

Full Length Research Paper

## Evaluation of the different diffusion capacity from a contaminated zone to adjacent areas, of two epidemic strains of *K. pneumoniae* OXA48–carbapenemase

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**Introduction:** Some disinfectants as detergent solutions, used in rooms with patients in isolation precautions, can seed to the patients' environment multi-resistant bacteria better than chlorinate products. This fact can be evaluated through new methods of surfaces disinfection. **Methods:** 1) "Immediate effect": Four disinfectants were compared using a glass germ-carrier and *Klebsiella pneumoniae* with carbapenemase OXA-48 (2 strains: ST 11 and ST405). Disinfectants were applied with microfiber cloths (unused or re-used 20-30 times). Log<sub>10</sub> reductions were calculated for colony forming units (CFU) obtained after 15 min of disinfectant application. 2) Also was assessed whether these microfiber cloths (unused or reused 20-30 times), could "transfer microorganisms" to adjacent areas. **Results:** Sodium hypochlorite, chlorine dioxide or the mixture alcohol + quaternary ammonium compounds (QUACs) produced complete destruction of all used microorganisms. Moreover diffusion of microorganisms to around area was none or very little. Nevertheless, the diluted quaternary ammonium permitted *K pneumoniae*-OXA48 diffusion to adjacent areas, through the re-used microfiber cloth. With unused microfiber, the results improve, but did not eliminate this diffusion to around area. **Conclusion:** Two types of tests should be performed before advising surface disinfectant of hospital rooms of patients on contact precautions: 1) direct effect and 2) evaluation of the possibility of transfer of microorganisms by the used or unused microfiber. In our case, chlorine dioxide, hypochlorite or alcohol + QUACs must be preferred to QUACs, in rooms' disinfection of patients with *K. pneumoniae* OXA48.

**Keywords:** Evaluation, surface-disinfection, microfiber, *K. pneumoniae*-OXA48 carbapenemase

## INTRODUCTION

The transmission of nosocomial pathogens occurs most often from the hands of medical personnel, however, instruments or surfaces are also important as a source of contamination or transmission of microorganisms like *Clostridium difficile*, Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-R *Enterococci spp.*, Gram negative bacteria, *Norovirus*, etc (Otter et al, 2011).

Factors that may contribute to bacterial transmission from surfaces are: survival of microorganisms in the environment (Kramer et al, 2006), virulence, frequent contamination from the patient's environment, capacity to be transmitted with very small doses, transient contamination of HCW's hands and resistance to disinfectants (Weber et al, 2010; Lawley et al, 2010).

The area with the highest probability of contamination by microorganisms is usually the closest to the patient (bed, rails, bedside table, etc.) or those frequently used as switches, knobs, etc. (Boyce et al, 2007; Barbut et al, 2009).

To prevent transmission of microorganisms from surfaces we must treat them, either by cleaning (removal of organic matter, including microorganisms) or by disinfection (microbial destruction). Cleaning products may be enhanced by new physical methods, such as using microfiber cloths, which trap microorganisms due to their electrostatic charge (Rutala et al, 2007). Even if the disinfectants are effective, there may be failures due to lack of access to the contaminated area, or because of errors in the cleaning technique (Eckstein et al, 2007; Dubberke et al, 2007). It is therefore not surprising that we obtain odds ratios of 1.4 to greater than 4, for a patient to become infected by the same microorganism present on the patient that was previously "housed" in that room (Huang et al, 2006; Rutala et al, 2007; Eckstein et al, 2007; Dubberke et al, 2007; Barbut et al, 2009) .

Assessment methods based on international standards (EN, EPA, etc.) with microorganisms on germ-carriers, are not similar to hospital reality because the microorganisms are covered by the disinfectant all the time, but in the disinfection of hospital surfaces, we apply disinfectants with cotton or microfiber cloth, and these products dry quickly on the surfaces. Therefore, microbial destruction will be less than expected by the International Norms. These standards are also unable to assess the possibility of spread of microorganisms to other adjacent surfaces, by the cloth used in disinfection. This can explain disinfection failures.

