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Diagnostic Approaches to Combat Antibiotic Resistance in Bacteria

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ABSTRACT

ALEX OLIVERI, ANSHUL ANUGU, SHADE SMITH: Diagnostic Approaches to Combat Antibiotic Resistance in Bacteria

(Under the direction of Dr. Colin Jackson)

The threat of antibiotic resistance is a major problem faced by the healthcare field affecting millions of people and costing tens of thousands of lives annually. Of the potential ways to mitigate this issue the field of antibiotic resistance testing presents an opportunity for significant improvement and benefits. Several methods of such diagnostic processes can yield more informative results than the current commonly used Kirby-Bauer test. However, there are benefits and limitations to each method. In the context of a clinically relevant diagnostic for antibiotic resistance, the microarray platform exhibits the necessary breadth with opportunities to overcome some limitations with further research and development. There is potential for innovation of this method to automate it and optimize its efficiency in a clinical setting for the purpose of antibiotic resistance diagnosis. This presents a hopeful tool that physicians can rely upon to combat antibiotic resistance and improve patient outcomes.

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LIST OF ABBREVIATIONS

CARD	Comprehensive Antibiotic Resistance Database
CDC	Centers for Disease Control and Prevention
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction

INTRODUCTION

The problem of antibiotic resistance is a major concern to global healthcare. There is a large and rapid emergence of antibiotic resistant bacteria occurring worldwide, threatening the efficacy of current antibiotics. This problem has created a substantial burden on hospitals, health care systems, insurance companies, families, and government. The CDC classifies this as an urgent issue and as one of the biggest public health challenges of our time, as each year, at least 2.8 million people in the United States contract an antibiotic resistant infection, which accounts for more than 35,000 deaths [1].

The emergence of this antibiotic resistance crisis can be traced to the abundant use of antibiotics [1]. National guidelines have been created to assist with proper stewardship of antibiotics, and many education programs are in place. However, the extent to which antibiotics are prescribed improperly is not well known. Data from 182,032 outpatient visits from 2010-2011 suggest that an estimated 154 million prescriptions are prescribed annually, or 506 per 1000 people [2]. Of those 506 instances of antibiotic prescription, experts on outpatient antibiotic use suggest that only 353 were likely appropriate [2].

Since that study, the CDC reports that education programs are working, and antibiotic use has been reduced [1]. However, antibiotics are still used in massive quantities and stewardship is still an issue. Regarding decreasing antibiotic use for common infections, there seems to be a balancing act. There is concern of doctors about issues such as suppurative complications and the desire of patients to prioritize their individual health that drives antibiotic prescription up [2]. Indeed, patients are less satisfied when not prescribed antibiotics [3]. In contrast, national and international antibiotic stewardship programs and governmental guidelines seek to drive

prescriptions down. However, even with appropriate antibiotic use, there would still be 107 million prescriptions annually in the United States alone [2]. This prolific use of antibiotics drives further resistance.

The COVID epidemic of 2019-2021 could also have a potential impact on antibiotic resistance, potentially through increased telehealth visits. Antibiotic stewardship practices are worse for telehealth: For example, for acute respiratory tract infections, 52% of telemedicine encounters resulted in antibiotic prescription as opposed to 31% of primary care and 42% of urgent care visits [4]. This is a supposed result of the lack of diagnostic procedures such as physical exams and laboratory tests [4].

Another study of 8,437 respiratory tract infection telemedicine encounters found that there is a significant correlation between physician rating and antibiotic prescription rate. Further, 66.1% of encounters resulted in an antibiotic prescription. Of the patients who received an antibiotic, 90.9% rated their care as five stars whereas only 72.5% rated their care five stars when not receiving a prescription, and there was a clear relationship between patient satisfaction and an individual physician's rate of prescribing antibiotic. [Fig. 1; 5]

The antibiotic resistance crisis cannot be solved through new antibiotics, as antibiotic development has slowed with most new drugs simply consisting of minor variations over old medications [6]. Barring development of an evolution-proof miracle drug, it does not seem antibiotic development will be a long-lasting solution to combat resistance. Instead, there exists an arms race between novel antibiotics and resistance mechanisms. For example, in the early 1980s many β-lactam antibiotics had become less effective due to resistance such as β-lactamase enzymes [7]. New extended-spectrum cephalosporins were released which were initially effective, but quickly extended-spectrum β-lactamases appeared. By 2010, over 200 different

