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Evaluation of the Nocolyse®-Nocospray® process for disinfecting surfaces contaminated by spores of *Clostridium difficile*

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Context

C. difficile is a gram-positive, anaerobic, spore-forming bacillus that is responsible for the development of 10 to 25% of antibiotic-associated diarrhoea and over 95% of cases of pseudomembranous colitis[1]. This bacterium is the principal enteropathogen responsible for nosocomial diarrhoea in adults [2]. *C. difficile* infections occur frequently as epidemics and since 2003, the incidence and severity of *C. difficile* infections have increased [3-5]. This development would appear to be related to the emergence and then the spread of a particularly hypervirulent clone, called « 027 » in reference to its profile by PCR ribotyping [6, 7]. This clone is largely predominant in North America and has already spread to several European countries (Great Britain, Belgium, The Netherlands and France) [8-10]. Contamination is oral-fecal and is transmitted either from the contaminated hands of care staff, or through the environment. *C. difficile* is isolated in **20 to 50% of environment samples** from a patient having diarrhoea infected with this germ [11]. *C. difficile* spores can persist for several weeks even months on inert surfaces [12]. Cleaning-disinfectant products normally used in hospital environments are only slightly or not effective on *C. difficile* spores, with the exception of bleach, which is currently recommended for disinfecting the rooms of infected patients[13].

The Nocolyse®-Nocospray® process (**figure 1**) is a technology that is used to disinfect hospital environments. Its principle is based on the dispersion of an aerosol of particles of hydrogen peroxide (disinfection by air). This process is used when there are no humans present.

The solution called Nocolyse® is 60 % made up of hydrogen peroxide, of complex silver salts ($AgNO_3$) playing the role of stabiliser and pure water (solvent).
This solution meets the French and/or European standards of bactericidia (EN 1040), virucidia (NF T 72-180), fongicidia (EN 1275) and sporicidia. However the standard of sporicidia validated is NF 72-230, which uses a test in a liquid environment and not the germ-carrier method.
The atoms of silver have a catalytic effect and increase the speed of action of the solution; they also have a residual effect because they inhibit the subsequent growth of micro-organisms newly deposited on the surfaces treated.

The Nocospray® is a turbine used to aerosolise and propel the Nocolyse® solution at 80 m/s and 37°C. The solution is concentrated by drying, creating an aerosol made of particles whose size does not exceed 5µ, leading to slow, uniform sedimentation on the surfaces treated,

without humidity or corrosion. The aerosol diffuses into all the volume treated, without any manual intervention by an operator.
The association of this speed and this temperature causes an increase in the activity of the aerosol by inducing the ionisation of the particles emitted and the degradation of the peroxide into very oxidising free radicals (« super oxide » ions), with a short life.

According to the manufacturer, the product is 99.9% rapidly biodegradable (in water and oxygen), in other words basically non-toxic and non-corrosive.

The appliance is designed to treat rooms with a volume between 20 and 500 m^3, diffusing 1 ml of disinfectant per m^3. The only adaptable parameter of the Nocospray® is the volume to be treated, which is determined with the aid of an adjusting screw, which is in fact a « timer », as a result only the diffusion time varies, with the flow being constant.
For a disinfection procedure not aimed at bacterial spores, the treatment of a « **standard** » room corresponds to a diffusion time of **4 minutes** plus a recommended contact time of 20 minutes, all of it making up the disinfection cycle. As we will see later, different diffusion times have been tested outside our study.

***Figure 1*:** Nocospray®-Nocolyse® couple

Objectives
To evaluate the activity *in vitro* of the Nocolyse®-Nocospray® for the disinfection of surfaces experimentally contaminated by *Clostridium difficile* **spores** (germ carrier method) and compare it to that of bleach (reference method). This evaluation was based on 2 tests:

- measuring the sporicide activity of the Nocolyse®-Nocospray® after different diffusion times (4 minutes, 22 minutes et 28 minutes)
- measuring the activity of the bleach 0.5% of active chlorine prepared extemporaneously (after contact times of 5 seconds and 10 minutes) (reference method)

Method

Preparation of the spores and germ carriers

We have used the germ carrier[14] method.
Three strains of *C. difficile* were studied:

- the strain 1067 (epidemic strain PCR ribotype 027, toxinotype III)
- the strain VPI 10463 (toxinotype 0)
- the strain CD196 (ATCC 43 596 strain, PCR ribotype 027 non epidemic)

Two materials were used for the evaluation of the bleach: vinyl resin (pieces 2 cm x 2 cm) and laminate board (pieces 2 cm x 2 cm). These materials were cleaned and sterilised prior to use.

