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1 **EXTENDED SPECTRUM  $\beta$ -LACTAMASE SHV-12-PRODUCING SALMONELLA**  
2 **FROM POULTRY**

3  
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13  
14 **ABSTRACT**

15 *Salmonella* strains isolated from poultry and poultry products over the period 2005-2006 have  
16 been investigated in order to ascertain the presence of Extended Spectrum Cephalosporins (ESC)  
17 resistance. Twelve (ESC)-resistant isolates (n=1 *S. Enteritidis*, n=1 *S. Braenderup* and n=10 *S.*  
18 *Livingstone*) were characterized as SHV-12-positive. The multi-drug resistant *S. Livingstone*  
19 SHV-12-producing isolates, untypeable by Pulsed-field gel electrophoresis (PFGE), showed a  
20 clonal relationship by Random Amplified Polymorphic DNA (RAPD) analysis. The SHV-12  $\beta$ -  
21 lactamase is reported for the first time in *Salmonella enterica* strains isolated from poultry in  
22 Italy. The results suggest poultry as a source of *Salmonella* carrying Extended-Spectrum  $\beta$ -  
23 lactamases (ESBLs) genes and highlights the need of monitoring animal productions to prevent  
24 spreading of (ESC)-resistant strains.

25 *Keywords:* Salmonella; Extended Spectrum  $\beta$ -Lactamases; PFGE; RAPD-PCR; poultry.

26

## 27 INTRODUCTION

28

29 Non-typhoid *Salmonella* are among the most important causes of food-borne gastroenteritis  
30 worldwide, and poultry is commonly recognized as a *reservoir* of this zoonotic agent. *Salmonella*  
31 *enterica* serovar Livingstone has recently become in the last two years the most frequent serovar  
32 isolated from poultry in the Veneto Region of Italy and has been also commonly reported in  
33 *Salmonella* isolates from poultry in UK (Liebana et al., 2001). Although human outbreaks of *S.*  
34 Livingstone are relatively uncommon (Old D. C et al., 1994; Guerin et al., 2004), this serovar is  
35 potentially zoonotic and genotypic relatedness between human and poultry isolates has been  
36 reported in past studies (Old et al., 1995). *Salmonella* Livingstone (ESC)-resistant has been  
37 recently reported in a nosocomial outbreak in a neonatal ward in Tunisia (Bouallègue-Godet et  
38 al., 2005). In recent years, we have seen an emergence of *Salmonella* resistant to ESC (Hasman  
39 H. et al. 2005; Arlet G., 2006; Bertrand S., 2006), creating concern because third-generation  
40 cephalosporins, such as ceftriaxone, are the drugs of choice for invasive *Salmonella* infections  
41 particularly in children where the use of quinolones are not indicated (Paterson D. L., 2006).  
42 Resistance to these antimicrobials is mainly mediated by Ambler class A Extended-Spectrum  $\beta$ -  
43 Lactamases (ESBLs) and plasmid-mediated Ambler class C enzymes. *Salmonella* (ESC)-resistant  
44 are isolated in particular from humans cases but also from animals and food, with poultry as  
45 primary food source (Riaño et al., 2006; Hasman et al. 2005; Bertrand at al., 2006). The finding  
46 of the same *bla* genes in *Salmonella* isolates from foodstuffs and humans suggests a possible  
47 source of human infection. However it is also possible that humans act as a *reservoir* of these  
48 genes in animals.

49 This study reports the characterisation of 12 strains of *Salmonella enterica* carrying a SHV-12  $\beta$ -  
50 lactamase isolated in poultry in Italy in 2005-2006. In particular, the study shows the presence of  
51 genetically related clones of *S. Livingstone* SHV-12-producing strains scattered in different farms  
52 in the north-east of Italy, which is the main region for poultry production (ISTAT Source  
53 [<http://www.istat.it>]: 38% of national chicken production, 52% of turkey production).

