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Be aware of Shiga-toxin 2f-producing Escherichia coli: case report and false-negative results with certain rapid molecular panels

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1 **Be aware of Shiga-toxin 2f-producing *Escherichia coli* : case-report and false negative**
2 **results with certain rapid molecular panels**

3

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19

20 **Running title: Misdiagnosis of Stx2f by molecular panel**

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22 **Abstract : 50 words**

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27

28 **Abstract**

29 We report a hemolytic uremic syndrome (HUS) case due to Stx2f-producing *E. coli*
30 illustrating the diagnostic difficulty of this Shiga-toxin subtype. Clinicians should be aware of
31 limits of certain rapid molecular panels that are increasingly being used and may play a role in
32 underestimating the global burden of such infections.

33

34 **Keywords:** enterohemorrhagic *Escherichia coli*, Shiga-toxin 2f, hemolytic uremic syndrome,
35 hybrid pathotype, rapid gastrointestinal molecular panels

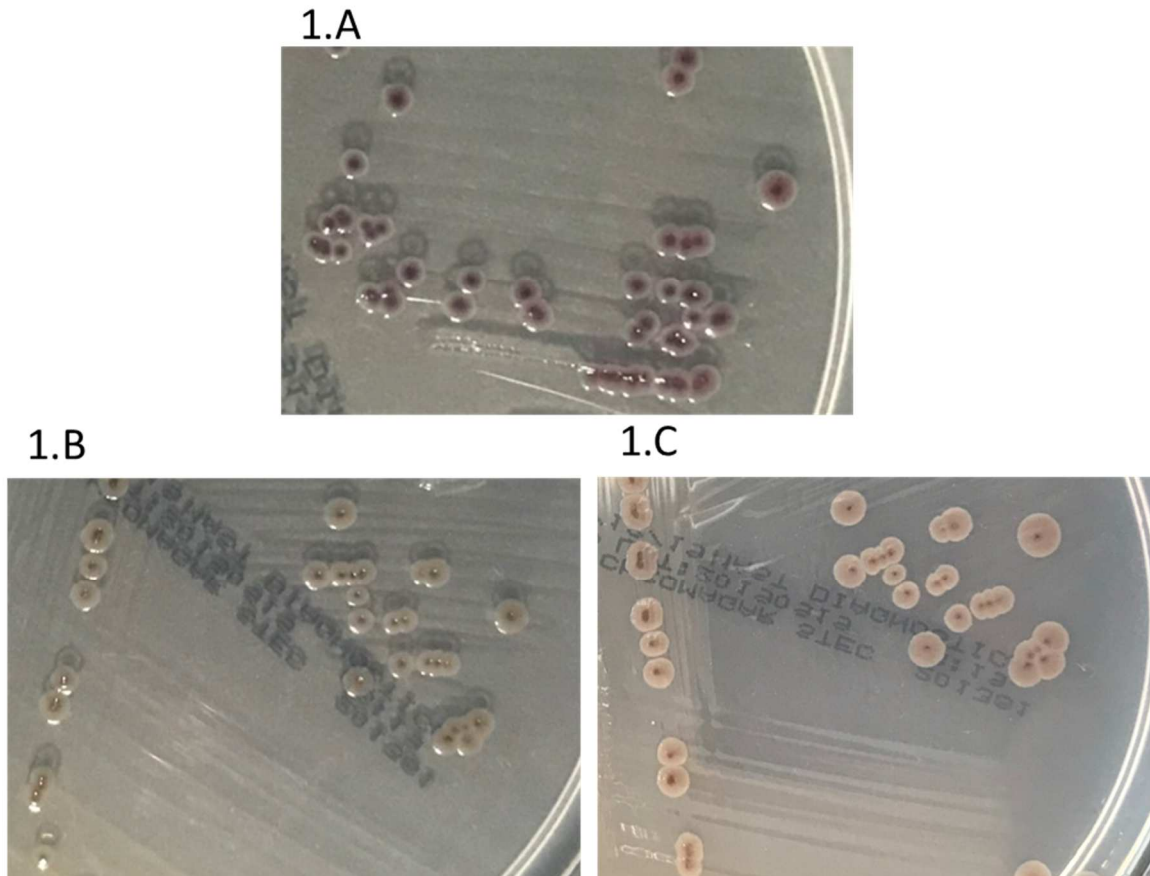
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39 Case report

