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ANTIMICROBIAL & ANTIFUNGAL STICKY MAT

Introduction.

In the recent past years, the problem of the infections acquired within the hospitals, the so called "hospital infections" (HI) or "medical care related infection", has arisen as one of the most prominent issues in the field of infection control.

Several different factors have influenced this increase: the appearance of multidrug resistant microorganisms and the extended use of invasive medical procedures for the diagnosis and treatment of patients are among the most important. In order to control the increment of the incidence of the HI, that greatly contributes to the raise of costs for the health management, many different strategies have been developed.

First of all, many hospitals set up a commission composed by ID doctors, microbiologists, nurses and statisticians that must elaborate the incidence data for HI and draw new interventional approaches for their control on a short time (hopefully weekly) base. The warning coming from the microbiology data about any possible epidemic outbreak provoked by a suspected microorganism needs to be handled by this commission in the shortest possible time, in order to break down the diffusion of the epidemic to a larger number of patients or wards. A limited set of well known microorganisms has been involved in the majority of the infection outbreaks in hospitals, even if the spectrum of the possibly so called "hospital pathogens" enlarges over time.

The most commonly identified pathogens in this group include methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus faecalis* (VRE). The bacteria differ from the "not resistant strains" belonging to the same genus and species only from the presence and expression of selected genes that confer these pathogens the capability to metabolize and survive in the presence of methicillin (a semi substituted derivative molecule of penicillin) and vancomycin (a glycopeptide antibacterial drug),

respectively. These particular phenotypes are more aggressive under the pathogenic point of view and are frequently involved in epidemic outbreaks within hospitals causing a prolongation of the "hospital stay" time and a worsening of the clinical pictures in the affected patients. Additional micro organisms that are commonly identified as a cause of HI include *Aspergillus fumigatus* (that can generate a pulmonary infection thus worsening the prognosis of immuno deficient neutropenic patients) and *Stenotrophomonas maltophilia* (that commonly originates from tap water within the Intensive Care Units (ICU) and can provoke severe lung infections, in particular in immuno compromised patients). *A. fumigatus* is generally sensitive to the majority of the antifungal drugs. On the other hand, this micro organism holds the capacity to grow within the host tissues without generating a clearly detectable clinical picture until a diffuse growth is present and this fact makes these infections deadly severe in immuno compromised patients, since once the fungus is detected by X-ray or by microbiological methods the patient is usually definitively compromised. Recently, clusters of hospital acquired pneumonia (HAP) caused by *Corynebacterium striatum* has been reported. This microorganism generally has a narrow antibacterial susceptibility pattern. The appearance of this bacterium in the aetiology list for hospital infections raised a major concern among microbiologists and epidemiologists. Another microorganism that recently started to play a role in the field of HI, is *Acinetobacter baumannii*: this Gram negative bacterium has been identified with a rapidly increasing frequency in urinary tract and respiratory infections acquired during hospitalization, in particular in post surgical patients within the ICUs.

In general, the best measure to avoid the insurgence of an outbreak of hospital infection is the strict environmental control of the causative agents, within the surgical wards, ICUs and, more widely, in all the areas where the patients are admitted for a long time. A special attention must be directed to the possibility that some common device, like for instance the sole of the shoes worn by the nursing personnel or the wheels of the trolleys used in the wards, could act as a tool for the spread of microbes from one room to another. In this field, several different approaches have been evaluated in order to decrease the environmental bacterial loads: the most effective has been proven the increased frequency of hands washing for all the medical and nursing personnel. In addition, the local application of chemical substance that exert an antimicrobial activity has been proven effective in order to decrease the microbial load over the time. Among the principal weakness of the free local application of antimicrobials in the hospital environment are:

1. the low residual activity that these substance can exert after a limited time from the application, since they generally are removed by standard cleaning procedure of the surfaces;
2. the leakage of the chemical compounds in the hospital environment, that can drive to a persistent pollution of the environment itself.

For the above and additional reasons, the use of chemical antimicrobials in the hospital environment is nowadays mainly performed with substances incorporated in or bound to different polymeric flat substrates. This method of application, in fact, ensures the persistency of the antimicrobial over a long period of time without a significant environmental leakage. In addition, the addition of a sticky property to these surfaces consistently enhance the capability of the material to block dust and micro particles, even those that adhere to the shoes or to wheels of the trolleys, that are important diffusion vehicles for the microbes.

In this study, the antimicrobial activity of the "antimicrobial and antifungal sticky mat – MULTIMAT STERILAB" has been evaluated using an in vitro method, performed following the ASTM E 2180 procedure.

Method.

Please refer to the above reported procedure for an y detailed information about the method used.

Results.

The findings of this study are reported in detail in the attached "TEST REPORT N° 1556/08 issued by BIOCHEM".

Discussion.

