Inappropriately disinfected hospital textiles can act as a vector for cross transmission of healthcare associated infections, which represent complications in the treatment of patients and cause economic damage. Through the surveillance program that includes controlling the hygiene and sampling of cleaned healthcare textiles, nosocomial infections are considered to be preventable. Until now various types of textile sampling methods were divided into non-destructive and destructive methods, which vary depending on the state of the fabric at the end of the process. In our research, the Morapex A device as a non-destructive elution based method, was introduced for textile hygiene testing. The efficiency of Morapex A device was compared to the swabbing and RODAC agar plate sampling as most common used methods for sampling of textiles. The low efficiency of capturing microorganisms due to the rough, uneven three-dimensional fabric surface turned out to be the downside of the swabbing and RODAC plate method. The Morapex A device has proved to be a better implementation and an adequate substitute for non-destructive textile hygiene testing.

Key words: hospital textiles, hygiene, cleaned healthcare textiles, textile hygiene testing, testing methods

1. Introduction

Garments of health care workers are an important aspect of the hospital environment that can easily become contaminated. It has been suggested that hospital textiles could be a source of healthcare acquired infections, contributing to the transmission of pathogens both through indirect contact, via hospital staff, endogenously, and by means of aerosols [1, 2]. Healthcare associated infections (HCAI) do not only represent complications in the treatment of patients in the hospital, but also cause economic damage [3]. Approximately 20 % to 30 % of nosocomial infections are considered to be preventable by intensive infection prevention and control programs including surveillance [4, 5]. One of these surveillance programs is controlling the hygiene of hospital textiles. The origin of infection can be an infected person or the environment. Microorganisms that cause the nosocomial infection can be part of the patient’s normal flora, which during the process of diagnosis, treatment and care of immunocompromised patients. The source may also be other patients or health workers who are infectious. Microorganisms are able to survive on environmental surfaces for periods of up to several weeks [6], providing a significant biotransfer / cross-contamination/ cross-infection potential [7] that should not be overlooked.

2. Theoretical basis

2.1. Hospital textiles

The characteristics of the textile in question, together with humidity and heat, can create the right conditions for the proliferation of numerous microorganisms [8, 9]. Inappropriately
disinfected textiles can be one of the possible sources of nosocomial pathogens and are a possible vehicle of nosocomial infections [10], since microorganisms are able to survive in the patient’s abiotic environment, such as contaminated equipment for care, diagnosis and treatment (textiles) and on surfaces [11]. Surveys show that hospital textiles can be the source of nosocomial infections with streptococci [12], enterococci [13], Bacillus cereus [14], staphylococci [15] and coliform bacteria [16]. Boyce [17] reported that 65% of nurses who had performed patient care activities on patients having MRSA in a wound or urine had contaminated their nursing uniforms or gowns with MRSA. Pathogenic bacteria such as P. aeruginosa and K. pneumoniae [18] and C. difficile [19] were also detected on uniforms of physicians and nurses.

2.2. Healthcare associated infections

On the basis of the Report on the Burden of Endemic Health Care-Associated Infection Worldwide that included data from results of systematic reviews of the literature on endemic HCAI from 1995 to 2010 in high- and low/ middle-income countries it is estimated that 4.131.000 patients are affected by approximately 4.544.100 episodes of HCAI every year in Europe. High frequency of infection is associated with the use of invasive devices, in particular central venous catheters, urinary catheters, and ventilators [20]. The impact of HCAI implies prolonged hospital stay, long-term disability, increased resistance of microorganisms to antimicrobials, a massive additional financial burden for health systems, high costs for patients and their families, and excess deaths. In Europe, HCAIs cause 16 million extra-days of hospital stay, 37.000 attributable deaths, and contribute to an additional 110.000 every year. Annual financial losses are estimated at approximately € 7 billion, including direct costs only. In the USA, approximately 99.000 deaths were attributed to HCAI in 2002 and the annual economic impact was estimated at approximately US$ 6.5 billion in 2004 [20]. In large studies conducted in France, Germany, and Italy included in above mentioned review, of 13.954 isolates, the most frequently reported pathogens in intensive care unit-acquired infections were: Staphylococcus aureus (21.8%); Enterobacteriaceae (20.2%); Pseudomonas spp. (17.2%); enterococci (10.0%); Escherichia coli (9.1%); Candida spp (8.8%); coagulase-negative staphylococci (7.0%); and Acinetobacter spp (5.1%) [21, 22, 23, 24].

