Molecular epidemiology and in vitro antimicrobial susceptibility of *Salmonella* isolated from poultry in Kashmir

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Summary
A total of 480 samples, comprising 429 faecal samples from healthy adult birds and 51 tissue samples from dead birds, were collected from four government poultry farms in the Kashmir valley from September 2007 to April 2008. In all, 33 *Salmonella* isolates were obtained. Of these, 28 (84.85%) isolates were *Salmonella* Gallinarum, 3 (9.09%) were *Salmonella* Enteritidis and the remaining 2 (6.06%) were *Salmonella* Typhimurium. All the isolates harboured the invA, sefA, stn and spvC virulence-specific genes. However, the sopB gene was found in only 90.9% of the isolates. Pulsed-field gel electrophoresis analysis of representative isolates revealed that the majority were related but a few belonged to different clones. The majority of the isolates were resistant to cefpodoxime, nalidixic acid and sulphadiazine and sensitive to chloramphenicol, cefotaxime and tetracycline. Isolation of multidrug-resistant *Salmonella*, including the zoonotically important serovars, revealed a potential threat not only to poultry but also to human health in Kashmir.

Keywords

Introduction
Salmonellae are well recognised as potential pathogens, causing diseases in a wide range of mammalian and avian hosts (6). To date, more than 2,541 serovars of *Salmonella* have been described (National Salmonella Reference Laboratory, Galway, Ireland). Some serovars of *Salmonella* show host-specificity, such as *Salmonella enterica* serovar Gallinarum (S. Gallinarum), which causes fowl typhoid (21). Other serovars, such as *Salmonella enterica* serovar Typhimurium (S. Typhimurium) and *Salmonella enterica* serovar Enteritidis (S. Enteritidis), are promiscuous and can cause infections in different species, including poultry and humans (31). Salmonellae are the leading cause of morbidity and mortality in poultry and lead to significant economic losses (5, 23). At present, S. Typhimurium and S. Enteritidis are the most prevalent causes of *Salmonella*-induced food poisoning in humans (27).

The virulence of salmonellae depends upon an array of factors, encoded by genes, that lead to their colonisation,
invasiveness, intracellular survival and damage to host tissues. The chromosomally located invasion (invA) gene is thought to trigger the invasion of salmonellae into cultured epithelial cells (13), while an operon spvRABCD, containing five genes, is present on plasmids commonly associated with some serotypes. The spv genes possibly have the ability to increase the severity of enteritis and allow infection and persistence at extra-intestinal sites (17). Other virulence genes include sefA, stn and sopB coding for Salmonella Enteritidis fimbriae, Salmonella enterotoxin and Salmonella outer protein, respectively (22, 29).

Molecular typing of salmonellae is valuable in determining the epidemiology and tracing the source of infection. In this context, pulsed-field gel electrophoresis (PFGE) analysis has proved to be a very versatile tool in genotyping Salmonella isolates (24), and is considered the gold standard for molecular subtyping of some serovars of S. enterica in epidemiological studies (3).

In recent years, antibiotic resistance in Salmonella has assumed alarming proportions worldwide. Monitoring drug resistance patterns among the Salmonella isolates not only gives vital clues to the clinician on the best therapeutic regime in each individual case, but is also an important tool in devising a comprehensive chemoprophylactic and chemotherapeutic drug schedule within a geographical area (20).

In India, although the isolation of various Salmonella serovars from poultry has been regularly reported from different parts of the country, information about the molecular characteristics of these serovars is scarce (20, 23). The present study was undertaken to determine the prevalence, serological diversity, virulence gene profile and in vitro antibiogram of salmonellae from poultry, as well as to study the genetic relatedness of the various serovars.

### Materials and methods

#### Sampling and isolating Salmonella

From September 2007 to April 2008, a total of 480 samples, comprising 429 faecal samples from healthy adult birds and tissue samples from 51 dead birds, were collected from four government poultry farms in the Kashmir valley, namely:

- Athwajan
- Hariparbat
- Umberhair
- the Government Poultry Project, Mattan.

Details of the samples are given in Table I. The tissue samples (liver, heart, blood and lungs) and intestinal contents were collected from dead birds. These samples were directly inoculated into 10 ml of tetrazyanate broth (TTB) for selective enrichment of salmonellae and incubated at 42°C for 48 h. The TTB-enriched culture was then plated on brilliant green agar and Salmonella Shigella agar (HiMedia, Mumbai, India). Suspected colonies of Salmonella were further purified by subculture and transferred onto nutrient agar slants. The isolates were then subjected to standard morphological and biochemical tests, as described by Holt et al. (14), to ascertain their identity as Salmonella.

