

Óbuda University

PhD Theses



Survival capability of multidrug-resistant bacteria on textiles: the role of environmental conditions and antibacterial agents

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Budapest

2018

I. Introduction

Healthcare-associated infections (HAIs) caused by multidrug-resistant bacteria (MDRB) are among the most serious public health problems globally. According to the data of the European Centre for Disease prevention and Control 4 million patients acquire a HAI in European hospitals annually, which leads to 16 million extra days of hospital stay and 7 billion euros of extra medical cost. HAIs cause 37 000 deaths in Europe each year and contribute to the death of another 110 000 patients (ECDC, 2015; ECDC, 2017; WHO, 2016).

The majority of HAIs are caused by a few MDRB, the so-called ‘ESKAPE bugs’. This acronym refers to six key pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species) which compromise medical care (Rice, 2008). These pathogens can cause a variety of clinical infections ranging from minor local skin and wound infections to life-threatening bacteraemia, pneumonia or meningitis (ECDC, 2015).

To reduce the spread of MDRB it is important to understand the correlation among hosts, bacteria and inanimate surfaces. Hospital textiles (e.g. bedclothes, pyjamas, towels, uniforms of

healthcare workers, clothes of visitors) can be contaminated with pathogens, even with multidrug-resistant bacteria during daily use (Manian, 2011; Mitchell, 2015). Touching these textiles can contribute to the further transmission of pathogens (Morgan, 2012; Stiefel, 2011; Wiener-Well, 2011). Contaminated surfaces can represent sources of outbreaks as well (Falk, 2000; Hardy, 2006; van't Veen, 2005; Zanetti, 2007). Some MDRB can preserve their viability even for months or years on textiles (Kramer, 2006; Otter, 2013). Environmental factors (e.g. presence of nutrients, type of textiles, values of temperature and relative humidity) can affect the duration of bacterial survival (Kramer, 2006; Neely, 2008). Nevertheless it is an open question how environmental factors influence the number of surviving bacteria in a period of a few hours or days, which are relevant in healthcare facilities. Quantitative studies can be also important to estimate the risk of infection caused by contaminated surfaces.

Antimicrobial finishing of textiles can inhibit the bacterial growth and prevent the spread of pathogens (Bhaskara, 2015; Borkow, 2008). Silver salts and Quaternary Ammonium Compounds (QACs) belong to the most common antimicrobials used for textile finishing (Alexander, 2009; Russell, 2004),

nonetheless only a few studies have evaluated their effectiveness against numerous, healthcare-associated, multidrug-resistant bacterial strains with known genetic background.

Use of antimicrobial agents – especially silver compounds – become more and more widespread not only in health care but in daily life (e.g. deodorants, underwear, sport and bed clothing, cosmetics, domestic water purification cartridges) as well (Silver, 2003). Due to the excessive and often unreasonable use of silver, concerns have been raised about the emergence of widespread silver resistance, which can reduce the antibacterial effectiveness of silver agents in health care settings. Toxic heavy metals can be found in nature also without human contribution, thus sophisticated and precisely regulated heavy metal homeostasis systems have evolved in bacteria. Frequent mechanisms in Gram-negative bacteria are the intra- and extracellular sequestration, the enzymatic detoxification and the active efflux (Bondarczuk, 2013). The principal role of chromosomally encoded Copper sensing copper efflux system (Cus-system) and the plasmid harboured Copper Homeostasis and Silver Resistance Island (CHASRI) is to maintain the homeostasis of copper, which is an essential, but potentially toxic micronutrient (Stahlin, 2016). Nevertheless these copper homeostasis systems can also protect the bacteria against the

toxic effect of silver due to the similar chemical and ligand binding properties of copper and silver ions. A single nucleotide polymorphism of Cus-system or CHASRI can be a sufficient condition to develop high level resistance against both copper and silver (Randall, 2015; Staehlin, 2016). The mechanism of silver resistance has been mainly studied on *Escherichia coli* isolates, but it is also important to investigate this phenomenon in case of multidrug-resistant *K. pneumoniae* strains.

II. Objectives

The systematic study of bacterial survival on textiles, the evaluation of antibacterial textiles against MDRB and the investigation of biocide resistance can contribute to the improvement of infection control protocols and thus reduce the risk of HAIs. In our opinion the use of a few randomly chosen standard strains or a few isolates with extremely good survivability are not suitable for these investigations. The systematic study of a well-characterized, healthcare-associated, multidrug-resistant bacterial collection is required in clinically relevant incubation periods.

- 1. Untreated textiles:** Our aim was to investigate quantitatively the effect of environmental conditions

(presence of nutrients, chemical composition or weaves of the textiles, temperature and level of relative humidity) to the survival capability of MDRB.

