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

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In the mid-1990s it was reported in Europe the emergence of a pandemic monophasic variant of *Salmonella* Typhimurium, *S. enterica* subsp. *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 tetraresistance pattern, showing co-resistance to ampicillin, streptomycin, sulphonamides 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) as recommended by the European Food Safety Authority[1]. 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), sulphamethoxazol (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 (BioMérieux) 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.[4] The study also confirms the importance of combining traditional serotyping methods with PCR, since misidentifications could have significant public health consequences.

References

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

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

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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 and 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. Screening of antimicrobial susceptibility of 56 Gram-negative isolates (20 *Escherichia coli*, 8 *Citrobacter* spp., 7 *Klebsiella* spp., 6 *Kluyvera* spp., 4 *Sphingomonas panni*, 2 *Enterobacter* spp., 1 *Acinetobacter johnsonii*, 3 *Aeromonas veronii*, 1 *Hafnia alvei*, 1 *Pantoea agglomerans,* 1 *Roultella ornithinolytica*, 1 *Serratia* sp., 1 *Stenotrophomonas maltophilia*), identified by 16S rRNA gene sequencing analysis using universal primers, was performed by disk diffusion method. Interpretative reading of susceptibilities allowed to direct the search for antibiotic resistant genes. PCR and sequencing were used to screen and identify beta-lactamase- and plasmid-mediated quinolone resistance (PMQRs)-encoding genes. All isolates were also screened for the presence of class 1 integrons. PCR-based replicon typing (PBRT) was used to type the resistance plasmids of the bla_{GES-5}-producing isolate among the major incompatibility (Inc) groups, specifically FIA, FIB, FIC, HI1, HI2, I1-Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA. 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/PF8/mlst/Kpneumoniae.html).

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. panni*, 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. maltophilia*, ImiS in three *A. veronii*, both MBLs, and OXA-type carbapenemase in one *A. johnsonii*, responsible for their intrinsic resistance; the class A GES-5-producing *K. pneumoniae* isolate belonged to a novel MLST sequence type, the ST961 (18-22-18-90-142-13-179). PBRT of the plasmid-carrying bla_{GES-5} gene showed that it did not belong to any of the Inc groups tested. No carbapenemases were found in the 4 *S. panni* isolates. The β-lactam resistance, carbapenem susceptibility, found in 33 isolates was justified by the presence of various Class A (12 bla_{TEM-1} with distinct promoters, 6 bla_{SHV}) and different Class C β-lactamase-encoding genes (bla_{CMY}, bla_{ACC}, bla_{ACT}), some here firstly described: bla_{CMY-65} (JF780936), bla_{CMY-89} (HE819403), bla_{CMY-90} (HE819404), bla_{ACT-13} (HE819402) and bla_{ACC-5} (HE819401). Class 1 integrons were detected among 6 of TEM-1-producing isolates. Together, the beta-lactamases identified explain the level of beta-lactam resistance. Besides quinolone resistance detected, none PMQR were identified, suggesting chromosomal alterations in the quinolone resistance-determining region.

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 mechanisms 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 inefficient to remove antibiotic resistant bacteria.

Keywords: Water, GES-5