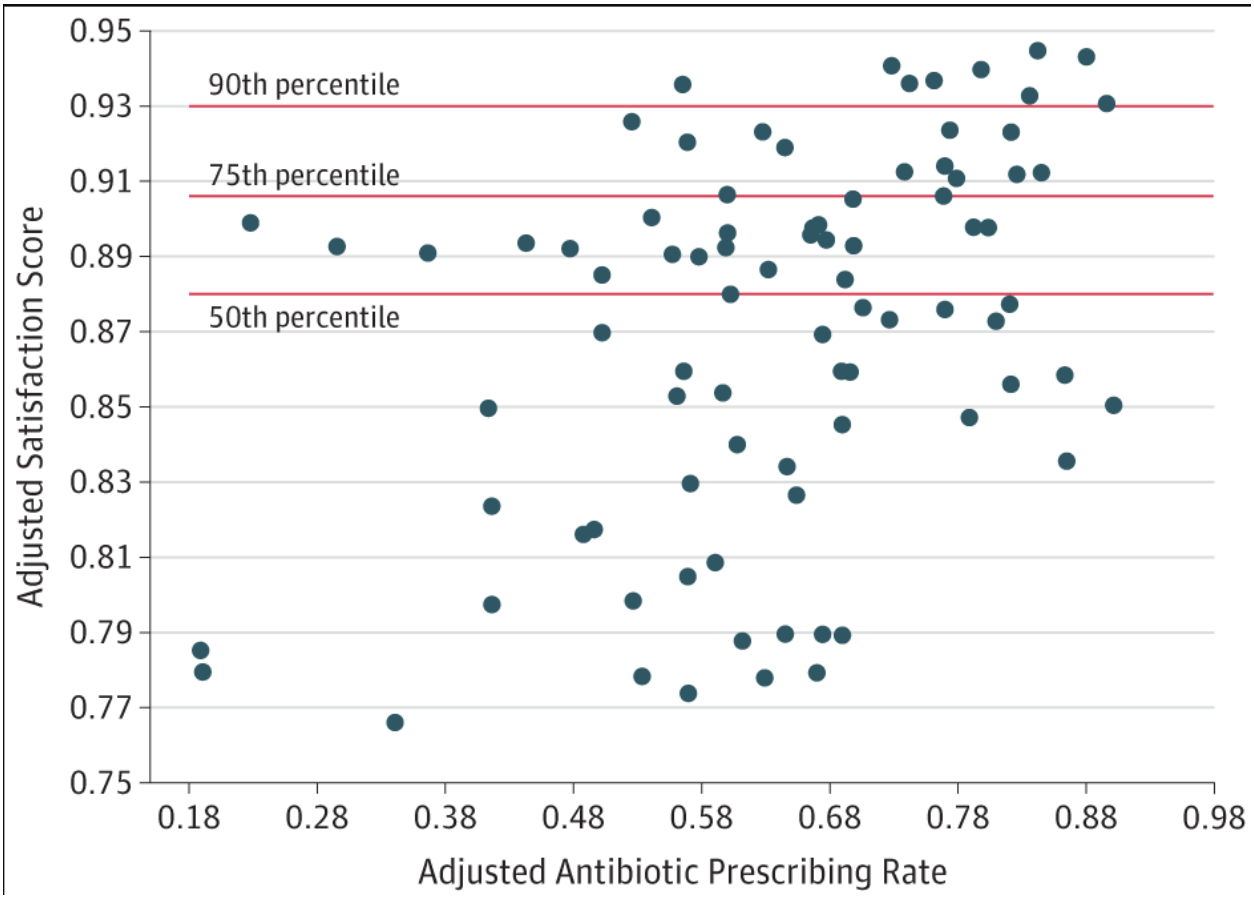

Fig.1: Prescription of antibiotics has a significant correlation to patient satisfaction rating. Almost all physicians in the 90th percentile had a prescription rate greater than 75% [5].

extended-spectrum β-lactamase had been discovered [7]. There are more examples like this where new, effective antibiotics have become ineffective because of the evolution of antibiotic resistance in bacteria (Fig. 2; 1). Thus, it is apparent that a more permanent solution to treat antibiotic resistance is required than simply developing novel antibiotics.

Germs Develop Antibiotic Resistance

Select Germs Showing Resistance Over Time

Since the discovery of penicillin more than 90 years ago, germs have continued to develop new types of resistance against even our most powerful drugs. While antibiotic development has slowed, antibiotic resistance has not. This table demonstrates how rapidly important types of resistance developed after approval and release of new antibiotics, including antifungals.

Antibiotic Approved or Released	Year Released	Resistant Germ Identified	Year Identified
Penicillin	1941	Penicillin-resistant *Staphylococcus aureus*[20, 21]	1942
		Penicillin-resistant *Streptococcus pneumoniae*[9,10]	1967
		Penicillinase-producing *Neisseria gonorrhoeae*[11]	1976
Vancomycin	1958	Plasmid-mediated vancomycin-resistant *Enterococcus faecium*[12,13]	1988
		Vancomycin-resistant *Staphylococcus aureus*[14]	2002
Amphotericin B	1959	Amphotericin B-resistant *Candida auris*[15]	2016
Methicillin	1960	Methicillin-resistant *Staphylococcus aureus*[16]	1960
Extended-spectrum cephalosporins	1980 (Cefotaxime)	Extended-spectrum beta-lactamase- producing *Escherichia coli*[17]	1983
Azithromycin	1980	Azithromycin-resistant *Neisseria gonorrhoeae*[18]	2011
Imipenem	1985	*Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae*[19]	1996
Ciprofloxacin	1987	Ciprofloxacin-resistant *Neisseria gonorrhoeae*[20]	2007
Fluconazole	1990 (FDA approved)	Fluconazole-resistant *Candida*[21]	1988
Caspofungin	2001	Caspofungin-resistant *Candida*[22]	2004
Daptomycin	2003	Daptomycin-resistant methicillin-resistant *Staphylococcus aureus*[23]	2004
Ceftazidime-avibactam	2015	Ceftazidime-avibactam-resistant KPC-producing *Klebsiella pneumoniae*[24]	2015

