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D3.1 Comprehensive study of the state of the art techniques & technologies, including patent search results

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Deliverable Description
This delivery is an analysis of the state of the art at worldwide level which is a key activity in order to understand what technologies are currently available and used and what R&D projects are being developed in the area of healthcare associated infections prevention, and particularly detection of multi-drug resistant bacteria.

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ABBREVIATIONS AND ACRONYMS

AMR	Anti-microbial Resistance
COPD	Chronic Obstructive Pulmonary Disease
ELISA	Enzyme Linked Immuno-Sorbent Assay
FISH	Fluorescent In Situ Hybridization
GC-IMS	Gas Chromatograph - Ion Mobility Spectrometer
GC-MS	Gas Chromatography–Mass Spectroscopy
HS-SPME-GC-MS	Head Space-Solid Phase Micro Extraction-GC-MS
IMR-MS	Ion Molecule Reaction-MS
LFA	Lateral flow assay
MALDI-TOF	Matrix Assisted Desorption Ionization - Time of Flight
MDRO	Multi Drug Resistant Organisms
NDM-1	New Delhi Metal beta-lactamase-1
PCR	Polymerase chain reaction
POC	Point of Care
PTR-MS	Proton Transfer Reaction-MS
qPCR	Real time PCR
RPA	Recombinase Polymerase Amplification
RT-PCR	Reverse transcriptase-PCR
SEA	Strand Exchange Amplification
SIFT-MS	Selected Ion Flow Tube-MS
SME	Small and medium enterprises
TD-GC-MS	Thermal Desorption GC-MS
VOC	Volatile organic compounds

TABLE OF CONTENTS

EXECUTIVE SUMMARY

A wide set of technologies are in clinical use for detection of MDRO. In this report we describe the basic principles for methods in use at central laboratories at hospitals such as culture based identification and resistance determination by biochemical methods or analyses of nucleic acid sequences. Most of the methods in use at the hospital central laboratories are less suited for use at point of care (POC), both regarding equipment and need of trained personnel. Different instruments have been developed/adapted for use at POC and would indeed be possible to apply close to the patient. Such instruments have sample preparation and assay highly integrated and automatized. Some of these instruments are using isotherm amplification and detection of nucleic acids, or detection of surface antigens/beta-lactamase activity. Even though such instruments are relatively simple to operate they do require a person to take a sample from the patient and load the sample into a disposable cassette and place it into the instrument, and thus, do require some hands-on.

This report has shown that diagnostics based on VOC, volatile organic compounds, has potential to become a screening tool for early detection of infectious agents. A fast and disseminated VOC-based diagnostics could thus be used for infectious disease management.

There are several alternative detection technologies for VOC available on the market today that could be applied for detection of VOC profiles from air samples. With an acquired VOC profile, it is possible to compare it to databases of known VOC profiles and thus detect presence of specific bacteria, such as *C. difficile*, *A. baumannii*, or *K. pneumonia*. Today the VOC sample is normally acquired from exhaled breath or head space over a sample of blood, urine or feces. The aim of the procurers to simplify sampling by probing air near the patient or in the whole patient room or patient toilet etc. would present new challenges regarding sensitivity and possible “cross contamination” from other environmental sources besides sample dilution. Detection of antibiotic resistance has been demonstrated in the literature for differentiation of MRSA versus MSSA but it is necessary to demonstrate this also for the other relevant bacteria and antibiotic resistances. Finally, to differentiate toxin A/B producing C. difficile from toxin non-producers, by VOC profiling, might be a challenge on the same level as for antibiotic resistance.

Thus, there is still a gap between the wishes from treating physicians and what the market can provide, especially regarding multianalyte detection, i.e. detecting a set of bacteria and a set of antibiotic resistances in one fast and simple to perform assay. We see that in the future the gap could potentially be filled by systems based on the development of analyses of volatile organic compound from bacteria that will allow non-invasive sampling or measurements in the environment.

1 INTRODUCTION

To complete a rigorous state of the art analysis, we have carried out searches in scientific and patent literature databases in the area of detection of multi-drug resistant bacteria. The patent information is a key source to technology information and can be used to find existing solutions to technical problems, to identify which actors are active, or to avoid colliding with the rights already protected by others (freedom to operate). In fact, they are the first source of published information on new technologies and about 80% of the information contained in patents is not published elsewhere. The search of public databases allows reviewing of research projects, solutions under development and testing of novel technologies. Analyses of the scientific and technical literature have been conducted in order to establish a list of technologies, classified in terms of different solutions answering to the identified needs. In addition, we aim to understand what are the most advanced solutions already adopted or under development by other public procurers or private sector customers on the EU Internal Market or in other parts of the world to address the same challenge as the one addressed by the PCP and what are the shortcomings of the state-of-the-art compared to the procurement needs of the buyers group.

2 REQUIREMENTS

The searches performed here has been to search for technologies that are related to detection of (multi-)drug resistant bacteria, potentially causing nosocomial (hospital-acquired) infections, on people (patients/non-patients), in human samples and/or hospital environment. The technology should be fast compared to procedures used today, be possible to handle in the clinics (point-of-care) by existing personnel (no lab technician required). If samples are required they should be non-invasive. The technology should also give an alert through the common journal system used and provide event-driven clinical decision support.

3 SEARCHES

The searches have been based on the list of requirements provided by the needs assessment performed by the procurers group in the Anti-superbugs project. The field of the searches has been limited to technologies that are related to detection of (multi-) drug resistant bacteria, potentially causing nosocomial (hospital-acquired) infections. In order to accommodate for the need of non-invasive and potentially environmental (non-sampling) detection the search was focused towards detection of volatile organic compounds (VOC).
Scientific literature has been searched for in four different databases: MEDLINE, EMBASE, CAPLUS (Chemical Abstracts), and BIOSIS (see Appendix 1 for search strings). Two different patent databases have been used in search for patent literature, Derwent Innovation and Espacenet. Patent search strategies can be found in Appendix 2 and 3.
This analysis of the literature has been divided based on the following criteria:

1) Commercially available technologies,
2) Emerging technologies based on information in scientific journals, and
3) Analysis of patent literature

The commercially available detection systems have been further divided based on the requirement of access to a full laboratory or if they are/can be used in primary care units. Emerging technologies are divided based on target for detection. The present state regarding detection in the environment and the possibility of integration of the output into journal systems has further been investigated.
The patent literature has been scanned for relevant techniques that will be the basis for next generation products.

4 COMMERCIALLY AVAILABLE TECHNOLOGIES

4.1 For use in hospital central laboratories

Presence of infectious agents and their possible resistance to antibiotics has been analyzed with various technologies. The oldest way, but still the golden standard in hospital central laboratories, is culturing bacteria on selective media followed by identification of bacteria/resistance by different means. In the past, bacteria were mainly identified by detecting different biochemical properties (e.g. enzyme activities and ability to use different carbon sources), today it is common to perform the identification by amplification of signature sequences of nucleic acids using PCR. Detection of resistance to antibiotics is traditionally determined by growth on media containing antibiotics. Thus, growth in presence of a specific antibiotic (i.e. carbapenem) will only occur if the bacteria are resistant to the concentration present in the culture media. In the same way as for identification of the pathogenic bacteria, detection of a gene encoding the enzyme breaking the beta-lactam ring of penicillin can be detected via analyses of the DNA. This can be done via amplification, such as PCR or isotherm amplification using primers for the specific gene, or it can be done via hybridization to the nucleic acid with a fluorescent probe such as in FISH (Fluorescent In Situ Hybridization). Other technologies used in central laboratories involve mass spectrometry to detect certain molecules that indicates the bacteria, and/or breakdown products from antibiotics indicating presence of a penicillinase, or ELISA (Enzyme Linked Immuno-Sorbent Assay) where e.g. a surface antigen from the bacteria is detected. These examples represent some of the more commonly used technologies in the central laboratories of modern hospitals. One can conclude that these technologies are dependent on relatively complex (and expensive) equipment that require handling by trained personnel.

In short, the central laboratories are able to detect all clinically relevant pathogens that can be cultured in a lab environment, i.e. to identify the bacterial pathogen, as well as define its antibiotic resistance profile. This is done with high sensitivity as well as specificity. However, to take these technologies to a POC device is still challenging, although, recent developments have shown it to be possible (https://www.alere.com/en/home/products-services/brands/alere-i.html, https://www.alere.com/en/home/products-services/brands/alere-q.html). Obstacles are to 1) make the instrumentation both compact and simplified, permitting it to be operated by “non-laboratory” personnel, 2) shorten the assay time, and 3) be affordable in relation to the cost in the central laboratory.

Today larger companies dominate the market of instrumentation for central bacteriological laboratories; for identification of microorganisms and detection of their (potential) resistance to antibiotics. In general, many of these instruments or systems are optimized to be used in a large laboratory and can analyse many samples in parallel. With this approach the input of man-power, and cost, can be held at a minimum. Thus, most of the technologies are therefore difficult to directly transfer

into a POC solution where relatively few samples will be handled at a time by non-laboratory personnel.

