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1 **Diagnostic challenge of gastrointestinal infection due to lactose-fermenting**

2 ***Salmonella enterica* subsp. *enterica* serovar 4,5:i:-**

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28 Color should be used for both figures.

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31 **Running title:** lactose-fermenting *Salmonella enterica*

32 **Abstract :** 47 words

33 **Text :** 1035 words

34 **Abstract:**

35
36 Here, we describe a case of a non-typhoidal *Salmonella* disease caused by a
37 *Salmonella enterica* serovar 4,5:i:- (monophasic *Salmonella* Typhimurium) which
38 acquired a Lac operon. This lactose-fermenting bacterium presents a major
39 challenge for phenotypical detection of *Salmonella*. Only specific agar plates or
40 molecular techniques allow reliable detection.

41

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

44

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46 **Case Report**

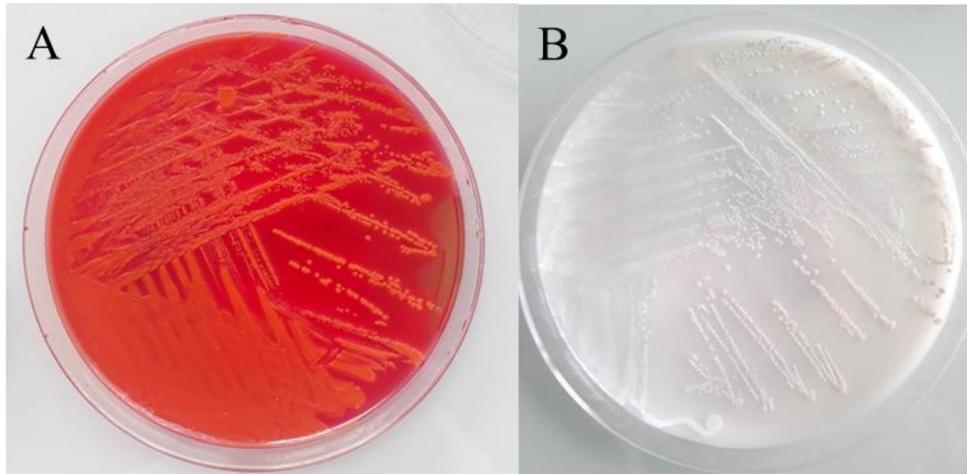
47
48 A 6 year-old girl with no relevant past medical history was admitted to the emergency
49 department for aqueous diarrhea with fever up to 39.5°C that had started three days
50 before. No history of travel was noted and no family members were reported ill. A
51 stool culture was then prescribed to search for *Salmonella*, *Shigella*, *Campylobacter*
52 and *Yersinia*.

53 Twenty-four and 48 hours later no enteric pathogen was isolated. In particular no
54 suspicious colonies were observed either on Hektoen agar (Biomérieux, Marcy-
55 l'Étoile) or on C8-esterase agar (ChromID *Salmonella*®, Biomérieux), two media
56 conventionally used for the detection of *Salmonella* (Figure 1). On Hektoen medium,
57 *Salmonella* appear as blue-green colonies due to their inability to ferment lactose,
58 and also generally have a black iron sulfite precipitate due to the reduction of
59 thiosulfate in H₂S, while lactose-fermenting Enterobacteria grow yellow to red, due to
60 the pH change caused by the lactose fermentation. On C8-esterase agar, *Salmonella*

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

63

64

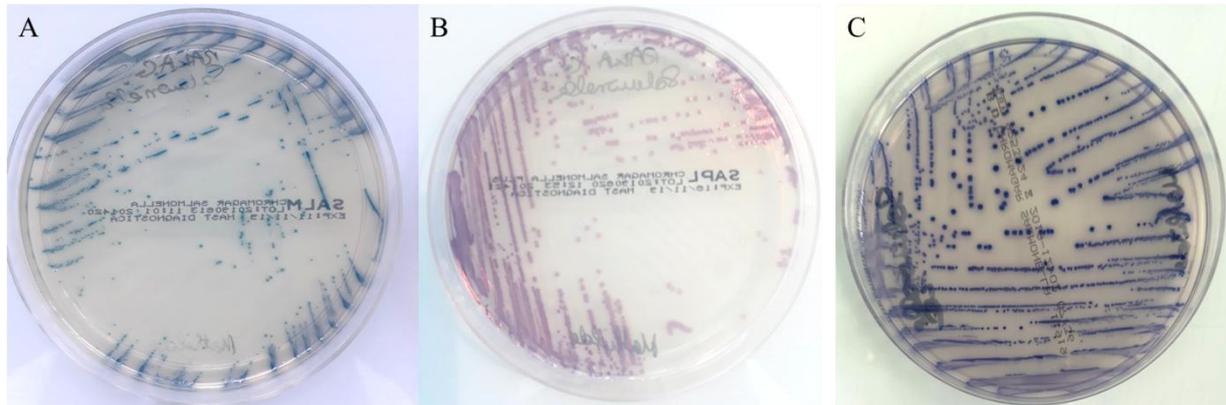


65 **Figure 1** : Atypical *Salmonella enterica* serovar 4,5:i:- isolate appearing (A) as
66 lactose-fermenting and H₂S non-producing colonies on Hektoen enteric agar medium
67 and (B) as C8 esterase non-producing on chromogenic *Salmonella* medium
68 (Biomérieux).
69

