Preplanned Studies

Salmonella Grumpensis Causing Diarrhea in Children — Shanghai Municipality, China, 2023

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Summary

What is already known about this topic?

Foodborne diseases are a growing public health concern with a notable disease burden in China.

What is added by this report?

Two children with diarrhea visited a healthcare facility within 24 hours on August 1 and 2, 2023. *Salmonella* Grumpensis was detected in their stool samples by the public health laboratory. Whole genome sequencing (WGS) analysis revealed characteristics typical of outbreak strains. Although the origin of the outbreak was unknown, the possibility of a hidden shared infection was deemed feasible.

What are the implications for public health practice?

It underscores the importance of thorough genomic surveillance to promptly detect emerging pathogens. Public health laboratories play a crucial role by utilizing advanced genomic technologies for accurate pathogen identification and timely warning systems.

In August 2023, the Shanghai CDC laboratory received diarrheal stool specimens from two pediatric hospitals in close succession. These specimens were identified and serotyped as *Salmonella enterica* serotype Grumpensis (*S.* Grumpensis). The rarity of this serotype was confirmed upon consulting the local Chinese *Salmonella* genome database (*1*), which contains no recorded instances, suggesting that it is an infrequent occurrence in China. Commonly, symptoms of salmonellosis emerge anywhere from 6 hours to 6 days following infection. The discovery of two instances of this unusual serotype within a 24-hour period signals a red flag for a possible outbreak and underscores the pathogen's transmission capability.

Two male children, aged 1 and 2 years, presented to the hospital on August 1and 2 with similar clinical symptoms of bloody diarrhea (>3 episodes in 24 hours) and abdominal pain (Table 1). Initially treated with cefdinir, patient G2's symptoms persisted despite a 5-day course, leading to a switch to azithromycin, which resulted in gradual improvement and ful recovery.

Epidemiological investigations play a crucial role in managing cases related to uncommon pathogens. Despite the initial findings showing no evidence of typical sources of infection such as dining out, travel, contact with symptomatic individuals, consumption of raw water, undercooked foods, or owning pets, it posed a challenge in determining the origin of the infection.

CDC laboratory personnel collected specimens from the household of individual G2. Adhering to the procedures specified in GB4789.4-2016, a diverse set of samples, including stool from family members, uneaten food, and environmental swabs, were gathered. Maintaining sterility and a constant temperature of 4 ℃, samples were transported to the lab for pathogen examination within two hours. Despite these precautions, no *Salmonella* was detected in any of the samples. Additionally, there were no further cases involving this particular *Salmonella* serotype reported at the same hospital. In our continued investigation of the two *S.* Grumpensis strains, we conducted a comprehensive analysis that included both antimicrobial susceptibility testing (AST) and whole genome sequencing (WGS). The AST employed the broth microdilution technique to assess the resistance against 22 antibiotics encompassing 11 classes, as listed in Supplementary Table S1 (available at https://weekly.chinacdc.cn/). This method was strictly in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines and the National Antimicrobial Resistance Monitoring System (NARMS) protocol, aiming to determine the Minimum Inhibitory Concentrations (MICs) for each antibiotic (*2*–*3*). The AST findings, as presented in Supplementary Table S1, indicated that the strains were susceptible to the full array of antibiotics tested.

Meanwhile, WGS results, detailed in Table 2, classified both isolates as *Salmonella* Grumpensis S.I (13, 23: d: 1,7). They shared multilocus sequence type (ST) 2060 and core genome multilocus sequence type (cgST) 175517, differing in only one allele, suggesting a strong genetic similarity. Their matching phenotypic

and genotypic resistance patterns, absence of plasmid replicons, and common genetic features indicate a close genetic relationship, typical for strains involved in outbreaks, pointing to a common source or transmission chain.

