Does molecular antibiotic resistance testing improve diagnostics and standard of care?

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Preface

At ECCMID 2013, held in Berlin in April, Curetis AG organized and sponsored an Integrated Symposium entitled “Does molecular antibiotic resistance testing improve diagnostics and standard of care?”

Since the event was very well attended and feedback on its content was enthusiastic, Curetis has decided to put these symposium proceedings together into a white paper.

The symposium was chaired by two leading scientists in the infectious disease area: Prof. Carl-Eric Nord and Prof. Christian Giske, both at the Karolinska Institute, Stockholm, Sweden. In addition to elaborating on the problem of antibiotic resistance, talks at the symposium covered clinical aspects of two disease areas, pneumonia and implant and tissue infections, as well as discussing the relevance of gene-based testing.

Prof. David Livermore, Norwich, England, and Prof. Gian Maria Rossolini of the University of Siena, Italy, delivered presentations on antibiotic resistance and summarized the current situation. Prof. Livermore focused on the growing problem of antibiotic resistance. He presented global data on various antibiotic resistance mechanisms. Prof. Rossolini discussed studies about the correlation of phenotype- and genotype-based antibiotic resistance testing and their underlying mechanisms. Their talks clearly demonstrated the challenges associated with the more complex resistance mechanisms that are currently emerging. Both experts agreed on the need and the added value of fast molecular testing.

Then another two internationally renowned expert teams comprising a microbiologist and a clinician / surgeon presented exciting case studies in the areas of pneumonia and prosthetic joint infections, respectively:

• Prof. Antoni Torres, University of Barcelona, Spain & Prof. Eiman Mokkadas, University of Kuwait City, Kuwait

• Prof. Andrej Trampuz, Charité, Berlin, Germany & Dr. Olivier Borens, Centre hospitalier universitaire vaudois, Lausanne, Switzerland

These talks indicated the need to rigorously evaluate antibiotic treatment in light of patients’ medical needs and the demands of antibiotic stewardship. The case histories provided hands-on examples of how physicians and microbiologists are joining forces in disease states as diverse as respiratory disease and joint implants to offer higher-quality and more cost-effective medical care. Both presentations pointed out the high potential of fast molecular testing. In the long run, deployment of new diagnostic tests such as multiplex PCR-based panels may point the way to new and better guidelines for the diagnosis and treatment of such patients. Initial clinical data from Kuwait supported the clinicians’ statements that the application of Curetis’ Unyvero™ P50 Pneumonia Tests has enabled physicians to adjust empiric treatment in a time frame of just a few hours.

All of these experts have agreed to contribute to Curetis’ ECCMID Symposium Proceedings in order to further disseminate their presentations. The management of Curetis would like to express our deep thanks to all of the authors. In addition, Curetis has included an additional chapter in this white paper that shows how health data can be used to demonstrate the potential medical and economic benefits of rapid molecular testing.

Sincerely yours,
Anne Thews
Medical Director
Curetis AG
Genotypes and phenotypes of emerging resistances

David M Livermore

Abstract
Remarkable shifts in antibiotic resistance have occurred in many important human pathogens over the last decade and a half. While some resistance types have declined in prevalence, others have accumulated sharply over the same timeframe. The implications for antibiotic therapy are significant and worrisome; infections due to multi-resistant strains are more difficult to treat and are not covered by standard empirical therapy, resulting in greater morbidity and mortality, and are often associated with higher treatment costs.

Since mechanisms of antibiotic resistance are numerous, it is important to keep track of their changing landscape at local and global levels. What is more, it is necessary to track both resistant pathogens and resistance genes and to understand the relationship between them. This article summarizes the temporal and geographic trends in phenotypic and genotypic changes for important pathogenic bacteria over the last decade and a half.

Keywords
Antibiotic resistance, resistance mechanisms, pneumonia
What is more consistent is the rise in resistance among Escherichia coli from bloodstream infections. There were dramatic increases in fluoroquinolone resistance in many countries from 2001 to 2011 and also in cephalosporin resistance, in the UK, uniquely - not elsewhere in Europe - there has been a levelling out of this resistance since around 2007-8, probably due to an intense effort to reduce the use of these antibiotics, largely owing to concern that they also selected Clos- tridium difficile, a pathogen that became a major politi- cal issue in the UK. Since the middle of the last decade, use of cephalosporins and quinolones has experienced a sixty to eighty percent drop in many UK hospitals.

The spread of cephalosporin resistance in Enterobacteriaceae has driven the greater use of carba- penems across Europe, with a corresponding rise in carbapenem resistance. In just three years, the prev- alence of resistance to carbapenems among blood- stream Klebsiella pneumoniae in Italy rose from less than five percent in 2008 to seventeen percent in 2010 and to twenty-nine percent in 2011 (Figure 2).

In the case of Pseudomonas aeruginosa, countries in the north and west of Europe have the lowest rates of resistance and countries in the south and east have the highest, regardless of which antibiotic class - amino- glycosides, cephalosporins, carbapenems or fluoro- quinolones - is considered.

Acinetobacter baumannii, an opportunistic species that is problematic in UK hospitals, already had ninety per- cent resistance to imipenem by year 2000. Nevertheless, in 2000, fewer than 2% of Acinetobacter isolates in the UK were resistant to imipenem. This proportion then rose stead- ily to nine percent in 2005 and twenty-six percent in 2010 and to twenty-nine percent in 2011 (Figure 2).

In the case of MRSA, a single acquired gene, meca, accounts for almost all resistance; it can be carried in a variety of chromosomally-inserted ‘cassette chromoso- mes’, which have penetrated different S. aureus clones. Transfer of the meca gene is a rare event and the greater problem is the spread of MRSA clones from patient to patient, hospital to hospital and country to country. The geographic distribution of these clones can be tracked by sequencing the spa gene. Studies by Grundmann et al. illustrate that particular MRSA strains are geograph- ically clustered in Europe. In the UK, the two predomi- nant MRSA strains are EMRSA15 (clonal complex 22) and EMRSA6 (clonal complex 39). These are prevalent also in Portugal, parts of central Europe, and in Australia.

In the UK, recent shifts in MRSA prevalence are largely due to changes in the occurrence of these two strains. In 2000, EMRSA 15, and 16 accounted for over ninety- five percent of all MRSA-related bacteremias with a rough two-thirds: one third split between them. How- ever, by 2003, the proportion of EMRSA-16 had drop- ped to less than twenty percent of MRSA bacteremias while EMRSA-15 increased. These changes preceded the intense public health effort to contain the spread of MRSA in the UK, which was followed by the more recent general decline - also affecting EMRSA-15.

Despite these successes new MRSA strains conti- nue to evolve, via the slow spread of meca. These strains pose new threats. In Europe, there are limit- ed problems with community-acquired MRSA but, in the US, a community-MRSA strain, USA300, has spread so widely that community-acquired MRSA is a greater problem than hospital-acquired MRSA. The same trend is occurring in parts of Australia and South East Asia, with different MRSA strains involved (Fig.3).