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**Table I**

<table>
<thead>
<tr>
<th>Source of samples</th>
<th>Samples examined for Salmonella</th>
<th>Samples testing positive for Salmonella</th>
<th>No. of samples testing positive for various serotypes of Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type of sample</td>
<td>No. of samples examined</td>
<td>No. of positive samples</td>
</tr>
<tr>
<td>Hariparbat</td>
<td>FS</td>
<td>248</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>VS</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Athwajan</td>
<td>FS</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>VS</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Umberhair</td>
<td>FS</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>VS</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>GPP Mattan</td>
<td>FS</td>
<td>106</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>VS</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total (%)</strong></td>
<td><strong>480</strong></td>
<td><strong>33 (6.875)</strong></td>
<td><strong>28 (84.85)</strong></td>
</tr>
</tbody>
</table>

FS: faecal sample
VS: visceral sample
GPP: Government Poultry Project
Identification and typing of Salmonella

All the isolates of Salmonella were tested by genus-specific polymerase chain reaction (PCR), using the 16S ribosomal RNA (rRNA) primers, as described by Lin and Tsen (18).

Serotype-specific PCR for S. Typhimurium was essentially similar to the protocol described by Alvarez et al. (1). For S. Enteritidis, the PCR protocol of Alvarez et al. (1) was followed, with minor modifications. The PCR conditions consisted of initial denaturation at 94°C for 2 min, followed by 35 cycles of 95°C for 1 min, 57°C for 1 min and 72°C for 2 min. The final extension was carried out at 72°C for 5 min. Details of the primer sequences used in this study are given in Table II.

Serotyping of Salmonella

All the isolates of Salmonella were referred to the National Salmonella and Escherichia Centre, Central Research Institute, Kasauli – 173204, Himachal Pradesh, India, for confirmation and serotyping.

Detection of virulence-specific genes (invA, sefA, stn, spvC and sopB) of Salmonella

A multiplex PCR protocol, described earlier (11), was used to detect invA and sefA virulence genes. Similarly, stn, sopB and spvC genes were detected with PCR protocols and primer sequences described by Rahman (25, 26) and Chiu and Ou (10), respectively.

Table II
Details of primers used in this study

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer sequence (5’ → 3’)</th>
<th>Primer concentration</th>
<th>Product size (base pair)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td>TGT GGT TAA TAA CCG CA</td>
<td>1 µM each</td>
<td>574</td>
<td>(18)</td>
</tr>
<tr>
<td></td>
<td>CAC AAA TCC ATC TCT GGA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>invA</td>
<td>TTGTTAGGCTTA TTT TGA CCA</td>
<td>0.2 µM each</td>
<td>521</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td>CTG ACT GCT ACC TTG CTG ATG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sefA</td>
<td>GCA GCG GTT ACT ATT GCA GC</td>
<td>0.2 µM each</td>
<td>330</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td>TGT GAC AGG GAC ATT TAG CG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stn</td>
<td>TTG TGT GCG TAT CAC TGG CAA</td>
<td>1 µM each</td>
<td>617</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td>ATT GAG AAC CGG CTC TGG TCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spvC</td>
<td>ACT CCT TGC ACA ACC AAA TGG GGA</td>
<td>1 µM each</td>
<td>571</td>
<td>(10)</td>
</tr>
<tr>
<td></td>
<td>TGT CTG CAT TTC GGC ACC ATC A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sopB</td>
<td>CAA CCG TTC TGG GTA AAC AGG AC</td>
<td>1 µM each</td>
<td>1,348</td>
<td>(26)</td>
</tr>
<tr>
<td></td>
<td>AGG ATT GAG CTC CTC TGG CCA T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis</td>
<td>TGT GTT TTA TCT GAT GCA AGA GGC</td>
<td>0.075 µM</td>
<td>304</td>
<td>(1)</td>
</tr>
<tr>
<td></td>
<td>TGA ACT AGA TTC TGT CTT CTG G</td>
<td>0.1 µM</td>
<td>401</td>
<td>(1)</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>TTG TTC ACT TTA TAC CCC TGA A</td>
<td>0.1 µM each</td>
<td>574</td>
<td>(18)</td>
</tr>
<tr>
<td></td>
<td>CCC TGA CAG CGG TTA GAT ATT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

rRNA: ribosomal ribonucleic acid

Pulsed-field gel electrophoresis analysis of Salmonella

Bacterial strains

Representative isolates of S. Gallinarum, S. Enteritidis and S. Typhimurium isolated from different farms were subjected to PFGE for genotyping, as described below. In all, six isolates of S. Gallinarum (three from Hariparbath, and one each from Athwajan, Umbherhair and Mattan) and all the isolates of S. Enteritidis (3) and S. Typhimurium (2) were included in the study.

