[Frontiers in Bioscience 12, 2658-2669, January 1, 2007]

Bacterial DNA microarrays for clinical microbiology: the early logarithmic phase

Marco Cassone [1,2], Antonio Giordano [1,3], Gianni Pozzi [2]

[1]Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, 1900 North 12th street, 19122 Philadelphia, PA, USA, [2]Department of Molecular Biology, Laboratory of Molecular Microbiology and Biotechnology, University of Siena, Viale Bracci 53100 Siena, Italy, [3]Department of Human Pathology and Oncology, University of Siena, Viale Bracci 53100 Siena, Italy

TABLE OF CONTENTS

1. ABSTRACT

In this era of coexistence of high-throughput sequencing technologies and serious difficulties in the management of both common and novel infectious syndromes, new techniques which improve the study of micro-organisms is timely. In bacteriology, the most important subjects are bacterial pathogenicity, discovery of the genomic complexity of bacteria, and the epidemiology of antimicrobial resistance traits. From the clinical point of view, genetic testing is flanking phenotypic testing for the assessment of new, difficult to test antibiotic resistance traits, and for correlations with the microbial behaviour *in vivo*. The demand for faster, comprehensive and highly parallel microbial diagnostics is also cogent even at the basic laboratory level, where the ultimate objective is saving lives. In this setting, DNA microarrays offer a pivotal contribution by allowing performance of hybridization experiments in highly parallel formats, with an increasing reliability. Not only they are useful in deciphering host and microbial pathophysiology, they can also make the difference in the management of prognostic and therapeutic aspects of many diseases. Here, we provide an overview of the current use and the potential of DNA microarrays in clinical bacteriology, and several applications and technical solutions are discussed.

2. INTRODUCTION

The availability of a large quantity of data on microbial genomes enables the scientists to address challenging questions about biological functions and correlations of genes and encoded proteins, their regulatory networks and, after all, on how all this works in an integrated manner. There are also other questions which have long been investigated, such as the presence and function of single genes or genetic elements in different strains or species, and the role of regulatory elements that still need to be answered as comprehensively as possible. DNA microarrays are the only technique capable of screening an entire genome or transcriptome from any organism in a single experiment. They can also be used to screen for genes belonging to different species, to characterize unknown samples in a much shorter turnaround time than any other currently used technique. The range of possible applications of DNA microarrays is also very broad, ranging from gene expression profiling to DNA sequencing, and sensitivity is improving year after year. For these reasons, this technique is emerging as a very useful tool to widen actual research and diagnostic testing capabilities in the field of infectious diseases and clinical microbiology.

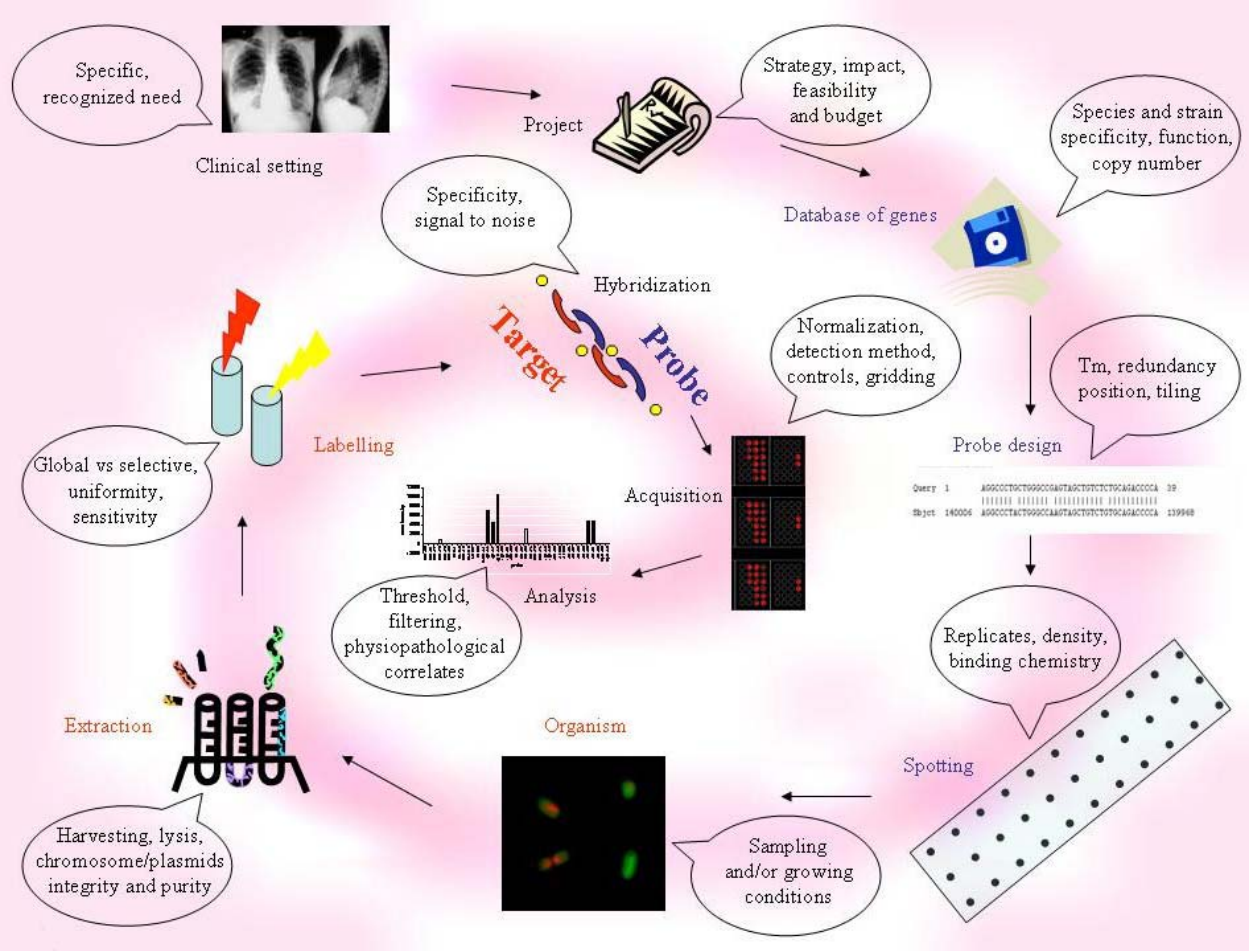

Figure 1. Workflow for development of Bacterial DNA Microarrays. The steps involved in the construction and use of DNA microarrays are shown in the same sequence as they are usually pursued. For each step, we outlined some of the most important aspects to be considered. The Chest radiogram is reproduced with permission from Virtual Hospital (Courtesy of Michael D'Alessandro, Department of Radiology, University of Iowa).

2. 1. What are DNA microarrays?

Generally speaking, they are used to assess whether certain nucleic acid sequences are present in a given template, or whether they are more abundant in one of two templates (Figure 1). The basic principle of DNA microarrays is the same as blotting procedures (1-3), in which the presence or absence of selected sequences in the tested nucleic acid template is ascertained by checking whether hybridization to complementary DNA probes occurs. The difference is that the probes are great in number, are "packed" on a suitable substrate in a relatively small area (e.g., 1 cm^2), and each of them is placed exactly in the desired position. Thanks to this positional tagging, the probes do not need to be labeled to allow recognition. It is the test target that is labeled and then allowed to hybridize with the probes. Also, more targets can be tested simultaneously in a competitive hybridization assay (Figure 2). The amount of hybridized target per each feature is estimated by signal intensity and can be acquired and managed as numerical data by a variety of methods depending on the purpose of the study.

Hybridizations can be performed on substrates obtained from commercial sources, but for large and medium-sized laboratories it may be more convenient and possibly cheaper to purchase the instrumentation to produce substrates *ad hoc*: this allows the investigator to have low-cost DNA microarrays with exactly the desired probes in the desired quantity and replicates. Alternatively, it is possible to order custom-designed microarrays.

