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Efficiency study of biocides used in hospitals on *Enterococci*

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Abstract

The present work explore the efficiency of some disinfectants used in hospitals in the operating theaters of the Maternity Department and the Neonatology Unit in the EHS of Tlemcen. Four biocides of different formulations were used: quaternary ammonium, glutaraldehyde, alcohols, and quaternary ammonium + alcohols. The disinfectant sensitivity test was carried out by determining the minimum inhibitory concentration of the different molecules on microplate with respect to eight reference strains and 50 strains of *Enterococci* isolated in a hospital environment. The results of the MIC revealed a good efficiency of quaternary ammoniums and alcohols on *Enterococci*. However, the glutaraldehyde product remained ineffective with respect to the majority of strains tested. In conclusion, the concentration of the disinfectant used the diversity of the active ingredient and the type of microorganism greatly influence the activity of the biocide.

Keywords: Biocide, Disinfection, *Enterococcus*, Sensitivity.

Introduction:

Infections related to medical care in hospitals continue to gain ground by daily morbidity and exponential death (Guimarães *et al.*, 2000), which generates an important additional cost. These infections are the result of several factors such as a dysfunction in therapeutic proceedings and an imbalance in hospital hygiene. Hospital surfaces are a microbial reservoir that can contaminate the hands of caregivers or patients directly (Carling et al., 2008) by microorganisms that are often pathogenic and multi-resistant to biocides.

A large number of infectious agents may be responsible for care-related infections. However, some of them are more frequently involved and are often found on supports; among these microorganisms, which are characterized by longevity on these supports, *enterococci* remain an edifying example (Kraemer et al., 2006). Therefore, it is essential to identify them and to know their preferential ecosystem in order to be able to master them. Optimizing the effectiveness of these biocides requires a better knowledge of resistance mechanisms. In this case, clinicians are helpless, especially when microorganisms constantly evolve genetically to accumulate mechanisms of adhesion resistance and especially persistence on different surfaces. In order to prevent and minimize these infections, it is necessary to control the microbial colonization of the supports after of a rational use of the biocides. However, these disinfection protocols are often undertaken empirically using biocides whose

effectiveness remains to be proven. The objective of this work was to verify the effectiveness of the biocides (disinfectant and antiseptics) used at the hospital of Tlemcen, by the determination of the level of sensitivity of the strains of *Enterococci* by looking for the minimal inhibitory concentrations through the technique of microdilution.

Materials and Methods:

Samples:

During one year (2015 to 2016), 80 samples were taken from surfaces in the operating theaters of the maternity ward and the neonatal unit in the EHS of Tlemcen. Samples were taken from the different inanimate surfaces (Table Mayo, Table Instruments, Cart Anesthesia, floor, door, Steribloc, surgical light). The sampling method involves rubbing a surface with a sterile saline solution (0.9% NaCl) using a sterile wet swab (Jomha et al, 2014).

The samples are then sent to the laboratory in a cooler and incubated in an enrichment broth at 37 ° C for 24 hours.

Identification

Identification was performed by conventional tests (Gram, catalase, growth on bile aesculin agar (Oxoid, Ltd) and on hypersalty broth of 6.5% NaCl and haemolytic activity); identification to the species was obtained by the Strep API system (Biomérieux, France).

Study of Resistance Level

Biocides Sensitivity (Rouillon et al., 2006) : Four products (P1', P2', P3 ', P4') used in the Algerian market (table 01) were tested on reference strains: *Escherichia coli* ATCC 25922; *Klebsiella pneumoniae* ATCC 700603; *Bacillus cereus*; ATCC 10876, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Acinetobacter baumanii* ATCC 19606; *Pseudomonas aeruginosa* ATCC 27853, and *Citrobacter freundii* ATCC 43864.

The efficiancy of four other products (P1, P2, P3, P4) (table 01) was studied on 50 strains of *Enterococcus sp*.

A serial dilution of stock solution of a disinfectant are carried out according to a geometric progression of ½ reason (final concentration: 5%, 2.5%, 1.25%, 0.625%, 0.25%, 0.125%, and 0.0625%). The diluent is BHIB and the stock solution is the sterile marketed disinfectant product with known concentration. The inoculum is prepared from a 24-hour culture in liquid medium (BHIB broth); the bacterial solution then diluted in physiological saline until an opacity of 0.5 Mac Farland is obtained. Inoculation is carried out on a 96-well microplate and the reading is realized after 24 hours of incubation at 37°C. The MIC value is the concentration of the cup that does not grow; it is expressed as a percentage.

