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Title: Emergence of CTX-M-1-producing Salmonella enterica serovar Napoli:
a novel "enzyme-pathogen association" in the Italian ESBL endemic context

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To S. Stefani
Editor in Chief
Journal of Global Antimicrobial Resistance

August 23, 2018

Enclosed please find the revised manuscript now entitled “*Emergence of CTX-M-1-producing Salmonella enterica serovar Napoli: a novel “enzyme-pathogen association” in the Italian ESBL endemic context*”, which I am resubmitting for possible publication as a *Letter to the Editor* in *Journal of Global Antimicrobial Resistance*.

In this manuscript, we describe the first isolation of CTX-M-1-producing *Salmonella enterica* serovar Napoli from a clinical sample. This finding is strictly related to the Italian ESBL endemic context, in which uncommon associations enzyme-pathogen can be discovered. This serovar, relatively uncommon in Europe, is among the most common serovars causing infection in Italy. Moreover, *Salmonella* Napoli, especially in children, is often associated to invasive infections. The acquisition of resistance determinants by a so-diffused pathogen is a major cause of concern, making difficult the treatment of related infections.

The manuscript has been revised according to reviewer’s comments and suggestions. The final version has been seen and approved by all the authors. The material is original, unpublished, and has not been simultaneously submitted to another medical journal.

Yours sincerely,

Francesco Luzzaro

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"First description of CTX-M-1-producing *Salmonella enterica* serovar Napoli: a new association enzyme-pathogen in the Italian ESBL endemic context"

Point-by-point response

Reviewer #1

The authors describe a case of salmonellosis with a CTX-M-1 producing strain of Salmonella Napoli in Italy. It is a pity that not more was done to determine the properties of the blaCTX-M-1 carrying plasmid.

R: Additional information about the plasmid carrying the *bla*_{CTX-M-1} gene has been added in the text (see also response to point #4).

All comments are mentioned below.

1. *Line 35: Please add the number of cases in this period of time in parenthesis.*

R: The number of cases has been added as requested.

2. *Line 34-38: Please shift this paragraph to the discussion below (e.g. line 60).*

R: The paragraph has been moved to the discussion.

3. *Line 50: Which method was used to determine the serovar?*

R: The method used to determine the serovar has been specified as requested.

4. *Line 59: How was the presence of only one plasmid confirmed without conjugation and plasmid isolation or plasmid number/size determination by S1 restriction and PFGE? PBRT just confirmed the presence of a replicon indicating the presence of at least one plasmid. Please rephrase.*

R: The sentence has been rephrased adding new information as follows: "Conjugation experiments using *E. coli* J53 recipient (rifampicin-resistant) performed at 37°C, and selecting on MacConkey plates containing ampicillin and rifampicin (both 50 µg/ml), were successful (efficiency, 4×10^{-3}) and confirmed the presence of an IncII plasmid along with *bla*_{CTX-M-1}".

5. *Line 69-71: An acquisition of the ESBL gene within the gut from other Enterobacteriaceae could be possible - where the stool samples tested for presence of other ESBL-producing Enterobacteriaceae?*

R: We agree with this consideration. The acquisition of the ESBL gene within the gut from other *Enterobacteriaceae* could be possible. However, only selected stool pathogens (i.e., *Salmonella*, *Shigella*, and *Campylobacter* species) were searched for (according to our diagnostic routine methods).

6. *Line 71-74: Please delete this sentence - it is redundant.*

R: The sentence has been deleted as requested.

7. *Line 78: Please discuss: Are 3rd generation cephalosporins used as treatment option for salmonellosis in children?*

R: The possible role of third-generation cephalosporins for treating salmonellosis in children has been briefly discussed as requested.

8. *Line 79-82: The role of IncII finding is not discussed - what is its relevance in the "Italian context"?*

R: The role of IncII and the relevance in the Italian context have been briefly discussed as requested.

9. *Table 1: Please always use the standard three-letter-code for antibiotic names.*

R: The standard three-letter-code has been used in Table 1 as requested.

Minor Comments

Title: Please modify: "Emergence of CTX-M-1-producing Salmonella enterica serovar Napoli: a novel "enzyme-pathogen association" in the Italian ESBL endemic context".

R: The title has been modified as requested.

Line 32: Please delete "first".

R: The word "first" has been deleted as requested.

Line 35: Please write "main cause".

R: The sentence has been modified as requested.

Line 45: Please delete "each month".

R: The sentence has been modified as requested.

Line 46: Please delete "having the same features".

R: The sentence has been modified as requested.

1 **Emergence of CTX-M-1-producing *Salmonella enterica* serovar Napoli: a novel**
2 **“enzyme-pathogen association” in the Italian ESBL endemic context**

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5 Endimiani ², Francesco Luzzaro ^{1*}

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24 *Sir,*

25 In a recent nationwide survey, Giani et al. reported on the spread of extended-spectrum beta-
26 lactamase (ESBL) enzymes among *Enterobacteriaceae* isolated from both inpatients and
27 outpatients, thus highlighting the Italian endemic context for these resistance determinants [1]. The
28 CTX-M-1 group was the most common among ESBL enzymes (>80% of ESBL-producing
29 enterobacterial isolates, including *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*).
30 In this endemic scenario, ESBLs could be sometimes found in uncommon associations with other
31 species. Here we describe the isolation of CTX-M-1-producing *Salmonella enterica* serovar Napoli
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33 On June 2017, we recovered a *Salmonella* Napoli from the stool culture of a one-year-old Italian
34 girl, presenting diarrhea, abdominal pain and vomiting. The infant showed leukocytosis (12,000/ μ l),
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50 1). The presence of an ESBL determinant was phenotypically evaluated on the basis of synergistic
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58 *Salmonella* Napoli is an emerging public health concern. During 2000–2006, a 140% increase in
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1 **Emergence of CTX-M-1-producing *Salmonella enterica* serovar Napoli: a novel**
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Table I. Characteristics and susceptibility profile of the CTX-M-1-producing *Salmonella enterica* serovar Napoli

Code	Source	Resistance determinant	MIC values (mg/L) of antimicrobial agents and clinical interpretation (S/I/R)*																
			AMC	TZP	ATM	CAZ	CTX	FEP	ETP	IPM	MEM	AMK	GEN	CIP	TGC	CST	SXT	CZA	CTT
LC0541/17	Stools	CTX-M-1	8/2 (S)	4/4 (S)	32 (R)	4 (I)	>8 (R)	4 (I)	≤0.12 (S)	≤0.5 (S)	≤0.12 (S)	≤4 (S)	≤0.5 (S)	≤0.06 (S)	≤0.25 (S)	0.5 (S)	≤1/19 (S)	≤0.5/4 (S)	1/4 (S)

*MIC values indicate minimal inhibitory concentrations, as obtained by standard broth microdilution method. Interpretation based on EUCAST criteria (version 7.1, 2017; <http://www.eucast.org>).

Abbreviations: AMC, amoxicillin-clavulanate; TZP, piperacillin-tazobactam; ATM, aztreonam; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; ETP, ertapenem; IPM, imipenem; MEM, meropenem; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; TGC, tigecycline; CST, colistin; SXT, trimethoprim-sulfamethoxazole; CZA, ceftazidime-avibactam; CTT, ceftolozane-tazobactam; S, susceptible; I, intermediate; R, resistant.