Fig. 2. Development of Antibiotic Resistance to New Antibiotics [1].

DEVELOPMENT OF IMPROVED DIAGNOSTICS

One area that has room for improvement and may lead to permanently better treatment is diagnostics. Better, more available diagnostics can lead to new treatment options. There are many strains of multidrug-resistant bacteria that have a dangerous resistance to many antibiotics. This potentially leads physicians to jump to last-resort antibiotics more often than necessary, reducing their efficacy overtime. Furthermore, there are many cases where a particular antibiotic or treatment would likely be effective, but this needs to be identified early on during diagnosis and treatment.

For example, a case study in 2012 looked at a potential treatment for respiratory tract infection by multidrug-resistant *Acetinobacter baumannii* [8]. It was found that inhaled colistin methanesulfonate was effective against the infection and significantly reduced hospital stays and enhanced patient recovery. However, colistin is a last-resort antibiotic, which should not be used more than necessary as some resistant strains have been documented, and there is some evidence of rare neuro and nephrotoxicity [9]. Thus, diagnostic methods are important to distinguish between necessary and unnecessary use of antibiotics such as colistin.

The CDC states that, “Diagnostics can be just as critical for fighting infections as antibiotics. These tools help human and animal healthcare providers identify infections as soon as possible and guide selection of the best treatment option for their patient. In addition, they help sound the alarm that alerts local infection control programs and health departments to emerging threats.” [1]. The CDC cites further benefits to improved diagnostic testing such as

improving accuracy and speed of diagnosis, improving appropriate antibiotic use, reducing unnecessary antibiotic use, and assisting epidemiological measures. More development is required to current technology because it is costly, time consuming, and does not detect emerging resistance markers or rapidly discriminate bacterial/fungal infections from viral ones [1].

The complexity and severity of antibiotic resistant forms appear in stark contrast to current diagnostic tools. Diagnostic tests for antibiotic resistance or susceptibility testing are seldom used clinically unless an infection is severe or not responding to treatment. When used, the primary method by clinics is to determine antibiotic resistance is culturing on Kirby-Bauer disk diffusion plates. This method has some major drawbacks. It takes up to two days to plate and isolate bacteria, and then another several hours to determine resistance via culture with antimicrobial agents [10]. During this time, patients will often already be prescribed an antibiotic, potentially an inappropriate one that contributes to resistance and makes it less effective for successive patients. Further, there is a tremendous amount of diversity among bacteria. Some species grow slowly or have special growth requirements and will not grow easily on the Mueller-Hinton agar used in Kirby-Bauer. In fact, it is estimated that only 2% of bacteria species are actually culturable [11], although the percentage is noticeably higher for pathogens. Additionally, there are several antibiotics such as vancomycin which are composed of large molecules that diffuse slowly. This slow diffusion makes the distinction between resistance and susceptibility a matter of millimeters which can be difficult to determine. Finally, in vitro susceptibility does not always translate to in vivo efficacy. For example, *Salmonella enterica* exhibits in vitro susceptibility to aminoglycosides. However, in an actual infection *Salmonella* invades host cells, protecting it from aminoglycosides which have poor membrane permeability

[12]. A better diagnostic method is needed to combat antibiotic resistance and improve patient outcomes. This diagnostic method must be sufficiently rapid, inexpensive, and must be capable of assessing susceptibility to a wider range of pathogens and antibiotics.

DIFFERENT APPROACHES TO DIAGNOSIS

Currently, there are several approaches from which one can attempt to create a diagnostic method fitting these criteria. As Sandle writes, "The use of genotypic approaches for detection of antimicrobial resistance genes has been promoted as a way to increase the rapidity and accuracy of susceptibility testing. Methods that employ the use of comparative genomics, genetic probes, microarrays, nucleic acid amplification techniques (such as polymerase chain reaction (PCR)), and DNA sequencing are capable of increased sensitivity, specificity, and speed in the detection of specific known resistance genes"[10]. Genotypic methods are now capable, especially with the ever-growing databases that catalogue research on genes denoting antibiotic resistance. For example, one of the largest and most maintained databases is the Comprehensive Antibiotic Resistance Database (CARD) that contains the sequences of 88 pathogens, 9560 chromosomes, and 21362 plasmids, as well as other data [13].