2.3. Methods for sampling of hospital textiles

Detecting microorganisms on textiles is useful for many purposes, for example to determine the bioburden on used textiles before laundering procedures, assessing the reduction of bacterial counts in connection with various laundry procedures, or trace transfer routes in infection control investigations [25]. In the recommendations of Centers for Disease Control and Prevention and the Healthcare Infection Control there is a report about the absence of microbiologic standards for laundered textiles, so no rationale exists for routine microbiologic sampling of clean healthcare textiles and fabrics. Sampling may be used as part of an outbreak investigation if epidemiologic evidence suggests that textiles, fabrics, or clothing are a suspected vehicle for disease transmission. Sampling techniques include aseptically macerating the fabric into pieces and adding these to broth media or using RODAC (replicate organism detection and counting) plates for direct surface sampling [26]. In the European Union, the Certificate of quality and hygiene in laundry published by the Research Institute Hohenstein, Germany and authorized by the German Institute for quality assurance and certification (RAL) serves as an important recommendation for hospital laundries [27]. It follows the Robert Koch Institute requirements that indicate textiles as one of the critical control points where the sampling with RODAC agar plates should be conducted [28].

Until now, two basic types of textile sampling methods, which vary depending on the state fabric at the end of the process, have been described:

- Non-destructive methods, in which the test fabric remains essentially unaltered after sampling:
  - impression sampling, specifically RODAC or contact plating [29],
  - release of fabric-bound dust and cellular particles onto sterile sampling surfaces by scraping with an inverted petri dish (sweep plating) [30],
  - sampling by means of nutrient agar cylinders - sausages [31],
  - sampling by impact upon the fabric surface (percussion sampling) [32],
  - the use of a Folin bubbler apparatus with attached funnel [33, 34].

- Destructive methods, in which the test fabric is rendered unsuitable for use after completion of the sampling process:
  - maceration [33, 35, 36] of fabric samples in a defined liquid medium,
  - agitation [37, 38, 39, 40] of fabric samples in a defined liquid medium,
  - overlaying fabric samples with agar [38, 41].

The German company, SedoTreepoint GmbH [42] reports about some typical and already used applications with the device called Morapex A for testing fabric (woven, nonwoven, knitted, yarn or fiber), paper and leather materials on a non-destructive basis. Typical Morapex A applications are pH measurement, control of wash procedure, wash and water fastness checks, perspiration fastness checks, residual analysis (including size, alkali, acids, salts, peroxide, for-
maldehyde, etc.) and conductivity analysis. Based on our research [43, 44, 45], Morapex A also has proved to be an adequate substitute for textile hygiene testing as it is a non-destructive elution based method.

3. Experimental

Our recent research work [43, 44, 45], has been focused on the efficiency of different textile sampling methods for detecting selected HCAI pathogens (i.e. Clostridium difficile, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa).

The experiment presented in this article is described in detail in previous articles and was carried out as follows. The sterile fabric pieces were artificially contaminated with a suspension of selected microorganisms (Staphylococcus aureus, ATCC 25923 and Klebsiella pneumoniae, ATCC 13883) and then left in the laminar flow cabinet for 24 hours to allow the applied suspension to dry. Inoculated swatches were sampled with four different methods:

- swabbing with cotton swabs pretreated by dipping into a prepared NaCl and Tween 80 solution;
- RODAC contact plates prepared with selective agar for each of the microorganisms;
- destructive elution method where a inoculated fabric was transferred to a centrifuge tube with prepared elution solution for shake-out method;
- Morapex A device where inoculated fabric was placed between two plates and the elution solution was pressed through the fabric.

4. Discussion

The efficiency of four sampling methods is represented graphically in Fig. 1 and Fig. 2 for S. aureus and K. pneumoniae.

Swabbing results: Summarizing the results, swabbing method provides the lowest efficiency for retrieving microorganisms from textiles. The efficiency of swabbing is reliant on the efficiency of the individual carrying out three aspects of the procedure: the removal of bacteria from the surface; the removal of bacteria from the swab, and cultivation of bacteria [46]. In addition, the properties of the surface (topography, wettability, porosity, etc.) can affect the efficiency of swabbing [7], which is also the case when taking samples from textiles. It was found that the cfu captured from inoculated fabric (with swabbing) was approximately 10^5 cfu / mL lower than the initial applied concentration of microorganisms before 24 hour drying. Although swabbing is a widely used sampling method, it lacks the standardization required to provide the level of reproducibility and, as our research shows, it also gives the lowest results among the methods tested in our survey for fabrics [43]. Lusardi et al. [47] carried out a laboratory investigation and validation of methods for sampling contaminated uniforms and work-wear; they report that the swabbing method gave low and inconsistent recoveries, which is probably because swabs are generally designed to access surface bacteria on wounds or work surfaces rather than reach contamination within the fibres of a material. Also the sampling head has a small surface area and may become overloaded.