54

## 55 MATERIALS AND METHODS

56

57 **Bacterial strains.** The Italian National Reference Laboratory NRL for salmonella at Istituto  
58 Zooprofilattico delle Venezie (Italy) receives *Salmonella* isolates from animals and foodstuffs for  
59 serotyping (approximately 2000 exams/year) through its network comprised of eleven diagnostic  
60 laboratories located in three regions of north-eastern part of Italy (Veneto, Friuli Venezia Giulia,  
61 Trentino Alto Adige). Moreover, it receives data and isolates for phage typing from all the  
62 national official veterinary laboratories. Isolates from poultry are mostly collected in the  
63 framework of mandatory salmonella monitoring and control programs according to Reg.  
64 2160/2003. Approximately 2162 isolates from poultry were serotyped in the two years period  
65 2005-2006.

66 **Serotyping and phage typing.** The isolates were serotyped on the basis of somatic O, phase 1  
67 flagellar and phase 2 flagellar antigens by agglutination tests with commercial antisera (Staten  
68 Serum Institut-Denmark) as specified by Kauffmann-White scheme (Popoff, 2001). Phage typing  
69 was performed according to the recommendations of Health Protection Agency Service  
70 (Colindale, United Kingdom).

71 **Antimicrobial susceptibility testing.** Antimicrobial susceptibility was assayed on 1840  
72 *Salmonella* isolated from poultry over the period 2005-2006 by the disk diffusion method

73 according to the recommendations of CLSI (formerly NCCLS). The following 16 antimicrobial  
74 drugs (Becton Dickinson Microbiology Systems Cockeysville, MD, USA) were tested: colistin  
75 (CL 10 µg), ampicillin (AMP 10 µg), tetracycline (TE 30 µg), kanamycin (KAN 30 µg),  
76 gentamicin (GEN 10 µg), trimethoprim-sulfamethoxazole (SXT 23,75+ 1,25 µg), nalidixic acid  
77 (NAL 30 µg), chloramphenicol (CHL 30 µg), streptomycin (STR 10 µg), ciprofloxacin (CIP 5  
78 µg), ceftazidime (CAZ 30 µg), amoxicillin /clavulanic acid (AMC 30 µg), cefotaxime (CTX 30  
79 µg), cephalotin (CF 30 µg), enrofloxacin (ENO 5 µg), sulfonamides (SUL 250 µg). All the  
80 isolates that showed resistant and/or intermediate values to cefotaxime and/or ceftazidime were  
81 confirmed for the ESBL phenotype by quantitative E-test special strips (AB, Biodisk, Solna,  
82 Sweden) testing the synergistic effect of clavulanate with ceftazidime, cefotaxime and cefepime.  
83 *E. coli* strain ATCC 35218 and *Klebsiella pneumoniae* ATCC 700603 were used as ESBL-  
84 negative and ESBL-positive controls, respectively. The isolates with ESBL-positive phenotype  
85 were further tested for antimicrobial susceptibility using a commercially available MIC technique  
86 (Sensititre, Trek Diagnostic Systems Ltd, UK) according to CLSI guidelines.

87 **Molecular characterization of β-lactamases.** Total DNA was extracted with InstaGene matrix  
88 kit (Bio-Rad) in accordance with the manufacturer's recommendation. The isolates with a ESBL  
89 phenotype were analysed by PCR for the presence of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M</sub> type genes using  
90 primers and PCR conditions previously described (Liebana et al., 2004; Weill et al., 2004).  
91 Identification of the *bla*<sub>SHV</sub> genetic variant was done initially by sequencing a 409-bp short  
92 amplicon. Further primers (P-fw 5' GATGTATTGTGGTTATGCGTT 3'; P-rv 5'  
93 GTGCTRCGRGCCGATAAC 3') were then designed in order to sequence the entire *bla*<sub>SHV</sub>  
94 gene. A 30-cycle program with annealing at 60°C was used, resulting in a 1022 bp amplicon. The  
95 purified PCR fragments were sequenced on both strands with the "BigDye terminator v3.1 cycle

96 sequencing” kit (Applied Biosystems) using the same primers used in PCR reactions. Sequence  
97 analysis was performed on an 3100-Avant Genetic Analyzer (Applied Biosystems) and studied  
98 using the software SeqScape v2.1.1. The obtained nucleotide sequences and the derived amino  
99 acid sequences were compared with those previously described from the GeneBank database  
100 [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov) and [www.lahey.org/studies/webt.html](http://www.lahey.org/studies/webt.html), respectively.

