

Assessment of the efficacy of Umonium³⁸ on multidrug-resistant nosocomial pathogens

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Key words

Antiseptic "resistance" • Nosocomial infections • Antimicrobial resistances

Summary

Introduction. We investigated the efficacy of a biocide Umonium³⁸ on multidrug-resistant strains by comparison with a chloride derivative (Decs).

Methods. *In vitro* susceptibility tests were performed by agar diffusion disk and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI). *In vitro* antibacterial efficacy of Umonium³⁸ and Decs over selected strains was evaluated according to European Standards protocol with or without organic substance.

Results. *In vitro* tests with Umonium³⁸ at 2.5% concentration demonstrated an overall drop in microbial and yeast charges after 5 min. contact without organic substance. The same results were obtained in presence of organic substance. *In vitro* tests with chloride derivative at 5% without organic substance also resulted in overall drop in bacterial and mycotic charges. Conversely, in

presence of organic substance, the hypochlorite reduced the initial 10⁸ UFC/ml to 10⁴ UFC/ml for all bacterial strains with a decrease of 4 log except for *Enterococcus faecalis* and *Candida albicans* whose reduction was 2 and 1 log units respectively.

Discussion. The organic substance in water requires large use of oxidising disinfectants (chloride, ozone) implying in the need for higher-than-standard concentrations. The disinfecting effect of chloride is only visible when the "requirement" of organic substance has been met. By contrast, Umonium³⁸ behaves like a powerful biocide even in presence of organic substance, as it is not "consumed" by possible organic residues.

Conclusions. Umonium³⁸ resulted beneficial and effective. It is to be stressed, however, that all these experiments were *in vitro* tests and still requires validation from a correct use of clinical practice.

Introduction

Nosocomial infections are a great hospital problem as they are associated with an increasing morbidity, mortality, and costs. In a one-day prevalence study (EPIC study) carried out in 1,417 Intensive Care Unit (ICU) West Europe on more than 10,000 in-patients, the prevalence of infections contracted during hospital stay was 21% [1]. No doubt that the ICUs are the departments where infection rate is highest [1], being connected with several factors depending either on patient (immunodepression, acute or chronic organic insufficiency, coma, malnutrition, hypotension, metabolic acidosis, diabetes, old age) or environment (operators hand, non-sterile tools, no glove change, contaminated circuits, reservoirs) or therapy (sedation, cortisone and cytotoxic substance, length of stay, prolonged or inappropriate use of antibiotics, use of anti-acids increasing G-colonisation) [2].

Active surveillance protocols were developed based on microbiological monitoring of in-patients and hospital environments in order to detect those subjects who showed greater susceptibility to infections, describe the incidence of those infections over time, identify epidemics, detect bacteria reservoirs and transmission mechanism, start a proper antibiotic therapy and assess the efficacy of sanitation procedures [3].

In the last few years, despite remarkable progress to the knowledge of risk factors and of prevention and control measures, the incidence of nosocomial infections has not decreased, also following the outbreak of new mul-

tidrug-resistant pathogenic agents which are selected by an excessive and often irrational use of antibiotics [4-6]. These micro-organisms are resistant to majority of antibiotics in use and even several disinfectants, resulting in increased environmental contamination [7-9]. In many cases it was demonstrated that the molecular mechanisms responsible for antibiotics-resistance are the same implied in "nonsusceptible" to biocides [8].

