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Characterization of a multidrug-resistant (MDR) Salmonella enterica serovar I 4,[5],12:i:- isolate associated with a 2015 foodborne outbreak from pork

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Introduction

Nontyphoidal *Salmonella* is a leading cause of bacterial foodborne disease in humans. *Salmonella enterica* serovar I 4,[5],12:i:- has emerged as the fourth most frequent cause of human salmonellosis in the U.S. based on 2015 National Antimicrobial Resistance Monitoring System (NARMS) data, and the most common multidrug-resistant (MDR; resistance to 3 or more antimicrobial classes) serovar with ~68% of isolates being considered MDR (CDC, 2018). An MDR serovar I 4,[5],12:i:- outbreak was linked to pork in 2015 with 188 infections and 30 hospitalizations (Kawakami, et al. 2016); 523,380 pounds of pork were recalled. The pork outbreak-associated isolates were resistant to ampicillin, streptomycin, sulfisoxazole, and tetracycline (R-type ASSuT). Colonization and pathogenesis of a pork outbreak-associated serovar I 4,[5],12:i:- isolate in swine was consistent with trials conducted with virulent serovar Typhimurium, indicating that the increased prevalence of serovar I 4,[5],12:i:- is not due to an increase in pathogenesis (Shippy, et al. 2018). We sought to characterize strain FSIS1503788 associated with the pork outbreak and its derivatives using genomic, transcriptomic, and phenotypic analysis.

Material and Methods

Salmonella serovar I 4,[5],12:i:- strain FSIS1503788 was recovered from the cecal contents of a pig postslaughter during investigation of the 2015 outbreak by FSIS. Strains SX240 (isolated from swine ileocecal lymph node following passage) and BBS 1270 [*Salmonella* Genomic Island 4 (SGI-4) deletion mutant] are derivatives of strain FSIS1503788. Recombineering was used to delete SGI-4 from FSIS1503788 by insertion of *neo*, resulting in a kanamycin resistant phenotype for BBS 1270.

The genome sequence of strain FSIS1503788 was assembled using PacBio sequencing reads and error corrected using Illumina data. Growth of strains FSIS1503788 and BBS 1270 were evaluated using Biolog Phenotype MicroArrays to determine metal tolerance. Transcriptional analysis using RNA-Seq was determined for FSIS1503788 and BBS 1270 following 60 minutes of growth during early log-phase in the presence or absence of 5 mM copper sulfate.

Swine were administered a diet with or without zinc oxide (2,000 mg/kg) and copper chloride (200 mg/kg; n=10/group) for 4 weeks prior to and 3 weeks post-inoculation with 8 x 10^7 CFU of SX240 via intranasal route. Fecal shedding of serovar I 4,[5],12:i:- was monitored at 2, 7, 14, and 21 days post-inoculation (dpi) with SX240. Colonization of the cecum, cecal contents, ileocecal lymph nodes, and Peyer's Patches region of the ileum by SX240 was determined at 21 dpi.

Results

Genome analysis of pork outbreak-associated serovar I 4,[5],12:i:- isolate FSIS1503788 indicates 2 large insertions compared to serovar Typhimurium. An ~28 kb module contains antimicrobial resistance genes for R-type ASSuT and mercury tolerance genes; the insertion deletes ~15 kb of the *fljB* genomic region, resulting in the monophasic phenotype. *Salmonella* genomic island 4 (SGI-4) is ~80 kb and encodes genes for metal tolerance (copper, silver, and arsenic) and DNA mobilization and transfer.

Growth in Biolog Phenotype Microarrays (Figure 1) indicate that strain FSIS1503788 has increased tolerance to copper and arsenic compounds compared to BBS 1270 (SGI-4 deletion mutant) or serovar Typhimurium (data not shown).

Exposure of SX240 to 5 mM copper for 60 minutes resulted in significant differential expression of 1,635 genes including transcriptional induction of metal tolerance genes for copper, arsenic, silver, and mercury; copper tolerance genes in both the core genome and SGI-4 were induced.