The different materials were experimentally contaminated by 0.1 ml spore suspension of *C. difficile* (around 5.5 log_{10}/germ-carier*)* prepared based on the Wullt technique *et al.* [15]. These supports were subjected either to the action of the Nocolyse®-Nocospray® process, or to disinfection with the bleach 0.5%.
After neutralising the disinfectant residue (thisosulfate 0.5% for the bleach, DNP [AES] for the hydrogen peroxide), the amount of bacteria present on each material was numbered (test, N1) and compared to the amount of bacteria present on the same type of material not exposed to the disinfectant product (control, N0).

The spores present on the « control » pieces were numbered by immersing and placing the pieces in 3 ml of neutralising agent in an ultrasonic bath. The absence of an inhibitory effect in the neutralising agent was checked beforehand. Each suspension (0.1 ml of suspension pure and diluted up to 10^{-4}) was then sown on a TCCA (taurocholate cycloserine, cefoxitine agar) medium and anaerobically incubated for 48 hours.
For the « test » pieces (i.e. subjected to the disinfection process), 1 ml of the suspension was sown on 2 TCCA agars to get a sensitivity threshold for the 3 spores /piece method.

The amount of spores was expressed in log_{10} of UFC/germ-carrier
For each test, the reduction factor (RF) was calculated by looking at the difference between the number of spores present on the control piece (N0) and on the piece subjected to the disinfection process (N1). The RFs obtained for each disinfection technique were compared by the ANOVA test using the GraphPrism software (San Diego, CA, USA).

Disinfection conditions with the Nocolyse®-Nocospray® process

To get close to the actual conditions of use, the tests were held in an unoccupied hospital room, made up of a main room and a bathroom (with toilets), with a total volume of around 50 m^3 (**figure 2**)
For each strain, 3 pairs of germ-carriers (two « test » pairs and one « control » pair) were arranged in 3 open Petri dishes. The 2 dishes containing the 2 « test » pairs were always placed at the same locations near the bed and in the bathroom; the 3rd dish (containing the « control ») pair was kept in the laboratory so that it would not be affected by the disinfectant. The door leading to the bathroom was left open.