For any potential genotypic method to detect antibiotic sensitivity, the first step of the diagnostic procedure regardless is to collect a sample from the patient. This can take many forms such as a throat swab, stool sample, blood sample, etc. This sample must be processed to remove as much human DNA as possible, as there is often a low ratio of pathogen to human DNA. Successful processing thus confers several advantages such as the ability to detect pathogens that would be undetectable in unprocessed samples [14].

Various methods can be used to separate pathogen DNA from human genetic material. The easiest ways often involve taking advantage of the size difference between eukaryotic cells and pathogen cells or viruses. One method is to filter a sample through a filter. This allows for

bacteria cells and viruses to pass through, while larger human cells remain behind. To illustrate with an example, consider a patient with a bloodstream infection. Most nucleated human blood cells range in diameter from around 10 to 20 microns [15]. In contrast, most bacteria range from 0.2 to 2 microns in diameter, and of course, viruses are much smaller. Thus, we speculate that a Millipore filter with a pore size around 10 microns should selectively filter pathogens from human cells. Then, DNA extraction can be performed on the filtrate.

Another method to selectively obtain the cells of a pathogen is separation by centrifugation. Differential centrifugation centrifuges a sample in a sucrose solution, with the rate of sedimentation almost entirely dependent on the size of particles. However, this method can have poor resolution as smaller particles near the bottom of the tube will pellet with larger particles. Furthermore, particles along the sides of the tube will move more rapidly than in the center [16]. Density gradient centrifugation solves some of these problems by using multiple layers of increasing sucrose concentrations to give a much higher resolution than simple differential centrifugation. Density gradient centrifugation offers two potential separation methods, rate separation and equilibrium separation. Rate separation slowly centrifuges the solution for a short time, so that larger particles travel farther than small ones. Equilibrium separation centrifuges the solution rapidly for a longer time. This causes particles to separate out by density rather than size. Centrifugation methods are easy and inexpensive to perform but may be difficult to efficiently isolate pathogens from human cells, especially if the pathogen size or density is unknown. Pathogens will vary greatly in size and density depending on species and, depending on the type of clinical sample, there can be similar variation in human cells. Thus, centrifugation appears to be much less of a one-size-fits-all method for separation than Millipore filtration.

Hasan, et al. (2016) assessed the effectiveness of other methods in processing of samples. They compared several detergents for the ability to selectively lyse human cells in spiked cerebrospinal fluid (CSF) and nasopharyngeal aspirate (NPA) aliquots as compared to the commercially available MolYsis kit (Molzym GmbH & Co. KG, D-28359, Bremen, Germany) which was found to be ineffective [17]. The detergent with the most success was Saponin, a glycoside with hemolytic activity. Saponin interacts with membrane-bound sterols, increasing plasma membrane permeability and destroying the cell [17]. At a concentration of 0.025%, Saponin was effective at enriching the concentration of microbial DNA (Fig. 3; 15). However, it appears less effective on Gram-positive bacteria such as *Streptococcus pneumoniae* and enveloped viruses such as HSVII

The procedure used in this study was simple and inexpensive. Saponin was added to aliquots to a 0.025% concentration. Samples were then vortexed for 10 seconds and incubated for 5 minutes at room temperature. DNAse was then added and the samples were incubated at 37℃ for 30 minutes. This represents yet another option for the selective enrichment of microbial DNA.

After selective isolation of microbial cells, DNA is extracted. It is not uncommon for procedures to involve enzymes, hazardous chemicals, fume hoods, and require a lot of time with many steps involved. However, in a clinical setting, it is important to have an easy and rapid extraction method. Recently, researchers have been looking into using hydrophilic ionic liquids. These are organic salts which are liquid below 100℃ and are effective at lysing cells. In the past, these liquids have had trouble with the hard peptidoglycan layer of Gram-positive bacteria, but a recent paper shows a procedure that seems to overcome this difficulty [18]. That study compared a simple procedure with ionic liquids to commercial DNA extraction kits and a traditional phenol

