

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# PCR Technique for the Microbial Analysis of Inanimate Hospital Environment

---

Urška Rozman and Sonja Šostar Turk

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/65742>

---

## Abstract

Discipline of molecular ecology and molecular techniques such as polymerase chain reaction (PCR) offers a possibility to study and reveal the microbial diversity in environmental settings with complicated mixed communities, non-culturable organisms, interfering contaminants and low levels of target DNA. Hospital environment represents a new ecological niche for clinically important nosocomial pathogens and antibiotic-resistant microorganisms, which have been commonly found on various hospital surfaces. Accurate characterization of microbial communities depends on several factors, starting with sample collection and conditional enrichment step. In the step of nucleic acid isolation and purification, the DNA, as a dominant signature molecule, is extracted followed by removing co-extracted impurities. PCR target sequences are often 16S rDNA gene, functional gene probes or species-specific probes, depending on the objective of the study. Furthermore, properly prepared PCR amplicons can serve as a basis for characterizing microbial community. The PCR technique is a powerful tool for the analysis of microbial diversity of environmental ecosystems. In a hospital environment, advantages of detecting pathogens and antibiotic-resistant bacteria need to be pointed out.

**Keywords:** microorganisms, hospital environment, inanimate surfaces, DNA extraction, PCR

---

## 1. Introduction

Characteristics of the hospital environments are very specific where inanimate environment can be colonized with a wide range of microorganisms [1, 2]. The cultured microorganisms represent only a small fraction of natural microbial communities, hence the microbial diversity

in terms of species richness and species abundance is grossly underestimated [3]. Therefore, the discipline of molecular ecology and molecular techniques such as polymerase chain reaction (PCR) offers a possibility to study and reveal the real-microbial population complexity and a possibility to overcome limitations of culture-based approaches. Due to the power of the PCR to amplify small amounts of DNA, organisms occurring in small numbers in an environment are now detectable [3]. Special challenges in environmental settings are complicated mixed communities, interfering contaminants and low levels of target DNA [4]. The specifics of environmental samples are low to medium concentration of target cells, low sample homogeneity and high degree of PCR inhibition [5].

Many types of pathogenic microorganisms have been found on various common hospital surfaces. Most common nosocomial bacteria present and detected on inanimate hospital surfaces, using specific marker genes, are presented in **Table 1**.

Microorganism	Locality	Marker gene
<i>Clostridium difficile</i>	Bed, sink, toilet, wall, rails, call button, stretcher [6–9]	<i>tcd<sub>D</sub></i> , <i>tcd<sub>E</sub></i> , <i>tcd<sub>C</sub></i> , <i>cdu<sub>2</sub></i> , <i>cdd<sub>3</sub></i> [10]
<i>Klebsiella pneumoniae</i>	Bed frame, over-bed table, bedcovers, drains, sinks, shower, drug trolley, blood pressure cuff, laryngoscope, stethoscope [11–13]	<i>bla<sub>TEM</sub></i> , <i>bla<sub>CTX-M</sub></i> [14] <i>bla<sub>SHV</sub></i> [15] <i>bla<sub>KPC</sub></i> [16]
<i>Staphylococcus aureus</i>	Air, bed, mattress cover, bathroom floor, bed linen, chairs, table, floor, shower, drug trolley, blood pressure cuff, laryngoscope, stethoscope [13, 17–20]	<i>spa</i> [21] <i>fem<sub>A</sub></i> [22, 23]
<i>Acinetobacter baumannii</i>	Bed rails, sinks, tables, curtains, door handles [24]	<i>bla<sub>OXA-23</sub></i> , <i>bla<sub>NDM-1</sub></i> [25] <i>omp<sub>A</sub></i> [26] <i>bla<sub>OXA</sub></i> , <i>bla<sub>VIM</sub></i> , <i>bla<sub>MBL</sub></i> [27]
<i>Pseudomonas aeruginosa</i>	Bed, tables, ward sinks and surgical equipment, shower, drug trolley, blood pressure cuff, laryngoscope, stethoscope, water [13, 28]	<i>las<sub>A</sub></i> [29]
<i>Escherichia coli</i>	Bed, shower, drug trolley, blood pressure cuff, laryngoscope, stethoscope, water [13]	<i>bla<sub>TEM</sub></i> , <i>bla<sub>CTX-M</sub></i> [14] <i>bla<sub>SHV</sub></i> [15] <i>bla<sub>KPC</sub></i> [16]
<i>Legionella pneumophila</i>	Drinking water [30]	<i>mip</i> [30]
<i>Vancomycin resistant enterococci [VRE]</i>	General areas in patients' rooms and toilets, light switch [31]	<i>van<sub>A</sub></i> [31]
<i>ESBL Enterobacteriaceae</i>	Mechanical ventilator, showers, beds, wall, sinks, toilet, hospital room [32, 33]	<i>bla<sub>SHV</sub></i> , <i>bla<sub>CTX-M</sub></i> , <i>bla<sub>CMY</sub></i> , <i>bla<sub>IMP</sub></i> , <i>bla<sub>VIM</sub></i> [32, 33]

**Table 1.** Most common nosocomial bacteria present and detected on inanimate hospital surfaces.

Hospital environment also represents a new ecological niche for clinically important antibiotic-resistant microorganisms. Along with identification through the amplification of conserved genomic sequences, PCR can also be used to detect antimicrobial resistance or virulence genes [34].