40 A two-year-old boy was admitted to the intensive care unit of Rouen University
41 Hospital (France) in December 2018 for oliguria and acute renal failure 10 days after the
42 onset of non-bloody diarrhea and vomiting spontaneously resolving. At admission, clinical
43 examination found a fever of 38.5°C. Laboratory findings showed signs of hemolytic uremic
44 syndrome (HUS) with anemia (hemoglobin level of 10 g/dL), thrombocytopenia (platelet
45 count of 108 G/L), acute renal failure (serum creatinine level of 306 µmol/L), and hemolysis
46 hallmarks (presence of schistocytes (1%), undetectable haptoglobin, and an LDH level of
47 2,402 UI/L). During hospitalization, the hemoglobin level decreased to 7.2 g/dL (maximum of
48 4% schistocytes) and the platelet count to 93 G/L but no blood transfusion was required. The
49 serum creatinine level increased to 659 µmol/L leading to 48 hours of anuria but did not
50 require dialysis. Investigation of the complement pathway showed normal C3, C4, and CH50
51 levels. Stool sample was sent to the National Reference Center (NRC) at Robert Debré
52 University Hospital (Paris, France) to search for an enterohemorrhagic *Escherichia coli*
53 (EHEC) and thus confirm the diagnosis of typical HUS. Classical multiplex PCR was
54 performed, following stool broth enrichment, using primers that allow the amplification of all
55 Shiga-toxin (Stx) subtypes, except *stx2f*, as well as intimin (*eae*) and enterohemolysin (*ehxA*)
56 genes (1). The PCR was positive for *eae* and *ehxA* but negative for *stx1* and *stx2*. The clinical
57 history and combined presence of the *eae* and *ehxA* genes suggested the presence of an EHEC
58 strain. Thus, the stool broth was further tested for the *stx*-subtype specific PCR (Istituto
59 Superiore di Sanità, Italy, <http://old.iss.it/vtec/>) and was positive for the *stx2f* subtype.
60 Bacterial culture confirmed the presence of an EHEC strain (PMK-45057) harboring the *eae*,
61 *stx2f*, and *ehxA* genes. On CHROMagar™ STEC medium, this strain has a particular color
62 (brownish) characteristic of O80 serogroup while most of STEC belonging to the major
63 serogroups appear purple (Figure 1).



64

65

66 Figure 1: Enterohemorrhagic *Escherichia coli* (EHEC) growth on CHROMagar™ STEC medium. Most
 67 EHEC serogroups appear purple as shown by the representative strain O157:H7 EDL933 (Figure 1.A)
 68 while O80 and O55 serogroups are stained in brownish. Appearance of the isolate O55:H9 from the
 69 case (PMK-45057) after 24 and 48 hours of incubation at 35°C (Figure 1.B and 1.C respectively)

70

71 PMK-45057 whole genome sequencing shows that it belongs to serotype O55:H9,
 72 ST-301 and harbors *fimH54*. Sequencing data confirmed the combined presence of the *eae-ξ*,
 73 *ehxA*, and *stx2f* subtype but also unexpectedly, genes characteristic of the pS88 plasmid, a key
 74 determinant of extra-intestinal pathogenic *E. coli* virulence (2). Moreover, *in silico* analysis
 75 showed the presence of multiple resistance genes, conferring resistance to beta-lactam
 76 (*bla*_{TEM-1B}), aminoglycoside (*aph*(3')-Ia, *strA*, *strB*, *aadA1*, *aadB*), sulfonamide (*sul1*, *sul2*),
 77 nalidixic acid (*gyrA* S83L), tetracycline (*tetA*, *tetB*), and phenicols (*floR*). This molecular
 78 resistance profile was correlated with phenotypic result (diffusion method). Together, these

79 features, except the O55 antigen and *stx2f*, are hallmarks of the emergent O80 EHEC hybrid
80 pathotype, responsible for HUS and bacteremia and now reported in several countries in
81 Europe (2).

82 We then evaluated the detection capacity of commercially available molecular panels
83 that detect major gastrointestinal pathogens, including EHEC. They are increasingly used in
84 microbiological laboratories and will very likely replace conventional culture-based methods
85 in the near future. We tested the stool broth with four major gastrointestinal panels available
86 in France: the BioFire FilmArray® Gastrointestinal Panel, Novodiag® Bacterial GE+,
87 DiagCORE® Gastrointestinal Panel, and BD MAX® Enteric Bacterial Panel. *E. coli*
88 pathovars targeted by these kits and the results obtained with the stool broth are summarized
89 in Table 1. Briefly, only the DiagCORE® and the Novodiag® detected the *stx2f* gene,
90 whereas the other two tests were negative for this gene (Table 1).