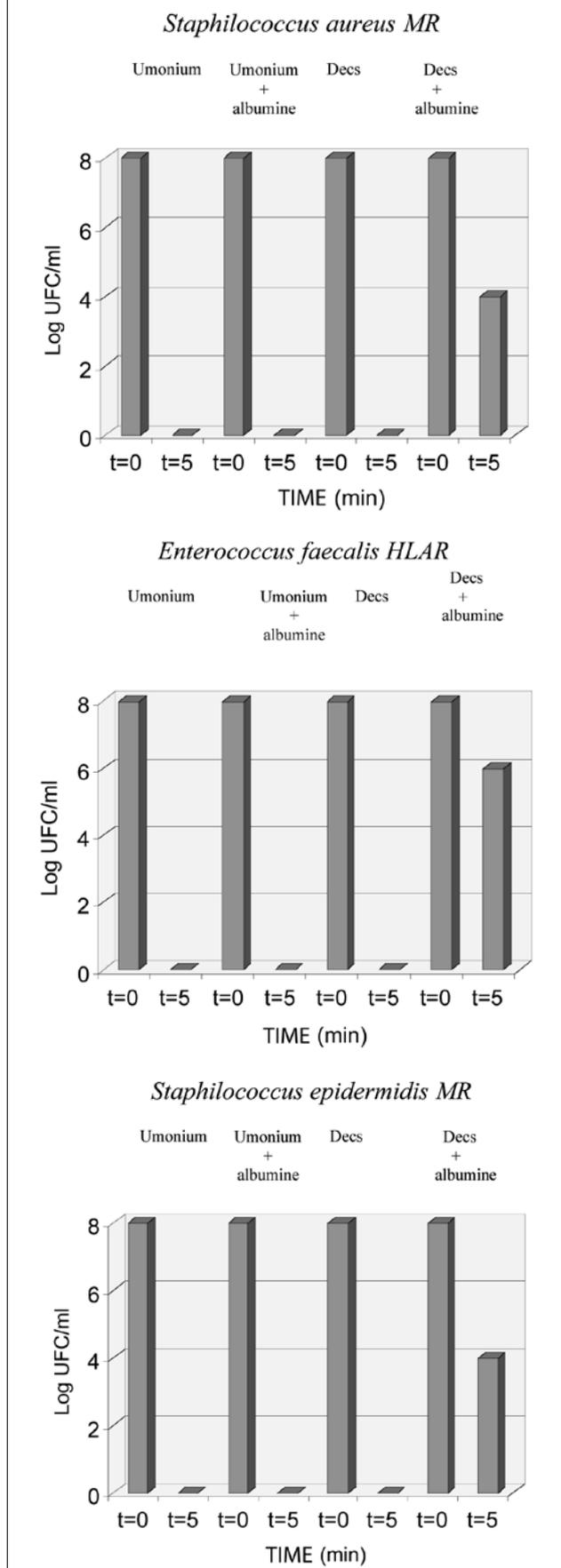
Aim of this study was to assess the efficacy of Umonium³⁸ as biocide on multidrug-resistant strains by comparison with a chloride derivative (Decs) widely common in clinical sanitation procedures.

Materials and methods

BACTERIAL STRAINS

Environmental epidemic strains (*Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* Methicillin-resistant, *Staphylococcus aureus* Methicillin-resistant, *Enterococcus faecalis* High Level Aminoglycoside Resistant, Extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae*, *Candida albicans*) were isolated from 2002 to 2005 in the Intensive Care Units of "Cotugno" Hospital and "Federico II" University Hospital during sanitation checks following nosocomial outbreaks. Brain-heart moistened swabs were used to sample horizontal surface and points of frequent hand contact as well as monitoring equipment, drug trolleys, respirators and washing

Fig. 1. Gram+: *in vitro* Umonium³⁸ and Decs activity in suspension and surface tests with or without organic substance.



sinks. Contact plates (Rodac PBI) with selective agar were used to sample the others surfaces (floor, walls, beds). Culture specimens were enriched over night at 37 °C in brain-heart infusion broth and then isolated in pure culture on agar plates. Environmental isolates were identified by a commercial microidentification system (API 20E; BioMerieux Marcy-L'Etoile, France).

SUSCEPTIBILITY TESTING

Isolated environmental antibiotypes were determined by antibiotic diffusion disk method on Muller Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) criteria for broth microdilution and disk diffusion methods [10]. Susceptibility or resistance was defined using CLSI criteria [10]. ESBL activity in *K. pneumoniae* was detected initially by double disk synergy test performed using cefotaxime, ceftazidime, ceftriaxone, aztreonam and amoxicillin-clavulanic acid on Muller Hinton agar plates and then by confirmatory test [10]. Gentamicin and streptomycin disks were used to screen resistance to high amino-glycoside resistance in *E. faecalis* at 120 and 500 respectively and finally methicillin and vancomycin were applied to test these resistance in *Staphylococci* [10].