101 **Nucleotide sequence accession numbers.** The nucleotide sequence reported in this study have  
102 been deposited in EMBL nucleotide sequence database under accession numbers EU137689 (*S.*  
103 *Braenderup* 1058/10); EU137690 (*S. Livingstone* 1124/2); EU137691 (*S. Livingstone* 1262/2);  
104 EU137692 (*S. Livingstone* 654/2); EU137693 (*S. Livingstone* 919/16); EU137694 (*S.*  
105 *Livingstone* 1987/3); EU137695 (*S. Livingstone* 2025/3); EU137696 (*S. Enteritidis* 2068/8);  
106 EU137697 (*S. Livingstone* 982); EU137698 (*S. Livingstone* 2030); EU137699 (*S. Livingstone*  
107 739/3); EU137700 (*S. Livingstone* 739/4).

108  
109 **Pulse-field gel electrophoresis (PFGE).** *S. Livingstone* SHV-12-producing isolates and a  
110 control strain (*S. Livingstone* LEP26 Salm-gene EQA panel) were subjected to PFGE analysis.  
111 The genomic DNA was prepared according to the PulseNet method ([www.cdc.gov/pulsenet](http://www.cdc.gov/pulsenet)) and  
112 digested with the restriction endonuclease *Xba*I (Roche Diagnostics, GmbH Mannheim,  
113 Germany). The *Salmonella enterica* serovar *Braenderup* H9812 (Pulse Net Standard) was used as  
114 the molecular size marker. In order to overcome problems related to DNA degradation, PFGE  
115 was also performed with the addition of 750  $\mu$ M thiourea (Sigma, T7875) to the electrophoresis  
116 PFGE buffer and agarose gel as previously described (Liesegang and Tschäpe, 2002; Bouallègue-  
117 Godet et al., 2005).

118 **RAPD-PCR (Random amplification polymorphism).** Sixteen *S. Livingstone* isolates were  
119 assayed by RAPD-PCR. Among these, ten were the SHV-12-producing isolates under study. Six

120 (ESC)-susceptible *S. Livingstone*, isolated from different sources (poultry carcasse, poultry meat  
121 and a quality control strain) and in different farms were also included in the study to test the  
122 discriminatory ability of RAPD analysis. Total DNA was extracted using the Wizard Genomic  
123 DNA purification System (Promega, Medison, WI). The RAPD-PCR reactions were performed  
124 using the Ready-To-Go RAPD analysis kit (GE Healthcare, UK) in accordance with the  
125 manufacturer's recommendations. All the samples were assayed with two different primers  
126 (primer#1: 5' GGTGCGGGAA 3'; primer#5: 5'AACGCGCAAC 3') and the 100 base-pair DNA  
127 marker (GE Healthcare, UK) was used as a molecular size standard.

128 To determine significant differences in the patterns, the reproducibility of results was assessed by  
129 repetition of three independent RAPD assays.

130 The cluster analysis of bands was carried out with InfoQuestFPSsoftware (Biorad) using Dice  
131 coefficient ( $S_d$ ) and unweighted average pair group method (UPGMA); the position tolerance  
132 was set at 1.5%.

133

## 134 **RESULTS**

135

### 136 **Antimicrobial resistance in *Salmonella* from poultry**

137 In 2005 and 2006, 789 and 1053 *Salmonella* poultry isolates were tested for antimicrobial  
138 resistance. Among these, 3 isolates in 2005 (0,4%) and 9 in 2006 (0,9%) were identified as  
139 (ESC)-resistant: 1 *S. Enteritidis*, PT6, 1 *S. Braenderup* and 10 *S. Livingstone*. All the strains were  
140 isolated from faeces and carcasses of poultry (chicken and turkey) but one from an environmental  
141 sample from a farm where animals were found positive as well. Over the period 2004-2006, *S.*  
142 *Livingstone* was the most common *Salmonella* serovar isolated from poultry in north eastern  
143 Italy. A marked increase of *S. Livingstone* isolates, rising from 51 (5,70 %) in 2002 to 329 (24,70



