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Microbiological inspections of different medical devices

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ABSTRACT

The microbiological quality control represents a cornerstone in the production process of pharmaceutical products and Medical Devices (MDs). In both the pharmaceutical and MD industries, along with evolving regulatory requirements, products of greater complexity are elevating the challenges related to maintaining microbiological integrity. For a given inactivation treatment, the probability of bacterial survival is determined by number and resistance of microorganisms and by the environment in which the organisms live. Nevertheless, the sterility of an individual item in a population of sanitized products cannot be ensured in the absolute sense.
For the first time, different MDs were analyzed from the microbiological viewpoint. Following health warnings, several kinds of MDs were examined for investigating their real compliance with the sterility requirements expected for the specific product category.
According to the European Pharmacopeia, mesophilic viable microorganisms, faecal bacteria, moulds and yeasts, *P. aeruginosa*, *S. aureus* and other microbial parameters of interest depending on the circumstances were determined. Culture methods and biochemical confirmation tests were performed. The sterility expected for some MDs under test, such as saline solution for contact lens and dentistry graft devices, was not always confirmed and pathogens were isolated and identified. The microbiological evaluation of other MDs for which the sterility was not required (auricular cones, vaginal douching and denture adhesive powder) was performed assimilating them to pharmaceutical product for topic use. In some cases, high numbers were observed and environmental potential pathogens were identified. These results emphasize the fundamental role of good manufacturing practices in this production area and stress the need for closely monitoring any step of the production chain.

1. Introduction

The term Medical Devices (MDs) covers a wide range of health or medical instruments used in the treatment, mitigation, diagnosis or prevention of a disease or abnormal physical condition [1,2]. Depending of the specific national legislation, there are different MDs definitions and regulation systems. However, the exclusive aspect of this products category, which diversified them from drugs, is that MDs do not achieve any of their primary intended purposes by chemical action within or on human body or by being metabolized.

To assess their safety, effectiveness and quality before being authorized for sale, MDs have to be reviewed by a notified body.

The directives concerning MDs, diversified among the countries, set a number of essential safety requirements which products must comply with [3–5]. None technical details for achieving compliance with these requirements are provided. General Requirements, address to the safety and intrinsic device performances and relate them to health and safety of patients and user, risk analysis, minimization of risks associated with the use, guarantee the performance of the device.

Requirements relating to design and construction are aimed at multiple aspects of technological devices. They are applicable, depending on the case, at the various types of product. They relate both to chemical, physical, mechanical, biological characteristics (suitability of materials, toxicity, released of substances, etc.), and infection/microbial contamination (in appropriate and strictly controlled conditions of production), as well as to sterilization using validated methods.

In line with the directives, the manufacturer must ensure that his products meet the essential requirements drafting a technical dossier concerning design, risk assessment, production, clinical data, etc.

To achieve the quality objective, it is necessary to control all stages of medical devices production, which individually or collectively influence the quality of a product, including raw materials, manufacturing process and evaluation of finished product. Microbiological quality assessment is one of the control levels of MD production. Microbiological characteristics can represent an important issue in the production process of medical devices, pharmaceutical and personal care products, as well as in borderline products and can affect the whole production chain.

Several factors may affect microbial occurrence in medical devices: the nature of the product that could support or not the microbial

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growth, the type of device that could require the application of different hygienic procedure (sterilization or sanitation), the possible presence of preservatives.

Handling, packaging and storage are other critical phases, potentially influencing microbial characteristics.

Microbiological assessment of MDs is particularly pertinent in view of the fact that microbial contamination can reduce their medical support effects or induce infections. In addition to risk infection, microbes may change chemical, physical and organoleptic properties of the devices, potentially converting the components to toxic products. Besides microorganisms can affect product characteristics as well as produce degradation of active ingredients, excipients, and factors themselves that should inhibit their proliferation.

For a given inactivation treatment, the probability of bacterial survival depends on both number and resistance of microorganisms and environment in which the organisms inhabit. It is accepted that sterility of an individual item in a population of sanitized products cannot be ensured in the absolute sense. Nevertheless, the microbial quality may contribute to modify the MD characteristics. Following health warnings, an investigation was performed on several kinds of MDs with the aim to evaluate their compliance with the microbial stability/sterility requirements expected for each specific product category.

2. Materials and methods

Following health warnings associated with reported microbial inadequacy of some types of MDs and/or with the occurrence of use-correlated health adverse effects, 35 samples of sterile and non-sterile MDs belonging to five different MDs types were sequestered (Table 1). The Italian Health Authorities performed the collection over national market for microbiological examinations: 60% in the Northern of Italy, 10% in the central area and 30% in the Southern. For each type of product, all the samples belonged to the same production lot. Product packaging conditions examined before analyses were regular.

Samples were analyzed following official methods published on European Pharmacopeia [11]. Depending on the nature of the examined MD, different analytical techniques were applied (membrane filtration for liquid samples, buffer dissolution for powders and solids, followed by culture method). Each type of sample was inoculated in Eugonic broth (1:10 v/v) and incubated for 5–7 days at 30 °C with the aim to neutralize preservative and antimicrobial agents.

