Detection of Clostridioides difficile in hospital environment by using C diff Banana Broth (TM)

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Short Communication

Detection of *Clostridioides difficile* in hospital environment by using C diff Banana Broth™

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ABSTRACT

116 environmental samples from a 504 bed clinical hospital obtained in 2017/19 were inoculated into C diff Banana Broth™. Six *C. difficile* and 12 *C. pefringens* strains were isolated. Antibiotic-resistant *Clostridium* spp. dominated in hospital environment. To determine *Clostridium* spp. in hospital environment suitable medium like C diff Banana Broth™ should be used.

Toxigenic strains of *Clostridioides difficile* are the leading cause of hospital antibiotic-associated diarrhea [1]. *Clostridium* spp. under unfavorable conditions transform into spores resistant to various factors including alcohol-based disinfectants [2]. Currently *C. difficile* constitutes the main epidemiological problem, responsible for 11 306 cases of infections and 33% of epidemic outbreaks in Poland in 2019 [3,4]. The domination of *C. difficile* belonging to RT 027 in hospitals of Eastern Europe was demonstrated [5]. *C. difficile* RT 027 strains overproduce toxins A, B and binary toxin, possess high epidemic potential, increased sporulation capacity and multidrug resistance. Infection with the RT 027 strain is associated with greater complications and higher mortality rate [6].

In our previous paper [7] domination of *C. difficile* RT 027 was stated in patients with *C. difficile* infection - CDI. To follow the transmission of *C. difficile* between patients we aimed to study the hospital environment for the presence of spores of *C. difficile* using a C diff Banana Broth™ (Hardy Diagnostics, Santa Maria, USA).

This study was conducted twice (2017/19) in a 504 bed clinical hospital. The incidence rate of CDI in this hospital was 6.4 per 10 000 patient/days with domination of RT 027 [7].

In 2017, 38 samples were collected in 4 wards, with noted cases of CDI: Internal Medicine, General Surgery, ICU, and Nephrology. In 2019, 78 samples were collected from 6 wards including Cardiology and Urology. In each case 2 additional broths were assigned as the positive/negative controls. Sterile swabs moistened with sterile PBS used for collection of environmental samples directly were inoculated to C diff Banana Broth™ and transported to the our laboratory. This medium is constructed to enable the germination of spores to the vegetative form, with a red color due to neutral red - an indicator of a pH changes, and turns yellow after spore germination [8]. The color change is very explicit and easy to distinguish (Fig. 1).

Swabs were collected from various and about the same size surfaces (10 × 10 cm template was used) evaluated by investigators collecting samples. The C diff Banana Broths™ were incubated at 37 °C for 2–14 days, checking color and transparency every 24 h. Positive broths were inoculated on *C. difficile* selective media - CLO and CDIFF (bioMérieux, Marcy L'Etoile, France) and Columbia Blood Agar, incubated for 48 h at 37 °C under anaerobic conditions (Whitley A35 Workstation, UK). In some cases different *Clostridium* spp. from the same sample were cultured. Biochemical identification was performed with the VITEK 2 Compact (bioMérieux). The

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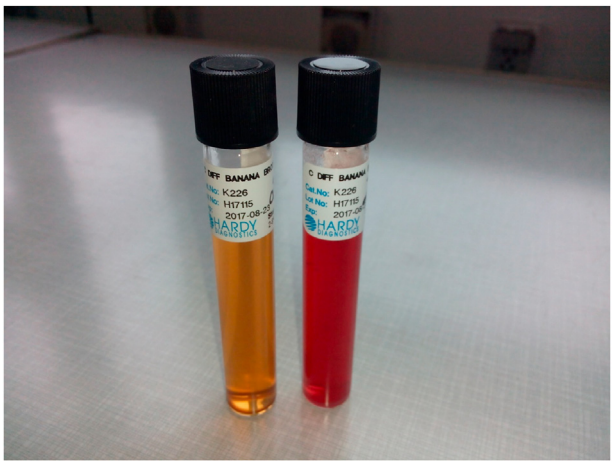