DISINFECTANTS

Umonium³⁸ (benzyl-dimethyl-ammonium chloride, isopropyl alcohol, lauro-myristic alcohol) and Decs (sodium hypochlorite) were used for this study.

Solutions of the above disinfectants were prepared by diluting both Umonium³⁸ and the chloride derivative in sterile water at the usual hospital concentration (2.5% and 5% respectively). The Umonium³⁸ and Decs were assessed by applying the European Standards protocol [11] with and without organic substance (bovine albumine at of 0.3 g/100 ml concentration).

IN VITRO TESTS

Bacterial physiological suspensions were prepared from fresh cultures of the above micro-organisms, being sub-cultivated in non-selective media (brain Hearth Infusion Agar for Gram-, *Enterococcus* and *Candida*, Triptone soya Agar for *Staphylococci*).

Final concentrations of the bacterial inoculums of 1×10^8 UFC/ml and of 1×10^6 UFC/ml for molds were measured through a spectrophotometer. Bacterial and yeast 100 μ l suspensions were added to 900 μ l of sterile physiological solution (control) and to 900 μ l of each one of disinfectant solutions in a 25 °C thermostatic bath. Each inoculum was prepared twice, with and without organic substance. After 5 min. incubation, 100 μ l were removed from each inoculum and soon smeared on Bacto D/E Neutralizing Agar (Becton Dickinson) through serial dilutions. Plates were incubated at 37 °C for 24 hours for bacterial strains and at 32 °C for 72 hours for mold strain. The test was also carried out on glass surfaces initially contaminated with the inoculums, for which the same concentrations were used as for the suspension test, respectively with and without organic substance. After 5 min. sanitation with both disinfectants, the surfaces were rinsed with a sterile physiological solution and 100 μ l were removed from the

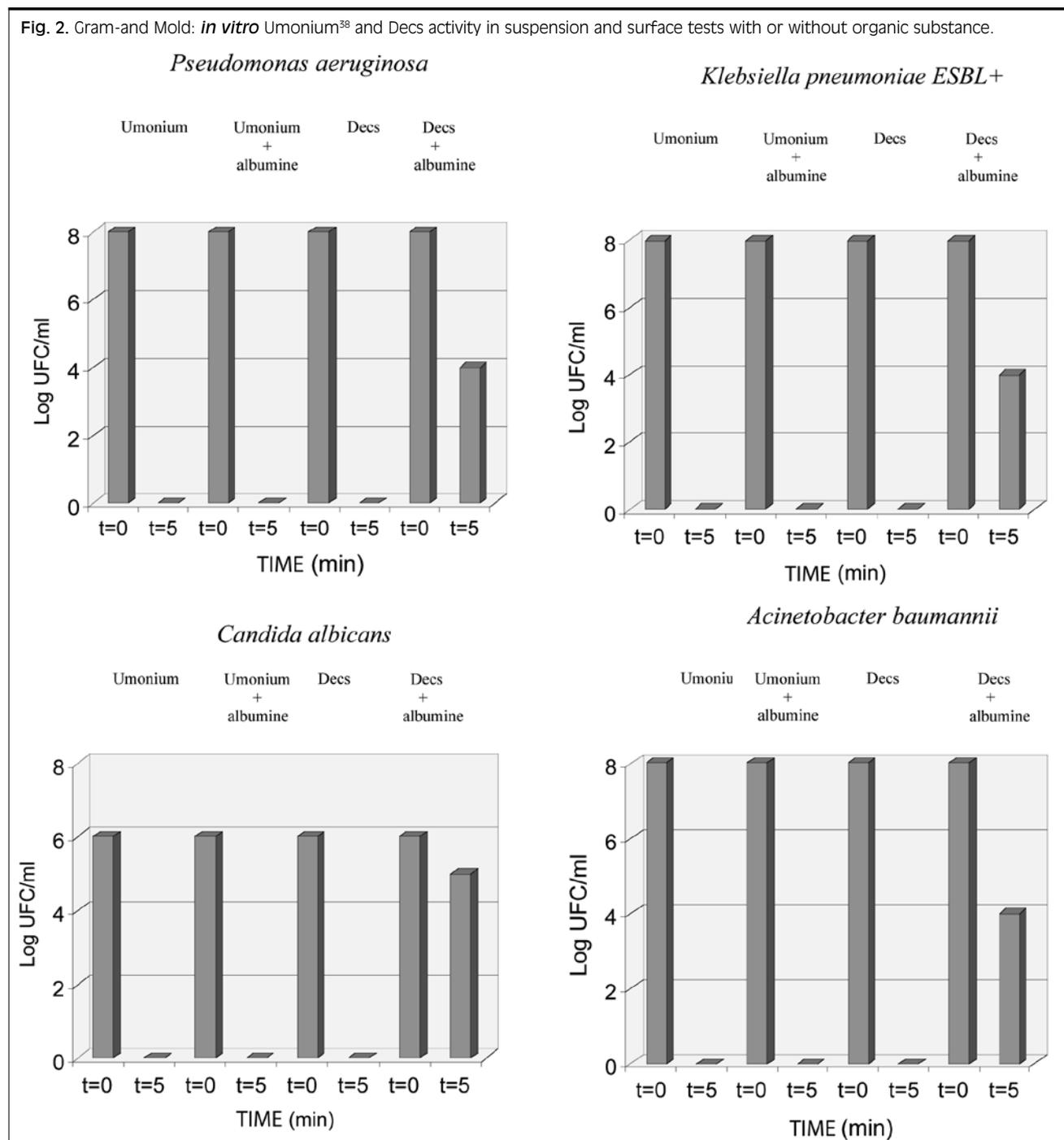
rinsing solution and soon smeared on D/E Neutralizing Agar using the serial dilutions. Plates was incubated at the same conditions as above.