The study analyzed the genomes of 51 *S.* Grumpensis available in the NCBI database (Supplementary Table S2, available at https://weekly.chinacdc.cn/), revealing its widespread across 11 countries and regions globally, with the highest numbers in Spain (*n*=20), the United Kingdom (*n*=15), and the United States (*n*=7). The strains were isolated between 2005 and 2023, with a surge from 2017 to 2023. Various sample types were identified, including human (*n*=42), food (*n*=2), plant (*n*=1), poultry (*n*=1), and unknown sources (*n*=7). Human samples primarily consisted of fecal specimens (*n*=39), as well as blood (*n*=2) and cerebrospinal fluid (*n*=1).

Phylogenetic analysis (Figure 1) identified ST2060 (*n*=37) and ST751 (*n*=13) as the predominant global STs among *S.* Grumpensis isolates. Most isolates harbored *acc(6')-Iaa* (98.1%) and *fosA7* (96.2%) genes. ST751 has been observed since 2016 in the UK, Canada, the USA, and Brazil, from both humans and poultry, notably lacks of multidrug resistance. Initially reported in 2006, ST2060 is mainly present in human samples (97.3%) and comprised of two genetic clades: 2060.1 and 2060.2, with the latter branching into three sub-clades (2060.2-1, 2060.2-2, 2060.2-3). The study conducted hierarchical single linkage clustering based on pairwise single nucleotide polymorphism (SNP) differences at different thresholds (100, 50, 25, 10, 5, 0). Two isolates from the study belonged to the 2060.2-1 sub-clade, genetically close (0–80 SNPs) to isolates from the UK, USA, and Senegal, and highly similar (0–4 SNPs) to a 2023 USA strain (SRR26351730). An intriguing finding was an isolate from Senegal, in the 2060.2-1 sub-clade, having 14 antibiotic resistance genes (ARGs) and originating from a cerebrospinal fluid sample.

DISCUSSION

This study highlights the crucial contribution of public health laboratories in identifying and addressing outbreaks of uncommon *Salmonella* serotypes. It underscores the common dilemma faced by public health departments in confirming outbreaks that exhibit typical characteristics but are challenging to confirm through regular surveillance due to few cases or unidentified sources.

A foodborne disease outbreak is defined when two or more cases of similar illness caused by the consumption of a common food (*4*). In the investigation, two young boys with identical clinical symptoms within a 24-hour period were involved. The pathogens isolated from their diarrheal stool samples were conclusively identified as *S.* Grumpensis, a serotype not been previously recorded in the Chinese local *Salmonella* genome database, highlighting its rarity (*1*). Unfortunately, the epidemiological investigation did not yield a probable source of infection due to limited data. Nevertheless, the absence of additional cases suggests that the event might have

TABLE 1. Medical history of pediatric diarrhea cases.

Case number	Date of admission	Hospital name	Age of patient	Gender	Presented symptoms
G2	August 1, 2023	SCMC	1 year	Male	Bloody diarrhea and abdominal pain
G5	August 2, 2023	SCH	2 years	Male	Bloody diarrhea and abdominal pain

Abbreviation: SCMC=Shanghai Children's Medical Center; SCH= Shanghai Children's Hospital.

TABLE 2. Genomic characteristics of the two *Salmonella* Grumpensis isolates.

Feature		Isolates	
		G2	G5
Serotype		*Salmonella* Grumpensis (13, 23: d: 1,7)	*Salmonella* Grumpensis (13, 23: d: 1,7)
Multilocus Sequence Type		2060	2060
Core Genome Multilocus Sequence Type		175517	175517
Antibiotic Resistance	Phenotype	All Sensitive	All Sensitive
	Genotype	*acc(6')-Iaa*, *fosA7*	*acc(6')-Iaa*, *fosA7*
Plasmid Replicons Detected		None	None

Note: Core genome multilocus sequence type is 1 allele difference.