In molecular approaches, for studying microbial population complexity, DNA is the dominant signature molecule that phylogenetically dissects microbial communities and substantially increases our insight into microbial diversity. This method does not provide a clear distinction between viable and non-viable organisms, so may not be an accurate reflection of the microbial load present on a surface [34]. On the other hand, accurate determination of the total bacterial load is important in many microbiological applications but cannot be obtained with traditional bacterial cultivation methods. These classical incubation methods based on phenotypic detection of microorganisms are also time-consuming and can work poorly with slow growing or viable but non-culturable (VBNC) organisms [35]. The PCR method clearly has potential in environmental studies where there may be low numbers of viable but non-culturable microorganisms [34] that can still be active cells and can maintain their infectivity in the case of pathogenic bacteria [36–38].

## 2. Sample collection

Accurate characterization of microbial communities depends upon several factors, starting with the sample collection step that is often ignored as a source of problem [3] in afterwards analysis. Three major obstacles are the sample volume, sample site accessibility and sample transport.

Sampling sites in hospitals can be divided in two large groups:

- Inanimate surfaces (stethoscope, hospital textiles, beds, sinks...)
- Hospital water (drinking water, waste water)

For each of the mentioned group, the appropriate sampling method is required.

### 2.1. Swabbing and elution

The swab-rinse sampling technique was first described in 1916 by Manheimer and Ybanez [39]. Current recommendations for surface sampling suggest the use of a pre-moistened flocked nylon swab [40], followed by a dry flocked nylon swab for soaking up any remaining liquids. Ekrami et al. [13] used cotton tipped sterile swabs moistened in sterile brain-heart infusion broth. Swabs for environmental surface sampling have been used in numerous studies, and they allow the recovery of microorganisms from hard-to-reach surfaces such as behind taps [41], drains [6] and bed rails [34, 42]. The swab can be transferred into eluting solution (e.g. 0.9% NaCl with 0.2% Tween 80) [43, 44] and vortexed to obtain the substrate for DNA isolation.

## 2.2. Contact plate sampling

For direct surface sampling, RODAC (replicate organism detection and counting) plate is a common choice [45]. Small Petri dishes are filled in order to provide a convex surface, optionally with a nutrient or selective growth medium. The plate is then pressed onto any flat surface and incubated. The resulting colony count can be expressed as cfu/area [46]. The efficiency of this method depends on the evenness of the surface tested [47]; therefore, Rabuza et al. [48] concluded that the RODAC plate method used for sampling microorganism on textiles has certain limitations due to the rough, uneven three-dimensional (3D) fabric surface. Regarding the use of molecular techniques, each selected colony of interest can be used for DNA isolation and subsequent PCR analysis.

## 2.3. Elution

When sampling hospital textiles, as a part of inanimate environment that can contribute to the transmission of healthcare associated infections, the elution method is the most effective. This method is based on the principle of eluting microorganisms from textiles; therefore, microorganisms that have penetrated into the deeper structure of the 3D structure of the textile material are also collected. Microorganisms trapped in the 3D structure of the material will not be detected by the RODAC plate method, neither by swabbing, but they can be captured by elution, either destructive or non-destructive [48].

## 2.4. Water samples filtration and concentration

Since a fundamental limiting factor in the assessment of microbial quality of waters, and especially drinking water, is often the very low number of each organism present, most samples need to be concentrated, usually by sterile filtration. Bacteria are generally recovered on membrane filters with porosities of 0.22–0.45  $\mu\text{m}$ . Subsequently, membrane filters may be incubated on the solid media or soaked in the liquid media [49].

## 2.5. Enrichment in growth medium

Generally, the purpose of environmental monitoring is to establish an aerobic colony count of bacteria from a surface, which can be processed without enrichment. This provides a direct enumeration of the level of microbial contamination of a surface [34]. On the other hand, enrichment steps can be used to increase detection in the case of identifying specific multi-resistant or virulent pathogens, which may be the cause of an outbreak. In this case, the number of organisms is not strictly required, but rather their presence or absence [34]. Typically, the number of pathogenic microorganisms is low [50] and their recovery is low because they are under stressed conditions. Therefore, the chances of detecting pathogenic bacteria will be greater by using an enrichment step. Usually enrichment step is also used prior to detection by PCR. Special attention must be paid to false positive results, since PCR does not discriminate between viable and non-viable organisms [49]. Again, disadvantages of DNA-based methods may be partly overcome by including a pre-enrichment step that allows organisms to multiply before gene probe tests are applied [51].

### 3. Nucleic acid isolation and purification

When collecting hospital environmental samples by methods described above, protocols for DNA isolation from water samples or pure cultures are usually successfully applied [52], but to date none have been accepted as a standard procedure [4]. The extracted DNA is a mixture of DNA referred as a community DNA, which is ideally a representative of all populations within the sample community; however, in reality, the extraction efficiency of different types of microorganisms can vary widely [52].