Results using RODAC contact plates: The surface sampling method using RODAC contact plates filled with appropriate agars is one of the common used surface (also textiles) sampling methods [29] for direct contact sampling of surface contamination. The RODAC plate method has found wide acceptance and use in a variety of areas where sanitation and contamination level are important, particularly in institutional areas, such as hospitals and food production and service facilities. The use of RODAC plates for surface sampling can be valuable in the field because of its simplicity, reliability, and transportability, particularly in estimating the bacterial contamination on flat surfaces [48]. The efficiency of this method depends on the evenness of the surface tested [49] and because the structure of a fabric, due to the mechanical combination of warp and weft, a relatively small contact area is available. This is clearly seen in results from our survey [43], where the cfu captured from the inoculated fabric (with RODAC plates) were also

![Fig. 1 Sampling Staphylococcus aureus with various textile sampling methods.](image1)

![Fig. 2 Sampling Klebsiella pneumoniae with various textile sampling methods.](image2)
approximately $10^5$ cfu / mL lower from initial applied concentration of microorganisms before 24 hour drying. Sampling with RODAC agar plates also has proved to be the least effective in our research [44] and has provided much less effective and also less reliable results. The lowest initial applied concentration of microorganisms before 24 hour drying that can still be detected with RODAC plate sampling was approximately $10^4$ cfu / mL [45]. Low counts of microorganisms covered by the RODAC plate technique appear in several studies [50]. B. Eriksson et al. [51] report much lower counts from impression plates (RODAC agar plates) than those from the elution method and therefore conclude that the contact plate technique in inappropriate for determination of bioburden on textiles. The other problem obtained with our work was that when sampling fabrics with higher initial concentration of microorganisms, counting the cfu on RODAC plates was very difficult or even sometimes impossible. This is why contact plates are more successful if a selective culture media is used for particular indicator microorganisms on a surface, as suggested by P. J. Eginton et al. [52].

If surfaces are rough or wet, the sampling is inaccurate, or the resulting growth on the agar may be confluent [52]. The usefulness of RODAC plates can be limited to surfaces with lower microorganism concentrations or for verifying whether a microorganism is present or not at all. Results of the elution methods: Much better results were obtained when using both the destructive elution method and the non-destructive elution methods using Morapex A device, where the cfu captured from the inoculated fabrics (with elution) were approximately $10^5$ cfu/mL lower than the initial applied concentration of microorganisms before 24 hour drying [43]. Viable plate counting after the destructive elution again proved to be the second most efficient out of three investigated methods [44]. According to H. J. Cody et al. [54], the elution method has excellent overall recovery efficiency, is easily performed, and yields reproducible results under a variety of test conditions, bacterial species, seed concentrations and fabric types. Our results also confirm these conclusions. The destructive elution method, although providing recovery data comparable to that of the maceration method, is simpler, quicker, and less expensive to use. H. J. Cody et al. [54] suggests that the elution method can be used as a simple and accurate method for enumerating fabric-associated bacteria and will permit assessment of bactericidal characteristics of laundry procedures. The downside of this method is that it belongs to a group of destructive methods, where a piece of fabric must be cut out of the sampling object, which means that the test fabric is rendered unsuitable for use after completion of the sampling process. So this method can be particularly useful in the laboratory criteria, but it cannot be used in a real environment.

The Morapex A device is able to analyse and therefore control either raw or dyed material in minutes rather than hours, compared to standard methods. Morapex A is a compact test device for use in production and can operate up to 95°C; the multi liquid option is able to operate with a wide range of wash liquids. When using the Morapex device for eluting microorganisms from inoculated fabrics [43], the efficiency was similar to the classical elution method. Cfu captured from inoculated fabric (with Morapex) were approximately $10^5$ cfu/mL lower than the initial applied concentration of microorganisms before 24 hour drying. The system worked according to the method of forced desorption, which means the inner condition of a fabric was revealed. The testing material was placed between two metal plates; the test liquid was pressed through the fabric and then collected in a tube. Such testing is possible at any stage of production, for example, on incoming fabrics, intermediate analysis during production, analysis of finished goods, research and development checks, the effect of process and equipment adjustments, etc. In our research, the device has also been tested for the detection of microorganisms; therefore it can also be used as an efficient and non-destructive method for checking the hygiene of textiles, which is an important aspect in preventing nosocomial infections. The obtained extract can be analysed quickly and easily and the tested fabric remains essentially unaltered after sampling and can therefore be reused in the real environment.

5. Conclusion
Detection limit of bacteria is very important since hospital laundry only needs to be ‘hygienically clean’, that is free of pathogenic microorganism in numbers sufficient to cause human illness [37]. Most methods for sampling microorganisms on textiles have certain limitations. The downside of the swabbing and RODAC plate method is the low efficiency of capturing microorganisms due to the rough, uneven three-dimensional fabric surface. The most applicable methods are the methods that work on the principle of eluting microorganisms from textiles as microorganisms in the fabric are also collected. The Morapex A device has proved to be a better implementation for textile hygiene testing in a real environment as it is a non-destructive elution based method.

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