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

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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.

Introduction:

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

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

remaining to be associated with the highest risk for CDI (Spigaglia, 2016; Banawas, 2018).

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

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

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

Results:

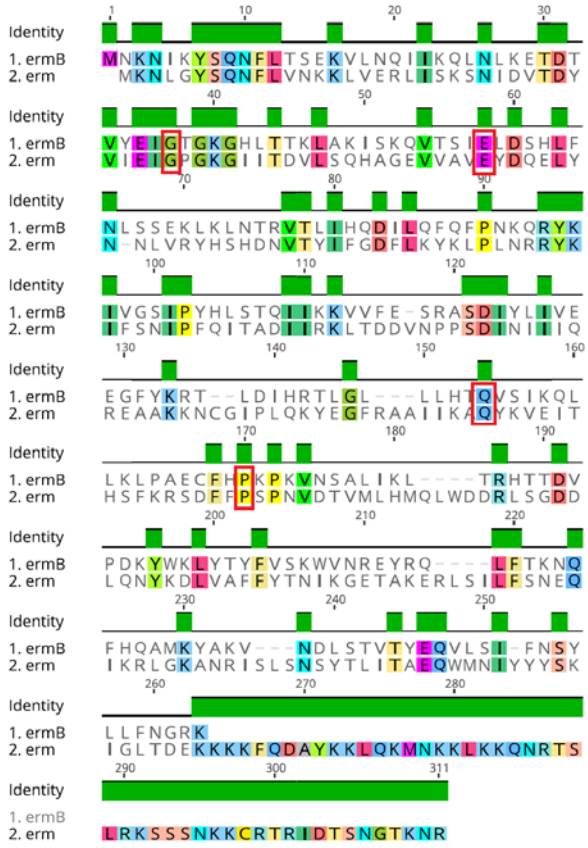

Figure 1. Alignment of *erm* genes. Marked in red boxes are the amino acids associated whit essential ribosome methylation activities.

The gene product analyzed showed a 96% of coverage and 26% identity when compared to ermB from Tn5398. In addition compared to ermB this putative erm protein presents 4 of 6 essential amino acids necessary for ribosome

methylation activity already described (Farrow *et al*, 2002).

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

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

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

Discussion:

Here we report the presence of a putative *erm* gen in isolates from Costa Rican Hospitals 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 have been associated to the ribosome methylation activity essential for the high levels of resistance to for MLS_B agents such as clindamycinin 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 mechanism snot 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-5700used in this work can be found under the accession numbersERR467550andERR467555 respectively at the SRA database. The genomes were assembly using EDENA (Hernandez *et al*, 2008) and annotated using the RAST platform (Aziz, 2008). The predicted erm protein (supplementary material 1) was aligned against ermB from Tn5398 in *C difficle* 630 genome (accession numberNZ_CP019870.1) using Genius platform (Kearse *et al*, 2012). Similarly, they were compared using pBLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) for determination of identity and coverage between the sequences.

Authors declare no conflict of interest.

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Suplemnetary material 1. Putative erm protein sequence in this study

MKNLGYSQNFLVNKKLVERLISKSNID
VTDYVIEIGPGKGIITDVLSQHAGEVVA
VEYDQELYNNLVRYHSHDNVTYIFGDF
LKYKLPLNRRYKIFSNIPFQITADIIRKLT
DDVNPPSDINIIIQREAAKKNCGIPLQK
YEGFRAAIIKAQYKVEITHSFKRSDFFPS
PNVDTVMLHMQLWDDRLSGDDLQN
YKDLVAFFYTNIKGETAKERLSILFSNEQ
IKRLGKANRISLSNSYTLITAEQWMNIY
YYSKIGLTDEKKKKFQDAYKKLQKMNK
KLKKQNRTSLRKSSSNKKCRTRIDTSN
GTKNR