Yersinia enterocolitica in Italy: A Case of Septicemia and Abdominal Aortic Aneurysm Infection

Donatella M. Rodio¹†, Alessia Bressan¹†, Cecilia Ambrosi¹,²†, Daniela Scribano¹, Rita Tolli³, Wassim Mansour⁴, Francesco Speziale⁴, Guido Antonelli⁵, Maria Trancassini¹† and Valeria Pietropaolo¹*

† These authors have contributed equally to this work.

INTRODUCTION

Yersinia enterocolitica is a non-sporo Gram-negative bacterium belonging to Yersinia genus and to the Enterobacteriaceae family. Among Yersinia species, only Y. pestis, Y. pseudotuberculosis, and some Y. enterocolitica strains are pathogenic for humans, whereas others are environmental species that might be opportunistic pathogens. Y. enterocolitica is a heterogeneous group of bacterial strains, classified into 6 biogroups (1A, 1B, 2, 3, 4, and 5) based on phenotypic characteristics, human/animal pathogenicity, and ecologic and geographic distribution (1). Serologically, Y. enterocolitica is further distinguished into more than 57 O serogroups, depending on their lipopolysaccharide-O-antigens. Only isolates belonging to biogroup 1A may be considered avirulent so far. Among the other 5 biogroups, strains belonging to serogroups O:3 (biogroup 4), O:5,27 (biogroups 2 and 3), O:8 (biogroup 1B), and O:9 (biogroup 2) are most commonly isolated from human samples worldwide (2, 3). However, the most prevalent Y. enterocolitica serogroup in
many European countries is serogroup O:3 followed by O:9, whereas the serogroup O:8 is mainly detected in the United States (1, 3). Despite a significant decrease between 2008 and 2016, human Yersiniosis represents the third most commonly reported bacterial food-borne zoonosis in all EU countries (https://ecdc.europa.eu/).

As a foodborne pathogen, *Y. enterocolitica* causes acute terminal ileitis and mesenteric lymphadenitis. Depending on both patient conditions and bacterial serogroup, it can spread at the systemic level (3, 4). Several septic complications have been described that can evolve, eventually, into endocarditis or infected aortic aneurysm, also known as mycotic aneurysm (3). At present, only 14 papers associated with *Y. enterocolitica* have been reported in literature (Table 1), including 13 cases of infected abdominal aortic aneurysms (AAA). The main pathogenic properties of *Y. enterocolitica* are due to the presence of the two chromosomally encoded genes, *ail* and *yst* (1, 19, 20). The *ail* gene encodes for the membrane-associated protein Ail, involved in adhesion to and invasion into eukaryotic cells, as well as serum resistance. Conversely, the *yst* gene encodes for thermostable enterotoxin Yst, whose presence is related with diarrheal illness, although its role remains largely controversial (21). Being the most common virulence genes of *Y. enterocolitica*, both are considered excellent PCR targets for bacterial detection and subtyping as well as for its pathogenicity potential (22).

Herein, we describe a case of *Y. enterocolitica* septicemia in a patient suffering from an AAA. Microbiological and molecular analyses have demonstrated the presence of a strain belonging to the O:9 serotype, carrying both the *ail* and *yst* genes and resistant to amoxicillin/clavulanate.

