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Prevalence and antimicrobial resistance pattern of *Escherichia coli* from clinical and non-clinical sources in selected regions of Bareilly, India

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Abstract
The present study was designed to understand the tetracycline and quinolone resistance pattern of *E. coli* isolates from Bareilly, India. For achieving the objectives, a total of 61 *E. coli* isolates from clinical and non-clinical sources were used in this study. Among 61 *E. coli* isolates, 40.98% were resistant to doxycycline and 41.18% to ciprofloxacin. Almost 65.3% of *E. coli* isolates were multidrug resistant. Genotypic detection of AMR shows that 24% and 12% of *E. coli* isolates were positive for *tet*A and *qnr*S genes, respectively. The *tet*A gene was more prevalent clinically (P-0.04) whereas detection of *qnr*S gene was not statistically significant among isolates from clinical and non-clinical samples (P>0.05).

Keywords: *E. coli*, multidrug, isolates

Introduction
Antibiotics have been the revolutionary invention which in medicine field has transformed the methods of treatment against bacterial infections. Their widespread application has systematically changed the healthcare sector both in terms of treatment methods and efficacy of therapies (Annunziato *et al*., 2019) [1]. However, mis-use of antibiotics has contributed to the emergence of AMR which has resulted in high mortality which is alarming (Simlai *et al*., 2016) [10]. Global mortality rate may reach up to 10 million with a reduction of 1.1-3.8% of overall economic growth due to antimicrobial resistance by 2050 (Wang *et al*., 2020) [14]. By 2030, due to AMR a total of 24 million people would be affected and as a consequence extreme poverty especially in low income countries may hamper the workforce (Lima *et al*., 2019) [6]. *E. coli* is one among the six priority pathogens identified by WHO and it is responsible for more than 250 000 deaths associated with AMR (Murray *et al*., 2022) [7]. With this view, the present study was conducted to know the antimicrobial susceptibility of *E. coli* isolates to most commonly used antibiotics such as doxycycline and ciprofloxacin.

Materials and methods
Revival and preliminary identification of *E. coli* isolates: *E. coli* isolates were revived from the repository of clinical Epidemiology laboratory, Division of Epidemiology, ICAR- Indian Veterinary Research Institute, Bareilly, Uttar Pradesh. Initial screening was done using MacConkey agar plates and incubated at 37 °C overnight. From each plate four isolated lactose fermenting colonies were inoculated on eosin methylene blue (EMB) agar medium for preliminary characterization and colonies showing characteristic metallic sheen on EMB agar were picked up and considered as presumptive *E. coli*. The purified cultures of presumptive *E. coli* were stored in nutrient agar for further identification by biochemical tests (IMViC pattern) and other studies.
The isolates were also tested for antibiotic susceptibility pattern with imipenem (IPM- 10µg), meropenem (MRP-10 µg), ertapenem (ETP10 µg), doripenem (DOR-10 µg), enrofloxacin (ENO-5 µg), ciprofloxacin (CIP-5 µg), doxycycline (DO-30 µg), minocycline (MO), tetracycline (TE-30 µg), ceftriaxone (CTR-30µg), cefoperazone (CFP-30 µg), gentamicin (GEN-10 µg), chloramphenicol (C-30 µg), sulphatrimethoprim (COT- 25µg), cefepime (FEP-30 µg), azithromycin (AZM-15 µg), cefoxitin (CX30 µg), nitrofurantoin (NIT-300 µg) and amoxycillin-clavulanic acid (AMC-30 µg) by disk diffusion method. Antimicrobial susceptibility results were interpreted by the following criteria established by the Clinical and Laboratory Standards Institute.

E. coli showing resistance to at least three classes of antibiotics were categorized as multidrug-resistant strains. The genomic DNA of *E. coli* isolates was extracted by QIAamp DNA Mini Kit (Qiagen India Pvt. Ltd., New Delhi, India). All phenotypically confirmed *E. coli* isolates were subjected to PCR amplification for identification of tetracycline resistance genes, *tet*A, *tet*B and quinolone resistance genes *qnr*A, *qnr*B, *qnr*S and *aac(6')-Ib-cr*. The primer details and their respective annealing temperatures and its amplification size are given in table 1.The amplified PCR product was visualized by a gel documentation system (UVP, UK) after electrophoresis in 1.5% (w/v) agarose gel containing ethidium bromide (0.5 µg/mL, Loba Chemie, India). The positive PCR amplicons were sent to commercial sequencing services (Eurofins Ltd., Bangalore) for further purification and sequencing by Sanger method. The homology searches were made using the BLAST algorithm available at http://blast. ncbi.nlm.ni.gov/Blast.cgi, and the representative sequences were submitted to GenBank for accession number.