DNA/RNA isolation and purification must be achieved through methods efficient enough for releasing DNA from the cells; too rigorous conditions could lead to highly fragmented nucleic acids [3]. DNA or RNA, which is not released from the cells, will not contribute to the final analysis of diversity. RNA extraction is a special case because of the possible RNases effect in the procedures [3] and also short half-life of mRNA [52]. Since DNA is mostly found in low concentrations and DNA solution also contains high concentration of saline, this is of concern in later disturbance of PCR. The most popular application for both cases is simple alcohol precipitation, where many varieties and also commercially available kits exist [53].

To remove co-extracted impurities one or more purification steps are often necessary. However, the number of purifications performed should be minimized as much as possible since large portions of the extracted DNA may be lost during the purification process [52].

### 4. PCR target sequence

Depending upon the objective of the study, gene probes to target various genes can be designed. Generally, the gene probes can be divided into the phylogenetic probes, to obtain information about taxonomy and phylogeny of microorganism; functional gene probes, to search for the unique activity of the microbial community; species-specific probes.

#### 4.1. Phylogenetic probes

PCR target sequence is often 16S rDNA gene due to the presence of variable and conserved regions used as a phylogenetic marker [54]. Full length 16S rDNA gene can be amplified either directly or after reverse transcription of rRNA with a set of primers binding to conserved regions of the 16S rRNA/rDNA [3]. On the other side, 16S rDNA gene often fails to discriminate between species and strains level; therefore, the 23S rRNA gene and the ITS regions are also well employed [4].

Clifford et al. [55] described a set of 16S rRNA real-time PCR primers, designed to have the same optimal annealing temperature, and displaying high specificity for four clinically important pathogens (e.g. *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*).



The amplification of 16S rDNA gene can also be useful for initial estimation or pre-screening of the microbial diversity by denaturing gel electrophoresis of amplified 16S rDNA products [3].

#### **4.2. Functional gene probes**

The target gene may code the production of a unique enzyme, where the positive gene probe indicates that the environmental sample contains the genetic potential for that particular activity [52]. In hospital environmental samples, this special activity usually refers to the antibiotic resistance. Szczepanowski et al. [56] designed and synthesized 192 resistance-gene-specific PCR primer pairs to detect plasmid-borne antibiotic-resistance genes in wastewater treatment plant bacteria. Perreten et al. [57] described rapid and efficient screening of Gram-positive bacteria for the presence of up to 90 of the most prevalent and transferable antibiotic resistance genes using microarray technology.

#### **4.3. Species-specific probes**

Primers can also be species specific, as for detecting environmental pathogens. The target sequences that are unique to a particular microbial species allow screening of an environmental sample for the presence of that specific microorganism [52]. There is a vast majority of literature describing species-specific primers for pathogen microorganisms usually find in the hospital environment and on top of that, primers described for identification of microorganisms in clinical samples are working equally well.

#### **4.4. mRNA**

Messenger mRNA is turned over rapidly in living bacterial cells, with very short half-lives inside the cell [58, 59] and has therefore been proposed as marker for cell viability [51]. mRNA is also desirable to target when detecting those microbial cells that are in a viable but non-culturable (VBNC), i.e. a dormant state in the environment, since live cells are considered those capable of cell division, metabolism (respiration) or gene transcription (mRNA production). There are several reports on the existence of many microorganisms, including human pathogens, in the environment in a VBNC state that are shown to be potentially infectious when suitable conditions prevail [36, 60–62].

### **5. Inhibition of PCR amplification**

Materials co-extracted with nucleic acids strongly inhibit DNA modifying. Sometimes dilution of the DNA template could be useful, but very low DNA concentrations may influence the PCR efficiency. Therefore, it is desirable to avoid the effects of PCR inhibitors in the amplification reactions. Although the methods described in chapter 'Nucleic acid isolation and purification' can remove the majority of the environmental contaminants and are useful for various molecular biological studies; there is no standard protocol for removing all possible

inhibitors that can be applied for all types of environmental samples [60]. In addition, various biotic and abiotic components of environment, such as blood, urine, feces, tissue, skin, bleach and detergent, can act as the source of PCR inhibitor [63–65], affect lysis efficiency and may interfere with subsequent DNA purification and enzymatic steps [3].

Finally, it should be noted that community DNA extracts may also contain non-microbial DNA, usually origination from humans (patients, hospital workers and visitors) [52] and co-extracted non-target DNA can also inhibit the PCR [66, 67].

## 6. PCR-based microbial complexity analyses

Properly prepared PCR amplicons can serve as a basis for further analysis of microbial populations.

Culture-independent methods based on amplification and sequencing of 16S rRNA genes allow identification of thousands of different bacteria in a single sample [68–70] when combined with high-throughput DNA sequencing, and hundreds of samples can be multiplexed simultaneously. Therefore, 16S rRNA gene has become a mainstay for characterizing microbial community structure [70, 71]. Hewitt et al. [72] used culture-independent next-generation sequencing to survey bacterial diversity in neonatal intensive care unit surfaces with amplification of the bacterial small subunit [16S] ribosomal RNA gene sequence using ‘universal’ barcoded primers. They found averaging approximately 100 bacterial genera per surface containing many known opportunistic pathogens, as well as abundant groups whose pathogenic potential and ability to resist antibiotic treatment are poorly understood [72].

