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Subtyping *Salmonella* enterica serovar Derby with multilocus sequence typing (MLST) and clustered regularly interspaced short palindromic repeats (CRISPRs)

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ABSTRACT

Salmonella Derby is one of the most prevalent serovars in pork and the most common serotype isolated from infants and toddlers. However, *Salmonella* Derby is also poorly understood, so we used Multilocus sequence typing (MLST) and Clustered regularly interspaced short palindromic repeats (CRISPRs) to subtype 100 *Salmonella* Derby isolates from pig farms, pig slaughterhouses, retail markets and humans that were collected during different years in Yangzhou, Jiangsu Province, China, in respect to the transmission of clonal groups of the serovar along the food chain. MLST analysis showed that two sequence type (ST) patterns (ST40 and ST71) were shared, and ST40 was the most common sequence type among isolates from four different sources. CRISPRs typing identified 32 different Derby CRISPR types (DCTs); ST40 and ST71 strains had 21 and 11 DCTs respectively, demonstrating the distinctiveness of the CRISPR regions among the isolates from the four sources during a seven-year period. It demonstrated that *Salmonella* Derby clones persisted in the same places and spread along the pork production chain. Overall, 100 spacers were analysed, including 61 for CRISPR1 (18 new) and 39 for CRISPR2 (17 new). Interestingly, we also found that the spacer arrangements were distinct between ST40 and ST71 strains, except for strain 13-S1. This analysis revealed that CRISPR genes are highly polymorphic even in the same serotype, which could be tremendously useful for bacterial subtyping during molecular epidemiological investigations.

1. Introduction

Salmonella Derby was first isolated from humans who were infected after ingesting contaminated pork pies (Peckham & Savage, 1923). In 1946, an outbreak of gastro-enteritis among infants in a hospital was caused by *Salmonella* Derby; sixty eight children were involved in this outbreak and ten died (MUSHLN, 1948). *Salmonella* Derby is now one of the most frequent *Salmonella* enterica serovars found in pigs across the EU, and pork is a major source of foodborne salmonellosis in the EU and many other countries (Hauser et al., 2011; Kerouanton, Rose, Weill, Granier, & Denis, 2013). *Salmonella* Derby is the most common serotype isolated from infants and toddlers in China (Cui et al., 2009). Therefore, as one of the largest pork producing and consuming countries in the world, great attention should be paid to the prevalence of *Salmonella* Derby with respect to pig farms, pig slaughterhouses, retail markets and humans in China.

The genetic diversity of *Salmonella* Derby strains has not been extensively investigated. Multilocus sequence typing (MLST), a recently developed methodology that requires minimal human input, has been used to type *Salmonella* strains (Kotetishvili, Stine, Kreger, Morris, & Sulakvelidze, 2002). The data available for *Salmonella* Derby (http://mlst.ucc.ie/mlst/mlst/dbs/Senterica/) indicate that the serovaris polyphyletic, having originated from more than one common ancestor, and it possesses several distantly related STs (Hauser et al., 2011). Clustered regularly interspaced short palindromic repeats (CRISPRs) are bacterial loci whose

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dynamic nature has made them ideal targets for molecular subtyping (Shariat & Dudley, 2014). CRISPRs have been shown to be better for identification and for distinguishing between *Salmonella* outbreak strains/clones than PFGE (Liu, Barrangou, et al., 2011), with the additional benefits of being faster and less costly. This study was conducted to gain a better understanding of the clonality of *Salmonella* Derby and of the subtypes actually transmitted to humans from pigs via pork in China. For this purpose two different sequence-based approaches were applied to a set of 100 *Salmonella* Derby strains from pig farms, pig slaughterhouses, retail markets and humans.

2. Materials and methods

2.1. Serotyping and DNA extraction

The 100 *Salmonella* Derby strains used for the analysis were collected in Yangzhou, Jiangsu Province, China from 2009 to 2015 (Table 1). Twenty-one *Salmonella* Derby strains were isolated from porcines, and 40 *Salmonella* Derby strains were isolated from pig slaughterhouses. Another 31 *Salmonella* Derby strains were isolated from pork. The remaining 8 *Salmonella* Derby strains were isolated from human gastroenteritis cases. All strains were serotyped according to the White–Kauffmann–Le Minor schemeby slide agglutination with O- and H-antigenspecific sera (SSI Diagnostika, Hiller, Denmark). Confirmed isolates were grown aerobically, overnight at 37 °C in LB broth with shaking. Then genomic DNA was extracted with the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

2.2. Multilocus sequence typing (MLST)

All strains were further characterized by MLST using seven housekeeping genes *aroC, dnaN, hemD, hisD, purE, sucA and thrA* (Kidgell et al., 2002). The seven housekeeping gene sequences for each isolate were uploaded to the MLST database for comparison (http://mlst.ucc.ie/mlst/dbs/Senterica), which allowed us to determine the sequence type (ST). When necessary, new alleles and sequence types were submitted to the website. A minimum spanning tree was generated using BioNumerics software version 7.6 (AppliedMaths, Kortrijk, Belgium) to analyse the distribution of STs in *Salmonella* Derby strains from pig farms, pig slaughterhouses, retail markets and humans.