144 %) in 2006, was noted. The 401 *S. Livingstone* strains isolated in the period under study were  
145 completely susceptible to ciprofloxacin, chloramphenicol, kanamycin and enrofloxacin.  
146 Percentages of resistance < 5% were detected for all the other antimicrobials tested. The results  
147 of colistin were not included in the analysis of antimicrobial resistance.

148 The quantitative E-test confirmed ESBLs production in all the twelve strains characterized by  
149 resistant or intermediate values to ceftazidime and/or cefotaxime. The results of antimicrobial  
150 susceptibility of the ESBLs-producing isolates were confirmed by a microdilution broth assay to  
151 determine MIC values (Table 1). All the *S. Livingstone* isolates showed co-resistance to  
152 gentamicin (MIC  $\geq$  16  $\mu\text{g/ml}$ ) and nalidixic acid (MIC  $\geq$  32  $\mu\text{g/ml}$ ); among these, eight strains  
153 showed additional resistance to sulfonamides (MIC  $\geq$  512  $\mu\text{g/ml}$ ) and streptomycin (MIC  $\geq$  64  
154  $\mu\text{g/ml}$ ). *S. Braenderup* was completely susceptible to all additional drugs tested and *S. Enteritidis*  
155 PT6 showed also resistance to Tetracycline (MIC  $\geq$  64  $\mu\text{g/ml}$ ).

#### 156 **Characterization of $\beta$ -lactamase genes.**

157 The twelve strains showing a ESBL phenotype were screened for the presence of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>,  
158 *bla*<sub>CTX-M</sub> genes. All of them only gave positive results for the *bla*<sub>SHV</sub> PCR. Sequencing, followed  
159 by BLAST searches of the expected 409 bp amplicons, showed 100% of identity with the SHV-  
160 12  $\beta$ -lactamase gene. The amino acid sequences derived from the nucleotide sequences of a 1022  
161 bp PCR product identified a SHV-12 enzyme.

162 **Molecular typing of *S. Livingstone*.** The clonal relatedness of SHV-12 producing *S. Livingstone*  
163 was assessed by PFGE using XbaI. The isolates were not really typeable by PFGE, also adding  
164 thiourea to the running buffer and agarose gel; even with this poor quality due to partial  
165 degradation of DNA some bands were still identifiable, making some comparisons possible. The  
166 use of thiourea not made possible to obtain clear PFGE profiles of the isolates under study. All  
167 the strains were subsequently submitted to RAPD-PCR, as an alternative typing method, using

168 two different primers. The RAPD patterns obtained with primer#5 showed 7-11 bands ranging in  
169 size between 0.3 Kb and 2.0 Kb; the RAPD analysis with primer#1 gave patterns containing 8-9  
170 bands ranging in size between 0.2 Kb and 1.6 Kb. The similarity in cluster analysis was  
171 calculated to be > 85 % in replicate RAPD-PCR for both primers tested. The threshold for RAPD  
172 types identification was therefore set to 85 %. The number of RAPD types generated by cluster  
173 analysis at this level was 3 for RAPD performed with both primer P#1 (A1, B1,C1) and primer  
174 P#5 (A5, B5,C5). When using RAPD, in both case RAPD types A1 e A5 included all the (ESC)-  
175 resistant *S. Livingstone* isolates ( $S_d$  100%); RAPD types B1 ( $S_d$  100%) e B5 ( $S_d$  93%) included  
176 the three *S. Livingstone* isolates susceptible to all antimicrobial tested and RAPD types C1 ( $S_d$   
177 100%) e C5 ( $S_d$  94%) included the following *S. Livingstone* isolates 879/11 (R-type=AMP-SXT-  
178 SUL), 1077/3 (R-type= AMP-CEF-STR-TE-SXT-SUL) and the QC strain LEP26. (Fig 1).