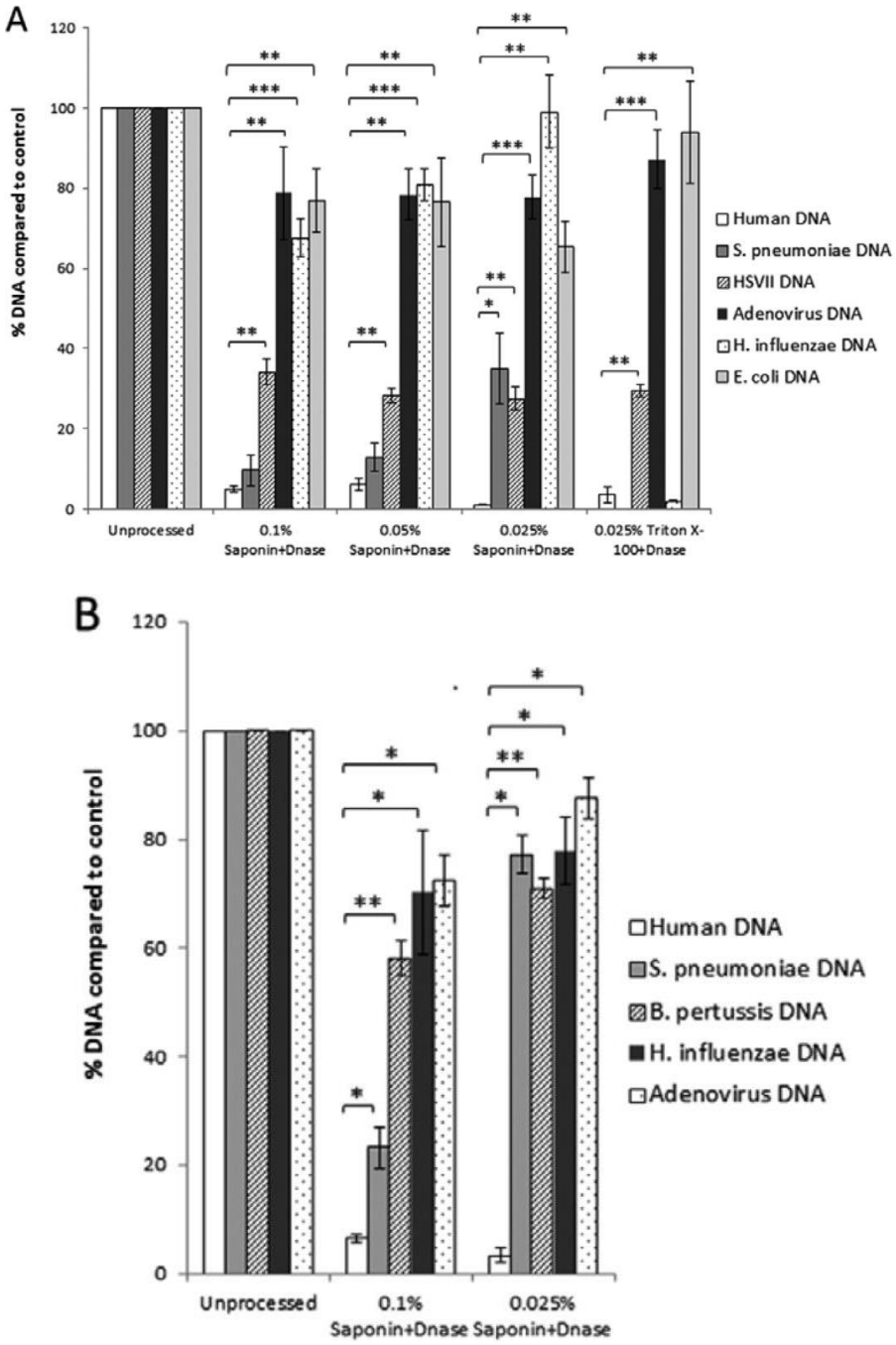

Fig. 3. Effectiveness of microbial DNA extraction methods using various detergents and concentrations are compared for their ability to selectively lyse human cells. Samples were processed and then analyzed with real-time PCR to measure the amount of nucleic acids present in the extract. These methods were compared for different pathogens [17].

chloroform/enzyme procedure. The most effective ionic liquid procedure was found to be adding either 90% w/w 1-Ethyl-3-methylimidazolium acetate or 50% w/w choline hexanoate in Tris pH 8 buffer to pelleted and resuspended cells, followed by incubation at 65℃ for 5 minutes to lyse cells. The authors used qPCR to quantitatively compare five different methods of extraction (Fig. 4; 16).

The ionic liquid procedures were never the best method for any strain of bacteria, but one or both liquids were always close to optimal (Fig. 4; 18). Considering the potential savings in time and money that the ionic liquid procedures provide, they may well be suitable for a clinical diagnostic test. However, a potential downside to using ionic liquids is that they can have an inhibitory effect on the amplification reaction in PCR. This inhibition can be mitigated by dilution, although dilution can reduce the sensitivity of the diagnostic test, so that other approaches such as the use of a DNA-binding column or silica beads to isolate DNA are recommended [18].

After DNA extraction, PCR may be performed as a diagnostic procedure for antibiotic sensitivity. Multiplex PCR has been used to identify antibiotic resistance and has proven effective [19-21]. Advantages of this approach include sensitivity, specificity, inexpensiveness, and rapidity. It certainly represents an improvement over traditional Kirby-Bauer or other similar phenotypic methods of testing antibiotic sensitivity and in clinical situations where it is important to rapidly or precisely identify antibiotic sensitivity, PCR represents a convincing alternative.