2.2. Types of DNA microarrays

DNA microarrays can be divided into several categories according to the nature and method of deposition of the probes and the purpose of utilization: *Oligo-micorarrays* are made with synthetic oligonucleotide probes designed *in silico* from a database, while *cDNA* microarrays are made of longer PCR fragments amplified from a reference sample and then purified. All these DNA sequences are subsequently printed on the substrate. *High density* microarrays are characterized by a very high number of closely packed features. The best performing high density microarrays are obtained by *photolithographic synthesis* directly on the slide (e.g., *Affymetrix GeneChip*

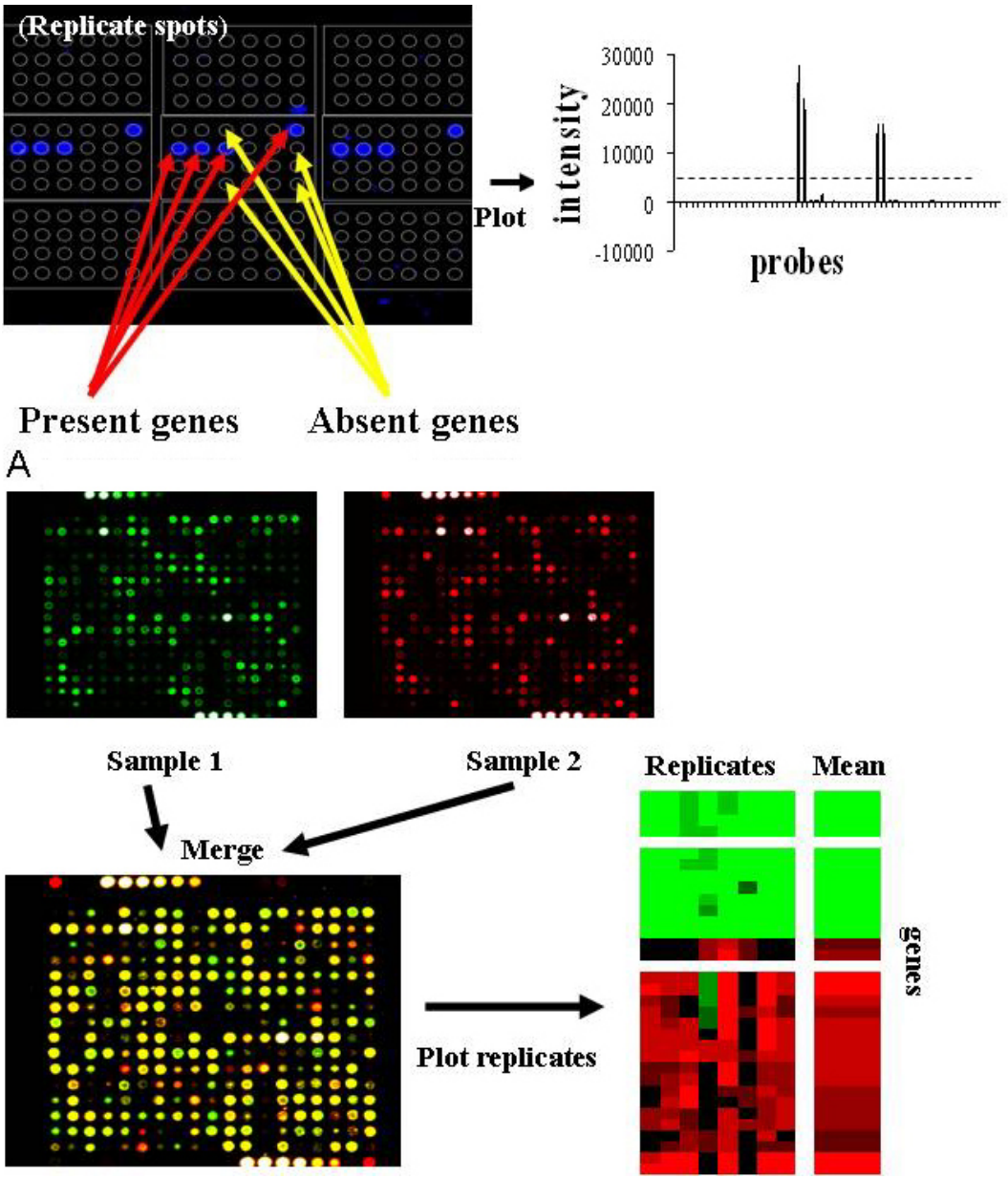

Figure 2. Gene-detection and gene-expression microarrays. A. Gene detection microarrays: a sample with unknown genotype is challenged: some genes are present in the template (red arrows), others are absent (yellow arrows). Probes are usually printed in many replicates for each slide. Plotting of data allows setting a presence threshold (dotted line).B. Gene expression microarrays: two samples with same genotype exposed to different environmental conditions are compared. Most genes are expressed in a similar way (yellow). Some of the genes (typically less than 10%) are differentially expressed in either sample (red or green). Among the different data plotting strategies, cluster analysis of replicate experiments allows detection of genes consistently overexpressed in one (red) or the other (green) sample.

proprietary technology), instead of synthesizing and then printing them. Oligonucleotides synthesized on the substrate are usually very short, about 25 nucleotides in length.

2.2.1. Gene detection microarrays

Gene detection microarrays have a direct application in clinical microbiology and can detect the presence of selected genes of interest (e.g., species-specific sequences, virulence island genes, antibiotic-resistance factors) from cultured or amplified templates (Figure 2A). They are used for the discovery of new genes and the detection and localization of genes in not fully characterized templates. Compared to the large variety of literature already present on gene expression microarrays, this field has evolved rather recently but is now growing expeditiously.

2.2.2. Gene expression microarrays

Gene expression microarrays are very popular and allow the comparison of two differently labeled

templates to verify whether some genes are more expressed in one of the two samples (Figure 2B). They are extensively used in many research fields, such as human oncology and pharmacogenomics. Bacterial gene expression microarrays often target the whole transcriptome and can be used, for instance, to compare two isogenic mutants, or a given strain under different environmental conditions (for instance, *in vitro* culture and in an experimental infection model). A crucial step in gene expression microarray experiments is extraction of total RNA or mRNA from the cells. RNA is then usually reverse-transcribed into cDNA and labeled during or after transcription. If the amount of starting RNA is very limited, a prior amplification step may be necessary. Among the many labeling substances, two fluorophores which respond to laser excitation with different absorption and emission wavelengths are often used. The labeled cDNAs from the two templates are then applied to the array, and each labeled sequence is allowed to diffuse over the array to find its complementary probe. After washing away all unbound labeled targets, an array is obtained which carries, for each feature, a certain quantity of signal depending on the quantity of its labeled target complementary to the feature's probe. The array is scanned and the signal intensity of each feature is measured and acquired. For each probe it is now possible to determine, through differences in the signal intensity, which labeled target is more or less abundant. Since a great number of different experiments are performed every day based on different design, platforms, substrates, protocols, and data analysis tools, to avoid an overwhelming publication of useless if not misleading data, the Microarray Gene Expression Data Society has developed the Minimal Information About a Microarray Experiment (MIAME) protocol to comply with when reporting and publishing microarray data (4).

The study of gene expression is the most frequent pursuit entrusted to DNA microarrays, not only because they can offer a simultaneous view of complex genetic networks, but also because they eliminate the bias generated by prior selection of genes believed to be involved in a certain event (5). Studies assessing the influence of global regulators (6), other main regulators such as two-component systems (7, 8), and sigma factors (9) on the whole bacterial genome are amongst the most common examples of the possibilities offered by this technology. Other interesting applications are the study and screening of operon organization (10), the response to metabolites and micro-elements (11), or to antibiotics and antibiotic-resistance mechanisms (12), the microbial adaptation to host response, and the switch to different growth modalities such as planctonic growth or biofilms (13). Although information obtained by gene expression microarrays will have a fundamental impact on the future of clinical microbiology and in the prevention and therapy of infectious diseases, at present they are not specifically pursuing a direct clinical use. For this reason, they will not be extensively reviewed here.