179  
180 **DISCUSSION**

181 (ESC)-resistant *Salmonella* strains from poultry, belonging to different serovars, have been  
182 isolated in animals and food in United States and Europe since late 90s. In the USA, poultry and  
183 poultry products are most frequently contaminated with CMY-2-producing *Salmonella* (White et  
184 al., 2001; Chen et al., 2004). However, the epidemiology of ESC-resistant *Salmonella* seems to  
185 be quite different in Europe, where strains isolated from poultry and poultry meat mostly harbour  
186 Ambler class A  $\beta$ -lactamases (Weill et al., 2004; Politi et al., 2005; Hasman et al., 2005).

187 Some recently published papers point to poultry as an important animal *reservoir* of  $\beta$ -lactamase  
188 genes (Brinās et al., 2003; Weill et al., 2004; Hasman et al., 2005; Bertrand et al., 2006). Poultry  
189 is also frequently implicated in foodborne outbreaks suggesting a possible route of (ESC)-  
190 resistant *Salmonella* transmission to humans via the food chain.

191 A marked increase in *S. Livingstone* frequency, compared to *S. Enteritidis* and *S. Typhimurium*,  
192 was noted among *Salmonella* isolated from poultry during 2004-2006 in north eastern Italy. This  
193 serovar showed susceptibility or low percentage of resistance (< 5%) to all the drugs routinely  
194 tested for antimicrobial resistance in our laboratory. The number of ESBL-positive *Salmonella*  
195 isolates poultry-associated increased from 3 (0,4%) in 2005 to 9 (0,9%) in 2006 in the Northeast  
196 of Italy. All twelve strains under study ( $n=1$  *S. Enteritidis* PT6,  $n=1$  *S. Braenderup* and  $n=10$  *S.*  
197 *Livingstone*) carried a SHV-12  $\beta$ -lactamase. The multi-drug resistant *S. Livingstone* isolates were  
198 rough and untypeable by PFGE analysis as previously reported (Murase T. et al., 2004; Liebana  
199 et al., 2001). The RAPD-PCR analysis was the method showing the close relatedness of the ten  
200 SHV-12-producing *S. Livingstone* isolates and made it possible to discriminate among these and  
201 two different groups of (ESC)-susceptible *S. Livingstone* isolates. This relation suggests a  
202 possible common origin of the isolates, e. g. an infected flock of breeders from which the  
203 different flocks of broiler originated. This origin could reduce the significance of an increase in  
204 the prevalence of (ESC)-resistant isolates, were these strains are related to a possible single  
205 outbreak. However, the application of molecular typing methods with a higher level of specificity  
206 and reproducibility than RAPD-PCR should be used in order to confirm the clonal origin of the  
207 isolates. Although *S. Livingstone* human outbreaks are relatively uncommon, the increased  
208 prevalence reported demands careful monitoring and possible control actions in order to avoid the  
209 dissemination of *bla* genes to other *Salmonella* serotypes of human importance. Concern also  
210 arises from the recent sanitary alert for an ESBL CTX-M-2-producing *Salmonella* Virchow clone  
211 isolated at first from poultry and subsequently in human gastroenteritis cases in Belgium  
212 (Bertrand S. et al., 2006).

213 The SHV-12  $\beta$ -lactamase has been recently found to be widespread among ESBLs-producing  
214 isolates in clinical settings in Italy (Perilli et al., 2002). Furthermore, studies reporting SHV-12-