However, PCR has disadvantages in that a level of technical knowledge and experience is required to optimize and perform the procedure. Furthermore, PCR requires a variety of different

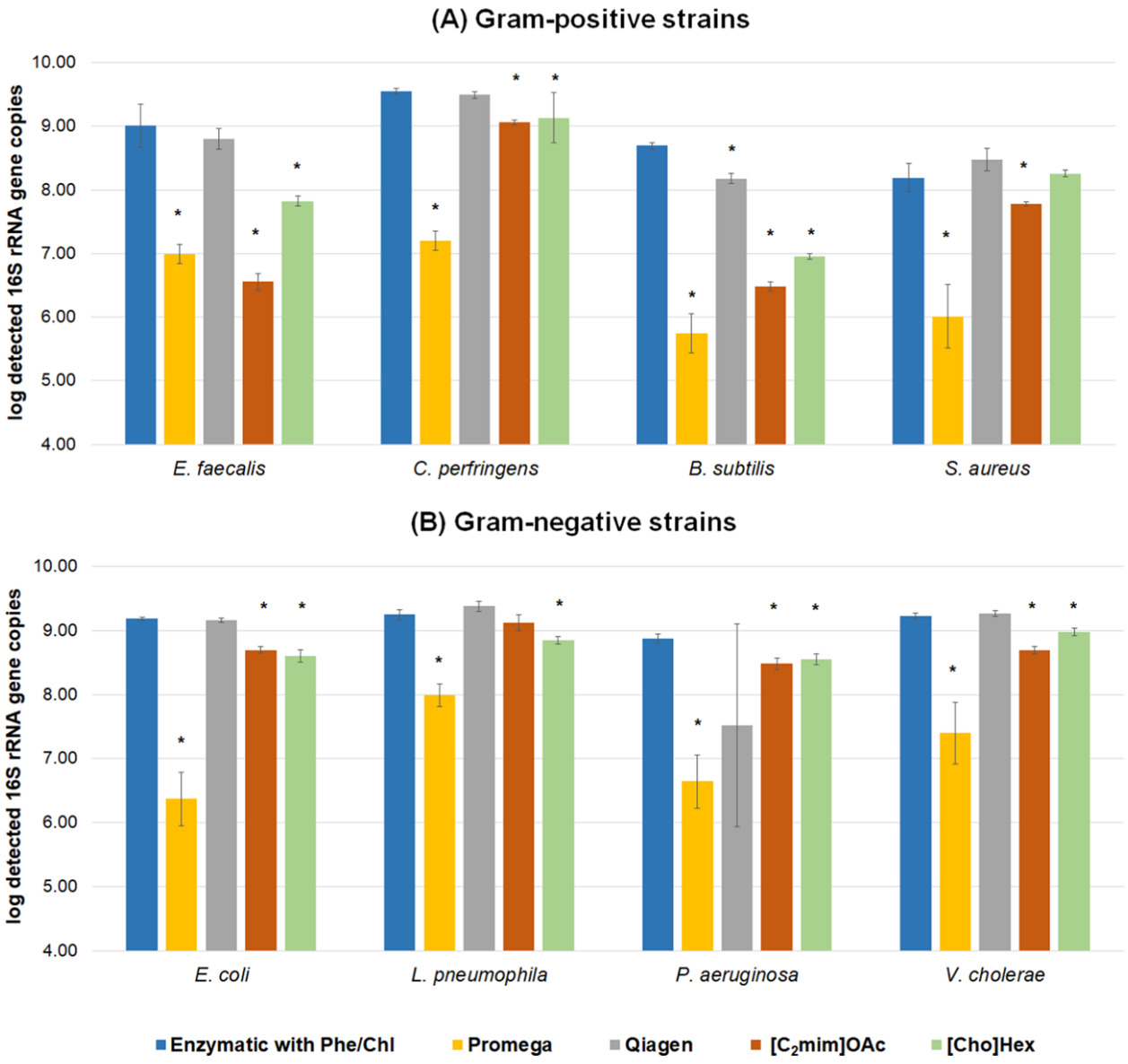

Fig. 4. Five DNA extraction methods were used and analyzed with qPCR to quantify the amount of nucleic acids extracted. These were compared across a range of both gram-positive and gram-negative pathogenic bacteria. [C₂mim]OAc abbreviates the ionic liquid 1-Ethyl-3-methylimidazolium acetate and [Cho]Hex is choline hexanoate. [18].

chemicals to be kept on-hand, most of which must be stored frozen and must be checked to ensure they are always in stock. These concerns may present a logistical headache for smaller clinics although it is possible to purchase pre-mixed PCR kits which would streamline the diagnosis process for clinics. The amount of technical knowledge required could also be mitigated through automation of the process. Ultimately, however, the greatest limitation to PCR for susceptibility testing does not appear to have a remedy. PCR is inherently limited in its scope of detection. As a general-purpose antibiotic susceptibility diagnostic, PCR does not have enough breadth to identify the incredibly numerous resistance forms.