The following microbial tests were performed:

Total aerobic microbial count (TAMC): incubation on Tryptic Soy Agar (Oxoid, England) at 30 °C for 5–7 days; all colonies were counted;

Total yeast and mold count (TYMC): incubation on Sabouraud Dextrose Agar (Oxoid, England) at 25 °C for 8 weeks; fungal isolates were enumerated and identified by classical phenotypic criteria;

Enterobacteriaceae: incubation on MakConkey Agar (Difco, France) at 37 °C for 48 h; all red colonies were counted;

Pseudomonas aeruginosa (P/A test): 10 μL of sample enrichment culture in Eugonic broth were transferred on Pseudomonas CN Agar (Oxoid, England) and incubated at 37 °C for 24 h. All fluorescent blue-green colonies were considered as *P. aeruginosa*; brown or fluorescent colonies were considered as presumptive *P. aeruginosa* and confirmed by biochemical tests (oxidase reaction, ammonia production from acetamide; fluorescence production);

Staphylococcus aureus: (P/A test): 10 μL of sample enrichment culture in Eugonic broth were transferred on Mannitol Salt Agar (Oxoid, Italy) and incubated at 35 °C for 24 h; colonies surrounded by bright yellow zones were considered presumptive coagulase-positive staphylococci and submitted to bacterial identification;

Salmonella sp. (P/A test): aliquots of sample enrichment culture in Eugonic broth were transferred in Rappaport Vassiliadis Broth (Biolife, Italy) and incubated at 42 °C for 24 h; 10 μL were then inoculated on Xylose-Lysine Deoxycholate Agar (Becton & Dickinson, Italy) at 36 °C for 24 h; all red colonies with black centers were isolated and identified;

Candida albicans (P/A test): 10 μL of sample enrichment culture in Eugonic broth were transferred on Biggy Agar (Oxoid, England) at 30 °C up to 5 days; smooth, circular brown-black colonies with slight mycelial fringe were isolated and identified;

Other parameters of interest have been detected depending on the circumstances:

Amoebae (for Saline solution for contact lens): culture on non-nutrient agar containing pre-inactivated *E. coli*, for 3–5 days at 37 °C; microscopic examination.

Bacterial identification: strains isolated by culture methods were typed by microscopic examination of Gram staining preparations and identified by VITEK 2 Compact (Biomerieux, France).

Table 1
Characteristics of the analyzed MDs.

Medical device	Samples (n.)	Material (type)	Package entirety
Auricolar cones	11	Solid	Intact
Vaginal douching	10	Liquid	Intact
Denture adhesive powder	8	Powder	Intact
Saline solution for contact lens	2	Liquid	Intact (sterile)
Dentistry graft devices	4	Powder	Intact (sterile)

3. Results and discussion

Table 2 shows the mean values obtained from the microbiological analyses of the MDs taken into consideration. An analysis of test results showed the percentage of non-compliant samples to be high (43%). In fact, the non-compliant samples with the Pharmacopeia criteria [11] exceeded for microbial counts and the presence of pathogens.

The dominant sub-group among the MDs under study was auricular cones made of beeswax that represented 31% of tested samples, while aqueous preparations constituted 34%.

Some of the analyzed MDs did not require sterility (83%). For this reason, they were assimilated to not-sterile pharmaceutical products according to the Pharmacopeia requirements [11]. For this category of MDs, the percentage of non-compliant samples was 66%. For auricolar cones and denture adhesive powder, the limits for TAMC exceeded the value recommended. In the other non-sterile products, the presence of environmental microorganisms with a potential pathogenic role was observed. All the identified microbial strains were environmental highly resistant microorganisms: endospore-forming bacteria belonging to the genus *Bacillus* and cocci. Among these latter, different species of *Staphylococcus* were isolated and identified. Members of the genus *Staphylococcus* are widespread as commensals of humans and animals where they colonize the skin or mucous membranes. Nevertheless, especially the coagulase-negative *S. epidermidis*, isolated from the vaginal douching, play a role as infectious agent. In recent years, this species has emerged as a common cause of catheter-associated infections and septicemia, particularly in immunocompromised patients. The other isolated *Staphylococcus* species are opportunistic pathogens and rather occasionally are observed as infectious agents of humans.

For the other category of MDs (17%), the sterility expected was not confirmed and pathogenic parasites and opportunistic pathogenic bacteria were isolated and identified. The percentage of non-compliant samples was 33%.

From the saline solution for contact lens, *Acanthamoeba* was isolated. This protozoan parasite is responsible of keratitis, an inflammation of the cornea and a potentially blinding corneal infection and represents a serious risk for humans. Microbial keratitis has frequently been linked with contaminated care products. The incidence of the disease in developed countries is approximately one to 33 cases per million contact lens wearers. This protozoan is able to survive for a long time in the

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