



RESEARCH ARTICLE

Molecular Screening of Resistant and Virulent Genes in *Salmonella* Enteritidis and *Salmonella* Typhimurium from Poultry in Khyber Pakhtunkhwa

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ABSTRACT

The current study was conducted to investigate the prevalence of *Salmonella* and its dominant serovars, *S. Enteritidis* and *S. Typhimurium* by phenotypic and molecular characterization in poultry. We reported the antimicrobial resistance profile, biofilm formation and presence of resistant and virulent genes of *S. Enteritidis* and *S. Typhimurium* of poultry origin in Peshawar. A total of 310 liver samples were collected from morbid and dead birds and processed both by direct plating and selective enrichment methods. Over all prevalence of *Salmonella* was found to be 21.6% in poultry liver. PCR based molecular screening of *Salmonella* isolates through serovar specific genes revealed that *S. Enteritidis* (74.11%) and *S. Typhimurium* (18.8%) were the most prevalent serovars. *Salmonella* isolates were found highly resistant to Tetracycline, Oxytetracycline, Nalidixic acid, Doxycycline, Sulfamethoxazole and Erythromycin. While susceptibility of *Salmonella* isolates was noted against Ceftriaxone, Cefotaxime, Meropenem, Amikacin, Norfloxacin and Ceftazidime. The prevalence of MDR strains were found to be 93.3% of the total isolates while 14.4% isolates were ESBLs producers. The most prevalent resistant genes in *Salmonella* were *sul1* (98.8%), *sul2* (97.6%), *gyrA* (95.2%), *strB* and *CTX-M* (80%). Four virulent genes i.e. *SpaN*, *CsgA*, *SipA* and *MsgA* were present in more than 80% of isolated strains of *Salmonella*. Hence, it was concluded that poultry birds in the Peshawar region are carrier of highly resistant and virulent *S. Enteritidis* and *S. Typhimurium* strains and are a potential public health risk. A routine surveillance and a regulated antimicrobial application are required for control and prevention of the current situation in the poultry.

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INTRODUCTION

Salmonella is an important food-borne pathogenic genus of the family *Enterobacteriaceae*. The genus *Salmonella* contains two species i.e. *S. enterica* and *S. bongori* with more than 2600 serovars causing

gastroenteritis, typhoid and sepsis (Mezal *et al.*, 2014). These serovars can be classified into two broad categories i.e. typhoidal and non-typhoidal *Salmonella* (Wang *et al.*, 2019). *S. Typhi*, *S. Paratyphi A*, *S. Paratyphi B* and *S. Paratyphi C* are included in typhoidal group while the rest of the serovars are classified as non-typhoidal including *S.*

Typhimurium, *S. Enteritidis*, *S. Gallinarum*, *S. Pullorum*, *S. Newport* and *S. Hader* (Smith, Seriki, and Ajayi, 2016). Food items like fruits, vegetables, pork, beef and poultry meat play crucial role in the transmission of *Salmonella* to human. Poultry is the significant reservoir for certain serovars such as *S. Typhimurium* and *S. Enteritidis* which can cause gastroenteritis in human. According to recent studies, *S. Typhimurium* and *S. Enteritidis* are the most prevalent serovar isolated from poultry meat and eggs, respectively (Freitas Neto *et al.*, 2010).

Due to excessive and persistent use of antibiotics in poultry industry resulted in the emergence of MDR (Multidrug-Resistance) and XDR (Extensively Drug Resistant) strains of *Salmonella* (Marshall and Levy, 2011). According to Food and Drug Administration, antimicrobial resistance is considered as a global threat. Resistance to antibiotics in non-typhoidal *Salmonella* was reported about four decades ago when MDR *S. Typhimurium* emerged in the United Kingdom. It was named as ACSSuT because it displayed resistance to Ampicillin, Chloramphenicol, Streptomycin, Sulfamethoxazole and Tetracycline. This resistant phenotype was followed by many resistance patterns in which *Salmonella* strains were resistant to two or more antibiotics of therapeutic importance (Wang *et al.*, 2019). In Pakistan, little is known about data regarding the prevalence, antibiotic resistance status, virulence and clonal relatedness of prevalent *Salmonella* strains in poultry sector. Moreover, there is no control and regulation on the use of antibiotics in the poultry industry that have accelerated the emergence and spread of antibiotic resistant genes in different serovars of non-typhoidal *Salmonella*. Hence, the current study was designed to investigate the prevalence and resistance of *Salmonella* in the local poultry population in the Peshawar region.

MATERIALS AND METHODS

The present study was conducted at Genomic Lab and Center of Microbiology and Biotechnology (CMB), Veterinary Research Institute (VRI), Peshawar from November 2018 to May 2019. A total of 310 liver samples were collected from morbid and dead broiler birds. Sample size was determined by using the formula:

$$N = 1.96^2 P_{\text{exp}} (1 - P_{\text{exp}}) / d^2$$

Where N= sample size, P_{exp} expected prevalence, d= desirable absolute precision.