Fig. 1. C diff Banana Broth™ (LEFT TUBE – YELLOW BROTH, DEMONSTRATES SPORES GERMINATION;
RIGHT TUBE - RED COLOR, NO GERMINATION OF SPORES). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

antibiotic susceptibility of the isolated strains was determined by E-test (bioMérieux) for 10 antibiotics. The MIC50/MIC 90 values were calculated for the *C. difficile* and *C. perfringens* isolates. Susceptibility results were interpreted according to the EUCAST (Version 10.0, valid 2020-01-01mk).

DNA was extracted from *Clostridium* spp. strains using the QIAamp DNA Mini Kit (Qiagen, USA). A PCR was performed to determine the presence of *ermB* gene, the multiplex PCR (mPCR) to detect genes encoding glutamate dehydrogenase, A, B and binary toxins also was done. The results of electrophoretic separation were analyzed in a G: BOX Chemi XR5 gel imaging system (Syngene, UK) [7]. Ribotyping of *C. difficile* strains was performed as previously described [7]. In *C. perfringens* isolates the toxin genes: alpha *cpa*, enterotoxin *cpe* and *cpb2* were detected as previously described [9].

In 2017 in 6/38 samples (15.8%) the presence of *Clostridium* spp. was confirmed, in 4/38 (10.5%) - *C. difficile* and in 5/38 (13.2%) *C. perfringens*. In 2019 in 12/78 (15.4%) the presence of *Clostridium* spp. strains was confirmed: in 2/78 (2.6%) - *C. difficile*, in 7/78 (9%) - *C. perfringens,* and in 3/78 (3.8%) *C. histolyticum*, *C. baratii* and *C. subterminale* (Table 1).

All *C. difficile* isolates, were sensitive to metronidazole, vancomycin, chloramphenicol and piperacillin/tazobactam, 66.7% of *C. difficile* were resistant to imipenem and clindamycin, 50% - to erythromycin, moxifloxacin and benzylpenicillin, 33.3% - to rifampicin. All *C. perfringens* isolates were sensitive to metronidazole, vancomycin, piperacillin/tazobactam and imipenem, 66.7% were resistant to rifampicin, 33.3% - to moxifloxacin and erythromycin, 25% to benzylpenicillin and 16.7% - to chloramphenicol and clindamycin (Table 2).

The *ermB* gene was detected in 4 *C. difficile* strains with erythromycin and clindamycin MIC>256 μg/ml. Genes encoding GDH antigen - *gluD* and toxin B - *tcdB* were found in all *C. difficile* isolates. Genes encoding toxin A - *tcdA* and the binary toxin - *cdtA* and *cdtB* were found in 4 strains. Four of *C. difficile* strains belonged to RT 027, and 2 - to RT 137. The *cpa* - alpha toxin gene was detected in all *C. perfringens* isolates, however *cpe* and *cpb2* genes were not found.

There are several ways to monitor the effectiveness of disinfection process in hospital environment: from the simplest as visual assessment, to those with the use of fluorescent markers, ATP bioluminescence or quantification of live microorganisms using RODAC contact plates [10]. None of these methods provides an accurate estimation of *Clostridium* spp. spores in the hospital environment. The great importance is to use an appropriate medium and the correct collection site. In this study we used C diff Banana Broth™ enabling the germination of spores. We conducted the study twice, obtaining 116 samples, over 30% of them demonstrated the growth of *Clostridium* spp. The same medium used Srinivasa et al. who demonstrated the presence of spores mainly in the Rehabilitation Unit, while ICU was negative and the spores were found mainly on the floor (>50% of positive results) [11]. In our study the spores mainly occurred in General Surgery, however it cannot be clearly stated that the spores dominated in one place (e.g. on the floor). In Spain, sterile sponges were used to collect samples from surfaces in hospital. The samples were collected from the same places, as we did, but at different time (at morning/evening). Only 4/100 samples were *C. difficile*-positive. Mostly positive results were obtained after full day work and 2/4 strains, were resistant to metronidazole [12], while we did not observe any metronidazole resistant *C. difficile*.

