Diagnostic challenge of gastrointestinal infection due to lactose-fermenting Salmonella enterica subsp. enterica serovar 4,5:I:-

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Color should be used for both figures.

Running title: lactose-fermenting *Salmonella enterica*

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Abstract:

Here, we describe a case of a non-typhoidal *Salmonella* disease caused by a *Salmonella enterica* serovar 4,5:i:- (monophasic *Salmonella* Typhimurium) which acquired a Lac operon. This lactose-fermenting bacterium presents a major challenge for phenotypical detection of *Salmonella.* Only specific agar plates or molecular techniques allow reliable detection.

Keywords : Monophasic-Salmonella ; Salmonella Typhimurium ; Lac operon ; Lactose fermenting Salmonella ; C8-esterase

Case Report

A 6 year-old girl with no relevant past medical history was admitted to the emergency department for aqueous diarrhea with fever up to 39.5°C that had started three days before. No history of travel was noted and no family members were reported ill. A stool culture was then prescribed to search for *Salmonella*, *Shigella*, *Campylobacter* and *Yersinia*.

Twenty-four and 48 hours later no enteric pathogen was isolated. In particular no suspicious colonies were observed either on Hektoen agar (Biomérieux, Marcy-l'Étoile) or on C8-esterase agar (ChromID Salmonella®, Biomérieux), two media conventionally used for the detection of *Salmonella* (Figure 1). On Hektoen medium, *Salmonella* appear as blue-green colonies due to their inability to ferment lactose, and also generally have a black iron sulfite precipitate due to the reduction of thiosulfate in H_2S, while lactose-fermenting Enterobacteria grow yellow to red, due to the pH change caused by the lactose fermentation. On C8-esterase agar, *Salmonella*

are expected to be pink to mauve due to C8-esterase activity, compared to white when there is no C8-estearase activity.

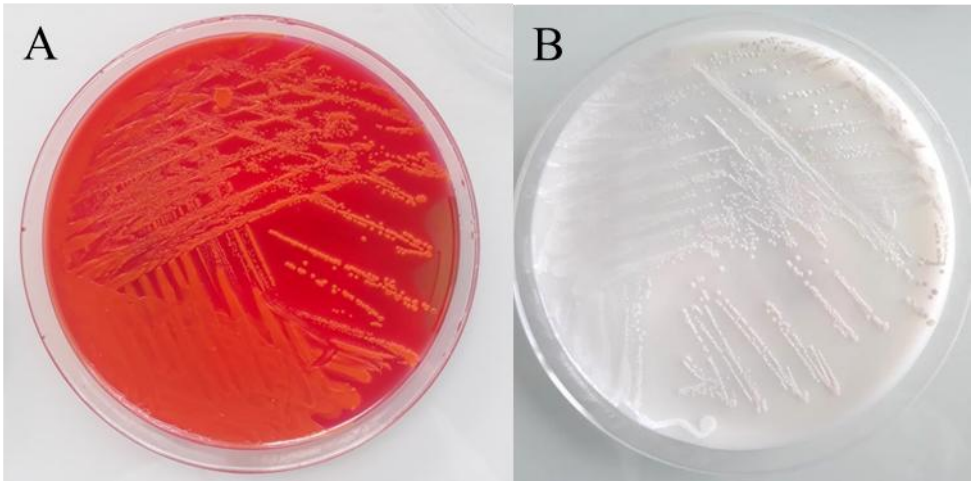

Figure 1 : Atypical *Salmonella enterica* serovar 4,5:i:- isolate appearing (A) as lactose-fermenting and H_2S non-producing colonies on Hektoen enteric agar medium and (B) as C8 esterase non-producing on chromogenic *Salmonella* medium (Biomérieux).

In order to search for other pathogens, a multiplex PCR (FilmArray GI panel, Biomérieux®) was performed on the stool and it detected *Salmonella spp.* as the sole pathogen. A bacterial identification performed by MALDI-TOF® technique (Brucker, Wissembourg, France) on the predominant lactose-fermenting (Lac+), H_2S negative (H_2S-), and C8-esterase negative (C8-) colonies, confirmed the presence of *Salmonella spp.* We then used three chromogenic agars: CHROMagar Salmonella®, CHROMagar Salmonella Plus® (CHROMagar, Paris, France) and BBL CHROMagar Salmonella® (Becton Dickinson, Le Pont-de-Claix, France) and only the CHROMagar Salmonella Plus® plate enabled the detection of the Lac+ *Salmonella* isolate (Figure 2).