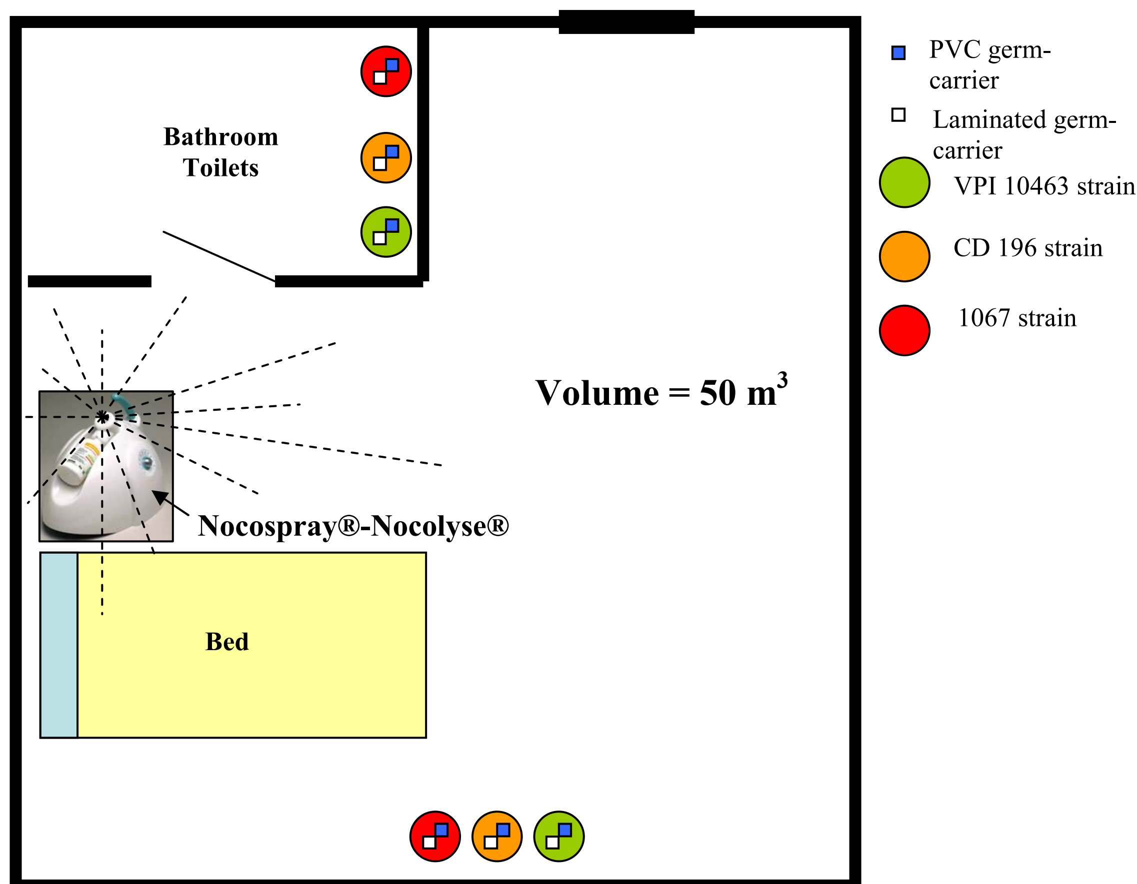

Figure 2: **Room with location of the germ carriers and the Nocospray®-Nocolyse®**

The Nocospray®-Nocolyse® appliance was then placed in the room (always in the same place), set on the « volume to be treated » (diffusion time), then started. Disinfection then took place with no humans present, with windows and the entrance door closed until the end of the disinfection cycle.

The tests were carried out in the service using 3 diffusion times:

- diffusion of the product for 4 minutes (then 20 min contact),
- diffusion for 22 minutes with a contact time of 60 minutes
- diffusion for 28 minutes with a contact time of 60 minutes.

Disinfection conditions with the bleach at 0.5% of active chlorine

For each strain tested, a pair of « control » germ carriers and a « test » pair were prepared.
Each « test » germ carrier was immersed in a pot containing bleach with 0.5% of active chlorine. Two contact times were evaluated: 10 seconds and 10 minutes. The germ-carrier was then drained and placed in a sterile pot, contaminated face upwards, to allow it to dry for 10 minutes.
Then, 3 ml of a solution of NaCl 0.9% containing 0.5% of thiosulphate were added in the pot to neutralise the action of the bleach.
Numbering of the spores deposited on the « control » germ-carrier and the surviving spores on the « test » germ-carrier was then completed as per the protocol described above.

Results

The number of spores deposited on each germ carrier does not vary significantly for each of the tests (spores recovered from « controls ») (Kruskall Wallis test, p=0.39)(***Figure 3***).