In the case of pneumococci, deployment of conjugate vaccines, initially 7-valent (PCV7) and latterly 13-valent (PCV13), has dramatically decreased the burden of invasive disease in young children. In the UK, PCV7 resulted in a large decrease in macrolide-resistant pneumococ- cal infections as it targeted serotype-14, which account- ed for about sixty-percent of all macrolide-resistant invasive infections. However, genotypic shifts occur over time and, in a German study published in 2011, Weil-Oliver found an increasing number of antibiotic-resistant pneumococcal infections due to serotype 19A, which is not covered by PCV7. Serotype 19A increased from a baseline of fewer than ten percent of infections to over twenty percent in just four years. It is covered by the new PCV13, vaccine but resistance is now being reported in other non-vaccine serotypes, notably 15A and 38B. Increasing cephalosporin resistance in E. coli is largely due to the spread of extended-spectrum β-lactamases (ESBLs). Until 2000, most ESBLs were mutants of clas- sical TEM and SHV plasmid-mediated penicillinas. These were found mostly in Klebsiella and rarely in E. coli. Since 2000 (earlier in South America) the greater problem has become the proliferation of Cefotaximase- Munich (CTX-M)-type ESBLs. These are chromosomal β-lactamases from Kuyvera spp. that have escaped onto plasmids, which have disseminated among Enterobacteriaceae, including E. coli. This type of genetic es- cape has happened repeatedly and different CTX-M ty- pes have become prevalent in different parts of the world. From India, throughout most of Europe, Canada, and increasingly in the US, CTX-M-15 is the dominant ESBL. It is also spreading into South America, partially displac- ing CTX-M-2, which originally was the prevalent CTX-M type in the southern cone of the continent (also in Israel). In the Far East and Spain, CTX-M-14 is the dominant ESBL.

A major factor in the success of CTX-M-15 is the com- monest ESBL worldwide - its association with a particular lineage of E. coli, designated sequence type (ST) 131. ST131 E. coli was circulating as a suscepti- ble lineage as early as the 1980s but then developed
Genotypes and phenotypes of emerging resistances

Figure 4: Predominant CTX-M ESBL by region

Figure 5: Genes encoded by plasmid pEK499 isolated from E. coli strain A, an ST131 variant prevalent in the UK. It carries three β-lactamase genes (CTX-M-15, OXA-1 and TEM-1), two aminoglycoside-modifying enzymes genes, one mutant so it can also modify some fluoroquinolones, including ciprofloxacin, along with common resistance genes affecting antibiotics, tetracycline and chloramphenicol.9

Many carbapenemase producers – whether Enterobacteriaceae or Acinetobacter spp. – are exceptionally resistant to antibiotics: among the few agents that typically remain active are colimin, fosfomycin and tigecycline. Even these may be compromised. Colistin-resistant variants of the KPC-carbapenemase-producing K. pneumoniae ST258 are circulating in Italy, and Horsey and colleagues described the mutational emergence of tigecycline-resistant variants of a UK-prevalent carbapenemase, KPC.3

These latter mutants had an upregulated efflux pump. Increased complexity, arising via mutation, was also seen in E. coli ST131, with CTX-M-15 β-lactamase. This strain is normally susceptible to ertapenem and other carbapenems, with MICs less than 0.25 mg per litre. On top of the three β-lactamases typical of the line-age, (CTX-M-15, OXA-1 and TEM-1), this variant also had acquired a CMY-23 β-lactamase and had lost the both outer membrane porins OmpC and F, reducing drug entry and tipping the balance towards ertapenem resistance.

Conclusions

We are seeing major shifts in both the phenotypes and genotypes of resistance. For MRSA and pneumococci, the situation is improving in many countries, reflecting better infection control and vaccine deployment, respectively, but, for gram-negatives from E. coli to gonococci, resistance problems are getting worse and, crucially, we are seeing a trend towards (i) greater genetic complexity in resistance and (ii) the rise of globally successful resistant lineages such as the E. coli ST131 with CTX-M ESBLs and K. pneumoniae ST258 with KPC carbapenemases.

The impact of increasing numbers of resistances has been studied in patients with bacteriaeas. An elegant study in Spain looked at the correlation between the numbers of resistances present in patients’ isolates with the likelihood of inappropriate empirical therapy being selected. If the isolate had no resistances, the doctor prescribed inappropriate antibiotic therapy in only nine cases out of three hundred. However, if the isolate contained three more resistances, the patient had a one in three chance of receiving an empirical antibiotic that proved inactive. As a consequence, greater mortality was observed in patients whose bacteria had three or more resistances.11

For the critically-ill patient, immediate effective antibiotics are critical to a successful outcome. Shifting resistance patterns and increasing complexity make this choice harder. New strategies, with early precise detection of pathogens and their resistances are urgently needed to guide patient treatments, and to provide a potential route to radically better antibiotic stewardship.

β-lactamase genes (ctx-M-15, Oxa-1 and tem-1), two aminoglycoside-modifying enzymes genes, one mutant so it can also modify some fluoroquinolones, including ciprofloxacin, along with common resistance genes affecting antibiotics, tetracycline and chloramphenicol.9

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David Livermore worked at the London Hospital Medical College from 1980 until 1997, when he joined the Health Protection Agency (HPA), becoming Director of its Antibiotic Resistance Monitoring and Reference Laboratory in 1998, where he remained until October 2011. Since then he has had a split role, as Professor of Medical Microbiology at the University of East Anglia and Lead on Antibiotic Resistance for the HPA. He has broad interests on the evolution, mechanisms and epidemiology of antibiotic resistance.

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Correlation between genotypes and phenotypes: clinical implications

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Abstract
Determining the genotype of antibiotic resistant pathogens is a crucial step in understanding the dynamic relationship between phenotype and genotype. Genotype information is increasingly being used to guide the selection of appropriate antibiotic therapy for critically ill patients. Yet the phenotype-genotype relationship is complex. There are a number of factors that can confound the analysis and lead to a misleading assessment, which can in turn introduce delays into the clinical management of patients with serious infections. This article will describe the advantages and challenges of genotypic analysis as it relates to the known phenotype of important human pathogens. In addition, the clinical and epidemiological implications for genotypic analysis will be discussed.

Keywords
Antibiotic resistance, resistance mechanisms, genotyping, molecular diagnostics

Introduction
Resistance mechanisms employed by important human pathogens are becoming increasingly complex and dynamic, as pathogens continue to evolve and become more resistant to our current suite of antibiotics. The genotypic detection of resistance markers can be used in making early antibiotic therapy decisions, with the caveat that there are several other factors, such as the level of resistance gene expression, the growing diversity of resistance genes, and other factors that can confound the correlation between the resistance genotype and phenotype. A weak correlation between genotype and phenotype complicates the clinical decision making process. New technology, capable of rapidly detecting genotypes, is in the early stages of being deployed in the clinical microbiology laboratory. Like any new technology, it is important to assess the reliability and accuracy of genotype results and to understand the advantages and disadvantages of the technology. However, although the technology is in the early days of clinical evaluation, it is emerging as a useful tool to enable better decision making for antibiotic therapy for the critically ill patient. As more clinical data become available over time, it will be possible to evaluate the correlation between genotype and phenotype more completely and to accurately determine the clinical impact of genotype analysis on patient outcomes.

