POTENTIAL DISSEMINATION OF ANTIMICROBIAL RESISTANCE FROM SMALL SCALE POULTRY SLAUGHTERHOUSES IN PAKISTAN

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Abstract. The importance of environmental reservoirs as a source from which antimicrobial resistance emerges and subsequently transferred is already established. To evaluate the role of small scale poultry slaughterhouses in resistance dissemination in Pakistan, a comparative genomic analysis of antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs) in the gut microbiome of broiler and household chickens (*Gallus gallus domesticus*) was performed. An array of 52 qPCR primers targeting the 16S rRNA gene, ARGs and MGEs was used in the experiment to analyse the abundance of selected genes in collected samples. A total of 22 ARGs and 7 MGEs were detected in all samples. The detection frequency of specific gene classes and diversity of genes was found to be higher in broiler as compared to household chicken. *Sul1* was the most abundant ARG with the highest percent relative abundance (2.4%). Total percent relative abundance of ARGs in broiler chicken was found to be significantly ($p < 0.05$) greater than household chicken fecal samples. A significant linear correlation ($R^2 = 0.89$) was found between relative abundances of *int1* and total ARGs. The clustering and correlation of selective ARGs with MGEs has implied that small scale poultry slaughterhouses can be a potential source for the dissemination of ARGs to other non-resistant environmental and/or clinical bacteria.
Keywords: *ARGs, MGEs, qPCR, AMR, microbiome*

Introduction

Antimicrobial resistance genes (ARGs) are emerging environmental contaminants causing serious public health concerns (Sanderson et al., 2016). The annual number of human deaths due to antimicrobial resistance is expected to reach up to 10 million by 2050 (de Kraker et al., 2016). Accumulating scientific evidence have suggested that the abuse of antibiotics in clinical and agricultural settings is mainly responsible for the emergence of antimicrobial resistance in microorganisms (Rather et al., 2017; York, 2017). Antibiotic resistant bacteria and associated ARGs can be transported from different environmental reservoirs contributing to the ever increasing global problem of antimicrobial resistance (Waseem et al., 2017a). The scientific community is already exploring new directions for evaluating the spread of the antimicrobial resistance from environmental routes to clinical pathogens (Andersson, 2015; Waseem et al., 2019; Williams et al., 2017). Fecal microflora from animals and birds harbours a vast variety

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of ARGs that can be transferred into other bacteria including human pathogens (Bonnedahl and Järhult, 2014; Zhao et al., 2018).

The poultry industry is one of the fastest growing agricultural sectors of the world. Use of antibiotics at therapeutic and sub-therapeutic levels as growth promoters have been employed in poultry for over half a century (Castanon, 2007). Although antibiotics utility for growth promotion has already been ostracized in many developed countries, but the situation is disquieting in developing countries like Pakistan and Egypt (Mund et al., 2017). In Pakistan, over the counter availability of antibiotics and less implementation of health and safety regulations in the poultry industry makes the challenge of antibiotic abuse and subsequent resistance dissemination far more intriguing. By 2030, the global consumption of antibiotics for cattle, chicken, and pigs is expected to reach 105,596 ± 3,605 tons (Van Boeckel et al., 2015). The consumption of antibiotics is continuously putting increasing pressure on environmental and clinical bacteria to acquire antimicrobial resistance. Apart from the resistance development under selection pressure for antibiotics, bacteria can also develop resistance by acquiring resistance genes via mobile genetic elements (MGEs) mediated horizontal gene flow (Oliveira et al., 2017).

One of the most useful strategies against the global antimicrobial resistance is to mitigate the spread of ARGs from environmental reservoirs. Surveillance and monitoring of such reservoirs are thus essential for curbing the dissemination of ARGs into the environment. Small scale poultry slaughterhouses are one of the potential reservoirs for antibiotic resistance. They are widely distributed throughout Pakistan. Chicken fecal waste from these small scale slaughterhouses, a source of resistant bacteria and ARGs, is generally being disposed of in the environment without any proper treatment. Chances of meat contamination with the antimicrobial resistant bacteria at the time of slaughtering in unhygienic conditions are also there (Amir et al., 2017). Additionally, ARGs can also be disseminated into the environment when poultry feces are used as organic fertilizers for fruits and vegetables.

Antibiotics can also influence ARGs to which they are not explicitly related (Foxman, 2012; Guo et al., 2018). Therefore, instead of antibiotic specific screening of ARGs in chicken *(Gallus gallus domesticus)* gut microbiota, analysis of ARGs from all major classes is recommended. Additionally, most of the research on antimicrobial resistance was focused on individual bacteria mainly foodborne pathogens (Lee et al., 2017; McMahon et al., 2007), which represents only a small fraction of the gut microbiome. Evaluation of resistance determinants from gut microbiome community can give a comprehensive analysis of dissemination and/ or fate of the ARGs into the environment.

To the best of our knowledge the status of AMR in gut microbiome of chicken in Pakistan yet remains elucidative to a greater extent. The primary purpose of the current study was to evaluate and compare the detection frequency, diversity, and abundance of selected ARGs from chicken fecal samples collected from small scale poultry slaughterhouses. The abundance and occurrence of selected MGEs have also been evaluated as they are believed to be involved in the dispersal of ARGs into non-resistant pathogenic bacterial strains. The possible correlations between ARGs and MGEs were also determined. Present research is a pilot scale study with only a limited sample set but it will likely provide first-hand information regarding ARGs dissemination from the small scale poultry slaughterhouses.