Preparation of genomic DNA

Pulsed-field gel electrophoresis was performed according to the protocol described by PulseNet USA, with little modification. In brief, the isolates were grown in Luria-Bertani broth at 37°C, overnight, with shaking in a shaking incubator (SI-300, Lab Companion, Jeio Tech, South Korea). Bacterial cells were harvested by centrifugation at 5,000 × g for 15 min. The cells were washed twice and re-suspended in cell suspension buffer (100 millimolars [mM] Tris-hydroxymethylmethylamine-hydrochloric acid [Tris-HCl], 100 mM ethylene diamine tetra-acetic acid [EDTA], pH 8.0), at a final concentration of 2 × 10⁹ bacterial cells per ml. Two hundred microlitres of the cell suspension was mixed with 10 µl of proteinase K (20 mg/ml), by inverting the tube 5 to 6 times in a 1.5 ml microcentrifuge tube, and kept at 37°C in a circulating water bath (CW05G, Lab Companion, Jeio Tech). The suspension was then mixed with 2% plug agarose (Bio-Rad, United States), in a 1:1 ratio, and kept in the water
bath at 55°C. The mixture was immediately pipetted into disposable plug moulds (Bio-Rad) and allowed to solidify. Cell lysis in the plugs was performed by 750 μl of cell lysis buffer (50 mM Tris-HCl, 50 mM EDTA, pH 8.0, and 1% sodium dodecyl sulphate) and 20 μl of proteinase K (20 mg/ml) at 54°C for 2 h. The plugs were washed twice with triple distilled water (TDW), at 54°C for 30 min each, and twice with Tris-EDTA (TE) buffer, as above, for 15 min each, under shaking. The washed plugs were stored in TE buffer at 4°C until use.

After washing, the TE buffer was carefully removed and the plugs were finally equilibrated in 225 μl of sterile TDW and 25 μl of 10 × XbaI fast-digest buffer at room temperature for 1 h. Eight units of XbaI fast-digest restriction enzyme (MBI Fermentas, Germany) were added to the digestion buffer and incubated at 37°C for 4 h.

Pulsed-field gel electrophoresis

The electrophoresis of the plugs was performed using the CHEF Mapper XA Chiller (Bio-Rad). In brief, after digestion, the plugs were loaded into a 1% PFGE-grade agarose gel (Bio-Rad), prepared in 0.5 × Tris-borate-EDTA (TBE) buffer and sealed with 1% agarose. Electrophoretic conditions were applied at a constant voltage of 6 v/cm; a pulse time of 2.16 to 54.0 sec; for 19 h, 0.5 × TBE running buffer, and the temperature was maintained at 14°C. Salmonella Braenderup Strain H9812 was used as a standard molecular size marker. After electrophoresis, the gel was stained with ethidium bromide (0.5 μg/ml) for 40 min, then de-stained 4 times with distilled water for a period of 15 min each time. The DNA bands were visualised and photographed by the gel documentation system.

Antibacterial sensitivity assay

The in vitro antimicrobial sensitivity patterns of the Salmonella isolates to various antimicrobial agents were determined by the disc diffusion technique (4). The antimicrobial discs (HiMedia, India) used were:
- ampicillin (10 micrograms [mcg])
- amoxicillin-clavulanic acid (30 mcg)
- cefotaxime (30 mcg)
- cefpodoxime (10 mcg)
- chloramphenicol (30 mcg)
- ciprofloxacin (5 mcg)
- enrofloxacin (10 mcg)
- gentamicin (10 mcg)
- nalidixic acid (30 mcg)
- streptomycin (10 mcg)
- sulphadiazine (300 mcg)
- trimethoprim (5 mcg)
- trimethoprim-sulphamethoxazole (23.75/1.25 mcg)
- tetracycline (30 mcg).