In this study, 4 of *C. difficile* isolates belonged to RT 027 and 2 to PCR RT 137. Strains of PCR RT 027 possess genes encoding toxin A (*tcdA*), B (*tcdB*) and a binary toxin (*cdtA* and *cdtB*), as well as *ermB* gene encoding MLS_B type resistance. Analysis of *C. difficile* RT 137 strains demonstrated *tcdA* (−)/*tcdB* (+), *cdt* (−), *ermB* (−) gene profile. The RT 137 is a ribotype that is not often reported in Europe. Freeman et al. [13] did not isolate any *C. difficile* strain belonging to RT 137, once again proving domination of *C. difficile* RT 027 in Europe and also in Poland [14], providing information on strains with reduced sensitivity to metronidazole and vancomycin, while vancomycin resistant strains - from Spain [13]. The RT 137 strain appeared in a Switzerland study in cows and calves, and in sheep and lambs - in Australia [15,16]. *C. difficile* RT 137 appears in a report from a study conducted in 2 regions of Australia among patients with CDI and the domination of RT 014/020 was demonstrated, while RT 137 tcdA (−)/*tcdB* (+) appears in <10% [17].

C diff Banana Broth™ is dedicated to *C. difficile* spore detection, but thanks to the prolonged incubation time, we managed to obtain the spore germination of other *Clostridium* spp, including *C. perfringens*, found mainly in General Surgery. About 5% of *C. perfringens* strains produce enterotoxin (*cpe* +) responsible for mild food poisoning and beta2 toxin (*cpb2*+), responsible for gastrointestinal symptoms related to post-antibiotic diarrhea [9,18]. All our *C. perfringens* isolates produced alpha toxin (*cpa* +) and no others.

Penicillin G is used for treatment of *C. perfringens* infection, however 25% of environmental *C. perfringens* isolates were resistant to benzylpenicillin and 16.7% - to clindamycin. We consider this quite alarming. In Hungary only 2.6% *C. perfringens* resistant to penicillin and 3.8% - to clindamycin were obtained [19]. Among our *C. perfringens*, no strains resistant to metronidazole and imipenem were noted similar to the Hungarian study.

Among the tested *Clostridium* spp. strains we observed high resistance to rifampicin (33.3% *C. difficile* and 66.7% *C. perfringens*) however in studied hospital rifampicin usage was not common. Reigadas et al. demonstrated a similar percentage of resistance to rifaximine during study in 1500 bed hospital. The most dominant ribotype was RT 001 and no correlation between *C. difficile* resistance to rifampicin and the severity of the infection or its recurrences was found [20].

We confirmed the presence of hyperepidemic *C. difficile* strains, belonging to RT 027 in hospital environment, also rarely occurred in Europe *C. difficile* strains belonging to RT 137.

All *C. perfringens* isolates were *cpa*-positive. We did not detect *beta2* toxin or *enterotoxin* in the environmental *C. perfringens* isolates tested, and we would like to confirm this in the future study.

Antibiotic susceptibility testing of anaerobic bacteria is not a

Table 1
The places in hospital environment from where strains of *Clostridium* spp. were cultured.