Two methods were adopted for isolation of *Salmonella* species from poultry hepatic tissues: 1) direct plating and 2) selective enrichment. Selective enrichment was done according to ISO-6579-I guidelines. The suspected black-colored colonies were subjected to further biochemical tests including Oxidase, Catalase, Citrate utilization test and Triple Sugar Iron test.

Antibiotic susceptibility testing of *Salmonella* isolates: Antimicrobial susceptibility testing of *Salmonella* isolates were performed by Kirby-Bauer disk diffusion method on Muller Hinton Agar (MHA) plates by using commercial

antibiotic discs (Oxoid[®], n=17) according to the guideline of Clinical & Laboratory Standards Institute (CLSI). Briefly, a single isolated colony of *Salmonella* was emulsified in sterile water and compared with 0.5 McFarland standard. A lawn was made with sterile cotton swabs over the surface of MHA plate and left for few minutes to dry. Antibiotics discs were then applied and incubated at 37°C for 18 hours. The isolates were grouped into sensitive, intermediate and resistant according to guidelines of CLSI.

Biofilm formation assay: Biofilm forming assay was performed in 96 well plate according to standard protocol. Biofilm formation was classified into categories based upon the ODs reading (Stepanović *et al.*, 2000). Sterile TSB was used as a negative control.

DNA extraction: DNA was extracted from all isolates using TE freeze thaw method. Briefly, a single colony was inoculated in Eppendorf tubes containing 1ml BHI (Oxoid, CM1135) broth and incubated at 37°C for 24 hours. The tubes were centrifuged at 14×10^3 rpm for 10 min. The supernatant was discarded without disturbing the pellet. The pellet was dissolved in 200µl TE buffer. The tubes were placed in the water bath for 5 min at 100°C. The tubes were then immediately placed in -80°C for 3 minutes. This freeze thaw cycle was repeated three times. Finally, the tubes were centrifuged at maximum speed for 15 minutes. The supernatant was used for molecular analysis of the isolates. The quantification of DNA was done using Nanodrop (Titertek Berthold[®]).

PCR screening using specific primers: The isolates of *Salmonella* were confirmed on molecular basis using PCR. A highly conserved Type III secretion system gene *invA* was targeted for genus confirmation. *S. Typhimurium* and *S. Enteritidis* were screened individually in two separate PCR reactions by targeting serovars specific genes i.e. *rfbJ*, *fliC*, *fljB* for *S. Typhimurium* and *ST11*, *SPV*, *SefA* for *S. Enteritidis* as mentioned in Table 1. The amplified PCR products were analyzed by 1.5% agarose gel electrophoresis, stained with Ethidium Bromide and visualized under gel documentation system (Fas-Digi[®]).

PCR detection of resistant and virulent genes: *Salmonella* Enteritidis and *S. Typhimurium* isolates were screened for the presence of resistant genes. A total of nineteen resistance genes i.e. *TEM*, *CTX-M*, *OXA*, *tetA*, *tetB*, *sul1*, *sul2*, *gyrA*, *gyrB*, *ParC* and *ParE*, *Cat1*, *Cat2*, *strA*, *strB*, *aadA1*, *QnrB* and *QnrS*; along with four virulent genes i.e. *msgA*, *csgA*, *spaN* and *sipA* were screened in *S. Enteritidis* and *S. Typhimurium* isolates through PCR reaction. The oligonucleotide sequences are mentioned in Table 1. The PCR products were then analyzed by 1.5% agarose gel electrophoresis and visualized by gel documentation system (Fas-Digi[®]).

Statistical analysis: The obtained data was analyzed through computer software SPSS version 21.0. Student t-test was applied for determining variation between groups. A *p*-value less than 0.05 was considered as statistically significant.

RESULTS

Isolation and biochemical identification: Out of 310 liver samples, 24 (7.74%) were positive for *Salmonella* through direct plating while 67 (21.6%) samples were found positive by selective enrichment. These two prevalence values were significantly different ($P < 0.05$). A total of 90 isolates were recovered from positive liver samples. The *Salmonella* isolates resulted in straw colored or colorless colonies with black centers on SS agar and red colored colonies with black centers on XLD agar. All *Salmonella* isolates were positive for catalase test, negative for Oxidase test and Citrate test. *Salmonella* isolates resulted in H_2S production in TSI agar slants and no gas production in the butt region. The slants were alkaline.