215 producing *Salmonella* and *E. coli* isolated from animals exist to date (Carattoli et al. 2005; Brinās  
216 et al., 2003; Riaño et al., 2006). *S. Livingstone* ESBL-SHV-12 producing isolates under study  
217 showed co-resistance to nalidixic acid, sulfonamides, streptomycin and gentamicin; it is worth  
218 noting that this latter resistance is reported rarely in *Salmonella* isolates. Both human and animal  
219 SHV-producing isolates have frequently shown additional resistance to these antimicrobials  
220 (Brinās et al., 2003; Riaño et al., 2006; Spanu et al., 2002). Although the use of ESC in poultry  
221 farms is infrequent in Italy, sporadic use of ceftiofur in breeder flocks, according to the allowed  
222 exception of the Directive 2004/28/EC, should be considered when examining possible reasons  
223 for the occurrence of this resistance. Amongst the isolates SHV-12 producing isolates reported in  
224 this study a *S. Enteritidis* was also identified and is a *Salmonella* of public health relevance,  
225 representing the serovar most frequently involved in human cases in Europe (The Community  
226 Summary Report 2005, EFSA). Careful surveillance and prudent use of antimicrobials in  
227 breeding is needed in order to prevent ESBLs-producing *Salmonella* dissemination and human  
228 transmission by food chain.

229

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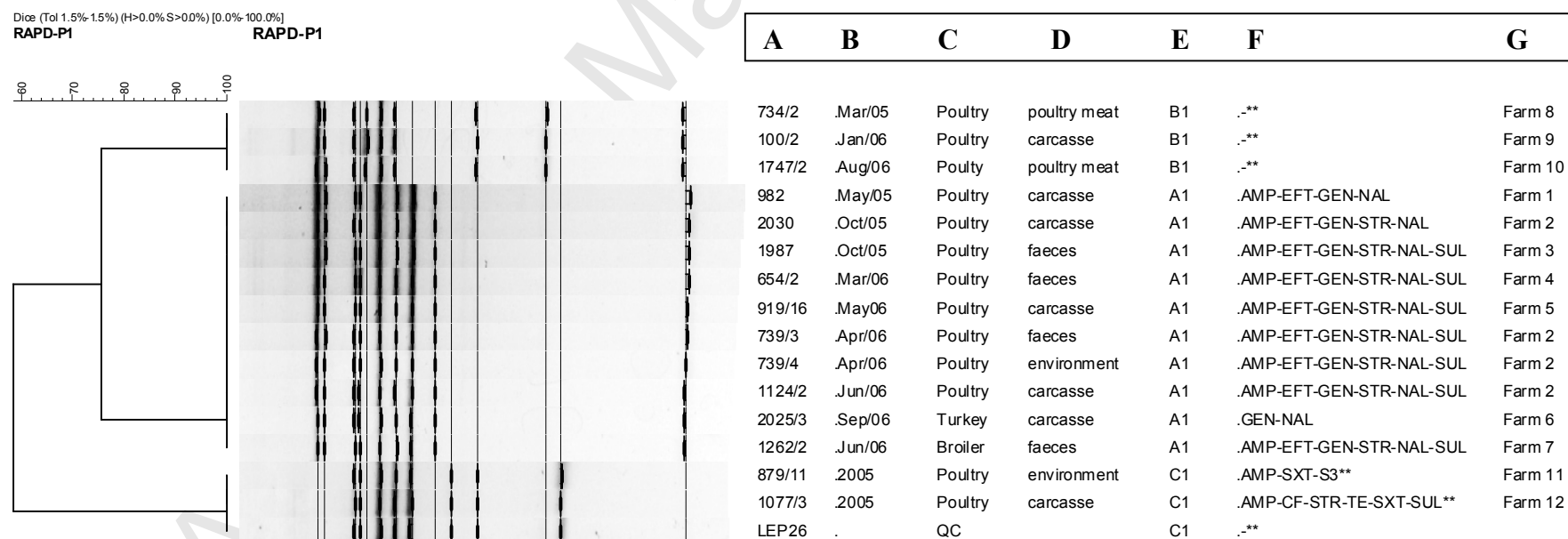
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1 Figure 1. Dendrograms showing the relatedness among RAPD types of *S. Livingstone* isolates (ESC)-resistant and (ESC)-susceptible  
 2 generated by primer#1 (I) and primer#5 (II) (InfoQuestFPSsoftware - Biorad).

