

**Detection of resistant and virulent genes of *Escherichia coli (E. coli)* and *Staphylococcus sp*. isolated from pneumo-enteritic goats in Chittagong of Bangladesh**

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Session: 2014-2015 (July-December)

**A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Medicine**

 **Department of Medicine and Surgery**

**Faculty of Veterinary Medicine**

**Chittagong Veterinary and Animal Sciences University**

**Chittagong-4225, Bangladesh**

**July, 2017**

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**DR. Sharmin Akter**

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**This is to certify that we have examined the above Master’s thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee will be addressed**

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**July, 2017**

***DEDICATED TO MY RESPECTED AND BELOVED PARENTS AND SISTERS***

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**List of Abbreviations**

|  |  |
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| Abbreviation | Elaboration |
| *E. coli* | *Escherichia coli* |
| Sp. | Species |
| BDT | Bangladeshi taka |
| PM | Post mortem |
| MRSA | Methicillin Resistant *Staphylococcus aureus* |
| MRS | Methicillin Resistant *Staphylococci* |
| AMR | Antimicrobial resistance |
| µm  | Micrometer |
| LT | Heat labile |
| ST | Heat stable |
| ETEC | Eenterotoxigenic *Escherichia coli* |
| EAEC | Enteroaggregative *Escherichia coli* |
| EIEC | Enteroinvasive *Escherichia coli* |
| EPEC | Enteropathogenic *Escherichia coli* |
| EHEC | Enterohaemorrhagic *Escherichia coli* |
| PAI | Pathogenecity islands |
| CFA | Colonizing factor antigen |
| STX | Shiga toxin |
| INV | Invasion |
| AAF | Aggregative adherence fimbrieae |
| DEAC | Diffusely adherent *Escherichia coli* |
| PRTC | Poultry research and training center |
| CVASU | Chittagong Veterinary and Animal Sciences University |
| SAQTVH | S.A. Quaderi Teaching Veterinary Hospital  |
| RAJ | Recto-Anal Junction |
| EMB | Eosin methylene blue |
| °C | Degree Celsius |
| BHIB | Brain Heart Infusion Broth  |
| h | hour |
| MSA | mannitol salt agar |
| EDTA | Ethylene Diamine Tetra Acetic Acid |
| BPA | Buffer Peptone Agar |
| µl | Microlitre |
| MHA | Mueller-Hinton agar |
| BaCl2 | Barium Choloride |
| H2SO4 | Sulphuric acid |
| PCR | polymerase chain reaction  |
| mL | MilliLitre |
| DNA | Deoxyribo Nucleic Acid |
| MS | Master of Science |
| % | Percentage |
| *et al.* | And his associates |
| etc. | Et cetera |
| pmol | Pico mol |
| bp | Base pair |
| gm | gram |
| NCBI | National Center for Biotechnology Information  |
| CI | Confidence interval |

**ABSTRACT**

An investigation was conducted to evaluate the prevalence of common bacterial pathogens specially *Staphylococcus sp., E.coli* in pneumoenteritic goats. For this, fecal and nasal swab samples were subjected to various cultural and biochemical tests to isolate these bacterial pathogens. Cultural sensitivity test followed by identification of resistant genes to evaluate the drug resistance phenomena of isolated bacterial pathogens and virulent genes specific to both organisms were also studied. From nasal swab samples 28% (42) were found to be positive for *Staphylococcus sp.* in all cultural and biochemical tests. *E. coli* isolates were screened out from both fecal and nasal swab samples that reflected 26% (78) of prevalence in affected goats. Subsequently, cultural sensitivity test revealed most bacterial isolates were resistant to commonly used antibiotics like ampicillin, oxacillin, trimethoprim-sulfamethoxazole and tetracycline but some isolates showed variable in sensitivity. The screening of the presence of Stx1 and Stx2 virulent genes as well as tetA, blaTEM, sul2 resistant genes in *E. coli* isolates and resistant genes mecA, vanA, tetM in Staphylococcal isolates was done by PCR. This study disclosed the isolation rates of Stx1 and Stx2 are 5% (95% CI 0.0013-0.249) and 30% (95% CI 0.119-0.543) respectively whereas the isolation rates of tetA, blaTEM and sul2 are 45 (95% CI 0.23-0.684), 45 (95% CI 0.23-0.684) and 70% (95% CI 0.457-0.881) in fecal *E. coli* and 50 (95% CI 0.118-0.881), 83.4 (95% CI 0.358-0.995) and 83.4% (95% CI 0.358-0.995) respectively in nasal *E. coli*. In Staphylococcal isolates, the prevalence of mecA, vanA and tetM are 42.9 (95% CI 0.176-0.711), 0 and 28.6% (95% CI 0.084-0.581) respectively. Gene sequencing was done commercially and the sequences were compared with other known sequences published in GenBank, and revealing highest nucleotide similarity showed 100% (Stx1). Phylogenetic tree was prepared using mega6 software. In this study, it was shown that, virulent and antibiotic resistant bacteria were isolated from pneumoenteritic goats indicating random uses of antibiotics or it might be cross infection from the environment. Therefore, awareness against random uses of antibiotics in food animals should be strengthened in commercial as well as smallholder goat farms.

**Key words**: Virulent gene, resistant gene, *Staphylococcus sp., E.coli,*pneumoenteritic goats, prevalence.