A few examples of systems designed for the large microbiology laboratory will be mentioned and commented. It should be stated that these are only examples of what is available on the market. Firstly, a system from Becton Dickinson, BD Phoenix™, that characterizes bacterial phenotype by automatic following of the growth of an inoculum of previously isolated bacteria (Figure 1). It determines antibiotic resistance and performs biochemical tests to identify the bacteria.

Figure 1. Automatic automated identification and susceptibility testing system BD Phoenix™.

This system is working with bacterial samples from agar plates. Thus, the bacteria must first be isolated, and colonies picked by hand, and inoculated. This system is saving both work and time in the central laboratory but requires larger scale analyses to be practical and economical. Similar systems are available from other companies such as Vitek 2 from BioMerieux, or Microscan from Siemens. This type of systems requires approximately 5-12 h following initial isolation of bacteria.[1]
Other systems are based on mass spectrometry (MS), where the bacterial sample is analysed by MALDI-TOF (Matrix Associated Laser Desorption Ionization Time-of-Flight). This gives a profile of proteins present in the sample. The recorded profile can be compared to a library of profiles of known bacteria and be used to identify the bacterial strain. The analyses take only a few minutes. However, the bacterial sample must first be isolated (normally on agar plate) and applied in a pure form.

Figure 2. Biomerieaux Vitek MS – an automated mass spectrometer.

Biomerieaux Vitek MS is an automatic mass spectrometry microbial identification system (Figure 2). Considering its size and weight it is not a mobile unit and requires a laboratory for isolation of bacteria before analyses. This, besides the cost, makes it more suitable for use in a central laboratory than for POC.

The given examples above, Vitek MS and BD Phoenix, both detects the phenotype of bacteria; the other major direction in detection is based on analyses of the bacterial genotype. Different forms of PCR, including standard, real time, and multiplex PCRs, are in use in central laboratories. Instruments are available from several producers, that, together with various kits from different manufacturers, allow for detection of a large variety of bacteria in combination with the presence of genes for resistance to antibiotics. As an example of a complex system that take care of the whole procedure with sample preparation and DNA amplification/target detection, real time PCR multiplexing, Siemens Versant kPCR can be mentioned (Figure 3).

Figure 3. Siemens Versant kPCR Molecular System.

Another example of an "easy-to-use" system is Chepheids GeneExpert, this is an automatic q-PCR system that can be used to genetically identify (and characterise) bacteria. Normally it is used on isolated cultured bacteria, but it can also be used on patient samples (e.g. from swabs). However, for this more demanding samples, the sensitivity goes down markedly according to Chepheid. This can be mentioned as a general remark, that the performance of nucleic acid-based tests is better the purer the (bacterial) sample is.

Next generation sequencing, i.e. large-scale and fast DNA sequencing can be used both to identify pathogenic bacteria and to indicate antibiotic resistance. Systems such as MiSeqDx from Illumina, are very competent instruments and could be used for characterization of pathogens. However, it would require certain handling, preamplification/library preparation that would require it to be located in a specialized laboratory.

In conclusion, central laboratories are well equipped for sophisticated analyses where both identity of bacteria, and their complete antibiotic resistance profile, can be characterized. However, these technologies are often dependent on initial isolation of bacterial colonies before identification/resistance determination. This procedure takes time even if the following analyses for bacterial ID and resistance profiling might be relatively fast. In addition, sending a sample for characterization in the central laboratory also add to the total time to get results. However, large-scale analyses with possibilities for high throughput, makes the central laboratory difficult to beat both regarding quality and cost. The technologies and equipment used in the central

laboratories often requires trained laboratory personnel, which make them less suitable for POC applications.

4.2 For use in small laboratories / primary care units

It should be noted that, at primary care units, there are normally not personnel, nor equipment available for more advanced analyses. Here, samples are rather collected, and sent off to a central laboratory for analyses. However, some easy-to-use analyses for infectious diseases are routinely performed, such as fast detection of "strep-Throat" by use of dip-sticks that are read manually, or with instruments such as QuickRead-go from Orion Diagnostics (based on immuno-turbimetric measurements, Figure 4). This instrument can store data, as well as communicate with patient data systems (HIS/LIS). However, the sample taken from the throat by a swab needs to be handled and reagents added sequentially before the processed sample is put into the instrument. The procedure is not difficult but needs "hands-on" work. As of today, this technology only detects streptococci and does not give opportunities for determination of antibiotic resistance.

Figure 4. QuickRead-go from Orion Diagnostics.

Other, more sophisticated analyses could also be performed at primary care units, such as isotherm amplification of DNA/RNA. Isotherm amplification of nucleic acids permits a simpler instrument that still can perform amplification in shorter time. One such instrument is Alere™i (Figure 5). Today this instrument can detect Group A streptococcus in 8 minutes directly from a patient sample on a throat swab. One can speculate that detection of other bacteria, as well as genetic detection of antibiotic resistance will be available in the future based on Isotherm amplification of nucleic acids with this, or a similar instrument.

Figure 5. Alere™i for isotherm amplification of nucleic acids.

Other instruments, based on isotherm amplification, are available on the market. One of these is Illumigene C. difficile (Meridian Bioscience) that detects toxigenic C. difficile in stool specimen using a loop mediated isotherm DNA amplification (LAMP). However, to perform a measurement, the stool sample has to be prepared manually before being put into the instrument that performs the analyses in 40 minutes.

Figure 6. Alere™ q automatic qPCR.

Alere™q, is an example of a fully automated real-time PCR system with multiplexing capability (Figure 6). The system can run on batteries for a limited time and communicate via GSM using add-on equipment. The system today appears to be focused on POC in developing countries (for HIV detection) but can also be regarded as an example of systems that could be applied for disseminated diagnostics of MDRO in clinical settings. For this it is necessary to adapt the reagents (primers) for new targets; bacteria/genes for resistance.

However, these more advanced systems for POC, have not been implemented to a larger scale. One reason might be that even though it is of high value to have an early warning, it would be a radical change to substitute the services of the central laboratory with a POC test. It would most likely be easier to implement it as a complement to the central laboratory services. This raises two concerns; one is the trust in a POC (specificity/sensitivity).[2] The other concern relates to the cost, both for the instrument and possibly disposables, but perhaps more importantly for increased need of man-hours and training of personnel. With this in mind, the practical demands on a novel detection system regarding sensitivity/specificity/cost/need of personnel are challenging, and as far as we can see not being fulfilled with systems on the market.

Next generation sequencing, i.e. large scale and fast sequencing of e.g. a whole genome can potentially also be implemented in POC environment to characterize infectious agents. Nanopore based sequencing has quickly developed into practically useful systems. The hand-held sequencing system MinIONS, from Oxford Nanopore Technologies (Figure 7), is a very small device that can be used to sequence the whole genome of an organism and thereby give bacterial identity and genomic information on resistance in approximately four hours, if the starting material is a bacterial isolate, including time for sample treatment and DNA purification/library construction that

must forego sequencing. The needed time for sequencing is dependent on the purity/homogeneity of the sample and will be much longer if one would sequence a sample with mixed bacteria. This is an affordable novel technology with minimal hardware (approximately 1000 USD) that was difficult to foresee only a few years ago, and open for totally new possibilities of analyses.

Figure 7. MinIONS handheld nanopore DNA/RNA sequencer from Oxford Nanopore Technologies.

Communication of data between POC devices and hospital patient data handling systems are in some cases far developed. AegisPOC (Alere) is one system for data communication that connects Alere POC instrumentation (as well as other brands of instrument using the same communication standard) with the regular patient data systems in use.

In conclusion, there are POC instruments on the market today that can perform some of the analyses we ask for, or could be modified to do so by change of specificity of target organism and/or complement identity with analyses of resistance. The challenge is to do these analyses fast, cheap, and with minimum need of personnel so it would be attractive to be used at POC. Furthermore, PCR-techniques all require a physical sample of some kind.

5 EMERGING TECHNOLOGIES

5.1 Technologies based on scientific literature

This section is limited to discuss the trends in research by looking at what has been made public by university researchers in form of published papers and conference reports. Since the field is very big, only a selection of technologies can be mentioned. One general observation is that university research is often focused on proof of principle rather than practical applicability. This is mentioned, not to reduce the value of their research, but to point at the fact that the implementation of the researcher findings as functional POC instruments can be long and difficult. SME, small and medium sized enterprises, are also mentioned here since they often have their origin in university-based research. Large funding has been channelled to promote development of early warning systems such as i-sense, an Interdisciplinary Research Collaboration funded by Engineering and Physical Science Research in UK that aims for mobile phone connected diagnostic tools.

Detection can be grouped, either after microbial target (genotype/phenotype) or after detection methodology. In the table below, it has been grouped after microbial targets. We can see that the targets are the same, or similar, to what is used in the central laboratory. The difference rather lies in how to achieve a faster read out of the signal with a device that also is possible to use in POC.