Surface disinfectants mainly used in patient-rooms had been diluted quaternary ammonium compounds or sodium hypochlorite.

In this study we used a new microbiological method, assessing not only the immediate effect, but also the

possibility of spreading microorganisms through the cloth (microfiber) used to apply the disinfectant, which may explain the above described increased risk of infection.

## MATERIAL AND METHODS

### a) Disinfectants:

1) Sanitbio® Lab Proder-Pharma: 1.6 g% benzyl chloride C12, C18 alkyldimethylammonium, 1.5 g% chlorine-didecyl dimethylammonium chloride and 1.6 g% of benzyl-C12, C14 alkyl dimethyl and <5 g of anionic surfactants. Used diluted 200 times. This product will be named "QUACs"

2) Sodium hypochlorite, Lab Guinama: 10% sodium hypochlorite diluted 100 times (1000 mg/L)

3) ) Tristel-duo®, Lab Vesimin: Chlorine dioxide (Cl O<sub>2</sub>). Undiluted

4) Bacoban®, Lab Adexano: benzalkonium chloride 0.71g %, sodium pyrithione 0.05g %, etanol 49.4 g % and isopropanol 7.1 g %. Used undiluted. This product will be named "QUAC+alc"

### b) Other products:

-Glass cover-slides: 12 x 35 mm, used as "standard surface" (Herruzo et al 2014).

-Glass beads: 0.5 mm in diameter.

-The cloth used may be standard cotton (control) or microfiber, either on first use, or reused more than 20 times, with the standard processing method (Hospital Laundry Service).

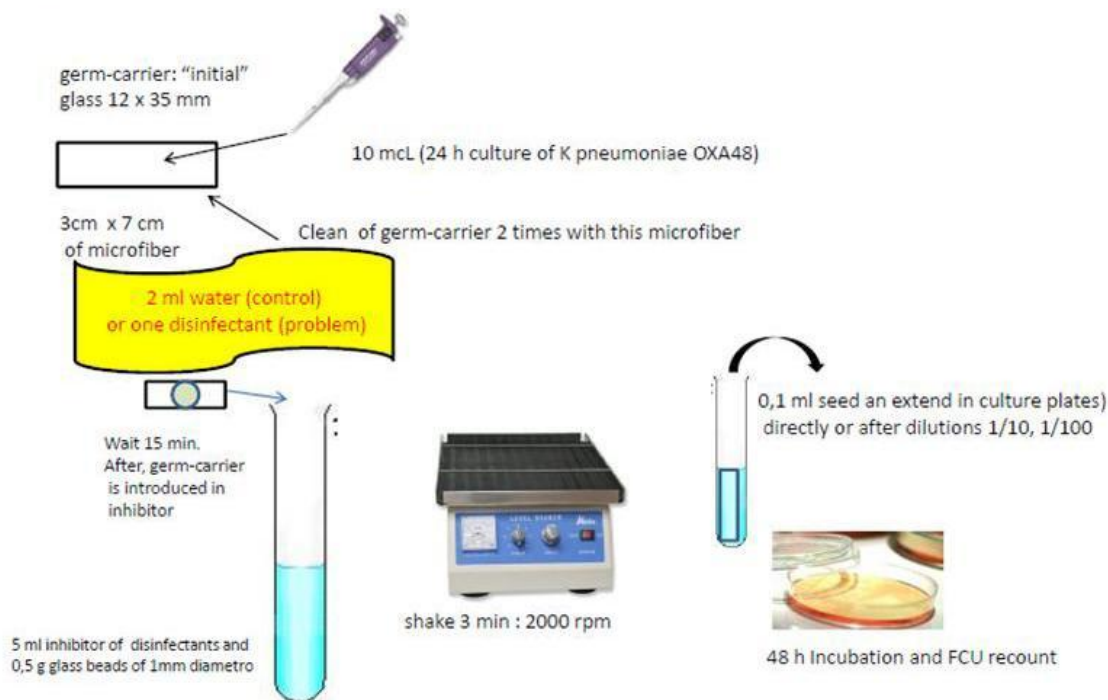
-Neutralizer of disinfectant activity (Herruzo et al, 2004; Herruzo et al, 2014) : Nutrient broth with Tween-80 at 6% + 0.5% sodium bisulphite + 0.5% sodium thiosulfate.