Oberauner et al. [73] used the 16S rRNA pyrosequencing approach to study the intensive care units (ICU) environmental microbiome. The phylogenetic spectrum combined species associated with the outside environment, taxa closely related to potential human pathogens and beneficials as well as included 7 phyla and 76 genera [73]. A similar methodology was obtained by Poza et al. [74], who amplified a hypervariable region of the bacterial 16S rRNA gene to explore the bacterial diversity at inanimate surfaces of the ICU wards. Detected microbiota contained a total of 3000 operational taxonomic units. The identified representatives were 16 canonical bacterial phyla, members of the phyla Firmicutes (mainly *Staphylococcus* and *Streptococcus*) and Actinobacteria (mainly *Micrococcaceae*, *Corynebacteriaceae* and *Brevibacteriaceae*), the phylum Proteobacteria (mainly by members of the families Enterobacteriaceae, Methylobacteriaceae and Sphingomonadaceae), the phyla Proteobacteria, Bacteroidetes, Deinococcus-Thermus and Cyanobacteria, Proteobacteria (mainly due to the high abundance of Enterobacteriaceae members) [74]. 16 S rDNA PCR and sequencing was also employed in study of Xu et al. [75], where 53 isolates from environmental water-associated sites in a haematology unit, and the outer surfaces of cleaning lotion containers sited throughout a tertiary referral hospital were investigated. Sequence analysis was able to identify 51 isolates, mostly Gram-positive bacteria. Nine different genera were identified from the haematology unit and 13 from the cleaning lotion containers [75].



For the estimation of the total microbial population, purified PCR 16S rRNA amplicons can be separated by denaturing high-performance liquid chromatography (DHPLC), with afterward sequencing of chosen fractions (outstanding peaks on DHPLC chromatograms). Described methodology was used by Rozman for estimation of the total microbial population complexity on hospital textiles, where 63 bacterial genera/species were identified, *Acinetobacter* spp., *Corynebacterium* spp., *Staphylococcus* spp., *Sphingomonas mucosissima* and *Stenotrophomonas maltophilia* being mostly abundant [76].

## 7. Conclusion

DNA molecules can survive in the environment for long periods of time [49] therefore the PCR technique is a powerful tool for the analysis of microbial diversity of environmental ecosystems that can solve many questions in the area of microbial ecology and microbial community structure. Offered advantages of detecting pathogens and antibiotic-resistant bacteria or resistant genes in environmental samples need to be pointed out. PCR has been available for three decades and has become a gold standard to use in microbiology; moreover, several modified PCR assays are the essential tool for use in environmental microbiology.

## Author details

Urška Rozman\* and Sonja Šostar Turk

\*Address all correspondence to: [urska.rozman@um.si](mailto:urska.rozman@um.si)

University of Maribor, Faculty of Health Sciences, Maribor, Slovenia

## References

- [1] Dancer S. How do we assess hospital cleaning? A proposal for microbiological standards for surface hygiene in hospitals. *J Hosp Infect* [Internet]. 2004 Jan [cited 2016 Jun 17];56(1):10–5. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0195670103003955>
- [2] Griffith CJ, Cooper RA, Gilmore J, Davies C, Lewis M. An evaluation of hospital cleaning regimes and standards. *J Hosp Infect* [Internet]. 2000 May [cited 2016 Jun 17];45(1):19–28. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S019567019990717X>

