Antimicrobial Resistance Pattern and Identification of Resistant Gene in Bacteria Isolated from Meat and Milk Collected from Local Market of CMA

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STATEMENT OF AUTHOR

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This is to certify that I 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 have been made

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List of Acronyms and Symbols Used

Abbreviation and symbols	Elaboration
AMR	Antimicrobial Resistance
MDR	Multidrug resistant
%	Percent
>	Greater than
<	Less than
≥	Greater than equal
≤	Less than equal
=	Equal to
℃	Degree Celsius
BHI	Brain Heart Infusion
bp	Base Pair
BPW	Buffered Peptone Water
CDC	Center for Disease Control and Prevention
CFU	Colony Forming Unit
CI	Confidence Interval
CLSI	Clinical and Laboratory Standards Institute
CRE	Carbapenem Resistant *Enterobacteriaceae*
CSE	Centre for Science and Environment
CS	Culture Sensitivity
CVASU	Chittagong Veterinary and Animal Sciences University
DAEC	Diffusely Adherent *E. coli*
DNA	De-oxy Ribonucleic Acid
EaggEC	Entero-aggregating *E. coli*
E. coli	*Escherichia coli*
EHEC	Enterohemorrhagic *E. coli*
EMB	Eosin Methylene Blue
ESBL	Extended Spectrum β-Lactamase
ETEC	Enterotoxigenic *E. coli*
Kb	Kilo Base

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SUMMARY

Antimicrobial resistance (AMR) is known as a silent pandemic; an emerging global public health issue. The problem of AMR is similarly salient and prevalent in animals. From a public health perspective, AMR in dairy cattle, poultry meat can also jeopardize human health by the potential dissemination of AMR pathogens to humans via consumption of infected dairy products or direct contact with infected poultry and animal meat. Harshly, the dairy and poutry industry is increasingly confronted with AMR *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Campylobacter* and *Salmonella*. This study was designed to investigate the multidrug- resistant pattern along with the most frequently isolated resistant genes of *S. aureus. E. coli, Campylobacter* and *Salmonella* from buffalo milk, buffalo meat, goat meat, beef meat, goat milk, layer meat and poultry meat in the Chittagong metropolitan area of Bangladesh. A total of 130 meat and milk samples including 40 poultry products (broiler & layer chicken meat) and 90 large (buffalo and cattle) and small (goat) animal products were collected from the study population.

Isolation and identification of *S. aureus, E. coli, Campylobacter* and *Salmonella* were done following the standard bacteriological method. The nuc gene,16s rRNA and ST11, ST15 gene were tabbed in molecular identification of *S. aureus, E. coli, Campylobacter* and *Salmonella respectively*.

In this study among 7 isolates of *S. aureus* four in broiler meat (20% , 95%CI 5.73 - 43.6%), one in beef meat (5%, 95%CI 0.12%- 24.8%), one in buffalo meat (10% , 95%CI 0.25%- 44.5%), one in Goat meat (5% , 95%CI 0.12%- 24.8%) were confirmed.

Whereas among 47 isolates five in broiler meat (25% , 95%CI 8.65%- 49.1%), nine in beef meat (45%, 95%CI 23.05%- 68.45%), four in buffalo meat (40%, 95%CI 12.15% - 73.76%), thirteen in goat meat (65%, 95%CI 40.78%- 84.60%), six in goat milk (24% , 95%CI 9.35%- 45.12%), seven in buffalo milk (46.67% , 95%CI 21.26%-73.41%), three in layer meat (15% , 95%CI 3.2%- 37.8%) were confirmed as *E. coli.*

Also twenty-one isolates were confirmed as *Campylobacter* strain out of 130 samples; Nine in layer meat (45% , 95%CI 23.05%-68.47%), twelve in broiler meat (60% , 95%CI 36.05%-80.88%). On the other hand, a total of 5 isolates were confirmed as *Salmonella* strain out of 130 meat and milk samples by PCR. One in beef meat (5% , 95%CI 0.12%- 24.8%), four in buffalo milk (26.67% , 95%CI 7.78%- 55.10%).