2. **Antibacterial agents:** Our aims were to investigate the antibacterial efficiency of cotton textile treated with widely used compounds (silver-chloride or QAC-containing agent) and to compare the results of MDRB and antibiotic-sensitive, quality control standard strains often used in efficiency tests.
3. **Silver resistance:** Our aims were to determine the silver susceptibility of *K. pneumoniae* strains belong to high-risk or minor clones and to investigate the inducibility, the molecular mechanisms, the transferability and the fitness cost of silver resistance.

III. Materials and methods

Bacterial strains

We examined a total of 60 bacterial strains that belong to four species frequently causing HAIs. The investigated collection consists of multidrug-resistant bacterial strains corresponding to global molecular epidemiological trends and originated from different areas of Hungary between 1998 and 2014. For

comparison we selected four antibiotic-sensitive quality control strains from the American Type Culture Collection (ATCC), which are often used in viability and antibacterial efficiency tests.

- **The Gram-negative isolates:** 15 multidrug-resistant *Klebsiella pneumoniae* (MRKP), 15 multidrug-resistant *Acinetobacter baumannii* (MACI), ATCC 25922 and ATCC 11105 *Escherichia coli*.
- **The Gram-positive isolates:** 15 methicillin-resistant *Staphylococcus aureus* (MRSA), 15 vancomycin-resistant *Enterococcus faecium* (VRE), ATCC 25923 and ATCC 6538 *Staphylococcus aureus*.

Antibacterial agents

- Sanitized T99-19 liquid (Sanitized AG, Burghof, Switzerland): 50 m/m% Dimethyltetradecyl (3-(trimethoxysilyl)propyl) ammonium-chloride, hereinafter referred to as **T99-19-QAC**
- Sanitized T27-22 Silver liquid: 2 m/m% AgCl and 8 m/m% TiO₂, hereinafter referred to as **T27-22-Silver**
- 0.1 M AgNO₃ liquid (Szkarabeusz Ltd., Pécs, Hungary)

Untreated textiles

We used the textiles of INNOVATEXT Textile Engineering and Testing Institute Co. (Budapest, Hungary):

- 100% cotton sheet, plain weaved, areal density: 104 g/m²
- 100% cotton terry, terrycloth weave, areal density: 539 g/m²
- 100% polyester sheet, plain weaved, areal density: 106 g/m²

Antibacterial finishing of the cotton sheet

The antibacterial finishing process of the 100% cotton sheet was implemented at the Department of Organic Chemistry and Technology, at Budapest University of Technology and Economics according to the manufacturer's (Sanitized AG, Burghof, Switzerland) instructions.

Investigation of antibacterial agents

The minimum inhibitory concentration and minimum bactericide concentration values of the bacterial strains were determined according to the EUCAST broth microdilution method (EUCAST, 2003).

Investigation of bacterial survival on textiles

We investigated the survival capability of the bacterial strains quantitatively according to the ISO 20743-2013

(ISO20743:2013(E), 2013). The results were analysed using IBM SPSS Statistics Data Editor, dependent t-test, one way analysis of variance (One Way ANOVA) and according to Antibacterial Activity Value (ISO20743:2013(E), 2013).

Environmental conditions

We examined quantitatively how the chemical composition of the textiles (100% cotton, 100% polyester), the weave of the textiles (plain weaved, terrycloth) or the presence of nutrients (physiological saline, nutrient broth - National Public Health Institute, Budapest, Hungary) affect the survival capability of the bacteria. We modelled a common environmental condition of a hospital ward ($T=25^{\circ}\text{C}$, $\text{Rh}=52\%$) and a condition in close contact with the patient's body ($T=35^{\circ}\text{C}$, $\text{Rh}=83\%$) to investigate the effect of temperature and relative humidity to the bacterial survival. Based on pilot studies we selected one day (MRKP), three days (MRSA) and seven days (MACI, VRE) long incubation for our comprehensive study (with 60 MDRB).

The efficiency of antibacterial textiles

To investigate the short and long time antibacterial efficiency of the T99-19-QAC and T27-22-Silver sheets we selected one hour and one day incubation times in case of each bacterial group. We used untreated, 100% cotton sheet as control.

Silver resistance

We examined the inducibility of silver resistance in case of MRKP and MACI strains using passage experiments with continuous exposure to increasing concentrations of AgNO_3 according to the method of Tóth et al. (Tóth, 2014). We investigated the molecular mechanisms of silver resistance via whole genome sequencing of five pairs of parent and silver-resistant derivative strains and two strains, which proved unable to evolve silver resistance. Sequencing was performed on a MiSeq system using the MiSeq reagent kit v2 (Illumina, San Diego, USA) generating 250-bp paired-end reads. Relative changes in the fitness of the parent and silver-resistant derivative strains were determined in propagation assays using three different AgNO_3 concentrations (0 μM , 5 μM and 315 μM Ag^+). To investigate the transferability of silver resistance genes conjugation experiments were performed by the filter mating procedure according to the modified method of Werner et al. (Werner, 2010).