2.2.3. SNP microarrays

SNP microarrays are used to detect a large number of single nucleotide mutations in a highly multiplexed fashion and without the need for sequencing (e.g., rapid detection of multi-drug resistant *M. tuberculosis*). *Sequencing microarrays* are high-density SNP microarrays. When the number of probes is sufficiently high, the sequence data obtained from the single probes can be collected and ordered with dedicated software to extrapolate the complete or partial sequence of the tested organism. With a very large set of probes, it is theoretically possible to obtain the complete sequence of any template

2.2.4. Comparative Genomic Hybridization (CGH) microarrays

Comparative Genomic Hybridization (CGH) microarrays are obtained by overlapping large DNA fragments selected from a library, or with high-density oligonucleotides; in both cases probes represent an entire genome or chromosome, but with different resolution power. They are used to compare the DNA sequences of entire genomes in order to check differences in the number of copies of chromosomal loci or, recently, even single genes (e.g., presence of complete or partial mobile genetic elements in bacteria or allele loss in human karyotype).

2.2.5. Cell microarray

Cell microarrays are the bacterial counterpart of human *Tissue microarrays,* and are made by immobilizing a different bacterium in each feature of the array; the array is then hybridized with a labeled probe, similar to a high yield, automated blotting. Printed bacteria can be used to detect molecules different from DNA, such as serum antibodies that are detected using secondary labeled antibodies (14). *Plate microarrays* are a series of 96 identical microarrays printed in the wells of microtiter plates. In each well a different sample can be deposited and hybridized in a high-throughput fashion following automated liquid handling procedures: they are useful for large and fast screenings.

In Electronic DNA Chips, the hybridization of the template to the probe is directly transduced in a quantitative signal by coupling to an electronic circuit. Although projecting and fabrication costs are high, the good sensitivity, the labeling-free template processing speed, and the ability to feature many post-hybridization controls make electronic chips good candidates for future diagnostic applications. Microarrays involving probes other than DNA have also been developed, although not well standardized. In the field of microbiology, protein, lipopolysaccharide and antibiotic microarrays have proven useful to detect the host's inflammatory response to infection, microbial antigens and also antibodies at least as accurately as conventional serology (15-22).

3. CLINICAL APPLICATIONS OF BACTERIAL DNA MICROARRAYS

3.1. Gene detection and high throughput typing

Genome typing is a basic research application, used to gain important information about the genomic content of a bacterium by comparison with a well known counterpart which is used as reference. At present, the

availability of complete genome sequences for some type-strains belonging to the most important pathogenic species allows us to obtain a very dependable reference for genomic investigations. A typical example of the use of DNA microarrays for whole genome typing implies the discrimination between highly variable and highly conserved genes among multiple isolates belonging to a single species (23). The genomic regions displaying higher and lower levels of homology can be the subject of further characterization, taking into account the information available on their biological function by phenotypic characterization, mutagenesis or at least by protein structural similarity searches. While the conserved genes are good candidates as species-specific virulence factors, antigens and drug targets, the highly variable regions are often addressed in studies on the evolution and/or the transfer of mobile antibiotic-resistance and virulence-associated genes (24). Another possibility exploited is detecting the presence of groups of several associated virulence factors which may constitute a fundamental attribute to the physiology and pathogenicity of environmental and human colonizing bacteria; those results can be then translated to downstream applications such as "pathotyping" (25-27). Pathogen typing microarrays are very promising from many points of view, since they are opening the doors for further investigations on a limited set of genes, with unlimited research and clinical diagnostic applications.

3.1.1. Genomic variability studies

Different strains of the same species may vary considerably (Fitzgerald et al. reported more than 20% variability in the genome of *S. aureus*) (24), but the complete sequence of only a few reference strains is available. By comparing templates to probes originating from sequenced reference strains, microarrays provide an as yet unparalleled tool to screen for the sequences of as many strains as desired without sequencing, as discussed in a comprehensive early review of this topic (28). A *Mycobacterium tuberculosis* high-density oligonucleotide microarray was implemented in 2001 to detect small-scale genomic deletions in clinical strains; the deletions pattern and quantity correlated respectively to the clonality and to the likelihood of cavity formation, thus proving particularly useful for epidemiological surveys (29). The versatility of in-house made microarrays was used for an inter-species study of large plasmids in *Salmonella enterica* and *Escherichia coli* (30). The authors constructed an array of 500-800 base-pair plasmid fragments, allowing assessment of the heterogeneity and also of some evolutionary aspects of these plasmids and their transmission between species. Bae and colleagues designed probes specific for unsequenced genes of *Salmonella* strains (31). They isolated specific sequences by means of a suppressive subtractive hybridization against sequenced reference strains. Those probes were used to obtain sub-species specific microarrays. In a recent study, the authors used the seven complete sequences of *Staphylococcus aureus* reported so far to develop a complete, multi-genomic array able to obtain sensitive discrimination of related isolates (23). Thanks to this approach, the authors offered a clear description of how isolates attributed to the same clone of epidemic methicillin-resistant *S. aureus* differ substantially in their carriage of mobile genetic elements, including virulence and antibiotic resistance genes. While other typing techniques such as Multi Locus Sequence Typing (MLST) and Pulsed Field Gel Electrophoresis (PFGE) are based on a limited set of targets such as some "house-keeping" genes or a restriction site, DNA microarrays can rely on a high number of probes. Their reproducibility, although not extensively tested, is not inherently limited by technical drawbacks such as in PFGE. In a recent study, the authors performed MLST-like typing using DNA microarrays with 30mer oligonucleotides based on the previous knowledge of polymorphic loci (32). A good performance of the test was reported, although still below the MLST gold standard. DNA microarrays cannot be considered as a substitute for classical typing techniques. Indeed, they offer a large body of relevant information from a different point of view. For instance, they have been used to analyze genetic similarities and differences between *Salmonella enterica* serovars, and between strains belonging to the same serovar, possibly allowing a better discrimination of traits contributing to pathogenicity (33). The authors of this study constructed an array carrying PCR products amplified from almost all the genome of a *S. typhi* and a *S. typhimurium* serovar, plus several virulence plasmids. Random priming allowed uniform labeling of the template for broad-range interrogation. Another group validated a *Y. pestis* whole-genome microarray consisting of 4,000 PCR products each representing an almost entire Open Reading Frame (putative translated region) (34). This array was used to hybridize the whole genome of *Yersinia* strains belonging to different species against reference *Y. pestis* strains. The authors were able to select a panel of 28 species-specific genes of *Y. pestis*.

3.1.2. Studies targeted on specific genes

Many studies have been carried out with microarrays that focused on a limited set of genes. Although information at the single-base level may be obtained if needed (35), a more common use of DNA microarrays in the clinical microbiology setting is to detect the presence and association of selected genes involved in the pathogenesis of infection, in resistance to antimicrobials and other genes or groups of genes directly involved in disease (25, 36-39). Arrays, falling in the category of "gene detection", are less expensive due to a more limited number of probes required, but can be very useful in studying specific aspects of microbial genetics. Although companies are now offering more and more "detection" microarrays, they may just as well be independently developed by a medium-sized laboratory to satisfy specific research needs, and the data they provide are easy to handle and share. Typing is usually aimed at the assessment of unknown patterns of genes among known isolates; on the other hand, species identification relies on the detection of a set of well-known genes on unknown isolates. The strategy in the earlier studies was to obtain labeled amplicons by PCR from conserved genes and subsequently hybridize them (36). Although similar information can be obtained by TaqMan Real-Time PCR,