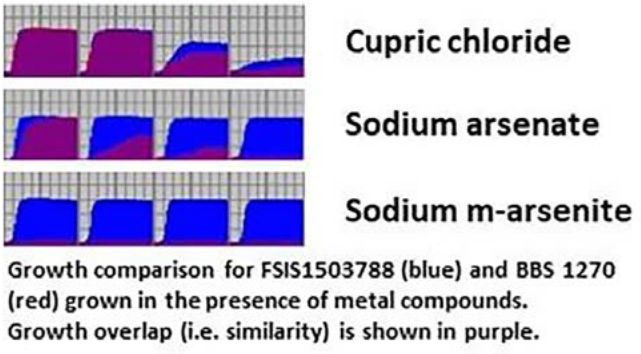

Figure 1. Reduced metal tolerance of BBS 1270 (SGI-4 mutant) compared to wildtype FSIS1503788.

Inclusion of copper and zinc as an antimicrobial in the swine diet did not significantly reduce quantitative fecal shedding (2, 7, 14, or 21 dpi) or intestinal tissue colonization (21 dpi; data not shown) of SX240 compared to control pigs receiving a diet without metals. A strong trend towards a significant increase (P=0.0572) in fecal shedding of SX240 was seen at 21 dpi in Zn/Cu fed swine compared to control pigs (Figure 2).

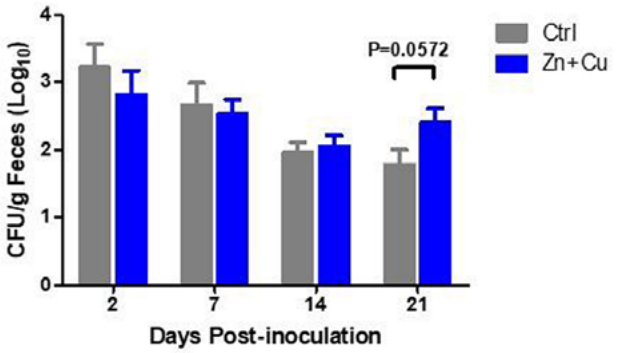

Figure 2: Swine fecal shedding following Salmonella serovar I 4,[5],12:i:- inoculation

Discussion and Conclusion

Salmonella serovar I 4,[5],12:i:- has emerged as a common cause of human salmonellosis and the most frequent MDR *Salmonella* serovar in the U.S. Serovar I 4,[5],12:i:- strain FSIS1503788 has 2 large DNA insertions conferring antimicrobial resistance (R-type ASSuT) and metal tolerance (copper, silver, arsenic, and mercury); this 2015 pork outbreak-associated isolate is genetically related to other MDR serovar I 4,[5],12:i:- strains that are globally distributed (Europe, Australia, and Japan).

Exposure of serovar I 4,[5],12:i:- to 5 mM copper for 60 minutes resulted in a metabolic shift with significant differential expression of >1,600 genes including induction of metal tolerance genes present in the core genome, SGI-4 (copper, silver, and arsenic), and the antimicrobial resistance module (mercury). This suggests that exposure of serovar I 4,[5],12:i:- to copper may co-select for the MDR phenotype of this strain due to induction of mercury tolerance genes located on the antimicrobial resistance module.

The inclusion of copper and zinc in the diet did not reduce swine fecal shedding or intestinal tissue colonization of serovar I 4,[5],12:i:-, and at 21 dpi, a strong trend for increased fecal shedding in pigs administered the metals was observed. The use of copper and zinc in swine feed as alternatives to antimicrobials to limit microbial pathogens may have the unintended consequence of selecting for the persistence of MDR *Salmonella* serovar I 4,[5],12:i:- in swine production.

The prevalence of MDR *Salmonella* serovar I 4,[5],12:i:- has increased globally and the combined presence of multiple antimicrobial resistance and metal tolerance genes may be beneficial for swine colonization or environmental survival.

References

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