IV. New scientific results

A. Survival capability of multidrug-resistant bacteria on untreated textiles

To the best of our knowledge we are the first to investigate quantitatively the survival capability of numerous MDRB that correspond to global molecular epidemiological trends in five common environmental conditions [1-3].

We found that each examined MDRB strain was able to survive on textiles in an extent to pose health risk. MACI and VRE strains showed the highest, MRKP strains the lowest survival capability (CFU/textile swatch).

We found that the environmental conditions (weave and composition of the textiles, presence of nutrients, values of temperature and relative humidity) can significantly affect the CFU values of surviving MDRB on textile surfaces. All the four bacterial groups we investigated survived in significantly higher CFU/swatch values on 100% cotton terry towel than on 100% plain weaved cotton sheet. The higher survival capability can be explained by the higher water absorbing ability of the towel. The slower drying of the bacterial cells on towel could increase the

viability via decreasing the damage of membranes, proteins and nucleic acids.

MRKPs and MRSA_s showed significantly higher survival capability inoculated in nutrient broth than in nutrition poor saline solution. The possible explanation is that organic compounds could cover the bacterial cells as a shield similarly to exopolysaccharides of biofilm producing bacteria. This shield could retain water molecules, slow down the dehydration of bacterial cells thus increase their viability. Our hypothesis is supported by the fact that inoculation in nutrient broth only increased the survival capability of the desiccation-sensitive MRKPs and MRSA_s, but did not influence the survival of desiccation-tolerant MACIs and VRE_s.

The chemical composition of the textiles affected the survival capability of the Gram-negative and Gram-positive strains differently. The Gram-positive MRSA_s and VRE_s showed significantly higher, the Gram-negative MACIs showed significantly lower CFU/swatch values on polyester than on cotton sheet.

Each MDRB showed the lowest CFU/swatch values in the condition that modelled a textile in close contact with the human body. One possible explanation is that bacteria are less active at

lower temperatures, their metabolism slows down, thus they can more easily reach a dormant state that help to survive on dry surfaces.

B. The efficiency of antibacterial textiles

According to our knowledge we are the first to investigate the efficiency of two antibacterial finishing agents against numerous healthcare-associated MDRB that correspond to global molecular epidemiological trends [4-6].

The silver-chloride containing T27-22-Silver sheet showed significant antibacterial activity against Gram-positive MRSA and VREs, but neither T27-22-Silver nor T99-19-QAC sheet were able to eliminate the Gram-negative MRKP and MACI strains.

The ATCC 25923 and ATCC 6538 *S. aureus* strains had similar survival capability and biocide-tolerance as MRSA, thus we can support the use of these standards in efficiency tests. However the investigation of other, better-surviving Gram-positive bacteria (e.g. VREs) is recommended as well.

The ATCC 25922 and ATCC 11105 *E. coli* strains showed lower survivability on all the investigated textiles than the multidrug-resistant Gram-negative strains. With the application of these

standards the risk of infection caused by surfaces contaminated with Gram-negative bacteria can be underestimated, on the other hand the efficiency of antibacterial agents can be overestimated.

For antibacterial efficiency tests we recommend the use of carefully selected MDRB. For the analysis of the data the Antibacterial Activity Value is recommended.

C. Silver resistance in multidrug-resistant *Klebsiella pneumoniae* strains

To the best of our knowledge we are the first to investigate the inducibility, the mechanism and the horizontal transferability of silver resistance in globally distributed, high-risk (ST15, ST11, ST258) and in minor (ST274, ST25) MRKP clones [7, 8].

We found that high level silver resistance evolved in high-risk (ST15 (n=6), ST11 (n=3), ST258 (n=2)) clones and in ST274 (n=1) minor MRKP clone as well. The silver-resistant phenotype remained stable in the absence of silver.

We first described mutations in chromosomally encoded *cusS* and *ompK36* genes causing silver resistance in a MRKP strain (ST274). According to our knowledge we are the first to describe the successful horizontal transfer of silver resistance genes from high-risk MRKPs (ST15, ST11) to an *E. coli* strain. With the

conjugative transfer of p5442 plasmid we proved the hypothesis of Sütterlin et al. according to one single nucleotide polymorphism in the *silS* gene (CHASRI system) is a sufficient condition to develop silver resistance.

We found that the silver resistance mediated either by the CHASRI system or the chromosomally encoded Cus-system had minimal impact on bacterial fitness in silver-free environment compared to the parent strains. In the presence of silver the carriage of CHASRI could be an important selective benefit for bacterial strains due to the higher growth rate and the multiple possibilities to develop a silver resistant phenotype.