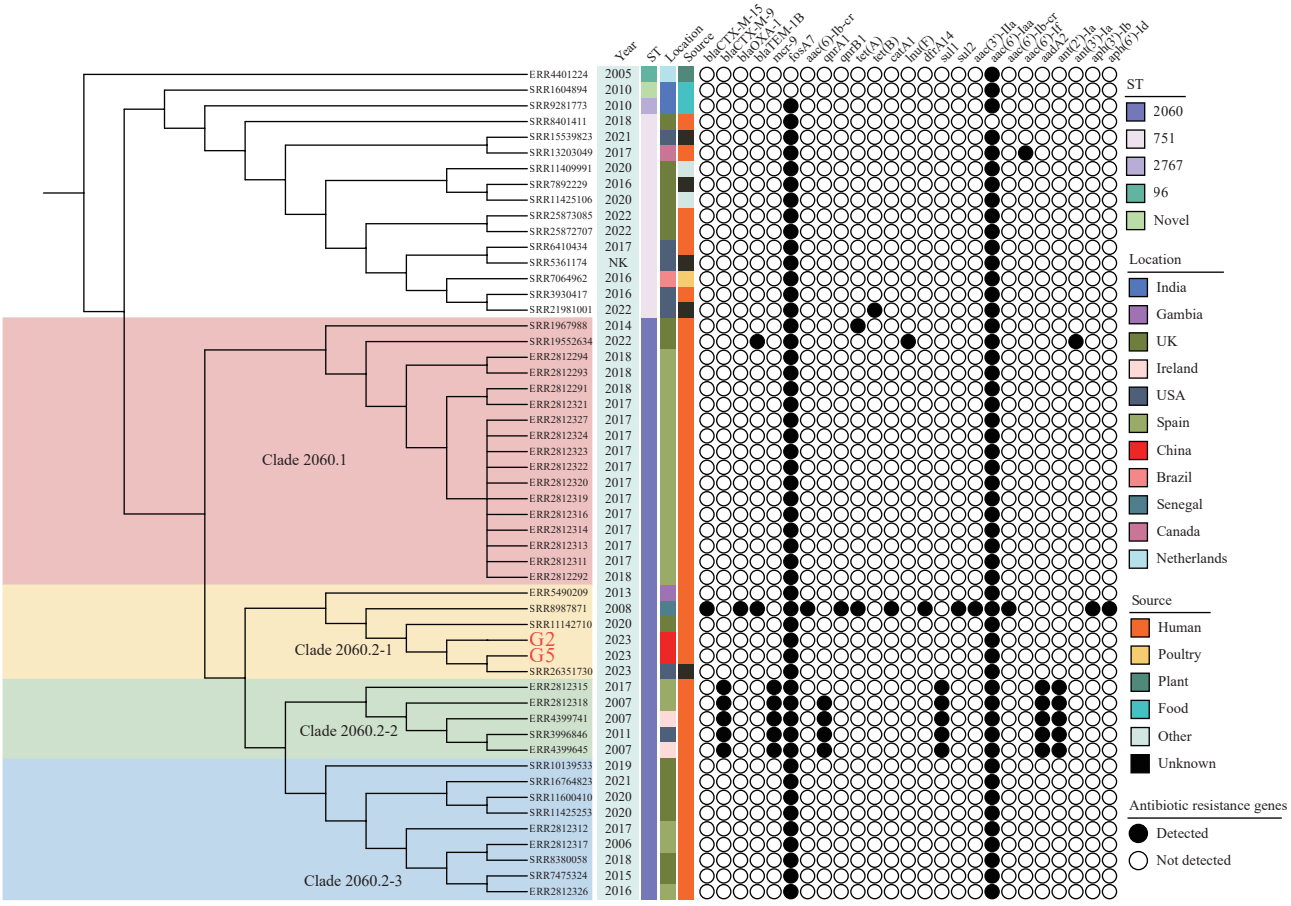

FIGURE 1. Genetic evolution and antibiotic resistance dissemination of 53 global *S.* Grumpensis isolates. Image source: https://itol.embl.de/tree/1391771982423341698626779#.

been a confined occurrence, unlikely to have propagated further.