Results and discussion:

Reference strains:

The reaction of the reference strains tested against the product (P1') varied from a total sensitivity of *Escherichia coli* to a resistance of certain species such as *E. faecalis* and *A. baumanii* to a concentration of the order of 1.25%. Considering that this product should be used at a concentration of 2%, our results showed a total effectiveness on the strains tested. This activity was also remarkable on *Staphylococcus aureus* and *Pseudomonas aeruginosa*, since a concentration of 0.125% inhibited their growth. On the other hand, these two species were characterized by respective MICs of the order of 2.5% and 5% compared to glutaraldehyde. But all other strains tested were able to grow at a concentration greater than 5%. These results do not reflect the inefficiency of this glutaraldehyde-based product because it must be used as a concentrate (100%). This is consistent with the work of some authors (Angellilo et al., 1998, Ghotaslou et al., 2012) who found that a glutaraldehyde product is both effective and faster against vegetative forms, spores and fungi.

Table 1: Composition of tested biocides

Biocides tested on reference strains			**Biocides tested on *Enterococcus* strains**		
initials	**Product**	**Use**	**initials**	**Product**	**Use**
P1'	Acetate propylene diamine guanidinium, and propionate N, N-didecyl-N-methylpoly ammonium	Cleaning and disinfection pretreatment of rigid and flexible medical instruments and endoscopes	**P1**	Didecyldimethylammonium chloride And N- (3-aminopropyl) -N-dodecylpropane-1,3-diamine	Aldehyde-free disinfectant for surgical instruments, instruments of health care and odontalgic instruments
P2'	Glutaraldehyde	Cleaning of medical instruments	**P2**	Glutaraldehyde	Sporicide
P3'	Didecyldimethylammonium chloride And N- (3-aminopropyl) -N-dodecylpropane-1,3-diamine	Pre-treatment cleanser and disinfectant medical and dental instruments	**P3**	Alcohol	Hand hygiene
P4'	Propane-2-ol, propane-1-ol-benzyl-C12-16-alkyldiméthylammonium chloride	Hand disinfection	**P4**	Propane-2-ol-propane-1-ol benzyl-C12-16-alkyldiméthylammonium chloride	Hygienic disinfection for hands

Compared to other products, quaternary ammoniums seem to be less active because with the exception of *S. aureus*, *P. aeruginosa* and *C. freundii*, all species were able to grow at a concentration of 2.5%, reflecting an inefficiency that affects more specifically *E. coli* and *B. cereus*. This product is recommended for the disinfection of surgical instruments with all that implies in terms of microbiological risk due to microorganisms that are refractory to the action of this molecule. This product is systematically at the origin of a leak of the intracellular components following damage in the plasma membranes (Tattawasart et al., 2000, McDonnell, 2007, Bragg et al., 2014). Its effectiveness is proportional to its concentration of use. Other authors even report that the concentration of a biocide should be considered as the most important factor in its efficiency (Russell and McDonnell, 2000). These results suggest a new reflection on use concentrations that must be well above 2%. The results obtained show that *Bacillus cereus* is the most resistant species to all the molecules tested, illustrating multidrug resistance. It should be noted that the bacterium that accumulates resistance mechanisms is also endowed with an organelle (spore) that contributes to the amplification of the resistance phenomenon. On the other hand, *Staphylococcus aureus* is the species most sensitive to all disinfecting solutions, this bacterium is sensitive to products that are based on biguanides and alcohols, its cell wall is mainly composed of teichoic acid and peptidoglycan, a structure that cannot act as an effective barrier against the entry of antiseptics and disinfectants (White and McDermott, 2001). Microorganisms in the hospital environment do not all react in the same way to disinfectants; these reactions are due to the peculiarity of each type of organism (Mc Donnell and Russell, 1999).The response of the other bacteria tested was shared, with relatively greater resistance in Gram-negative bacilli than in Gram-positive cocci; which is consistent with the Spaulding classification (McDonnell and Burke, 2011). This variable resistance is due to a difference in the structure of their cell walls.

One of the main reasons for this increase in resistance in Gram-negative bacteria is the outer membrane that acts as a barrier to permeability, so that absorption into the cell is greatly reduced (Russell, 2003).Our results show that the type of antiseptic and disinfectant and their concentrations could have a

direct impact on the antibacterial effects of these molecules, which seems to be in agreement with those reported by Saha et al (2014). Moreover, the different species tested vary in their response to different types of active ingredients, which greatly complicates the choice of molecules.

The product based on quaternary ammoniums and biguanide was the most effective because this molecule regains its activity once associated with biguanides (Jomha et al., 2014). This could imply that the combination of several active ingredients increases the effectiveness of disinfectants.