Antimicrobial susceptibility testing: The antimicrobial susceptibility testing was performed using Kirby-Bauer disk diffusion method. The *Salmonella* strains were interpreted as sensitive, intermediate and resistant based upon the formation of zone of inhibition using Clinical and Laboratory Standard institute (CLSI). The *Salmonella* Isolates were found to be highly resistant to the commonly used antibiotics in poultry industry. Highest resistance was noted against Tetracycline (90%), Oxytetracycline (94.4%), Sulfamethoxazole (84.4%), Nalidixic acid (77.7%), Doxycycline (77.7%) and Ciprofloxacin (47.7%). All *Salmonella* isolates were found susceptible to Amikacin, while 96.67% were found susceptible to Meropenem. The isolates that were resistant to at least three antibiotics belonging to different classes or subclasses were considered MDR. We found MDR prevalence in 93.3% of the total *Salmonella* isolates in our collected samples. The *Salmonella* isolates having 5mm difference between zones of inhibition of Cefotaxime and Cefotaxime/Clavulanic acid were considered ESBL (Extended Spectrum Beta-lactamases) positive and these were found to be 14.4% of the total *Salmonella* isolates. At least 36 different antibiotic resistance phenotypic profiles were observed in all *Salmonella* isolates as presented in Table 3. Antibiotic sensitivity data is presented in Fig. 1 and Table 2.

Capacity of biofilm formation: Biofilm forming capacity was assessed through 96 well microtiter plate assay. *Salmonella* isolates were categorized into non-adherent, weak, moderate and strong biofilm producers. The strains isolated from the collected liver samples had a strong tendency towards biofilm formation. Strong and moderate biofilm producers were 52.94 and 39.02% respectively. Only 7.31% isolates were weak producers, while 1.21% isolates were non-adherent. An overall, 98.79% isolates were biofilm producers. The data is presented in Fig. 2.

Molecular analysis: PCR screening of 90 biochemically confirmed *Salmonella* isolates revealed that only 85 (94.4%) isolates were positive for *invA* gene and were then used for further molecular analysis. The isolates were screened for *S. Typhimurium* and *S. Enteritidis* specific genes in two separate multiplex PCR reactions. Out of selected 85 isolates, 63 (74.11%) were identified to be *S.*

Enteritidis while 16 (18.8%) were *S. Typhimurium*. While only six isolates were not identified through multiplex PCR. Figure 3 represents gel electrophoresis of *invA* gene in *Salmonella* isolates while Figure 4 represents gel electrophoresis of *S. Enteritidis* specific genes i.e. *ST11*, *sefA* and *SPV*. All *S. Typhimurium* isolates were positive for *sul1* and *sul2* genes; whereas, 98.4% of *S. Enteritidis* isolates were positive for *sul1* and 95.2% were positive for *sul2* gene. Among Tetracycline resistant genes, *tetA* was detected in only 90.4% of *S. Enteritidis* isolates while *tetB* was detected in 63.4% isolates. *S. Typhimurium* isolates carried 87.5% and 50% of *tetA* and *tetB* respectively. Among beta-lactamases, *CTX-M* was the most prevalent followed by *OXA* and *TEM*. *CTX-M* was detected in 79.3% of *S. Enteritidis* and 75% of *S. Typhimurium* isolates. The prevalence of *TEM* was high in *S. Enteritidis* (28.5%) as compared to *S. Typhimurium* (6.2%). Chloramphenicol resistance genes i.e. *cat1* and *cat2* were found in 74.6% and 71.4% isolates of *S. Enteritidis* respectively. The prevalence of *cat1* and *cat2* was found less in *S. Typhimurium* as compared to *S. Enteritidis*. Among aminoglycoside resistance genes, *strA*, *strB* and *aadA1* was detected in 22.2%, 93.6% and 54.4% isolates of *S. Enteritidis* respectively. Efflux pumps i.e. *QnrS* and *QnrB* were positively amplified in 54% and 47.6% of *S. Enteritidis* isolates respectively. Prevalence of *QnrS* and *QnrB* was slightly higher as 62.5% and 56.2%, respectively compared to *S. Enteritidis*. *Class 1 integron* was detected in 23.8% of *S. Enteritidis* isolates while 6.25% isolates of *S. Typhimurium* were positive for *Class 1 integron*. The prevalence of these resistant genes of *S. Enteritidis* and *S. Typhimurium* is graphically presented in Figure 8. The figure 5, 6 and 7 represents gel electrophoresis of *TEM*, *gyrB*, *QnrS*, *tetB*, *cat1* and *cat2* respectively. The resistant genes *csgA*, *msgA*, *sipA*, and *spaN* were detected in more than 80% of *Salmonella* isolates. The *spaN* was amplified in 90.5% isolates. *CsgA* was second most prevalent gene which was present in 87.05% isolates. The *msgA* and *sipA* were detected in 82.3% and 80% *Salmonella* isolates respectively.