### Microorganisms

We used recently isolated microorganisms from different patients in the ICUs of our hospital, to avoid increased susceptibility to disinfectants caused by adaptation to the laboratory (Herruzo et al, 2004): multi-R *Klebsiella pneumoniae* (with carbapenemase OXA-48, strains ST11 and ST 405) were causing an outbreak in our hospital.

### Method A: Assessment of the "immediate effect" of 4 liquid disinfectants on surfaces

Our germ-carrier standard of surfaces, were placed horizontally on parallel glass bars, which were previously disinfected by flame. After placing the cover slides, we poured 10 microL of a 24 h culture media (diluted to 1/20 or undiluted) of one strain of *K. pneumoniae* with carbapenemase OXA-48 , (ST11 or ST 405) into the centre



**Figure 1:** Direct effect (on the "initial" germ-carrier )

of each one and allowed them to dry completely (one hour). With flame-sterilized forceps we held one of these cover slides and wiped it with a piece (of 3x5 cm) of microfiber (new or 20-30 times used microfiber), in which we had placed 3 mL of disinfectant (see above), leaving it to soak for 10 seconds. With one of these cloths, we rubbed the glass surface of one germ-carrier, five successive times. The disinfectant was left to act for 15 min and then we introduced this glass germ-carrier in a test tube with five mL of a disinfectant activity neutralizer and 0.5 g of sterile glass beads. This was vortexed for 3 min at 2000 rpm to elute surviving CFU (figure 1).

Next, two 0.1 mL aliquots of the supernatant liquid were cultured on MacConkey agar and incubated at 37°C for 48h, after which we took the CFU count. In order to make counting easier, we made dilutions at 1/10 and 1/100 of a third sample of 0.1 mL of this same liquid that were treated and counted in the same way.

The CFU for the control were also calculated similarly, but using 3 mL of sterile distilled water instead of disinfectant on the cloth. Three samples of 0.1 ml were sown on MacConkey plates: 0.1 mL directly from the control, and two from dilutions at 1/100 and 1/10000 (if not, the CFU number would be too high for proper counting).

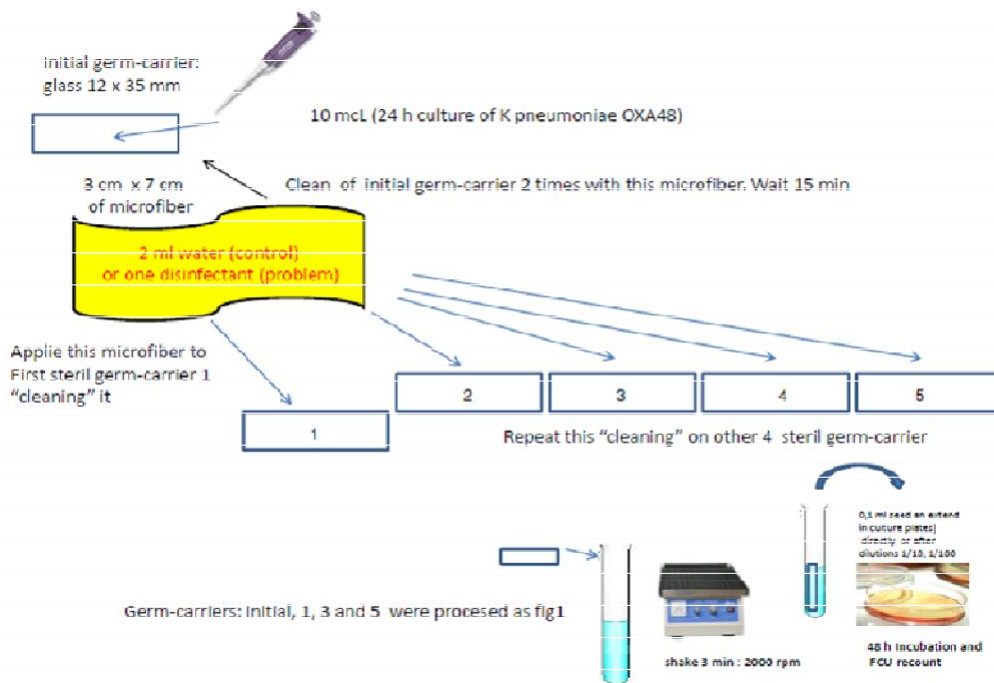
**Method B: Transfer of bacterial contamination to other surfaces** during the cleaning process (figure 2): we used five cover slides for each disinfectant (and microorganism) tested, but we did not contaminate them. They were held