WGS is an essential tool for investigating outbreaks. In our study, both strains were found to have the same multilocus sequence type and core genome multilocus sequence type, differing by only one allele or four SNPs, indicating a close genetic relationship. Despite the absence of a direct epidemiological link, several factors strongly suggest that the cases were part of an outbreak of *S.* Grumpensis stemming from a single source. These factors encompass the temporal proximity of the cases, the high genetic similarity among isolates, consistent antibiotic resistance patterns, and the rarity of the *S.* Grumpensis serotype.

In the management of patient G2, a discernible divergence was observed between the ineffectiveness of cefdinir and the subsequent clinical improvement with azithromycin. This contrasted with the results indicated by AST and WGS. This variance could be due to a confluence of factors, such as drug pharmacokinetics, pharmacodynamics, and individual patient characteristics like immune function or microbiota. We also considered the possibility that adverse effects of cefdinir, rather than its antimicrobial ineffectiveness, might have contributed to the patient's condition. Commonly, cefdinir is associated with diarrhea in pediatric patients at therapeutic doses (*5*). Additionally, occurrences of reddish stools have been noted, which are thought to result from a nonabsorbable complex forming between cefdinir or its metabolites and dietary iron in the gut (*5*). The absence of follow-up stool testing complicates the ability to discern whether the continuing symptoms were due to an uncontrolled infection or a reaction to cefdinir. This underscores the importance of thorough follow-up testing to accurately assess treatment outcomes.

In this study, we identified the presence of the *acc(6')-Iaa* and *fosA7* genes in *S.* Grumpensis isolates, illuminating their potential contribution to antibiotic resistance. The *acc(6')-Iaa* gene codes for an aminoglycoside acetyltransferase, which can confer resistance to aminoglycoside antibiotics, frequently observed in *Salmonella* species (*6*–*7*). It is vital to note

that this gene usually remains inactive in *Salmonella* genus (*7*). Thus, the mere presence of the *acc(6')-Iaa* gene does not necessarily result in aminoglycoside resistance. On the other hand, the *fosA7* gene codes for a metal-dependent glutathione-S-transferase, conferring fosfomycin resistance (*8*). Originating chromosomally, possibly through gene islands or prophages, it aids in spreading *fosA7* among various *Salmonella* serovars (*8*). In China, the frequency of the *fosA7* gene ranges from 10% to 15% (*9–10*), with its presence being inconsistent among certain serotypes. Furthermore, most *fosA7*-positive *Salmonella* isolates demonstrate only low-level resistance to fosfomycin (*8*). Notably, our study discovered a higher prevalence of the *fosA7* gene in *S.* Grumpensis isolates.

The limited availability of information on *S.* Grumpensis globally highlights a gap in understanding its sources and transmission patterns. Utilizing WGS data and comparing it with international databases can help identify similar strains worldwide, aiding in pinpointing common origins. In our study, we combined publicly accessible genomes from the NCBI database with our own strains to conduct a thorough analysis of the global distribution and genetic characteristics of *S.* Grumpensis. This serotype has been prevalent in 11 countries and regions globally since 2005, with sources ranging from animal products to plant-based items like chili powders. However, the scarcity of non-human-origin genomes in the phylogenetic tree indicates a significant surveillance gap, especially in the food chain. Therefore, enhancing surveillance to encompass diverse sources beyond human clinical samples is essential.

For *S.* Grumpensis, two STs are notable: ST2060 and ST751. ST2060 has been prevalent in human samples since 2006 and has evolved into two genetic clades: 2060.1 and 2060.2, with the latter further dividing into three sub-clades. The two strains in this study are categorized under sub-clade 2060.2-1, similar to strains found in the UK, USA, and Senegal. Comparing the genetic profiles, the G5 isolate matches strains from the USA, while the G2 isolate varies by only 4 SNPs, suggesting a close genetic relationship and a possible common origin or shared transmission chain that might span international boundaries. However, the origin of the USA strain remains undisclosed, highlighting the necessity for robust genomic surveillance to monitor the global spread and evolution of pathogens like *S.* Grumpensis. Additionally, an isolate from Senegal within sub-clade 2060.2-1 was observed to contain 14 ARGs and show fewer than 100 SNP disparities compared to other ST2060.2-1 strains. The global spread of sub-clade 2060.2-1 indicates genetic adaptations influenced by various local conditions, leading to differences in ARG prevalence across regions. This highlights the crucial role of global cooperation and the implementation of comprehensive genomic surveillance networks.

