

Molecular Identification and Antimicrobial Resistance Assessment of Bacterial Isolates Collected from Sheep Carcasses in Honduran Harvest Facilities

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I. INTRODUCTION

Shiga-toxin producing *Escherichia coli* (STEC) causes over 2.8 million human illnesses globally each year [3]. Commonly found in the feces of ruminants, STEC from the hides or GI tract often contaminate carcasses during the harvest process [2]. MLG 5B.05 and 5.09 guidelines are commonly used to isolate and identify STEC isolates in processing plant environments. However, whole genome sequencing provides a confirmation standard for identity. However, the identity often doesn't correlate to the MLG 5B.05 and 5.09 guideline [1] results of presumptive positives. Frequently associated with STECs, antimicrobial resistance genes (AMR) on the chromosome or acquired through mobile elements in the food chain, are a growing concern to public health [4]. The preliminary objective of this study was to confirm presumptive positive STEC isolates, observe distribution of virulence factors and AMR genes through whole genome sequence (WGS) analysis of isolates collected from sheep hides, pre-evisceration carcasses and carcasses going into the cooler in Honduran slaughter facilities.

II. MATERIALS AND METHODS

A total of 10 presumptive STEC isolates were collected following the MLG reference methods from sheep hides, pre-evisceration carcasses and carcasses ready for chilling in Honduran slaughter facilities and further characterized by WGS. Each presumptive STEC was confirmed through Latex Agglutination and BAX with varying O serotypes. Frozen isolates were recovered on brain heart infusion (BHI) agar at 37°C for 24 hours. Overnight cultures of one isolated colony in 3ml of BHI broth, were subject to genomic DNA extraction using GenElute™ Bacterial Genomic DNA. Pure gDNA extractions were quantified using a Qubit 2.0 Fluorometer and library preparations were performed using the Nextera XT DNA Library Preparation Kit following manufacturer's recommendations. Whole genome sequencing was performed on Illumina MiSeq DNA platform using 1ng of gDNA. Raw sequencing reads were assembled using SPADES pipeline [8] and annotated through Rapid Annotation using Subsystem Technology [6]. Assemblies were processed through the Center for Genomic and Epidemiology [5] website and analyzed to confirm genus and species using SpeciesFinder, and KmerFinder, virulence factors through VirulenceFinder, and investigated AMR genes using ResFinder and KmerResistance.

III. RESULTS AND DISCUSSION

After analysis, all 10 samples were not identified as *Escherichia coli* even though they complied with MLG standards for presumptive positive isolates. VirulenceFinder did not observe O serotypes on any isolate. The genera and species identified after whole genome analysis were *Providencia stuartii* (n=1), *Morganella morganii* (n=3), *Klebsiella quasipneumoniae* (n=1), and *Citrobacter freundii* (n=1) which belong to the Enterobacteriaceae family (Table 1). *Aeromonas hydrophila* (n=4) was also identified and isolated from hide and pre-evisceration samples of different animals. Despite the lack of confirmation of presumptive STECs, multiple AMR genes were observed on each isolate. Resistance genes were found for aminoglycoside, phenicol, tetracycline, β -lactams, colistin, and quinolone antimicrobials as shown in table 1. Multi-drug resistance genotypes were observed on all samples. β -lactam resistance genes were present on 9/10 isolates, potentially correlated to the availability of over-the-counter antibiotics and the heavy use in human and animal health industries in Honduras. Several AmpC-like genes, providing resistance to β -lactam inhibitors, penicillins, and first, second, and third-generation cephalosporins, were detected, including *bla*_{MOR-2}, *bla*_{CMY-10}, *bla*_{CMY-65} and *bla*_{MOX-6}. Furthermore, colistin resistance gene, *mcr-3*, likely on a plasmid as previously described in literature [3], was observed on two *Aeromonas hydrophila* isolates. Antimicrobial resistance genes could be present on the chromosome or easily transferred by mobile elements such as plasmids or transposons.

IV. CONCLUSION

After WGS analysis, presumptive positive STEC isolated from hides, pre-evisceration and final carcasses from Honduran sheep, after isolated using MLG guidelines and confirming through Latex Agglutination and BAX were found to not be *Escherichia coli*. Based on the results of this study, a more stringent confirmation process for presumptive positive STEC utilizing the MLG 5B.05 and 5.09 guidelines should be performed to ensure *E. coli* are the species being analyzed. Nevertheless, various gram-negative bacterial species were identified and characterized for AMR genotypes. β -lactam resistant genes were observed, encoding for AmpC-like and carbapenemase enzymes along with colistin resistance gene, *mcr-3*. To our knowledge, this is the first report of colistin resistance genes in bacterial isolates from the food chain in Honduras. Having AMR genes present in the food chain causes an increasing concern for consumers and public health agencies. With the possible transfer of these AMR genes to foodborne pathogens such as STECs, proper interventions should be performed in the harvesting chain to decrease this risk.

Table 1. Antimicrobial Resistance Genes of Whole Genome Sequenced Isolates Collected from Honduran Sheep Hides, Pre-evisceration, and Final Carcasses. (N=10)

Type	Animal	Species	AMR ² Gene	AMR ²
Hide	1	<i>Providencia stuartii</i>	<i>aac(2')-Ia</i>	Aminoglycoside
			<i>catA3</i>	Phenicol
			<i>tet(B)</i>	Tetracycline
Hide	2	<i>Aeromonas hydrophila</i>	<i>ampH</i>	Beta-lactam
			<i>cphA8</i>	Beta-lactam
			<i>mcr-3.6</i>	Colistin
			<i>mcr-3.4</i>	Colistin
Hide	3	<i>Morganella morganii</i>	<i>blaMOR-2</i>	Beta-lactam
			<i>catA2</i>	Phenicol
			<i>tet(D)</i>	Tetracycline
Hide	4	<i>Morganella morganii</i>	<i>blaMOR</i>	Beta-lactam
			<i>catA2</i>	Phenicol
			<i>tet(D)</i>	Tetracycline
Pre-evis	1	<i>Aeromonas hydrophila</i>	<i>ampH</i>	Beta-lactam
			<i>cphA8</i>	Beta-lactam
			<i>mcr-3.6</i>	Colistin
			<i>mcr-3.4</i>	Colistin
Pre-evis	2	<i>Morganella morganii</i>	<i>blaDHA-2</i>	Beta-lactam
			<i>catA2</i>	Phenicol
			<i>tet(D)</i>	Tetracycline
Pre-evis	3	<i>Klebsiella quasipneumoniae</i>	<i>blaOKP-B-5</i>	Beta-lactam
			<i>fosA</i>	Fosfomycin
			<i>oqxA</i>	Quinolone
			<i>oqxB</i>	Quinolone
Pre-evis	4	<i>Aeromonas hydrophila</i>	<i>blaOXA-504</i>	Beta-lactam
			<i>blaCMY-10</i>	Beta-lactam
Final	5	<i>Aeromonas hydrophila</i>	<i>blaOXA-504</i>	Beta-lactam
			<i>blaMOX-6</i>	Beta-lactam
			<i>tet(E)</i>	Tetracycline
Final	6	<i>Citrobacter freundii</i>	<i>blaCMY-65</i>	Beta-lactam
			<i>qnrB38</i>	Quinolone

¹ Shiga-Toxin producing *Escherichia coli*

² Antimicrobial Resistance Genes

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