### TABLE 1 | Data summarizing previously reported cases of *Y. enterocolitica* mycotic aneurysms.

| References | Age | Sex | First isolated from | GI symptoms | Endocarditis | Site of aneurysm | Serogroup/Biogroup | Antibiotic therapy | Outcome |
|------------|-----|-----|---------------------|-------------|--------------|----------------|--------------------|-------------------|---------|
| (5)        | 53  | M   | Stool and blood     | Yes         | No           | Carotid artery | Biotype 4          | Cefamandole       | Good    |
| (6)        | 79  | M   | Blood               | Yes         | No           | AAA*           | Serotype O:3, Biotype 4 | Ampicillin and gentamicin | Fatal    |
| (7)        | 76  | M   | Aneurysm wall       | Yes         | No           | AAA*           | Serotype O:9, Biotype 2 | Gentamicin and Co-trimoxazole | Good    |
| (8)        | 59  | M   | Blood               | Yes         | No           | AAA*           | Biotype 4          | Cefuroxime and metronidazole | Fatal    |
| (9) (6 cases) | 73  | M   | Blood               | No          | No           | AAA* and Popliteal Artery | Serotype O:9 | Ciprofloxacin and Co-trimoxazole | Fatal (2/6) |
| (10)       | 57  | M   | Resected aorta      | No          | No           | AAA*           | Serotype O:3, Biotype 4 | Ciprofloxacin and aztreonam | Fatal    |
| (11) (3 cases) | 70  | M   | Blood               | No          | No           | AAA*           | Serotype O:9       | Ciprofloxin/tetracycline and Ceftriaxone | Fatal (2/3) |
| (12)       | 64  | M   | Blood               | Yes         | No           | Femoral artery and AAA* | Unspecified       | Unspecified       | Fatal    |
| (13)       | 78  | M   | Blood               | No          | No           | AAA*           | Serotype O:9, Biotype 2 | Ofloxacin          | Good    |
| (14)       | 55  | M   | Blood               | No          | No           | AAA*           | Serotype O:3, Biotype 4 | Gentamicin and ceftriaxone | Fatal    |
| (15)       | 70  | M   | Vascular graft      | No          | No           | AAA*           | Serotype O:3, Biotype 4 | Cefotaxime and ciprofloxacin | Good    |
| (16)       | 74  | M   | Blood               | Yes         | Yes          | AAA*           | No serogroup/Biotype 2 | Amoxicillin, metronidazole and gentamicin | Good    |
| (17)       | 78  | M   | Blood               | No          | Yes          | AAA*           | Specified          | Ciprofloxacin     | Good    |
| (18)       | 68  | M   | Blood               | No          | No           | AAA*           | Specified          | Piperacillin-tazobactam | Good    |

*AAA, abdominal aortic aneurysms.*
CASE REPORT

Ethics Statement

The subject gave a written informed consent in accordance with the Declaration of Helsinki.

A 63-year-old patient was admitted to the Vascular Surgery Department of Umberto I Hospital (Rome, Italy) for an AAA.

At admission, the patient presented with low-grade fever (37.2°C) and reported a history of asthenia and weight loss in the previous months. No abdominal symptoms, vomiting or diarrhea were referred. In September 2017, the patient performed an abdominal ultrasound and blood tests, including three sets of peripheral blood culture that were sent to the Microbiology laboratory. Blood tests showed an erythrocyte sedimentation rate of 48 mm/h, a white blood cell (WBC) count of 13.94 x 10^9/L and a C-reactive protein (CRP) level of 185 mg/L. An empirical therapy was therefore started with the intravenous administration of daptomycin (500 mg die), ertapenem (1 g die), and fluconazole (400 mg die). Abdominal ultrasound identified described an abdominal aortic aneurysm with a diameter of 6.42 cm. The computed tomography (CT) scan confirmed the presence of the AAA with a peri-aortic inflammation (Figure 1). Moreover, trans-thoracic echocardiogram ruled out active endocarditis. The patient therefore underwent endovascular repair. After 24 h of incubation in the automatic Virtuo BacT/ALERT (bioMérieux, Inc. France), two sets of blood cultures became positive. Microscopic examination revealed motile Gram-negative coccobacilli. Colonies on MacConkey agar were small, round and non-lactose fermenting. Colonies on MacConkey agar, Extended Spectrum Beta-Lactamase (ESBL) Gram staining. In parallel, isolates were seeded on Blood agar, CIN agar plates and CIN agar plates and incubated at 28°C for 24 h. Clinical isolates were identified by VITEK 2 System (bioMérieux, Inc. France) and by MICROSCAN WalkAway System 96 Plus (Beckman Coulter S.r.l.) revealed that the Y. enterocolitica isolate was susceptible to all antibiotics tested, except to amoxicillin/clavulanate (Table 2). Serogrouping analysis using specific Y. enterocolitica antisera evidenced that the Y. enterocolitica isolate belonged to the O:9 serotype (biogroup 2). Therefore, we concluded that the same Y. enterocolitica isolate was colonizing the patient. The antibiotic therapy was modified; the patient suspended daptomycin and fluconazole and was treated intravenously with ertapenem (1 g die) and tigecycline (100 mg for first day and 50 mg for following days) for 4 weeks. Over this period, all the sets of blood culture were negative. The patient was discharged home 8 weeks after surgery.