Results

Antimicrobial susceptibility analysis showed a common multidrug-resistant antibiotype for all the isolated micro-organisms. In particular, the screening evidenced extended-spectrum beta-lactamase activity in *K. pneumoniae* with a resistance to third-generation cephalosporins, high amino-glycoside resistance in *E. faecalis*

and methicillin-resistance in *Staphilococci* with either positive or negative coagulase.

Results of *in vitro* tests (Figs. 1 and 2) were interpreted in agreement to European Standards [11]. According to E.S. the biocide efficacy must reduce the initial charges by 4 or 5 log units and is estimated by micro-organisms UFC/ml starting solution-UFC/ml neutralization solution ratio. *In vitro* tests with Umonium³⁸ at 2.5% concentration demonstrated an overall drop in the microbial and yeast charges after 5 min. contact without organic substance. The same results were obtained in presence of organic substance. In-vitro tests with chloride derivative at 5% without organic substance also resulted in



overall drop in bacterial and mycotic charges. Conversely, in presence of organic substance, the hypochlorite reduced the initial 10⁸ UFC/ml to 10⁴ UFC/ml for all bacterial strains with a decrease of 4 log except for *E. faecalis* (10⁸ UFC/ml to 10⁶ UFC/ml) and *C. albicans* (10⁶ UFC/ml to 10⁵ UFC/ml) whose reduction was 2 and 1 log units respectively.

Discussion

Umonium³⁸ resulted beneficial and effective (overall drop in microbial and yeast charges after 5 minutes contact without organic substance used at 2.5% concentration and the same results were obtained in presence of organic substance ever used at 2.5% concentration) [11].

This can be explained by considering that organic substance in water requires large use of oxidising disinfectants (chloride, ozone) implying in the need for higher-than-standard concentrations. The disinfecting effect of chloride is only visible when the "requirement" of organic substance has been met. However, an increase in chloride concentration is not entirely riskless [12]. By contrast Umonium³⁸ (quaternary ammonium added to isopropyl and lauro-myristic alcohols) behaves like a powerful biocide even in presence of organic substance, as it is not "consumed" by possible organic residues.

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