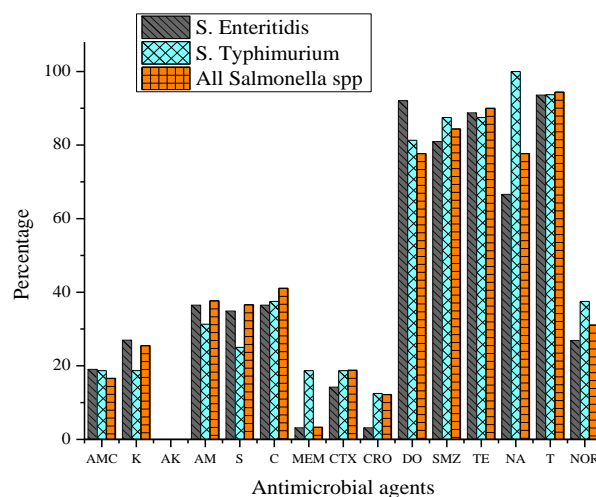


Fig. 1: Antibiotic susceptibility of *Salmonella* isolates for various antimicrobial agents (AMC=Amoxicillin/Clavulanic acid, K=Kanamycin, AK=Amikacin, AM=Ampicillin, S=Streptomycin, C=Chloramphenicol, MEM=Meropenem, CTX=Cefotaxime, CRO=Ceftriaxone, DO=Doxycycline, SMZ=Sulfamethoxazole, TE=Tetracycline, NA=Nalidixic acid, T=Oxytetracycline, NOR=Norfloxacin).

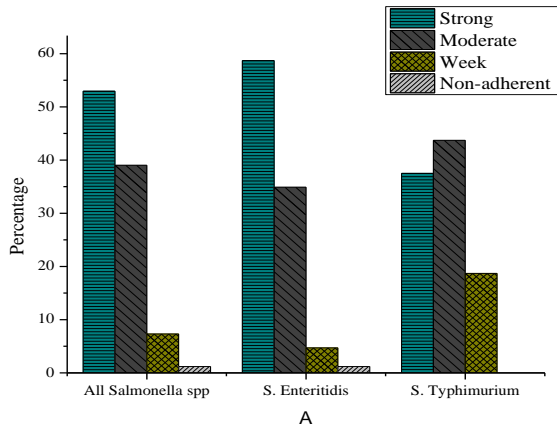


Fig. 2: Tendency of biofilm formation of *Salmonella* Enteritidis, *Salmonella* Typhimurium and all *Salmonella* isolates.

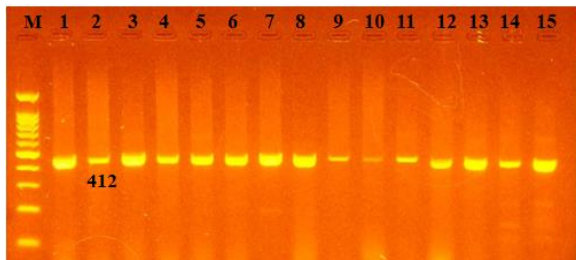


Fig. 3: Migration of PCR product of *invA* gene of various samples by Agarose gel electrophoresis. M represents 100bp DNA ladder. Lane 2 represents migration of PCR amplified product (412 bp) of *invA* gene of various samples.

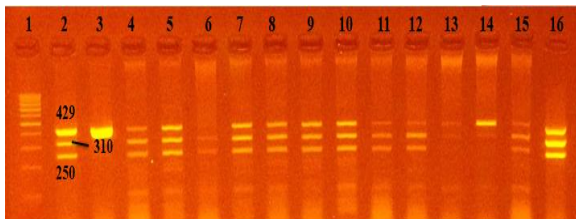


Fig. 4: Agarose gel electrophoresis of *S. Enteritidis* and *S. Typhimurium* specific genes. Lane 1 represents 100bp ladder. Lanes 2-16 show the amplified products of *ST11* (429bp), *SefA* (310bp) and *SPV* (250bp).

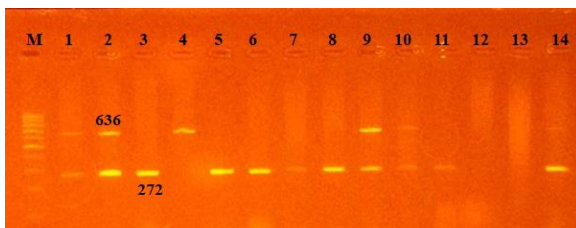


Fig. 5: Agarose gel electrophoresis of *TEM* and *gyrB*. Lane M represents 100bp ladder. Lanes 2 and 3 show the amplified products of *TEM* (636bp) and *gyrB* (272bp) respectively.

