CAN SHOE SOLES CONTRIBUTE TO *C. DIFFICILE* DISSEMINATION IN HOSPITAL ENVIRONMENT?

Sandra Janezic[1,2], Ines Blazevic[2], David Eyre[3,4], Aleksander Kocuvan[2], Maja Rupnik[1,2]

[1]National Laboratory for Health, Environment and Food, Maribor, Slovenia, [2]University of Maribor, Faculty of Medicine, Maribor, Slovenia, [3]Nuffield Department of Medicine, University of Oxford, Oxford, UK, [4]Big Data Institute, University of Oxford, Oxford, UK

NACIONALNI LABORATORIJ ZA ZDRAVJE, OKOLJE IN HRANO

sandra.janezic@nlzoh.si

Univerza v Mariboru
Medicinska fakulteta

BIG DATA INSTITUTE
UNIVERSITY OF OXFORD

INTRODUCTION AND AIM

Shoe soles are shown to have a dense pathogenic load and shoes can be a reservoir and a vector that spreads bacteria through different environments, posing threat to human infections (Rashid *et al*., 2016). Two previous community studies demonstrated high rate of *C. difficile* contamination of shoe soles (up to 43 %) and high prevalence of PCR ribotypes that are commonly isolated from humans, animals and different natural environments (Janezic *et al*., 2018, Alam *et al*., 2014).

The aim of this study was to evaluate the presence of *C. difficile* on shoe soles to explore their possible contribution to the dissemination of *C. difficile* spores in hospital environment.

METHODS

SAMPLING	Single large teaching hospital - different departments. Shoe soles swabs were taken from health-care staff (physicians, nurses, cleaning staff and paramedics), medical students and a visitor. Sampling was performed in May, July and August in 2014.
ISOLATION OF *C. difficile*	Shoe soles: surfaces were swabbed with pre-moistened Polywipe™ sponges (MWE) and placed into 50 ml sterile centrifuge tubes. Sponges were incubated in enrichment medium BHI supplemented with 0.1 % L-cystein, 0.5% yeast extract, 0.1% taurocholic acid sodium salt and *C. difficile* selective supplement (SR0096E, Oxoid). After enrichment and ethanol shock *C. difficile* was isolated on selective plates chromID™ *C. difficile* (BioMerieux). Clinical samples: *C. difficile* isolated from stool samples from the same hospital in routine diagnostic laboratory were included in the comparison. All isolates were identified with MALDI-TOF MS (Bruker).
TYPING	All isolates were characterized with PCR ribotyping (Janezic and Rupnik, 2010).
WHOLE GENOME SEQUENCING AND SNV ANALYSIS	For whole genome sequencing, bacterial DNA was isolated with QIAamp DNA Mini Kit (Qiagen) following protocol for isolation of DNA from Gram-positive bacteria. Paired-end libraries were generated with Nextera XT Library preparation kit (Illumina) following manufacturer's protocol. Libraries were sequenced on MiSeq (Illumina) with 600-cycle MiSeq Reagent Kit v3, aiming for the theoretical coverage of 100x. SNV analyis was prerformed as described previously (De SIlva *et al.,* 2016).

RESULTS

- Almost **two-thirds (62 %, 34 out of 55)** of shoe swabs were **positive** for *C. difficile*. (Table 1)
- **Eleven different PCR ribotypes** were identified. Most **common** were **PCR ribotypes 010** (n=8), **027** (n=8), **014** (n=7), **018** (n=5) which are also PCR ribotypes that are commonly isolated from patients.

Table 1. Number of positive *C. difficile* shoe sole swabs and PCR ribotypes found across different departments within a single hospital

Department	# of swabs tested	# of *C. difficile* positive swabs	# of PCR ribotypes	PCR ribotypes
Dept. of Gastroenterology	9	4	3	010, 027, SLO 253
Dept. of Infectious Disease and Febrile Conditions	27	19	7	010, 012,014, 027, 018, SLO 029, SLO 222
Dept. of Internal Intensive Med.	11	6	4	010, 014, 027, 176
Dept. of Cardiology	8	5	4	002, 014, 023, 027

- Fourteen shoe isolates and 39 matching (temporal and spatial) clinical isolates of PCR ribotypes 027 (n=31), 014/020 (n=15) and 002 (n=7) were selected for whole genome sequencing and high-resolution single nucleotide variant (SNV) analysis to determine their relatedness on a whole genome level.
- With core genome **SNV analysis 12 out of 14 (86 %)** of **shoe sole** isolates **had ≤2 SNVs** difference from one or more human **clinical isolates** which is consistent with recent **transmission** (Figure 1).
- Genetically related pairs of clinical and shoe strains were isolated from 3 days to 20 weeks apart and were found both within the same departments as well as in different departments.