Enterococcus sp:

As shown in Table 2, all the enterococci tested had a MIC greater than 5% with respect to Quaternary Ammonium (P1). This rate remains unacceptable in view of the concentrations of use of this molecule which are between 0.25% and 2%. This result seems consistent with external studies that have revealed a high frequency of resistance (Jomha et al., 2014). This acquired resistance, which is considered a tolerance by some authors, is the result of an acquisition of plasmids or transposing either of a mutation (Hegstadet al., 2010).

Glutaraldehyde (P2) was effective on 11 strains at a concentration of 1.25%. This efficacy was greater on three strains since a concentration of 0.0625% was able to inhibit them. Moreover, 50% of the strains were able to grow at a concentration of 5%. This result does not seem to agree with the properties of this product (bactericidal, fungicidal, tuberculocidal, virucidal and sporicidal) which normally should be used at 2%.

In a study conducted in Brazil, all enterococci strains tested were sensitive to glutaraldehyde. This sensitivity also concerned sodium hypochlorite and even the combination of quaternary ammonium-formaldehyde and ethyl alcohol (Guimarães et al., 2000).Taking note that the use of formaldehyde in countries such as Brazil is less expensive than glutaraldehyde, this molecule is often chosen for use in public health centers (Penna et al., 2001). Despite its toxicity (corrosive and carcinogenic), it has a considerable advantage because it is active in the presence of organic matter and non-reactive with natural and synthetic materials (Mazzola et al., 2003).

Compared to other molecules such as oxidants (peroxygen), this product is both effective and faster against vegetative forms, spores and fungi (Angelillo et al., 1998). This efficacy on bacteria in the plankton state is not obvious on strains in the sessile state, indeed some authors have found that *E. faeacalis* and *E. faecium* adher strongly to supports at optimal temperatures of 25° and 39°C respectively (Fernandes et al., 2015). In a study conducted in Sweden on hen farms, disinfection with a combination of steam and formaldehyde was highly effective against vancomycin-resistant enterococci (Nilsson et al., 2013).

Although the alcohols were used according to the recommendations without dilution, they showed activity against 13 strains at a concentration of 0.5%. It should be noted that 6 strains were inhibited at a concentration ranging from 0.0625% to 0.25%. Among the molecules tested, this product is distinguished by remarkable activity on 31 strains resulting in low MICs ranging from 0.0625% to 1.25% This efficacy seems to be relative since 19 strains were able to grow at a concentration of 5%.

It should be noted that alcohols are widely used in the disinfection of surfaces by air, premises and medical devices. Their spectrum remains very wide reaching both Gram-positive, Gram-negative, mycobacterium, yeasts and some viruses (Boyce, 2018) .The mechanism of action of these molecules lies in the rapid release of intracellular components that disrupt the membrane probably as a result of the penetration of the solvent into the hydrocarbon portion of the phospholipid bilayer (Chiouet al., 2004). Thus, ethanol, isopropanol, phenylethanol and phenoxyethanol are disrupters of the cytoplasmic membrane generating the loss of function (Ingram and Buttke, 1984; Boyce, 2018). At low concentrations, they cause proton translocations in *E. coli* (MacDonnell and Russell, 1999).

Our results are in agreement with a study carried out in Lebanon, which recorded an 8.7% resistance rate of gram-positive bacteria to alcohols once again demonstrating a satisfactory activity of these products (Jomha et al., 2014). .

Table 2: Results of the MICs of *Enterococci* to the disinfectants tested:

	Products			
Strain	P1	P2	P3	P4
E.faecalis	>5%	<0.0625	<0.0625%	<0.0625%
E.faecalis	>5%	>5%	=0.0625%	=0.25%
E.faecalis	>5%	>5%	=0.0625%	=1.25%
E.faecalis	>5%	>5%	=0.5%	>5%
E.faecalis	>5%	>5%	>5%	>5%
E.faecalis	>5%	=1.25%	>5%	>5%
E.faecalis	>5%	=1.25%	=0.5%	=0.25%
E.faecalis	>5%	<0.0625%	=1.25%	>5%
E.faecalis	>5%	=1.25%	<0.0625%	<0.0625
E.faecalis	>5%	>5%	=0.5%	>5%
E.faecalis	>5%	>5%	>5%	>5%
E.faecalis	>5%	=5%	>5%	>5%
E.faecalis	>5%	=1.25%	=0.5%	=0.25%
E.faecalis	>5%	>5%	=0.25%	>5%
E.faecalis	>5%	=0.5%	=0.0625%	=0.25%
E.faecalis	>5%	=1.25%	=0.5%	=0.25%
E.faecalis	>5%	>5%	=0.5%	=0.25%
E.faecalis	>5%	>5%	>5%	>5%
E.faecalis	>5%	<0.0625%	>5%	>5%
E.faecalis	>5%	>5%	<0.625%	=0.25%
E.faecalis	>5%	>5%	=0.5%	=0.25%
E.faecalis	>5%	>5%	=0.5%	>5%
E.faecium	>5%	>5%	<0.625%	=0.25%
E.faecium	>5%	>5%	=0.5%	>5%
E.faecium	>5%	>5%	<0.625%	>5%
E.faecium	>5%	>5%	>5%	>5%
E.faecium	>5%	>5%	=0.25%	=0.25%
E.faecium	>5%	=1.25%	=0.5%	=0.25%
E.faecium	>5%	=1.25%	=1.25%	>5%
E.faecium	>5%	=1.25%	>5%	=0.25%
E.faecium	>5%	=5%	>5%	=0.25%
E.faecium	>5%	>5%	=0.5%	>5%
E.faecium	>5%	>5%	=0.625%	>5%
E.faecium	>5%	>5%	>5%	>5%
E.faecium	>5%	>5%	>5%	=0.25%
E.faecium	>5%	>5%	>5%	>5%
E.faecium	>5%	=5%	>5%	>5%
E.faecium	>5%	>5%	>5%	=1.25%
E.faecium	>5%	=5%	=0.625%	>5%
E.faecium	>5%	=1.25%	=0.625%	=0.25%
E.faecium	>5%	>5%	=0.5%	=0.25%
E.faecium	>5%	>5%	=0.625%	>5%
E.faecium	>5%	>5%	>5%	>5%
E.avium	>5%	=0.25%	0.5%	=0.25%
E.avium	>5%	=0.25%	0.5%	=0.25%
E.durans	>5%	=1.25%	=1.25%	>5%
E.durans	>5%	>5%	>5%	>5%
E.durans	>5%	>5%	>5%	>5%
E.gallinarium	>5%	>5%	>5%	>5%
E.viridans	>5%	=1.25%	=1.25%	>5%

Unlike some studies that report that the activity of alcohols decreases during their dilutions in water (Reybrouck et al., 1998), our results have shown an interesting activity even by solubilizing the product in water. In Canada, studies have shown that the effectiveness of an alcohol in very high concentrations

would be less interesting for a disinfection of the hands because to denature the proteins, the alcohol must react with a part of the water (CINQ, 2010) The alcohols/quaternary ammonium combination gave a good activity on 20 strains at a concentration of 0.25%, it was enough of a concentration of 0.0625% to inhibit a strain of *Enterococcus*. The reaction of the strains of enterococci tested was different because at a concentration greater than or equal to 5%, 25 strains could grow and develop.

All these results made it possible to classify the disinfectants according to their effectiveness. The product P4 seems to be among the most active knowing that it must be used concentrated (100%). It has been effective on a large number of strains.

These antiseptics are very sensitive to environmental conditions: they are less active in alkaline medium; their activity is reduced by 50 to 150 times in the presence of organic matter or soap; their effectiveness is reduced in the presence of hard water and anionic or non-anionic compounds (McDonnell and Russell, 1999, Williamson et al, 2017).

Quaternary ammoniums are surfactants: therefore, they have a foaming and detergent power that allows their use in bath or on large surfaces. Being cationic, they are antagonistic with soaps and anionic surfactants. Their antiseptic activity is low: they have a bacteriostatic effect on gram-positive bacteria, but there is resistance of certain strains of *S. aureus* to these agents. They have no residual effect and no data are available on a possible cumulative effect. They are inactivated by organic materials. Like any surfactant, they can be irritating and caustic, especially in folds and mucous membranes (Gerba, 2015).

The bacterial response to biocides is determined primarily by the nature of the chemical agent and the type of organism involved. Other factors such as contact temperature, environmental pH and the presence of organic matter can have a significant effect on the activity of an antimicrobial agent (Russell, 1997).

Conclusion:

The fight against infectious diseases imperatively requires an awareness of the phenomenon of resistance that continues to evolve and spread, so it is essential to periodically assess the levels of sensitivity of different microorganisms to biocides while focusing on the efficiency and rigor in the use of these molecules. For this, it is necessary to develop protocols for optimizing biocide yields in well-defined conditions thus crystallizing a response tailored to each situation. In order to limit this major risk and avoid any resistance, it is necessary to know the type and environment of each microorganism while choosing the right disinfectant. Compliance with hygiene rules combined with a program against the spread of multidrug-resistant bacteria in the hospital could contribute significantly to the fight against these phenomena.

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Author's Contributions

M. Rahmoun and S.A Rebiahi carried out the experiment and wrote the manuscript. W. Khazini, M. Kaouadji and A. Mahi performed the sampling and sensitivity tests. S. Benamar did the translation.

Ethics:

No ethical issues.

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