Among the positive *S. aureus* isolates, showed resistance against all tested antibiotics except Meropenem (MEM). Highest number of isolates (100%) in broiler meat was resistant against Tetracycline (TE), followed by 75% to Oxytetracycline (OT) and 50% to Ceftriaxone (CRO), Oxacillin (OX) and others. All *S. aureus* isolates were multi drug resistant, 57.14 % of the isolates had resistance against 4 to 6 tested antimicrobials and 42.85% had resistance to more than 7 antimicrobials.

E. *coli* isolates were characterized with the presence of AMR genes. Afterward, the positive isolates were screened against antimicrobials using the disc diffusion technique. At a fleeting glance, alarmingly in buffalo milk, broiler meat, cattle meat and goat meat, 100% *E. coli* isolates of CMA showed resistance to Sulphathiazole- trimethoprim (SXT), Erythromycin (ERE), Tetracycline (TE) and Ampicillin (AMP), followed by in buffalo milk 85.71% against Ciprofloxacin (CIP), 57.14% against Gentamycin (CN), and Neomycin (N). In buffalo meat, 100% resistance showed only against Ciprofloxacin (CIP) and Ampicillin (AMP). All 47 *E. coli* isolates were multi drug resistant. 57.44% isolates were resistant to 4-6 antimicrobials and 40.42% were resistant to more than 7 tested drugs.

In Layer meat 100% *Campylobacter* isolates of Chattogram area showed resistance to Erythromycin (ERE), Streptomycin (S) and Azithromycin (AZM) followed by 88.88% against Ciprofloxacin (CIP) and Sulphamethoxazole-trimethoprim (SXT), following 77.77% resistance against Cefotaxime (CTX), Ceftriaxone (CRO) and Ceftazidime (CAZ). In broiler meat, 100% *Campylobacter* isolates showed resistance to Erythromycin (ERE), Streptomycin (S) and Azithromycin (AZM) followed by 83.33% against Ciprofloxacin (CIP) and Sulphamethoxazole-trimethoprim (SXT). All 21 *Campylobacter* isolates were multi drug resistant. 23.81% isolates were resistant to 4-6 antimicrobials and 76.19% were resistant to more than 7 tested drugs.

Salmonella spp. isolates of the study area from buffalo milk showed 100% resistance against Ciprofloxacin (CIP), followed by 75% against Tetracycline (TE), Sulphamethoxazole-Trimethoprim (STX), Streptomycin and 25% against Erythromycin (ERE) and Gentamycin (CN). All 5 *Salmonella* isolates were multi drug resistant. 40 % isolates were resistant to 2-3 antimicrobials and 60% were resistant to more than 4-6 tested drugs.

Among the 7 *S. aureus* isolates had ESBL producing 2 *blaz* gene in broiler meat, cattle meat and 1 in goat meat and Erythromycin producing 1 *Erm (B*) gene in broiler meat and 1 in buffalo meat. Whereas, 1 *Erm (C)* gene in broiler meat, 1 in buffalo meat and 1 in goat

meat was identified. Tetracycline producing 3 *Tet (K)* gene found in broiler meat and 1 in buffalo meat.

Within 47 isolates of *E. coli* 4 *Sul1* gene(sulfonamide) gene and 1 *Tet (B)* (tetracycline) and 1 *AAC(IV)* gene were identified in goat meat and 2 *Sul1,* 1*Tet (B)* and1 *AAC(IV)* gene were identified in buffalo milk. Also found 3 *blaCMY* gene, 4 *Sul1, 2Tet (B), 3AAC(IV)* and 1 *ERE* gene in cattle meat. In layer meat, only 1*Sul1* and 1*Tet (B)* gene found. In goat milk, 2 *Sul1,* 3*Tet (B),* 2 *Tet (C)* and1 *AAC(IV)* gene were identified in *E. coli* isolates.

Very few *Campylobacter* isolates of Chattogram area was identified with antimicrobial resistant gene. 3 isolates had *blaTem* gene both in broiler and layer meat. 2 isolates in broiler meat had *Tet (C)* gene.

2 *Sul1* gene (sulfonamide) and 1 *Tet(A)*gene was identified in *Salmonella* spp.isolates in buffalo milk whereas 1 *blaSHV,*1 *blaCMY* and 1 *Sul1* identified in cattle meat.

Conclusively, the study findings will provide ample statistical evidence to develop strategies for improvement of antimicrobial stewardship, rejuvenate the antimicrobial drug channel and to develop efficacious and sustainable alternative approaches to tackling AMR crisis both in humans and livestock.