Table 1. Detection grouped after microbial targets.

Bacterial component	Target #1 Bacterial Identity	Target #2 Resistance	Sensor principles
Bacterial surface antigens	Whole cell detection	PBP (penicillin binding protein)	Fluorescence SERS SPR
Bacterial internal antigens	Soluble antigens after lysis		LFA Immunoturbidity
Nucleic acids DNA/RNA	DNA/RNA	DNA (e.g. B-lactamase gene) rRNA	PCR or Isotherm amplification followed by detection of target
Enzymes/metabolites/metabolism	Enzymes VOC	B-lactamase activity	Metabolic activity Fluorescence MS Electronic nose
Whole bacteria	Fluorescent spectral analyses		Spectral analysis
Whole bacteria	ID via bacterial phage receptor	Metabolic activity in presence of any antibiotic	Fluorescence (Recombinant bacteriophage)

Even though some targets are the same, there is variation in strategies for detection between the standard methods used in the hospital central laboratories and what is implemented in POC sensors, or assays, suggested for POC sensors.
Examples of research in the field are given below. Here has also examples been given for sensor research that possibly can be adapted to detect MDRO. This is done to demonstrate the large variations in technologies suggested for detection of MDRO.

5.1.1. Nucleic acid analyses for detection of bacterial identity and/or detection of presence of genes for antibiotic resistance

PCR and qPCR are commonly used in hospital central laboratories where they perform very well, and highly automatic systems exist that performs the whole procedure after the sample has been loaded (manually) into a disposable cassette that subsequently is inserted into the large machine. However, these systems have limitations. Besides size, price, and need of service they are normally used on more or less pure bacteria from an agar plate or from liquid culture. The performance is less reliable when used on mixed cultures directly from the patient or environmental samples via e.g. swabs.
Large efforts has been put on different strategies to design methods/instruments that can be used in a POC setting with limited possibilities for sample preparation and "hands-on" input from the healthcare personnel. In general, the trend has been that isotherm amplification has been gaining popularity over PCR since it makes it possible to design less complicated instruments compared with the earlier preferred PCR that requires multiple temperature cycling's. The time needed for PCR amplification is mainly dependent on the time needed for temperature changes. Thus, one can generalize and say that a faster PCR machine is more challenging to construct and will be more expensive than a slower one. In later years, isotherm amplification of nucleic acids has become increasingly popular. This is mainly dependent on the need of only one constant temperature during amplification. Besides that the temperature control for isotherm amplification of nucleic acids is easier to achieve than temperature cycling, it also has the advantage that amplification is fast. This combination of features is well suited for POC applications.
Different variants of Isothermal amplification have been used, such as loop mediated amplification (LAMP), rolling circle amplification, and later RPA, Recombinase Polymerase Amplification that has gained popularity among researchers in the field of nucleic acid-based detection/diagnostics.
Some recent examples of applications of isotherm amplification of nucleic acids used for detection of pathogenic bacteria and/or antibiotic resistance are given below.
Strand exchange amplification (SEA), this method has the advantage over LAMP that it can perform amplification of RNA with a single step without need of reverse transcription. An application of SEA is described by Zhang et al, where they do rapid detection of Listeria using strand exchange amplification.[3]
However, the need of sample handling or pre-treatment is a problem in a POC setting and some research groups are aiming to present more complete systems that minimize the needs of hands-on work. In a paper by Law et al. the combination of RPA amplification of DNA with centrifugal handling of microfluidics appears to have been successful.[4]

In another resent publication Recombinant Polymerase Amplification (RPA) is integrated in a disposable chip that combines detection with sample preparation using a microfluidic system run by an integral diffusion-based vacuum system.[5] It should be noted that the authors here have combined a relatively advanced sample preparation, i.e. separation of blood cells, lysis of bacteria, isotherm DNA amplification, and detection, all inside a disposable chip.
Goothenberg et al. has developed a novel use of CRISPR, and CRISPR associated enzymes for detection of specific DNA/RNA sequences, as well as point mutations, where the read-out can be as simple as a dip-stick.[6] The method, called SHERLOCK, has potential to be used outside a laboratory but requires today an initial DNA purification and for highest sensitivity an additional RPA amplification (Figure 8).

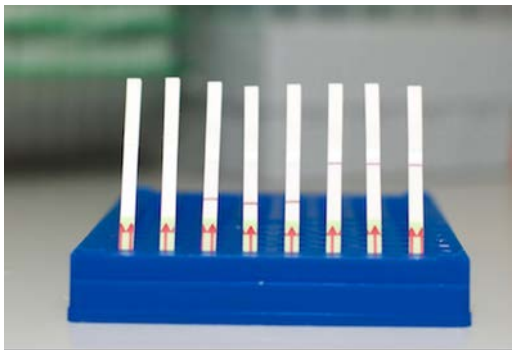

Figure 8. A set of CRISPR based SHERLOCK tests.

Another CRISPR-based detection uses dCAS9/sgRNA complex in an antibody-like fashion for optical detection of MRSA in a DNA FISH (fluorescent in situ hybridization) assay. Guk et al. proposes this assay to be used in a POC setting where it should be able to in half an hour distinguish between MRSA and MSSA, as well as other pathogens/resistance.[7] However, the method still requires bacterial lysis and design of an integrated supportive system.

5.1.2 Phenotype: The other main group of technologies are targeting the phenotype

VOC - Volatile Organic Compounds
The electronic nose has been proposed as a tool to detect certain type of bacteria. Saviauk et al. demonstrated the possibility to differentiate a number of different cultured bacteria (e.g. MRSA, E. coli, and Pseudomonas aeringuosa) using their eNose.[8] This work was done using cultivated bacteria and if the same “sniffing” would be done on mixed bacterial cultures, or directly on the patient or on environmental samples, it would obviously be a more challenging, but hopefully possible task.
A similar approach but using a trained dog instead of the electronic nose to detect specific bacterial contamination on surfaces in a health care setting has been found successful in Canada.[9]

Enzymatic activity
In the central laboratories classical tests with a set of biochemical and enzymatic reactions has been used for a long time. Reis et al. implemented some of these tests in

micro capillary tubes (Lab-on-a-stick) and could distinguish fermentation of different sugars and inhibition of growth by antibiotics.[10] However, here pure cultures were used for this demonstration that also required several hours to establish antibiotic sensitivity.
A more specific detection of presence of ESBL beta lactamase activity in bacteria was presented by Mao et al.[11] Here they demonstrated a fluorescent probe that increases its fluorescence 2500 times when activated specifically by an ESBL enzyme. Using this ratiometric fluorescent test they could distinguish ESBL producing clinical isolates from other in one hour.

PAD polarization anisotropy diagnostics
Ki Soo Park et al. describes the use of polarization anisotropy for optical (fluorescent) detection of specific nucleic acid sequences after RT- PCR amplification.[12] They have developed a modular system that has all the needed functionality with sample preparation (cell lysis and RNA preparation) as well as amplification and detection. The compact system is controlled by a smartphone via Bluetooth.
New technologies are often developed at small companies with (intellectual) base from university research. The large diagnostic companies are constantly looking for small companies with new prosperous technologies, also in the field of MDRO detection and several recent acquisitions have been seen in the area. This can be a way for smaller companies, with a good product, to reach a market that otherwise would be almost impossible to penetrate. One example of such a small company is GeneWEAVE that was acquired by the much larger Roche last year. GeneWEAVE had developed a generic technology for detection of drug resistant living bacteria by selectively transforming specific pathogens with a gene for luciferase that literary makes them shine, and be detected by an instrument. To what level the technology is specific enough to differentiate MDRO is unclear, as well as the transfection efficiency of bacteria present in clinical samples. In addition, the same technique can also be used to determine resistance to antibiotics by observing luminescence in the presence of different antibiotics. The procedure is said to be very simple and the assay not to take more than a few hours using a small instrument. The technology, that is patented, was valued to several hundred million dollars upfront and more after reached milestones.

6 DETECTION IN ENVIRONMENT

6.1 Surface detection- available techniques

In order to reduce dissemination of MDRO in the hospital environment it would be beneficial to detect surfaces contaminated with such bacteria, or at the very least to detect organic material on surfaces such as door handles, water faucets or other surfaces patients, personnel, or visitors naturally come in contact with in the hospital environment. So far, we are not aware of attempts to make an integrated system for automatic surveillance of environmental surfaces to detect multi drug resistant organisms without any manual hands-on work from an operator, as well as sample preparation.

A common practice is to make wipe tests, where a wipe is used to sample the surface and subsequently handled aseptically and transported to a central laboratory for culture. This takes time, both for sampling/handling/transportation and for culture and identification.

An alternative is to analyse presence of bacterial ATP in a test wipe followed by fast enzymatical detection. However, this will only show the presence of bacteria or bacterial remains, and not give bacterial identity and resistance profiling. One can regard this latter example as a test for efficiency of cleaning procedure and cleaning products. Another way to obtain similar “clean check” is to look for remains of organic material by use of autofluorescence after illumination with short wavelength light.

