

## ORIGINAL RESEARCH

Isolation of *Salmonella* species of public health concern from commonly fed dried meat dog treatsGenever Morgan<sup>1</sup>  | Mikhela Saal<sup>1</sup> | Aoife Corr<sup>1</sup> | Claire Jenkins<sup>2</sup> | Marie Anne Chattaway<sup>2</sup> | Gina Pinchbeck<sup>1</sup> | Nicola Williams<sup>1</sup><sup>1</sup>Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Neston, UK<sup>2</sup>Gastrointestinal Bacteria Reference Unit, United Kingdom Health Security Agency, London, UK

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## Abstract

**Background:** Dried non-heat-treated meat treats, such as ears, skin and tails, are popular supplementary dog foods. Previous studies have demonstrated *Salmonella* spp. contamination on treats, particularly in pig ears and chicken products. This small, exploratory, cross-sectional study investigated *Salmonella* spp. presence in dried treats available in the UK.**Methods:** A selection of dried treats from local pet shops and online retailers underwent bacterial culture for *Salmonella* spp. and subsequent antimicrobial susceptibility testing, with *Salmonella* serotype determined by whole genome sequencing.**Results:** Eighty-four samples were tested, with 16% being *Salmonella* spp. positive. Five *Salmonella* serotypes were identified, each associated with specific treat types. An antimicrobial-resistant phenotype was identified in 39% of isolates. All serotypes identified are known to cause human infection.**Limitations:** This study was limited by a small sample size and limited number of retail sources.**Conclusion:** *Salmonella* spp. of public health concern were present in some dried dog treats in this study. Dog owners, pet food retailers and veterinary professionals should be aware of the potential zoonotic disease risk associated with these treats, and appropriate hygiene measures, including thorough hand washing, should be utilised if they are fed.

## KEYWORDS

dog, *Salmonella*, dried treats

## INTRODUCTION

Non-heat-processed meat items, which include raw meat diets (RMD) and air dried, freeze dried or dehydrated treats, are an increasingly popular diet choice for dogs.<sup>1</sup> These foodstuffs have not undergone any cooking or heat treatment as part of the production process; however, the process used for treat production must have been proven in sampling tests to destroy *Salmonella*.<sup>2</sup> Items used as treats or chews may include body parts, such as ears, snouts, tendons, skin, trachea, tails, penis, hooves and feet, from a range of animals.<sup>3,4</sup> Previous studies have demonstrated that dog owners who choose to feed non-processed meat items do so as they believe them to be a more natural and healthier choice for their pet.<sup>5–8</sup> They may

also believe that these items provide benefits such as mental stimulation and increased satisfaction in food, and allow the dog to exhibit more natural chewing behaviour.<sup>7,9,10</sup>

Dried, non-processed dog chews are composed of category 3 animal by-products (ABPs), as per Defra regulation, and may include raw abattoir material that passed as fit for human consumption but was unwanted due to commercial reasons and material from animals that passed an antemortem test but was deemed unfit for human consumption.<sup>2</sup>

While there is an increasing body of research examining RMDs for dogs, there remains relatively limited evidence regarding the microbiological risks of ABPs used as dog treats. *Salmonella* spp. contamination has previously been reported in dried and dehydrated

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treats in the UK and elsewhere,<sup>4,11–18</sup> with pig ear treats, raw hide and chicken products frequently represented, even where they were expected to have been heat treated.<sup>17</sup>

The present small exploratory cross-sectional study aimed to investigate the presence of *Salmonella* spp. in a selection of dried natural dog treats readily available in the UK.

## MATERIALS AND METHODS

A selection of dried natural dog treats was purchased from a convenience sample of suppliers. Treats were purchased in-person from an independent pet shop (supplier A) and a large nationwide chain pet shop in Merseyside (supplier B), and also from two nationwide-supplying online retailers (suppliers C and D), during September–October 2021. Treat type selection was opportunistic and at random, depending on availability at the time of the shop or website visit. Purchases were made on two visits 2 weeks apart from supplier A, whereas one-time purchases were made from suppliers B, C and D. Information regarding packaging type and labelling was recorded.

Whole treats were placed into individual, sterile, sealable bags and homogenised with 25 ml of buffered peptone water. The broth was then poured into a sterile universal tube and incubated overnight at 37°C, after which 100 µl was added to 5 ml of Rappaport-Vassiliadis broth (RVB) and incubated overnight at 42°C.