In essence, identifying two cases of a rare *Salmonella* serotype in close proximity, both geographically and temporally, highlights the vital role of public health laboratories in early outbreak detection and response. By utilizing advanced diagnostics and genomics, these laboratories produce crucial scientific data essential for robust surveillance and efficient alert systems, crucial in densely populated urban areas where outbreaks can rapidly escalate, leading to significant public health threats. Embracing the "One Health" approach underscores the importance of breaking down barriers between sectors like human health, veterinary science, agriculture, and food production to enhance communication and collaboration. Additionally, establishing a comprehensive WGS database is key to fortifying a more vigilant and responsive public health infrastructure. The integration of cutting-edge laboratory techniques with a collaborative public health strategy forms the cornerstone of an effective surveillance and response mechanism, setting the stage for a healthier future.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. The AST results of the two strains in the study.

CLSI class	Antimicrobial agent	MIC interpretive standard (μg/mL)			Result (MICs)			
		Susceptible	Intermediate	Resistant	G2		G5	
Penicillins	Ampicillin (AMP)	≤8	16	≥32	≤1	S	≤1	S
β-lactamase inhibitor combinations	Ampicillin/Sulbactam (AMS)	≤8/4	16/8	≥32/16	≤1/0.5	S	≤1/0.5	S
	Ceftazidime/avibactam (CAZ)	≤8/4	NA	≥16/8	≤2/1	S	≤4/2	S
Cephems	Cefazolin (CFZ)	≤2	4	≥8	1	S	2	S
	Cefuroxime (CXM)	≤4	8–16	≥32	2	S	2	S
	Cefotaxime (CTX)	≤1	2	≥4	≤0.25	S	≤0.5	S
	Ceftazidime (CAZ)	≤4	8	≥16	0.5	S	1	S
	Cefepime (CPM)	≤2	NA	≥16	≤1	S	≤1	S
	Cefoxitin (CFX)	≤8	16	≥32	2	S	4	S
Penems	Imipenem (IMP)	≤1	2	≥4	≤0.25	S	≤0.25	S
	Meropenem (MEM)	≤1	2	≥4	0.25	S	0.25	S
	Ertapenem (ETP)	≤0.5	1	≥2	≤0.25	S	≤0.25	S
Aminoglycosides	Gentamicin (GEN)	≤2	4	≥8	≤1	S	≤1	S
	Streptomycin (STR)*	≤32	NA	≥64	8	S	8	S
	Amikacin (AMK)	≤4	8	≥16	≤2	S	≤2	S
Macrolides	Azithromycin (AZI)	≤16	NA	≥32	4	S	4	S
Monobactams	Aztreonam (ATM)	≤4	8	≥16	4	S	2	S
Tetracyclines	Tetracycline (TET)	≤4	8	≥16	≤1	S	≤1	S
Quinolones	Nalidixic acid (NAL)	≤16	NA	≥32	4	S	8	S
	Ciprofloxacin (CIP)	≤0.06	0.12–0.5	≥1	≤0.015	S	≤0.015	S
Phenicols	Chloramphenicol (CHL)	≤8	16	≥32	4	S	4	S
Folate pathway inhibitors	Trimethoprim/sulfamethoxazole (SXT)	≤2/38	NA	≥4/76	≤0.25/4.75	S	≤0.25/4.75	S

Abbreviation: NA=not applicable.
* STR is streptomycin breakpoints followed NARMS Standard, while other antibiotics used CLSI guideline.

SUPPLEMENTARY TABLE S2. The 53 genomes of S. Grumpensis used in the study.