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Materials and methods

Sample collection and DNA extraction

Sample collection was performed in early spring of 2018 from a small scale poultry slaughter house in Johar Town, Lahore, Pakistan. Pools of fresh fecal samples from different cages of broiler and household chickens were collected (*Fig. A1* in the *Appendix*). A total of 6 fecal samples 3 from broiler chickens and 3 from household chickens were collected in 30 ml falcon tubes using a sterile spatula. Samples were thoroughly mixed and homogenized before DNA extraction (*Fig. 1*). DNA was extracted from collected samples by using QIAGEN DNeasy PowerSoil kit (QIAGEN Inc., MD, USA) as per manufacturer's instruction. Extracted DNA was stored at -20 °C until the amplification was performed. All the experiments in our study were performed at Environmental Microbiology Laboratories, Department of Microbiology, Quaid-i-Azam University, Islamabad. The concentration of DNA from each sample was normalized to 10 ng/µl before using the DNA for qPCR reactions.

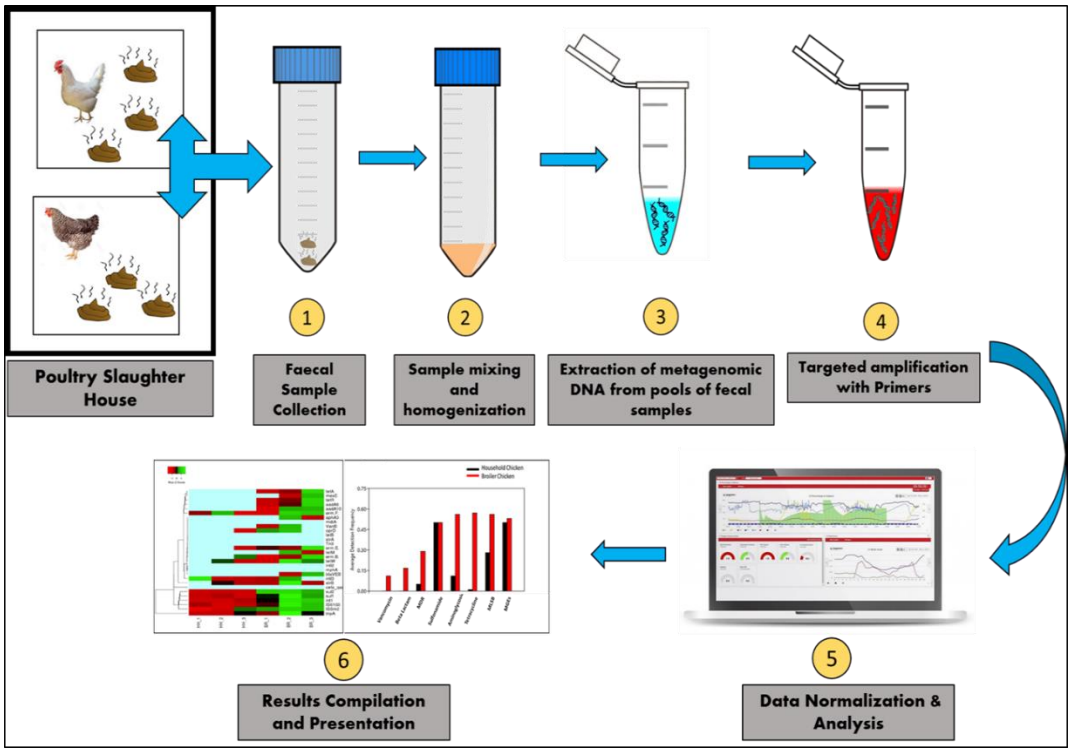

Figure 1. *Diagrammatic flowchart of the whole experimental design*

Primers and qPCR

A total of 52 primers were used in this study for the evaluation of ARGs and MGEs abundance in fecal samples. The primers used in our study are a subset of qPCR Primer set 2.0 (Stedtfeld et al., 2018). The sequences of the qPCR primers can be found in the *Appendix* (*Table A1*). The primers were designed, validated and assayed as described in previous studies (Johnson et al., 2016; Karkman et al., 2016; Stedtfeld et al., 2017, 2018). The design protocol and parameters of each primer set are to target sequence diversity within a gene to analyse the environmental resistome. The array covered seven

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major antibiotic classes including MDR (7 genes), tetracyclines (7 genes), aminoglycosides (6 genes) beta-lactams (6 genes), macrolide-lincosamide-streptogramin B (MLSB) (6 genes), Vancomycin (6 genes) sulfonamides (3 genes) involved in different resistance mechanisms (*Fig. 2*).