The interpretation of the isolates as sensitive and/or resistant was carried out as per the manufacturer's instructions.

Results

A total of 33 Salmonella isolates from 33 samples were obtained from four farms (Table I). Out of 33 isolates, 10 (30.30%) were from faecal samples while 23 (69.70%) were from tissue samples. All the isolates amplified 16S rRNA gene-specific 574 base pairs (bp) product.

Table III

<table>
<thead>
<tr>
<th>Salmonella serovars</th>
<th>No. of isolates tested</th>
<th>16S rRNA</th>
<th>Typh.</th>
<th>Ent.</th>
<th>invA</th>
<th>sefA</th>
<th>stn</th>
<th>spvC</th>
<th>sopB</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Gallinarum</td>
<td>28</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>33</td>
<td>2</td>
<td>3</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>30</td>
</tr>
</tbody>
</table>

rRNA: ribosomal ribonucleic acid
Typh.: Typhimurium
Ent.: Enteritidis

The interpretation of the isolates as sensitive and/or resistant was carried out as per the manufacturer's instructions.

Table III

| Distribution of specific genes among different serovars of Salmonella from the Kashmir valley |
|-----------------------------------------------|-------------------|------------------|-----------------|---|---|---|---|---|
| No. of isolates | 16S rRNA | Typh. | Ent. | invA | sefA | stn | spvC | sopB |
| S. Gallinarum  | 28 | 28 | 0 | 0 | 28 | 28 | 28 | 28 | 26 |
| S. Typhimurium | 2 | 2 | 2 | 0 | 2 | 2 | 2 | 2 | 1 |
| S. Enteritidis | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 3 | 3 |
| Total           | 33 | 33 | 2 | 3 | 33 | 33 | 33 | 33 | 30 |

The PFGE analysis revealed that the S. Gallinarum isolates from Hariparbath belonged to two different clonal types, since they exhibited two distinct profiles, as depicted in Fig. 1a:
- profile I (lanes E and F)
- profile II (lane G).
The isolates from Mattan (lane B) and Umberhair (lane C) resembled profile I. However, the isolate from Athwajan (lane D) did not belong to either of these clones.

Similarly, PFGE analysis of the three isolates of *S.* Enteritidis (Fig. 1b) revealed that the isolates from Athwajan (lane D) and Hariparbat (lane E) gave similar band patterns. However, the isolate from Mattan (lanes B and C) differed from the other two isolates. Likewise, the two *S.* Typhimurium isolates from Hariparbat and Mattan showed different band patterns under PFGE (Fig. 1c).

An antibiotic sensitivity assay revealed that all the isolates of *S.* Gallinarum were resistant to cefpodoxime, sulphadiazine and nalidixic acid. Sensitivity to other antimicrobials varied, as shown in Table IV.

Among the two *S.* Typhimurium isolates, the isolate from Hariparbat was sensitive to all the antibacterial agents tested, except cefpodoxime and nalidixic acid, while the isolate from Mattan was sensitive only to amoxicillin-clavulanic acid, cefotaxime, cefpodoxime, chloramphenicol and ciprofloxacin. Similarly, all the *S.* Enteritidis isolates were resistant to cefpodoxime, sulphadiazine and nalidixic acid. In addition to these antibacterials, one isolate was also resistant to enrofloxacin while another was resistant to trimethoprim.

**Discussion**

Salmonellae are among the major bacterial pathogens causing huge economic losses to the poultry industry, as well as posing a significant threat to public health. Preventing *Salmonella* infection thus becomes crucial but this can be achieved only through good monitoring and screening programmes (32). In this study, all four organised poultry farms were found to be loaded with different serovars of *Salmonella*. The overall prevalence of salmonellosis was 6.88%, lower than that observed in other studies (20), one of which reported a *Salmonella* prevalence rate of 14.7% in cloacal swab samples from diarrhoeic poultry in north-east India. This could be
attributed to the fact that, in the present study, sampling was carried out at random, eliminating the bias of selecting samples from birds with diarrhoea.

The predominant prevalence of S. Gallinarum could be attributed to its host adoption. These findings are in agreement with those of earlier workers (16, 20, 23).

