

1 Article type: Letter to Editor

2 **Possible contribution of shoes to *Clostridioides difficile* transmission within the**
3 **hospitals**

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17 To the Editor:

18 *Clostridioides difficile* remains the most common cause of healthcare associated intestinal
19 infection. Hospitalized patients aged more than 65 years and receiving antibiotic therapy are the
20 population most at risk of *C. difficile* infection (CDI).

21 The main sources of *C. difficile* spores in hospitals are likely to be symptomatic patients
22 and asymptomatic carriers, with contamination of the hands of healthcare workers a main route
23 of transmission from patient to patient. However, exposure to unknown sources or to the
24 healthcare environment is also a commonly documented possible transmission route [1].

25 *C. difficile* spores can be detected on different surfaces within hospital environment;
26 toilets, beds, socks, bed sheets and medical equipment [2]. Hospital floors are another surface
27 that are often contaminated with spores and could potentially play a role in *C. difficile*
28 transmission, but this possibility has so far not been fully explored. In particular the role that
29 shoes contaminated with *C. difficile* spores play in hospital transmission has not been reported
30 [2], while for domestic environment it has previously been demonstrated that shoe soles are
31 among sites with highest *C. difficile* contamination rates (up to 43%) [3].

32 Because of the high reported positivity rates in domestic environment, we conducted
33 between May and August 2014 a similar study where we tested swabs of shoe soles of healthcare
34 staff (physicians, nurses, physiotherapists, cleaning staff and paramedics) and medical students
35 from different departments of two hospitals in Slovenia. No specific selection was used and all
36 personnel present at the department at the time of sampling were invited to participate. We used
37 PCR ribotyping and whole-genome sequencing to determine the genetic relationships between

shoe sole isolates and those from temporally and spatially linked clinical *C. difficile* isolates to further explore possible contribution of shoes to the dissemination of *C. difficile* within hospitals.

Altogether 98 shoe sole swabs were collected; a high proportion of shoe swabs were positive for *C. difficile* in both a general (6/40; 15%) and teaching hospital (35/58; 60%). All shoe isolates were PCR ribotyped, which demonstrated that isolates from shoe soles were diverse and similar to contemporaneous clinical *C. difficile* isolates (Supplementary Material; Table 1). On shoes we found a high prevalence of PCR ribotypes 014/020 and 027 that were common in patients of one of the hospitals at the time of sampling. At the same time, these are also among the top five PCR ribotypes found in Slovenian patients in general and throughout the last decade. Given their common distribution and known heterogeneity within a ribotype, we performed whole-genome sequencing to confirm possible clonality.

Fourteen shoe sole isolates and 39 temporally and spatially matched clinical isolates of PCR ribotypes 027 (n=31), 014/020 (n=15) and 002 (n=7) were selected for whole-genome sequencing (Supplementary material; Table 2). Genomes were sequenced on MiSeq (Illumina). Single nucleotide variants (SNVs) were identified following mapping to the 630 reference genome as described previously [4]. All sequenced strains originated from the teaching hospital as *C. difficile* isolates from this hospital were routinely PCR ribotyped in our laboratory.

The SNV analysis of sequenced genomes found several clusters of indistinguishable strains with ≤ 2 SNVs difference (a cut-off of ≤ 2 SNVs has been previously proposed as an indication of a recent direct or indirect transmission event [4]). Clusters contained only shoe isolates or shoe and patient isolates. These clusters were either confined to a single department or found in different departments, suggesting that footwear can be a vehicle to disseminate spores within

60 and between hospital departments. We sampled only health care workers and not the visitors.
61 But based on a high contamination rate of shoe soles with *C. difficile* spores reported in domestic
62 environment we speculate that footwear soles of the visitors could also be a vehicle to
63 disseminate spores between hospital and community environment.

64 Moreover, genetically related pairs of shoe sole and clinical strains were isolated up to 19
65 weeks apart, suggesting long-term persistence or multiple introductions of clones to the hospital
66 environment. Eyre et al. [4] analysed 957 *C. difficile* strains and based on genomic identity found
67 that 333 patients had strains with at least one closely genetically-related match. Of those, 126
68 patients had documented close hospital contact with another patient, while 120 patients had
69 none. Although the study was not performed in a single hospital, it shows that large proportion
70 of clonal clusters do not have known transmission routes such as patient/patient contact. It is
71 possible, that at least in the cases that shared hospital, but not ward, overlaps that shoes could
72 have contributed to transmission.

73 Based on our data we cannot assess directionality of transmission, but both scenarios are
74 possible; spores can be transferred from patients to the shoe soles or vice versa.

75 Shoes are usually not considered as a vehicle for infectious diseases transmission and
76 shoes are also not typically considered a route of *C. difficile* nosocomial transmission [5]. The
77 presence of *C. difficile* spores on the footwear could only be temporary. However, our results
78 suggest that footwear might be considered in *C. difficile* infection control management guidelines,
79 at least in some situations.

Data availability

Raw sequence reads were submitted to the Sequence Read Archive under the BioProject accession number: PRJNA658847.

Author contributions

MR conceived the study and contributed to writing and revising the manuscript. IB performed the sampling, isolation and molecular characterization of isolates. SJ performed the genome sequencing, analyzed the data and wrote the first draft. DWE performed the WGS data analysis and contributed to revising the manuscript. TR and BKK performed sampling, contributed to data acquisition and revising the manuscript. All authors have approved the final version of the manuscript.

Declarations

DWE declares lecture fees from Gilead, outside the submitted work. MR reports personal fees from Ferring, outside the submitted work. All other authors had no potential conflicts.

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127 **Figure legend**

128 **Figure 1. Recombination adjusted maximum likelihood trees.** Trees are scaled in SNV and color-
129 coded according to source: Green—shoe isolates, blue – clinical isolates. For genetically related
130 isolates isolation date is specified. AbdSurg - Department of Abdominal and General Surgery; Card
131 - Department of Cardiology and Angiology; CardSur - Department of Cardiac Surgery; Gastro -
132 Department of Gastroenterology; Inf - Department of Infectious Disease and Febrile Conditions;
133 Inf-amb - Department of Infectious Disease and Febrile Conditions (outpatients); ThorSurg -
134 Department of Thoracic Surgery; Traum - Department of Traumatology.