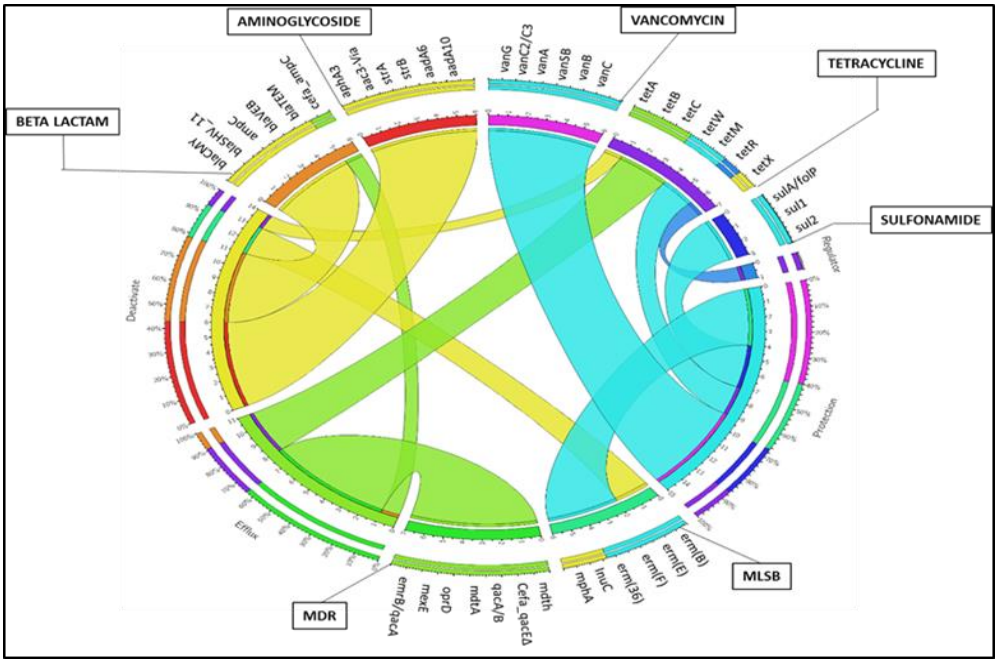

Figure 2. *Circos plot showing the antibiotic resistance class and mode of resistance mechanism of all 41 ARGs included in our array. The plot is made by using Cir-cos software (Krzywinski et al., 2009)*

A real-time qPCR thermocycler (Mastercycler® ep *realplex;* Eppendorf) was used to perform DNA amplification in 25 µl reaction volume. The contents used in qPCR are as follows: 2x Power SYBR Green PCR Master Mix (12.5 µl), 10 µm primer mix (1.25 µl), sample DNA (1 µl), and water (10.25 µl). Cycling conditions for qPCR reactions were as follow: an initial activation step at 95 °C for 10 min followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. Gene copy number was estimated from the Cycle threshold (Ct) values based on the method previously described (Looft et al., 2012). A Ct value of 29 was used as the cut-off limit. All sample-primer assays were performed in triplicates. A negative control without sample DNA was also included for every assay. Genes detected from at least two out of three technical replicates were considered as true positive and all false positive results were removed. Overall a total of 1248 qPCR reactions were performed during the whole study.

Data analysis

Relative abundance was calculated by dividing the estimated gene copy number of the targeted gene with the gene copy number of 16S rRNA. A multivariate principal component analysis (PCA) of ARG profiles between sample types was performed in Paleontological Statistics Software Package for Education and Data Analysis (PAST: v3.20) (Hammer et al., 2001). The scale of the principal components was transformed into eigenvalues. Basic data processing of qPCR results was performed on Microsoft

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Excel 2010. A student t-test was applied to the sum of total ARGs and MGEs abundance in order to test the significant differences between two different types of chicken. Shannon diversity calculations and scatter plots were also made in PAST v3.20 using percent relative abundance of genes in order to see the diversity of the ARGs in different samples. A heat map based on the natural log-transformed values of the relative abundance of ARGs and MGEs was made by using heat mapper software (Babicki et al., 2016) to evaluate the presence of individual genes in fecal samples. Complete linkage clustering method and Euclidean distance measurement method was used to cluster the rows representing genes in the heat map (Yeung et al., 2003).

Results

Detection and diversity of ARGs and MGEs

For evaluating the ARGs of 6 fecal samples from 2 different types of chicken (broiler vs. household), a qPCR sub-array of 52 primer sets targeting 16S rRNA gene, antibiotic resistance genes (ARGs) and transposase genes related to horizontal gene transfer (HGT) were used in the present study (*Fig. 2*). Out of 52 targeted genes, a total of 22 ARGs were detected in the fecal samples. The detected ARGs represented every targeted class of antibiotics (*Fig. 3*). In addition to ARGs 7 MGEs were also detected in both groups. The detected MGEs belonged to insertion sequences, transposase, and integrase groups are believed to play a role in HGT. Out of 29 detected ARGs and MGEs 8 genes were detected all (6/6) samples while 6 genes were detected in only 1 out of 6 samples. Two genes conferring resistance to aminoglycosides (*aadA6*, *aadA10*) and two tetracycline resistant genes (*tetA*, *tetR*) were absent in all three samples from household chicken but were detected in all three samples from broiler chicken. Overall more genes were detected in broiler chicken samples as compared to household chicken samples.

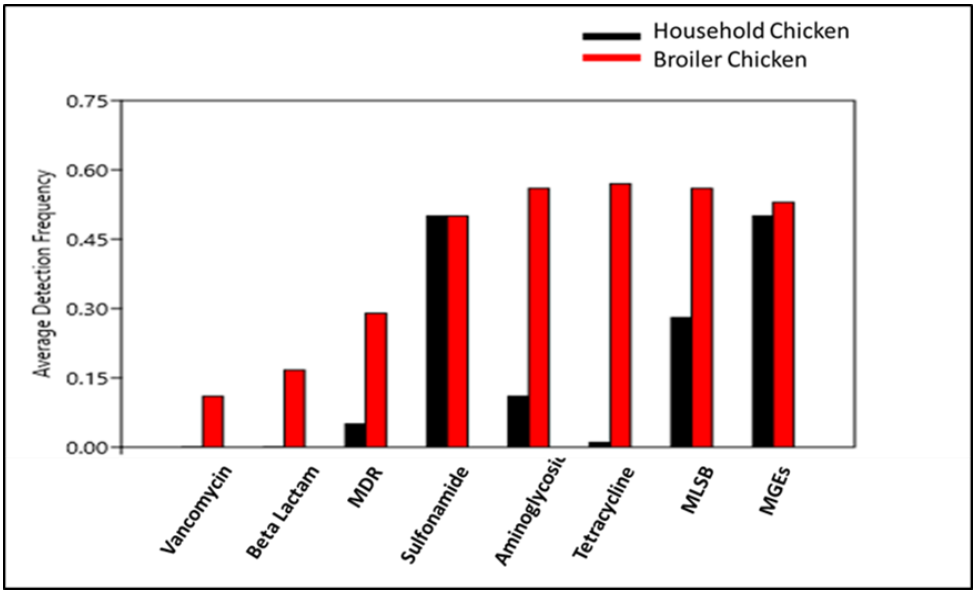

Figure 3. *Antibiotic resistance class wise average detection frequency. Average detection frequency is an average number of samples in which genes within a class is detected divided by total number of samples against which the genes are tested for one or more genes conferring resistance to that AR class*