SRA	BioSample	location	Sequence type	Year	Source 1	Source 2
ERR2812291	SAMEA4939521	Spain	2060	2018	Human	
ERR2812292	SAMEA4939522	Spain	2060	2018	Human	
ERR2812293	SAMEA4939523	Spain	2060	2018	Human	
ERR2812294	SAMEA4939524	Spain	2060	2018	Human	
ERR2812311	SAMEA4939541	Spain	2060	2017	Human	
ERR2812312	SAMEA4939542	Spain	2060	2017	Human	
ERR2812313	SAMEA4939543	Spain	2060	2017	Human	
ERR2812314	SAMEA4939544	Spain	2060	2017	Human	
ERR2812315	SAMEA4939545	Spain	2060	2017	Human	
ERR2812316	SAMEA4939546	Spain	2060	2017	Human	
ERR2812317	SAMEA4939547	Spain	2060	2006	Human	
ERR2812318	SAMEA4939548	Spain	2060	2007	Human	
ERR2812319	SAMEA4939549	Spain	2060	2017	Human	

Continued

SRA	BioSample	location	Sequence type	Year	Source 1	Source 2
ERR2812320	SAMEA4939550	Spain	2060	2017	Human	
ERR2812321	SAMEA4939551	Spain	2060	2017	Human	
ERR2812322	SAMEA4939552	Spain	2060	2017	Human	
ERR2812323	SAMEA4939553	Spain	2060	2017	Human	
ERR2812324	SAMEA4939554	Spain	2060	2017	Human	
ERR2812326	SAMEA4939565	Spain	2060	2016	Human	
ERR2812327	SAMEA4939566	Spain	2060	2017	Human	
ERR4399645	SAMEA7113136	Ireland	2060	2007	Human	
ERR4399741	SAMEA7113232	Ireland	2060	2007	Human	
ERR4401224	SAMEA7114717	Netherlands	96	2005	Plant	
ERR5490209	SAMEA8227021	Gambia	2060	2013	Human	
SRR10139533	SAMN12783652	UK	2060	2019	Human	
SRR11142710	SAMN14161829	UK	2060	2020	Human	
SRR11409991	SAMN14443813	UK	751	2020	Other	
SRR11425106	SAMN14449695	UK	751	2020	Other	
SRR11425253	SAMN14449709	UK	2060	2020	Human	
SRR11600410	SAMN14681283	UK	2060	2020	Human	
SRR13203049	SAMN16927787	Canada	751	2017	Human	
SRR15539823	SAMN20880130	USA	751	2021	Unknown	
SRR1604894	SAMN02678803	India	Novel	2010	Food	
SRR16764823	SAMN22867027	UK	2060	2021	Human	
SRR19552634	SAMN28885006	UK	2060	2022	Human	
SRR1967988	SAMN03478079	UK	2060	2014	Human	
SRR21981001	SAMN31382628	USA	751	2022	Unknown	
SRR25872707	SAMN31209171	UK	751	2022	Human	
SRR25873085	SAMN31169200	UK	751	2022	Human	
SRR26351730	SAMN37769924	USA	2060	2023	Unknown	
SRR3930417	SAMN05389757	USA	751	2016	Human	
SRR3996846	SAMN05464603	USA	2060	2011	Human	
SRR5361174	SAMN06481001	USA	751	Unknown	Unknown	
SRR6410434	SAMN08199243	USA	751	2017	Human	
SRR7064962	SAMN08951167	Brazil	751	2016	Poutry	
SRR7475324	SAMN09610265	UK	2060	2015	Human	
SRR7892229	SAMN10104853	UK	751	2016	Unknown	
SRR8380058	SAMN10662455	UK	2060	2018	Human	
SRR8401411	SAMN10698050	UK	751	2018	Human	
SRR8987871	SAMN11533635	Senegal	2060	2008	Human	CSF
SRR9281773	SAMN02678803	India	2767	2010	Food	
G2		China	2060	2023	Human	
G5		China	2060	2023	Human	