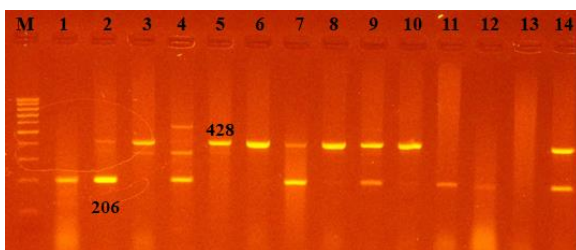


Fig. 6: Agarose gel electrophoresis of *QnrS* and *tetB* genes. Lane M represents 100 bp ladder. Lanes 2 and 5 show the amplified products of *tetB* (206bp) and *QnrS* (428bp) respectively.

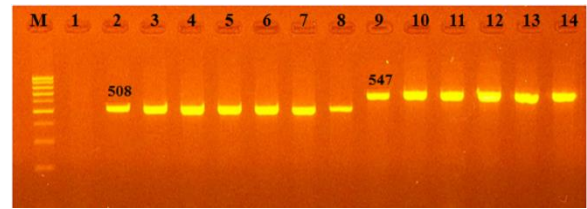


Fig. 7: Agarose gel electrophoresis of *catI* and *cat2* genes. Lane M represents 100bp ladder. Lanes 2 and 9 show the amplified products of *catI* (508bp) and *cat2* (508bp) respectively.

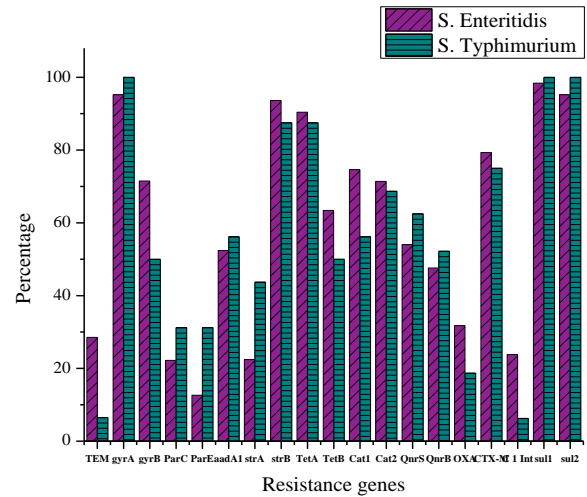


Fig. 8: Prevalence of various resistant genes of *S. Enteritidis* and *S. Typhimurium*.

DISCUSSION

Food-borne salmonellosis is a global problem resulting in 93 million illnesses and 155,000 deaths annually worldwide (Balasubramanian *et al.*, 2019). Moreover, it is estimated that food-related salmonellosis causes 1 million illnesses and 400 deaths per year in USA (Chiller, 2019). The problem causes fatality in children of age less than 4 years by *S. Typhimurium* and *S. Enteritidis* (Chlebicz and Śliżewska, 2018). Food products of animal origin especially poultry are deemed to be the main mode of transmission of salmonellosis to human. *S. Enteritidis* and *S. Typhimurium* are the two main serovars implicated in majority of food-borne salmonellosis. The prevalence and characterization of *S. Enteritidis* and *S. Typhimurium* has rarely been investigated in Peshawar and to the best of our knowledge, this is the first study on estimation of prevalence and molecular characterization of dominant *Salmonella* serovars in poultry in Khyber Pakhtunkhwa region, Pakistan.

In the present study, the prevalence of *Salmonella* in poultry liver was found to be 21.6% in Peshawar region. Previously, a similar study was conducted in Kohat wherein the authors found 26.6% prevalence of *Salmonella* in poultry (Asif *et al.*, 2017). Antibiotic resistance is ever since increasing in non-typhoidal *Salmonella* due to increased use of antimicrobials in broiler industry for a better growth performance and prevention of infections (Mehdi *et al.*, 2018). Due to this excessive use of antibiotics, the *Salmonella* isolates are found to be highly resistant to routinely used antibiotics i.e. Oxytetracycline (96.6%), Tetracycline (90%), Sulfamethoxazole (84.4%), Doxycycline (77.7%), Ciprofloxacin (47.7%) and Chloramphenicol (41.1%).