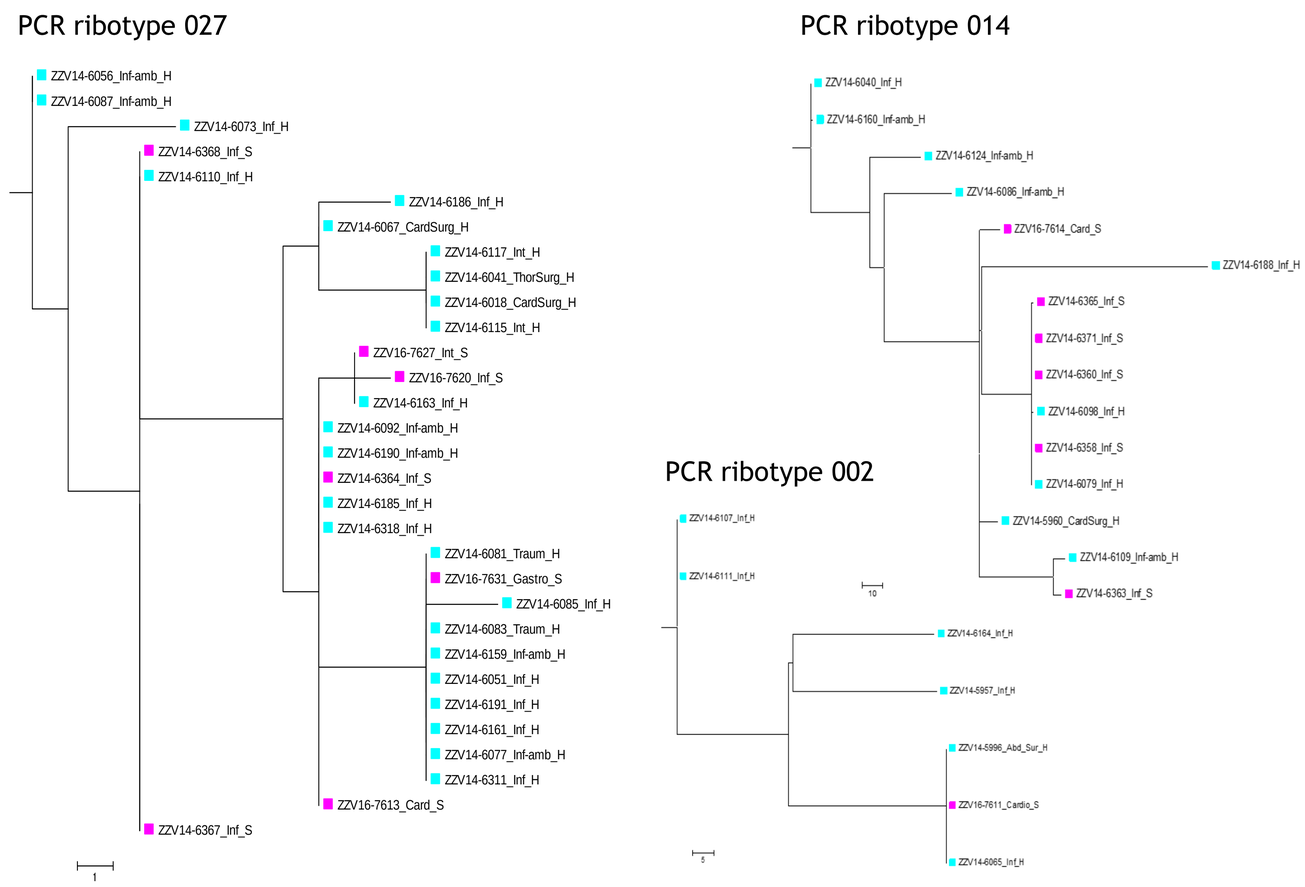

Figure 1. Recombination adjusted maximum likelihood trees. Trees are scaled in SNV and color-coded according to source: fuchsia – shoe isolates, light blue – clinical isolates.

CONCLUSION

We demonstrated a high rate of *C. difficile* contamination of shoe soles in hospital environment and high prevalence of PCR ribotypes that are commonly isolated from humans. This study also shows that shoe soles could play an important part in the dissemination of *C. difficile* spores in hospital environment.

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