Detection and characterization of extended spectrum $\beta$-lactamase producing *Escherichia coli* from poultry of eastern India

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BETA LACTAM RING

BETA LACTAMASES enzymes that inactivate the beta-lactam ring

PENICILLIN

CEPHALOSPORIN
Extended-spectrum $\beta$-lactamases

• ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem or imipenem).

• It is the major cause of treatment failure for the hospitalized patients, specially those are admitted to intensive care unit (ICU), immunocompromised patients and patients undergoing chemotherapy.

• Recent times have witnessed ESBL producers being detected outside the hospital premises in the community set-up. Even the livestock were also detected to harbor these pathogens.

• ESBL producing *Enterobacteriaceae* were found to cause sever infection in animals including persisting and chronic intramammary infection of bovine and haemorrhagic diarrhea in poultry.
Altogether, 22 isolates were ESBL producers and two were positive for both ESBL and ACBL as confirmed by disc diffusion and E-test.

The genes $\text{bla}_{\text{CTXM}}$, $\text{bla}_{\text{SHV}}$, and $\text{bla}_{\text{TEM}}$ were detected in 14, 17 and 21 isolates, respectively.

18 of them carried class 1 integron and 90% of the class 1 integron carrying isolates were also positive for sulphonamide resistance gene ($\text{sul}1$).

Several ESBL producers were found to carry PMQR ($\text{qnrA}$, $\text{qnrB}$ & $\text{qnrS}$) and virulence genes of extra-intestinal pathogenic $E. \text{coli}$ commonly detected in human patients with UTI related problems.

All the PCR amplified products were cloned in PGMT-Easy and PTZ57/R vectors, sequenced and subjected to homology search in BLAST algorithm for confirmation.
Drug resistance pattern of ESBL producing *E. coli* isolates from poultry birds of Eastern India.

ESBL producers exhibited resistance to most of the oxyimino cephalosporins and monobactam including ceftriaxone (CTR), ceftazidime (CAZ), cefotaxime (CTX), aztreonam (AT), cefpodoxime (CPD), cefepirome (CFP). Resistance was also frequently noted to other antibiotics as well, like amoxicillin (94%), piperacillin+tazobactam (94%), cotrimoxazole (88%), ciprofloxacin (83%), tetracycline (77%), cefepime (66%) and chloramphenicol (61%).
PCR amplification of major ESBL genes – bla\textsubscript{TEM} (867 bp), bla\textsubscript{CTX-M} (540 bp), and bla\textsubscript{SHV} (393 bp) genes among the ESBL producing \textit{E. coli} isolates from diverse food producing animals of eastern India.

Restriction analysis for confirmation of successful cloning of class I integron (1009 bp), PMQR (qnrB) (476 bp) and astA (119 bp) genes of ESBL producing \textit{E. coli} isolated from diverse food animals from Odisha and West Bengal using pGMET-Easy and pTZ57/R vectors.
Organization of gene cassette in the class 1 integron of MDR ESBL producing *E. coli* from poultry

KJ624023, 1915 bp
*E. Coli* isolate from Odisha

KJ775803, 1009 bp
*E. Coli* isolate from WB

KJ851207, 1664 bp
*E. Coli* isolate from Jharkhand
Schematic representation of the genetic environment of *blaCTX-M* gene

- ATG
- TAA
- TAA
- ISEcp1
- tnpA
- *blaCTX-M-15*
- W 48 bp
- ORF 477/IS903 tnpA

KJ851207, 1664 bp
*E. Coli* isolate from Duck, WB
Phylogenetic analysis of ESBL producing *Escherichia coli* isolates isolated from eastern India. The neighbour-joining method was used to summarize the similarity of ERIC-PCR profiles of the strains in a dendrogram containing 3 major clusters.
Conclusions:

- Poultry birds seem to be a potential reservoir of ESBL producing *E. coli*.
- This may be a major cause of frequent therapeutic failure for controlling the bacterial infectious diseases in poultry like diarrhea, colibacillosis and septicaemia.
- The infection may also be transmitted to human via consumption of contaminated poultry products.
- Genetic similarity of the isolates from three states indicate that the isolates from distant geographical regions are clonally related.
THANK YOU