Table 1: List of primers for the detection of various *Salmonella* genes

Gene	Oligonucleotide Sequence	AT	Size	Reference
<i>InvA</i>	GCTGGTTTTAGGTTGGCGG CAAAGGTGACGCTATTGCCG	65°C	412	This study
<i>rflB</i>	CCAGCACCAGTTCCAATTGATAC GGCTTCCGGCTTTATTGGTAAGCA	65°C	663	(Moosavy <i>et al.</i> , 2015)
<i>fliC</i>	ATAGCCATCTTACCAGTTCCCCC GCTGCAACTGTTACAGGATATGCC	65°C	183	(Moosavy <i>et al.</i> , 2015)
<i>fljB</i>	ACGAATGGTACGGCTTCTGTAACC TACCGTCGATAGTAACGACTTCGG	65°C	526	(Moosavy <i>et al.</i> , 2015)
<i>ST11</i>	GCCAACCATTGCTAAATTGGCGCA GGTAGAAATCCCAGCGGGTACTGG	65°C	429	(Moosavy <i>et al.</i> , 2015)
<i>SPV</i>	GCCGTACACGAGCTTATAGA ACCTACAGGGGCACAATAAC	65°C	250	(Moosavy <i>et al.</i> , 2015)
<i>SefA</i>	GACGCGGTTACTATTGCAGC TGTGACAGGGACATTTAGCG	65°C	310	(Moosavy <i>et al.</i> , 2015)
<i>aadA1</i>	TTATTTGCCGACTACCTTGGTG ATGAGGGAAGCGGTGATCG	56°C	792	(Farsiani <i>et al.</i> , 2015)
<i>cat1</i>	CTTGTCGCCTTGCATATAAT ATCCCAATGGCATCGTAAAG	56°C	508	(Odoch <i>et al.</i> , 2018)
<i>cat2</i>	AACGGCATGATGAACCTGAA ATCCCAATGGCATCGTAAAG	56°C	547	(Odoch <i>et al.</i> , 2018)
<i>CTX-M</i>	GCTCAATACCGCCATTCCAG TTTTATCGCCCACTCCCCAT	60°C	200	This study
<i>gyrA</i>	AAATCTGCCCCGTGTCGTTGGT GCCATACCTACGGCGATACC	54°C	344	(Zurfluh <i>et al.</i> , 2014)
<i>gyrB</i>	GAAATGACCCGCCGTAA ACGACCGATACCACAGCC	54°C	272	(Zurfluh <i>et al.</i> , 2014)
<i>OXA</i>	GCTGTAGACCCGCAAGT CACCCAACCTTATCTAGCACAT	54°C	151	This study
<i>ParC</i>	CTGAATGCCAGCGCCAAATT GCGAACGATTTCCGATCGTC	54°C	168	(Zurfluh <i>et al.</i> , 2014)
<i>ParE</i>	CTGAAGTCTGGCGGAGATG GCGGTGGCAGTGGCAGCTAA	54°C	483	(Zurfluh <i>et al.</i> , 2014)
<i>QnrB</i>	GGMATHGAAAATTCGCCACTG TTTGCGYGYCCGAGTCGAA	58°C	264	(Song <i>et al.</i> , 2018)
<i>QnrS</i>	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	56°C	428	(Odoch <i>et al.</i> , 2018)
<i>sul1</i>	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTCCG	65°C	433	(Li <i>et al.</i> , 2020)
<i>sul2</i>	CAACCGCCTTGCCTTGATC GTGTGTGCGGATGAAGTCAG	65°C	244	This study
<i>TEM</i>	CTTCTGTTTTTGCTCACC AGCAATAAACCCAGCCAGC	58°C	636	(Feizabadi <i>et al.</i> , 2010)
<i>strA</i>	AGCAGAGCGCGCTTCGCTC CCAAAGCCCACTTCACCGAC	60°C	684	(Li <i>et al.</i> , 2020)
<i>strB</i>	ATCGTCAAGGGATTGAAACC GGATCGTAGAACATATTGGC	60°C	504	(Li <i>et al.</i> , 2020)
<i>tetA</i>	GCGCGATCTGGTCACTCG AGTCGACAGYRGG CCGGC	54°C	164	(Farsiani <i>et al.</i> , 2015)
<i>tetB</i>	CGTGAATTTATTGCTTCGG ATACAGCATCCAAAGCGCAC	54°C	206	(Farsiani <i>et al.</i> , 2015)
<i>Cl Integron</i>	GGCATCCAAGCAGCAAGC AAGCAGACTTGACCTGA	60°C	1000	(Odoch <i>et al.</i> , 2018)
<i>spaN</i>	AAAAGCCGTGGAATCCGTTAGTGAAGT CAGCGCTGGGGATTACCGTTTTG	60°C	504	(Tarabees <i>et al.</i> , 2017)
<i>msgA</i>	GCCAGGCGCACGCGAAATCATCC GCGACCAGCCACATATCAGCCTCTTCAAAC	60°C	189	(Tarabees <i>et al.</i> , 2017)
<i>sipA</i>	CCAGATACCTCCACACCGTT TCCCTTTTAGCCTGGTCAG	60°C	248	This study
<i>csgA</i>	CGGATCCACGTTGAGCATT TTAGCGTTCCACTGGTCGAT	60°C	200	This study