Genetic factors that confound the correlation between phenotype and genotype
Although any phenotype depends on the genotype, the correlation may be variable due to: i) the level of expression of the resistance gene product; ii) the presence of co-factors expressed along with the resistance gene; iii) the possibility that different resistance genotypes result in common resistance phenotypes.

The following section provides some examples for the variable correlation between resistance genotypes and phenotypes.

Same resistance genotype associated with different phenotypes and same resistance phenotype associated with different genotypes
The main resistance mechanism to ampicillin and amoxicillin in Escherichia coli is due to the production of TEM-1, a plasmid-encoded β-lactamase, which is sensitive to β-lactamase inhibitors such as clavulanic acid. E. coli isolates producing TEM-1 are resistant to ampicillin and amoxicillin, but are normally susceptible to amoxicillin-clavulanic acid. Resistance to amoxicillin-clavulanic acid (and to other β-lactamase inhibitor combinations) can be due to overproduction of TEM-1, providing an example of how the same resistance genotype may be associated with different phenotypes. On the other hand, resistance to amoxicillin-clavulanic acid can also be due to the production of an inhibitor-resistant variant of TEM-1 (e.g. TEM-30), or to the production of a class D (OXA-type) or class C (AmpC-type) β-lactamase which are resistant to inhibitors, giving an example of how the same resistance phenotype may be associated with different genotypes.

Cofactor expression
The influence of cofactor expression can impact genotype expression of resistant organisms and account for phenotypic differences in strains with the same β-lactamase genotype. In a 2006 publication by Elliott et al., cofactor expression was the reason for resistance in a patient with pneumonia caused by Klebsiella pneumoniae producing CTX-M-15, a prevalent Extended Spectrum β-lactamase (ESBL). The first isolate analyzed from the patient revealed cephalaxine and ceftriaxone resistance but good sensitivity to ertapenem. However two subsequent isolates from the same patient, although they belonged to the same strain, had lost the ampCβ gene, which encodes a porin in the outer membrane. Loss of the porin affected antibiotic permeability resulting in acquisition of resistance to ertapenem.

The influence of reduced outer membrane permeability on the β-lactam resistance phenotype can also result in a similar resistance phenotype for strains with different β-lactamase genotypes. An example is represented by K. pneumoniae isolates producing the CTX-M-15 ESBL in presence of an outer membrane permeability defect or co-producing the CTX-M-15 ESBL and the OXA-48 carbapenemase. In both cases the isolates exhibit resistance to expanded-spectrum cephalosporins and decreased susceptibility or resistance to carbapenems, and only genotypic analysis allows identification of the resistance mechanisms.

The relevance of genotyping analysis for clinical epidemiology
The possibility that the same multiresistant phenotype may be associated with a different set of resistance genes underscores the importance of genotypic analysis for clinical epidemiological purposes. For instance,
the first Italian isolates of K. pneumoniae producing the OXA-48 carbapenemase were detected almost simultaneously from two patients in two different hospitals, who were seemingly unrelated. However, the two patients had been transplanted with organs from the same donor, and genotypic analysis confirmed that the two isolates were actually identical in terms of clonality and resistance genes, strongly suggesting that in both cases cross-infection via transplanted organs had occurred.4

Therefore, screening organ donors for the presence of multiple drug resistance strains could be an important application of rapid molecular testing methodologies.

Silent genotypes

In Acinetobacter baumannii, carbapenem resistance is caused primarily by the expression of acquired oxacillinase, but in some strains, carbapenemases. Strains that have picked-up the blaKPC-2 carbapenemase gene are increasing in frequency worldwide and accounts for the majority of carbapenem resistance.

In a study to determine the primary source of the blaKPC-2 gene, Poirot et al. examined by PCR fifty Acinetobacter spp. strains for the presence of blaKPC-2. The results showed that Acinetobacter radioreisistens is the source of the blaKPC-2 gene, but in this species, the gene is chromosomal and is not expressed at significant levels. This species of Acinetobacter is a commensal bacterium, which resides on the skin of healthy people and hospitalized patients. Importantly, it is an antibiotic-susceptible genotype, as A. radioresistens remains susceptible to carbapenems.4

As another example of a silent resistance genotype, Gianne et al. examined the genotype of an isolate of Proteus mirabilis, taken from a patient with a blood stream infection.5 The strain was largely antibiotic resistant, showing resistance to cefotaxime, cefazidime, amikacin, gentamicin and ciprofloxacin, while retaining susceptibility to carbapenems, etrapenem and meropenem as well as piperacillin / tazobactam. The genotype was determined and the strain was ESBL negative and blaTEM-1 positive. However, the surprising and worrisome finding was that the strain was also positive for the blaoxa-23 gene. The blaoxa-23 gene and flanking regions showed the gene was identical to the blaoxa gene from Klebsiella pneumoniae but in this strain there was no detectable carbapenemase activity. This finding is worrisome since similar organisms could act as a silent reservoir of blaoxa genes. Moreover, if expressed, this would render P. mirabilis totally drug resistant (since this species is naturally resistant to tigecycline and colistin).

Making the case for genotyping

Higher reliability of genotyping for detection of some resistance determinants

An important factor that must be considered in making the case for genotypic detection of resistance determinants is when genotyping can be more reproducible and robust than phenotyping. For instance, Giakkoupi’s laboratory in Greece confirmed the results of susceptibility testing of VIM-1-producing Klebsiella pneumoniae isolates, and observed that results of carbapenem susceptibility exhibited remarkable discrepancies when using different assay systems, including Etest®, disk diffusion, Vitek®2, Phoenix®12 and Microscan®.10 In similar cases, genotyping of carbapenemase genes can be more reliable for detection of the presence of resistance determinants and assessment of the risk of a carbapenem-resistant phenotype.

Established use of genotyping to predict for resistance phenotypes: the case of MRSA

MRSA is one of the most important resistant pathogens, and the presence of the mecA gene is considered the gold standard for detection of the MRSA phenotype. However, low-level expression of mecA has been detected in some mecA-positive strains (e.g., CC-SCCmecIV, which is prevalent in Taiwan) which can result in an MSSA phenotype and in an erroneous assignment as MRSA by genotypic analysis.10 Moreover, several different mec alleles are associated with a mecA-positive phenotype, type, ranging from 62 to over 98 percent nucleotide identity to mecA, have been detected in staphylococci.9 In particular, a mec allele, named mecC, which is only seventy percent identical to mecA, was recently identified in Staphylococcus aureus from both human and bovine origin in the UK and Denmark.11 In this report, mecC positive isolates were identified as mecA negative, when in fact the isolates were resistant to methillin. These findings underscore the potential limitations of genotypic analysis, since novel genes can go undetected, and the importance of updating genotyping assays on a regular basis.