There are methods aiming to detect bacteria/organic substance on surfaces. However, the sensitivity and specificity regarding detection of MDRO might be relatively low using methods based on non-contact optical analyses (no samples collected). Consequently, detection of resistance to antibiotics will also be very limited even if a combination between technologies where fluorescence from antibiotic derivatives is activated by beta-lactamases combined with fluorescence read out from the surface. In addition, most optical methods, with or without addition of reagents to the surface to be analysed, are best performed in total darkness using UV light for excitation and analysing very weak (fluorescence) signals. This makes them unsuitable for use in normal light if not sophisticated measurements are done in the time domain.

Some work has been done to make use of principal analyses on the signal from autofluorescence claiming the ability to distinguish the type of bacteria. However, to do this identification in a complex mixture of bacteria in a matrix of e.g. sputum would be very challenging and has not been reported.

In general, for the auto-fluorescent detection, the sensitivity is limited without a pre-concentration. For detection of possible antibiotic resistance, the same question of sensitivity comes up, especially for a mixed bacterial community.

6.2 Detection of bacterial pathogens via their VOC profiles

For thousands of years, detection of VOC has been done by the human nose, i.e. the smell, to diagnose a patient.[13] Dogs, and even rats, have been trained to detect disease related “bad” smell indicating, e.g. infection by *M. tuberculosis* or *C. difficile*, but also detect other diseases such as diabetes or cancer.[9] One system, based on a trained

springer spaniel dog has been successful in using its nose to detect c. difficile in hospital environment.[14] This is mentioned to point at the possibilities to use VOC for identification of bacterial contamination.

Detection of infection is based on profiling the VOC produced by infectious agents, such as *Klebsiella pneumonia* or *Clostridium difficile*. In Table 2 examples of VOCs associated with different pathogens are presented. The VOCs typical for one type of bacteria should be regarded as a set of different VOC´s making up a fingerprint that could be represented by an analysis profile of spectra. However, often there might very well be a partial overlap between such a set of VOC´s from one type of bacteria with that of another type of bacteria. This is obviously a complication, but is addressed by data signal processing. In order to comprehensively assess the volatile metabolic fingerprints of important bacterial and fungal pathogen groups, one must consider both a wide range of groups as well as a diverse collection of isolates in each group.[15]

Table 2 VOC originating from different bacteria (adapted from Hong-Geller and Adikari 2018).

Pathogen	Inf. disease	VOC marker	ref
A Baumannii	VAP	1-undecene, nonanal, decanal, 2,6,10 trimethyl-dodecane, 5-methyl-5-propyl-nonane, longifolene, tetradecane, 2-butyl-1-octanol	16
C. Difficile	Ulcerative colitis diarrhea	Ethanol, Butanol, Isopropanol	17
K. Pneumoniae	Bronchitis, pneumonia	Butaraldehyde, octyl acetate, tridecanol, dodecanal, butanoic acid	18

The VOC profile can relatively easily be determined, using laboratory instruments, from a pure culture of the microorganism. The profiles of different bacteria are possible to differentiate from each other. There are several useful databases of VOC in relation to bacterial metabolic activity. Firstly, mVOC, the Microbial organic compound database (www.bioinformatics.charite.de/mvoc). Secondly KNApSAcK, a database over species metabolite relationship (www.kanaya.naist.jp/KNApSAcK)

The VOC profile in a sample from a patient (blood, saliva, urine, faeces, breath) will obviously also be influenced by the commensal flora and the patient himself. However, the use of data processing and principal component analyses can sift out the relevant signals.

One technique that has been used to analyse VOC from bacteria is Surface plasmon enhanced Raman spectroscopy. This technology is interesting but requires addition of nano (gold) particles to enhance the otherwise weak Raman signal. This can be achieved by immobilization of such nanoparticles on a sensor surface which is used to analyse the VOC sample.[19]

The question of antibiotic resistance profiling via VOC is more complex, and might also present possible use of repeated VOC sampling to analyze the effect of antibiotics on the pathogen. However, it has been shown that differentiation can be achieved between MRSA and MSSA, i.e. methicillin resistant versus methicillin sensitive *S. aureus*. Another example has been found in wound management research, attaining rapid detection of the most relevant bacteria causing wound infections and differentiating MRSA from MSSA utilizing gaseous headspace sampling with an eNose.[8]
Detection per se, can be performed with different detection technologies that also have various sensitivity/specificity, in Table 3 different technological solutions are listed with advantages and limitations. It is for example common to use a combination of gas chromatography and mass spectrometry (GC/MS). A parallel for the use of this combination of techniques is found in airports for detection of explosive materials.
A technology, that is appealing by its relative simplicity, is the electronic nose (e-nose). This can consist of a panel of electrodes, each reacting differently on different VOC. The set of electrodes can thus be used to create a map of VOC's in the sample. Another detection technology is based on Surface Enhanced Raman Spectroscopy (SERS). Here, the relatively insensitive Raman spectroscopy is strongly enhanced by gold nanostructures that forms localized plasmon hot-spots with very strong electromagnetic fields resulting in enhancement of the Raman response with five orders of magnitude. The SERS method has been reported to detect one colony forming unit, in the gas phase over an agar plate after 20 hours of culture.[19]
Yet another, very sensitive detection method, is based on mid infra-red incoherent cavity ring-down spectrometry (mid-IR I CRDS) where the design of the optical path inside the instrument is 1000 meters giving very low detection levels of VOC's (Los Gatos Research/ ABB). This instrument can detect VOC at low ppb or even sub-ppb levels.
The use of a pre-concentration device that binds VOC from the sample, e.g. exhaled air, can be introduced to the instrument to provide a higher concentration to the instrument. However, a pre-concentration device will most likely have different affinity for different VOC's and must therefore be selected with regard to the analyte to detect, and the output need to calibrated to the potential shift in the bacterial fingerprints. One common variant is the Solid Phase Micro-Extraction (SPME) where VOCs are adsorbed onto a coated micro-fiber. Other variants are based on Metallo Organic Frameworks (MOFs) that potentially can be more selective in their binding properties.[20]
The sensitivity to detect VOC per se can be determined and is normally given in ppm or ppb. However, the important subject of sensitivity and specificity of different methods to detect bacteria via their VOCs is very elusive since it also is dependent on the conditions when the sample is collected, what sample is used - exhaled air, head space over saliva, urine, feces etc. as well as if the bacteria are isolated and cultured. These differences in how experiments have been performed contribute to the complexity and force us to present mainly sensitivity as ppm/ppb.

Table 3. Technologies for detection of VOC.

Technique	Description	Advantages	Limitations	Ref
GC–MS	GC–MS combines separation, GC and MS. Separation is typically performed by a capillary column, with compounds being separated by their boiling point and polarity. As compounds are eluted, they are detected by the mass spectrometer as a function of their mass to charge ratio. Different MS detectors are available, with Time Of Flight (TOF) and tandem quadrupoles (MS–MS) being the most common.	• Good sensitivity (ppm-ppt) HS-SPME-GC-MS gave 83 % sensitivity and 100 % specificity for C. difficilie in faeces, but after 18 hrs. • Separates, identifies and quantifies VOCs all in one • High chromatographic resolution achievable • Highly reproducible results • Can analyze VOCs from complex mixtures • Can tentatively identify unknown compounds based on comparison to known mass spectra	• Often requires sample pre-concentration • Lengthy processing and analysis times • Reference library required • Requires a supply of pure inert carrier gas	21
GC-IMS/ IMS	GC-IMS combines separation, GC and IMS. Separation may be performed using standard GC capillary columns or multi-capillary columns. Dual separation occurs first through the column and then in the detector according to the compound's gasphase ion mobility. Ionized molecules are accelerated by an electric field towards a Faraday plate, where the impact of single ions is detected.	• High sensitivity (ppb-ppt) • Rapid results • Best for identifying differences between non-identical samples • Simple to use on site • Can use ambient air as the carrier gas	• Detection is compound specific and depends on the ion's mass and charge • Limited dynamic range for quantitation • Reference library required • Confusing mass spectra can arise when high levels of solvents are present	22 Owlstone Medical: Lonestar FAIMS voc analyser
Direct detection	These methods include SIFT-MS, IMR-MS, PTR-MS. They are popular for their sensitivity, rapid analysis times, and ability to extract target compounds from samples with little or no pre-separation.	• High sensitivity (sub-ppb) • Rapid results • Absolute quantification • Can detect trace compounds in mixtures	• Expensive • Reference library required • PTR-MS only suitable for compounds with higher proton affinity than water	
E-nose	A variety of E-nose detectors exist today. They generally consist of a micro-array of sensors which differ from each other in polarity. The sample passes through the array, and compounds adsorb to varying degrees on the different sensors depending on their	• 80 % sensitivity for eNose • Best for identifying the differences between nonidentical samples • Rapid results • Does not require sample separation or pre-concentration. • Relatively small, portable, and simple to use on site	• Cannot quantify VOCs • Can only identify known patterns of VOCs stored in its database • Reference library required • Sensitive to high ambient temperature	23 The eNose company Sensigent : Cyranose – an electronic nose with

	composition. Compound adsorption on sensors changes the mass or resistance of each sensor, and this change is detected to provide different outputs.		and humidity	32 organic polymer nano composite sensorarrays Owlstone Medical: Lonestar FAIMS voc analyser,
SERS	Surface Enhanced Raman Spectroscopy. Here VOC are stuck on gold nanostructures and can be detected by Raman spectroscopy because of surface plasmon enhancing of sensitivity	• High sensitivity (PP?) one CFU on agar plate can be detected in after 20 hrs. • Small portable equipment • Easy to use	• Can only identify known patterns of VOCs • Unsuitable for screening unknown compounds	19
mid-IR I CRDS	mid infra-red incoherent cavity ring-down spectrometry (mid-IR I CRDS) long optical path inside the instrument is 1000 meters giving very low detection levels of VOCs	• High sensitivity -sub-ppb • Rapid results 5-15 min • Does not require sample separation or pre-concentration • Simple to use on site • Can use ambient air as the carrier gas	• Heavy -50 kg • Cost?	Los Gatos Research/ ABB