Table 1: Details of the primer sequence, annealing temperature and product size for *E. coli* PCR assay

Gene		Primer Sequence (5'-3')	Annealing Temp. ºC	Length	Reference
*qnr*A	F	CAGCAAGAGGATTTCTCACG	54	630	Cattoir *et al*.,2007 [15]
	R	AATCCGGCAGCACTATTACTC			
*qnr*B	F	GGCTGTCAGTTCTATGATCG	54	488	Cattoir *et al*.,2007 [15]
	R	GAGCAACGATGCCTGGTAG			
*qnr*S	F	GCAAGTTCATTGAACAGGGT	54	428	Cattoir *et al*.,2007 [15]
	R	TCTAAACCGTCGAGTTCGGCG			
aac(6')-Ib-cr	F	TTGGAAGCGGGGACGGAM	54	260	Robicsek *et al*.,2006 [16]
	R	ACACGGCTGGACCATA			
*tet*A	F	GTAATTCTGAGCACTGTCGC	60	956	Mayanard *et al*., 2004 [17]
	R	CTGCCTGGACAACATTGCTT			
*tet*B	F	CTCAGTATTCCAAGCCTTTG	60	414	Mayanard *et al*., 2004 [17]
	R	ACTCCCCTGAGCTTGAGGGG			

Results and Discussions

In the present study, a total of 61 *E. coli* isolates from clinical and non-clinical sources were revived from repository of clinical Epidemiology laboratory, were confirmed by biochemical characteristics (Edwards and Ewing, 1972) [5]. All the 61 isolates conformed to the typical *E. coli* except two strains not producing gas on utilizing lactose (Caprioli *et al*., 1994) [4]. Though gas production from lactose is one of the important characters of *E. coli*, non-gas producers *E. coli*, especially biochemically inactive *E. coli* is not uncommon in environment (Bueschkens *et al*., 1984) [2]

Antibiotic sensitivity assay of *E. coli* isolates revealed high resistance rates for tetracycline (78.68%), sulpha-trimethoprim (72.13%) and ceftrioxone (65.57%). This may indicate the fact that ciprofloxacin, third generation cephalosporins and tetracyclines are the prime antibiotics used in India for treatment of bovines with mastitis. Tetracyclines are also among the most commonly used antibiotics in poultry farming in India. Low resistance rates for minocycline (22.95%), nitrofurantoin (22.95%) and chloramphenicol (8.19%). The difference might be linked with the overall decline in the use of these antibiotics in India since 2000.

A total of 49.18% of the *E. coli* isolates were resistant to ciprofloxacin reported 45.5% enrofloxacin resistance in *E. coli* isolates, which is similar to the findings of the present study reported ciprofloxacin resistance in 82% of the *E. coli* isolates. Nirupama *et al*., (2018) [8] reported ciprofloxacin resistance rate of 52.6% from *E. coli* isolates of animal origin reported ciprofloxacin (57.14%) resistance in *E. coli* isolated from street vended foods. Of the 49 (80.32%) multidrug-resistant (MDR) *E. coli* isolates, 29 (59.1%) and 20 (40.81%) isolates were from clinical and non-clinical samples, respectively.

Of the 27 doxycycline resistant isolates screened for the presence of *tet*A and *tet*B genes, 6 (24%) isolates had *tet*A gene and all were of clinical origin. None of the isolate was positive for *tet*B gene. Of the 31 ciprofloxacin resistant isolates screened for presence of *qnr*A, *qnr*B, *qnr*S and *aac-61-Ib-cr* genes, 3 (12%) isolates were positive for *qnr*S gene, of those 2 were of nonclinical and one was of clinical origin. None of the isolates were positive for *qnr*A, *qnr*B and *aac-61-Ib-cr*. Nirupama *et al*., (2018) [8] screened the ESBL producing isolates (n=243) for plasmid mediated quinolone resistance (PMQR) genes revealed that 37.9%, 46.1% and 20.2% isolates harboured *qnr*A, *qnr*B and *qnr*S genes, respectively. Many factors are responsible for development of drug resistance such as unrestricted access to antibiotics, poor animal husbandry practices, inadequate disease control practices and lack of compliance in implementing regulations on use of antibiotics in animals (Van Boeckel *et al*., 2017) [13].

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