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The number of detected genes ranged from 8 to 13 in fecal samples from household chicken whereas the genes detected in fecal samples of broiler chicken ranged from 18 to 24. Shannon diversity indicating the richness and abundance of genes in the samples was calculated by taking an average of the relative abundance of the three technical replicates for each of the 6 samples. Shannon diversity of the broiler chicken samples was found to be higher than the household chicken (*Fig. 4*).

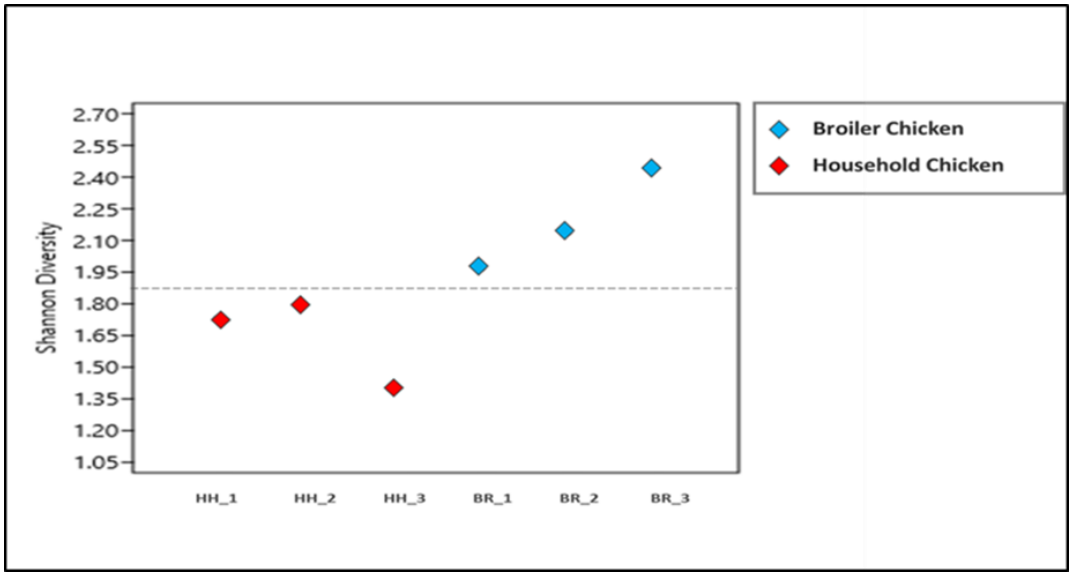

Figure 4. *Scatter plot showing the Shannon diversity of the ARGs and MGEs in 6 fecal samples*

The abundance of the ARGs and MGEs

To minimize the sampling variations, the estimated gene copy number of ARGs and MGEs was normalized to 16S rRNA gene copy number to achieve the relative abundance. The relative abundance of different genes varied significantly among the samples (*Fig. 5a*). The most abundant gene was *tnpA*, a transposase from IS-6 family detected in all 6 samples with percent relative abundance ranging from 0.52 to 4.68 whereas the least abundant gene was an ARG *vanB* from vancomycin group, the only gene detected from this group, ranged from 2.4×10^{-3} to 1.3×10^{-2}. Overall the summed relative abundance of ARGs ($p < 0.05$) and MGEs was higher in broiler chicken fecal samples than in broiler chicken samples (*Fig. 5b*).

Gene cluster patterns were observed by using ordination analysis. Relative abundances of the ARGs were used to perform multivariate PCA analysis in order to assess the distribution of ARGs among two chicken types (broiler vs. household). The PCA plot has revealed that the ARGs profile of fecal samples from two chicken groups is different from each other (*Fig. 6*).

Correlations among different gene classes were also evaluated in our study. Significant correlations were observed between total abundances of aminoglycosides, sulphonamides and tetracycline with *int1* and/ or total MGEs. The highest coefficient of determination (R^2) values was observed for aminoglycosides with MGEs and int1 (*Table 1*). The abundance of *int1* was also considerably correlated to the total relative abundance of the ARGs based on the linear regression analysis (*Fig. 7*).