Having a robust molecular detection assay for MRSA has both clinical and epidemiological implications:

• Rapid detection of MRSA colonization for infection control purposes

• Rapid detection of MRSA infection in clinical speciﬁc treatment guidance

The standard identiﬁcation and susceptibility proﬁle of staphylococci from blood cultures takes up to forty-eight hours using phenotypic culture methods. However in the past decade, a number of rapid PCR-based assays have been set up with the goal of rapidly detecting MRSA in blood samples suspected of containing S. aureus.12

As part of an initial validation study, Herdman and colleagues compared the results from the mecA rapid PCR assay with the standard culture-based resistance analysis. The advantages included a rapid turnaround time (hours versus days) and high sensitivity. Increased cost and high false positive rate were the main disadvantages.13 Other publications have corroborated the usefulness of a rapid PCR-based assay for MRSA.

Hallin’s group evaluated the clinical usefulness of a PCR assay that can discriminate between S. aureus and the less pathogenic Coagulase-negative staphylococci (CoNS), which reside on normal skin. In this study of twenty-eight patients with a blood stream infection, seven benefited from a modification of their antimicrobial chemotherapy based on the rapid PCR results. The remaining seventy-five percent of patients received the appropriate empirical therapy.13

In a larger study of approximately one hundred and twenty patients with bacteraemia, Rümü et al. in a North German University Hospital, investigated whether results from a rapid real-time PCR assay for S.aureus would have resulted in a change of therapy compared with the actual therapy the patients received. The results showed that out of eight patients, three were MRSA positive and thirty-six patients (thirty-six percent) could have received more effective therapy based on the results obtained from the PCR assay.13

In contrast, a study by Cattoir et al. revealed no significant difference in patient outcomes comparing a conventional phenotypic microbiology culture assay with real-time PCR. In this study, conducted over a twelve-month period, two groups of patients with positive blood cultures received antibiotic therapy based on results from either a conventional phenotypic assay or a real-time PCR assay. At the twelve-week follow-up time point, patients were assessed for clinical outcomes. Overall, out of ninety-seven positive blood cultures, the prevalence of S. aureus was twenty four percent with the remaining being CoNS. The fraction of patients with a clinically successful outcome was roughly the same in both groups: successful clinical outcomes were obtained in sixty percent of patients evaluated with a conventional phenotypic assay and fifty-eight percent of patients with a rapid PCR assay.14

Multi-drug resistant Gram-negatives: the emerging plague

The increasing prevalence of multi-drug resistant Gram-negative pathogens poses a serious public health threat as only a few antibiotics remain effective. The organisms of greatest concern include:

• ESBL-positive Enterobacteriaceae

• Carbapenem-resistant Enterobacteriaceae (CRE) expressing KPC, MBL, OXA-48 carbapenemases

• Carbapenem resistant Acinetobacter

• MBL-positive Pseudomonas aeruginosa

The epidemic situation in Greece is a good example of the seriousness of the problem. Figure 1 illustrates the proportion of carbapenem-resistant K. pneumoniae isolates from bloodstream isolates in recent years in Greece and Italy. From 2005 to 2007, Greece dealt with an epidemic of carbapenem resistant VIM-producing K. pneumoniae.15 This was immediately followed by new epidemic of K. pneumoniae expressing KPC-type beta-lactamasmes. A similar trend has been observed in Italy, where the proportion of carbapenem-resistant isolates was very low until 2009 and since this time has rapidly increased up to approximately thirty percent in 2011 (EARS-Net database), with most isolates producing KPC-type carbapenemases.
In preparation for dealing with this growing threat, several assays have been developed for rapid genotypic detection of ESBL and CRE. Rapid identification of these organisms could be one of the best approaches to improve patient screening, enhance infection control practices in hospitals as well as reduce the use of inappropriate antibiotic use. The flowchart shown in Figure 2 illustrates how molecular testing for carbapenemase genes is currently implemented in our laboratory, which serves a large hospital experiencing a high-level endemicity of CRE.

The workflow foresees that positive blood cultures and lower respiratory tract specimens (broncho-alveolar lavage, bronchial aspirate) from critical care patients are subjected to rapid molecular testing for the presence of KPC producing bacteria. In case of positivity, the empiric treatment is modified to include colistin and tigecycline (which are among the few agents that usually retain activity against KPC-producing Klebsiella), while in the case of negativity the empiric treatment, which is normally based on carbapenems, is left unchanged.

Conclusions
Genotyping assays can rapidly identify antimicrobial resistance genes and have an important role in the treatment of serious infections, although more clinical evidence is needed. In conclusion, this article has covered the following important points:
- The correlation between anti-microbial resistant phenotypes and genotypes is variable
- Genotyping confirms the resistance mechanism but may not always predict the phenotype
- Genotyping is faster and more sensitive but only detects genes that are targeted by the input probe sequences
- Genotyping is essential for molecular epidemiology and infection control and is an established use of genotyping methods
- Genotyping is a very promising method to help guide and make adjustments in antibiotic treatment and improve clinical outcomes.

4 Rossolini et al., unpublished
Clinical relevance of fast genetic identification of microorganisms and antibiotic resistance genes – a case study

Eiman Mokaddas and Antoni Torres

Abstract

The continuing rise in the number of antibiotic resistant microorganisms coupled with the declining number of effective antibiotics have made caring for patients with serious infections increasingly challenging. Nevertheless, clinicians must follow antibiotic stewardship guidelines and choose the appropriate empiric antibiotic drug at the right dose at the right time for the right duration. This is because in most of the occasions clinicians do not know which pathogen is the causal microbe.

This article traces a challenging case study of a sixty-year old Chronic Obstructive Pulmonary Disease (COPD) patient with multiple exacerbations. This case was chosen to highlight the complex diagnostic options that clinicians face. It also illustrates the impact those decisions have not only for the individual patient but also for the community at large. The emergence of a new technology that can identify both pathogens and resistances in hours rather than days is changing clinical practice and making stewardship both more practicable and more effective.

Introduction

Nine years ago, the Infectious Disease Society of America (IDSA) published a provocative article. Entitled “Bad Bugs, No Drugs,” the article warned about the rise in threatening microorganisms coupled with stagnation of new antibiotic drug discovery. Unfortunately, the situation today has grown considerably worse. Apart from some new therapeutic options for MRSA, there is a paucity of new antibiotics for treating pathogens on the ESKAPE list (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.). Already responsible for most nosocomial infections, ESKAPE organisms are becoming increasingly resistant to our current set of antibiotics. There are no new drugs at all for multi-drug resistant (MDR) Gram-negative organisms such as A. baumannii and P. aeruginosa. Gram-negative bacterial infections are particularly concerning since, they now account for the majority of infections in intensive care units (ICUs), comprising a full sixty-three percent in 2007 compared with 39.1% in 1995.

There are published guidelines intended to assist the clinician in selecting appropriate antimicrobial therapy for patients in acute care hospitals. The goals of antimicrobial stewardship are twofold: first, to optimize clinical outcomes and minimize unwanted toxic side effects for the patient; and second, to limit the selection pressure on pathogenic organisms and thereby minimize the generation of resistance.

