Distribution of Multidrug Resistant isolates of *Salmonella* 1,4,[5],[12]:i- in Portugal, the new pandemic serovar

Rui Seixas1, Filipa Dias1, Jorge Machado1, Isa Serrano1, Miguel Castanho1, Fernando Bernardo1, Cristina Lobo Vilela1, Manuela Oliveira1

1 CIISA/Faculty of Veterinary Medicine, Technical University of Lisbon, Avenida da Universidade Técnica, 1300-477, Lisbon, Portugal.
2 The National Health Institute Doutor Ricardo Jorge (INS), Avenida Padre Cruz, 1649-016 Lisbon, Portugal.
3 Institute of Molecular Medicine, School of Medicine, University of Lisbon, Portugal.

In the mid-1990s it was reported in Europe the emergence of a pandemic monophasic variant of *Salmonella Typhimurium*, *S. enterica* subspp. *enterica* serovar 1,4,[5],[12]:i-, presently considered one of the major serovars responsible for human salmonellosis worldwide. The incidence of antimicrobial resistant 1,4,[5],[12]:i- strains has been escalating. The most frequent Multidrug Resistant (MDR) pattern, isolated from 30% of the human infection cases and from farming animals, is the ASSuT tetratressistance pattern, showing co-resistance to ampicillin, streptomycin, sulfonamides and tetracyclines.

This study aimed to characterize the distribution of ASSuT MDR *Salmonella* 1,4,[5],[12]:i- isolates in Portugal. The collection comprised 187 monophasic isolates obtained from 15 districts located in Portugal during a six years period (2006-2011) at the National Health Institute Doutor Ricardo Jorge. They were all previously serotyped and their identification was confirmed by multiplex PCR (mPCR) recommended by the European Food Safety Authority1. Each monophasic isolate confirmed by mPCR corresponds to a different clinical case, with the exception for three environmental isolates. They were evaluated for the presence of ASSuT profile using the disc diffusion method as recommended by the Clinical Laboratory Standards Institute guidelines, using the following antimicrobial compounds: ampicillin (AMP, 30 µg. Oxoid), streptomycin (S, 25µg. Oxoid), sulfamethoxazol (RL, 10 µg. Oxoid) and tetracyclin (TE, 30 µg. Oxoid).2 The isolates’ MDR profile was confirmed by Minimal Inhibitory Concentration determination using E-test (Biomerieux) as recommended by WHO’s Global Salm-Surv.3

From the 187 serotyped isolates, 133 (71.1%) were confirmed by mPCR as monophasic strains. These isolates (n=133) revealed an ASSuT profile prevalence of 63.9% (n=85). MDR isolates distribution through Portugal, evaluated by district, showed that Porto has the higher percentage of cases (25%), followed by Setúbal (14%) and Aveiro (13%). It is important to refer that in the vast majority of the districts included in this study, more than half of the Salmonellosis cases evaluated were promoted by ASSuT isolates.

This study shows the high incidence of monophasic *S. Typhimurium* isolates in Portugal, which are widely distributed from north to south of the country. It is important to characterize the distribution of these highly pathogenic isolates to prevent their dissemination to non-problematic districts and adequate the regulatory measures to their true prevalence. Similar or higher percentages of ASSuT profile frequency have been detected in other European countries.2 The study also confirms the importance of combining traditional serotyping methods with PCR, since misidentifications could have significant public health consequences.

**Diversity of β-lactamase-encoding genes among Gram-negative isolates from water samples in Northern Portugal**

V. Maneiro1, E. Ferreira1, V. Figueira1, C. M. Manaia1 and M. Caniça1

1 National Research Laboratory of Antimicrobial Resistances, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge. Av. Padre Cruz, 1649-016 Lisbon, Portugal
2 CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, 4200-072 Porto, Portugal

**Background**: Water has been recognized as a reservoir for antibiotic resistance genes (ARG), where the presence of mobile genetic elements, including plasmids, favors their dissemination. It is noteworthy that non-pathogenic environmental organisms, where plasmids encoding multiple ARG are prevalent, can provide resistance to most classes of antimicrobials including β-lactams, aminoglycosides, chloramphenicol, trimethoprim, streptomycin, fosfomycin, quinolones, among others. The main goal of this study was to evaluate the presence of ARGs, related with β-lactam and quinolone resistance, in Gram-negative bacteria isolates from surface and raw treated waste water environments.

**Methods**: Water samples were collected from different environments within an urban water cycle in the region of Northern Portugal, which included treated and raw wastewater, water to the consumers and water surface. Sampling was performed at different locations, with water sample collection in parallel to other independent sampling, in order to avoid introducing biases in the environmental background. A total of 50 water samples were collected from different locations within the region of Northern Portugal, which included treated and raw wastewater, water to the consumers and water surface.

**Results**: Overall, 16/56 isolates were multidrug-resistant (MDR), i.e. presenting a reduced susceptibility to 3 or more structurally unrelated antibiotics, suggesting a great diversity of resistance mechanisms. Noteworthy, 10 isolates (4 S. *pnni*, 1 A. *johnsonii*, 3 A. *veronii*, 1 K. *pneumoniae*, and 1 S. *maltophilia*) showed nonsusceptibility to carbapenems, which constitutes one of the last resorts on the antimicrobial therapy. Their phenotypic and molecular characterization revealed the expression of several enzymes: the naturally occurring carbapenemase in one *S. panni* producing isolate among the major incompatibility (Inc) groups, specifically FIA, FIB, FIC, HII, HII-1, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FII. Multilocus sequence typing (MLST) of the GES-5 *K. pneumoniae*-producing isolate was performed according to the Institute Pasteur scheme (http://www.pasteur.fr/recherche/genopole/PFS/mlst/Kpnepneumoniae.html).

**Conclusion**: This study identified ARGs related not only to commonly used antibiotics, but also to carbapenems, providing, at our knowledge, the first description of a GES-5-producing *Enterobacteriaceae* recovered in an environmental setting. The study highlights the need of surveillance of these antibiotic resistance environments in environmental backgrounds, since it represents a liable reservoir of potential pathogenic resistant bacteria. Worryingly, recent studies demonstrated that while the WWTP reduced the bacterial load, the treatment is insufficient to remove antibiotic resistant bacteria.

**Keywords**: Water, GES-5

---

**References**

5 External resistance, ASSuT, Portugal, *Salmonella* 1,4,[5],[12]:i-.

---

**Keywords**: antimicrobial resistance, ASSuT, Portugal, *Salmonella* 1,4,[5],[12]:i-.