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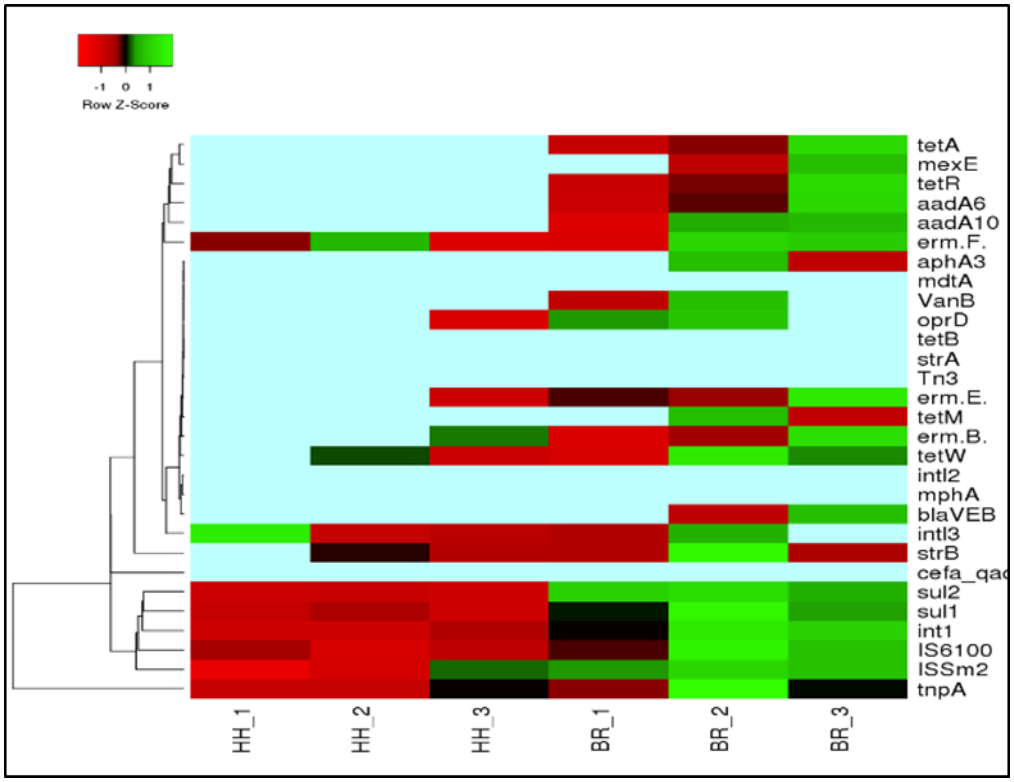

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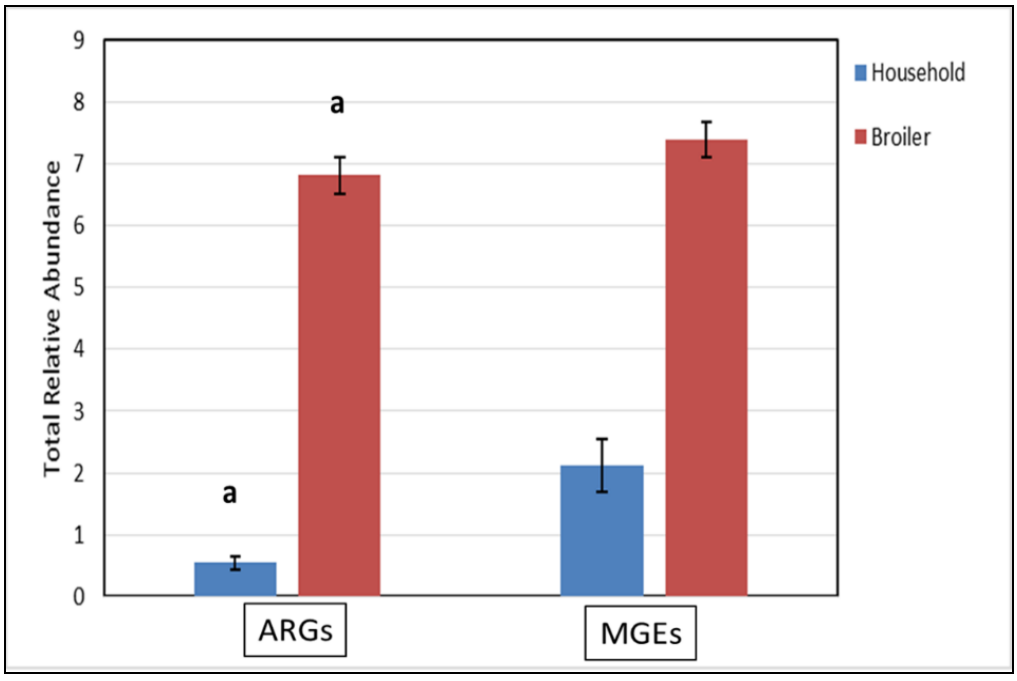

B

Figure 5. a *Heat Map showing the ARGs and MGEs detected in 6 fecal samples (3 technical replicates each). Relative abundance (log transformed) was used for the formation of the heat map. Complete linkage clustering is showing that the sul genes are clustered with many MGEs including int1.* ***b*** *Sum of percent relative abundances of ARGs and MGEs in two types of chicken measured by 6 pools of fecal samples (3 technical replicates each). Letter (a) shows that the difference is significant at 95% confidence of interval. Error bars are showing the standard error of the calculated mean*

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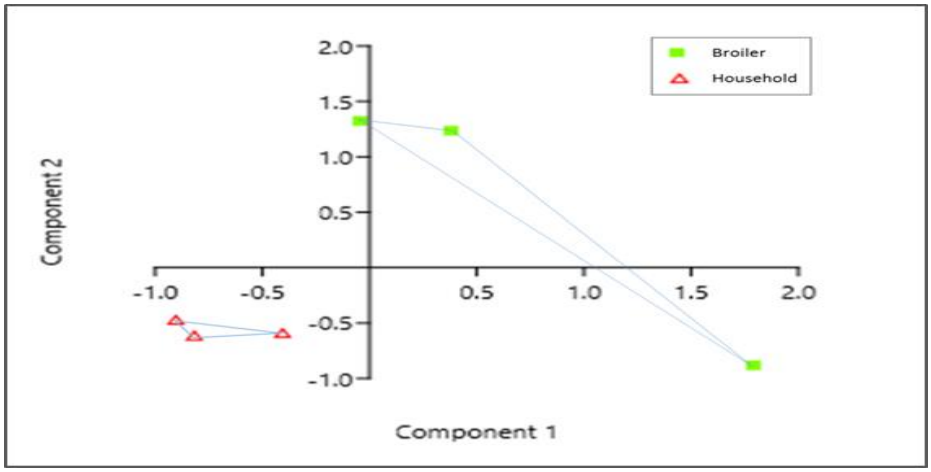