Harlequin chromogenic agar for *Salmonella* esterase (CASE) (Neogen, UK) was inoculated with the RVB and incubated for 18–20 hours at 37°C. CASE plates were examined for turquoise colonies characteristic of suspected *Salmonella* spp., and if present, two individual colonies were then plated onto nutrient agar and incubated overnight at 37°C. Confirmation of *Salmonella* spp. was undertaken using matrix-assisted laser desorption/ionisation-time of flight mass spectrometry.

Isolates underwent whole genome sequencing (WGS) at the Gastrointestinal Bacteria Reference Unit within the United Kingdom Health Security Agency (UKHSA). For isolates confirmed as *Salmonella* spp., DNA was extracted using the QIAamp DNA mini kit (Qiagen, Crawley, UK). Following DNA extraction, isolates were prepared for WGS with Nextera XT DNA preparation kits, and sequenced on the Illumina HiSeq 2500 platform in rapid run mode to produce 100 bp paired-end reads. Trimmomatic v0.40<sup>19</sup> was used to quality trim FASTQ reads with bases removed from the trailing end that fell below a Phred score of 30. The Metric Orientated Sequence Type v1<sup>20</sup> was used for sequence type (ST) assignment, and serotype was assigned using a combination of the *Salmonella* multi-locus sequence type (MLST) database and SeqSero2.<sup>21–23</sup>

FASTQ sequences were deposited in the National Center for Biotechnology Information Sequence

Read Archive under the BioProject accession number PRJNA248792 ([www.ncbi.nlm.nih.gov/bioproject/?term=248792](http://www.ncbi.nlm.nih.gov/bioproject/?term=248792)). Raw sequence data files of isolates from this study were uploaded to Enterobase (<https://enterobase.warwick.ac.uk/>), and short reads were assembled by Enterobase using the then current backend pipelines (versions 3.61–4.1), including core genome MLST (cgMLST) analysis to produce a core genome ST (cgST), as previously described,<sup>24</sup> using the cgMLST v2 HierCC v1 algorithm.<sup>25</sup> All 13 isolates met the cgMLST quality parameters for *Salmonella* (minimum size 4000 kbp, maximum size 5800 kbp, minimum N50 20 kbp, maximum number contigs 600, maximum low-quality sites 5%, minimum taxonomic purity 70%<sup>26</sup>) for analysis. Hierarchical clustering (HierCC of cgMLST) is a multi-level clustering scheme for population assignments based on cgMLSTs,<sup>26</sup> and previous studies have shown that analysing strains at the 5 allelic threshold is appropriate to detect clusters or closely related clones.<sup>24,27,28</sup> Therefore, HierCC was analysed at the 5 allelic level (HC5—strains linked within five cgMLST alleles) for microbiologically linked human cases. The minimum spanning tree was created in Enterobase for each pathogen using the MSTree v2 algorithm and visualised on GrapeTree.<sup>25</sup>

*Salmonella* spp. isolates underwent antimicrobial susceptibility testing via disc diffusion. Antimicrobials tested were ampicillin 10 µg, amoxicillin–clavulanate 20 µg/10 µg, ciprofloxacin 5 µg, tigecycline 15 µg, trimethoprim–sulphamethoxazole 1.25 µg/23.75 µg, amikacin 30 µg and meropenem 10 µg (MAST Group, Liverpool, UK). Isolates were inoculated into sterile saline to 0.5 McFarland units and a 5 µl loopful was spread on to Muller–Hinton agar (Neogen). Discs were placed and plates were incubated aerobically for 18–20 hours at 37°C. Following incubation, the zones of inhibition were measured and susceptibility was interpreted. Breakpoints and screening concentration criteria used for interpretation were as recommended by the European Committee on Antimicrobial Susceptibility Testing.<sup>29</sup> Data processing and descriptive statistics were carried out using Microsoft Excel 2016.

No animal subject, human participant, or personal data collection was required for this study; hence, ethical approval was not required.

## RESULTS

Eighty-four samples were tested from a selection of treat types. Animal proteins represented were buffalo/bison ( $n = 25$ ), chicken ( $n = 19$ ), beef ( $n = 13$ ), lamb ( $n = 4$ ), pork ( $n = 4$ ), duck ( $n = 3$ ), rabbit ( $n = 3$ ), camel ( $n = 3$ ) and other unspecified sources sold as ‘bronchos’, tendons and ‘pizzle sticks’ ( $n = 10$ ). Full data regarding treat type, supplier and *Salmonella* spp. presence are provided in Table S1.

