A putative *erm* gene is present in *ΔermB Clostridioides difficile* isolates showing high levels of resistance to clindamycin

Viviana Ramírez-Hernández[1], Gabriel Ramírez-Vargas[2]

[1]Hospital "Dr. Rafael Ángel Calderón Guardia", San José, Costa Rica

[2]Hospital Nacional de Niños "Dr. Carlos Sáenz Herrera", San José, Costa Rica

[2]Corresponding author: garava291@hotmail.es

Abstract:

Clostridioides difficile has become the leading cause of hospital-acquired diarrhea. Clindamycin is a member of the macrolide-lincosamide-streptogramin B family of protein synthesis inhibitors. Been the ribosomal methylation the most widespread mechanism of resistance to these antibiotics in *C difficile erm* genes usually produce high levels of resistance to these drugs. In this short report we present evidence about the presence of an unreported putative *erm* gene in *C difficile* isolates that despite of presenting a Δ*ermB* genotype maintain high levels of clindamycin resistance.

Key words: *Clostridiodes difficile*, *erm*, clindamycin.

21 **Introduction:**

22 *Clostridiodes difficile* is a Gram-positive, sporulating and anaerobic bacterium that has
23 become the leading cause of hospital-acquired diarrhea (Banawas, 2018; Guery *et al*, 2019).
24 *C difficile* infection (CDI) is associated with the exposure of the normal intestinal microbiota
25 to antibiotics. The resulting disruption of this normal intestinal microflora allows the
26 multiplication and establishment of *C difficile* in the large intestine causing the pathology
27 (Banawas, 2018; Guery *et al*, 2019).

28

29 Antibiotic resistance is crucial in spreading CDI among hospitalized patients, particularly in
30 the older ones (Banawas, 2018). Antibiotics used for treatment of any type of infection
31 could potentially promote (CDI) and resistant to multiple agents may have a selective
32 advantage for the bacteria (Spigaglia, 2016). Most of the antibiotics associated with CDI
33 appearance, including clindamycin, remaining to be associated with the highest risk for CDI
34 (Spigaglia, 2016; Banawas, 2018).

35

36 Clindamycin and erythromycin are members of the macrolide-lincosamide-streptogramin B
37 (MLSB) family of protein synthesis inhibitors. Ribosomal methylation is the most
38 widespread mechanism of resistance to these antibiotics in *C difficile*. *erm* genes usually
39 produce high levels of resistance to these drugs this by modifying the ribosomal 23S rRNA
40 (Spigaglia, 2016; Banawas, 2018).

41

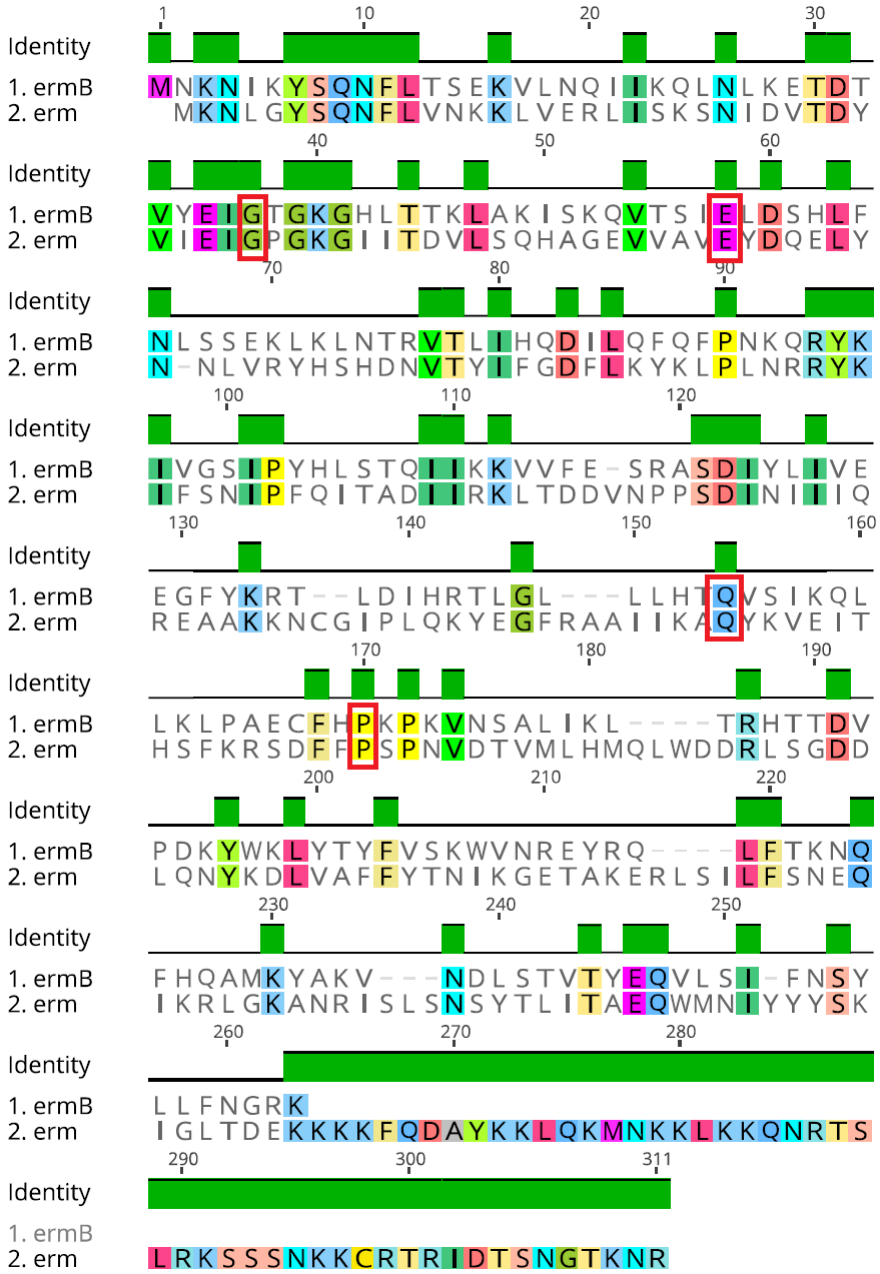
42 The *ermB* gene can be located in the transposon Tn5398, a mobilizable nonconjugative
43 element of 9.6 kb found in *C difficile* genomes and contains two copies of *ermB* (Spigaglia,
44 2016; Banawas, 2018). Isolates whit a truncated version of Tn5398, containing only one
45 copy of *ermB*, are called Δ*ermB* isolates and fail in inducing the MLS_B resistance phenotype
46 (Hussain *et al*, 2005).