MATERIALS AND METHODS

Peripheral blood culture bottles, for both aerobic and anaerobic microorganisms, were incubated for 24 h in the automatic Virtuo BacT/ALERT (bioMérieux, Inc. France). Aneurysm specimens were incubated in BHI at 37°C for 24 h. Clinical isolates were examined under light microscope (400x) for motility and upon Gram staining. In parallel, isolates were seeded on Blood agar, MacConkey agar, Extended Spectrum Beta-Lactamase (ESBL) agar (bioMérieux, Inc. France), and CIN agar plates and incubated at 28°C for 24 h. Colonies were identified by VITEK 2 System (bioMérieux, Inc. France), by MALDI-TOF MS System (Bruker Daltonics, Inc.) and by 16S rDNA gene sequencing, as previously described respectively in Febbraro et al. (24) and in Yu et al. (25). Antimicrobial susceptibility testing was performed by VITEK 2 System (bioMérieux, Inc. France) and by MICROSCAN WalkAway System 96 Plus (Beckman Coulter S.r.l.). The resulting minimum inhibitory concentration (MIC) values were classified into clinical categories of susceptible, intermediate or resistant, following the EUCAST recommendations (26). Serogrouping analysis was performed by slide agglutination using O:3, O:5, O:8, and O:9 antisera (Biogenetics, s.r.l. Italy).
following the recommendation of the manufacturer. Real-time PCR was performed as previously described (21). Both analyses were carried out at the Experimental Zooprophylactic Institute of Lazio and Tuscany “Mariano Aleandri” (Rome, Italy).

**DISCUSSION**

There has been an increasing body of evidence showing that *Y. enterocolitica* serogroups O:3 and O:9 are a cause of mycotic aneurysm and septicaemia, mainly in males over 50 years, with risk factors for cardiovascular disease (10, 14). Herein, we report a case of *Y. enterocolitica* serogroup O:9 (biogroup 2) septicemia in a patient with an AAA. It is important to note that this is the first study reporting the identification of *Y. enterocolitica* both in the peripheral blood and in the specimens collected from the aneurysmatic aortic wall.

It has been reported that, following ingestion, virulent *Y. enterocolitica* crosses the intestinal mucus layer and binds to the M cells overlying Peyer’s Patches. Thereafter, bacteria penetrate these cells to gain access to and multiply in subjacent tissue. Among others, attachment and invasion of M cells is promoted by the membrane-associated Ail protein, encoded by the chromosomal invasion *ail* gene, thereby facilitating the spread to several tissues, as well as bloodstream (1). Also in this environment, Ail provides strong protection to complement killing bactericidal activity, thereby allowing survival of bacteria in the blood and increasing its spreading (1). Due to *Y. enterocolitica* predilection for vascular tissues, it is highly likable that bacteria colonize the aneurysmatic aorta during bacteremia. On the other hand, the thermostable enterotoxin Yst, encoded by the *yst* gene, is mainly related with diarrheal illness (21).
Although its role remains largely controversial, it has been reported in an animal model that Yst can cause weight loss without the occurrence of diarrhea, possibly depending on the degree of host immune competency.

Our clinical and microbiological data support the hypothesis that Y. enterocolitica firstly colonized the patient's intestine. Accordingly, the patient referred a weight loss in the previous months. Thereafter, the inflammatory process of the small intestine, in tightly conjunction to the aneurysm, allowed Y. enterocolitica translocation into the aneurysm, thereby shortening the period of bacteraemia. Y. enterocolitica septicemia occurs commonly in patients suffering from underlying disease(s) (1). Although our patient did not seem to have predisposing factors, the high degree of inflammation of the small intestine might have even favored Y. enterocolitica translocation into the aneurysm.

