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DETERMINATION OF DECIMAL REDUCTION TIME (D-VALUE) OF CHEMICAL AGENTS USED IN HOSPITAL DISINFECTION

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ABSTRACT

Prior to selecting disinfectant for low, intermediate and high (sterilizing) levels, the decimal reduction time, D-value, for the most common or persistent bacteria identified on a medical device or at a health care facility should be determined. The D-value was determined by inoculating 100 mL of disinfecting solution with 1 mL of a bacterial suspension. At regular intervals, 1 mL aliquots of this mixture were transferred to 8 mL of growth media containing a neutralizing agent, and incubated at optimal conditions for the microorganism. *B. stearothermophilus* and *E. coli* were the most resistant bacteria for the disinfecting and sterilizing procedures.

Key words: decimal reduction time, sanitizers, desinfection.

INTRODUCTION

To evaluate the efficacy of the chemical agents for hospital use against the bacteria (*S. aureus*, *E. coli*, *E. cloacae*, *S. marcescens* and *A. calcoaceticus*) involved in the outbreaks of infections in hospital nurseries in Brazil, the resistance of these bacteria to the particular disinfectant was investigated and expressed in decimal reduction time (D-value). The confidence levels were set for 6 to 12 $\log_{10}$ reduction of the initial population of bacterium in order to a predicted probability of a surviving microorganism of 10^{-1} or better (1).

MATERIALS AND METHODS

The bacterial strains, obtained from lyophilized culture collection at the Adolfo Lutz Institute (IAL, SP, Brazil), were *E. cloacae*; *S. marcescens*; and *A. calcoaceticus*. The reference bacteria used were *E. coli*, *S. aureus*, *B. subtilis*, and *B. stearothermophilus*.

The chemical agents were: chlorhexidine digluconate, sodium dichloroisocyanurate, glutaraldehyde, formaldehyde, a mixture of peracetic acid (and hydrogen peroxide plus acetic acid (Minncare®) were used (1,2,3).

The agents used to inactivate the test disinfectants in solutions at 1% concentration, were: polysorbate 80 (chlorhexidine), glycine (glutaraldehyde and formaldehyde), catalase (Minncare®); sodium thiosulphate (sodium dichloroisocyanurate) (1,3,4).

Decimal reduction time (D-value) is the interval of time required, under a defined set of conditions, to provide a one decimal logarithm (1-$\log_{10}$) or 90% reduction in the initial viable bacterial population (bioburden). The determination of the D-value for the test disinfectant consisted in the transference of 1.0 mL of a 24 h suspension of the test bacterial strain into 100 mL of the disinfectant solution that is kept in constant agitation at a controlled temperature room (at 25ºC ± 1.0ºC), at time zero. At regular intervals a sample of the 1.0 mL mixture was transferred to 8 mL of TSB containing 1 mL of a inactivating agent at 1% concentration to guarantee a complete inactivation of the disinfectant without interfering with survivor growth. Using TSA pour plates, the survivors were evaluated (1).

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RESULTS

The vegetative strains which showed the best resistance to the solution of 0.4% chlorhexidine were *E. cloacae* (D=8.3 min) and *S.aureus* (D=5.9 min); the more sensitive ones were *A. calcoaceticus* (D=4.1 min), *S. marcescens* (D=4.0 min) and *E.coli* (D=3.0 min). A time interval of 3 to 4 minutes was enough to reduce 90% of the population of *E. coli*, *S. marcescens* and *A. calcoaceticus*; a $3\log_{10}$ reduction in bioburden for these species varied between 9 to 12 minutes. The spore strains exposed to 2% chlorhexidine showed close D-values among themselves D=9.1 min for *B. stearothermophilus* and D=6.7 min for *B. subtilis*.

In a solution of 5000 mg/L (0.5%) of formaldehyde, the spore formers *B. stearothermophilus* and *B. subtilis* exhibited similar D-values of 10.9 min and 11.8 min, respectively, showing twice the resistance of *A. calcoaceticus* (D=5.2 min) and *E. cloacae* (D=4.5 min); and five-fold resistance as determined for *S. marcescens* (D=2.1 min), the most sensitive species. Both *B. subtilis* and *B. stearothermophilus* have proven to be adequate BIs in the evaluation of the efficacy of formaldehyde in the immersion of medical devices. Periods from 65.3 min to 141.3 min are needed to reduce 6 and $12\log_{10}$ of the initial spore population.