7 INTEGRATION OF INSTRUMENT WITH THE DIGITAL PATIENT RECORD SYSTEM

7.1 Surveillance and local support systems

Many countries have regional or national surveillance systems in place to keep track of HAI and AMR occurrence and development. Núñez-Núñez *et al.* have reviewed the methodology used in publicly available data.[24] 56 systems from 20 different countries were included in the study and of these 33 (58.9%) target HAIs and 45 (80.3%) target AMR; 22 target both. The authors found publicly available information on important methodologic aspects and indicators measured frequently lacking. Furthermore, there is a large heterogeneity across countries or regions regarding methodologic, and in the case of AMR, few of the systems include indicators based on incidence and clinical information.

In the EU 7th Framework Large scale project DebugIT advanced data mining of data collected and stored in electronic Clinical Information Systems (CIS) was utilized.[25] Results of data-mining are stored in a Knowledge Repository, which also includes existing guidelines and expert opinions. For the decision support workflow relevant medical knowledge is used which has already been collected and stored in a Knowledge Repository. Monitoring of ongoing care activities and outcomes, may help to predict future outcomes to give additional support to treatment decision on individual patients and for populations. One of the partners of DebugIT, Agfa healthcare (https://global.agfahealthcare.com/main/) feature commercial systems for clinical support and data handling (ORBIS) and enterprise management (ECM).

There are numerous smart phone apps that are directed towards the healthcare sector. In 2014 Schnall and Iribarren screened almost 3000 apps and found only 17 that were related to infection prevention and available in English language.[26] The apps were found to be limited in functionality and coverage of HAI prevention. The authors suggested increase of the functionality to include feedback or tailored reminders for HAI prevention measures. As development is fast in the field of Smart phone apps it is expected that the number of available apps and their functionality has developed considerably since this review was performed. However, as data and regulations vary between regions and countries they are likely developed for local markets. One example is Bug Wise by Pharma Dynamics which is a customizable point of care tool for local antimicrobial stewardship, both in hospitals and in primary care medicine.[27] It contains information for patients as well as healthcare providers relating to infections (diagnosis), microbes and antimicrobials (bugs and drugs), including antibiotics and antifungals. Although, this app contains medication and surveillance data from South Africa, it demonstrates that the technique is there. Another example is MRSA Infection Control and Epidemiology: Daily Cleaning of Resident Room mobile app by GoCanvas with the purpose to evaluate the level of MRSA risk within an LTC facility and to develop facility- and unit-specific strategies to reduce MRSA transmission risk to residents, staff, and visitors.[28]

7.2 Integration of instrument with the digital patient record system

The commercially available POC instruments mentioned above that has passed the FDA criteria are in most cases possible to connect to a data communication system as other laboratory instruments, e.g. the Alere™i instrument for isotherm amplification of DNA can be connected to a regular digital hospital patient record system using the AegisPOC functionalities.

For instruments that have still not come out on the market, or are not yet productified, it is less clear how far they can be integrated with the existing hospital patient data handling system. In general, one can conclude that practically all instruments will either have an integrated on-board computer or will be connected with a cellular phone to use hardware for analyses (e.g. camera or processing power) and/or for the communication of data to a central service for data processing/patient data storage.

8 PATENT LITERATURE ANALYSIS

8.1 General outcome of searches

Patent searches have been performed in several different patent databases and the search strategies can be found in Appendix 2 and 3.

Several of the large companies active in the field and today producing of equipment for hospital laboratories or point of care systems (see section 4) are found among the top assignees in the searches performed (Figure 9, Figure 10). Some universities and smaller companies are also present among those with high recent activity. It should be noted that searches in Derwent Innovation do not include any Asian patent collections, and thus, the results are focused on USA and Europe. When looking at results from searches in Espacnet, there is a high degree of Asian (primarily Chinese) patents included. These have to a large extent not been analysed thoroughly, due to that usually only title and abstract are machine translated to English, which is commonly not enough to grasp the content of the invention. Thus, it cannot be ruled out that relevant information have been missed by this, as the number is indicating a high activity in the field.

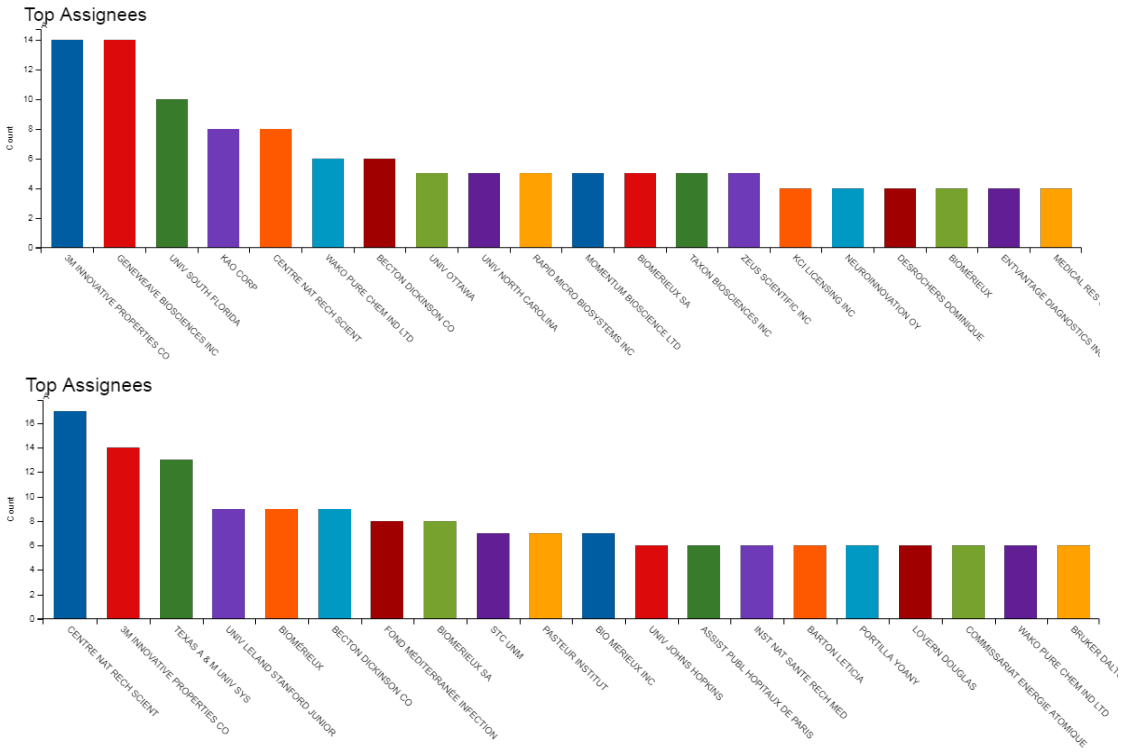

Figure 9. Top 20 Assignees in Derwent Innovation search G (Detection of bacteria in hospital environment), and b) top 20 Assignees in Derwent Innovation search I (Detection of volatile organic compounds from bacteria).

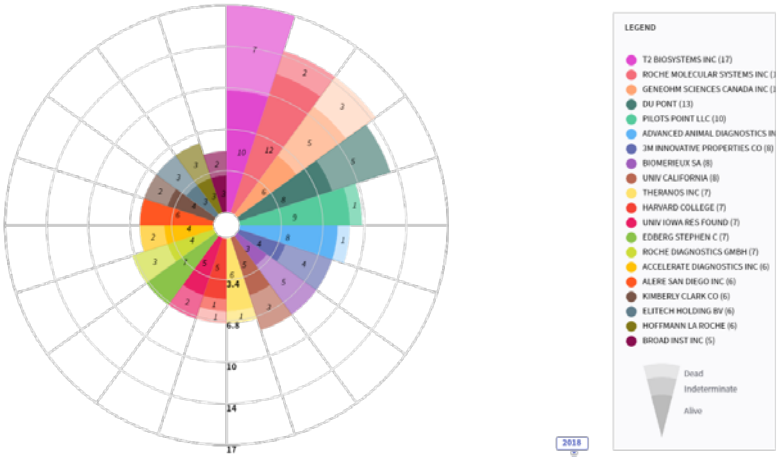

Figure 10. Number of patents assigned to the top 20 assignees in Derwent Innovation search B+D (Testing of most important bacteria + Quick OR Point of care testing).