2017				
No.	Place	*C. difficile*	*C. perfringens*	*Clostridium* spp
Internal Medicine				
1	tap in the room		yes	
General Surgery Department – toilet - bathroom in front of room 32				
2	shower cabin handle		yes	
3	tap	yes	yes	
4	shower head	yes	yes	
5	shower tray	yes	yes	
Nephrology Department – isolation room				
6	hole in the bed frame (dust)	yes		
2019				
General Surgery Department				
1	treatment room - floor under the radiator		yes	
2	toilet - bathroom in front of room 32 –shower tray		yes	
3	room 32- clean bed (without a patient) – bed side rail		yes	
4	room 32 rail with media above the bed		yes	
5	room 32 - windowsill		yes	
6	room 32 - radiator			*C. subterminale*
Nephrology Department				
7	room 218- rail with media		yes	
8	isolation room - light switch			*C. histolyticum*
ICU- isolation room, after cleaning (without a patient)				
9	rail with media above the bed		yes	
Department of Cardiology - Intensive Cardiological Supervision Room/R-room				
10	patient table	yes		
Urology Department - the room that the CDI patient had left, before cleaning				
11	Bed side rail	yes		
12	chair			*C. baratii*

Table 2
MIC50, MIC90, Geometric Mean (GM), range and % of antibiotic resistant strains of *C. difficile* ($n = 6$) and *C. perfringens* ($n = 12$).

Antibiotic	*C. difficile*					*C. perfringens*					EUCAST [μg/mL][a]
	MIC_{50} [μg/mL]	MIC_{90} [μg/mL]	GM	Range [μg/mL]	% strains resistant [EUCAST]	MIC_{50} [μg/mL]	MIC_{90} [μg/mL]	GM	Range [μg/mL]	% strains resistant [EUCAST]	
Metronidazole	0.25	1.5	0.27	0.016–1.5	0	0.75	1.5	0.8	0.38–1.5	0	>2
Vancomycin	0.25	0.25	0.23	0.094–0.5	0	0.5	0.75	0.57	0.5–1	0	>2
Moxifloxacin[b]	1	32	4.80	0.5–32	50	0.25	8	0.85	0.25–32	33.3	4
Erythromycin	2	256	16.32	0.75–256	50	3	256	9.16	2–256	33.3	IE
Clindamycin[c]	6	256	30.50	2–256	66.7	2	256	3.04	0.064–256	16.7	>4
Piperacillin/ Tazobactam[c]	1.5	2	1.51	0.5–4	0	0.094	1.5	0.18	0.023–2	0	>16
Imipenem[c]	16	32	11.54	1.5–32	66.7	0.125	0.5	0.17	0.094–0.75	0	>4
Benzylpenicillin[c]	0.5	0.75	0.46	0.094–1	50	0.094	1.5	0.16	0.032–1.5	25	>0.5
Chloramphenicol[c]	1	4	1.26	0.125–4	0	4	8	4.8	2–16	8.33	>8
Rifampicin[b]	0.002	32	0.05	0.002–32	33.3	0.006	0.008	0.005	0.002 –0.008	66.7	0.004

Range [μg/mL] = range of antibiotic susceptibility test results from minimum to maximum.
[a] Resistance according EUCAST (The European Committee on Antimicrobial Susceptibility Testing); IE-lack of limit value.
[b] ECOFF (epidemiological cutoff value) for *C. difficile* was used, because lack of them according EUCAST.
[c] MICs for Gram-positive anaerobes were used, because lack of them according EUCAST.

routine test, but the fact that in hospital environment we found 25% penicillin resistant *C. perfringens* strains, seems very important. Our study suggests that antibiotic susceptibility testing should be performed for each *Clostridium* isolate when outbreak situation is confirmed. The presence in the hospital environment *C. baratti*, *C. histolyticum*, *C. subterminale* is also important finding from the medical point of view, and maybe a subject of future studies.

In this study, we have proven that spores of *Clostridium* spp. are widespread in the hospital environment. Epidemiological supervision of hospital cleanliness should include appropriate tests for spores of anaerobic bacteria, appropriate collection technique and media as C diff Banana Broth™ to enable the germination of *Clostridium* spp. spores. This is very important, especially if outbreak among patients is confirmed and environmental transmission of infection is suspected.

Declaration of competing interest

All Authors: None conflicts.

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