Overall reads' quality, total bases, total reads, GC (%) and basic statistics are calculated.
2. Preprocessing: In order to reduce biases in analysis, FastQC and quality filtering process are performed. And filtered reads' quality, total bases, total reads, GC (%) and basic statistics are calculated again.

Analysis

1. Mapping: The filtered reads are mapped to reference genome. In this process, sufficient read depth is needed for a more accurate analysis. After mapping, duplicated reads are removed.
2. Variant Analysis: Variants (SNPs and short Indels) are captured through aligned reads' information.
3. Annotation: The variants are classified by each chromosomes or scaffolds, and the information of the location is marked

RESULT

Antimicrobial resistance gene responsible in *E. coli* isolated from chickens. Following antibiotics were investigated in the current study shown in table 1.

Table 1: Antibiotic markers used to identify resistant genes in *E. coli*

Sr. No	Gene	Antibiotic resistance
1	rarD	Choramphenicol
2	fsr	Fosmidomycin
3	emrE	Methylviologen
4	tehB	Tellurite selenium resistance
5	bcr	Bicylomycin resistance
6	cysA	Chromate resistance
7	bacA	Bacitracin resistance
8	acrF	Acridine resistance
9	ampC	Penicillin resistance
10	emrD	Multidrug resistance
11	marR	Multidrug resistance
12	marA	Multidrug resistance
13	marB	Multidrug resistance
14	mdtk	Multidrug resistance
15	emrA	Multidrug resistance
16	emrB	Multidrug resistance

Table 2: Genomewide distribution of resistant genes in *E. coli*

	source	feature	Start	end
NC_000913.3	RefSeq	CDS	119281	120135
NC_000913.3	RefSeq	CDS	503476	504696
NC_000913.3	RefSeq	CDS	516583	517362
NC_000913.3	RefSeq	CDS	568315	568647
NC_000913.3	RefSeq	CDS	1501562	1502155
NC_000913.3	RefSeq	CDS	1619120	1619554
NC_000913.3	RefSeq	CDS	1619574	1619957

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Conflict of interest

Authors do not have any conflict of interest.

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