CONCLUDING REMARKS

Our case contributes in enriching epidemiological data concerning Y. enterocolitica infections, which might represent severe complications in patients suffering from cardiovascular diseases. The most common site of infections in these patients is the aortic aneurysm, causing the mycotic aneurysm. Therefore, colonization of the aneurysm aorta due to bacteraemia can lead to vessel rupture due to inflammation and necrosis, but also to endocarditis, due to the contiguity of the infection site (1, 18). Finally, together with the previous reports, our case underlines the importance of the microbiological surveillance of Y. enterocolitica. Regrettfully, no routine screening test for detecting Y. enterocolitica for cardiovascular disease patients is available at the moment. No reliable data of monitoring circulating Yersinia spp. on food and animals are available, since it is not mandatory for Zooprophylactic Institutes to report data (27). Therefore, all case reports on Y. enterocolitica involved in cardiovascular diseases should be regarded as valuable and useful tools to monitor the rate of infections worldwide.

AUTHOR CONTRIBUTIONS

DR, AB, CA, MT, and VP contributed equally to this work. DR, AB, MT, and VP collected clinical data and guided the testing and experiments. DS and CA guided the 16S rDNA gene analyses. WM and FS collected clinical samples and provided clinical data. All of authors discussed the results and implications. DR, AB, MT, DS, CA, and VP wrote the manuscript. All the authors revised and approved the final manuscript.

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REFERENCES

1. Bottone EJ. Yersinia enterocolitica: overview and epidemiologic correlates. Microbes Infect. (1999) 1:323–33. doi: 10.1016/S1286-4579(99)80028-8
2. Asplund K, Johansson T, Siitonen A. Evaluation of pulsed-field gel electrophoresis of genomic restriction fragments in the discrimination of Yersinia enterocolitica O:3. Epidemiol Infect. (1998) 121:579–86. doi: 10.1017/S095026880001629
3. Fábrega A, Vila J. Yersinia enterocolitica: pathogenesis, virulence and antimicrobial resistance. Enferm Infect Microbiol Clin. (2012) 30:24–32. doi: 10.1016/j.eimc.2011.07.017
4. Fredriksen-Abomaa M, Stolle A, Korkea H. Molecular epidemiology of Yersinia enterocolitica infections. FEMS Immunol Med Microbiol. (2006) 47:315–29. doi: 10.1111/j.1574-695X.2006.00095.x
5. Plotkin GR, O’Rourke JN, Jr. Mycotic aneurysm due to Yersinia enterocolitica. Am J Med Sci. (1981) 281:35–42. doi: 10.1097/00000441-198101000-00006
6. Verhaegen J, Dedeyne, G, Vansteenbergen W, Vandepitte J. Rupture of vascular prosthes in a patient with Yersinia enterocolitica bacteremia. Diagn Microbiol Infect Dis. (1985) 3:451–4.

7. Van Noyen R, Peeters R, Van Dessel F, Vandepitte J. Mycotic aneurysm of the aorta due to Yersinia enterocolitica. Contrib Microbiol Immunol. (1987) 9:122–6.
8. Knudsen JD, Arpi M, Jensen KT. A fatal case of Yersinia enterocolitica septicemia associated with mycotic aortic aneurysm. Infection (1993) 21:338–9.
9. Prentice MB, Fortineau N, Lambert T, Voinnesson A, Cope D. Yersinia enterocolitica and mycotic aneurysm. Lancet (1993) 341:1535–6. doi: 10.1016/0140-6736(93)90672-4
10. Hagensee ME. Mycotic aortic aneurysm due to Yersinia enterocolitica. Clin Infect Dis. (1994) 19:801–2. doi: 10.1093/clinids/19.4.801
11. Mercie P, Morlat P, N’Gako A, Pheline P, Besian MC, Gorin G, et al. Aortic aneurysms due to Yersinia enterocolitica: three new cases and a review of the literature. J Mal Vasc. (1996) 21:68–71.
12. Donald K, Woodson J, Hudson H, Menzoian JO. Multiple mycotic pseudoaneurysms due to Yersinia enterocolitica: report of a case and review of the literature. Ann Vasc Surg. (1996) 10:573–7. doi: 10.1007/BF02000446
13. La Scala B, Musso D, Carta A, Piquet P, Casalta JP. Aortoabdominal aneurysm infected by Yersinia enterocolitica serotype O:9. J Infect. (1997) 35:314–5. doi: 10.1016/S0163-4453(97)93536-2
14. Tame S, Wit D, Meek A. *Yersinia enterocolitica* and mycotic aneurysm. *Aust N Z J Surg.* (1998) **68:**813–4. doi: 10.1111/j.1445-2197.1998.tb04689.x