In general, there are many patents/patent applications that directly or indirectly deal with detection of HAI and MDRO. However, most are focused on the principles for detection in a more controlled sample and have not been developed into practically useful systems. Today it is, as far as we can see, no fully integrated surveillance systems that non-invasively are able to detect multi-drug resistant bacteria. However, promising developments in the area of VOC analysis may in the future potentially be implemented as a fully automatic and ICT integrated POC system.

8.2 Patent examples

Many patents and patent applications targeting MDRO can be grouped under following categories, based on bacterial targets:

- **Genotype, detection of nucleic acids– DNA or RNA** - This is by far the most active area and can be split up into subareas
  - PCR
  - Isothermal amplification (e.g. LAMP RPA)
  - Hybridization
  - In situ hybridization
- **Phenotype, detection of antigens**
  - Surface antigens (e.g. adhesins, LPS…)
  - Intracellular antigens (used in dipstick detection of strep troat)
  - (Volatile organic compounds (target for “electronic nose” or MS
- **Phenotype, detection of bacterial enzymatic activity**
  - Beta lactamase enzyme – breaks down penicillin
  - Other enzymes not related to resistance to antibiotics
  - (Volatile organic compounds (target for “electronic nose” or MS

Detection, and read-out of detection of mentioned targets can be done in a variation of ways, electrochemical, optical, auto-fluorescence, fluorescent or luminescent marker, or magnetic marker to mention the most obvious alternatives.
For this study, it is important to focus on fast, practical, and preferentially "labor free" tests. The lack of these characteristics is, so far, the weakest point in many patents/solutions. One more general observation is that, in later years, Chinese patents/patent applications have increased strongly.

To give an overview of the wide field of patents, a selection of patents/patent applications is mentioned under the headline of respective targets.

In summary, there are lots of patents/patent applications that directly or indirectly deal with detection of HAI and MDRO. However, most are focused on the principles for detection in a more controlled sample and have not been developed into practically useful systems. In addition to the sections on bacterial targets below, **a section on patent literature on non-contact detection has been added**.

8.2.1 Detection of nucleic acids – DNA or RNA

A large number of patent, and patent applications, deals with design of primers/probes for detection of pathogenic bacteria and possible presence of genes for antibiotic resistance. Nucleic acid analyses and technologies to perform analyses, has long been, and still is, a very active area and can be split up into subareas based on used technologies.

PCR
PCR requires a more sophisticated device, then the isotherm forms of amplification, to fast cycle temperatures up and down to generate DNA amplification. The limits of PCR for POC use are mainly time for temperature cycling on a small and affordable device, and automatic sample handling and detection of amplified product. A patent application from Uni Texas (US2018080067 A1) claims to have designed a microfluidic chip that should permit fast detection (of Pertussis) without need of specialized instrument and be suitable for POC.

Isotherm amplification
LAMP, Loop-mediated isothermal amplification is proposed by Chinese PLA Inst of disease control and prevention (WO2017116220A1). Here they disclose a LAMP kit and primers for detection of NDM-1 (New Delhi metallo-beta-lactamase) in pure bacteria or patient samples. In another patent application from China, University of Beijing, CN 106498033 from 2017, is described a method for on-sight quick detection of air and respiratory pathogenic microorganisms. DNA is extracted from patient samples (exhaled air or swabs) and bacteria detected by RPA.
Regarding Recombinase Polymerase Amplification (RPA), the activity in form of patent applications has increased strongly in the last years. Here, especially Alere Inc. has been most active, only in the last months of 2017 three applications from Alere became public US2017342473, US2017321262, and WO2017152122. These

applications have wide claims that could potentially interfere with new methods based on RPA. The same is true for Life Technologies patent application that became public in December 2017 (WO2017214561 A1). However, many patent applications are describing specific methods and applications for RPA such as very fast mobile detection of Shigella in food (CN107287315 A). This type of analyses could obviously be modified for detection of HAI, and resistance.

Hybridization

DNA chips and microarrays are used to simultaneously screen (amplified) DNA to arrays of immobilized nucleic acid probes to which they can hybridize and be detected, often by fluorescent signal (US2009239758A1).

Other means of detection has also been suggested, such as electrochemical detection upon target hybridized to probes sitting on the electrode A patent application (WO20160621101A1) from one of the Chinese army military medical universities, describes modified electrodes and set up for hybridization of a target sequence to a probe (NDM-1 specific) for detection of the gene for New Delli Metallo Beta-lactamse 1.

Another earlier variant of an electrochemical sensor was proposed to be used for both bacterial identification and detection of resistance gens. It combines hybridization of bacterial 16S rRNA to immobilized probes on the electrode, followed by a second hybridization of detector probe (conjugated to an enzyme) to other target on same 16S rRNA. The sandwich built up in this way would be electrochemically detection in analogy to the common glucose sensor, this patent was filed by University of California and Department of Veterans Administration (US2011/0027782A1).

In situ hybridization

A variation of hybridization is done inside bacteria, where a labeled DNA probe is allowed to hybridize to target sequences to give identity and/ or detect presence of resistance genes. The patent, filed by SeaPro Theranostics (US7005257 B1), describes the procedure to use in situ hybridization to 23S rRNA for detection of antibiotic resistance in Helicobacter pylori bacteria and suggests this to be used generally.

Alternative methods

CRISPR-CAS related techniques have been occurring frequently in the literature, and lately also for diagnostic procedures (mentioned earlier in this report). However, since the technology is very new and there is a delay between filing for patent and the applications becomes public, we expect to find patents in this field soon.

Other patents are dealing with read-out of a PCR reaction to facilitate design of equipment for use in POC. An application from 2016**,** from FluxErgy (US 2016/0369324 A1) describes the equipment for PCR using a camera for read out, aiming for use in a POC for identification of infectious agents.

Melting point analysis, i.e the determination of the melting point of a DNA – DNA hybrid is proposed to detect point mutations resulting in resistance to rifampicin in M. tuberculosis (Rutgers State University of New Jersey, US2017321258 A1).

8.2.2 Detection of antigens

Surface located antigens (e.g. adhesins, PBP, LPS, bacteriophage receptors)
Surface located molecules can be used to stain bacteria with e.g. antibodies. The Bacteriophages are very selective regarding what bacteria they infect. With this in mind, designed recombinant bacteriophages encoding a reporter gene has been used for binding to target cells for detection/labeling of specific bacteria by expression of luminescence or fluorescence. (US2009/0155768 A1)
GeneWEAVE has a partly similar approach, they aim to specifically transform the living bacteria to be detected (e.g. an E. coli) in a (complex) sample with genes that will make the bacteria glow in the dark, and subsequently be detected by their instrument. The same analyses performed in presence of antibiotics will also give information on drug resistance. A few examples of GeneWEAVE patents are (US2017327910 A1, US2017152576 A1). However, GeneWeave uses artificial vesicles as carriers for reporter genes instead of bacteriophages.
Different strategies have been used to enhance the signals from receptor-ligand interactions used to identify bacteria on their surface molecules. Forster Resonance Energy Transfer) or BRET (Bio-luminescence Resonance Energy Transfer) has been suggested to detect close proximity between pairs (ligands) (MX 2010005974 A). In a patent (US 9816990 B2), the combined use of microwaves is used to speed up receptor-ligand interactions, and enhanced fluorescent detection levels obtained by MEF, Metal-enhanced fluorescence.
Surface enhanced raman spectroscopy SERS in combination with gold particles was proposed to be used to detect and kill MDRB in food Jackson state university (US2012302940 A1).

8.2.3 Detection of bacterial enzymatic activity

Beta-lactamases – enzymes destroying B-lactam antibiotics
Beta-lactamases breaks down beta-lactam antibiotics. Detection of beta-lactamase activity can be done in different ways. The break down products can be detected by a change in its molecular weight using mass spectrometry. Another alternative is to use a derivative of the antibiotics that becomes fluorescent upon activity of the enzyme to mention two alternatives.
Siemens healthcare diagnostics have a patent application (WO2015013177A2) that aims to rapidly detect carbapenemase / beta-lactamase producing bacteria via exposure to antibiotics + stain and observe color change as a consequence of bacterial enzyme activity.
Another patent application, from Korea institute for science & technology (WO2017010759A1), proposes the use of analogs to antibiotics that turn fluorescent in the presence of specific beta-lactamase. Detection of degradation of antibiotics by MS (Mass spectrometry), as a consequence of bacterial (beta-lactamase) activity is proposed in (US 2012/0196309A1).
Other are targeting the resistance phenotype by directly detect carbapenemase activity via pH change (WO2017089823, WO2015013177A2), or simply by observing growth in presence of carbapenemase (US2013/0330756 A1)

In an application from Georgia state university, (US2017/0102349A1) Electrochemical detection of enzymes indicative for an infectious agent has been proposes for POC detection of both identity and resistance.

