The *Clostridium difficile* Epidemic and Its Prevalence in Two New Urban, International Environments

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ABSTRACT

Background: *Clostridium difficile* is a disruptive pathogen of global concern, particular among children, elderly, and immunosuppressed patients. While many studies have been done in United States and Europe, we looked at areas with high population density in third world countries. This provides a preliminary step to understand the prevalence of this virulent pathogen in parts of the world which have not yet received attention.

Methods: We collected non-hospital, environmental swabs from shoe bottoms and high-touch surfaces in Mumbai, India and Nuevo Laredo, Mexico. All positive samples for *C. difficile* were tested for toxins A and B, ribotyped, and compared with each other for further toxigenic analysis.

Results: Fairly similar percentages were produced for *C. difficile* prevalence in both urban environments. Also, very close percentages of toxigenic *C. difficile* were produced when both locations had the same ribotypes and when the cities each had their own unique ribotypes.

Conclusions: The toxigenic strains specific to both Mumbai and Nuevo Laredo and toxigenic strains unique to both environments had resembling prevalence indicating the ubiquity of the pathogen *C. difficile* in all environments regardless of population or size.

OBJECTIVE

To prove patterns and relationships between C. difficile prevalence in international environments, leading to proofing of the notion that C. difficile is ubiquitous and potentially a future epidemic based on present toxigenic strains.

INTRODUCTION

[2]*Clostridium difficile* infection (CDI) is the most common cause of death caused by gastroenteritis in the United States and costs up to $4.8 billion each year in health care costs. [3]More than 500,000 people contract CDI every year and over 29,000 lose their life to this bacterium. *C. difficile* is considered to be ubiquitous, meaning it is present in almost every environment. However, *C. difficile* best thrives in warm, anaerobic habitats, like the human gastrointestinal tract. [3]*C. difficile* is highly contagious because of its spores' tough resilience to commonly used disinfectants. These spores persist on surfaces for months and can easily spread through direct contact with contaminated objects. [1]This poses a great challenge for hospitals, as they must work constantly to reduce contamination to protect patients who may be more susceptible to CDI, such as children, elderly, and immunosuppressed patients. To investigate *C. difficile's* spread, we conducted a study by collecting shoe bottom and environmental samples in Mumbai, India and Nuevo Laredo, Mexico. To our knowledge, this is the first report of the prevalence of *C. difficile* in a non-hospital environment in these two countries. Our goal was to isolate *C. difficile* for toxigenic, virulent, and hyper-virulent strains from both environments and compare the prevalence of *C. difficile* in them.

METHODS

Preparing for Sampling

Wearing gloves, pour about 10-15 mL of distilled water onto two pieces of gauze and place them together in a plastic bag. Seal the bag and repeat for all desired samples.

Sample Collection

Wearing gloves, take out the gauze and swab the shoe bottom or environmental high-touch area by rubbing the gauze up and down for about 7-10 seconds. Place the gauze back into the bag and seal, leaving minimal air in the bag.

Isolation of *C. difficile*

Transfer each of the collected samples into individual 50 mL tubes using gloves. Prepare Brain Heart Infusion broth and pour approximately 25 mL into each tube with sample. Place in an incubator in an anaerobic chamber for 48 hours then plate enriched samples on CCFA plates. Incubate the plates for 48 hours and stock all samples into individual cryovials if the growth is pure. If growth is impure, isolate the colonies by plating each onto 5% blood agar plates. Repeat incubation and isolation steps until colonies are isolated and able to be stocked as pure.

PCR Ribotyping

For PCR, work in a sterile biosafety cabinet and prepare mixture with corresponding primers 2 µL of the corresponding sample into each well on a 96-well plate. Next, place the plate into a ThermoCycler and run with the scheduled program. Prepare a 2% agarose gel for gel electrophoresis with 10 µL ethidium bromide. Load the samples to their corresponding well on the gel and run the gel at a voltage of 225 amps and stop after 40-45 minutes. Analyze the results and input the data in the database.