47

48 In this short report we present evidence about the presence of an unreported putative *erm*
49 gen in *C difficile* isolates that despite presenting a Δ*ermB* genotype maintain high levels of
50 clindamycin resistance.

51

52 **Results:**



53

54 Figure 1. Alignment of *erm* genes. Marked in red boxes are the amino acids associated whit

55 essential ribosome methylation activities.

56

57 The gene product analyzed showed a 96% of coverage and 26% identity when compared to
58 ermB from Tn5398. In addition compared to ermB this putative erm protein presents 4 of 6
59 essential amino acids necessary for ribosome methylation activity already described
60 (Farrow *et al*, 2002).

61

62 Table 1. Features of analyzed C difficile isolates.

Isolate	*cfrC*	Δ*ermB*	CLI MIC (µg/ml) *
LIBA-5707	Yes	Yes	>256
LIBA-5701	No	Yes	>256

63 *CLI MIC: clindamycin minimum inhibitory concentration from Ramírez-Vargas *et al*, 2017.

64

65 Lacking *cfrC* gene (another ribosome methylating enzyme) and having Δ*ermB* genotype,
66 LIBA-5701 is reported to be resistant to clindamycin (MIC >256 µg/ml) according to CLSI
67 guides (Ramirez-Vargas *et al*, 2017; Stojković *et al*, 2019).

68

69 **Discussion:**

70 Here we report the presence of a putative *erm* gen in isolates from Costa Rican Hospitals
71 recovered from CDI patients in the 2000s. The isolates showed similarly pathologic

capabilities than other known *C difficile* epidemic isolates like NAP1 (Quesada-Gómez *et al*, 2015; López-Ureña *et al*, 2016).

This putative *erm* gen presents the key amino acids that has been associated to the ribosome methylation activity essential for the high levels of resistance to for MLS_B agents such as clindamycin in other erm proteins (Farrow *et al*, 2002). Mutagenesis showed that changes in these amino acids result in lower minimum inhibitory concentration for erythromycin, another agent belonging to the MLS_B group (Farrow *et al*, 2002).

In Δ*ermB C difficile* isolates the MLS_B resistance phenotype is lost (Hussain *et al*, 2005). So, other resistance mechanism should explain the resistance to clindamycin LIBA-5701. As reported before (Ramirez-Vargas *et al*, 2017; Stojković *et al*, 2019) lack of the element containing *cfrC* did not result in changes in the clindamycin resistance. Using the CARD database (Alcock *et al*, 2020) no other resistance genes associated to MLS_B resistance genotype were found (data not shown). At this point we cannot exclude the possibility of other new resistance mechanisms not detected here and the methylation activity of the putative erm gen product must be probe.

Materials and methods:

Raw Illumina SRA files for LIBA-5701 and LIBA-5700 used in this work can be found under the accession numbers ERR467550 and ERR467555 respectively at the SRA database. The

93 genomes were assembly using EDENA (Hernandez *et al*, 2008) and annotated using the
94 RAST platform (Aziz, 2008). The predicted erm protein (supplementary material 1) was
95 aligned against ermB from Tn5398 in *C difficle* 630 genome (accession number
96 NZ_CP019870.1) using Genius platform (Kearse *et al*, 2012). Similarly, they were compared
97 using pBLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) for determination
98 of identity and coverage between the sequences.

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100 Authors declare no conflict of interest.

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168 **Suplemnetary material 1. Putative erm protein sequence in this study**

169 MKNLGYSQNFLVNKKLVERLISKSNIDVTDYVIEIGPGKGIITDVLSQHAGEVVAVEYDQELYNNLVRYH
170 SHDNVTYIFGDFLKYKLPLNRRYKIFSNIPFQITADIIRKLTDDVNPPSDINIIIQREAAKKNCGIPLQKYEG
171 FRAAIIKAQYKVEITHSFKRSDFFPSPNVDTVMLHMQLWDDRLSGDDLQNYKDLVAFFYTNIKGETAK
172 ERLSILFSNEQIKRLGKANRISLSNSYTLITAEQWMNIYYYSKIGLTDEKKKKFQDAYKKLQKMNKKLKKQ
173 NRTSLRKSSSNKKCRTRIDTSNGTKNR