91

Pathovar targeted ^b and detection of <i>stx2f</i> in this case report	Rapid gastrointestinal molecular panels ^a			
	Novodiag® Bacterial GE+ (Mobidiag, Espoo, Finland)	BioFire FilmArray® Gastrointestinal Panel (BioMérieux, Marcy l’Etoile, France)	DiagCORE® Gastrointestinal Panel (Stat Dx Qiagen, Barcelona, Spain)	BD MAX® Enteric Bacterial Panel (Beckton Dickinson, Franklin Lakes, USA)
EAEC	×	×	×	
EHEC, <i>stx1</i>	×	× ^c	× ^c	× ^c
EHEC, <i>stx2</i>	×	× ^c	× ^c	× ^c
<i>E. coli</i> O157		×	×	
EIEC/ <i>Shigella</i> spp	×	×	×	×
EPEC	×	× ^d	× ^d	
ETEC	×	×	×	×
<i>stx2f</i> detection in the EHEC of the case report ^e	pos	neg	pos	neg

^aa cross means that the target is included in the test

^bEAEC, Enteroaggregative *E. coli*; EHEC, Enterohemorrhagic *E. coli*; EIEC, Enteroinvasive *E. coli*; EPEC, Enteropathogenic *E. coli*; ETEC, Enterotoxigenic *E. coli*.

^cdistinction between *stx1* and *stx2* does not appear in the result provided by these tests

^dthe presence of *eae* gene is masked when *stx1* or *stx2* are detected

^epos: *stx2f* detected; neg: *stx2f* not detected.

92 Table 1. *E. coli* pathovars targeted by four rapid gastrointestinal molecular panels and ability to
93 detect the Stx2f-producing *E. coli* by each test in the case reported.

94 Enterohemorrhagic *Escherichia coli* (EHEC), a subset of Shiga-toxin-producing
95 *E. coli* (STEC), is a major foodborne pathogen that can cause hemolytic uremic syndrome
96 (HUS), the most common cause of acute renal failure in young children.

97 Shiga-toxin, essential for the development of HUS, can be divided into two types:
98 Stx1, composed of the three subtypes, Stx1a, 1c, and 1d; and Stx2, composed of seven
99 subtypes: Stx2a-g. Stx2f is one of the last to be described, first isolated from the feces of feral
100 pigeons (3) and long considered a minor public health concern. Data on human illness due to
101 Stx2f-producing *E. coli* were scarce (4–6) until recent reports from Germany (7), Belgium (8),
102 and the Netherlands (9), showing that this subtype is increasingly associated with diarrhea and
103 sporadic HUS cases in Europe, making it an emerging human pathogen. Indeed, in Belgium
104 (8) and the Netherlands (9), Stx2f-producing *E. coli* infections are more common than would
105 be expected, representing an estimated 12% and 20%, respectively, of all STEC infections.

106 Although the reports of this subtype are currently increasing, it has been rarely reported in
107 HUS cases and in a limited number of serotypes (7, 9, 10). Here, we reported an *stx2f* subtype
108 in an O55:H9 strain that had never been described previously to our knowledge, except in one
109 strain responsible for a case of HUS in Italy in 2014 (10). Unexpectedly, sequencing data of
110 PMK-45057 showed this O55:H9 EHEC strain to be very close to EHEC of the O80
111 serogroup, a major clonal group considered as a hybrid pathotype due to the presence of
112 extra-intestinal virulence factors and the description of bacteremia in several cases (2).
113 Although no extra-intestinal spread occurred in our case, this O55 EHEC can also be
114 considered to be a hybrid pathotype, perfectly equipped to induce HUS, as well as life-
115 threatening invasive infections, making its diagnosis essential.

116 The Stx2f subtype is the most divergent (by nucleotide and amino acid sequence) of the
117 known Stx2 subtypes (11), showing very low homology with other subtypes and explaining

118 the difficulty to detect it. The FilmArray®, which is widely used, failed to detect this subtype,
119 as previously shown (12). To our knowledge, it is the only rapid molecular panel previously
120 evaluated for its performance on *stx2f* detection. Here, we show that only two of the other
121 three major panels used in microbiological laboratories, both in the community and the
122 hospital, were able to detect *stx2f*, the DiagCORE® Gastrointestinal Panel and Novodiag®
123 Bacterial GE+. These results highlight the fact that biologists and clinicians should be aware
124 of the performance of these new commercial tests, especially concerning the detection of
125 *stx2f*, an emerging subtype involved in HUS.

126

127 In conclusion, the Stx2f-subtype, first described in strains isolated from pigeons, has long
128 been considered to be a minor public health concern. Here, we described the first report of a
129 *stx2f*-producing EHEC of serotype O55:H9 responsible for HUS and perfectly equipped to
130 induce invasive infections. The varying ability of commercial tests to detect *stx2f* requires
131 increased vigilance on the part of microbiologists and clinicians for interpreting a negative
132 result in the presence of strong clinical suspicion of HUS. In this case, the laboratory should
133 use another method or send stools to a reference laboratory, especially if the intimin encoding
134 gene is detected. Microbiologists should at least clearly indicate to clinicians the ability of
135 their tests to detect *stx2f* gene.