Figure 6. *Ordination of ARGs with 6 fecal samples from two different chicken types. Red triangles represented fecal samples of household chickens. Green squares represented fecal samples from broiler chickens*

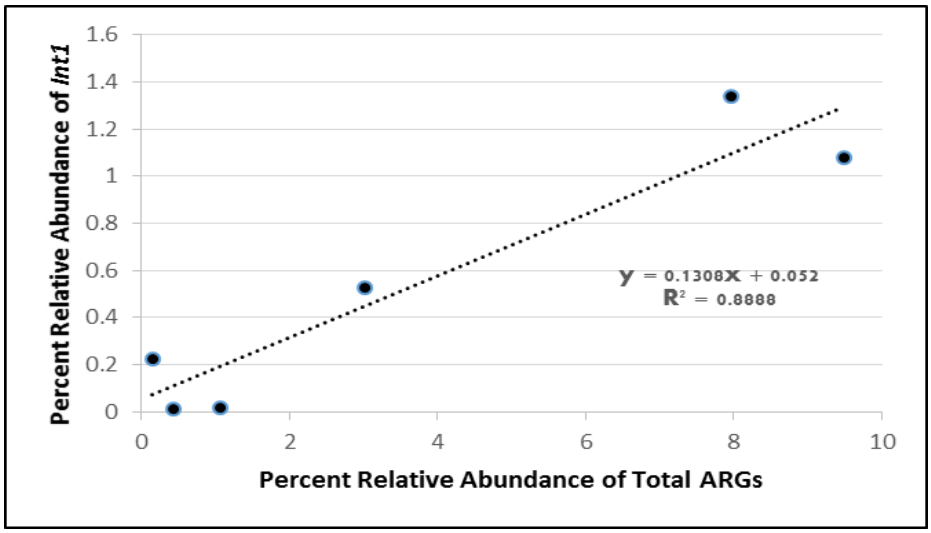

Figure 7. *Linear regression curve is showing a significant correlation between int1 and total ARGs abundances in 6 samples*

Table 1. *Coefficient of determination R^2 values between different gene classes*

Sr. no.	Gene class 1	Gene class 2	R^2 value
1	Total ARGs	Total MGEs	0.67
2	Aminoglycoside	int1	0.93
3	Aminoglycoside	MGEs	0.88
4	Sulfonamide	Int1	0.85
5	Sulfonamide	MGEs	0.78
6	Tetracycline	int1	0.53
7	Tetracycline	MGEs	0.29
8	MDR	int1	0.26
9	MDR	MGEs	0.08
10	MLSB	Int1	0.4
11	MLSB	MGEs	0.25

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Discussion

A large body of literature has provided information regarding the threats of antimicrobial resistance using conventional methods (Álvarez-Fernández et al., 2013; Khalili et al., 2012). In recent years, dissemination of ARGs via environmental routes has gained much attention. Characterization of mobile genetic elements along with other genes is becoming essential in antimicrobial resistance research (Waseem et al., 2017b). In our study, we have opted a relatively broader and comprehensive approach of targeting multiple genes (ARGs and MGEs) in the gut metagenomes of broiler and household chickens (*Gallus gallus domesticus*). Our data is useful for assessing the role of small scale poultry slaughterhouses, which are widely distributed throughout the country, in ARGs dissemination. Most of the conventional small scale slaughterhouses have improper blood and wastewater drainage system (Sohaib and Jamil, 2017) which further intensifies the threat of ARGs dissemination into the environment.

Fecal samples from broiler chickens, which get routine antimicrobial treatments, were found to have higher abundance and diversity of ARGs compared to the household chickens which had been on organic antibiotic-free feed. Our results are in accordance with many different studies where the resistance abundance or detection frequency of resistant genes was significantly higher in antibiotics treated chickens, pigs and cattle as compared to the controls (Cameron and McAllister, 2016; Guo et al., 2018; Looft et al., 2012). Our findings demonstrated that the detection frequency of MGEs was almost equal in two sample types but the total percent relative abundance was higher in broiler chicken. Similar results of elevated MGEs were also observed in small scale production birds as compared to household birds during a study of small scale production poultry operations in rural Ecuador (Moser et al., 2018). This signifies that apart from the selection of ARGs under the selection pressure of antibiotics, higher abundance of MGEs could also be a critical factor in the transfer of genes within chromosomes, across bacteria and even in between different species (Stokes and Gillings, 2011).

The most frequently detected ARGs (at least 5/6 samples) in our study were *sul1, sul2, ermF, strB, tetW.* The presence of ARGs in household chicken type is not surprising. The same genes were also detected in *Gallus gallus domesticus* feces and many other environmental matrices without antibiotic treatment (Berglund, 2015; Sui et al., 2016; Zhang et al., 2013). This implies some resistance genes could be ubiquitously present in the environment without antibiotic's selection pressure. For example, a diverse intrinsic antibiotic resistome was recently studied in bacteria isolated from a cave (Pawlowski et al., 2016). Such genes can provide a competitive advantage to environmental bacteria harbouring them over others.