All the isolates were found to carry the invA, sefA, stn and spvC virulence genes. The detection of the stn gene in all the isolates is in accord with the findings of Murugkar et al. (19), who also reported the presence of the stn gene in all isolates of Salmonella from poultry. The presence of the invA gene agrees with the findings of de Dias et al. (12), who reported the presence of the invA gene in all the Salmonella isolates recovered from chicken abattoirs in Brazil. The prevalence of sefA and spvC genes among all the Salmonella isolates was more or less similar to the findings of Castilla et al. (9), who reported the presence of sefA and spvC genes in a majority (90.1%) of Salmonella isolates from broiler chicks in Brazil. In the present investigation, the sopB gene was detected in 90.9% of the Salmonella isolates. This finding is similar to that of Skyberg et al. (29), who reported the presence of the sopB gene in more than 98% of Salmonella isolates recovered from both healthy and sick birds in the United States.

The similar PFGE profiles of S. Gallinarum isolates from Umberhair, Mattan and Hariparbath indicated that the same clonal type of S. Gallinarum was circulating on these two farms. The S. Gallinarum infection in Mattan and Umberhair could be attributed to vertical transmission of the infection from Hariparbath, as the chicks for these two farms were supplied from Hariparbath. The PFGE profile of the Athwajan isolate of S. Gallinarum was quite dissimilar from the profiles of the Hariparbath isolates, showing that a different clonal type of S. Gallinarum was prevalent in Athwajan. This may be due to horizontal transmission. This seems to demonstrate that S. Gallinarum infection on these farms originated from multiple sources, for example, water, feed or vertical transmission.

The similarity of the PFGE profiles of the S. Enteritidis isolates from both Hariparbath and Athwajan suggests that the same clonal type of S. Enteritidis was circulating on these farms. The birds at Athwajan might have received the infection through vertical transmission from Hariparbath, as the chicks were supplied from the Hariparbath farm.

The PFGE profile of the two S. Enteritidis isolates from Mattan differed from those of Hariparbath and Athwajan, suggesting that different clonal types of S. Enteritidis are circulating in Mattan.

Two isolates of S. Typhimurium from Hariparbath and Mattan revealed two different PFGE patterns, suggesting circulation of two different clonal types on these two farms. Genetic analysis of Salmonella isolates by PFGE has been carried out by other workers (15), who reported that the PFGE patterns of Salmonella isolates recovered from humans and chickens in Seoul (South Korea) were extremely similar and pointed out that a single clone of Salmonella was responsible for the disease in both humans and chickens. Similarly, Thong et al. (30) reported that the PFGE patterns of S. Enteritidis isolates from hospital outbreaks in Switzerland and Malaysia were similar, suggesting that the same clone of Salmonella could have been carried from one place to another during those outbreaks.

The detection of S. Typhimurium and S. Enteritidis in the present study indicates that poultry could be a source of infection for humans in India. Thus, all poultry should be thoroughly cooked before being consumed. The authors suggest that routine molecular typing of animal and human isolates should be carried out, to trace routes of infection. They further suggest that regular surveillance of Salmonella should be undertaken in poultry to ensure that it is safe for human consumption.

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### Table IV

**In vitro antimicrobial susceptibility pattern of Salmonella isolates from poultry in the Kashmir valley**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. tested</th>
<th>A</th>
<th>Ac</th>
<th>Ce</th>
<th>Cep</th>
<th>C</th>
<th>Cl</th>
<th>Ex</th>
<th>G</th>
<th>Na</th>
<th>S</th>
<th>Sz</th>
<th>Tr</th>
<th>T</th>
<th>Co</th>
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<tbody>
<tr>
<td>S. Gallinarum</td>
<td>28</td>
<td>21 (7)</td>
<td>21 (7)</td>
<td>27 (1)</td>
<td>0 (28)</td>
<td>26 (2)</td>
<td>20 (6)</td>
<td>23 (5)</td>
<td>16 (12)</td>
<td>0 (28)</td>
<td>14 (14)</td>
<td>0 (28)</td>
<td>16 (12)</td>
<td>28 (0)</td>
<td>14 (14)</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>02</td>
<td>1 (1)</td>
<td>2 (0)</td>
<td>2 (0)</td>
<td>0 (2)</td>
<td>2 (0)</td>
<td>2 (0)</td>
<td>1 (1)</td>
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<td>0 (2)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>03</td>
<td>3 (0)</td>
<td>3 (0)</td>
<td>3 (0)</td>
<td>0 (3)</td>
<td>3 (0)</td>
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<td>3 (0)</td>
<td>2 (1)</td>
<td>3 (0)</td>
<td></td>
</tr>
</tbody>
</table>

Figures in parentheses indicate the number of resistant strains.