Sample packaging varied greatly. Supplier A treats ( $n = 43$ ) were provided unpackaged with no labelling or traceability information present. Treats were in separate baskets based on treat type and purchased by

**TABLE 1** Treat number and type, *Salmonella enterica* serotype identification, sequence type (ST), hierarchical clustering of core genome multi-locus sequence type number at the 5 allelic level (HierCC HC5) and associated antimicrobial susceptibility testing results for isolates confirmed as *Salmonella* in this study

Treat no.	SRA		Visit no.	ST	HierCC HC5	<i>Salmonella</i> serotype	Antibiotic type						
	accession number	Treat type					Aug	Amp	Tig	TMS	Ami	Cip	Mer
13	SRR18529427	Pizzle stick	1	10	301902	Dublin	S	S	S	S	S	S	S
14	SRR18529420	Pizzle stick	1	10	301891	Dublin	S	S	S	S	S	S	S
15	SRR18488403	Bison ear	1	40	298030 <sup>a</sup>	Derby	S	S	R	S	S	S	S
16	SRR18488404	Bison ear	1	682	298030 <sup>a</sup>	Derby	S	S	R	S	S	S	S
21	SRR18488367	Furry rabbit ear	1	32	301731	Infantis	S	S	S	S	S	S	S
22	SRR18488418	Furry rabbit ear	1	32	301762	Infantis	S	S	R	S	S	S	S
34	SRR18488400	Bison ear	2	682	67536 <sup>a</sup>	Derby	S	S	S	S	S	S	S
35	SRR18488407	Bison ear	2	682	165407 <sup>a</sup>	Derby	S	S	S	S	S	S	S
36	SRR18488414	Bison ear	2	682	301761	Derby	S	S	S	S	S	S	S
37	SRR18488402	Bison ear	2	682	67536	Derby	S	S	S	S	S	S	S
43	SRR18529396	Chicken treat	2	64	301899	Anatum	S	S	S	S	S	S	S
44	SRR18488364	Chicken treat	2	34	1597 <sup>a</sup>	Typhimurium (monophasic)	S	R	S	S	S	S	S
47	SRR18545478	Chicken treat	2	34	1597 <sup>a</sup>	Typhimurium (monophasic)	S	R	S	S	S	S	S

Note: All isolates were isolated from treats from the same supplier obtained over two separate visits.

Abbreviations: Ami, amikacin; Amp, ampicillin; Aug, amoxicillin–clavulanate; Cip, ciprofloxacin; Mer, meropenem; R, resistant; S, sensitive; SRA, Sequence Read Archive; Tig, tigecycline; TMS, trimethoprim–sulphamethoxazole.

<sup>a</sup>Contains genetically linked human cases.

placing into paper bags. Supplier B treats ( $n = 4$ ) were individually wrapped in branded plastic sealed packets. Supplier C treats ( $n = 21$ ) were delivered in a box comprising some loose unpackaged ear treats and other items provided in branded sealed bags. Treats purchased from supplier D ( $n = 16$ ) presented as multiple items in clear plastic bags with no labelling. Country of origin was unknown for the majority of treats (70%, 59/84), although 5% (4/84) stated they were produced in the UK, and 25% (21/84) stated materials were sourced from the UK and Europe on their website (Table S1).

*Salmonella enterica* was isolated from 16% (95% confidence interval [CI] 7.8–23.2;  $n = 13$ ) of the treats tested. The types of treats that tested positive for *S. enterica* were dried bull's penis 'pizzle sticks' (67%, 95% CI 20.8–93.9;  $n = 2/3$ ), bison ears (24%, 95% CI 11.5–43.4;  $n = 6/25$ ), furry rabbit ears (67%, 95% CI 20.8–93.9;  $n = 2/3$ ) and dried chicken treats (60%, 95% CI 23.1–88.2;  $n = 3/5$ ). All treats that had *Salmonella* spp. isolated were purchased from the same independent pet shop and purchased on two separate visits.