- [3] Wintzingerode F V., Göbel UB, Stackebrandt E. Determination of microbial diversity in environmental samples: Pitfalls of PCR-based rRNA analysis. *FEMS Microbiol Rev.* 1997;21(3):213–29.
- [4] Bridle H. *Waterborne Pathogens: Detection Methods and Applications.* Elsevier; 2014.
- [5] Hedman J, Rådström P. Overcoming Inhibition in Real-Time Diagnostic PCR. In: Mark W, editor. *PCR Detection of Microbial Pathogens.* 2013. p. 17–48.
- [6] Martirosian G. Recovery of *Clostridium difficile* from hospital environments. *J Clin Microbiol* [Internet]. 2006 Mar [cited 2016 Jun 21];44(3):1202–3. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16517932>
- [7] Otter JA, French GL. Survival of nosocomial bacteria and spores on surfaces and inactivation by hydrogen peroxide vapor. *J Clin Microbiol* [Internet]. 2009 Jan [cited 2016 Jun 21];47(1):205–7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18971364>
- [8] Dubberke ER, Reske KA, Noble-Wang J, Thompson A, Killgore G, Mayfield J, et al. Prevalence of *Clostridium difficile* environmental contamination and strain variability in multiple health care facilities. *Am J Infect Control* [Internet]. 2007 Jun [cited 2016 Jun 21];35(5):315–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17577478>
- [9] Mutters R, Nonnenmacher C, Susin C, Albrecht U, Kropatsch R, Schumacher S. Quantitative detection of *Clostridium difficile* in hospital environmental samples by real-time polymerase chain reaction. *J Hosp Infect* [Internet]. 2009 Jan [cited 2016 Jun 21];71(1):43–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19041162>
- [10] Titov L, Lebedkova N, Shabanov A, Tang YJ, Cohen SH, Silva J. Isolation and molecular characterization of *Clostridium difficile* strains from patients and the hospital environment in Belarus. *J Clin Microbiol* [Internet]. American Society for Microbiology (ASM); 2000 Mar [cited 2016 Jul 5];38(3):1200–2. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10699022>
- [11] Touati A, Brasme L, Benallaoua S, Madoux J, Gharout A, de Champs C. *Enterobacter cloacae* and *Klebsiella pneumoniae* isolates producing CTX-M-15 recovered from hospital environmental surfaces from Algeria. *J Hosp Infect* [Internet]. 2008 Feb [cited 2016 Jun 21];68(2):183–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18192081>
- [12] Hobson RP, MacKenzie FM, Gould IM. An outbreak of multiply-resistant *Klebsiella pneumoniae* in the Grampian region of Scotland. *J Hosp Infect* [Internet]. 1996 Aug [cited 2016 Jun 21];33(4):249–62. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/8864938>
- [13] Ekrami A, Kayedani A, Jahangir M, Kalantar E, Jalali M. Isolation of common aerobic bacterial pathogens from the environment of seven hospitals, Ahvaz, Iran. *Jundishapur J Microbiol.* 2011;4(2):75–82.
- [14] Rivera-Jacinto M, Rodriguez-Ulloa C, Flores Clavo R, Serquen Lopez L, Arce Gil Z. TEM and CTX-M extended-spectrum beta-lactamase in *Klebsiella* spp and *Escherichia*

*coli* isolates from inanimate surfaces of hospital environments. Rev Peru Med Exp Salud Publica. 2015;32(4):752–5.

- [15] Dziri R, Klibi N, Alonso CA, Jouini A, Ben Said L, Chairat S, et al. Detection of CTX-M-15-producing *Escherichia coli* isolates of lineages ST131-B2 and ST167-A in environmental samples of a Tunisian Hospital. Microb Drug Resist [Internet]. 2016 Mar 9 [cited 2016 Jul 5]; 2016, 22:399–103. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26958744>
- [16] Tofteland S, Naseer U, Lislevand JH, Sundsfjord A, Samuelsen O. A long-term low-frequency hospital outbreak of KPC-producing *Klebsiella pneumoniae* involving Inter-genus plasmid diffusion and a persisting environmental reservoir. PLoS One [Internet]. 2013 [cited 2016 Jul 5];8(3):e59015. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23536849>
- [17] Rohr U, Kaminski A, Wilhelm M, Jurzik L, Gatermann S, Muhr G. Colonization of patients and contamination of the patients' environment by MRSA under conditions of single-room isolation. Int J Hyg Environ Health [Internet]. 2009 Mar [cited 2016 Jun 21];212(2):209–15. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18667356>
- [18] Shore AC, Rossney AS, Kinnevey PM, Brennan OM, Creamer E, Sherlock O, et al. Enhanced discrimination of highly clonal ST22-methicillin-resistant *Staphylococcus aureus* IV isolates achieved by combining spa, dru, and pulsed-field gel electrophoresis typing data. J Clin Microbiol [Internet]. 2010 May [cited 2016 Jun 21];48(5):1839–52. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20335411>
- [19] Hardy KJ, Oppenheim BA, Gossain S, Gao F, Hawkey PM. A study of the relationship between environmental contamination with methicillin-resistant *Staphylococcus aureus* (MRSA) and patients' acquisition of MRSA. Infect Control Hosp Epidemiol [Internet]. 2006 Feb [cited 2016 Jun 21];27(2):127–32. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16465628>
- [20] Asoh N, Masaki H, Watanabe H, Watanabe K, Mitsusima H, Matsumoto K, et al. Molecular characterization of the transmission between the colonization of methicillin-resistant *Staphylococcus aureus* to human and environmental contamination in geriatric long-term care wards. Intern Med [Internet]. 2005 Jan [cited 2016 Jun 21];44(1):41–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15704661>
- [21] Faires MC, Pearl DL, Ciccotelli WA, Straus K, Zinken G, Berke O, et al. A prospective study to examine the epidemiology of methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile* contamination in the general environment of three community hospitals in southern Ontario, Canada. BMC Infect Dis [Internet]. BioMed Central; 2012 [cited 2016 Jul 5];12:290. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23136936>
- [22] Hu H, Johani K, Gosbell IB, Jacombs ASW, Almatroudi A, Whiteley GS, et al. Intensive care unit environmental surfaces are contaminated by multidrug-resistant bacteria in biofilms: combined results of conventional culture, pyrosequencing, scanning electron