70

71

72 In order to search for other pathogens, a multiplex PCR (FilmArray GI panel,
73 Biomérieux®) was performed on the stool and it detected *Salmonella spp.* as the sole
74 pathogen. A bacterial identification performed by MALDI-TOF® technique (Brucker,
75 Wissembourg, France) on the predominant lactose-fermenting (Lac+), H₂S negative
76 (H₂S-), and C8-esterase negative (C8-) colonies, confirmed the presence of
77 *Salmonella spp.* We then used three chromogenic agars: CHROMagar Salmonella®,
78 CHROMagar Salmonella Plus® (CHROMagar, Paris, France) and BBL CHROMagar
79 Salmonella® (Becton Dickinson, Le Pont-de-Claix, France) and only the
80 CHROMagar Salmonella Plus® plate enabled the detection of the Lac+ *Salmonella*
81 isolate (Figure 2).



82
 83 **Figure 2** : The atypical *Salmonella enterica* serovar 4;5:i:- isolate appears (A) non-
 84 detectable on CHROMagar Salmonella® (blue instead of pink), (B) detectable on
 85 CHROMagar Salmonella Plus® (pink color expected of *Salmonella*) and (C) non-
 86 detectable on BBL CHROMagar® Salmonella (blue instead of pink).
 87

88 The bacterial isolate was sent to the French National Reference Center for enteric
 89 pathogens (Institut Pasteur), where it received the reference number 201904327.

90 The biochemical tests and serotyping performed identified *Salmonella enterica*
 91 subsp. *enterica* serovar 4,5:i:-. Whole genome sequencing (short-read sequence
 92 data available at <https://www.ebi.ac.uk/ena/data/view/PRJEB36519>) using an

93 Illumina platform (NextSeq 500) revealed the presence of both *fliC* “i” and *fljB* “1,2”
 94 alleles and multilocus sequence type (ST) 34. Using the Enterobase cgMLST
 95 scheme (Zhou et al., 2020), the isolate belonged to HC900I2, HC20I2 and
 96 HC10I19I52I. No other isolates sharing HC10I19I52I were identified in the
 97 Enterobase database containing 248 956 *Salmonella* genomes by January 25th 2020.

98 No antibiotic resistance genes were detected using the Resfinder tool
 99 (<https://cge.cbs.dtu.dk/services/ResFinder/>). The genomic analysis allowed the

100 identification of a Lac operon, 100% identical to the chromosomal Lac operon of
 101 *Enterobacter hormaechei*, as well as an IncHI1 plasmid also related to *E. hormaechei*
 102 plasmid (CP010380.1). Genes encoding the C8-esterase were complete.

103 Complementary C8-esterase spot test (MUCAP® test, Biomérieux) confirmed the
 104 C8-esterase activity of this isolate.

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

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

119 In the laboratory, the *Salmonella* genus is traditionally identified on Hektoen agar
120 thanks to its biochemical properties leading to Lac- and H₂S+ colonies. Lactose non-
121 fermentation is a key point in rapid *Enterobacteriaceae* screening and *Salmonella*
122 identification. Only *S. enterica* subspecies *diarizonae* and *arizonae* are known to be
123 naturally Lac+ with respectively approximately 75% and 25% of their serotypes being
124 Lac+ (Grimont et al., 2007). Since the 90s, detection of *S. enterica* subsp. *enterica* in
125 human stools has been improved by detecting the C8-esterase activity, specific to
126 this *Salmonella* subspecies (Cooke et al., 1999; Gaillot et al., 1999).

127 Lac+ *S. Typhimurium* human isolates were first reported during a prolonged outbreak
128 in São Paulo, Brazil in the early 1970s (Falcão et al., 1975). Six other outbreaks
129 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

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

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162 **Declarations of interest:** none

163

164 **Author contributions:**

165 Mathilde Payen : Writing - Original Draft

166 François-Xavier Weill : Writing - Original Draft

167 Aurélie Cointe : Visualization, Writing - Review & Editing

168 Alix Massiot : Writing - Review & Editing

169 Philippe Bidet : Writing - Review & Editing

170 María Pardos de la Gándara : Sequencing and annotation of the strain, Writing -

171 Review & Editing

172 Stéphane Bonacorsi : Supervision, Writing - Review & Editing

173

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175

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