DNA microarrays are much better suited to this task when the number of probes is high. In an early study, an oligonucleotide-based array was developed to screen for the presence of selected species-specific sequences in six *Listeria* genes, allowing the univocal distinction of six different species (40). In another study, 18 different microorganisms chosen from potential biowarfare agents were identified and typed by hybridizing fragments of about 100 bases obtained from genomic DNA or retroviral RNA to 50,000 oligonucleotide probes (35). Thanks to a high number of probes, it was possible to target many species-specific sequences with a redundancy of all but the last base, so as to gain a wealth of information on the nucleotide sequence to be drawn; this last aspect reflected the need to differentiate warfare strains from other environmental strains. A study focused on sensitivity rather than on broad spectrum addressed the three class A bacterial biowarfare agents (*B. anthracis*, *F. tularensis*, and *Yersinia*) by developing a microarray able to detect amplified DNA from 50 bacteria/ml of blood (41). In another study aimed at obtaining a good sensitivity, the authors identified specific 23s sequences amplified from *P. aeruginosa* and *A. baumanii* cultured from clinical samples (42). They reported comparable or superior sensitivity to microscopy and cultures, with 100% specificity. Using a similar approach, Lehner and colleagues validated an oligonucleotide array specifically aimed at the identification of enterococcal species (43). In a study carried out to discriminate Chlamidiae at species level, the Authors targeted the most variable part of a conserved ribosomal operon sequence to detect single base variations (44). They hybridized control strains to a high density photolitographic array, selected the best performing probes and used them to construct a much less expensive, low-density spotted array. In a recent study, a microarray was developed with 19mer probes targeting variable regions in the 5'-end of 16s RNA genes of some of the most frequent blood isolates (45). Total RNA from sub-cultured bacteria was chemically labeled, and hybridization data were analyzed using an artificial intelligence-based algorhytm instead of comparing intensities of single probes. Marlowe and colleagues (46) were also able to identify single and multiple isolates from a large number of blood cultures with 100% sensitivity and 96% specificity using specific rRNA probes. Since univocal specific probes could not be designed for each of the many targeted species, the authors implemented a flux diagram to interpret the data and report correct identification results.

Specific detection of genes related to pathogenicity is another important application of "gene detection" microarrays. For instance, an oligonucleotide microarray was used for detection of species-specific sequences of 16s RNA genes as well as virulence genes from *B. anthracis* and other bacteria (47). The authors amplified templates using either a conventional PCR with specific primers and a three-step random-primed PCR, which proved to be less sensitive but more discriminatory. In a recent study, *Neisseria meningitidis* and other *Neisseria* isolates were hybridized to a microarray with the whole genomes of the four sequenced *Neisseria* strains, to identify genes present in *N. meningitidis* strains and absent in the other species as candidates for a pathogenetic role in meningitis (27). A membrane array-based approach had been used by another group in 2002 for a similar purpose (48). Rasooly and colleagues (49) targeted several enterotoxin genes of *S. aureus*. Since multiplex PCR with several primer pairs was not efficient, the authors performed a PCR amplification step with universal primers and a subsequent single-strand, fluorescently labeled DNA synthesis. In an interesting study of *Escherichia coli*, 40 isolates were screened with 32 gene probes specific for extraintestinal as well as intestinal pathotypes (26). The template was amplified with a multi-step procedure, with random priming followed by biotinylation with specific primers. The small microarray was placed and hybridized directly in the 1.5 ml polypropylene reaction tube containing the labeled template. The authors observed a high heterogeneity regarding the presence of virulence and pathogenicity genes. Also, a direct link between presence of selected genes and clinical presentation was not always observed. Bekal and colleagues developed an array aimed at typing pathogenic *E. coli* from random primers, uniformly amplified templates and composed of 105 PCR amplicons targeting virulence genes (25). The probes were also divided into discrete subarrays according to their usual association in "pathotypes"; thus just by watching the hybridization image it was possible to have a preliminary glance of the strain's pathotype, and the presence of genes belonging to different pathotypes.

3.2. Drug resistance and epidemiology

DNA microarrays can be used to screen for the presence of a much larger number of antimicrobial resistance genes than any other molecular method. Also, they are complementary to phenotypic screening since they can allow the specific distinction of different genes responsible for an undistinguishable *in vitro* phenotype, but different *in vivo* outcomes. For basic research purposes they have been utilized to infer much interesting and sometimes unexpected information about the response of bacteria to drugs. Those results offer a general view of the transcriptomic response to drugs, and require a comprehensive evaluation and time-consuming confirmatory studies before a clinical or pharmacologic conclusion can be drawn. Although not strictly inherent to clinical microbiology, gene expression microarrays are the first step in increasing our knowledge of the mechanism of action of antibacterial drugs. Whole-genome surveys of the microbial response to antibiotics can display unexpected findings even for "best-seller" and very commonly used and studied drugs. Just as an example, two research groups studied the *in vitro* response of different bacterial species to fluoroquinolones. In one of those studies, *Pseudomonas aeruginosa* showed a uniform upregulation of the pyocins chromosomal region (about 35 Open Reading Frames) when challenged with ciprofloxacin (50). Construction of mutants resulted in the increase of MIC for the antibiotic. While pyocins seemed to be involved in the susceptibility to this antibiotic, DNA gyrase expression did not show significant changes. In the other study, investigating

Haemophilus influenzae, the expression of DNA gyrase showed modifications only with an antibiotic concentration of ten-fold the MIC, while other distinct, antibiotic-specific pathways were induced in response to two different antibiotics both targeting DNA gyrase (51). In a recent study, a database of gene expression profiles from *Bacillus subtilis* exposed to 14 different antibiotics as well as from antibiotic susceptible mutants was acquired (52). Against this database, the authors compared data obtained from *Bacillus* treated with two new antibiotics, with the aim of uncovering their mechanism of action. From an epidemiological point of view, DNA microarrays offer the opportunity to comprehensively and definitively screen large numbers of isolates from collections, to obtain data about the prevalence and diffusion of resistance determinants over time and geographic areas or different clinical settings. A multiplex PCR-based technique was used by the Rasooly's group for identification of six erythromycin resistance genes in *S. aureus* (53). Each gene was targeted by as many as seven oligonucleotide probes. Call and colleagues (37) used microarrays with PCR products from 17 tetracycline resistance genes as probes to detect resistance in the uniformly labeled total genomic DNA from several different species of bacteria. Broad species-range oligonucleotide microarrays have also been developed for a comprehensive detection of 65 genes conferring resistance to macrolide antibiotics (39). Using this approach, the authors were able to detect the presence of unexpected resistance determinants even in previously characterized strains, highlighting the epidemiological usefulness of a test which is not limited to the most known and frequently found resistance genes. The specificity of the system was high as a result of the direct labeling of the extracted DNA without PCR amplification Another group was able to identify 90 amplified antibiotic resistance genes common in Gram positive bacteria using a 30mer oligonucleotide microarray with two different probes for each gene (54). Van Hoek and colleagues constructed a 50-60mer oligonucleotide microarray for detection of antimicrobial resistance genes in cultured Salmonella strains (55). Also in this case, the authors were able to avoid PCR amplification of the template. In the few cases of non correspondence between the phenotypic resistance and microarray results, the authors pointed out the possible occurrence of mutations or deletions which may be detected only with dedicated microarrays carrying more (and shorter) probes covering the whole gene. However, this would be very expensive since the cost of oligonucleotides is the main limiting factor for in-house spotted oligonucleotide microarrays. In another study underscoring the link between a high number of specific probes and amplification-free labeling methods, 61 antimicrobial resistance genes were detected in 51 different species (56). More sharply focused oligonucleotide-based microarrays have been developed to detect resistance due to single nucleotide mutations. An important application is detection of Extended Spectrum Beta-Lactamases (ESBL) or inhibitor resistance phenotype (57). Another research group targeted selected *M. tuberculosis rpoB* gene sequences to detect single nucleotide mutations causing rifampicin resistance in an amplified portion of this gene (58). Yu and colleagues were able to correctly detect mutations in the amplified DNA gyrase gene in *E. coli* conferring resistance to quinolone antibiotics (59). Although they selected just two mutation sites, more than 50 19-mer probes were designed to take into account all the possible silent and resistance-causing mutations.