The inducibility and the transferability of silver resistance in globally distributed, high risk MRKP clones carrying CHASRI can endanger the efficient application of biocides in health care.

V. Exploitation possibilities of the results

Our results about the bacterial survival on untreated textiles can contribute to the better understanding of bacterial dissemination in hospital environment, could be useful by searching the source of outbreaks and in improving the infection control measures.

Our recommendations resulting from antibacterial efficiency tests might be useful for future researches in the development of methodology.

Our findings improve the knowledge of the emergence, mechanism and horizontal transferability of silver resistance in high risk MRKP clones and draw attention to the prudent use of silver as antimicrobial compound.

VI. Bibliography

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VII. Scientific publications of the candidate related to PhD theses

A. Survival capability of multidrug-resistant bacteria on untreated textiles

- 1. Hanczvikkell, A.;** Berta, B.; Tirczka, T.; Bayoumi, H. Hosam and Tóth, Á.: *Survival of high risk, multiresistant bacterial strains in abiotic environment*, in 5th ICEEE-2014 International Conference: Global Environmental Change and Population Health: Progress and Challenges, 19-21 November 2014. Óbuda University, Budapest, Hungary. ISBN 978-615-5460; pp. 60-70.
- 2. Hanczvikkell, A. and Tóth, Á.:** *The role of fabric composition, nutrients, temperature and humidity in the survival capability of multidrug-resistant bacterial pathogens*, in 9th Central European Conference (Fibre – Grade Polymers, Chemical Fibres and Special Textiles). 11-13 September 2017. Technical University of Liberec, Czech Republic. ISBN 978-80-7494-356-0; pp. 120-121.

3. **Hanczvikkell, A.** and Tóth, Á.: *Quantitative study about the role of environmental conditions in the survival capability of multidrug-resistant bacteria*, Journal of Infection and Public Health, pp. 1-6, Published online: 18 May 2018, <https://doi.org/10.1016/j.jiph.2018.05.001> **IF: 2.1**

B. The efficiency of antibacterial textiles

4. **Hanczvikkell, A.;** Víg, A.; Bayoumi, H. Hosam and Tóth, Á.: *Multirezisztens, nozokomiális baktériumtörzsek túlélése antibakteriális hatóanyagokkal kezelt pamutszöveten*. Magyar Textiltechnika, TMTE. 2016. **LXVIII**. HU ISSN 2060-453X; pp. 2-10.
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6. **Hanczvikkel, A.**; Víg, A. and Tóth, Á.: *Survival capability of healthcare-associated, multidrug-resistant bacteria on untreated and on antimicrobial textiles*. Journal of Industrial Textiles, pp. 1-23, Published online: 24 January 2018 <https://doi.org/10.1177/1528083718754901> **IF: 1.3**

C. Silver resistance in multidrug-resistant *Klebsiella pneumoniae* strains

7. **Hanczvikkel, A.**; Tóth, Á.: *Silver-susceptibility of multidrug resistant nosocomial Gram-positive and Gram-negative pathogens*. Acta Microbiologica et Immunologica Hungarica, 2015. **62** (Suppl), DOI: 10.1556/030.62.2015.Suppl.2. p. 156.
8. **Hanczvikkel, A.**; Füzi, M.; Ungvári, E.; and Tóth, Á.: *Transmissible silver resistance readily evolves in high-risk clone isolates of *Klebsiella pneumoniae**; Acta Microbiologica et Immunologica Hungarica, pp. 1-17, Published online: 25 July 2018, <https://doi.org/10.1556/030.65.2018.031> **IF: 1.1**

VIII. Further scientific publications of the candidate

Hanczvikkell, A.; Tirczka, T.; Berta, B.; Bayoumi H. Hosam and Tóth, Á.: *Serotyping Streptococcus pneumoniae strains isolated from invasive pneumococcal disease and investigating their antimicrobial resistance*; in 4th ICEEE–2013 International Conference: To Protect our Global Environment for Future Generations, 20-22 November 2013, Óbuda University, Budapest, Hungary. ISBN: 978-615-5018-93-0; pp. 171-180.

Nagy, E.; **Hanczvikkell, A.** and Telegdi, J.: *Biologically active polyesters for biomedical applications*. in ICDLIT International Conference on Design and Light Industry Technologies, 19-20 November 2014, Óbuda University, Budapest, Hungary (poster)

Németh, Á.; Kocsis, V.; Rakhimova, S.; Víg, A.; Gombkötő, J. and **Hanczvikkell, A.:** *New Testing Method for the Evaluation of Biocide Efficacy of Antimicrobial Finished Fabrics*. in IFATCC XXIV International Congress, 13-16 June 2016, University of Pardubice, Czech Republic. ISBN: 978-80-906086-8-9; pp. 396-398.