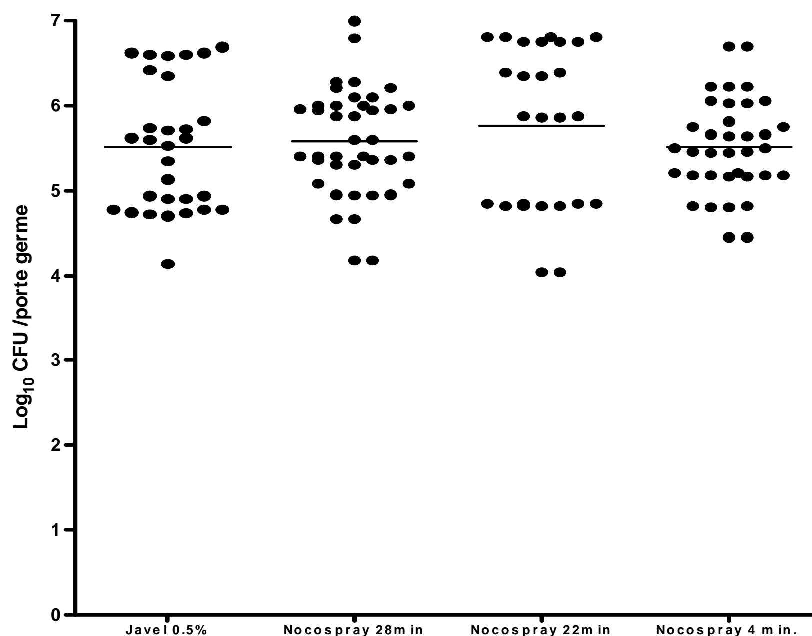

Figure 3: Quantity of spores deposited on each piece based on the different tests

The average reduction factors (RF) obtained are summarised in **table 1** and **figure 4**.

	Nocospray® **4 minutes** (n=36)	**Nocospray®** **22 minutes** (n = 26)	**Nocospray®** **28 minutes** (n = 40)	**Bleach 0.5%** **10 seconds** (n = 30)	**Bleach 0.5%** **10 minutes** (n = 24)
Average ± variance type	0.58 ± 0.52	2,14 ± 2,15	4.90 ± 0.90	1.76 ± 0.96	4.03 ± 1.09
Median	0.35	2.27	5.01	1.71	4.39
Minimum	0.03	0	2.18	0.16	1.93
Maximum	2.39	6.28	6.28	3.93	5.44

Table 1**:** Reduction factors ($\log_{10}$) obtained for each disinfectant

One will notice the absence of sporicide effectiveness in the Nocospray®-Nocolyse® at 4 minutes and the very large variability in the results obtained for the protocol with 22 minutes.