3.3. Diagnostics

PCR is an invaluable tool in many fields of bacterial diagnostics, such as the detection of slowly growing or uncultivable organisms such as *Mycobacterium* or *Chlamydia*, or to demonstrate the presence of very small loads of infectious agents in sterile tissues like liquor or blood. PCR diagnostics also require a very limited turnaround time, but only a few sequences can be amplified and detected at a time. For this reason, it is used only if one or a few infectious agents are suspected in the differential diagnosis. An appealing feature of DNA microarrays in the diagnostic setting is that, thanks to the highly miniaturized and parallel nature of this technology, the presence of many infectious agents can be simultaneously assessed. However, very few studies involving the use of microarrays in a diagnostic setting have been made because the complexity and/or the limited yield of samples pose, at present, severe limitations to the high throughput of this technique. For this reason, it is no surprise that most published diagnostic microarrays have been designed to detect a few sequences, and have to be viewed as preliminary works demonstrating the feasibility of the microarray-based diagnostic approach, rather than as ready-to-use diagnostic platforms, which probably need a few more years to be on the market. To overcome inherent sensitivity limitations, amplification of selected sequences is almost mandatory, although it may cause a degree of bias that may be not acceptable in a clinical setting. To match the high sensitivity of amplification with definite primer pairs to the specificity of target species-specific sequences, most authors have addressed small variable regions in the 16s or 23s rRNA genes by amplification of their conserved flanking regions. Although this approach is satisfactory for some applications and appears to be quite feasible, it has inherent limitations. The first limitation lies in the necessity of designing short probes (20-25 nucleotides), which may suffer from possible, unexpected single nucleotide mutations. Another drawback is the impossibility to develop a high multiplexed technique, since it is very difficult to select several groups of short variable sequences with common flanking regions that correctly span a high range of pathogens. In complex samples like most of the clinical ones, short oligonucleotides would suffer from a lack of relative discriminatory sensitivity. Of some concern, in view of a clinical use, is also the fact that rRNA genes are present in a high and variable number of copies. This would create many difficulties at the time of providing a semi-quantitative analysis, whether absolute or relative. This would especially be a problem with assays designed for detection of antibiotic resistance genes, many of which are present in single copy in the bacterial genome. In an early study, 20 predominant human intestinal bacterial

species were identified by amplifying 16s RNA gene sequences in genomic DNA extracted from stool samples and targeting them to 60 spotted oligonucleotide probes (3 for each species) (60). Similar to other authors, their work stressed the importance of designing multiple probes for each target gene/sequence, since each probe yields a different fluorescence intensity which is difficult to predict in advance at the design stage. However, with a correct test design a satisfying sensitivity can be obtained. In one study (61), as little as 60 fg of *Campylobacter* DNA was detected in fecal swabs after amplification of both 23s and species specific genes. Another study focused on a single bacteria (*S. pyogenes*) along with its more relevant antibiotic resistance determinants, was carried out to develop a narrow-focused, re-sequencing assay for the screening of this pathogen in clinical samples (62). One of the few papers aimed at multi-species detection targeted variations in the DNA gyrase gene amplified from DNA extracted from sputum samples as a means of identifying 14 *Mycobacterium* species (63). Roth and colleagues developed an array targeting variable regions of the topoisomerase genes of nine species representing the majority of bacteria causing upper respiratory infections (64). Another group successfully detected and characterized at the species or genera level several agents of sepsis from blood culture amplification of 16s rRNA genes and amplicon hybridization (65). Shi et al. (66) performed a multiplex asymmetrical PCR with fluorescently labeled primers differentially detecting *N. gonorrhoeae*, *C. trachomatis* and *U. urealyticum* in clinical samples by hybridization to oligonucleotide-based arrays. This valuable approach is exemplary of the diagnostic performance of DNA microarrays, and underscores a potential application whereby microarrays may aid in coping with difficult tasks for the microbiology laboratory. In the above study, intracellular bacteria which are difficult or impossible to grow and/or isolate with common laboratory techniques, and which may be present in low number though being the sole causative agent of disease, were detected and accurately identified. Vora *et al.*, 2005, set up a single multiplex PCR to amplify 95 sequences. Hybridization of the amplified template on long oligonucleotide microarrays allowed reliable identification of *Vibrio* species along with their antibiotic resistance profiles and made it possible to detect the presence of mobile genetic elements (67). Another research group addressed the problem of quantitative profiling of complex microbial populations, which is a fundamental item in the microbiology of samples from non-sterile sites, such as the upper respiratory tract, urine and feces (68). They developed a medium-length oligonucleotide microarray and used it for comparative hybridization of clinical fecal samples with reference samples of known composition.

Some studies have been carried out to validate automated methods for processing diagnostic samples. Although promising, those methods, which are based on pre-hybridization amplification strategies, have not been tested to parallel identification of complex samples, in which they may encounter the same technical difficulties as manual methods. A fully automated device was developed for extraction, PCR amplification, hybridization and detection of bacterial DNA from clinical sample fluids (69). The device was tested for detection of 10^3- 10^6 spiked *E. coli* cells in 1 ml of rabbit blood. Straub and colleagues validated another integrated method relying mainly on the ability to purify and concentrate environmental samples, and the ability to amplify and identify DNA from 100 bacterial cells, but reported some bias due to amplification (70). They used their device to address this problem by direct extraction and hybridization of RNA, but with lower sensitivity compared to conventional PCR detection. Another group used a microarray-format TaqMan PCR plate, encompassing a post-hybridization amplification strategy, allowing the parallel performance of a great number of reactions in separate microchambers, 40 nL each (71). An indirect quantification of the sample DNA was made possible by processing several dilutions of the templates in the many reaction chambers.

4. PERSPECTIVE

DNA microarrays are a fully validated and now largely used technique for detecting and comparing many genes in parallel. The recent increase in efforts made to refine their reliability and reproducibility, and the fast growing interest by top life science and medical companies is justified by great expectations of the role of this technique in the management of most diseases. Bacteriologists have used DNA microarrays to study pathogenicity, virulence, host-pathogen interactions, epidemiology, drug resistance, diversity, evolution, intra- and inter-species interactions, and more. Many are under development for clinical and diagnostic use. Different applications need different project and development strategies. The main and most discriminating attribute of any microarray is the probe set. Hence, in the preliminary phase of the project, the most important choice to be made is that of using short (20-30mers), longer oligonucleotide probes, or PCR amplicons. PCR amplicons offer the best hybridization-signal intensity, since their length is comparable to the mean length (200-500 bases) (72) of the target labeled fragments which contribute more to the hybridization process. However, they are not suitable to detect small sequence variations in different target templates, and they require much work and expense for amplification, purification, quantification and sequencing. PCR product-based arrays are scarcely represented among company product lists, but those developed in academic settings and research laboratories are widely used. Conversely, microarrays carrying short oligonucleotides are less sensitive, but allow detection of single base mutations. These characteristics have proven to be very useful in many fields of research and diagnostics, to be exploited to develop multi-probe features with single-mismatch internal controls (Affymetrix), multiple diagnostic or typing probes targeting a variable region of a single conserved sequence such as the 16s rRNA gene, or to gain indirect sequence information (35). Short oligo microarrays can also prove easy to construct, and the probes cast the same as PCR primers. Needless to say, long oligonucleotide (50-70mers) microarrays show intermediate features, approaching PCR

amplicons in terms of sensitivity and short oligo arrays in terms of cost and ease of fabrication, but lack single-base discriminatory capabilities. Indeed, long oligonucleotides are often the best choice for many applications, especially for differential expression studies, are easily printable and there is a wide choice available from companies. Whatever their nature, the probes must have a unique, highly specific sequence to eliminate biases due to non-specific hybridizations. A second crucial, but sometimes overlooked, step in the choice of the DNA microarray is the need to take into careful account the differences in the sequence of the target to be studied and the sequenced genome of the bacterial strain from which the probes have been developed (73, 74). If a microarray designed to a strain isogenic to our study strain is not available, it is mandatory to compare their sequences before carrying out the microarray experiment, to get an estimate of the differences in the presence or sequence of each gene, since that will obviously alter the meaning of hybridization results. When the published data are not enough to allow *in silico* analysis, it is possible to obtain a good estimate *in vitro* by comparing the two strains' DNAs in a competitive hybridization experiment. Another critical point is the choice of template amplification procedure: PCR amplification with specific primers exponentially increases the quantity of target genes, but is a source of bias due to different efficiencies of the reaction with the different primer pairs, and is not feasible when the probes are numerous, and may also introduce contamination artifacts. Random priming produces a much lower increase of the total template DNA, but uniformly acts on the whole template, maintaining the original variety of sequences that can be interrogated by as many probes as desired. For this reason it is currently used in most cases, especially if the starting sample is quantitatively adequate.