- microscopy, and confocal laser microscopy. *J Hosp Infect* [Internet]. 2015 Sep [cited 2016 Jun 17];91(1):35–44. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0195670115002583>
- [23] Wu A-H, Li C-H. [Antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* in hospital environment and inpatients]. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* [Internet]. 2008 Oct [cited 2016 Jul 5];30(5):525–30. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19024378>
- [24] Weber DJ, Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, *Clostridium difficile*, and *Acinetobacter* species. *Am J Infect Control* [Internet]. 2010 Jun [cited 2016 Jun 21];38(5 Suppl 1):S25–33. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20569853>
- [25] Zenati K, Touati A, Bakour S, Sahli F, Rolain JM. Characterization of NDM-1- and OXA-23-producing *Acinetobacter baumannii* isolates from inanimate surfaces in a hospital environment in Algeria. *J Hosp Infect* [Internet]. 2016 Jan [cited 2016 Jun 17];92(1):19–26. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0195670115003989>
- [26] McConnell MJ, Pérez-Ordóñez A, Pérez-Romero P, Valencia R, Lepe JA, Vázquez-Barba I, et al. Quantitative real-time PCR for detection of *Acinetobacter baumannii* colonization in the hospital environment. *J Clin Microbiol* [Internet]. American Society for Microbiology (ASM); 2012 Apr [cited 2016 Jul 5];50(4):1412–4. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22301021>
- [27] Bedenić B, Beader N, Godič-Torkar K, Vranić-Ladavac M, Luxner J, Veir Z, et al. Nursing home as a reservoir of carbapenem-resistant *acinetobacter baumannii*. *Microb Drug Resist* [Internet]. 2015 Jun [cited 2016 Jul 5];21(3):270–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25599131>
- [28] Gad GF, El-Domany RA, Zaki S, Ashour HM. Characterization of *Pseudomonas aeruginosa* isolated from clinical and environmental samples in Minia, Egypt: prevalence, antibiogram and resistance mechanisms. *J Antimicrob Chemother* [Internet]. 2007 Nov [cited 2016 Jun 21];60(5):1010–7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17906321>
- [29] Anbazhagan D, Mui WS, Mansor M, Yan GOS, Yusof MY, Sekaran SD. Development of conventional and real-time multiplex PCR assays for the detection of nosocomial pathogens. *Braz J Microbiol* [Internet]. SBM; 2011 Jun [cited 2016 Jul 21];42(2):448–58. Available from: [http://www.scielo.br/scielo.php?script=sci\\_arttext&pid=S1517-83822011000200006&lng=en&nrm=iso&tlng=en](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1517-83822011000200006&lng=en&nrm=iso&tlng=en)
- [30] Morio F, Corvec S, Caroff N, Le Gallou F, Drugeon H, Reynaud A. Real-time PCR assay for the detection and quantification of *Legionella pneumophila* in environmental water samples: Utility for daily practice. *Int J Hyg Environ Health*. 2008;211(3):403–11.

- [31] Souli M, Sakka V, Galani I, Antoniadou A, Galani L, Siafakas N, et al. Colonisation with vancomycin- and linezolid-resistant *Enterococcus faecium* in a university hospital: molecular epidemiology and risk factor analysis. *Int J Antimicrob Agents*. 2009;33(2):137–42.
- [32] Chouchani C, Marrakchi R, Ferchichi L, El Salabi A, Walsh TR. VIM and IMP metallo- $\beta$ -lactamases and other extended-spectrum  $\beta$ -lactamases in *Escherichia coli* and *Klebsiella pneumoniae* from environmental samples in a Tunisian hospital. *APMIS* [Internet]. 2011 Oct [cited 2016 Jul 5];119(10):725–32. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21917010>
- [33] Touati A, Benallaoua S, Djoudi F, Madoux J, Brasme L, De Champs C. Characterization of CTX-M-15-producing *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from hospital environments in Algeria. *Microb Drug Resist* [Internet]. 2007 [cited 2016 Jul 5];13(2):85–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17650958>
- [34] Galvin S, Dolan A, Cahill O, Daniels S, Humphreys H. Microbial monitoring of the hospital environment: why and how? *J Hosp Infect* [Internet]. 2012 Nov [cited 2016 Jun 17];82(3):143–51. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0195670112002174>
- [35] Alsharif R, Godfrey W. *Bacterial Detection and Live/Dead Discrimination by Flow Cytometry*. San Jose, CA; 2002.
- [36] Roszak DB, Colwell RR. Survival strategies of bacteria in the natural environment. *Microbiol Rev* [Internet]. American Society for Microbiology (ASM); 1987 Sep [cited 2016 Jul 5];51(3):365–79. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/3312987>
- [37] Barcina I, González JM, Iriberry J, Egea L. Survival strategy of *Escherichia coli* and *Enterococcus faecalis* in illuminated fresh and marine systems. *J Appl Bacteriol* [Internet]. 1990 Feb [cited 2016 Jun 27];68(2):189–98. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/2108110>
- [38] Grimes DJ, Atwell RW, Brayton PR, Palmer LM, Rollins DM, Roszak DB, et al. The fate of enteric pathogenic bacteria in estuarine and marine environments. *Microbiol Sci* [Internet]. 1986 Nov [cited 2016 Jun 27];3(11):324–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/2856614>
- [39] Manheimer WA, Ybanez T. Observations and experiments on dish-washing. *Am J Public Health (N Y)*. 1916;7(7): 614–8.
- [40] Hedin G, Rynbäck J, Lore B. New technique to take samples from environmental surfaces using flocked nylon swabs. *J Hosp Infect*. 2010;75(4):314–7.
- [41] Kac G, Podglajen I, Vaupré S, Colardelle N, Buu-Hof A, Gutmann L. Molecular epidemiology of extended-spectrum beta-lactamase-producing Enterobacteriaceae isolated from environmental and clinical specimens in a cardiac surgery intensive care