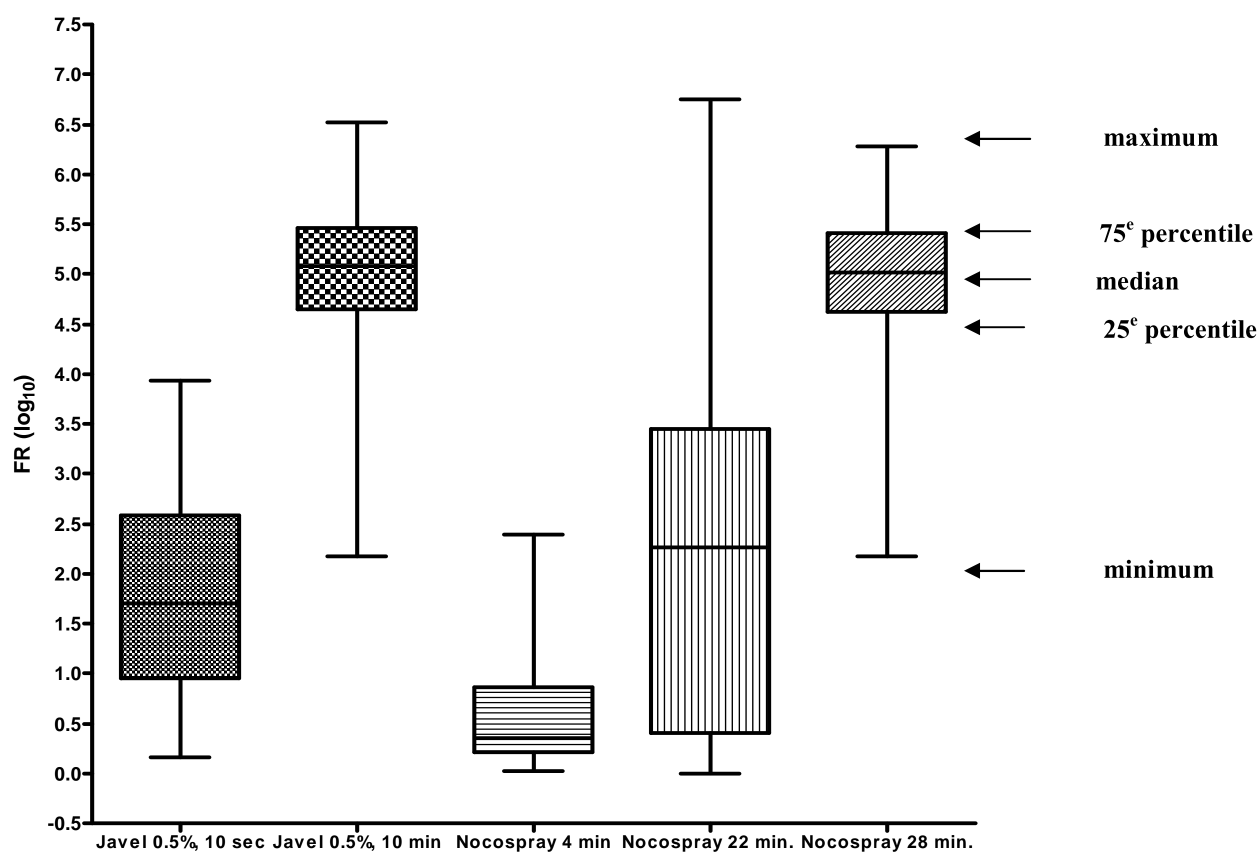

Figure 4: Comparison of the RF obtained for each disinfectant

Influence of the type of strain

For the Nocospray®-Nocolyse® (28 min) no significant variation is detected in the sporicidia, nor between the 3 strains, nor between the strains compared two by two (***Figure 5***).

Figure 5: RF obtained based on the strain for Nocospray®-Nocolyse® 28 min

Influence of the type of material making up the germ carrier

The Nocospray®-Nocolyse® (28 min) is more effective on PVC than on laminates (FR 5.3 ± 0.7 log_{10} and 4.7 ± 1 log_{10} respectively, p = 0,037) (**figure 6**)

Figure 6: RF obtained based on the type of material for the Nocospray®-Nocolyse® (28 min)

Influence of the location of the germ carrier in the room

The study of this parameter only concerned the Nocospray®-Nocolyse® diffused for 28 min.
No significant difference was found between the sporicide activities observed on the germ carriers placed in the room and the WCs (***Figure** 7*).

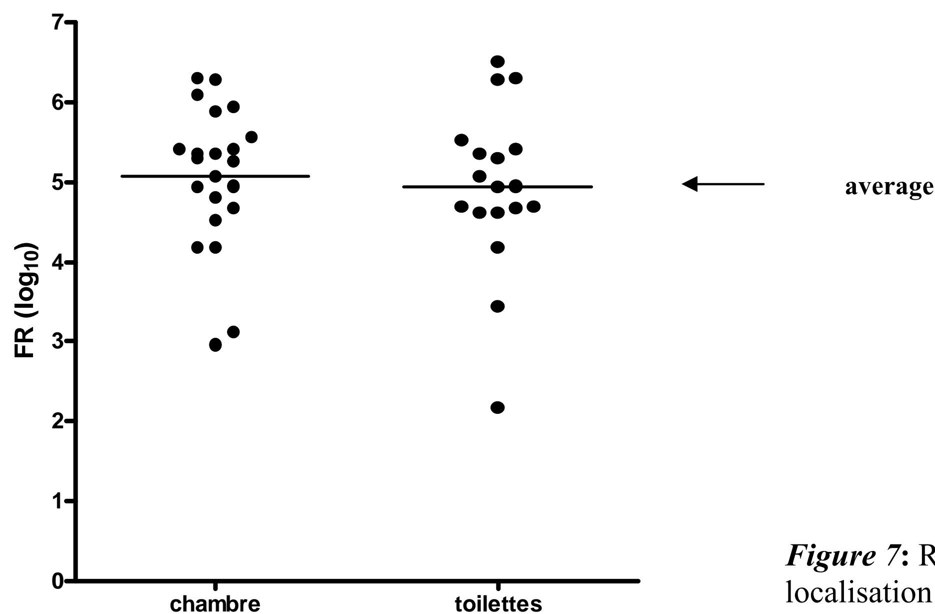

***Figure** 7*: RF obtained based on the localisation of the germ carriers in the room, for Nocospray®-Nocolyse® 28 min

Conclusion: After a diffusion time of 28 min in a 50 m^3 room, the couple NOCOSPRAY / NOCOLYSE proved effective *in vitro* vis à vis *Clostridium difficile* spores

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