In a solution of 2% glutaraldehyde, the spore formers *B. subtilis* and *B. stearothermophilus* both exhibit the same D-value, D=25.0 min, which is approximately 3 to 4 times greater than that determined for the most resistant vegetative species of *E. coli* (D=7.1 min) and *E. cloacae* (D=6.7 min). The more sensitive vegetative strains were *A. calcoaceticus* (D=4.7 min), *S. marcescens* (D=5.0 minutes), *S. aureus* (D=5.9 minutes) and *E. cloacae* (D=6.7 minutes). *B. subtilis* and *B. stearothermophilus* proved to be BIs appropriate for the evaluation of glutaraldehyde as a sterilizing agent, requiring exposure times of 300 min (5h), to undergo a $12\log_{10}$ reduction in viable sporeforms. Glutaraldehyde is also appropriate in the disinfection of semi-critical articles, requiring 150 min exposure for a $6\log_{10}$ reduction, respectively, in populations of *B. subtilis* and *B. stearothermophilus*. Comparing the sterilizing effect of 4% formaldehyde and 2% of glutaraldehyde, the necessary time for decay n=12 cycles of B. subtilis varied from 92.4 min (1h 32min) to 300 min (5h), respectively.

The bacteria the more resistant bacteria to the solution of 1% Minncare (0.45% peracetic acid plus 2.2% of hydrogen peroxide) were *B. stearothermophilus* (D=9.1 minutes), *E.coli* (D=6.7 minutes) and *B. subtilis* (D=5.9 minutes). The most sensitive strains with similar resistance were *A. calcoaceticus* (D=3.4 minutes), *E. cloacae* (D= 3.5 min) and *S. aureus* (D=3.6 minutes).

The most resistant vegetative strains to a 1000 mg/L (0.1%) solution of NaDCC (sodium dichloroisocyanurate) were *A. calcoaceticus* (D=5.9 min) and *E.coli* (D=5.9 min). The most sensitive vegetative strains were *S. marcescens* (D=4.3 min), *E. cloacae* (D=4.7 min) and *S.aureus* (D=5.0 min). *E.coli* was the most appropriate BI for the evaluation of NaDCC as a disinfectant agent (n=6 ycles) for an interval of 35.4 min. To reduce a log cycle of *B. stearothermophilus*, an exposure time of 4.4 min to 0.2% NaDCC was necessary. For *B. subtilis*, an exposure time of 3.8 min was required to provide a reduction of a logarithmic cycle. The high level disinfection parameter, n=12, required exposure times of 53.3 min (*B. stearothermophilus*) and 26.6 min (*B. subtilis*).

DISCUSSION

A successful disinfection at low and high levels (sterilization aim) depends upon the selection of the correct chemical agent associated with a proper disinfecting procedure. A thorough understanding of the unique characteristics of each chemical agent, including their limitations and appropriate applications, is necessary. It is also essential that the chemicals used in commercial products and in the preparation of the disinfecting solutions meet established quality requirements.

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RESUMO

Determinação do tempo de redução decimal (valor D) dos agentes químicos empregados em desinfecção hospitalar

Para selecionar o agente sanitizante de acordo com o nível (baixo, intermediário, alto ou esterilizante) é necessário determinar o tempo de redução decimal (valor D) para os microrganismos comumente identificados em equipamentos médico-hospitalares. O valor D é determinado inoculando-se 1 mL da suspensão de microrganismo em 100 mL da solução desinfetante. Em intervalos constantes, alíquotas de 1 mL da mistura devem ser transferidas para 8 mL de meio de cultura contendo agente neutralizante. *B. stearothermophilus* e *E. coli* se mostraram os microrganismos mais resistentes para soluções esterilizantes e desinfetantes.

Palavras-chave: tempo de redução decimal, sanitizantes, desinfecção.

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