

ABSTRACT

Antimicrobial resistance (AMR) is a global concern, particularly in the poultry industry. In Pakistan, AMR surveillance system has been implemented at the National Reference Laboratory for Poultry Diseases. This study aimed to detect Extended Spectrum β -Lactamase (ESBL) Shiga Toxin producing *Escherichia coli* (*E. coli*) and evaluate antimicrobial resistance in healthy poultry. Total 175 samples were collected from different chicken shops of Pakistan. 142 confirmed *E. coli* subjected to antimicrobial sensitivity test (AST) and 36 ESBL isolates analyzed by polymerase chain reaction (PCR) against 11 virulence factors. More than 50% resistance observed against 12 out of 31 antibiotics analyzed by AST which include Tetracycline (100%), Quinupristin/ Dalfopristin (100%), Penicillin (100%), Teicoplanin (100%), Linezolid (99%), Ampicillin (97%), Nalidixic Acid (95%), Erythromycin (87%), Streptomycin (87%) Chloramphenicol (79%), Sulfamethoxazole-Trimethoprim (73%), Ciprofloxacin (73%), Trimethoprim (71%), Ceftazidime (8%), Ertapenem (1%). Highest sensitivity for isolated *E. coli* samples was observed against Ertapenem (99%), Cefoxitin (88%), Azithromycin (87%), Ceftazidime (85%), Cefotaxime (80%), Gentamicin (75%), and Meropenem (53%). 36 isolates were analyzed by PCR revealed that all samples were negative for shiga toxin producing genes *stx1* and *stx2*. Only 2 isolates from avian pathogenic *E. coli* were positive for *stx1* and *stx2* which were from Layyah and Multan. The most prevalent genes were *bla*TEM-1 (28%), with *mcr-9* (22%) found in maximum isolates and *mcr-2* (8%) and *mcr-5* (8%) also found in some isolates but interestingly *mcr-1*, *bla*SHV, *bla*FOX, *bla*CTX-M8, and *bla*OXA were not found in any isolate. The study found that Pakistan retail chicken shops have a high prevalence for multi-drug resistant *E. coli*, possibly due to antibiotic misuse and persistent exposure. This can affect the health of chickens and humans, as they consume chicken meat. Public health authorities should control antibiotic misuse in poultry production and establish a strong surveillance network to monitor AMR conditions. Further evaluation of genotypic and phenotypic AMR profiling and husbandry practices is needed for effective containment measures.

Key Words: *Escherichia coli*; Shiga toxin; beta-lactamase gene; mobile colistin resistance gene