3 I



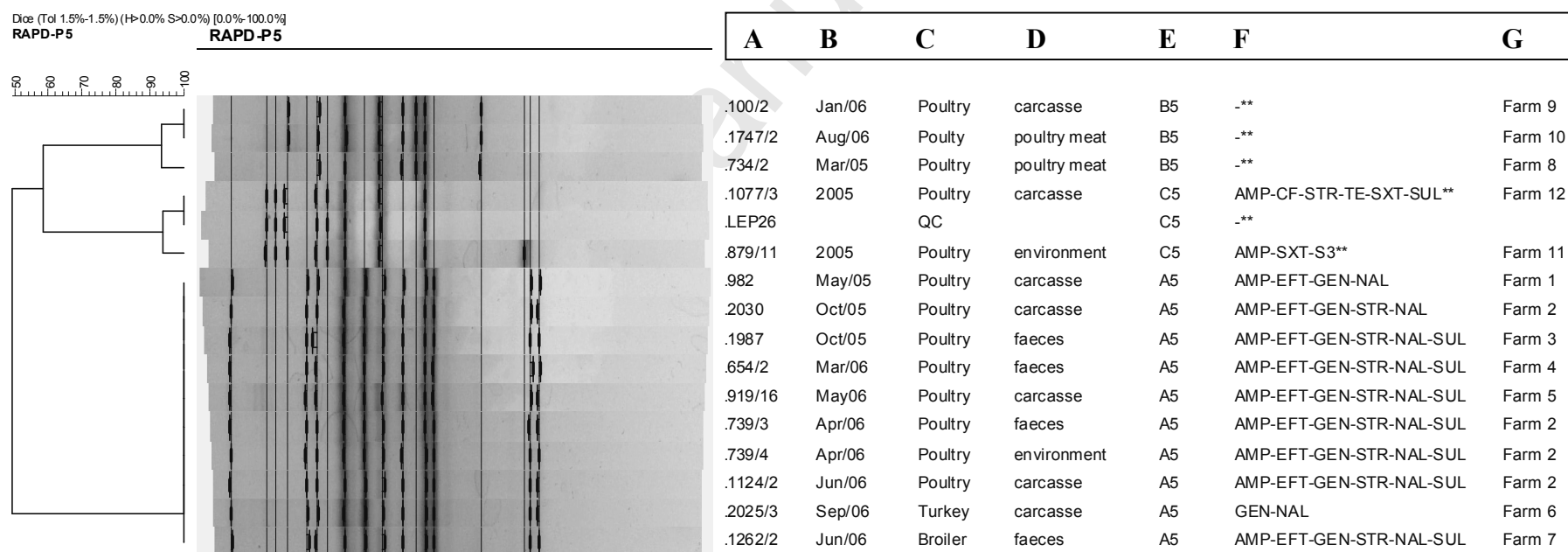
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8 II



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10 The figures I and II report the following information: A, isolate number; B: collection date; C-D: source of the isolate; E: RAPD-type;  
 11 F: antimicrobial resistance pattern; G: origin of the sample. Symbols: AMP: ampicillin; EFT: ceftiofur; GEN: gentamicin; NAL:  
 12 nalidixic acid; STR: streptomycin; SUL-S3: sulfonamides; TE: tetracycline; CEF: cephalothin; SXT: trimethoprim-sulfamethoxazole.  
 13 \*\*S. Livingstone ESC-susceptible isolates included in the study.

Table 1. MIC values for the twelve Salmonella SHV-12-producing isolates collected from poultry at NRL for Salmonella (2005-2006).