with the flame-sterilized forceps and wiped with the same cloth that was used to clean the initial contaminated germ-carrier (in method A). The five sterile cover slides were "cleaned" in the same manner as was done with the first germ-carrier, but only three were cultured on MacConkey plates; cover slide numbers 1, 3 and 5. The CFU was counted as in method A (Numbers 2 and 4 were discarded in order to reduce the work load). In this way we have a study of the amount of the microorganisms that may be transmitted by means of the cleaning cloth, after contamination in everyday contact with other areas within the patient's surroundings.

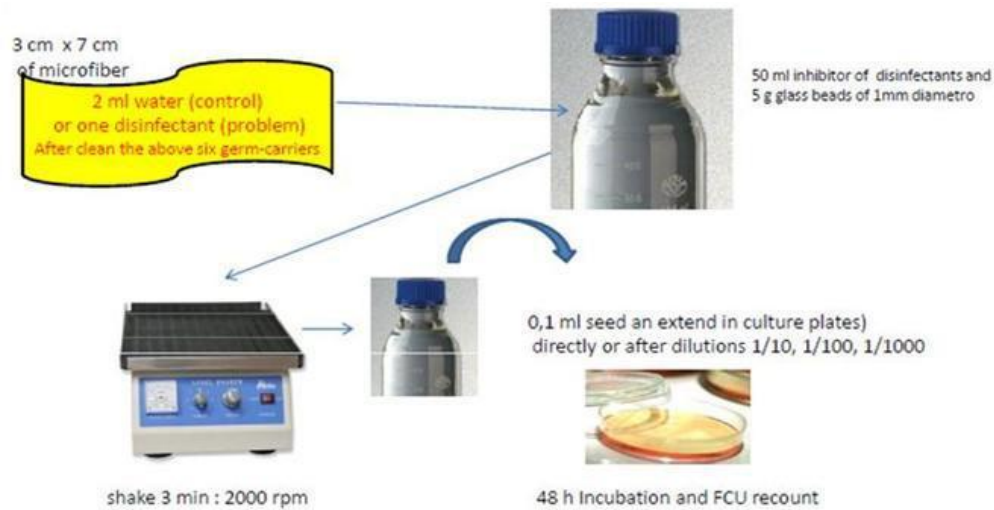
Finally (figure 3), after performing the procedures described above each piece of "used cloth" was introduced in a flask with 5 g of glass beads and 50 mL of inhibitor broth. It was vortexed at 2000 revolution/min for 3 minutes and two aliquots of 0.1 mL were then cultured on MacConkey plates, as was also done with the 1/100 and 1/10000 dilutions of a third aliquot. In this way we assessed the number of microorganisms that were trapped in the cloth.

All these experiments were repeated three times with each type of microfiber (new or used) and each liquid disinfectant and inoculums (two concentrations: undiluted and 1/20 dilution) of each *K. pneumoniae* (ST11 or ST405).