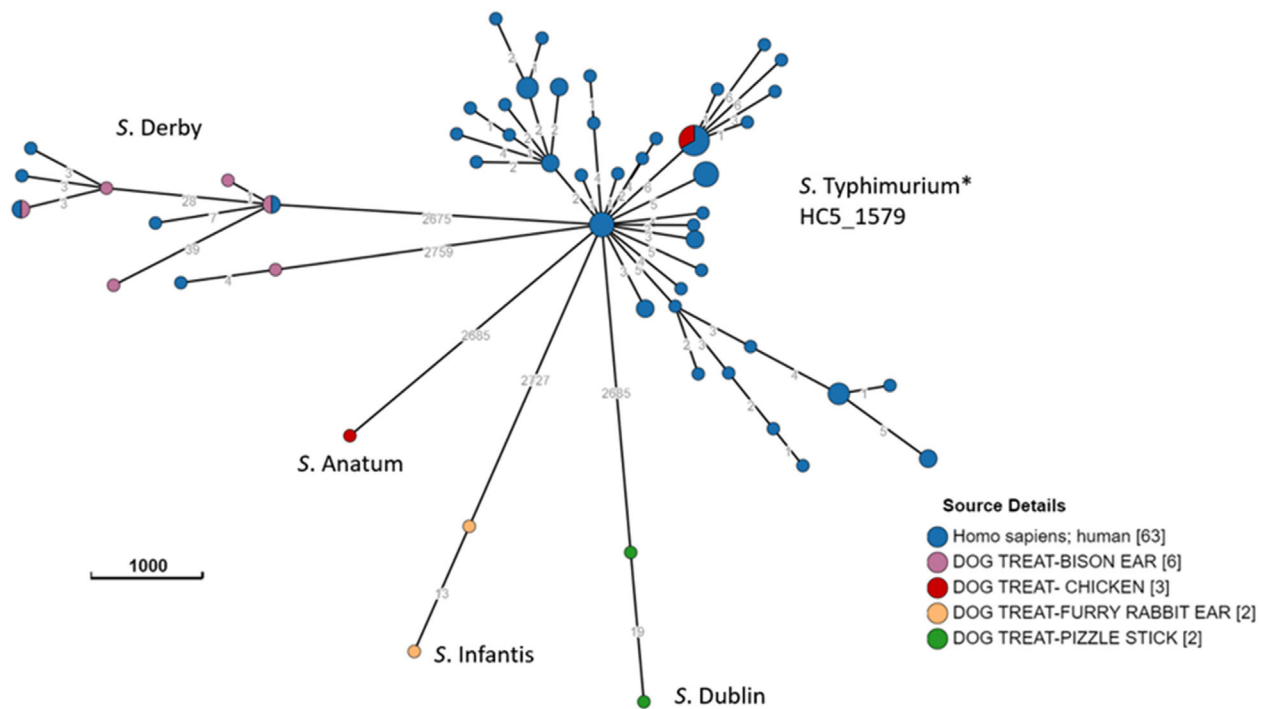
Five different *S. enterica* serotypes were identified via WGS (Table 1): *S. Anatum*, *S. Derby*, *S. Dublin*, *S. Infantis* and *S. Typhimurium* (monophasic). Each specific serotype was isolated from a single treat type only. Data were compared to all sequences in the UKHSA database. All serotypes detected were known to cause human infection.<sup>30</sup> The most frequently isolated serotype was *S. Derby* (46%, 6/13), isolated from bison ears, with *S. Dublin* identified in two pizzle stick samples. As well as identifying a diverse range of serotypes via WGS, HierCC analysis (cluster analysis for population assignments based on the core

genome) indicated that, even within serotypes, the populations were genetically diverse (Table 1). Figure 1 shows the population structure of *Salmonella* species isolated from different dog treats that have also been identified in human cases at the HierCC 5 allelic level. Dog treat types were associated with single specific *S. enterica* serotypes with the exception of chicken, which was associated with two serotypes. Isolates associated with human clinical cases were found in two of the five serotypes.

Of the confirmed isolates of *S. enterica*, 39% (5/13) demonstrated resistance to at least one antibiotic class. Resistance to tigecycline was observed in 23% (3/13) of isolates, which were serotypes Derby and Infantis. Ampicillin resistance was detected in 15% (2/13) of isolates, which were serotype Typhimurium (monophasic). No resistance was observed to other antibiotics.