- unit. Infect Control Hosp Epidemiol [Internet]. 2004 Oct [cited 2016 Jul 19];25(10):852–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15518028>
- [42] Eckstein BC, Adams DA, Eckstein EC, Rao A, Sethi AK, Yadavalli GK, et al. Reduction of *Clostridium Difficile* and vancomycin-resistant *Enterococcus* contamination of environmental surfaces after an intervention to improve cleaning methods. BMC Infect Dis [Internet]. BioMed Central; 2007 [cited 2016 Jul 19];7:61. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17584935>
- [43] Estill CF, Baron PA, Beard JK, Hein MJ, Larsen LD, Rose L, et al. Recovery efficiency and limit of detection of aerosolized *Bacillus anthracis* Sterne from environmental surface samples. Appl Environ Microbiol [Internet]. 2009 Jul [cited 2016 Jun 27];75(13):4297–306. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19429546>
- [44] Barkovskii AL, Fukui H. A simple method for differential isolation of freely dispersed and particle-associated peat microorganisms. J Microbiol Method [Internet]. 2004 Jan [cited 2016 Jun 27];56(1):93–105. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/14706754>
- [45] Hall LB, Hartnett MJ. Measurement of the bacterial contamination on surfaces in hospitals. Public Health Rep [Internet]. 1964 Nov [cited 2016 Jun 27];79:1021–4. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/14212586>
- [46] Hacek DM, Trick WE, Collins SM, Noskin GA, Peterson LR. Comparison of the Rodac imprint method to selective enrichment broth for recovery of vancomycin-resistant enterococci and drug-resistant Enterobacteriaceae from environmental surfaces. J Clin Microbiol [Internet]. 2000 Dec [cited 2016 Jun 27];38(12):4646–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11101613>
- [47] Maunz U, Kanz E. Contribution to the quantitative evaluation of imprinted cultures. Health care and disinfection. 1969;(61):129–42.
- [48] Rabuza U, Sostar-Turk S, Fijan S. Efficiency of four sampling methods used to detect two common nosocomial pathogens on textiles. Text Res J [Internet]. 2012;82(20):2099–105. Available from: <http://www.scopus.com/inward/record.url?eid=2-s2.0-84870454258&partnerID=tZOtx3y1>
- [49] Köster W, Egli T, Ashbolt N, Botzenhart K, Burlion N, Endo T, et al. Analytical Methods for Microbiological Water Quality Testing. In: Doufur A, Snozzi M, Koster W, Bartram E, Ronchi E, Fewtrell L, editors. Assessing microbial safety of drinking water: Improving approaches and methods. World Health Organization; 2003. p. 237–92.
- [50] Emde KME, Finch GR. Detection and Occurrence of Waterborne Bacterial and Viral Pathogens. Res J Water Pollut Control Fed. 1991;63(4):730–5.
- [51] Sheridan GE, Masters CI, Shallcross JA, MacKey BM. Detection of mRNA by reverse transcription-PCR as an indicator of viability in *Escherichia coli* cells. Appl Environ



- Microbiol [Internet]. 1998 Apr [cited 2016 Jun 28];64(4):1313–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9546166>
- [52] Pepper IL, Gerba CP, Gentry TJ. Environmental microbiology. Amsterdam; 705 p.
- [53] Mülhardt C. Molecular Biology and Genomics. 4th ed. Amsterdam: Elsevier; 2007.
- [54] Nocker A, Burr M, Camper AK. Genotypic microbial community profiling: a critical technical review. Microb Ecol [Internet]. 2007 Aug [cited 2016 Jun 30];54(2):276–89. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17345133>
- [55] Clifford RJ, Milillo M, Prestwood J, Quintero R, Zurawski D V., Kwak YI, et al. Detection of Bacterial 16S rRNA and Identification of Four Clinically Important Bacteria by Real-Time PCR. Willson RC, editor. PLoS One [Internet]. Public Library of Science; 2012 Nov 6 [cited 2016 Jul 4];7(11):e48558. Available from: <http://dx.plos.org/10.1371/journal.pone.0048558>
- [56] Szczepanowski R, Linke B, Krahn I, Gartemann K-H, Gutzkow T, Eichler W, et al. Detection of 140 clinically relevant antibiotic-resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics. Microbiology [Internet]. Microbiology Society; 2009 Jul 1 [cited 2016 Jul 4];155(7):2306–19. Available from: <http://mic.microbiologyresearch.org/content/journal/micro/10.1099/mic.0.028233-0>
- [57] Perreten V, Vorlet-Fawer L, Slickers P, Ehricht R, Kuhnert P, Frey J. Microarray-based detection of 90 antibiotic resistance genes of gram-positive bacteria. J Clin Microbiol [Internet]. American Society for Microbiology (ASM); 2005 May [cited 2016 Jul 4];43(5):2291–302. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15872258>
- [58] Rauhut R, Klug G. mRNA degradation in bacteria. FEMS Microbiol Rev. 1999;23(3):353–70.
- [59] Belasco J. mRNA Degradation in Prokaryotic Cells: An Overview. In: Belasco J, Brawerman G, editors. Control of Messenger RNA Stability. San Diego, CA: Academic Press, Inc.; 1993. pp. 3–12.
- [60] Bej AK, Mahbubani MH. Applications of the polymerase chain reaction in environmental microbiology. PCR Method Appl. 1992;1(1054-9803 SB - IM):151–9.
- [61] Colwell RR, Brayton PR, Grimes DJ, Roszak DB, Huq SA, Palmer LM. Viable but Non-Culturable *Vibrio cholerae* and Related Pathogens in the Environment: Implications for Release of Genetically Engineered Microorganisms. Nat Biotechnol [Internet]. Nature Publishing Group; 1985 Sep [cited 2016 Nov 2];3(9):817–20. Available from: <http://www.nature.com/doifinder/10.1038/nbt0985-817>
- [62] Hussong D, Colwell RR, O'Brien M, Weiss E, Pearson AD, Weiner RM, et al. Viable *Legionella pneumophila* Not Detectable by Culture on Agar Media. Nat Biotechnol