**Statistical analysis:** Log10 reductions in CFU for each disinfectant against all microorganisms (2) were calculated,



**Figure 2:** Difussion of microorganisms through microfiber



**Figure 3:** Residual contamination in a sample of microfiber, after to clean the germ-carriers

obtaining their centralization measures: mean or median, Standard Deviation (SD) or percentile 50 and statistical significance if there were significant differences by Analysis of Variance (ANOVA) or the Friedman and Wilcoxon tests.

## RESULTS

First, we have demonstrated a similitude between CFU from the inoculum (10 mL of nutrient broth) and CFU recovered from inoculated germ-carriers, after vortexing at

**Table 1:** Direct effect and diffusion of microorganisms\* to other zones, after to apply the detergent or disinfectant with a microfiber, on a contaminated standard-surface-carrier. Results in log<sub>10</sub> of survival microorganisms\*.

a) Unused microfiber

Inoculum Mean ±SD	disinfectant	initial carrier after “clean”	microfiber after “clean” initial Carrier	microorganisms’ difussion through microfiber to:		
				surf-1	surf-3	surf-5
6.5± 0.6	0.5% QUACs	2-3	6-6.5	2-3	0	0
7.7± 0.8	0.5% QUACs	4-5	7-8	5-6	4	4
6.5± 0.6	1000 Na Hyp	0	3	0	0	0
7.7± 0.8	1000 Na Hyp	0	4-5	3	0	0
6.5± 0.6	Chl -diox	0	0	0	0	0
7.7± 0.8	Chl-diox	0	0	0	0	0
6.5± 0.6	QUAC+alc	0	4-4.5	0	0	0
7.7± 0.8	QUAC+alc	0	4-4.3	0	0	0

b) Used microfiber

Inoculum Mean ±SD	disinfectant	initial carrier after “clean”	microfiber after “clean” initial Carrier	microorganisms’ difussion through microfiber to:		
				surf-1	surf-3	surf-5
6.5± 0.6	0.5% QUACs	3-4	6-7	3-4	1-2	1
7.7± 0.8	0.5% QUACs	5-6	7-8	5-6	4-5	4-5
6.5± 0.6	1000 Na Hyp	0	3-4	0	0	0
7.7± 0.8	1000 Na Hyp	0	5-6	3-4	3	3
6.5± 0.6	Chl -diox	0	0	0	0	0
7.7± 0.8	Chl-diox	0	0	0	0	0
6.5± 0.6	QUAC+alc	0	4-5	0	0	0
7.7± 0.8	QUAC+alc	0	4-5	0	0	0

\**K. pneumoniae* OXA48 (ST 11 or ST405);

Disinfectants: 0.5% QUAC = 0.5% Sanitbio; 1000Na Hyp = Sodium hypochlorite 1000 mg/L; Chl-diox= Tristel-duo; QUAC+alc= Bacoban

2000 rpm with glass beads (media 6.65 log<sub>10</sub> vs 6.7 log<sub>10</sub>). Thus this surface model is easy and reproducible.

a) With the minor inoculum on the germ-carriers (6-7 log<sub>10</sub>) the results were (Table 1): when we used QUACs at 0.5%, more than 6 log<sub>10</sub> remained in the cloth, and the initial germ-carrier was not completely disinfected, as it retained 3-4 log<sub>10</sub>

In addition, the five sterile cover slides that were “cleaned” with the contaminated cloth, used on the initial germ-carrier, became contaminated. This indicates an ability to contaminate areas that were initially free of microorganisms by the cleaning process.

With unused microfiber cloths, the results improve: the first of these five germ-carriers had only 2-3 log<sub>10</sub> survivors and there were no surviving microorganisms after the third contact.

With sodium hypochlorite or QUAC+alc, other all results, except contamination of the cloth, were 0. That is, there was no contamination left on the initial contaminated carrier, nor was it transmitted by the cloths to the five uncontaminated cover slides, except with sodium hypochlorite, being greater when used microfiber was applied on the germ-carrier. The better product was

chlorine-dioxide, because it reduced to 0 the contamination of all studied points (cloth and germ-carriers).

b) As a functional test we introduced inoculums 20 times higher: Table 1. When using 0,5% QUACs the transfer of microorganisms by the microfiber (even on first use) increased, both in number of microorganisms and during more contacts (all five cover slides). This transfer was slightly lower (significant differences) with hypochlorite, and too, there were differences between this and the other two products, indicating they were also effective in conditions of very high microorganism concentration.