## DISCUSSION

This small exploratory study provided further evidence that dried natural dog treats available in the UK can be contaminated with *S. enterica*. Previous studies globally have demonstrated a wide range (2%–51%) of *Salmonella* spp. prevalence in such treats, frequently from raw hide and pig ears.<sup>4,12,13,15–18</sup> Non-processed dog treats derived from raw animal material contaminated with *S. enterica* are known to be a source of gastrointestinal infectious disease in humans; there have been at least three outbreaks of human salmonellosis linked to dog treats in the United States and Canada, attributed to *S. enterica* serotypes Infantis,<sup>13</sup> Thompson<sup>11</sup> and Newport.<sup>31</sup> Dried 'natu-



**FIGURE 1** Grape tree illustrating the population structure of *Salmonella* isolated from dog treats in this study and which have been identified in human cases at the hierarchical clustering 5 allelic level. *S. Typhimurium* HC5\_1579 is a genetically diverse cluster, and dog treats from this study were highly genetically similar to the subcluster HC2\_299262. Dog treat types were associated with single serotypes with the exception of chicken, which was associated with two serotypes. Isolates from two serotypes (*S. Derby* and *S. Typhimurium*) were also associated with human clinical cases

ral' dog treats are an increasingly popular supplementary food choice, and the types of dried treats available are diverse; the present study has demonstrated the presence of *Salmonella* spp. in a range of commonly selected treats other than pig ears and raw hide.

A variety of *Salmonella* spp. serotypes were identified in this study. *S. Typhimurium* and *S. Infantis* are among the top five serotypes resulting in human infection reported to the UKHSA.<sup>30</sup> However, the most commonly isolated in this study was *S. Derby*, all isolated from bison ear treats, and several strains were found to be genetically highly similar to human cases, alongside *S. Typhimurium* strains (Table 1 and Figure 1). Additionally, *S. Typhimurium* is regularly reported in the top five reported serotypes in human cases in Europe<sup>32</sup> and is most commonly associated with pigs and poultry. Indeed, *S. Typhimurium* and *S. Derby* have been previously isolated from pork and poultry foodstuffs intended for pet food production in Italy.<sup>33</sup> *S. Derby* is a common cause of human salmonellosis in France<sup>34</sup> and was implicated in a foodborne disease outbreak in Germany, linked to the consumption of raw pork products.<sup>35</sup>

While *Salmonella* spp. infection typically causes self-limiting gastroenteritis in otherwise healthy humans, it poses a much higher risk in the immunocompromised, young and elderly, and can result in severe infections. *S. Dublin* is a cattle-adapted serotype, isolated in this study from pizzle stick treats. Although no microbiologically linked human cases were detected, this serovar is capable of causing severe invasive illness in humans that can result in

septicaemia, hospitalisation and death.<sup>36</sup> Antimicrobial resistance within *Salmonella* spp. is also of concern, and while resistance to ampicillin and tetracycline was identified in some isolates in the present study, no multidrug resistance was observed.

The risk of transmission to humans has been linked to lack of appropriate hand hygiene following handling of the dog treats and/or contact with animals that may shed *S. enterica* in their faeces after consuming the treats.<sup>11,13,31</sup> It is well documented that dogs can be asymptomatic carriers of *Salmonella* spp. and infected dogs often appear clinically well.<sup>37–39</sup> Previously identified risk factors for *Salmonella* spp. carriage include the feeding of offal and raw animal products,<sup>8,40–44</sup> and dogs have been shown to asymptotically shed *Salmonella* spp. in their faeces for up to a week following ingestion of infected food.<sup>14</sup> There is also a clinical disease risk to dogs, including diarrhoea,<sup>39,40,45</sup> and reports of non-enteric infections in dogs with additional comorbidities.<sup>46–48</sup>

This study has highlighted a potential 'one health' concern regarding natural treat products, with some isolates from these products being genetically highly similar to human case isolates, although epidemiological investigations would be needed to establish exposures. These items are often provided to pet dogs both as treats and as a popular natural alternative to traditional anthelmintics. Rehydration (via saliva during chewing) of treats may reactivate foodborne pathogens inactive in the dehydrated state. These treats may also take more time to chew and consume than conventional cooked treats, so they may be in the household environment for a prolonged period,

posing an elevated risk of contamination. Studies have shown that few pet owners perceive dry food items, including dried natural treats, to pose a microbiological risk,<sup>49</sup> and that owners who feed raw animal products generally perceive their diet choice as low risk for foodborne illness.<sup>8,50–52</sup>

