Detection of Virulence Plasmids and Antimicrobial Resistance in Salmonella Isolated from Cattle — Project Report

Non-typhoidal *Salmonella* are zoonotic pathogens of major public health concern (GBD 2017 Non-Typhoidal *Salmonella* Invasive Disease Collaborators, 2019). Comprised of over 2,600 serovars, the serovar of the infecting *Salmonella* dictates disease type i.e., gastroenteritis, which is contained within the intestine, or non-typhoidal systemic disease with bacteraemia (dos Santos, Ferrari, and Conte-Junior, 2019). The virulence factors possessed by the infecting *Salmonella* can dictate disease severity. For example, the *Salmonella* virulence plasmid (pSV), which is associated with several serovars, plays a role in intracellular survival and systemic spread of the infection (Guiney and Fierer, 2011). Antimicrobial resistance (AMR) profiles can also affect disease severity and it is crucial to determine AMR profiles of infecting *Salmonella* to facilitate treatment.

The aims of this project were: (1) to test 37 *Salmonella* cattle isolates belonging to 10 different serovars—Agona, Anatum, Cerro, Dublin, Kentucky, Meleagridis, Montevideo, Reading, and Typhimurium—for the presence of pSV, and (2) to determine their susceptibility to a range of clinically-relevant antibiotics—ampicillin, chloramphenicol, kanamycin, nalidixic acid, streptomycin, and tetracycline.

To determine the presence of pSV, the native plasmids of each isolate were extracted by lysing the bacterial cells, removing the cell contents by centrifugation, and then washing away any smaller contaminants with isopropanol and 70% ethanol (Delaney, Murphy, and Walsh, 2018; QIAGEN, 2020). Subsequently, PCR was performed for the *spvABC* genes of the *spv* operon, which is carried on all pSV. pSV was detected in 48.6% (n = 18) of isolates, which belonged to the following serovars: Anatum (n = 4), Cerro (n = 4), Dublin (n = 3), Meleagridis (n = 3), Montevideo (n = 1), and Typhimurium (n = 3). The presence of pSV in Anatum, Cerro, Meleagridis, and Montevideo were interesting findings as these serovars are not routinely reported in the literature to carry pSV.

To determine antibiotic susceptibility, the agar dilution method was used. The bacteria were plated in different antibiotic concentrations, any subsequent growth was analysed qualitatively, and based on the MIC breakpoints published by the CLSI and NARMS, the susceptibility of each isolate was determined (Table 1). The results obtained were equally interesting and concerning in that multiple drug resistance (MDR)—defined as resistance to at least one antibiotic in three or more categories—was noted in 32.4% (n = 12) of isolates (Magiorakos *et al.*, 2011). Furthermore:

* Four isolates (Agona, n = 1; Dublin, n = 2; Newport, n = 1) were resistant to all six antibiotics. From these, one Dublin isolate was found to carry pSV.
* Five isolates (Dublin, n = 1; Montevideo, n = 1; Newport, n = 1; Typhimurium, n = 2) were resistant to all antibiotics except kanamycin. However, according to the CLSI (2020), for *Salmonella* spp., aminoglycosides may appear to inhibit growth *in vitro* but are not effective in a clinical setting. Therefore, it is very likely that some or all five of these isolates are, in fact, resistant to all antibiotics tested as well. From these, three isolates were found to carry pSV (Dublin, n = 1; Montevideo, n = 1; Typhimurium, n = 1).
* Of the remaining three isolates that displayed MDR (Anatum, n = 1; Kentucky, n =1; Meleagridis, n = 1), only the Anatum isolate was found to carry pSV.

**Table 1. *Summary of antibiotic susceptibility testing results.*** (\*) These percentages may not be indicative of the true proportion of isolates that is susceptible to this aminoglycoside in a clinical setting.

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| --- | --- | --- | --- | --- |
| **Antibiotic** | **Percentage (%)** | | | |
| **Susceptible** | **Intermediate** | **Resistant** | **?** |
| **Ampicillin** | 16.2 | 45.9 | 32.4 | 5.4 *(n = 2)* |
| **Chloramphenicol** | 64.9 | 2.7 *(n = 1)* | 29.7 | 2.7 *(n = 1)* |
| **Kanamycin** | *64.9\** | 13.5 | 16.2 | 5.4 *(n = 2)* |
| **Nalidixic acid** | 48.6 | — | 51.4 | — |
| **Streptomycin** | *2.7\* (n = 1)* | — | 97.3 | — |
| **Tetracycline** | 64.9 | — | 35.1 | — |

When I was awarded a UKRI BBSRC-funded Research Experience Placement by the EASTBIO Doctoral Training Partnership to work on this project under the supervision of Dr. Prerna Vohra, I was very honoured and excited. My main goals going into this project were to broaden my knowledge in the field of bacteriology, improve my research and wet lab skills as well as get a good grasp of what a career in research would entail. At the end of my project, not only had I met all of my personal goals but I was also pleasantly surprised by how far along I had come in my research. I was even introduced to bioinformatics: I designed primers, performed gene alignments to look for variation and had the opportunity to hunt for the sequences of the *spvABC* genes in the genomes of the Dublin isolates using Artemis. This was not something I thought I would get to try during my project and although there was not enough time to do more work than that, I am glad to have been introduced to this field as well. While I am sad that my time working in the Vohra lab has come to an end, I look forward to continuing with my studies and even pursuing a PhD degree after completing my undergraduate one.

References

CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

Delaney, S., Murphy, R., and Walsh, F. (2018) ‘A Comparison of Methods for the Extraction of Plasmids Capable of Conferring Antibiotic Resistance in a Human Pathogen From Complex Broiler Cecal Samples’, *Frontiers in Microbiology,* 9. doi: <https://doi.org/10.3389/fmicb.2018.01731>

dos Santos, A. M. P., Ferrari, R. G., and Conte-Junior, C. A. (2019) ‘Virulence Factors in *Salmonella* Typhimurium: The Sagacity of a Bacterium’, *Current Microbiology,* 76, pp. 762-773. doi: <https://doi.org/10.1007/s00284-018-1510-4>

GBD 2017 Non-Typhoidal *Salmonella* Invasive Disease Collaborators (2019) ‘The global burden of non-typhoidal salmonella invasive disease: a systematic analysis for the Global Burden of Disease Study 2017’, *The Lancet Infectious Diseases,* 19(12), pp. 1312-1324. doi: <https://doi.org/10.1016/S1473-3099(19)30418-9>

Guiney, D. G. and Fierer, J. (2011) ‘The role of the *spv* genes in *Salmonella* pathogenesis’, *Frontiers in Microbiology,* 2. doi: <https://doi.org/10.3389/fmicb.2011.00129>

Magiorakos, A. P., Srinivasan, A., Carey, R. B. *et al.* (2012) ‘Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance’, *Clinical Microbiology and Infection,* 18(3), pp. 268-281. doi: <https://doi.org/10.1111/j.1469-0691.2011.03570.x>

NARMS (2019) *Antibiotics Tested by NARMS* [online]. Available at: <https://www.cdc.gov/narms/antibiotics-tested.html> [Accessed: 23/08/2023].

QIAGEN (2020) *QIAprep® Miniprep Handbook*. Available at: <https://www.qiagen.com/us/resources/download.aspx?id=22df6325-9579-4aa0-819c-788f73d81a09&lang=en> [Accessed: 26/07/2023].