While good antibiotic stewardship is critically important, from a clinician’s point of view it is difficult to implement. Figure 1 describes a typical decision tree for selecting appropriate antibiotic therapy for a critically ill patient as well as the modifications that may be necessary several days later when culture results are available. The decision-making process using standard microbiological culture-based assays takes several days, potentially delaying the implementation of targeted antibiotic therapy. But this delay is no longer inevitable given the rise of rapid, molecular-based diagnostic tools that can identify a panel of resistant pathogens and resistance markers in hours rather than days.

Being able to identify resistant microorganisms quickly with a rapid multiplex PCR assay has the potential to achieve both goals mentioned above. The impact is shown in Figure 3. The turnaround time using rapid molecular diagnostics is approximately 4.5 hours as illustrated by the vertical green bar, while conventional culture based microbiology results for the same pathogens ranges from a minimum of twenty hours for Enterobacter sp., E. coli, Serratia marcescens and Acinetobacter baumannii up to approximately forty hours for the remaining organisms.

Figure 2: Impact of a rapid multiplex PCR assay - de-escalation in critically-ill patients

Case Study

A case history will be used to illustrate the complexity of the decision-making process in a real-life situation involving a COPD patient with multiple exacerbations. Both treatment choices and the rationale behind them will be described.

The 60-year old male smoker (50 packs a year) presented with a five-year history of dyspnea. In addition, the patient suffered from diabetes type II, arterial hypertension and chronic cardiac insufficiency. COPD was diagnosed on the basis of a FEV1 (forced expiratory volume at 1s) of 44% and FEV1/VC (FEV1 / vital capacity) of 55%.
Clinical relevance of fast genetic identification of microorganisms and antibiotic resistance genes — a case study

The first episode of an acute exacerbation in December, 2011, was characterized by cough, increase of dyspnea and high volume of expectoration, while the chest-X-ray showed no consolidations. Based on the major laboratory parameters including a leucocyte count of 7,300/mm³, a CRP (C-reactive protein) of 1.5 mg/dL and PaO₂ of 70 mmHg and a PaCO₂ of 43 mmHg and a pH of 7.38, a further diagnostic was not indicated and therefore the patient was not admitted to the hospital.

The second episode began two months later with progressive, dry cough that was persistent for five days, increased dyspnea and expectoration volume, however no sign of sputum purulence. At admission to the hospital, the respiratory rate was 20 bpm, no fever, a heart rate (HR) of 90 bpm min with a blood pressure (BP) of 130/75 mmHg. Leucocytes were at 8,500/mm³ with a CRP of 1.6 mg/dL and blood gases with a PaO₂ of 65 mmHg; a PaCO₂ of 43 mmHg and a pH 7.37. The X-ray showed no sign of opacities or consolidation. The patient was diagnosted with an Anthonisen Type II COPD exacerbation.

The patient was not hospitalized because lack of severity of the exacerbation. Antibiotics were not prescribed due the lack of purulence of the sputum.

The initial culture showed heavily mixed microorganisms, while a two day subculture resulted in a pure growth of alpha-hemolytic colonies which were opitochin sensitive. The report to the ward at day two indicated a S. pneumonia (Penicillin susceptible by E-test). Empiric treatment was narrowed down to monotherapy with penicillin. At this stage, rapid molecular testing delivering a result in less than five hours would have enabled earlier therapy adjustment.

Conclusions

Despite the lack of new antibiotic therapies, practicing effective antibiotic stewardship is more important than ever. Rapid PCR-based diagnostic tools offer one possible solution to the problem by speeding up the identification process. The use of a rapid PCR-based assay can provide results in hours rather than days. Having data quickly enables an early diagnosis and faster administration of appropriate antibiotic therapy and avoidance of inappropriate therapies, which can lead to resistance. Rapid PCR-based molecular testing also provides an opportunity to make adjustments in anti-microbial adjustment during the course of therapy, which should result in improved medical and economic outcomes. Rapid detection of resistance markers allows early identification of patients with multi-drug resistance organisms, which in turn leads to infection control and overall good antimicrobial stewardship.

In summary, multiplexed rapid molecular identification of resistance organisms, which in turn leads to the short- and long-term management of patients, like COPD patients, who are at risk for pneumonia. However, further clinical randomized clinical studies are needed.

Patients with COPD exacerbations should receive antibiotics if the exacerbation is severe and requires invasive or non-invasive mechanical ventilation. (Guidelines for the management of adult lower respiratory tract infections ERS/ECMD 2011). Initial empiric treatment in this case must cover S. pneumoniae, H. influenzae and P. aeruginosa as the patient has risk factors for a P. aeruginosa infection with his recent hospitalization and administration of antibiotics. Therefore the patient was started on ciprofloxacin IV. Antibiotic treatment was changed to piperacillin/tazobactam 72 h when obtaining cultures and the antibiogram. The patient stayed ten days in intensive care and was discharged after a total of twenty days of hospitalization.

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21 Antimicrobial resistance is a major public health concern worldwide, with growing resistance in many communities. This trend is particularly worrying in countries where access to antibiotics is widespread and use is high. The World Health Organization (WHO) estimates that at least 70% of community-acquired respiratory infections are now treated with antibiotics. The overuse and misuse of antibiotics contribute to the development of antibiotic resistance, which can make infections more difficult and expensive to treat. The increasing prevalence of antibiotic-resistant bacteria poses a significant threat to public health, as it reduces the effectiveness of antibiotic therapy and increases the risk of infection-related complications and mortality. Moreover, the development of antibiotic resistance can lead to increased healthcare costs and prolonged hospital stays. Therefore, it is crucial to implement measures to promote the prudent use of antibiotics and to develop new strategies to combat antibiotic resistance.

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Lung infection is a severe problem in acutely ill patients. Infections are exacerbated by the emergence of multi-drug resistant bacteria. New molecular technology like multiplexed molecular assays will have an increasingly large impact on both the initial diagnosis of severe pneumonia as well as on the therapeutic decisions that result from this diagnosis.

The study described here illustrates the advantages of using rapid multiplex assays in the identification of pneumonia-causing pathogens and antibiotic resistance markers compared to conventional culture techniques.

The study included thirty-nine patients from two intensive care units (ICUs) and one organ transplant department in Kuwait. The patients were seen between November, 2012 and March 2013. All were suspected of suffering from pneumonia. Their diagnoses included severe community-acquired, hospital-acquired, and ventilator-associated pneumonias (sCAP, HAP and VAP, respectively). Three different pneumonia sample types, – sputum, endotracheal tubes and bronchial lavage (BAL) samples – were analyzed by rapid PCR assays as well as by conventional culture methods. All specimens, regardless of their different compositions and viscosities, were prepared according to the Unyvero™ lysis protocol. A broad range of pathogens and resistance markers were detected with this PCR-based assay (figure 1).

[Table: Pathogens and resistance markers detected by the multiplex PCR assay]

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Resistance</th>
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<tbody>
<tr>
<td><em>S. pneumoniae</em></td>
<td>lac</td>
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<tr>
<td><em>Haemophilus influenzae</em></td>
<td>sfr, hfr</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>cfr, cr, AM</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>lest, sfr, hfr</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em></td>
<td>sfr, hfr</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em></td>
<td>gmr, A</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>paeC</td>
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Of the thirty-nine patients, only a few were diagnosed with community-acquired pneumonia (CAP). These were all renal transplant patients. The treatment of those patients started empirically with piperacillin in combination with tazobactam and ofloxacin as recommended by the protocol of the department. The multiplex PCR was able to detect *Streptococcus pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* as well as antibiotic resistances. Those results could be obtained 48 to 72 hours earlier compared to the conventional culture methods. Due to this early diagnosis, therapy for these patients could be adjusted precisely at an initial stage.