Another diagnostic method for antibiotic sensitivity is a microarray system. There are several disadvantages to the microarray platform for such a diagnostic system. It can be expensive, user unfriendly, time-consuming, and probes must be carefully designed [22]. Despite the disadvantages, it offers a major advantage in that the scope of a microarray diagnostic is huge. Commercially available high density microarray chips can be spotted with up to 80,000 probe sequences. This would allow for broad diagnostic capability for many antibiotic resistance genes, limited most by the time required to design probes and the ability to mitigate the disadvantages of the platform.

To illustrate the use of a microarray, consider the most widely used class of antibiotics, β-lactams. β-lactams function by binding to and inactivating penicillin-binding proteins, enzymes that are required for the construction and maintenance of the bacterial cell wall. There are four ways that bacteria resist β-lactams. (1) They can produce β-lactamases, which are enzymes that directly degrade the antibiotics. (2) Gram-negative bacteria can also have efflux pumps that keep the antibiotics from reaching the cell wall. (3) Some bacteria have a horizontally transferred gene for a penicillin-binding protein that has an active site that does not recognize β-lactams. (4) Some

Gram-negative bacteria have point or insertion mutations in genes encoding porin proteins in the outer membrane, and the loss of these porins means antibiotics cannot access the cell wall. However, research indicates that this last type of resistance is not significant enough to cause resistance unless it is coupled with one of the other three mechanisms of resistance [7]. For those three mechanisms (β-lactamases, penicillin binding proteins, and efflux proteins) there are many gene sequences available in CARD. Thus, complementary probes to these sequences could be designed, taking into account a small amount of non-complementary pairing to accommodate genetic drift, particularly in third codon positions. While this approach could not be used to detect single nucleotide mutations to porin genes, that would not matter because that mechanism of resistance must be coupled with a resistance mechanism that could be detected. The large scope of a microarray device means that these concepts could be applied to many other classes of antibiotics simultaneously, and there are several areas in which the typical microarray procedure could become easier to perform in a clinical setting.

In many microarray experiments, the next step after extraction of genetic material is PCR amplification. PCR is performed to enhance the sensitivity of the microarray diagnostic by amplifying DNA before microarray hybridization. In some cases, DNA is randomly amplified while in other cases primers specific to resistance genes have been used [23-25]. However, in the majority of clinical samples with a problematic infection, PCR amplification should not be necessary. Microarrays, especially high-density ones, have an abundance of probes and can detect small amounts of complementary genetic material. Studies suggest that the detection limit of microarrays without amplification is close to that of real-time PCR [26]. While the addition of amplification could increase the sensitivity 100- to 1000-fold, this is not likely to be relevant enough to justify PCR and the associated drawbacks in a clinical setting. As discussed

previously, a diagnostic method for antibiotic resistance must be rapid, inexpensive, and easy to use. A PCR amplification would involve additional time, cost, and expertise required. If increased sensitivity is needed, it could occur through sample preparation involving as little dilution as possible and procedures to remove human DNA contaminants.

PCR can also be used to label a sample, and labeling could present a challenge without PCR. Biotin is often used to label DNA for microarray procedures, but is a time-consuming process [27]. One option may be to label DNA retroactively after hybridization. After hybridization and the subsequent washes, a pre-prepared protein that has already been labeled outside of the clinic could be added. If this protein binds selectively to double-stranded DNA or is large enough to be incapable of binding to the relatively short probe sequences, then PCR is unnecessary for labeling.

MICROARRAY PLATFORM INNOVATION

The microarray process of hybridization and subsequent washes can take time and requires expertise. Automation could be used to enable individuals with less technical knowledge to perform the process. Many diagnostic tests are a self-contained system, which enables their ease of use. In this case, a single device could be designed that is capable of automating the procedures necessary to run extracted microbial DNA from a clinical sample on a high density microarray chip. The device should be small and house a central bay for insertion of a single or small amount of glass microarray slides. Once the microarray chip is inserted, the device would be activated to perform a standardized procedure for the antibiotic sensitivity test. This would consist of hybridization, wash, and reading results subroutines. The device would be connected to a computer where compatible software would analyze and interpret results.

Such a device would represent a significant improvement over previous equipment for microarray procedures which are either large, expensive machines performing a singular function, or less expensive machines that require manual operation. A microarray diagnostic procedure using such existing equipment is not practical enough to achieve prevalence in clinical use because of the expertise and expense necessary to conduct microarray procedures as they are today. However, a smaller device capable of automating the process from hybridization through data interpretation, would be much more palatable to clinics.