| N. isolate<br>and collection<br>date | MIC ( $\mu\text{g/ml}$ ) |            |      |           |     |          |              |           |           |          |            |                  |          |           |           |
|--------------------------------------|--------------------------|------------|------|-----------|-----|----------|--------------|-----------|-----------|----------|------------|------------------|----------|-----------|-----------|
|                                      | FOX                      | AMK        | CHL  | TET       | CRO | AMC      | CIP          | GEN       | NAL       | EFT      | SUL        | SXT              | KAN      | AMP       | STR       |
| 982/May05                            | 2                        | $\leq 0,5$ | 4    | $\leq 4$  | 16  | 2/1      | 0,12         | $\geq 16$ | $\geq 32$ | $\geq 8$ | 32         | $\leq 0,12/2,38$ | $\leq 8$ | $\geq 32$ | $\leq 32$ |
| 2030/Oct05                           | 2                        | $\leq 0,5$ | 4    | $\leq 4$  | 32  | 2/1      | 0,12         | $\geq 16$ | $\geq 32$ | $\geq 8$ | $\geq 512$ | $\leq 0,12/2,38$ | $\leq 8$ | $\geq 32$ | $\geq 64$ |
| 1987/Oct05                           | 2                        | $\leq 0,5$ | 4    | $\leq 4$  | 16  | 2/1      | 0,12         | $\geq 16$ | $\geq 32$ | $\geq 8$ | $\geq 512$ | $\leq 0,12/2,38$ | $\leq 8$ | $\geq 32$ | 64        |
| 654/2/Mar06                          | 2                        | 1          | $<2$ | $\leq 4$  | 16  | 2/1      | 0,12         | $\geq 16$ | $\geq 32$ | $\geq 8$ | $\geq 512$ | $\leq 0,12/2,38$ | $\leq 8$ | $\geq 32$ | $\geq 64$ |
| 919/16/May06                         | 4                        | $\leq 0,5$ | 4    | $\leq 4$  | 16  | 2/1      | 0,12         | $\geq 16$ | $\geq 32$ | $\geq 8$ | $\geq 512$ | $\leq 0,12/2,38$ | $\leq 8$ | $\geq 32$ | 64        |
| 739/3/Apr06                          | 2                        | 1          | 4    | $\leq 4$  | 8   | $<1/0.5$ | 0,12         | $\geq 16$ | $\geq 32$ | $\geq 8$ | $\geq 512$ | $\leq 0,12/2,38$ | $\leq 8$ | $\geq 32$ | 64        |
| 739/4/Apr06                          | 2                        | 1          | 4    | $\leq 4$  | 8   | 2/1      | 0,12         | $\geq 16$ | $\geq 32$ | $\geq 8$ | $\geq 512$ | $\leq 0,12/2,38$ | $\leq 8$ | $\geq 32$ | 64        |
| 1124/2/Jun06                         | 4                        | 2          | 8    | $\leq 4$  | 16  | 2/1      | 0,12         | $\geq 16$ | $\geq 32$ | $\geq 8$ | $\geq 512$ | $\leq 0,12/2,38$ | $\leq 8$ | $\geq 32$ | 64        |
| 2025/3Sept06                         | 2                        | 1          | 4    | $\leq 4$  | 16  | 2/1      | 0,12         | $\geq 16$ | $\geq 32$ | $\geq 8$ | $<16$      | $\leq 0,12/2,38$ | $\leq 8$ | $\geq 32$ | $<32$     |
| 1262/2/Jun06                         | 2                        | 1          | 4    | $\leq 4$  | 16  | 2/1      | 0,12         | $\geq 16$ | $\geq 32$ | $\geq 8$ | $\geq 512$ | $\leq 0,12/2,38$ | $\leq 8$ | $\geq 32$ | $\geq 64$ |
| 1058/10/ Jun06                       | 2                        | 1          | 4    | $\leq 4$  | 16  | 2/1      | $\leq 0.015$ | $<0.25$   | 1         | $\geq 8$ | 64         | $\leq 0,12/2,38$ | $\leq 8$ | $\geq 32$ | $<32$     |
| 2068/8/Sept06                        | 2                        | 1          | 4    | $\geq 16$ | 16  | 2/1      | $\leq 0.015$ | $<0.25$   | 2         | $\geq 8$ | 64         | $\leq 0,12/2,38$ | $\leq 8$ | $\geq 32$ | $<32$     |

FOX: cefoxitin; AMK: amikacin; CHL chloramphenicol; TE: tetracycline; CRO: ceftriaxone; AMC: amoxicillin-clavulanic acid; CIP: ciprofloxacin; GEN: gentamicin; NAL: nalidixic acid; EFT: ceftiofur; SUL: sulfonamides; SXT: trimethoprim-sulfamethoxazole; KAN: kanamycin; AMP: ampicillin; STR: streptomycin