Our results are also in line with other findings who found 89% of *Salmonella* isolates of poultry origin were resistant to Tetracycline (Uddin *et al.*, 2018). Similarly, in line with our results, 49.4% resistance to Ciprofloxacin, 41.5% resistance to Chloramphenicol and 92.2% resistance to Tetracycline has also been found in another study (Ellerbroek *et al.*, 2010; Klemm *et al.*, 2018). In our study, *invA* was targeted to identify *Salmonella* genus which encodes a type III secretion system. Due to its conserved nature, it is universally used for molecular identification of *Salmonella* genus in a short span of time compared to lengthy and time consuming enrichment

procedures (Abdel-Aziz, 2016). According to our findings, *invA* gene was amplified in only 85 (94.4%) isolates as previously this gene was present in 36 isolates (94.7%) out of 38 food samples screened for *Salmonella* strains (Taşkale Karatug *et al.*, 2018) that is very close agreement with our findings.

Salmonella Enteritidis was found to be the most prevalent serovar (74.11%) isolated from poultry liver in the present study as previously found from 77.7% (Firoozeh *et al.*, 2012) to 80% (Akhtar *et al.*, 2010). For *S. Typhimurium* three sets of primers i.e. *fliC*, *fljB* and *rflB* were used that together make a unique antigenic

Table 2: Antimicrobial susceptibility profile of *Salmonella* isolates (here write the names of all these antimicrobial agents with their abbreviation as mentioned in this table)

Antibacterial agent	Salmonella isolates								
	S. Enteritidis			S. Typhimurium			All Salmonella spp.		
	R	I	S	R	I	S	R	I	S
CIP	28 (44.4)	20 (31.7)	15 (23.8)	7(43.7)	9 (56.3)	-	43 (47.7)	20 (22.2)	27 (30)
CAZ	5 (7.93)	23 (36.5)	35 (55.5)	3 (18.7)	7 (43.7)	6 (37.5)	10 (11.1)	20 (22.2)	60 (66.6)
AMC	12 (19.04)	17 (26.9)	34 (53.9)	1 (18.7)	(18.7)	12 (75)	15 (16.6)	14 (15.5)	61 (67.7)
K	17 (26.98)	25 (39.7)	21 (33.3)	3 (18.7)	(68.75)	2 (12.5)	23 (25.5)	36 (40)	31 (34.4)
AK	-	15 (23.8)	48 (76.2)	-	-	16 (100)	-	7 (7.7)	83 (92.2)
AM	23 (36.5)	14 (15.5)	26 (41.3)	5(31.3)	(31.3)	6 (37.5)	34 (37.7)	16 (17.7)	40 (44.4)
S	17 (34.9)	23 (36.5)	23 (36.5)	4(25)	6 (37.5)	6 (37.5)	33 (36.6)	17 (18.8)	40 (44.4)
C	23 (36.5)	16 (25.4)	24 (38.09)	6(37.5)	1 (6.3)	9 (56.3)	37 (41.1)	14 (15.5)	39 (43.3)
MEM	2 (3.17)	18 (28.6)	43 (68.3)	3(18.7)	4 (25)	9 (56.3)	3 (3.3)	14 (15.5)	73 (81.1)
CTX	7 (14.2)	17 (26.9)	39 (61.9)	3(18.7)	4 (25)	9 (56.3)	17 (18.8)	19 (21.1)	54 (60)
CRO	2 (3.17)	16 (25.4)	45 (71.4)	2(12.5)	2 (12.5)	12 (75)	11 (12.2)	4 (4.4)	75 (83.3)
DO	58 (92.06)	-	5 (7.9)	14 (81.3)	1 (6.3)	1 (12.5)	70 (77.7)	6 (6.6)	14 (15.5)
SMZ	51 (80.95)	9 (14.2)	3 (4.7)	15 (87.5)	1 (12.5)	-	76 (84.4)	10 (11.1)	4 (4.4)
TE	56 (88.8)	3 (4.7)	4 (6.34)	14(87.5)	-	2 (12.5)	81 (90)	3 (3.3)	6 (6.6)
NA	42 (66.6)	21 (33.3)	-	16 (100)	-	-	70 (77.7)	13 (14.4)	7 (7.7)
T	59 (93.6)	3 (4.7)	1 (1.6)	15(93.7)	1 (6.3)	-	85 (94.4)	3 (3.3)	2 (2.2)
NOR	17 (26.9)	13 (20.6)	33 (52.4)	6(37.5)	3 (18.7)	7 (43.7)	28 (31.1)	14 (15.5)	42 (46.6)

CIP: Ciprofloxacin, CAZ: Ceftazidime, AMC: Amoxicillin / Clavulanicacid, K: Kanamycin, AK, Amikacin, S: Streptomycin, C: Chloramphenicol, MEM: Meropenem, CTX: Cefotaxime, CRO: Ceftriaxone, DO: Doxycycline, SMZ, Sulfamethoxazole, TE: Tetracycline, NA: Nalidixic acid, T: Oxytetracycline, NOR: Norfloxacin.