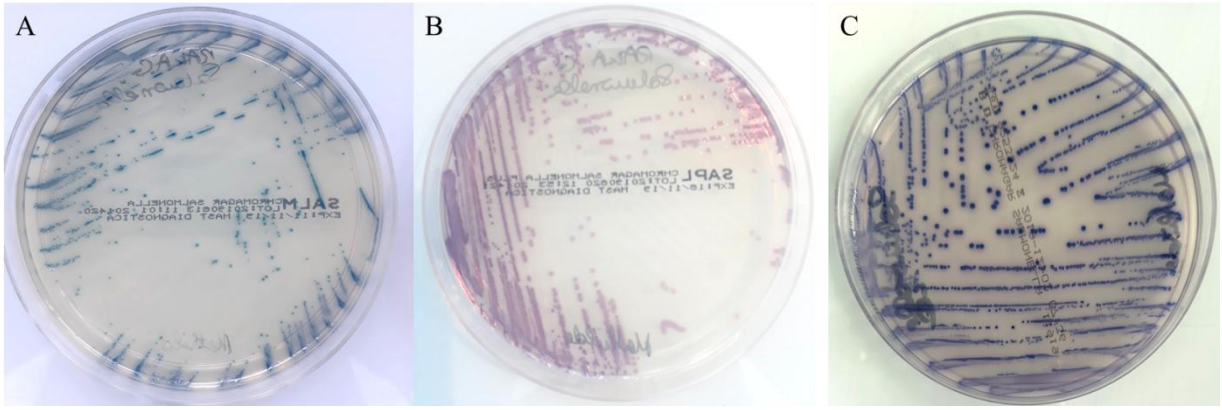

Figure 2 : The atypical *Salmonella enterica* serovar 4;5:i:- isolate appears (A) non-detectable on CHROMagar Salmonella® (blue instead of pink), (B) detectable on CHROMagar Salmonella Plus® (pink color expected of *Salmonella*) and (C) non-detectable on BBL CHROMagar® Salmonella (blue instead of pink).

The bacterial isolate was sent to the French National Reference Center for enteric pathogens (Institut Pasteur), where it received the reference number 201904327. The biochemical tests and serotyping performed identified *Salmonella enterica* subsp. *enterica* serovar 4,5:i:-. Whole genome sequencing (short-read sequence data available at https://www.ebi.ac.uk/ena/data/view/PRJEB36519) using an Illumina platform (NextSeq 500) revealed the presence of both *fliC* “i” and *fljB* “1,2” alleles and multilocus sequence type (ST) 34. Using the Enterobase cgMLST scheme (Zhou et al., 2020), the isolate belonged to HC900I2, HC20I2 and HC10I191521. No other isolates sharing HC10I191521 were identified in the Enterobase database containing 248 956 *Salmonella* genomes by January 25th 2020. No antibiotic resistance genes were detected using the Resfinder tool (https://cge.cbs.dtu.dk/services/ResFinder/). The genomic analysis allowed the identification of a Lac operon, 100% identical to the chromosomal Lac operon of *Enterobacter hormaechei*, as well as an IncHI1 plasmid also related to *E. hormaechei* plasmid (CP010380.1). Genes encoding the C8-esterase were complete. Complementary C8-esterase spot test (MUCAP® test, Biomérieux) confirmed the C8-esterase activity of this isolate.

Salmonella are Gram-negative, rod-shaped bacteria, belonging to the *Enterobacteriaceae* family. The *Salmonella* genus comprises two species, *S. bongori* and *S. enterica*. The *S. enterica* species is itself classically subdivided in 6 subspecies, including *S. enterica* subsp. *enterica*, a food-borne pathogen isolated from numerous warm-blooded animals (Havelaar et al., 2015). This subspecies contains many serovars which can be classified in 2 groups depending on clinical symptoms: typhoidal serovars and non-typhoidal serovars.

Salmonella enterica serovar Typhimurium (hereafter referred as to S. Typhimurium) and its monophasic variant are non-typhoidal serovars responsible of gastrointestinal disease in humans. Non-typhoidal *Salmonella* infection is characterised by fever, diarrhoea, abdominal pain and sometimes vomiting. Most of the time, this gastroenteritis is uncomplicated in immunocompetent patients. However, bacteraemia can occur in vulnerable patients and in this case antibiotics can be prescribed.

In the laboratory, the *Salmonella* genus is traditionally identified on Hektoen agar thanks to its biochemical properties leading to Lac- and H_2S+ colonies. Lactose non-fermentation is a key point in rapid *Enterobacteriaceae* screening and *Salmonella* identification. Only *S. enterica* subspecies *diarizonae* and *arizonae* are known to be naturally Lac+ with respectively approximately 75% and 25% of their serotypes being Lac+ (Grimont et al., 2007). Since the 90s, detection of *S. enterica* subsp. *enterica* in human stools has been improved by detecting the C8-esterase activity, specific to this *Salmonella* subspecies (Cooke et al., 1999; Gaillot et al., 1999).