2.3. Clustered regularly interspaced short palindromic repeats (CRISPRs)

CRISPR locus 1 (CRISPR1) was amplified using forward primer A1 (5′-GTTGGTAAAAGAGCTGGCGA-3′) and reverse primer A2 (5′-GATGGACTTAGATTAGTTTC- 3′). CRISPR locus 2 (CRISPR2) was amplified using forward primer B1 (5′-CAATACCCTGATCCTTAACG-3′) and reverse primer B2 (5′-ATTGTTGCGATTATGTTGGT-3′). The PCR amplicons were sequenced by Nanjing GenScript Biotech Co. (Nanjing, China). Spacers were identified for CRISPR1 and CRISPR2 using CRISPRfinder (http://crispr.u-psud.fr/Server/) (Grissa, Vergnaud, & Pourcel, 2008). Each spacer was queried against the Institut PasteurCRISPR database for *Salmonella* (http://www.pasteur.fr/recherche/genopole/PF8/crispr/CRISPRDB.html) to obtain the spacer and directrepeat (DR) names. For spacers or DRs that had no exact match in the DR and spacer dictionary, a new name was assigned in accordance with spacer nomenclature (Fabre et al., 2012).

Table 1
Salmonella Derby strains used for molecular analysis in this study.

Strain no.	MLST (ST)	DCTs	Year of isolation	Source
09-S79	40	DCT24	2009	H
10-S57	40	DCT30	2010	R
11-S56	40	DCT26	2011	H
11-S58	40	DCT24	2011	R
11-S59	40	DCT29	2011	R
11-S60	40	DCT22	2011	R
11-S61	40	DCT24	2011	H
12-S40	40	DCT28	2012	S
12-S43	40	DCT22	2012	S
12-S44	40	DCT22	2012	S
13-S41	40	DCT24	2013	S
13-S42	40	DCT19	2013	S
13-S45	40	DCT18	2013	S
13-S46	40	DCT14	2013	S
13-S47	40	DCT16	2013	S
13-S48	40	DCT12	2013	S
13-S49	40	DCT14	2013	S
13-S50	40	DCT20	2013	S
13-S51	40	DCT17	2013	S
13-S52	40	DCT28	2013	S
13-S53	40	DCT22	2013	S
13-S54	40	DCT24	2013	S
13-S55	40	DCT22	2013	S
13-S24	40	DCT31	2013	S
13-S25	40	DCT24	2013	S
13-S28	71	DCT4	2013	S
13-S29	71	DCT5	2013	S
13-S1	71	DCT11	2013	R
13-S2	40	DCT17	2013	R
13-S3	40	DCT14	2013	R
13-S4	40	DCT14	2013	R
13-S5	40	DCT21	2013	R
14-S26	40	DCT22	2014	S
14-S27	40	DCT28	2014	S
13-S30	71	DCT4	2014	S
14-S31	71	DCT4	2014	S
14-S32	71	DCT4	2014	S
14-S33	71	DCT4	2014	S
14-S34	40	DCT28	2014	S
14-S35	71	DCT4	2014	S
14-S36	71	DCT4	2014	S
14-S37	71	DCT4	2014	S
14-S80	40	DCT27	2014	S
14-S81	40	DCT14	2014	H
14-S82	40	DCT14	2014	H
14-S83	40	DCT14	2014	H
14-S38	40	DCT18	2014	H
14-S39	40	DCT14	2014	H
14-S6	40	DCT14	2014	R
14-S7	40	DCT18	2014	R
14-S8	40	DCT15	2014	R
14-S9	71	DCT7	2014	R
14-S10	71	DCT10	2014	R
14-S11	40	DCT18	2014	R
14-S12	71	DCT2	2014	R
14-S13	71	DCT5	2014	R
14-S14	71	DCT8	2014	R
14-S15	71	DCT7	2014	R
14-S16	71	DCT6	2014	R
14-S17	40	DCT17	2014	R
14-S18	71	DCT9	2014	R
14-S19	71	DCT10	2014	R
14-S20	71	DCT1	2014	R
14-S21	71	DCT9	2014	R
14-S22	71	DCT10	2014	R
14-S23	71	DCT9	2014	R
14-S62	40	DCT28	2014	R
14-S63	40	DCT24	2014	R
14-S64	71	DCT3	2014	R
14-S65	40	DCT31	2014	R
14-S66	40	DCT32	2014	R
15-S67	40	DCT23	2015	F
15-S68	40	DCT31	2015	F

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