Table 3: Phenotypic profiles observed in *Salmonella* isolates

Phenotypic Profile	No of isolates	MARI
AM,C,CAZ,CIP,CRO,CTX,DO,NA,S,SMZ,T,TE	1	0.705
AM,C,CAZ,CIP,CTX,K,NA,SMZ,T,TE	2	0.588
AM,CIP,CTX,DO,NA,S, SMZ,T,TE	2	0.529
C,CRO,CTX,DO,K,NA,S,SMZ,T,TE	1	0.588
AM,CIP,CTX,DO,NA,S,R,TE,SMZ	3	0.529
CIP,AM,NA,NOR,SMZ,T,TE,DO	3	0.470
AM,CRO,CTX,DO,NA,SMZ,T,TE	1	0.470
AM,AMC,CIP,NA,SMZ,T,TE,K,S	4	0.529
AM,DO,NA,NOR,SMZ,T,TE,K	2	0.470
C,CIP,DO,NA,NOR,SMZ,TE,T	3	0.470
CIP,DO,NA,NOR,SMZ,T,TE	2	0.411
AM,C,DO,K,NA,S,SMZ,T,TE	2	0.529
AMC,CIP,DO,NA,SMZ,T,TE	3	0.411
CAZ,CIP,DO,NA,SMZ,T,TE	3	0.411
AM,CIP,DO,NA,SMZ,T,TE	2	0.411
C,CIP,DO,K,NA,SMZ,T,TE	3	0.470
AMC,DO,K,NA,SMZ,T,TE	2	0.411
AM,AMC,CIP,K,NA,T,TE	4	0.411
CIP,DO,NA,S,SMZ,T,TE	3	0.411
C,CIP,DO,NA,SMZ,T,TE	2	0.411
DO,MEM,NA,SMZ,T,TE	3	0.352
C,CIP,DO,NA,SMZ,T,TE	2	0.411
DO,NA,S,SMZ,T,TE	3	0.352
C,DO,NA,SMZ,T,TE	6	0.352
CAZ,DO,SMC,T,TE	1	0.294
NA,SMZ,T,TE	2	0.235
CIP,AM,AMC	2	0.176
DO,NA,T,TE	3	0.235
NA,SMZ,TE	2	0.176
DO,NA,SMZ	1	0.176
AM,AMC,T	1	0.176
DO,SMZ,T	3	0.176
DO,T,NA	3	0.176
SMZ,T	4	0.11
T,TE	2	0.11
SMZ	1	0.05

combination of O4: H1: i and O4: H2: i for phase 1 and phase 2 flagellar proteins, respectively. In our results, the prevalence of *S. Typhimurium* was found to be 18.8% while other studies have reported a relatively high prevalence rate i.e. 38.4% (Wajid *et al.*, 2019) of *S. Typhimurium* in poultry. The most prevalent genes were found to be *sul1* (98.8%), *sul2* (97.6%), *gyrA* (95.2%), *strB* (92.9%), *tetA* (87.05%) and *CTX-M* (80%) in our

study. These findings are in close agreement to previous studies wherein the authors reported 100% prevalence of *tetA* gene in *Salmonella* (VV, Waghmare, and Yeotikar, 2018). Similarly, (Zishiri, Mkhize, and Mukaratirwa, 2016) reported 83% prevalence of both *tetA* and *sul1* genes. According to our data, *CTX-M* was found in 80% *Salmonella* isolates. The high prevalence of *CTX-M* in *Salmonella* is a highly alarming issue because it could be due to horizontal transmission from the recently emerged XDR *Salmonella* Typhi strains in Pakistan (Klemm *et al.*, 2018).

Among the virulent genes, we found *spaN* as the most prevalent one (90.5%) as previously reported as 83.3% (Kagirita *et al.*, 2017) that is close to our findings. Moreover, the phenotypic data of biofilm forming capacity is also close to molecular screening of biofilm associated gene *csgA* as 87.05%. The prevalence of *msgA* and *sipA* was found to be 82.3% and 80% respectively. Other authors reported a 100% prevalence of these genes in the *Salmonella* isolates (Mezal *et al.*, 2014).

In conclusion, poultry birds are carrier of highly resistant strains of *Salmonella* in the Peshawar region that is potentially a high public health risk. Moreover, the dominant serovars like *S. Enteritidis* and *S. Typhimurium* are highly resistant and virulent and are of great public health concern. A comprehensive surveillance system is urgently needed that could ensure a routine sampling pattern from different regions of KPK to have a clear picture of the resistant and virulent status of the *Salmonella* isolates. Moreover, a regulated use of antimicrobials in the poultry is required for control and prevention of the current situation.

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