A: amoxicillin (10 micrograms [mcg])
Ac: amoxicillin-clavulanic acid (30 mcg)
Ce: cefotaxime (30 mcg)
Cep: cefpodoxime (30 mcg)
C: chloramphenicol (30 mcg)
Cf: ciprofloxacin (5 mcg)
Cl: cefuroxim (5 mcg)
Co: clindamycin (1 mcg)
Ex: enrofloxacin (10 mcg)
G: gentamicin (10 mcg)
Na: nalidixic acid (10 mcg)
S: streptomycin (10 mcg)
Sz: sulphadiazine (300 mcg)
Tr: trimethoprim (5 mcg)
T: tetracycline (30 mcg)
A: ampicillin (10 micrograms [mcg])
G: gentamicin (10 mcg)
S: streptomycin (10 mcg)
Cf: ciprofloxacin (5 mcg)
Cl: cefuroxim (5 mcg)
Co: clindamycin (1 mcg)
Ex: enrofloxacin (10 mcg)
G: gentamicin (10 mcg)
Na: nalidixic acid (10 mcg)
S: streptomycin (10 mcg)
Sz: sulphadiazine (300 mcg)
Tr: trimethoprim (5 mcg)
T: tetracycline (30 mcg)
A: ampicillin (10 micrograms [mcg])
G: gentamicin (10 mcg)
Na: nalidixic acid (10 mcg)
S: streptomycin (10 mcg)
Cf: ciprofloxacin (5 mcg)
Cl: cefuroxim (5 mcg)
Co: clindamycin (1 mcg)
Ex: enrofloxacin (10 mcg)
At present, antimicrobial therapy is one of the primary control measures for reducing morbidity and mortality from pathogens, including *Salmonella* organisms. However, there is a need to use antimicrobials judiciously to maintain effective treatment and to avoid the development of drug resistance among the clinical bacterial isolates. In this study, the multi-drug resistance of the isolates may be due to the indiscriminate use of antibacterials in clinical practice. These resistance levels are comparable to those previously reported for *Salmonella* isolates by other investigators. For instance, Carramiñana et al. (8) reported that *Salmonella* isolates from a poultry slaughterhouse in Zaragoza (Spain) showed resistance to sulphadiazine, and Al-Zenki et al. (2) reported resistance in *Salmonella* isolates to ampicillin and nalidixic acid at a poultry farm and processing plant in Kuwait. Furthermore, the sensitivity levels recorded in this study are in accord with those observed by Shaheen et al. (28), who reported that all the *S. Enteritidis* isolates collected from poultry meat and eggs in Islamabad (Pakistan) were highly sensitive to chloramphenicol.

**Conclusion**

If antimicrobials are to remain effective treatments for *Salmonella* and other pathogens, then vigilance must be kept over the development of drug resistance in these strains, and using *in vitro* antibiotic sensitivity tests to determine resistance among clinical isolates is a useful tool.
Epidemiología molecular y sensibilidad in vitro a los antimicrobianos de salmonelas aisladas en aves de corral en Cachemira

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Resumen
Entre septiembre de 2007 y abril de 2008 se recogieron en explotaciones avícolas públicas del valle de Cachemira un total de 480 muestras: 429 muestras de heces de adultos sanos y 51 de partes enfermas de aves muertas. En total se aislaron salmonelas en 33 muestras, de las que 28 (un 84,85%) correspondían a *Salmonella Gallinarum*, 3 (un 9,09%) a *Salmonella Enteritidis* y las 2 restantes (6,06%) a *Salmonella Typhimurium*. Todos los microorganismos aislados contenían los genes específicos de virulencia *invA*, *setA*, *str* y *spvC*. El gen *sopB*, en cambio, estaba presente en el 90,9% de ellos. El análisis por electroforesis en gel de campo pulsante de cepas representativas de los microorganismos aislados puso de manifiesto que la mayoría estaban emparentadas, pero unas pocas pertenecían a clones diferentes. La mayoría eran resistentes a la cefpodoxima, el ácido nalidíxico y la sulfadiazina y sensibles al cloranfenicol, la cefotaxima y la tetraciclina. El aislamiento de salmonelas multirresistentes, algunas de ellas pertenecientes a variantes séricas importantes por su potencial zoonótico, descubre una posible amenaza para la salud no sólo de las aves de corral, sino también de las personas, en Cachemira.

Palabras clave
References