In the course of this study, the treatment of twelve of the thirty-nine patients was modified based on the rapid multiplexed PCR test. Of the VAP diagnosed patients, six out of eighteen received a modified therapy. Of the ten HAP and the eleven sCAP diagnosed patients, the therapy was modified in two and four cases, respectively, because of this rapid diagnosis. In aggregate, this is equivalent to 31 percent.

As shown above, the availability of a rapid and an accurate molecular diagnostic test can result in a reduction of inappropriate and inadequate antibiotic treatments as well as in successful infection control. By appropriately interpreting the results of such tests and correlating them with the clinical condition of patients, over-diagnosis and over-treatment of pneumonia can be avoided.

Implant and Tissue Infections
Olivier Borens and Andrej Trampuz

Abstract
Total hip and total knee arthroplasties are well-established surgical procedures that can increase mobility and alleviate pain associated with damaged or diseased joints. One complication, albeit an infrequent one, is a deep prosthetic joint infection. Such infections are very difficult to treat. One key reason for this difficulty is that the infectious organisms form a biofilm that then becomes attached to the prosthesis. Such pathogens growing in biofilms cannot be adequately identified and treated using standard microbiological procedures. This inability to detect these microorganisms complicates the diagnostics and therapy in time and can lead to repeat surgeries or even amputation.

The approach described here, one that we have successfully pioneered, is to combine the expertise of the orthopedic surgeon and the clinical microbiologist, working as a team. Together, we have improved the identification of biofilm microorganisms by the addition of an implant sonication step prior to microbiological culture. In addition, we are now evaluating the combination of biofilm microorganisms by the addition of an implant sonication step prior to microbiological culture. In addition, we are now evaluating the combination of biofilm microorganisms by the addition of an implant sonication step prior to microbiological culture. In addition, we are now evaluating the combination of biofilm microorganisms by the addition of an implant sonication step prior to microbiological culture.

Introduction
Hip and knee replacements are exemplars of surgical efficacy and efficiency. Arthroplasty has had a major impact on improving quality of life for many thousands of patients. In 2010, approximately 800,000 total hip arthroplasty (THA) procedures were performed in the US.1 Yet in conjunction with the increased mobility benefits conferred by arthroplasty comes a small but significant risk – that of post-transplant infection. A small number of patients who receive implants develop a deep infection on the surface of their prosthesis. It is too all easy for orthopedic surgeons to miss vital clues if they are not experts in clinical microbiology. The latent infection is due to a change in the physical form of the bacteria, a change which makes the detection of the bacteria almost impossible, and sometimes entails further surgery, with all the associated pain, costs and risks. In the presence of a solid implant surface, bacteria can no longer exist as individual planktonic cells. Instead, they accumulate in a firm, extracellular mass called a biofilm. Microorganisms residing in biofilms grow as localized communities attached to the implant in an amorphous, extracellular secreted matrix. Not only are these bacteria extremely difficult to detect, they are also relatively resistant both to antibiotic therapy and to host immune defenses. Furthermore, they are adapted at living under extreme conditions and at extreme temperatures. By the time the infection is correctly diagnosed, therapy options may be limited to removing the implant or even amputating the limb. Successful treatment depends on the ability to detect the biofilm infection early, an ability that until now has been nearly non-existent. For an approach that integrates microbiology with orthopedic surgery to succeed, a new treatment paradigm is needed.

A concept for success
Our multidisciplinary team has developed a treatment concept that produces good results and is based on the following parameters.2
• Good teamwork
• Good diagnostics
• Flexible treatment options

By working together as a team, clinical microbiologists and orthopedic surgeons can develop a deeper understanding of the problem. Together, they can apply good diagnostic methods with flexible treatment options for the patient, increasing the likelihood for a successful outcome. Orthopedic surgeons understand the importance of biomechanics and surgical debridement while microbiologists understand the complexity of choosing the appropriate antibiotic therapy and the threat of antimicrobial resistance.

Real-life examples of treating patients with implant infections
As examples, we have selected several case studies that highlight points that must be considered when treating patients with implant infections. These case studies will show that microbiological diagnosis is very difficult in patients with implant infections, as standard microbiology techniques often fail to identify organisms growing on the surface of the prosthesis.

Case Number One:
An eighty year old male patient complained about hip pain. The patient received a stage 1 surgical revision, with prosthesis removal, tissue debridement and immediate joint replacement. During the procedure, a tissue fluid sample was obtained and cultured, which had shown coagulase-negative Staphylococcus. The implant infection was characterized as follows; a sinus tract fistula and purulence with wound secretion pus around the prosthesis. Tissue histology revealed acute inflammation with between one and ten neutrophils per high-power field.3

Given that the patient had had surgery ten years earlier, it would be very unlikely for the infection to be related to the implant. Low-grade infections associated with the implant itself can occur at one, two or three years following implant surgery but very rarely at ten years. It is much more likely that the infection originated in the patient’s bloodstream.

Other diagnostic options, including preoperative C-reactive protein (CRP) levels, bacterial species testing and bone and leukocyte scintigraphy, while they provide useful data, are not as helpful in selecting the best course of treatment. Microbiology is important for identifying the organism and potential resistances but should optimally be performed on several swabs taken from the infected area and after sonicating the prosthesis.

Other cell measurements:
• Leukocyte counts in synovial fluid
  • Neutrophils: >5 x 10^9/L
  • Lymphocytes: >4 x 10^9/L
  • Monocytes: >1 x 10^9/L

• Microbial growth
• Synovial fluid
• 2a periprosthetic tissue (for low-virulent organisms) >1 positive
• Sonication fluid >20 CFU/mL
Implant and Tissue Infections

Case Number Two:
A sixty-seven year old person had increasing pain following knee implantation surgery in 2008 shown in Figure 2. Following surgery, he received standard antibiotic prophylaxis and had an uneventful post-operative follow-up. A couple of months later, he started to have severe radicular pain in his lower back with the onset of partial paralysis. He underwent several operations to stabilize his lower back but two days after the last operation, he was symptomatic with fever, chills and an elevated CRP level. There was lumbar drainage of pus from his spine, which was treatable, but the major concern was that his knee joint had become a little red and painful.

Microbiology blood culture revealed the presence of MSSA. The spine was debrided in the Operating Room with a satisfactory result. The biggest concern was the knee. If there are positive blood cultures, there is a thirty percent chance of preserving the total knee. If there are positive blood cultures, there is a thirty percent chance of preserving the total knee. If there are positive blood cultures, there is a thirty percent chance of preserving the total knee.