Other enzymes or metabolites typical for the bacteria to be identified/characterized
Point of Care Diagnostics LLC (US) 2017 (US2017/0175161 A1), measures released ATP (as bioluminescence) as a marker for metabolic activity in bacteria +/- antibiotics to determine resistance based on less ATP being produced by sensitive bacteria.
Kimberly- Clark proposes the use of functionalized ISFET-transistors to sense bacteria or metabolites (US 2012/0143027 A1)

Whole bacteria detection
Also devices and methods for use of optical fibers (implanted) are suggested to be used for (continuous) detection of specific auto-fluorescence from pathogenic bacteria and thus be give continuous (but invasive) surveillance Kiberly-Clark WO2009/074892 A2).
Detection of bacteria by handheld surface enhanced Raman scattering spectrophotometer Labguide Co Ltd (TW201725376A). System for detection of bacteria using surface enhanced raman spectroscopy (SERS) (CA2668259A1, US2009/0082220 A1).

8.2.3 Non-contact Detection of bacteria

Contactless and sample-free detection and identification of bacteria on surfaces is a very appealing concept. In performed patent searches, two major group of technologies stand out as candidates to solve this quest.

1) Optical analyses, based on determining the auto-fluorescence of bacteria upon excitation with shorter wavelengths, in combination with detection of emission at longer wavelengths. The surface to be analyzed is illuminated and the auto-fluorescence (-profile) recorded in one or several images representing different emission wavelengths. Identification is suggested to be done by comparison with known fluorescence footprints. In a cruder way, detection of faeces, by its auto-fluorescence, is suggested to be used for detection of contamination of agricultural products (patent KR 101793913 (B1)). This is mentioned as an alternative to detect dirt rather than specific pathogens. In a more defined way, and in a medical setting, fluorescent based imaging is used to monitor stem cells and cancer cells after implantation, as well as for detection of contamination of non-biological surfaces (patent application JP 2017189626 (A)). For bacterial detection, and identification, (mainly for food-related surfaces) auto-fluorescence imaging is suggested to be used in combination with optical footprints from known bacteria for fast detection/identification (Patent application NL1041809 (A)). The same methodology can obviously also be used for detection of pathogens in a hospital environment and on patients.

More patents exist in this field, as well as scientific publications. Open questions regarding detection via auto-fluorescence are mainly regarding sensitivity; i.e. how many bacteria are needed for detection, and if it is possible to detect specific bacteria in a mixed population.

2) Detection of volatile organic compounds (VOC), or simply put – detection by the smell, can be achieved using an electronic nose. In an analogy to the auto-fluorescent footprints of bacteria mentioned above, the electronic nose can visualize a footprint of the volatile organic compounds that bacteria produce. By matching the VOC footprint between an unknown sample and a library of footprints it can be possible to identify the bacteria. Procedures for detection of gynecological infections by electronic nose has be described in a patent application (WO2017178032 (A1)), as well as description of apparatus and method for fast sampling and measurement was described in patent application (US2013061692 (A1)). The use of certain VOC, or combinations of a set of VOC´s in relation to Clostridium difficile associated diarrhea, is described in a patent application from The Brigham and Women´s Hospital Inc. Boston (US 2017/0227429 A1). Other patents/patent applications are dealing with detection of VOC per se, such as “Method of detecting volatile organic compounds (US 9,429,537 B2) from 3M, or use of Surface enhanced Raman scattering for detection of VOC´s (WO 2015/026297 A1).

9 IDENTIFICATION OF RELEVANT TECHNIQUES AND ACTORS

A number of companies and research groups, active in the field and with technology which potentially could be developed to fulfil the requirements of the Antisuperbugs buyers have been identified in the searches of scientific and patent literature (Table 4). These would be a starting point for further dissemination and open market consultation activities.

Table 4. Possible actors to consult, companies with products/development, and researchers in the field of sensor development or medical diagnostic applications

Technology	Companies	Researchers
GC-MS	Smiths Detection Inficon (HAPSITE)	Sophia Koo, Brigham and Women's Hospital, Boston, MA 02115
GC-IMS	Environics Inc. Owlstone Medical G.A.S. Gesellschaft für analytische Sensorsysteme mbH	Niku Oksala, University of Tampere, Finland John R Dean, Northumbria University, UK
Electronic Nose	The E-Nose company Sensigent – Cyranose Owlstone Medical	Huib Kerstjens, Univ of Groningen, Holland Oriol Sibila, Univ. Aut. Barcelona, Spain Hossam Haick, Israel Institute of Technology, Haifa, Israel
SERS	HP inc.	Viktor Shkolnikov, HP Inc
Mid IR-ICRDS	Los Gatos Research/ ABB	

10 SUMMARY

Health care workers wish to have access to a surveillance system for detection of MDRO that work continuously all around the clock with no, or minimal hands on time, and at no higher cost than today. In addition, the system should also communicate data to the central digital patient data handling system, as well to local (hospital) authorities to inform of presence of MDRO on surfaces that can play a role in spread of infections.

In general terms, the diagnostic services performed in the hospital central laboratories are today very streamlined and cost efficient. They are based both on classical culture of bacteria on selective media and a variety of analytical methods, e.g. biochemical/enzymatic/MS/chromatographic methods. In the last decades, analyses of signature DNA (or RNA) sequences has become increasingly common, both for detection of organisms and for detection of genes encoding antibiotic resistance. The predominant technology is PCR and real time PCR for amplification and detection of minute amount of nucleic acids as markers for presence of pathogens and genes giving antibiotic resistance. However, in later years the use of other methods for amplification of DNA has become more popular, such as isotherm amplification. This means that temperature cycling can be avoided, making the instrumentation simpler, smaller, and more affordable. This kind of instruments is now being marketed by the large companies for point of care applications.

Other type of POC instruments can be described as miniaturized ELISA, where the presence of certain antigens, or enzymes (e.g. beta-lactamases), are detected.

By mentioning the two later examples of instruments, where integration of sample handling and assay leads to faster detection and reduced need of trained personnel a general trend is illustrated; to take the analyses from the central laboratory to give new tools for an agile treatment of patients.

VOC, volatile Organic Compounds-based diagnostics has potential to become a screening tool for early detection of infectious agents. A fast and disseminated VOC-based diagnostics could thus be used for infectious disease management.

There are several alternative detection technologies for VOC available on the market today that could be applied for detection of VOC profiles from air samples. With an acquired VOC profile, it is possible to compare it to databases of known VOC profiles and thus detect presence of specific bacteria, such as *C. difficile*, *A. baumannii*, or *K. pneumonia*. Today the VOC sample is normally acquired from exhaled breath or head space over a sample of blood, urine or feces. The aim of the procurers to simplify sampling by probing air near the patient or in the whole patient room or patient toilet etc. would present new challenges regarding sensitivity and possible “cross contamination” from other environmental sources besides sample dilution. Detection of antibiotic resistance has been demonstrated in the literature for differentiation of MRSA versus MSSA but it is necessary to demonstrate this also for the other relevant bacteria and antibiotic resistances. Finally, to differentiate toxin A/B producing C. difficile from toxin non-producers, by VOC profiling, might be a challenge on the same level as for antibiotic resistance.

Thus, there is still a gap between the wishes from treating physicians and what the market can provide, especially regarding multianalyte detection, i.e. detecting a set of bacteria and a set of antibiotic resistances in one fast and simple to perform assay. We see that in the future the gap could potentially be filled by systems based on the development of analyses of volatile organic compound from bacteria that will allow non-invasive sampling or measurements in the environment.

11 APPENDICES

1) Search strategies for scientific literature
2) Search strategies for Espacenet
3) Search strategies for Derwent Innovation

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MEDLINE; Medicinskt inriktad
EMBASE; Läkemedel, farmakologi
CAPLUS (Chemical Abstracts); Kemiskt inriktad
BIOSIS; Biologi, mikrobiologi

Search string:

L1 MULTI DRUG RESIST? BACTERIA OR ANTISUPERBUG OR (ANTIMICROBIAL# RESIST?)(A)(BACTER? OR ORGANISM?) OR AMR

L2 CARBAPENEM RESIST? GRAM NEGATIVE OR ESBL OR KLEBSIELLA OR ACINETOBACTER OR E COLI OR CLOSTRIDIUM OR MRSA OR NOSOCOMIAL

L3 DETECT? OR SPOTT? OR RECOGNIT? OR OBSERV? OR DISCLOSE? OR IDENTIF? OR REVEAL? OR UNCOVER?