- [Internet]. Nature Publishing Group; 1987 Sep [cited 2016 Nov 2];5(9):947–50. Available from: <http://www.nature.com/doifinder/10.1038/nbt0987-947>
- [63] Bessetti J. Detection of Inhibitors. Madison, Wisconsin: Promega. 2007. pp. 9–10.
- [64] Sigler V, Hensley S. Persistence of mixed staphylococci assemblages following disinfection of hospital room surfaces. *J Hosp Infect* [Internet]. 2013 Mar [cited 2016 Nov 2]; 83(3):253–6. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0195670113000030>
- [65] Harris KA, Thacker CR, Ballard D, Court DS. The effect of cleaning agents on the DNA analysis of blood stains deposited on different substrates. *Int Congr Ser.* 2006;(1288): 589–91.
- [66] Tebbe CC, Vahjen W. Interference of humic acids and DNA extracted directly from soil in detection and transformation of recombinant DNA from bacteria and a yeast. *Appl Environ Microbiol* [Internet]. American Society for Microbiology (ASM); 1993 Aug [cited 2016 Jun 29];59(8):2657–65. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/7690221>
- [67] Rozman U, Mentges M, Mathis B, Šostar Turk S. Implementing the Morapex A device for evaluating hygiene of hospital textiles. In: Pajnkihar M, editor. *International Scientific Conference Research and Education in Nursing*. Maribor: University of Maribor, Faculty of Health Sciences; 2016. pp. 204–10.
- [68] Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* [Internet]. 1995 Mar [cited 2016 Jul 5];59(1):143–69. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/7535888>
- [69] Pace NR. A molecular view of microbial diversity and the biosphere. *Science* [Internet]. 1997 May 2 [cited 2016 Jul 5];276(5313):734–40. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9115194>
- [70] Tringe SG, Hugenholtz P. A renaissance for the pioneering 16S rRNA gene. *Curr Opin Microbiol.* 2008;11(5):442–6.
- [71] Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* [Internet]. 2012 Aug [cited 2016 Jul 5];6(8):1621–4. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22402401>
- [72] Hewitt KM, Mannino FL, Gonzalez A, Chase JH, Caporaso JG, Knight R, et al. Bacterial Diversity in Two Neonatal Intensive Care Units (NICUs). Ravel J, editor. *PLoS One* [Internet]. Public Library of Science; 2013 Jan 23 [cited 2016 Nov 2];8(1):e54703. Available from: <http://dx.plos.org/10.1371/journal.pone.0054703>
- [73] Oberauner L, Zachow C, Lackner S, Högenauer C, Smolle K-H, Berg G. The ignored diversity: complex bacterial communities in intensive care units revealed

by 16S pyrosequencing. *Sci Rep* [Internet]. 2013;3:1413. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3593336&tool=pmcentrez&rendertype=abstract>

- [74] Poza M, Gayoso C, Gómez MJ, Rumbo-Feal S, Tomás M, Aranda J, et al. Exploring Bacterial Diversity in Hospital Environments by GS-FLX Titanium Pyrosequencing. Horn M, editor. *PLoS One* [Internet]. Public Library of Science; 2012 Aug 29 [cited 2016 Nov 2];7(8):e44105. Available from: <http://dx.plos.org/10.1371/journal.pone.0044105>
- [75] Xu J, Smyth C., Buchanan J., Dolan A, Rooney P., Millar B., et al. Employment of 16 S rDNA gene sequencing techniques to identify culturable environmental eubacteria in a tertiary referral hospital. *J Hosp Infect*. 2004;57(1):52–8.
- [76] Rozman U. Molecular methods for monitoring contamination of hospital textiles: doctoral thesis. University of Maribor, Faculty of Natural Sciences and Mathematics; 2014.