While there may be multiple ways to develop this automated device, we present an alternate microarray system that we have designed to meet the goals of easier automation, efficiency, and improved DNA hybridization to the complementary probes. Hybridization can be done in as little as a single hour without amplification and detect as low as 2ng or 15.6fM of

DNA [26]. With better hybridization, this could be even more sensitive. Our alternate process makes use of the physical advantage of different geometric shapes. In a regular microarray, a flat, rectangular piece of glass (the microarray chip) must oscillate for hours. The flat glass design for probes often complements an agitation apparatus shaped like a basket. Such a configuration for agitating solutions across the microarray chip is somewhat bulky, which limits the number of probes that can occupy a machine at one time. By changing the probe shape from a flat plane to a ring, there is more opportunity to utilize the same amount of space more efficiently. The result would be an increased number of microarray chips which can simultaneously occupy a machine of similar size, thus allowing more clinical samples to be processed at one time.

Instead of a flat sheet of glass or polymer, this novel type of "ring microarray" would be manufactured by immobilizing probe sequences on a long, thin, flat polymer surface which is then curved and fused to form a ring structure. A ring microarray takes advantage of many physical benefits. The first part of the process is the hybridization of genetic material to probes on the microarray surface. The ring microarray has an advantage in this process because of its circular shape. Current microarray hybridization machines are expensive, in part, because they must ensure that the hybridization buffer and genetic material are evenly spread across the flat surface and encounters each probe while simultaneously incubating. This is difficult and leads to long hybridization times otherwise hybridization efficiency is detrimentally affected. A thin, ring microarray could just be rotated to achieve the same purpose of contacting every probe. This is significantly easier to automate. The hollow center of the ring could be used to provide a heating source and uniformly incubate the array. Furthermore, the genetic material is more likely to be in close proximity to its complementary probe because the chip has much less width and the probe

will encounter sample genetic material every time the ring makes a full circle. This should increase hybridization efficiency allowing for a more sensitive and rapid procedure (Fig. 5).

Following hybridization, the next process in a microarray procedure is to wash off non-complementary genetic material so that the chip can be scanned for fluorescence to "read" it. This is a sensitive procedure and requires other expensive automated equipment or an experienced and talented person to perform. A ring system, however, could take advantage of gravity in that the ring, in an upright position, will enable gravity to pull contents to the bottom but genetic material hybridized to the immobilized probe sequences will travel to the top. Thus, a minimal or even no wash procedure could suffice if the reading system was located at the top of the ring. Only hybridized material would make it to the top of the ring and be scanned for fluorescence. Thus, because of the shape and positioning of components, the entire procedure of hybridization, washing, and reading could be automated through a simple, slow circular motion of the ring. Such an automated system would have ease of use, and be much more tractable for a small to medium sized clinic to operate than any current system.

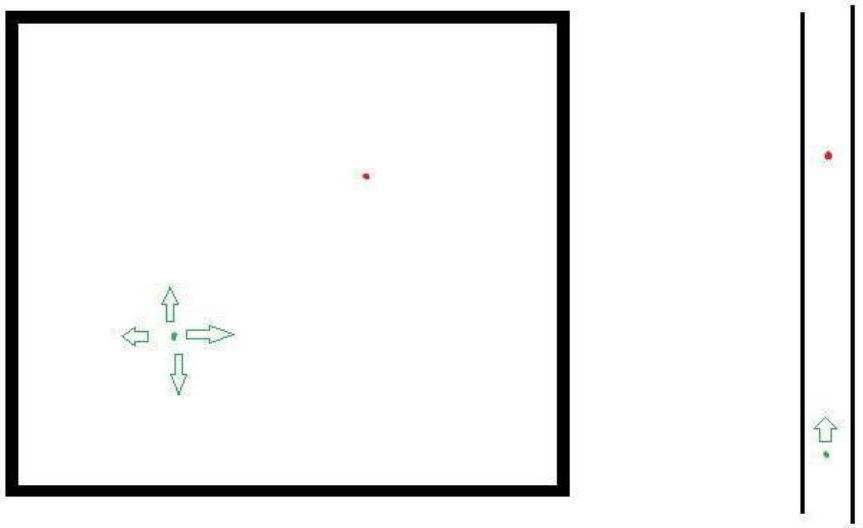

Fig. 5. The concept of a ring microarray. Green dots represent sample DNA and arrows represent their movement. Red dots represent the complementary probe hybridized to the microarray surface. As shown, the probability of sample DNA encountering its complementary probe is much higher on a ring than the traditional square layout.

CONCLUSION

The ongoing and growing problem of antibiotic resistance requires development of a broad, sensitive antibiotic susceptibility test. Of the technology available today the microarray procedure is the closest to achieving this. However, microarrays have inherent disadvantages of time and expense. The time can be significantly reduced at each stage of operation through careful selection of procedures such as hydrophilic ionic liquids for DNA extraction, avoidance of biotin labeling and PCR, and rapid hybridization. Expense can be reduced through automation and, significantly, more efficient design of the microarray platform. With more research and development, microarrays can fulfill the ambitious vision of a rapid, comprehensive, and genetic-based antibiotic susceptibility diagnostic for clinical use.

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