Having these technical precautions and developing perspectives in mind, it is an accepted opinion that some of the many DNA microarray platforms currently available will in the near future have an important role in infectious disease and microbiology laboratories, or even directly at the patient's bed. More platforms, mostly encompassing electronic microarrays, silica-based microarrays and even highly-multiplexing techniques different from microarrays, are under development by biotechnology companies and also by very large chemical and electronics companies new to this market. It is also common sense that for any microarray assay to prove competitive in a clinical setting, four conditions must be met: ease of use, cost-effectiveness, short turnaround time, and sensitivity-specificity, comparable to, or exceeding the current gold standards. Although often required by current protocols, amplification and labeling of the samples can have a negative impact on all these mandatory conditions. This can be a major source of bias, since the efficiency of amplification (especially by PCR) and of many labeling methods on different sequences is unpredictable, leading to poor specificity. Vora and colleagues addressed this issue by comparing specificity and sensitivity of 70mer oligonucleotide microarrays for detection of enterohemorragic *E. coli* DNA amplified and labeled with different procedures (75). Regarding sensitivity, they reported an important increase in sensitivity by performing two rounds of random primer-based isothermal amplification, with labeling performed during the second step only. Using this protocol they obtained the same sensitivity (about 1,000 copies from complex samples) of PCR-based methods with 450-fold less amplification-related bias. Labeling and amplification also affect turnaround time, which is of vital importance in infectious diseases. The labeling technique during PCR amplification has the most impact on bias, while labeling with random primers requires the longest time. Labeling is also expensive, and in many protocols it accounts for more than half of the management cost after the initial, *start-up* costs of probe synthesis are covered. Amplification can also be very expensive depending on the used protocol, for example in post-hybridization amplification protocols for SNP detection, or in protocols involving multiple primer pairs or even amplification of the template directly on the substrate (71). Both steps, finally, require trained specialized personnel, so 24h-7d service may be a problem in small laboratories and hospitals. To eliminate those potential drawbacks, many companies and institutions are developing new generation, labeling- or amplification-free microarrays, relying on newly developed technologies or technologies already used in other scientific areas. Easier and more reliable labeling techniques will also be developed by companies. What is most needed, however, is a better insight into the features and implications of the biological scenarios to be addressed, along with its very specific requirements for developing targeted diagnostic and epidemiologic techniques, which will inevitably prove to be the joint task of scientists and technical investigators.

5. ACKNOWLEDGEMENTS

The authors wish to thank Marco Maria D'Andrea, Marie Basso, Paraskevi Vogiatzi and Caitlin Logan for critical discussion and for technical and formal counselling. The authors also wish to thank Michael D'Alessandro for granting the permission to reproduce the chest radiogram image embedded in Figure 1. This work was supported in part by Grants from the Cava Oggi Foundation, the Sbarro Health Research Organization, and the University of Siena.

The Authors declare that they have no conflict of interest.

6. REFERENCES

1. M. Schena, D. Shalon, R.W. Davis, and P.O. Brown: Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270(5235), 467-70 (1995)

2. E.M. Southern**:** Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98(3), 503-17 (1975)

3. E.M. Southern: DNA microarrays. History and overview. *Methods Mol Biol* 170, 1-15 (2001)

4. A. Brazma, P. Hingamp, J. Quackenbush, G. Sherlock, P. Spellman, C. Stoeckert, J. Aach, W. Ansorge, C.A. Ball, H.C. Causton, T. Gaasterland, P. Glenisson, F.C. Holstege, I.F. Kim, V. Markowitz, J.C. Matese, H. Parkinson, A.

Robinson, U. Sarkans, S. Schulze-Kremer, J. Stewart, R. Taylor, J. Vilo, and M. Vingron: Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. *Nat Genet* 29(4), 365-71 (2001)
5. Y. Dharmadi, and R. Gonzalez: DNA microarrays: experimental issues, data analysis, and application to bacterial systems. *Biotechnol Prog* 20(5), 1309-24 (2004)
6. R.M. Berka, J. Hahn, M. Albano, I. Draskovic, M. Persuh, X. Cui, A. Sloma, W. Widner, and D. Dubnau: Microarray analysis of the Bacillus subtilis K-state: genome-wide expression changes dependent on ComK. *Mol Microbiol* 43(5), 1331-45 (2002)
7. T. Mascher, D. Zahner, M. Merai, N. Balmelle, A.B. de Saizieu, and R. Hakenbeck: The Streptococcus pneumoniae cia regulon: CiaR target sites and transcription profile analysis. *J Bacteriol* 185(1), 60-70 (2003)
8. S.N. Peterson, C.K. Sung, R. Cline, B.V. Desai, E.C. Snesrud, P. Luo, J. Walling, H. Li, M. Mintz, G. Tsegaye, P.C. Burr, Y. Do, S. Ahn, J. Gilbert, R.D. Fleischmann, and D.A. Morrison: Identification of competence pheromone responsive genes in Streptococcus pneumoniae by use of DNA microarrays. *Mol Microbiol* 51(4), 1051-70 (2004)
9. C.K. Sung, and D.A. Morrison: Two distinct functions of ComW in stabilization and activation of the alternative sigma factor ComX in Streptococcus pneumoniae. *J Bacteriol* 187(9), 3052-61 (2005)
10. B. Tjaden, D.R. Haynor, S. Stolyar, C. Rosenow, and E. Kolker: Identifying operons and untranslated regions of transcripts using Escherichia coli RNA expression analysis. *Bioinformatics* 18(S1), S337-44 (2002)
11. A.T. Ulijasz, D.R. Andes, J.D. Glasner, and B. Weisblum: Regulation of iron transport in Streptococcus pneumoniae by RitR, an orphan response regulator. *J Bacteriol* 186(23), 8123-36 (2004)
12. K.J. Shaw, N. Miller, X. Liu, D. Lerner, J. Wan, A. Bittner, and B.J. Morrow: Comparison of the changes in global gene expression of Escherichia coli induced by four bactericidal agents. *J Mol Microbiol Biotechnol* 5(2), 105-22 (2003)
13. M. Whiteley, M.G. Bangera, R.E. Bumgarner, M.R. Parsek, G.M. Teitzel, S. Lory, and E.P. Greenberg: Gene expression in Pseudomonas aeruginosa biofilms. *Nature* 413(6858), 860-4 (2001)
14. N.R. Thirumalapura, A. Ramachandran, R.J. Morton, and J.R. Malayer: Bacterial cell microarrays for the detection and characterization of antibodies against surface antigens. *J Immunol Methods* 309(1-2), 48-54 (2006)
15. L.G. Mendoza, P. McQuary, A. Mongan, R. Gangadharan, S. Brignac, and M. Eggers: High-throughput microarray-based enzyme-linked immunosorbent assay (ELISA) *Biotechniques* 27(4), 778-88 (1999)
16. T. Bacarese-Hamilton, A. Ardizzoni, J. Gray, and A. Crisanti: Protein arrays for serodiagnosis of disease. *Methods Mol Biol* 264, 271-83 (2004)
17. T. Bacarese-Hamilton, J. Gray, A. Ardizzoni, and A. Crisanti: Allergen microarrays. *Methods Mol Med* 114, 195-207 (2005)
18. C.J. Campbell, and P. Ghazal: Molecular signatures for diagnosis of infection: application of microarray technology. *J Appl Microbiol* 96(1), 18-23 (2004)
19. X. Duburcq, C. Olivier, F. Malingue, R. Desmet, A. Bouzidi, F. Zhou, C. Auriault, H. Gras-Masse, and O. Melnyk : Peptide-protein microarrays for the simultaneous detection of pathogen infections. *Bioconjug Chem* 15(2), 307-16 (2004)
20. K.H. Rubins, L.E. Hensley, P.B. Jahrling, A.R. Whitney, T.W. Geisbert, J.W. Huggins, A. Owen, J.W. Leduc, P.O. Brown, and D.A. Relman: The host response to smallpox: analysis of the gene expression program in peripheral blood cells in a nonhuman primate model. *Proc Natl Acad Sci U S A* 101(42), 15190-5 (2004)
21. N.R. Thirumalapura, R.J. Morton, A. Ramachandran, and J.R. Malayer: Lipopolysaccharide microarrays for the detection of antibodies. *J Immunol Methods* 298(1-2):73-81 (2005)
22. T.Z. Wu, C.C. Su, L.K. Chen, H.H. Yang, D.F. Tai, and K.C. Peng: Piezoelectric immunochip for the detection of dengue fever in viremia phase. *Biosens Bioelectron* 21(5), 689-95 (2005)
23. A.A Witney, G.L. Marsden, M.T. Holden, R.A. Stabler, S.E. Husain, J.K. Vass, P.D. Butcher, J. Hinds, and J.A. Lindsay: Design, validation, and application of a seven-strain Staphylococcus aureus PCR product microarray for comparative genomics. *Appl Environ Microbiol* 71(11), 7504-14 (2005)
24. J.R. Fitzgerald, D.E. Sturdevant, S.M. Mackie, S.R. Gill, and J.M. Musser: Evolutionary genomics of Staphylococcus aureus: insights into the origin of methicillin-resistant strains and the toxic-shock syndrome epidemic. *Proc Natl Acad Sci USA* 98(15), 8821-8826 (2001)
25. S. Bekal, R. Brousseau, L. Masson, G. Prefontaine, J. Fairbrother, and J. Harel: Rapid identification of Escherichia coli pathotypes by virulence gene detection with DNA microarrays. *J Clin Microbiol* 41(5), 2113-25 (2003)
26. B. Korczak, J. Frey, J. Schrenzel, G. Pluschke, R. Pfister, R. Ehricht, and P. Kuhnert: Use of diagnostic microarrays for determination of virulence gene patterns of Escherichia coli K1, a major cause of neonatal meningitis. *J Clin Microbiol* 43(3), 1024-31 (2005)
27. R.A. Stabler, G.L. Marsden, A.A. Witney, Y. Li, S.D. Bentley, C.M. Tang, and J. Hinds: Identification of pathogen-specific genes through microarray analysis of pathogenic and commensal Neisseria species. *Microbiology* 151(Pt 9), 2907-22 (2005)
28. G.K. Schoolnik**:** Functional and comparative genomics of pathogenic bacteria. *Curr Opin Microbiol* 5(1), 20-6 (2002)
29. M. Kato-Maeda, J.T. Rhee, T.R. Gingeras, H. Salamon, J. Drenkow, N. Smittipat, and P.M. Small: Comparing genomes within the species Mycobacterium tuberculosis. *Genome Res* 11(4), 547-54 (2001)
30. D.R. Call, M.S. Kang, J. Daniels, and T.E. Besser: Assessing genetic diversity in plasmids from Escherichia coli and Salmonella enterica using a mixed-plasmid microarray. *J Appl Microbiol* 100(1), 15-28 (2006)
31. J.W. Bae, S.K. Rhee, Y.D. Nam, and Y.H. Park: Generation of subspecies level-specific microbial diagnostic microarrays using genes amplified from subtractive suppression hybridization as microarray probes. *Nucleic Acids Res* 33(13), e113 (2005)