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142 **Conflict of Interest**

143 None to declare.

144 **Ethics**

145 Written informed consent from parents of the child involved in this case was obtained.

146

147 **Author contributions**

148 Aurélie Cointe : Conceptualization, Resources (acquisition, analysis and interpretation of
149 data), Writing - original draft

150 André Birgy : Conceptualization, Supervision, Validation, Writing - review & editing

151 Alice Pascault : Resources (acquisition of data), Writing - review & editing

152 Ferialle Louillet : Resources (acquisition of data), Writing - review & editing

153 Alice Dufougeray : Resources (acquisition of data), Writing - review & editing

154 Patricia Mariani-Kurkdjian : Supervision, Validation, Writing - review & editing

155 Stéphane Bonacorsi : Conceptualization, Supervision, Validation, Writing - review & editing

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157 **References**

- 158 1. Paton AW, Paton JC. 1998. Detection and Characterization of Shiga Toxigenic
159 *Escherichia coli* by Using Multiplex PCR Assays for stx1, stx2, eaeA, Enterohemorrhagic
160 *E. coli* hlyA, rfbO111, and rfbO157. J Clin Microbiol 36:598–602.
- 161 2. Cointe A, Birgy A, Mariani-Kurkdjian P, Liguori S, Courroux C, Blanco J, Delannoy S,
162 Fach P, Loukiadis E, Bidet P, Bonacorsi S. 2018. Emerging Multidrug-Resistant Hybrid
163 Pathotype Shiga Toxin-Producing *Escherichia coli* O80 and Related Strains of Clonal
164 Complex 165, Europe. Emerging Infect Dis 24:2262–2269.
- 165 3. Schmidt H, Scheef J, Morabito S, Caprioli A, Wieler LH, Karch H. 2000. A New Shiga
166 Toxin 2 Variant (Stx2f) from *Escherichia coli* Isolated from Pigeons. Appl Environ
167 Microbiol 66:1205–1208.
- 168 4. Sonntag A-K, Zenner E, Karch H, Bielaszewska M. 2005. Pigeons as a possible reservoir
169 of Shiga toxin 2f-producing *Escherichia coli* pathogenic to humans. Berl Munch Tierarztl
170 Wochenschr 118:464–470.
- 171 5. Etoh Y, Murakami K, Ichihara S, Sera N, Hamasaki M, Takenaka S, Horikawa K,
172 Kawano K, Takeishi T, Kuwana Y, Inoue A, Nagatsu Y, Hira Y, Takahashi M, Ito K.
173 2009. Isolation of Shiga toxin 2f-producing *Escherichia coli* (O115:HNM) from an adult
174 symptomatic patient in Fukuoka Prefecture, Japan. Jpn J Infect Dis 62:315–317.
- 175 6. Friesema IHM, Keijzer-Veen MG, Koppejan M, Schipper HS, van Griethuysen AJ, Heck
176 MEOC, van Pelt W. 2015. Hemolytic Uremic Syndrome Associated with *Escherichia coli*
177 O8:H19 and Shiga Toxin 2f Gene. Emerg Infect Dis 21:168–169.

- 178 7. Prager R, Fruth A, Siewert U, Strutz U, Tschäpe H. 2009. *Escherichia coli* encoding
179 Shiga toxin 2f as an emerging human pathogen. *Int J Med Microbiol* 299:343–353.
- 180 8. De Rauw K, Jacobs S, Piérard D. 2018. Twenty-seven years of screening for Shiga toxin-
181 producing *Escherichia coli* in a university hospital. Brussels, Belgium, 1987-2014. *PLoS*
182 *One* 13.
- 183 9. Friesema I, van der Zwaluw K, Schuurman T, Kooistra-Smid M, Franz E, van Duynhoven
184 Y, van Pelt W. 2014. Emergence of *Escherichia coli* encoding Shiga toxin 2f in human
185 Shiga toxin-producing *E. coli* (STEC) infections in the Netherlands, January 2008 to
186 December 2011. *Eurosurveillance* 19:20787.
- 187 10. Grande L, Michelacci V, Bondì R, Gigliucci F, Franz E, Badouei MA, Schlager S, Minelli
188 F, Tozzoli R, Caprioli A, Morabito S. 2016. Whole-Genome Characterization and Strain
189 Comparison of VT2f-Producing *Escherichia coli* Causing Hemolytic Uremic Syndrome.
190 *Emerg Infect Dis* 22:2078–2086.
- 191 11. Skinner C, McMahon S, Rasooly R, Carter JM, He X. 2013. Purification and
192 Characterization of Shiga Toxin 2f, an Immunologically Unrelated Subtype of Shiga
193 Toxin 2. *PLoS One* 8.
- 194 12. De Rauw K, Detemmerman L, Breynaert J, Piérard D. 2016. Detection of Shiga toxin-
195 producing and other diarrheagenic *Escherichia coli* by the BioFire FilmArray®
196 Gastrointestinal Panel in human fecal samples. *Eur J Clin Microbiol Infect Dis* 35:1479–
197 1486.

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