Sulfonamide resistance in bacteria usually arises by acquiring sul genes, encoding dihydropteroate synthase that is not inhibited by the antibiotics (Antunes et al., 2005). Sul genes have been most consistent targets in poultry and animal live stocks studies (Kozak et al., 2009; McKinney et al., 2010). Both of these genes were detected in all 6 samples. Considerable differences were found in the relative abundance of these genes within each sample. The difference in the abundance of these closely related genes can be ascribed to the differences in their dissemination mechanism. *Sul2* is normally located on Inc. Q family of plasmids whereas *sul1* is believed to be distributed with the help of integrons (He et al., 2014). Similar observation of different relative abundance of these related genes has been reported in other environmental matrices as well (Cheng et al., 2013; Sui et al., 2016; Zhang et al., 2013). A study had reported the prevalence and distribution of sulfonamide resistance genes in soils around poultry and livestock

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farms in Jiangsu Province, Southeastern China. Distribution frequency of *sul* genes and most frequent combination of *sul*1 and *sul*2 was studied (Wang et al., 2014). Our results are in close agreement with this study as the type of animal and waste can affect the distribution of ARGs in the environment.

Transposon specific gene *tnpA*, integron specific gene *int1* along with two other MGEs have been clustered together with sulphonamide resistant genes (*Fig. 4a*). Transposons and integrons have been investigated in-silico (Loot et al., 2017) and also in environmental matrices (Stokes and Gillings, 2011) to evaluate the possible enrichment of ARGs. *TnpA* association with antibiotic resistance is also highlighted in many studies (Carnelli et al., 2017; Feng et al., 2017). The *int*1 gene is believed to be responsible for the evolution and dissemination of multiple antibiotic resistant bacteria and the spread of mobile genetic elements in the environments (Moura et al., 2012). In our recent publication *int*1 gene as a proxy for environmental surveillance of ARGs has also been reconfirmed (Stedtfeld et al., 2017). An important observation in our present study is the strong correlation among the relative abundance of *int*1 gene and total ARGs. The reason behind this correlation could be co-occurrence of *int*1 and ARGs on a single plasmid and/ or chromosome. Our results are supported by the finding of a recent study about distribution and characterization of class 1 integrons where it was reported that these integrons harboured genes belonging to class aminoglycosides (Sung and Oh, 2014).

*tnp*A and *int*1 genes were two of the most abundant genes in broiler chicken which is in accordance with the results from a study of ARGs analysis in production chicken in rural Ecuador where the relative abundance of these two genes were highest among others (Guo et al., 2018). The clustering of *intI*1 and *tnp*A with ARGs has implied that broiler chickens in small scale slaughterhouses can negatively play their role by spreading ARGs in clinically important strains and thus can produce multiple antibiotic resistant strains which can cause serious public health concerns. For example, Talebiyan and colleagues have isolated 318 multiple resistant *E. coli* from commercial broiler flocks that have been given different doses of antibiotics (Talebiyan et al., 2014). This signifies that the broiler chickens in small scale slaughterhouses can provide an appropriate environment for the proliferation of ARGs through horizontal and/ or vertical gene transfer. Our results about the abundance and detection of ARGs in chicken fecal samples are in accordance with the trends of ARGs abundance observed in other gut microbiomes (Waseem et al., 2019).

Concrete measures should be opted in order to mitigate the threat of antimicrobial resistance. In this regard, strict AMR centric environmental strategies should be implemented on commercial poultry farms so that the use of antibiotics in the poultry industry should be regulated. Small scale poultry slaughterhouses, especially located in clusters, should be encouraged to develop their own waste and water management systems. Use of untreated chicken feces and litter as fertilizer should be discouraged. Apart from formulating and implementing rules and regulations, general public awareness campaigns about antimicrobial stewardship should be launched. This can also increase the demand for organic meat and/ or household chicken thus forcing the poultry farm owners to restrict antimicrobials use as growth promoters.

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Conclusion

Our study has provided an effective assessment and potential insight into the role and potential risk of small scale poultry slaughterhouses with the perspective of antimicrobial resistance. More clinically important ARGs were detected from the samples collected from cages of broiler chicken. Broiler chicken mostly raised in large scale commercial farms are usually located in outskirts of communities. Their transport poses a risk in the introduction of exogenous ARGs in the environment since such small scale poultry slaughterhouses are distributed widely throughout the country. Our research will aid in curbing the dissemination of antimicrobial resistance into the environment from such environmental reservoirs. Further research with larger sample size, more ARG and MGE targets along with bacterial community analysis will be helpful for validating the trends of ARGs spread in the environment.

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APPENDIX

Figure A1. a & b *Collection and transfer of household chicken fecal samples in centrifuge tubes;* ***c & d*** *Collection and transfer of broiler chicken fecal samples in centrifuge tubes*

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Table A1. *Primer sequences of 16S rRNA gene, 41 ARGs and 10 MGEs*

