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Multidrug resistant and extended spectrum β-lactamase producing *Salmonella* spp. in red meat of cattle, Bangladesh

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Background: Globally, the wide spread of drug resistant bacteria contaminated via meat poses a serious public health hazard. Red meat of cattle may be contaminated with bacteria mostly during slaughtering both in rural and urban area. To recognize the public health risk attributed to red meat of cattle, we estimated the burden of multidrug resistant and extended spectrum β-lactamase (ESBL) producing *Salmonella* spp. We also investigated the presence of quinolone resistance genes as well as ESBL-producing encoding genes.

Methods and materials: A total of 240 meat samples collected aseptically from different butcher shops of 5 divisions (out of 8) during July 2018 to September 2019. Standard bacteriological methods and PCR assay used for isolation and identification of *Salmonella* spp. Antimicrobial susceptibility test was performed using Kirby-Bauer disk diffusion method with 20 antibiotics of 9 classes and ESBL producing *Salmonella* were screened by double disc synergistic test. The quinolone resistance and ESBL encoding genes were detected by mPCR.

Results: The prevalence of *Salmonella* was 40.8% ($n = 98$). The multidrug resistant (MDR) isolates were 77.6% ($n = 76$), and 58.2% ($n = 57$) were ESBL-producer. The highest resistance showed to oxytetracycline (85.7%) and colistin sulphate (79.6%) followed by imipenem (57.1%). Five isolates were possible extensively drug-resistant (pXDR), which showed resistance to at least one antibiotic of each class of 8 and/or 9 classes of antimicrobials. Moreover, 25.5% (25/98) isolates were resistant to eight or more antibiotics irrespective of classes that are commonly used in animal and human therapeutics. Quinolones resistance gene, *qnr*S and *qnr*B were detected in 10.3% and 3.4% isolates, respectively, while none of the isolates was positive for *qnr*A. Furthermore, ESBL-producing encoding gene *bla*TEM was found in 100% isolates but none of isolates were positive for *bla*SHV, *bla*CTXM-1 and *bla*CTXM-2 genes.

Conclusion: This study showed that the red meat of cattle was extensively contaminated with MDR and pXDR *Salmonella*. The evidence of resistance to colistin sulphate and imipenem indicate that the possibilities of cross-resistance as these antimicrobials are not used in large animal production system in Bangladesh.

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Modifications of mgrB instigate colistin resistance in poultry gut-isolates in Bangladesh

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Background: Acquisition of colistin resistance by a plasmid-mediated gene, *mcr-1*, was described for Enterobacteriaceae in many countries. We identified *mcr-1*-carrying colistin-resistant poultry gut-bacteria in Bangladesh and observed a group of colistin-resistant isolates without harboring *mcr-1*. We sought to investigate the genetic factor conferring colistin-resistance in those *mcr-1*-naive isolates. Several recent studies have reported inactivation of a chromosomal gene, *mgrB*, to be responsible for acquired colistin-resistance in absence of *mcr-1*. In this study, we investigated any potential deletion or mutation in *mgrB* gene to link the colistin-resistance phenotype.

Methods and materials: Different selective culture media were used to isolate various bacteria from chicken dropping. Conventional biochemical procedures were followed by API 20E kit (BioMèrieux, Durham, NC) for identification of bacteria. A part of bacterial identification was validated further by genotyping using 16S rDNA analyses. Disc-diffusion and minimal inhibitory concentration (MIC) measurement were performed to determine the colistin susceptibility of the isolates. *mgrB* gene was amplified by polymerase chain reactions (PCR). Any alterations of nucleotides and/or amino acids in the *mgrB* gene clones were checked primarily by single sequence blasting with online database and multiple sequence alignment analysis by ClustalW program.

Results: Among 99 isolates 87.5% showed phenotypic colistin-resistant by disc-diffusion assessment. All the resistant isolates showed MIC level, between >8 μg/mL to >256 μg/mL. Among these resistant isolates only 36.2% were harboring *mcr-1* gene. The identified *mcr-1* gene showed complete harmony to phenotypic colistin-resistance. To determine the *mgrB* alteration, 15 isolates were analyzed in three groups: group-1 was colistin-resistant carrying *mcr-1* gene; group-2 was colistin-resistant without *mcr-1* gene; and group-3 was colistin-susceptible isolates. Analyses of nucleotide and amino acid sequences revealed deletion mutation and shortening of MgrB protein in all the colistin-resistant isolates. In addition, a novel single amino acid substitution at 28 position of MgrB from phenylalanine (F) to cysteine (C) was observed associating colistin-resistance.

Conclusion: Modulation of *mgrB* gene expression was the key factor for the acquired resistance to colistin. The deletion or non-synonymous substitution of the *mgrB* gene was shown to associate colistin resistance in gram negative bacteria. These findings attested the regulatory role of *mgrB* leading to phenotypic polymyxins-resistance.

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ID 19-53: Multi-drug resistant and extended spectrum β-lactamasesproducing *Salmonella* spp. in red meat of cattle

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Introduction: Globally, MDR and extended spectrum β-lactamases producing (ESBL)*Salmonella*is highly risk resulting foodborne illness associated with meat products reported widely in human and animals. We estimated the prevalence, resistance pattern and ESBL producing *Salmonella* spp.in beefcollected from butcher shopfromten districts of five divisions in Bangladesh.

Methods: During July, 2018 to September, 2019, a total of 240 samples aseptically collected and tested using standard bacteriological methods like colony characteristics, staining, biochemical reaction and sugar tests. Antimicrobial susceptibility test was performed using Kirby-Bauer disc diffusion test using 20 antibiotics of 9 classes and ESBL producing *Salmonella* were screening by double disc synergistic test.

Results: Theoverall prevalence of *Salmonella* spp. was 40% (96/240)of which 40.9% were ESBL producing. Moreover, 37(38.5%) of the isolates were MDR and exhibited resistant to oxytetracyclin (85.5%), polymyxin B(80.2%), colistinsulphate(79.2%) and imipenem (56.3%). The highest classes of antibiotic resistant weredetected 77.1%in polymyxin,74% in tetracyclineand 36.5 %in sulfonamides. All isolates exhibited resistant to seven or more potential antimicrobial like colistinsulphate (79.2%), polymyxinB (80.2%), imipenem (56.3%), oxytetracyclin (85.5%) nalidixic acid (32.3%) and gentamicin(16.7%).

Conclusion: The emergence of MDR and ESBL producing *Salmonella*in beef of butcher shop may constitute a public health concern, adequate cooking and reinforce good hygiene practice of butcher shop to avoid cross-contamination. Further, serotyping and resistance gene identification is strongly recommended.

Keywords: Drug resistant, ESBL producing*Salmonella*spp, antibiotics, resistance gene