Lac+ *S*. Typhimurium human isolates were first reported during a prolonged outbreak in São Paulo, Brazil in the early 1970s (Falcão et al., 1975). Six other outbreaks involving Lac+ but C8+ (referring to spot test not to chromogenic agars)

130 *S*. Typhimurium were also described in US cattle in 2000 (McDonough et al., 2000).
131 According to this latter study, the black iron sulfite precipitate is dissolved due to acid
132 production in the media during lactose hydrolysis, explaining the H2S- phenotype.
133 Other authors postulated that color intensity on chromogenic C8-esterase agar might
134 also be affected by the acidic environment (Cooke et al., 1999).
135 A recent study described the Lac operon genetic support in 13 Lac+ *S. enterica*
136 belonging to different non-Typhimurium serovars. The Lac operon was found either
137 on a plasmid similar to one carried by other *Enterobacteriaceae*, or on the
138 chromosome, flanked by IS elements (Leonard et al., 2015).
139
140 Due to the difficult detection of Lac+ *Salmonella* on conventional agar media, it is
141 possible that such isolates may not be so rare. Querying the full Enterobase
142 database for the presence of the Lac operon (Luhmann et al., 2020) might allow
143 accurate estimation of the proportion of the Lac+ *S. enterica* subsp. *enterica* isolates
144 that have been detected, sequenced and submitted to this genomic database.
145 In our laboratory, this Lac+ *Salmonella* isolate represented a major challenge and we
146 believe that without a molecular approach – here used in a second step to search for
147 other pathogens – the diagnostic would have been missed. Multiplex molecular
148 panels are increasingly replacing conventional methods for the detection of enteric
149 pathogens from stool samples. Our study did not aim to validate this approach.
150 Instead, we recommend the systematic use of an agar medium able to detect Lac+
151 *Salmonella*, like CHROMagar Salmonella Plus®, in case of negative reflex
152 conventional cultures for *Salmonella*. The systematic use of an agar able to detect
153 Lac+ *Salmonella* in clinical laboratories that only rely upon culture-based methods
154 might not be indicated given the apparent rarity of Lac+ *Salmonella* isolates and the

lack of specificity data with these media. They can, however, be used in a second line if conventional media do not show Lac- colonies, whereas there is a strong suspicion of salmonellosis.

Declarations of interest: none

Author contributions:

Mathilde Payen : Writing - Original Draft

François-Xavier Weill : Writing - Original Draft

Aurélie Cointe : Visualization, Writing - Review & Editing

Alix Massiot : Writing - Review & Editing

Philippe Bidet : Writing - Review & Editing

María Pardos de la Gándara : Sequencing and annotation of the strain, Writing - Review & Editing

Stéphane Bonacorsi : Supervision, Writing - Review & Editing

References:

Cooke, V.M., Miles, R.J., Price, R.G., Richardson, A.C., 1999. A novel chromogenic ester agar medium for detection of Salmonellae. Appl. Environ. Microbiol. 65, 807–812. PMID: 9925620

Falcão, D.P., Trabulsi, L.R., Hickman, F.W., Farmer, J.J., 1975. Unusual Enterobacteriaceae: lactose-positive Salmonella typhimurium which is endemic in São Paulo, Brazil. J. Clin. Microbiol. 2, 349–353. PMID: 1102562

Gaillot, O., di Camillo, P., Berche, P., Courcol, R., Savage, C., 1999. Comparison of CHROMagar Salmonella medium and hektoen enteric agar for isolation of salmonellae from stool samples. J. Clin. Microbiol. 37, 762–765. PMID: 9986847

Grimont, P. A., Weill F.-X. Antigenic Formulae of the Salmonella Serovars. 9th. Paris, France: WHO Collaborating Center for Reference and Research on Salmonella, Institut Pasteur; 2007.
Accessible at: https://www.pasteur.fr/sites/default/files/veng_0.pdf

Havelaar, A.H., Kirk, M.D., Torgerson, P.R., Gibb, H.J., Hald, T., Lake, R.J., Praet, N., Bellinger, D.C., de Silva, N.R., Gargouri, N., Speybroeck, N., Cawthorne, A., Mathers, C., Stein, C., Angulo, F.J., Devleesschauwer, B., on behalf of World Health Organization Foodborne Disease Burden Epidemiology Reference Group, 2015. World Health Organization Global Estimates and Regional Comparisons of the Burden of Foodborne Disease in 2010. PLOS Med. 12, e1001923. https://doi.org/10.1371/journal.pmed.1001923

Leonard, S.R., Lacher, D.W., Lampel, K.A., 2015. Acquisition of the lac operon by Salmonella enterica. BMC Microbiol. 15, 173. https://doi.org/10.1186/s12866-015-0511-8

Luhmann, N., Holley, G., Achtman, M., 2020. BlastFrost: Fast querying of 100,000s of bacterial genomes in Bifrost graphs (preprint). Bioinformatics. https://doi.org/10.1101/2020.01.21.914168

McDonough, P.L., Shin, S.J., Lein, D.H., 2000. Diagnostic and public health dilemma of lactose-fermenting Salmonella enterica serotype Typhimurium in cattle in the Northeastern United States. J. Clin. Microbiol. 38, 1221–1226. PMID: 10699026

Zhou, Z., Alikhan, N.-F., Mohamed, K., Fan, Y., the Agama Study Group, Achtman, M., 2020. The EnteroBase user's guide, with case studies on *Salmonella* transmissions, *Yersinia pestis* phylogeny, and *Escherichia* core genomic diversity. Genome Res. 30, 138–152. https://doi.org/10.1101/gr.251678.119