In summary, chlorine dioxide was the better product, but only differs from QUAC+ alcohol in residual contamination of microfiber.

## DISCUSSION

Our current method of assessing the effectiveness of surface disinfectants is an evolution of one in which we disinfected the lab bench and analyzed the disinfected area, sampling with a swab, which is a commonly used indirect method for assessing hospital microbiological surface disinfection (Moore and Griffith, 2006). However, we found it was an inefficient method: sampling with a moist swab only recuperated 1% of the microorganisms that were in an area (e.g. a contaminated glass slide). This is why we thought it best to design a method in which we could culture directly from a surface. We chose a glass cover slide of 12 x 35 mm, which can be introduced in a test tube with glass-beads.

The immediate effect seen with diluted QUACs was worse than with other 3 products: From an initial inoculum of 6 log<sub>10</sub> *K. pneumoniae* on the surface, after cleaning, there were 3-4 log<sub>10</sub> (> 99% effectiveness) and of these, after 24 hours, 2 log<sub>10</sub> still survived. Moreover, it permits the transfer of microorganisms by the microfiber (used or unused). These findings explain the increased risk (e.g. OR of 1.4 to more than 4, in some papers) of a patient being contaminated by a microorganism from the patient previously in their room, even after a thorough cleaning (Huang et al, 2006; Drees et al, 2008). But the "first use" of a microfiber cloth obtain significant better results that reused microfiber (lower possibility of microorganisms' diffusion).

With 1000 mg/L sodium hypochlorite no microorganisms remained on the previously contaminated area after cleaning (immediate effect) nor were they transferred to other surfaces with subsequent contacts, independently of the type of microfiber's cloth (used or unused) solving the problem referred to above: patient contaminated by microorganisms from the patient previously in the same room. But this good effect disappears, with high inoculums on the initial surface to disinfect (table 1).

Thirty, the data obtained in this experiment predict that may also be useful surfaces disinfection performed twice:

quaternary ammonium (which will reduce the number of microorganisms on the germ-carrier, as shown in Table 1) is first applied, and then, on the same surface, hypochlorite is applied. Thus, the results will be even better than with hypochlorite alone, completely destroying the initial contamination and avoiding the diffusion of multi-resistant microorganisms to adjacent surfaces. This dual application could be especially useful when there is visible dirt on the surface to be disinfected.

Fourthly, other "double" disinfectants application, QUACs+alc, was very useful on the initial surface to disinfect, (total destruction of the inoculums, included those in conditions of higher contamination) and does not permitted the diffusion of microorganisms to other surfaces. These results corroborate our indication in neonates, of a double disinfection of surfaces, first QUACs and after isopropyl-alcohol, because hypochlorite vapors could be a respiratory irritant to these patients.

Finally, chlorine dioxide appears as the better product in surface disinfection of rooms with patients in contact precautions, because eliminate the possibility of to find microorganisms in the disinfected surface and diffusion of germs to the other areas.

## CONCLUSIONS

- Two types of tests should be performed before advising a surface disinfectant for room of contact precaution in hospitals: direct effect and evaluation of microorganism transference by the microfiber.
- Diluted QUACs can permit transfer microorganisms to adjacent areas through the microfiber used, that is, the antithesis of a true disinfectant. However, these ammoniums are the products most commonly used for surface disinfection in many countries.
- Due to their direct effect (even for grand number of microorganisms), the mixture QUAC and alcohol or sodium hypochlorite (1000 mg/L), were better choices than diluted QUACs for disinfection of surfaces.
- The best disinfectant against *K. pneumoniae* OXA48 in hospital surfaces was the chlorine dioxide.

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