32. H. Swiderek, H. Claus, M. Frosch, and U. Vogel: Evaluation of custom-made DNA microarrays for multilocus sequence typing of Neisseria meningitidis. *Int J Med Microbiol* 295(1), 39-45 (2005)
33. S. Porwollik, E.F. Boyd, C. Choy, P. Cheng, L. Florea, E. Proctor, and M. McClelland: Characterization of Salmonella enterica subspecies I genovars by use of microarrays. *J Bacteriol* 186(17), 5883-98 (2004)
34. D. Zhou, Y. Han, E. Dai, Y. Song, D. Pei, J. Zhai, Z. Du, J. Wang, Z. Guo, and R. Yang: Defining the genome content of live plague vaccines by use of whole-genome DNA microarray. *Vaccine* 22(25-26), 3367-7 (2004)
35. W.J. Wilson, C.L. Strout, T.Z. DeSantis, J.L. Stilwell, A.V. Carrano, and G.L.Andersen: Sequence-specific identification of 18 pathogenic microorganisms using microarray technology. *Mol Cell Probes* 16(2), 119-27 (2002)
36. V. Chizhikov, A. Rasooly, K. Chumakov, and D.D. Levy: Microarray analysis of microbial virulence factors. *Appl Environ Microbiol* 67(7), 3258-63 (2001)
37. D.R. Call, M.K. Bakko, M.J. Krug, and M.C. Roberts: Identifying antimicrobial resistance genes with DNA microarrays. *Antimicrob Agents Chemother* 47(10), 3290-5 (2003)
38. G. Vernet, C. Jay, M. Rodrigue, and A. Troesch: Species differentiation and antibiotic susceptibility testing with DNA microarrays. *J Appl Microbiol* 96(1), 59-68 (2004)
39. M. Cassone, M. D'Andrea, F. Iannelli., M.R. Oggioni, G.M. Rossolini, and G. Pozzi: DNA Microarrays for Detection of Macrolide Resistance Genes. *Antimicrob Agents Chemother* 50(6), 2038-2041 (2006)
40. D. Volokhov, A. Rasooly, K. Chumakov, and V. Chizhikov: Identification of Listeria species by microarray-based assay. *J Clin Microbiol* 40(12), 4720-8 (2002)
41. K. Tomioka, M. Peredelchuk, X. Zhu, R. Arena, D. Volokhov, A. Selvapandiyan, K. Stabler, J. Mellquist-Riemenschneider, V. Chizhikov, G. Kaplan, H. Nakhasi, and R. Duncan: A multiplex polymerase chain reaction microarray assay to detect bioterror pathogens in blood. *J Mol Diagn* 7(4), 486-94 (2005)
42. K.C. Keum, S.M. Yoo, S.Y. Lee, K.H. Chang, N.C. Yoo, W.M. Yoo, J.M. Kim, J.Y. Choi, J.S. Kim, and G. Lee: DNA microarray-based detection of nosocomial pathogenic Pseudomonas aeruginosa and Acinetobacter baumannii. *Mol Cell Probes* 20(1), 42-50 (2006)
43. A. Lehner, A. Loy, T. Behr, H. Gaenge, W. Ludwig, M. Wagner, and K.H. Schleifer: Oligonucleotide microarray for identification of Enterococcus species. *FEMS Microbiol Lett* 246(1), 133-42 (2005)
44. K. Sachse, H. Hotzel, P. Slickers, T. Ellinger, and R. Ehricht: DNA microarray-based detection and identification of Chlamydia and Chlamydophila spp. *Mol Cell Probes* 19(1), 41-50 (2005)
45. P. Francois, Y. Charbonnier, J. Jacquet, D. Utinger, M. Bento, D. Lew, G.M. Kresbach, M. Ehrat, W. Schlegel, and J. Schrenzel: Rapid bacterial identification using evanescent-waveguide oligonucleotide microarray classification. *J Microbiol Methods* 65(3), 390-403 (2006)
46. E.M. Marlowe, J.J. Hogan, J.F. Hindler, I. Andruszkiewicz, P. Gordon, and D.A. Bruckner: Application of an rRNA probe matrix for rapid identification of bacteria and fungi from routine blood cultures. *J Clin Microbiol* 41(11), 5127-33 (2003)
47. J.E. Burton, O.J. Oshota, E. North, M.J. Hudson, N. Polyanskaya, J. Brehm, G. Lloyd, and N.J. Silman: Development of a multi-pathogen oligonucleotide microarray for detection of Bacillus anthracis. *Mol Cell Probes* 19(5), 349-57 (2005)
48. A. Perrin, S. Bonacorsi, E. Carbonnelle, D. Talibi, P. Dessen, X. Nassif, and C. Tinsley: Comparative genomics identifies the genetic islands that distinguish Neisseria meningitidis, the agent of cerebrospinal meningitis, from other Neisseria species. *Infect Immun* 70(12), 7063-72 (2002)
49. N. Sergeev, D.,Volokhov, V. Chizhikov, and A Rasooly: Simultaneous analysis of multiple staphylococcal enterotoxin genes by an oligonucleotide microarray assay. *J Clin Microbiol* 42(5), 2134-43 (2004)
50. M.D. Brazas, and R.E. Hancock: Ciprofloxacin induction of a susceptibility determinant in Pseudomonas aeruginosa. *Antimicrob Agents Chemother* 49(8), 3222-7 (2005)
51. H. Gmuender, K. Kuratli, K. Di Padova, C.P. Gray, W. Keck, and S. Evers: Gene expression changes triggered by exposure of Haemophilus influenzae to novobiocin or ciprofloxacin: combined transcription and translation analysis. *Genome Res* 11(1), 28-42 (2001)
52. C. Freiberg, H.P. Fischer, and N.A. Brunner: Discovering the mechanism of action of novel antibacterial agents through transcriptional profiling of conditional mutants. *Antimicrob Agents Chemother* 49(2), 749-59 (2005)
53. D. Volokhov, V. Chizhikov, K. Chumakov, A. Rasooly: Microarray analysis of erythromycin resistance determinants. *J Appl Microbiol* 95(4), 787-98 (2003)
54. V. Perreten, L. Vorlet-Fawer, P. Slickers, R. Ehricht, P. Kuhnert, and J. Frey: Microarray-based detection of 90 antibiotic resistance genes of gram-positive bacteria. *J Clin Microbiol* 43(5), 2291-302 (2005)
55. A.H. van Hoek, I.M. Scholtens, A. Cloeckaert, and H.J. Aarts: Detection of antibiotic resistance genes in different Salmonella serovars by oligonucleotide microarray analysis. *J Microbiol Methods* 62(1), 13-23 (2005)
56. J.G. Frye, T. Jesse, F. Long, G. Rondeau, S. Porwollik, M. McClelland, C.R. Jackson, M. Englen, and P.J. Fedorka-Cray: DNA microarray detection of antimicrobial resistance genes in diverse bacteria. *Int J Antimicrob Agents* 27(2), 138-51 (2006)
57. V. Grimm, S. Ezaki, M. Susa, C. Knabbe, R.D. Schmid, and T.T. Bachmann: Use of DNA microarrays for rapid genotyping of TEM beta-lactamases that confer resistance. *J Clin Microbiol* 42(8), 3766-74 (2004)
58. J. Yue, W. Shi, J. Xie, Y. Li, E. Zeng, L. Liang, and H. Wang: Detection of rifampin-resistant Mycobacterium tuberculosis strains by using a specialized oligonucleotide microarray. *Diagn Microbiol Infect Dis* 48(1), 47-54 (2004)
59. X. Yu, M. Susa, C. Knabbe, R.D. Schmid, and T.T. Bachmann: Development and validation of a diagnostic DNA microarray to detect quinolone-resistant Escherichia coli among clinical isolates. *J Clin Microbiol* 42(9), 4083-91 (2004)
60. R.F. Wang, M.L. Beggs, L.H. Robertson, and C.E. Cerniglia: Design and evaluation of oligonucleotide-