15. Abad C, Ponce G, Elices R. Early vascular graft infection due to *Yersinia enterocolitica* after repair of an abdominal aortic aneurysm. *J Cardiovasc Surg.* 1998;**45:**161–2.

16. Kumar P, Zhang J, Miller S, Wilton G. *Yersinia enterocolitica*: a rare cause of infected aortic aneurysm successfully treated with antibiotics and endovascular repair. *JMM Case Rep.* (2014). doi: 10.1099/jmmcr.0.002626

17. Mason JC, Lal P, Torella F, Sharma A, Cooke R, Anson J. *Yersinia enterocolitica*: a rare cause of infective endocarditis and mycotic aneurysm. *Clin Microbiol Newsl.* (2014) **36:**2. doi: 10.1016/j.clinmicnews.2014.01.002

18. Kumar HR, Eskandari MK. The benefit of early repair for a mycotic aortic aneurysm due to *Yersinia enterocolitica* infection. *J Vasc Surg Cases* (2015) **1:**61–4. doi: 10.1016/j.jvusc.2014.12.001

19. Miller VL, Falkow S. Evidence for two genetic loci in *Yersinia enterocolitica* that can promote invasion of epithelial cells. *Infect Immun.* (1988) **56:**1242–8.

20. Pierson DE, Falkow S. The ail gene of *Yersinia enterocolitica* has a role in the ability of the organism to survive serum killing. *Infect Immun.* (1993) **61:**1846–52.

21. Peruzy MF, Murru N, Perugini AG, Capuano F, Delibato E, Mercogliano R, et al. Evaluation of virulence genes in *Yersinia enterocolitica* strains using SYBR Green real-time PCR. *Food Microbiol.* (2017) **65:**231–5. doi: 10.1016/j.fm.2017.03.004

22. Petsios S, Fredriksson-Ahomaa M, Sakkas H, Papadopoulou C. Conventional and molecular methods used in the detection and subtyping of *Yersinia enterocolitica* in food. *Int J Food Microbiol.* (2016) **237:**59–72. doi: 10.1016/j.ijfoodmicro.2016.08.015

23. Stephan R, Cernela N, Ziegler D, Pflüger V, Tonolla M, Ravasi D, et al. Rapid species specific identification and subtyping of *Yersinia enterocolitica* by MALDI-TOF mass spectrometry. *J Microbiol Methods* (2011) **87:**150–3. doi: 10.1016/j.mimet.2011.08.016

24. Febbraro F, Rodio DM, Puggioni G, Antonelli G, Pietropaolo V, Trancassini M. MALDI-TOF MS versus VITEK 3: comparison of systems for the identification of microorganisms responsible for bacteremia. *Curr Microbiol.* (2016) **73:**843–50. doi: 10.1007/s00284-016-1121-x

25. Yu J, Zhou XE, Yang SJ, Liu WH, Hu XF. Design and application of specific 16S rDNA-targeted primers for assessing endophytic diversity in *Dendrobium officinale* using nested PCR-DGGE. *Appl Microbiol Biotechnol.* (2013) **97:**9825–36. doi: 10.1007/s00253-013-5294-y

26. EUCAST. *The European Committee on Antimicrobial Susceptibility Testing*. Breakpoint tables for interpretation of MICs and zone diameters. (2017). Available online at: http://www.eucast.org

27. EFSA. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA J.* (2017) **15:**3077. doi: 10.2903/j.efsa.2017.5077

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The reviewer MF declared a shared affiliation, though no other collaboration, with several of the authors DR, AB, CA, DS, MT, and VP to the handling Editor.

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