In addition to improving the yield of bacteria culture from tissue, sonication also improves sensitivity using multiplex PCR. In a study published by Achermann (Figure 4), multiplex PCR was used to detect microbial DNA in sonication fluid obtained from patient implants during surgery. As shown in Figure 4, in a total of thirty-seven implant infections analyzed, pathogens were cultured from sonication fluid in twenty-three cases (62% sensitivity) and were identified by multiplex PCR in twenty-nine patients.

Case Number Three:
An orthopedic surgeon was diagnosed with Parkinson’s disease in 2001 and retired three years later to enjoy hunting and fishing. Experiencing knee pain, he underwent knee implant surgery on several occasions. However, the culture results continued to be negative even though CRP levels were still elevated. Following the additional surgeries, the patient was still not doing well. He had persistent swelling of soft tissues and the knee was warm. An X-ray confirmed that the position of the implants were properly in place, with no signs of infection or loosening of the prosthesis. The patient was started on amoxicillin and clavulinate and the conclusion was that the redness and swelling were due to slow healing.

Two months later, he was doing a little better and could walk one kilometer but the knee was still warm to the touch. CRP levels were still elevated. Antibiotics were stopped, and treatment was ended for the patient.

One month later, the patient was still experiencing persistent knee pain, swelling and warmth and had difficulty walking. His CRP levels were still chronically elevated and he sought another opinion from an orthopedic surgeon. The diagnosis was that he most likely had an infection. Recommended treatment options included a one-step exchange of the prosthesis or the preferred option of performing an arthrodesis, which consists of fusing the bones and removing the joint altogether. Understandably, the patient did not want to undergo an arthrodesis and sought a third opinion from us.

We chose to go directly for surgery and removed the Total Knee Arthroplasty and inserted a bone cement spacer made from Palacos® bone cement infused with gentamycin and vancomycin. Standard histology did not show anything useful and standard fourteen-day tissue culture results were negative as before. However, this time, we subjected the prosthesis to sonication. The results revealed the presence of greater than one thousand colony-forming units per milliliter of Propionibacterium acnes, an anaerobic gram-positive bacterium, which grows very slowly in culture and therefore gives a result very late. It also grows predominantly as a biofilm and only rarely as a planktonic organism as it cannot survive in an aerobic environment. After we diagnosed the source of the infection by sonicating the prosthesis, we proceeded to treat the infection and succeeded at clearing it. The patient made a full recovery after several months and went back to fishing and hunting with no pain or swelling and a low to no CRP level.

An improved method for the diagnosis of biofilm infections.

The surgical cases summarized above highlight the difficulties of treating implant infections. Standard microbiology methods typically fail to detect infectious organisms present in implant infections as they grow as a biofilm, firmly attached to the implant surface. However, we are using a simple procedure that greatly improves our ability to accurately identify the infection. Figure 3 describes the procedure of sonication followed by standard culture. By subjecting the implants to sonication, which can be done easily by placing them into a sterile vial and sonication for one minute at forty kHz, there is a dramatic increase in the number of organisms that can be grown in culture compared with the standard method. Identifying organisms from sonication fluid has better sensitivity (eighty to ninety percent), is more quantitative and specific, is faster and less expensive and produces a larger volume of fluid for additional investigations and analyses. Moreover, it is better at identifying mixed infections, which can account for thirty percent of infected implants.

Figure 1: Using cut-off values of cell count results obtained from synovial fluid specimens, it is possible to diagnose a high likelihood of infection.

Case Number Three:

Microbiology blood culture revealed the presence of MSSA. The spine was debrided in the Operating Room with a satisfactory result. The biggest concern was the knee. If there are positive blood cultures, there is a thirty percent chance of preserving the total knee. If there are positive blood cultures, there is a thirty percent chance of preserving the total knee. If there are positive blood cultures, there is a thirty percent chance of preserving the total knee. If there are positive blood cultures, there is a thirty percent chance of preserving the total knee.

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As shown in Figure 4, in a total of thirty-seven implant infections analyzed, pathogens were cultured from sonication fluid in twenty-three cases (62% sensitivity) and were identified by multiplex PCR in twenty-nine patients.

A sixty-seven year old person had increasing pain following knee implantation, which had taken place one year earlier.

Several preoperative tests might conceivably have been used to detect this infection: Serum CRP levels; synovial fluid Gram stain and culture; synovial fluid leukocyte count and differentiation; conventional X-ray or even PET/CT scan. Since the surgery occurred only one year earlier, the most important first step was to obtain a synovial fluid leukocyte count and cell differential. Figure 1 describes a set of cell count results obtained from synovial fluid specimens taken preoperatively from the knees of 133 patients. Of the 133 patients; a joint infection was diagnosed in thirty-four patients. A leukocyte count of greater than 1,700 per microliter correlated with infection with 94% sensitivity (Fig. 1A) and a cell differential of greater than sixty-five percent neutrophils had a sensitivity of ninety-seven percent (Fig. 1B). Using these cut-off values, it is possible to diagnose accurately a high likelihood of infection. If surgery is performed, it is important to do tissue culture from three or more tissue specimens, using no swabs. Culture specificity is between sixty to eighty percent and is most accurate after prolonged periods of culture incubation from seven to fourteen days.

Figure 2: X-ray of a TKA (see text case number three)

Figure 3: Sonication procedure for diagnosis of biofilm infections

Figure 4: In a study published by Achermann (Figure 4), multiplex PCR was used to detect microbial DNA in sonication fluid obtained from patient implants during surgery.
Among nineteen patients receiving antibiotics, multiplex PCR analysis of sonication fluid was positive in all nineteen samples (100 percent sensitivity) compared with sonication culture, which identified the organism in eight patients (forty-two percent sensitivity). There was no significant difference in results if patients were not receiving antibiotics. Since the PCR panel used in this study did not contain *P. acnes* or *Corynebacterium* species primers, which were present in eight cases, it is worth noting that with additional primer sets, there is room for continued sensitivity improvement. In another study, by Portillo and colleagues, multiplexed PCR performed on sonication fluid identified infectious organisms in twenty three out of twenty four cases of prosthetic joint infections, (96 percent sensitivity) compared with sixteen out of twenty four identified with sonication followed by microbial culture (67 percent sensitivity).6

**Conclusion**

Culture of tissue samples taken from implants with suspected infection is prone to limited sensitivity and specificity due to the presence of bacterial biofilms, which escape detection using standard microbiological techniques. We have described several surgical case studies in which good teamwork coupled with good diagnostics resulted in favorable outcomes for the patients. As we continue to focus on understanding better how to improve diagnostic and treatment options for patients with implant infections, we are participating in the European Implant Cohort Study (EICS). The EICS was established by the Foundation for Implant-Associated Infections, whose mission is to improve the management and outcome of prosthetic joint infections and develop diagnostic consensus guidelines across Europe. In the future, we expect that better teamwork between surgeons and microbiologists will help many more patients and reduce repeat admissions and the associated costs.

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**Figure 4:** Multiplex PCR (SeptiFast) in sonication fluid compared to sonication fluid culture of PJIs.
Abstract
The introduction of new diagnostic tests and new diagnostic systems in hospitals is a remarkable challenge in times of cost cutting in health care budgets. This is even the case where benefits and savings have already been demonstrated by the implementation of the new system. Unyvero™ is an example of such a solution.