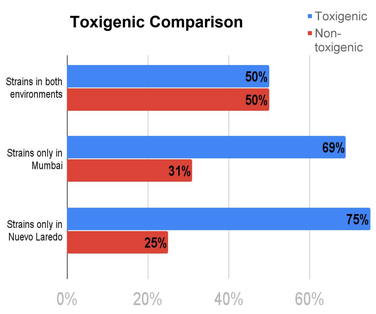
RESULTS

	Mumbai, India		Nuevo Laredo, Mexico		
Sample type	Shoe bottom		Shoe bottom	Environment	Both
Sample size (n)	**n=187**		**66**	**80**	**n=146**
+ for tpi*	**101**/187 (54.01%)		**22**/66 (33.33%)	**6**/80 (7.5%)	**28**/146 (19.18%)
+ for toxin A & B	**67**/101 (66.33%)		**10**/22 (45.45%)	**3**/6 (50.0%)	13/28 (46.43%)

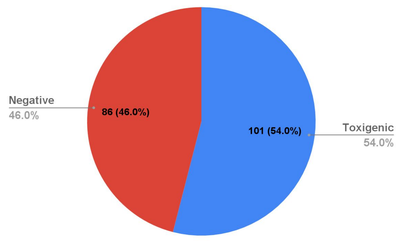
*triosephosphate isomerase (growth indicator for *C. difficile*)

Positive *C. difficile* growth

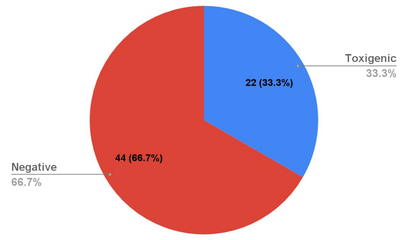
Toxigenic Comparison



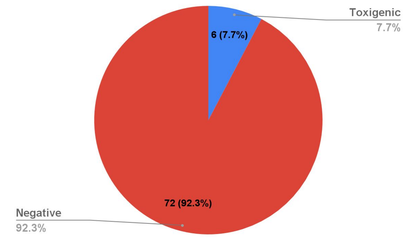
Mumbai shoe bottoms



Nuevo Laredo shoe bottoms



Nuevo Laredo environment



DISCUSSION

The data in the graphs and tables display how strains of C. difficile have similar distributions in Mumbai and Nuevo Laredo and how toxigenic strain prevalence is similar across both environments as well. The Mumbai shoe bottom samples were 54.01% positive for C. difficile (n=187) through presence of tpi, while the Nuevo Laredo shoe and environmental samples were 19.18% positive (n=146). The number may be comparatively low, but the fact that the Mumbai samples were solely shoe samples while the Nuevo Laredo samples were environmental high touch and shoe samples both skewed the outcome. Shoe bottoms are known to be very good strongholds of C. difficile, which is likely why the shoe bottom samples more frequently tested positive for C. difficile in both locations (54.01% positive in Mumbai and 33.33% positive in Nuevo Laredo). This further explains why the percent tpi is higher in Mumbai rather than Nuevo Laredo. When analyzing ribotypes common to both environments, 50% of C. difficile strains came back toxigenic, while 50% were non-toxigenic. Meanwhile, for ribotypes specific to the Mumbai samples, 69% of C. difficile strains came back toxigenic, while 31% came back non-toxigenic. Similarly, ribotypes specific to Nuevo Laredo samples came back 75% back toxigenic, while 21% came back non-toxigenic. The toxigenic strains had very similar percentages (50%, 69%, and 75%, respectively) regardless of what environment they came from. The significance of this is that the data could potentially be indicating that the similarity could have some correlation between location and toxigenic strain prevalence.

CONCLUSIONS

The ubiquity of C. difficile isolates noticed through the data analysis reinforces the known fact that C. difficile is everywhere. Current U.S. and Europe data on C. difficile and its prevalence in various European and American environments has shown that there have been almost equivalent distributions of C. difficile isolates in those areas. The fact that the toxigenic strains specific to both Mumbai and Nuevo Laredo and toxigenic strains unique to both environments had similar concentrations could be a good indicator of uniformity of the pathogen C. difficile in all environments regardless of population or size. This is consistent with the notion that C. difficile is ubiquitous, and so are its toxigenic strains, further indicating that virulent and hypervirulent strains positive for both toxins A and B could potentially be isolated from the toxigenic batch. The inference brings about future interest in research about discoveries of new toxigenic and even virulent strains and prevention of a toxigenic C. difficile epidemic.

REFERENCES

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