The panel of microorganism selected and used in this study is based on the etiological data of the HI in developed countries. All the bacteria and fungi isolates have been obtained from the American Type Culture Collection (ATCC) and each single isolate has been passed in vitro less than four times before being used for the test: this fact ensures that the phenotypic characteristics of each individual isolate are well conserved at the moment of the study. This panel includes 4 different Gram positive bacteria (*C.striatum*, MRSA, *E.faecalis* and VRE), two fungi (*C.albicans* and *A.fumigatus*) and three Gram negative organism (*E.coli*, *A. baumannii* and *S. maltophilia*). The antimicrobial susceptibility of these strains is widely variable from one to another: VRE and MRSA has the narrowest

spectrum of antibiotic susceptibility. The choice of this panel was mainly based on the role played by these micro organisms in the field of HI (see Introduction above) and on the high pathogenic capability that most of them hold, in particularly in selected groups of patients, included the recipients of solid organ transplant or the neutropenic subjects that underwent stem cells transplantation. Based on these considerations, the control of environmental diffusion of the micro organisms belonging to this panel has a great potential efficacy in the process of HI control.

The capability of MULTIMAT (the sticky material containing benzoisothiazoline 3-one) to reduce the bacterial loads was greatly variable, depending on the time of incubation and on the micro organism evaluated. As a negative control of the antimicrobial activity due to the intrinsic property of the plastic polymer, a Control MAT (not treated with benzoisothiazoline 3-one) was used for each individual experiment.


At time 0 (i.e. after the shortest possible contact with the materials, indicatively few seconds, depending on the ability of the operator) the lowest rate of reduction detected for MULTIMAT was for *A. fumigatus* (4.35%). The highest reduction was, on the contrary, identified for *C.striatum* (56.7%). These data clearly demonstrate that the antibacterial activity of MULTIMAT is highly variable and exerted within a very short time, (varying from 56,7% for *C.striatum* to 5.66% for *E.coli*) without a defined correlation with the Gram positivity or negativity of the micro organism evaluated. It is noteworthy that the reduction rate demonstrated for the three "Gram positive cocci" (*E.faecalis*, MRS and VRE) used in this study was quite identical (ranging from 28.23% for MRSA to 24.14% *E.faecalis*). This fact is not surprising, since the molecular structure of the outer envelopes of these bacteria is almost similar. The capability of MULTIMAT to reduce the fungal loads showed again a large degree of variability, since *C.albicans* was reduced by about 33% whereas *A.fumigatus* showed only a little 4% decrease. Again this fact is not surprising, since the architecture of the conidia of filamentous fungi (like *A.fumigatus*) is different from that of moulds (like *C.tropicalis*), being the first more resistant in the environment when compared to fungi of the genus *Candida*.

After 24 hours of incubation, the antimicrobial effect of MULTIMAT was greatly enhanced, for all the isolates of the panel, with the only exceptions of *C.tropicalis* (reduction rate was 27.29%, well comparable with that detected at time 0) and VRE (reduction at time 24 hours was 16.6%). These findings demonstrated that MULTIMAT is capable to immediately, but to a limited extend, destroy the vitality of these two micro organisms and that an increased exposure to the biocide substance does not enhance the killing rate. For all the remaining pathogens evaluated, the reduction rate was increased after 24 of

incubation in the presence of MULTIMAT. In particular, *E.coli* and *A. baumannii* were completely killed (reduction 100%), but also the vitality of *A.fumigatus*, MRSA, *E.faecalis* and *C. striatum* was highly affected (reduction rate > 90%). These results clearly demonstrate that a prolonged contact with the biocide contained within the MULTIMAT is required to kill most of the micro organism evaluated in this study: the presence of a sticky layer is probably relevant to ensure this necessary prolonged contact between the dust and the particles harbouring bacteria and fungi and the biocide. A peculiar case was that of *S.maltophilia*. This growth rate of this bacterium was decreased by 15.5% at time 0. After 24 hours of incubation no growth was detectable both after the incubation over the MULTIMAT and over the negative control material (common mat). A possible explanation for this result could be the incapability of this micro organism to survive for 24 hours under the experimental conditions used in this study, independently on the presence or on the absence of a biocide material. It is likely that the bacterial load is immediately reduced due to the high temperature (45°C +/- 2°C) required to keep the agar slurry (containing the *S.maltophilia* CFUs) melted for the purpose of making the plates used for colonies counting. Consequently the number of CFUs could be too low to be detectable after 24 hours of incubation. In the case of *S. maltophilia* a possible suggestion is that MULTIMAT could have a biocidal effect of these micro organism (reduction at time 0: 15.53%) but the available data do not allow to draw any conclusion about the effect of a more prolonged exposition of these bacteria to the MULTIMAT.

In conclusion, MULTIMAT demonstrated a high capability to reduce the bacterial load for a large spectrum of bacteria involved in the aetiology of HI. The results obtained in this in vitro study suggest that MULTIMAT is a useful tool to reduce the load of pathogenic micro organisms in all the locations where a decreased presence of bacteria and fungi is required to maintain the diffusion of "environmental borne" infections under control.

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