The data presented in this article is based on a health economic model derived from literature as well as actual clinical performance data for the Unyvero™ solution. Additional post marketing studies and a future randomized trial to further evaluate the medical and economic benefits of rapid molecular tests are already planned. These studies are expected to further strengthen the health economic case for an earlier diagnosis of pneumonia.

Introduction
Diagnosis and treatment of pneumonia represents a huge cost burden to the healthcare system. Among other issues, hospitals and other healthcare facilities face increasing financial liability for patient readmissions due to persistent infections. These infections are an insidious threat in part due to the rise of antibiotic resistance, a challenging trend for which few new antibiotics and limited technical solutions are available. Demonstrating economic benefit is an increasingly necessary part of the process of applying new diagnostics in routine hospital care.

Fortunately, new diagnostic modalities can augment traditional approaches such as microbiology culture in ways that both save patient lives and hold the line or even reverse the trend in healthcare cost increases. This article will describe the nature of the challenge clinicians and their institutions face in diagnosing and correctly treating severe disease. It will highlight the inherent limitations involved in administering the current standard of care diagnostic regime which consists primarily of microbiology culture. It will then demonstrate the impact on both patient health and provider cost of earlier, accurate pneumonia testing based on an extensive analysis carried out by Curetis.

Hospitalized pneumonia – a serious, life-threatening disease
The respiratory tract is the most common source of infection in acutely ill patients and is a leading cause of death among these patients. The frequency of lung infections ranged from 64-68%, respectively, in the EPIC II and SOAP studies. Five to ten cases of hospital-acquired pneumonia (HAP) are diagnosed in every one thousand patient admissions into hospital and are associated with other issues, hospitals and other healthcare facilities face a huge cost burden to the healthcare system. Among other issues, hospitals and other healthcare facilities face increasing financial liability for patient readmissions due to persistent infections. These infections are an insidious threat in part due to the rise of antibiotic resistance, a challenging trend for which few new antibiotics and limited technical solutions are available. Demonstrating economic benefit is an increasingly necessary part of the process of applying new diagnostics in routine hospital care.

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New challenges: Gram-negative bacteria, mixed infections and drug resistance
Gram-negative infections are increasing with a worldwide mean value of 47% for Gram-positive and 62% for Gram-negative organisms. German data for nosocomial pneumonia are in line with this, showing an incidence of Gram-positives of 24% compared to Gram-negatives of 64%.

In the case of gram-negative infections, Pseudomonas was the most frequent organism found in infected ICU patients (20%), followed by Klebsiella (13%) and Acinetobacter (9%). The high values for Pseudomonas infections are troubling because Pseudomonas is difficult to treat and is associated with increased mortality in ICU patients.

Keywords
HE model, multiplexed molecular testing, cost reduction, Pneumonia
Antibiotic therapy in a timely fashion is critically important and complications of multi-drug resistant pathogens further increase the risk of empiric treatment failure rates. The rate of drug-resistant pathogens in pneumonia is challenging. While the rate of MRSA infection is stable at 35%, multidrug resistant pathogens are on the rise and represent 56% of gram-negative bacteria involved in these infections. Infections by resistant strains result in greater morbidity and mortality because, in those cases, empiric therapy is more likely to prove ineffective, leading to an increase in the cost of treatment. The SOAP trial demonstrated the presence of a polymicrobial or mixed infection in 23% of cases observed. A Spanish study showed that in single pathogen infections, 90% of patients received appropriate empiric antibiotic therapy, while only 40% of patients with more pathogens received appropriate therapy.

Limitations of current standard of care

The ultimate goal of pneumonia therapy is to apply the appropriate antibiotic regimen that is effective against the infectious pathogens. Therapy should start as early as possible at the right dose via the correct route of administration to maximize exposure of the antibiotic at the infection site.

However without the knowledge of the causative pathogens, initial antibiotic therapy is often inadequate. Numerous published studies have demonstrated that inadequate antibiotic therapy increases the mortality rate and mean duration of hospital stay significantly (Figures 1 and 2). The increasing presence of mixed infections and the ever-present and increasing probiotic bacteria and microorganisms or mixed infection in 23% of cases observed. A Spanish study showed that in single pathogen infections, 90% of patients received appropriate empiric antibiotic therapy, while only 40% of patients with more pathogens received appropriate therapy.

Hypothesis confirmed: Early identification leads to faster antibiotic adjustment, which in turn leads to a better outcome.

The model described below is based on published data comparing medical and economic outcomes in patients having received inadequate antibiotics. The hypothesis is that the results of the rapid molecular-based testing would enable earlier antibiotic treatment and therefore has the potential to reduce costs and save lives.

The model confirmed that potential benefits of rapid molecular diagnostic testing include faster diagnosis and treatment, shorter length of hospital stay and decreased overall cost of treatment.

Inadequate treatment in 38.8% of patients can increase the length of hospital stay as well as time spent in the intensive care unit, which results in increased healthcare costs. Based on the literature, the model used length of stay (LOS) data and costs of EUR 21,700 and EUR 27,240 for adequate and inadequate initial antibiotic therapy, respectively.

A meta-analysis of seven publications showed an average mortality of 28.9%. Based on the assumptions described above for the Curetis model, benefits can be shown in both, mortality and cost. In terms of mortality, in 172 patients treated either adequately or inadequately with antibiotic therapy, the addition of the Unyvero™ platform in addition to microbiology culture would result in 11 fewer deaths (Figure 6). In terms of cost, adoption of Unyvero™ added only marginally (less than 2%) to the overall cost of treating those patients who were adequately treated. At the same time, the use of Unyvero™ early in the process of diagnosis and treatment would lead to a significant cost reduction of EUR 266,815 in the treatment of patients who were initially treated inadequately (Figure 7).
Conclusion
The introduction of new equipment into hospital laboratories is a process that almost inevitably increases costs of care. It is the rare technology that can find its place in a hospital and immediately begin to both improve the medical standard of care and at the same time help reduce cost. Unyvero™ is an example of such a technology. It has the potential to make a strong impact on the economics of healthcare providers as once difficult- or impossible-to-treat- infections are managed in their early hours through application of Unyvero™.

The data that underlie the model described here derive from the literature combined with actual clinical performance data for the Unyvero™ Solution. Even better would be a study based on data that are collected prospectively and are standardized in terms of the protocols applied. Curetis has initiated such a study in order to learn about two aspects of pneumonia-care in Europe: firstly, how many of the initial treatments currently offered in Europe are inadequate and, secondly, how often clinicians will change the treatment of their patients based on the results from microbiology and what reasons the clinicians give for any change. The study includes a chart review by an independent physician that asks, if Unyvero™ data had been available, how the clinician would have changed the therapy, for example by escalating or de-escalating it. The data can be used to design a randomized study to further evaluate the medical and economic benefit of rapid molecular testing. These studies are expected to support an even stronger health economic case for earlier diagnosis of the pathogens related to cases of acute pneumonia and for determination of antibiotic resistance markers.

Unyvero™ Health Economic Data

Curetis Symposium ECCMID Berlin 2013

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