Sr. no.	Targeted gene	Forward sequence	Reverse sequence
1	16S rRNA	GGGTTGCGCTCGTTGC	ATGGYTGTCGTCAGCTCGTG
2	vanG	TGTTTCGCAGAACCGTGTCAA	CCCTGCACTGTTCCATCTTCTC
3	vanC2/vanC3	TGACTGTCGGTGCTTGTGA	GATAGAGCAGCTGAGCTTGTTC
4	vanA	GGGCTGTGAGGTCGGTTG	TTCAGTACAATGCGGCCGTTA
5	vanSB	GAAGATAAAGAGGGAAGCGTACTC	CCGAATTGTCAGCCCTTGATAA
6	VanB	TTGTCGGCGAAGTGGATCA	AGCCTTTTTCCGGCTCGTT
7	vanC	CCTGCCACAATCGATCGTT	CGGCTTCATTCGGCTTGATA
8	blaCMY	AAAGCCTCATGGGTGCATAAA	ATAGCTTTTGTTTGCCAGCATCA
9	cefa_ampc	CAGGATCTGATGTGGGAGAACTA	TCGGGAACCATTTGTTGGC
10	blaSHV-11	TTGACCGCTGGGAAACGG	TCCGGTCTTATCGGCGATAAAC
11	ampC	CTGGCGCATACCTGGATTAC	GCCAGTTCAGCATCTCCCA
12	blaVEB	CCCGATGCAAAGCGTTATG	GAAAGATTCCCTTTATCTATCTCAGACAA
13	blaTEM	CGCCGCATACACTATTCTCAG	GCTTCATTCAGCTCCGGTTC
14	mdth	ATGCTGGCTGTACAAGTGATG	CACTCCAGCGGGCGATA
15	cefa_qacEdelta	TAGTTGGCGAAGTAATCGCAAC	TGCGATGCCATAACCGATTATG
16	qacA/B	AAGGGCCACTGCATTAGCTG	CCAGTCCAATCATGCCTGCA
17	mdtA	ACAAGCCCAGGGCCAAC	CCTTAATGGTGCCTTCGGTTTC
18	oprD	ATGAAGTGGAGCGCCATTG	GGCCACGGCGAACTGA
19	mexE	GGTCAGCACCGACAAGGTCTAC	AGCTCGACGTACTTGAGGAACAC
20	sulA/folP	CAGGCTCGTAAATTGATAGCAGAAG	CTTTCCTTGCGAATCGCTTT
21	sul2	TCATCTGCCAAACTCGTCGTTA	GTCAAAGAACGCCGCAATGT
22	sul1 NEW	GCCGATGAGATCAGACGTATTG	CGCATAGCGCTGGGTTTC
23	aphA3	AAAAGCCCGAAGAGGAACTTG	CATCTTTCACAAAGATGTTGCTGTCT
24	aac3-Via	GTGTCCGTCGCCAAGGA	GGTGACGGCCTTGTCGA
25	strA	CCGGTGGCATTTGAGAAAAA	GTGGCTCAACCTGCGAAAAG
26	strB	GCTCGGTCGTGAGAACAATCT	CAATTTCGGTCGCCTGGTAGT
27	aadA6	CCATCGAGCGTCATCTGGAA	CCCGTCTGGCCGGATAAC
28	aadA10	ACAGGCACTCAACGTCATCG	CGCGGAGAACTCTGCTTTGA
29	tetA	CTCACCAGCCTGACCTCGAT	CACGTTGTTATAGAAGCCGCATAG
30	tetB	AGTGCGCTTTGGATGCTGTA	AGCCCCAGTAGCTCCTGTGA
31	tetW	ATGAACATTCCCACCGTTATCTTT	ATATCGGCGGAGAGCTTATCC
32	tetX	AAATTTGTTACCGACACGGAAGTT	CATAGCTGAAAAAATCCAGGACAGTT
33	tetC	ACTGGTAAGGTAAACGCCATTGTC	ATGCATAAACCAGCCATTGAGTAAG
34	tetR	CCGTCAATGCGCTGATGAC	GCCAATCCATCGACAATCACC
35	tetM	GGAGCGATTACAGAATTAGGAAGC	TCCATATGTCCTGGCGTGTC
36	lnuC	GGGTGTAGATGCTCTTCTTGGA	CTTTACCCGAAAGAGTTTCTACCG
37	emrB/qacA	CTTTTCTCTAACCGTACATTATCTACGATAAA	AGAACGTAGCGACTGATAAAATGCT
38	erm(B)	GAACACTAGGGTTGTTCTTGCA	CTGGAACATCTGTGGTATGGC
39	erm(E)	GTCACGCAGCTGGAGTTCG	CGGTGAAGCACAGCTCGAC
40	mphA	TCAGCGGGATGATCGACTG	GAGGGCGTAGAGGGCGTA
41	erm(F)	TCTGATGCCCGAAATGTTCAAG	TGAAGGACAATTGAACCTCCCA
42	erm(36)	GGCGGACCGACTTGCAT	TCTGCGTTGACGACGGTTAC
43	intl3	CAGGTGCTGGGCATGGA	CCTGGGCAGCATCACCA
44	ISCR1	ATGGTTTCATGCGGGTT	CTGAGGGTGTGAGCGAG
45	Tn3	GCTGAGGTGTTCAGCTACATCC	GCTGAGGTAGTCACAGGCATTC
46	IS6/257	ATATCGTGCCATTGATGCAGAG	ACCATTGCTACCTTCGTTGAAG
47	tnpA	CCGATCACGGAAAGCTCAAG	GGCTCGCATGACTTCGAATC
48	int1	CGAAGTCGAGGCATTTCTGTC	GCCTTCCAGAAAACCGAGGA
49	intl2	TGCTTTTCCCACCCTTACC	GACGGCTACCCTCTGTTATCTC
50	ISPps1	CACACTGCAAAAACGCATCCT	TGTCTTTGGCGTCACAGTTCTC
51	ISSm2	TGGATCGACCGGTTCCAT	GCTGACCGAGCTGTCCATGT
52	IS1111	GTCTTAAGGTGGGCTGCGTG	CCCCGAATCTCATTGATCAGC

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