L4 (TECHNOLOGY OR JOURNAL SYSTEM) OR DATA(W)(COMMUNICAT? OR HANDL?) AND (HOSPITAL OR HEALTH CARE OR CLINICS)

L5 (HUMAN OR PATIENTS OR PERSONNEL)

L6 (FAST OR RAPID OR QUICK?)

Combination of L1 AND L2 AND L3 AND L4 AND L5 AND L6 gave **102** references in four different scientific databases. Duplicates are removed

Appendix 2

Patent serach strategies for searches in Espacenet and USPTO

SEARCH TIMEFRAME

The search was performed on March 2018.

DATABASES

Patents: Espacenet, upsto.gov

SEARCH STRATEGIES:

ESPACENET (European Patent Office) [Patents]

Search type: Title

Results:

resistant bacteria 417
nosocomial infection 27
antibiotic resistance 390
antibiotic resistant 341
multi drug resistant bacteria 11
bacteria detection 1120
resistant bacteria detection 12
multi drug resistant bacteria detection 0

ESPACENET (European Patent Office): 78 results

(bacteria* OR nosocomial* OR antibiotic* OR “gram-negative” OR carbapenem OR antimicrobial OR superbugs) AND detection AND resist* in the title AND 1998-2018 as the publication date AND C12Q* OR C12M* OR G01N* as the IPC classification

C12Q MEASURING OR TESTING PROCESSES INVOLVING ENZYMES OR MICRO-ORGANISMS (immunoassay G01N 33/53); COMPOSITIONS OR TEST PAPERS THEREFOR; PROCESSES OF PREPARING SUCH COMPOSITIONS; CONDITION-RESPONSIVE CONTROL IN MICROBIOLOGICAL OR ENZYMOLOGICAL PROCESSES [3]

C12M APPARATUS FOR ENZYMOLOGY OR MICROBIOLOGY (installations for fermenting manure A01C 3/02; preservation of living parts of humans or animals A01N 1/02; brewing apparatus C12C; fermentation apparatus for wine C12G; apparatus for preparing vinegar C12J 1/10) [3]

G01N INVESTIGATING OR ANALYSING MATERIALS BY DETERMINING THEIR CHEMICAL OR PHYSICAL PROPERTIES

ESPACENET (European Patent Office): 527 results

Appendix 2

(bacteria* OR nosocomial* OR antibiotic* OR “gram-negative” OR carbapenem* OR antimicrobial OR superbugs) AND detection AND resist* in the title AND 2008-2018 as the publication date

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ESPACENET (European Patent Office)

The ”environment” and the ”medical device” is considered in the IPC A61B, AG1K, G06Q50/22.

a) (bacteria* OR nosocomial* OR antibiotic* OR gram OR carbapenem* OR antimicrobial* OR superbug*) AND detect* AND resist* AND environment* in the title or abstract AND 2008-2018 as the publication date AND C12Q* OR C12M* OR G01N* OR A61B OR A61K OR G06Q50/22 44 results

b) (bacteria* OR nosocomial* OR antibiotic* OR gram OR carbapenem* OR antimicrobial* OR superbug* OR pathogen*) AND detect* AND environment in the title or abstract AND 2008-2018 as the publication date AND C12Q* OR C12M* OR G01N* OR A61B OR A61K OR G06Q50/22 360 results

c) (bacteria* OR carbapenem* OR antimicrobial* OR superbug* OR pathogen*) AND detect* AND (environment* OR hospital OR nosocomial* OR contamin*) in the title or abstract AND 2008-2018 as the publication date AND C12Q* OR C12M* OR G01N* OR A61B OR A61K OR G06Q50/22 912 results

Search c: (bacteria* OR carbapenem* OR antimicrobial* OR superbug* OR pathogen*) AND detect* AND (environment* OR hospital OR nosocomial* OR contamin*) in the title or abstract AND 2013-2018 as the publication date AND C12Q* OR C12M* OR G01N* OR A61B OR A61K OR G06Q50/22 as the IPC classification 496 results

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ESPACENET

Search d

(bacteria* OR cell* OR pathogen*) AND detect* AND (environment* OR surface* OR non-contact*) AND (hospital* OR contamin*) in the title or abstract AND 2013-2018 as the publication date 123 results

USPTO

ABST/(bacteria OR pathogen) AND detection AND (environment OR surface OR non-contact) AND (hospital OR contamination) OR ACLM/(bacteria OR cell OR pathogen OR resistant OR resistance OR biofilm) AND detection AND (environment OR surface OR non-contact OR fomite OR fomites OR hair OR skin) AND (hospital OR contamination OR colonization OR nosocomial) AND ISD/1/1/2013->20/3/2018) 10.347 results (the first 500 results in the Excel)

ESPACENET: 2 results

Appendix 2

(bacteria* OR cell* OR pathogen*) AND detect* AND (surface* OR non-contact*) AND hospital* in the title or abstract AND 2013-2018 as the publication date AND G01N* OR G06Q50/22

USPTO (United States Patent and Trademark Office): 560 results ABST/(bacteria OR cell OR pathogen) AND detect AND (surface OR non-contact) AND hospital OR ACLM/(bacteria OR cell OR pathogen) AND detect AND (surface OR non-contact) AND hospital AND ISD/1/1/2013->20/3/2018 AND ICL/G01N$ OR ICL/G06Q050/22

Appendix 3

Patent serach strategies for searches in Derwent Innvation

SEARCH TIMEFRAME

The search was performed in March 2018.

PATENT COLLECTIONS:

DWPI, US Granted, US Applications, European Granted, European Applications, WIPO Applications

SEARCH STRATEGIES:

S.No	Search Concept	Search Concept	Results*
A	Detection of drug or antibiotic resistant bacteria	((test* or detect* or screen* or measur* or diagnos* or identif*) NEAR10 (((Drug* or (multi ADJ drug*) or (multi-drug*) or antibiotic* or Methicillin or Carbapenem or ESBL or "extended spectrum beta-lactamase*" or "extended spectrum beta lactamase*" or "beta-lactamase*" or (beta ADJ lactamase*)) NEAR3 resist*) NEAR10 (bacteria* or pathogen* or organism* or microb* or microorganism*))) **Title, Abstract or Claims**	(1550)
B	Detection of most important bacteria	((test* or detect* or screen* or measur* or diagnos* or identif*) NEAR3 (superbug* or MDRO or "MDROs" or "Klebsiella pneumonia*" or (K ADJ2 pneumonia*) or "Acinetobacter baumannii*" or ("A" ADJ2 baumannii*) or "Escherichia coli" or "E.coli" or Ecoli or ("E" ADJ2 Coli) OR (Clostridi* ADJ2 difficile) or C.diff* or ("C" ADJ2 diff*)) OR MRSA or (Staphylococ* ADJ aureus) or ("S" ADJ2 Aureus)) **Title, Abstract or Claims**	(35549)
C	Detection of hospital infection causing bacteria	((test* or detect* or screen* or measur* or diagnos* or identif*) NEAR10 ((Hospital* or nosocomial) NEAR3 infect*)) **Title, Abstract or Claims**	(213)
D	Quick **OR** Point of care testing	((quick* or instant* or (real ADJ time) or real-time or fast* or accelerat* or rapid* or speed* or swift* or minute* or min or "Point of care" or (Point-of-care) or (Point ADJ of ADJ care) or POCT OR "Point of need" or (Point-of-need) or (Point ADJ of ADJ need) or POC or phone or smartphone* or (smart ADJ phone)) NEAR3 (test* or detect* or screen* or measur* or	(312360)

Appendix 3

		diagnos* or identif*)) **Full Patent Description**	
E	Restriction of String A, B and C by String D (detection should be fast or point of care)	A AND D B AND D C AND D	120 (331) 436 (1554) 5 (15)
F	Restriction of String A, B and C by String D (detection should be fast or point of care)	(A or B or C) AND D	517 (1694)
G		Detection of bacteria in hospital environment SSTO="DETECTION OF BACTERIA" "HEALTH CARE ENVIRONMENT" **Derwent Smart search**	353 (1000 most relevant)
H		Surveillance system for rapid detection of multi-drug resistant bacteria in hospital environment AND Detection of bacteria SSTO=("HEALTH CARE ENVIRONMENT" "RESISTANT" "DETECTION OF MULTI" "RAPID DETECTION" "MULTI DRUG" "HOSPITAL" "BACTERIA" "SURVEILLANCE") AND SSTO=("DETECTION OF BACTERIA"); **Derwent Smart search**	27 (54)
I		Detection of volatile organic compounds from bacteria SSTO=("DETECTION OF VOLATILE" "VOLATILE ORGANIC COMPOUNDS" "BACTERIA"); **Derwent Smart search**	422 (1000 most relevant)
J		I (2013-2018) AND antibiotic resistance SSTO=("DETECTION OF VOLATILE" "VOLATILE ORGANIC COMPOUNDS" "BACTERIA") AND SSTO=("ANTIBIOTIC RESISTANCE") **Derwent Smart search**	68

* No of results for year 2013-2018 (all years).