The government guidelines for the packaging of ABPs as treats state that dog chews must be packed in unused packaging. However, the treats contaminated with *Salmonella* spp. in this study were sold as loose items that could be picked up by hand and purchased in paper bags. Again, this is a public health concern and demonstrates the need for further education regarding safe storage and handling of ABPs. Furthermore, for many treats, no country of origin was indicated, which potentially poses a risk of importation of *Salmonella* spp. serotypes not commonly reported in the UK via these products and highlights the importance of clear package labelling for traceability. Defra guidelines state that the production process for dog chews must be proven by testing to destroy *Salmonella*, and an ABP will fail Defra testing if any *Salmonella* spp. colonies are identified within tested samples ([www.gov.uk/guidance/laboratory-testing-requirements-for-animal-by-products-abps#how-much-bacteria-your-samples-can-contain](http://www.gov.uk/guidance/laboratory-testing-requirements-for-animal-by-products-abps#how-much-bacteria-your-samples-can-contain)). Therefore, the treats identified as contaminated with *Salmonella* spp. in this study would be expected to fail testing at a Defra-accredited laboratory.

There are some limitations to this study. It was a small, exploratory investigation, and while UK-wide online suppliers were sought, in-person visits to independent pet shops were only carried out in a small area. Therefore, the findings may not accurately represent the prevalence of *Salmonella* spp. contamination in treats available across the UK. All contaminated treats were from the same independent pet shop, which could represent a localised problem, but could also potentially be a result of contamination at the suppliers or within the supply chain, and without further environmental sampling, it would not be possible to identify where within the production chain contamination occurred. However, cross-contamination within the shop itself was deemed unlikely for a number of reasons; treats were separated within the shop into separate baskets based on treat type and were purchased on separate occasions, and importantly, the serotypes identified were treat specific, and there was genetic diversity within the population (Figure 1). Additional limitations were that treats were picked opportunistically and only a small number of some types were selected depending on availability at the time of visits. Finally, the method of isolating *Salmonella* spp. using chromogenic agar is likely to have only selected for *Salmonella* subsp. *enterica*; therefore, a small number of other *Salmonella* subsp. may have been missed.

Nevertheless, this study has demonstrated the presence of *Salmonella* spp. contamination in dried natural dog treats that are readily available and commonly purchased by dog owners. Larger studies are required to quantify the risk further. Veterinary staff,

retailers and dog owners should be made aware of these risks. Efforts should be made to educate dog owners further regarding the potential risks posed by these treats if they choose to feed them, especially in households with higher risk individuals present, such as immunocompromised individuals or young children. The importance of hygienic practice surrounding their use should be stressed ([www.gov.uk/guidance/raw-pet-foods-handling-and-preventing-infection](http://www.gov.uk/guidance/raw-pet-foods-handling-and-preventing-infection)), particularly regarding hand washing after use and consideration against feeding them within the home environment.

## AUTHOR CONTRIBUTIONS

Laboratory work at the University of Liverpool was undertaken by Mikhela Saal, Aoife Corr, Genever Morgan and Nicola Williams. Whole genome sequencing was performed and analysed by Claire Jenkins and Marie Anne Chattaway at UKHSA. Nicola Williams and Genever Morgan supervised the project. All authors discussed the results. Genever Morgan wrote the manuscript with support from Nicola Williams, Gina Pinchbeck, Marie Anne Chattaway and Claire Jenkins.

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## CONFLICTS OF INTEREST

Claire Jenkins and Marie Anne Chattaway are affiliated with the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Gastrointestinal Infections and Genomics and Enabling Data at University of Liverpool and University of Warwick, respectively, in partnership with the United Kingdom Health Security Agency (UKHSA). Claire Jenkins and Marie Anne Chattaway are based at UKHSA. The views expressed are those of the author(s) and not necessarily those of the NIHR, the Department of Health and Social Care or the UK Health Security Agency.


## DATA AVAILABILITY STATEMENT

Data that support the findings of this study are available in the Supporting Information of this article. Additionally, FASTQ sequences from this study are available in the National Center for Biotechnology Information Sequence Read Archive under the BioProject accession number PRJNA248792 ([www.ncbi.nlm.nih.gov/bioproject/?term=248792](http://www.ncbi.nlm.nih.gov/bioproject/?term=248792)). Raw sequence data files of isolates from this study have been uploaded to EnteroBase (<https://enterobase.warwick.ac.uk/>)

## ETHICS STATEMENT

No animal subject, human participant or personal data collection was required for this study; hence, ethical approval was not required.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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