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Detection of Multidrug Resistant *Salmonella* with Next Generation Sequencing

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Summary

We used whole-genome sequencing (WGS) technology to identify known antimicrobial resistance genes in 20 samples of *Salmonella enterica* Serovar Infantis (Enterobacteriales Enterobacteriaceae) and correlated genomic data with susceptibility phenotypes to evaluate the possibility of surveillance of multidrug resistant strains by means of WGS sequencing. Twenty *Salmonella* samples were selected among retail chicken meat and human clinical isolates that were tested for susceptibility to 15 antimicrobials using broth microdilution. The MIC for each drug was used to categorize isolates as susceptible or resistant based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute clinical breakpoints (CLSI). Each isolate was subjected to whole-genome shotgun sequencing according to Illumina protocol. Raw reads were assembled by means of software Spades ver. 3.11 and resistance genes were identified from assembled sequences through software ResFinder ver. 3.0. A total of 127 plasmidic resistance genes were identified in all the samples. We found a good concordance between phenotypic resistance profiles and genotypic data.

Key Words

Salmonella; contaminated food; WGS technology.

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Introduction

Salmonella enterica Kauffmann et Edwards, 1987 (Enterobacteriales Enterobacteriaceae) is one of the most common causes of bacterial foodborne illnesses worldwide. In fact, manipulation, transportation, and marketing of poultry products in some case do not meet the standards of good manufacturing practices, promoting contamination of food product with *Salmonella* strains (Cortes Vélez et al., 2017). The majority of *Salmonella* Lignieres, 1900 infections are attributed to consumption of contaminated food of animal origin such as eggs, chicken, pork, etc. (Hur et al., 2016). For decades, the development of antimicrobial multidrug resistance in many *Salmonella* isolates often caused by misuse of antibiotics for treatment of non bacterial diseases or viral infections has been occuring. According to the American Centers for Disease Control and Prevention, up to one-third to one-half of antibiotic use in humans is unnecessary or inappropriate.

Recently, the occurrence of *Salmonella* isolates resistant to quinolones, fluoroquinolones or extended-spectrum cephalosporins antibiotics has increased among food and human isolates.

Recently, the public health commision was established to track resistance in *Salmonella*, *Campylobacter*, and other foodborne bacteria by comparing strains from food-producing animals, raw retail meats, and human infections (McDermott et al., 2016).

Material and Methods

The bacterial suspensions of *Salmonella enterica* isolates were inoculated in Mueller-Hinton (Oxoid, Germany) agar plates and cultured at 37 ºC for 24 hours. The susceptibility to various antibiotics molecules was evaluated according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2015). The agar plates containing the isolates were subjected to an antimicrobial microdilution suscepti-

bility test by using the BD PhoenixTM NMIC/ID-132 panels (Becton Dickinson, Sparks, MD, USA).

Considering three level of antimicrobial concentration, *Salmonella* strains were classified in resistant, intermediate, and susceptible (European Committee for Antimicrobial Susceptibility Testing: EUCAST, 2003).

Sequencing of the *Salmonella* isolates was performed at Molecular Biology Department of Experimental Zooprophylactic Institute of Sicily, Palermo. Genomic libraries were prepared according to Nextera DNA library preparation kit protocol (Illumina, San Diego, CA). The library pools were subjected to sequencing on a MiSeq platform (Illumina), generating 150-bp length paired end reads. (Faksri et al., 2016).

Reads were assembled using a local installation of Spades software, a de novo assembler that uses

Phenotype	***Resistance gene***
Aminoglycoside resistance	aadA1
	aadA2
	aph(3'')-Ib
	aph(3')-Ia
	aph(3')-Ic
	aph(3')-III
	aph(6)-Id
Beta-lactam resistance	blaCTX-M-1
	blaTEM-1B
	blaTEM-1D
Colistin resistance	mcr-1
Macrolide resistance	erm(B)
	mef(B)
Phenicol resistance	cat
	catA1
	cmlA1
Sulphonamide resistance	sul1
	sul2
	sul3
Tetracycline resistance	tet(A)
	tet(J)
Trimethoprim resistance	dfrA1
	dfrA14

Table 1. List of resistance genes found in *Salmonella enterica* samples through bioinformatics analysis.

Mutation	Nucleotide change	Resistance
gyrA p.D87G	GAC ➡ GGC	Quinolones

Table 2. Point mutation in chromosomal gene of various *Salmonella enterica* strains.

short read sets as input (e.g. Illumina Reads), and applies the de Bruijn graphs algorithm for generating longer contigs for following elaboration. Assembled reads were queried against Resfinder database ver. 3.0 (Center for Genomic Epidemiology, DTU), containing a list of 2215 resistance genes for most diffuse microbial organism and currently updated with new antibiotic resistance genes (Zankari et al., 2012).

Results

In our result we found a total of 23 resistance genes transmitted by means of plasmid vectors between bacterial strains and 1 point mutation involving a chromosomal gene that is involved in Quinolone resistance. The analysis pipeline involved the whole genome sequencing by means of Illumina Miseq NGS platform and a bioinformatic analysis pipeline developed by Center for Genomic Epidemiology of Danmark CGE - DTU. A detail of resistance genes are shown in Table 1. The point mutation found in the samples of *Salmonella* species is shown in Table 2.

This result were comparable with classical microbiological phenotypic resistance test. In particular the techniques of agar antibiotic disk and that of minimum inhibitory concentration (MIC) were tested against the *Salmonella enterica* isolates. Disks containing antibiotics solution or strips with a gradient of antimicrobial solution were placed onto agar plates to detect the minimum concentration of antimicrobial at which bacteria cannot grow. All samples showed resistance producing genes like blaCTX-M-1, blaTEM-1B, and blaTEM-1D for beta-lactamase, or tet(A) and tet(J) genes for tetracycline resistance, and other showed in Table 1.

The data were confirmed by phenotypic resistance test including resistance to antibiotics as beta-lactam antibiotics (Ampicillin, Ticarcillin, Ampicillin), Tetracycline antibiotics (Tetracycline, Doxycycline), Quinolones (Nalidixic acid), Phenicol resistance (Chloramphenicol) and Sulphonamide resistance genes.

Discussion and Conclusions

The phenotypic MDR pattern of *Salmonella* showed good correlation with the genotypic analysis. Many resistance genes were present and correlated positively with the resistance phenotype.

The presence of a resistance gene in a bacterial strain does not mean that it is biological active (e.g. may not be transcribed or translated) but it can be very indicative of the presence of phenotypic resistance in a particular strain.

The presence of a particular gene was tested with classical Polymerase Chain reaction PCR emplying specific primers.

The advantage of NGS technics is low cost per sample, standardized library preparation, possibility to detect unknown genes or mutations in known genes, the possibility to store the data produced for further elaboration like transcriptional analysis, metagenomic analysis (to study more species and resistance in the same samples), and many other.

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