microarray method for the detection of human intestinal bacteria in fecal samples. *FEMS Microbiol Lett* 213(2), 175-82 (2002)
61. G. Keramas, D.D. Bang, M. Lund, M. Madsen, S.E. Rasmussen, H. Bunkenborg, P. Telleman, and C.B. Christensen: Development of a sensitive DNA microarray suitable for rapid detection of Campylobacter spp. *Mol Cell Probes* 17(4), 187-96 (2003)
62. L. Davignon, E.A. Walter, K.M. Mueller, C.P. Barrozo, D.A. Stenger, and B. Lin: Use of resequencing oligonucleotide microarrays for identification of Streptococcus pyogenes and associated antibiotic resistance determinants. *J Clin Microbiol* 43(11), 5690-5 (2005)
63. M. Fukushima, K. Kakinuma, H. Hayashi, H. Nagai, K. Ito, and R. Kawaguchi: Detection and identification of Mycobacterium species isolates by DNA microarray. *J Clin Microbiol* 41(6), 2605-15 (2003)
64. S.B. Roth, J. Jalava, O. Ruuskanen, A. Ruohola, and S. Nikkari: Use of an oligonucleotide array for laboratory diagnosis of bacteria responsible for acute upper respiratory infections. *J Clin Microbiol* 42(9), 4268-74 (2004)
65. S. Shang, G. Chen, Y. Wu, L. DU, and Z. Zhao: Rapid diagnosis of bacterial sepsis with PCR amplification and microarray hybridization in 16S rRNA gene. *Pediatr Res* 58(1), 143-8 (2005)
66. G. Shi, S.Y. Wen, S.H. Chen, and S.Q. Wang: Fabrication and optimization of the multiplex PCR-based oligonucleotide microarray for detection of Neisseria gonorrhoeae, Chlamydia trachomatis and Ureaplasma urealyticum. *J Microbiol Methods* 62(2), 245-56 (2005)
67. G.J. Vora, C.E. Meador, M.M. Bird, C.A. Bopp, J.D. Andreadis, and D.A. Stenger: Microarray-based detection of genetic heterogeneity, antimicrobial resistance, and the viable but nonculturable state in human pathogenic Vibrio spp. *Proc Natl Acad Sci U S A* 102(52), 19109-14 (2005)
68. C. Palmer, E.M. Bik, M.B. Eisen, P.B. Eckburg, T.R. Sana, P.K. Wolber, D.A. Relman, and P.O. Brown: Rapid quantitative profiling of complex microbial populations. *Nucleic Acids Res* 34(1), e5 (2006)
69. R.H. Liu, J. Yang, R. Lenigk, J. Bonanno, and P. Grodzinski: Self-contained, fully integrated biochip for sample preparation, polymerase chain reaction amplification, and DNA microarray detection. *Anal Chem* 76(7), 1824-31 (2004)
70. T.M. Straub, B.P. Dockendorff, M.D. Quinonez-Diaz, C.O. Valdez, J.I. Shutthanandan, B.J. Tarasevich, J.W. Grate, and C.J. Bruckner-Lea: Automated methods for multiplexed pathogen detection. *J Microbiol Methods* 62(3), 303-16 (2005)
71. Y. Matsubara, K. Kerman, M. Kobayashi, S. Yamamura, Y. Morita, and E. Tamiya: Microchamber array based DNA quantification and specific sequence detection from a single copy via PCR in nanoliter volumes. *Biosens Bioelectron* 20(8), 1482-90 (2005)
72. E. Southern, K. Mir, and M. Shchepinov: Molecular interactions on microarrays. *Nat Genet* 21(1 Suppl), 5-9 (1999)
73. A.E. Murray, D. Lies, G. Li, K. Nealson, J. Zhou, and J.M. Tiedje: DNA/DNA hybridization to microarrays reveals gene-specific differences between closely related microbial genomes. *Proc Natl Acad Sci U S A* 98(17), 9853-8 (2001)
74. M. Gadgil, W. Lian, C. Gadgil, V. Kapur, and W.S. Hu: An analysis of the use of genomic DNA as a universal reference in two channel DNA microarrays *BMC Genomics* 6(1), 66 (2005)
75. G.J. Vora, C.E. Meador, D.A. Stenger, and J.D. Andreadis: Nucleic acid amplification strategies for DNA microarray-based pathogen detection. *Appl Environ Microbiol* 70(5), 3047-54 (2004)

Key Words: Microarrays, Clinical, Diagnostic, Bacteria, Antibiotic, Resistance, Infection, Molecular, Biotechnology, DNA Chip, Review

Send correspondence to: Marco Cassone, M.D., Ph.D., College of Science and Technology, Center for Biotechnology , Temple University, BioLife Science Building, Suite 333, 1900 N 12th Street, Philadelphia, PA 19122-6099, USA, Tel: 215- 204-9524, Fax: 215-204-9522, E-mail: cassone@temple.edu

http://www.bioscience.org/current/vol12.htm