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Rapid Detection of Bacterial Isolates in Wound and Body Fluid Cultures from Abdominal Surgery

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Abstract

Abdominal surgeries produce the highest number of Surgical Site Infections (SSI) than any other surgical procedures. Pathology specimens from these procedures (either fluid or tissue) have been analyzed for bacterial isolates in diagnostic labs using various plating and culture methods for years. While these methods have been effective, newer technology and tests based on whole genome sequencing have shortened the time for microbial identification. 6 different molecular diagnostic platforms: quantitative Reverse Transcription-PCR (RT-qPCR) Laboratory-Developed Test (LDT), a COBAS SARS-CoV-2 high-throughput system, 3 direct RT-qPCR kits, and Reverse Transcription loop-mediated isothermal AMPlification (RT-LAMP) plus rapid antigen tests were evaluated for their diagnostic capacity to detect SARS-CoV-2 RNA. 103 SARS-CoV-2 positive patient samples were tested with these 7 methods and viral RNA being detected between 50.5%-81.6% of samples on molecular platforms. Antigens were detected only 11.7% of samples when tested by rapid antigen test. Despite varying sensitivities on the different platforms, each platform was verified as a reliable detection tool for the virus with rapid antigen testing being a less reliable option for detecting coronavirus RNA. Increased precision and sensitivity from molecular testing platforms provide more accuracy and efficiency when looking for pathogenic bacteria causing surgical site infections in recovering patients. Early detection of bacterial isolates in surgical incisions post-surgery is imperative to the recovery of patients after a procedure. This project will investigate which molecular genomic platform is better at detecting pathogenic bacteria after abdominal surgery.

Keywords: Whole genome sequencing • Molecular testing • Surgical site infections • Abdominal surgery

Introduction

Overview of doctoral capstone project

Bacteria that cause disease in patients are called pathogenic bacteria. When strains of these bacteria are found in areas where a surgical procedure has happened, the resulting infection hinders the recovery process of the patient tremendously [1]. Infections inside surgical incisions are called Surgical Site Infections (SSI) and they are common hospital-acquired infections that plague surgical recovery units in hospitals. Approximately 21.7 million surgeries happen in the USA annually which is 4% of all hospital admissions on average [2]. Incidence of surgical site infections from abdominal surgery ranges from 1.2% to 5.2% depending on the procedure [3]. Emergency abdominal surgery has a higher probability of surgical site infections than many elective surgical procedures [4]. Bacterial plate culturing has been the standard for identifying microorganisms in samples sent from operating rooms, surgical recovery wards, and intensive care units for a long time now. In this test, a small portion of a patient's specimen (urine, blood, etc.) is streaked across a petri dish filled with growth media and placed in an incubator to grow. It takes anywhere from 72 hours to 96 hours for bacterial colonies to grow then each colony is examined under a microscope to determine the genus and specific strain of bacteria. This method has been effective; however, multi-drug resistant organisms that constantly evolve like influenza, MRSA, and beta coronaviruses (MERS, SARS-CoV-2, etc.) have increased the need for rapid detection of these bacteria and viruses.

Alignment to the specialization

The global pandemic has caused a shift in diagnostic practices to include Whole Genomic Sequencing (WGS) and molecular techniques for information on an evolving threat. Coronaviruses have the inane ability to stockpile different evolutionary traits and nucleotide mutations as it adapts to a new environment or a new host organism. Gene regulation is important for viral cells because it allows for a certain degree of versatility, adaptability, and immunity to various external stimuli in different environments to ensure survival. Molecular diagnostics have become especially useful in the detection of pathogenic bacteria with the development of newer, more accurate techniques and equipment. Gel electrophoresis is an established method of separating nucleic acid and proteins by size and charge to measure length of DNA or RNA strands ranging from 100 base pairs to 25 kilo-base pairs. However, patient samples sometimes contain small amounts of genetic material and may not be sufficient for gel electrophoresis working well with large strands of nucleic acid and macromolecules. Polymerase Chain Reaction (PCR) allows for DNA amplifications in small amounts making it useful in detecting bacteria because plasmids in bacterial genetics are not large enough for gel electrophoresis therefore, the process of DNA amplification allows for rapid identification of bacterial isolates [5]. Furthermore, Real-Time PCR technology like GeneXpert, BD-Max, and TaqMan assays amplified DNA is measured at each step in the reaction cycle (Real-Time) as opposed to conventional PCR which accumulates amplified DNA called "amplicons" then measures them producing an end-point analysis.

Problem statement and purpose of the deliverable

Plate culturing takes too much time, increases the chance of contamination, and is not as accurate in identification since it relies on morphology when other tests can detect a wide array of potential organisms. Subtyping is used to study the transmission of disease-causing microorganisms in epidemiology investigations and determine similarities between separate isolates from the same strain of bacteria [6]. Bacterial Whole Genome Sequencing (WGS) is an adaptive and innovative technology that uses subtyping to accurately detect bacterial while identifying strains with precision. There are other subtyping methods; however, whole genome sequencing can discern between bacterial strains better than other subtyping methods. Public health, diagnostic, and biomedical research laboratories use this technique frequently due to the speed (average time between 45 minutes to 120 minutes) and accuracy (Correct results 94% to 96% of the time).

DNA amplification techniques like Polymerase Chain Reaction (PCR) have been used on molecular diagnostic platforms to examine patient samples for infection. PCR can immediately test samples from patients through increasing the amount of genetic material available, scanning

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for certain genes and proteins in the genome, and providing an accurate identification of disease-causing microorganisms. The PCR process begins by isolating a segment of RNA then creating a complementary strand of DNA from a template for the RNA to bind to. Multiple copies of the strand are then synthesized, and the entire genome is analysed in real time providing detailed information about every gene present on the loci. Plate culturing normally takes days to complete while PCR can be done from anywhere from 45 minutes to 2 hours.

Evidence/data used to establish rationale for deliverable

Alkaaki conducted a prospective cohort study from February 2016 to July 2016 to study the bacteriology, risk factors, and incidence of surgical site infection in patients who had abdominal surgery [7]. Patients undergoing vascular, gynaecological, urological, or plastic surgeries were excluded from the cohort. Focusing solely on abdominal surgeries for the cohort limits the amount of relevant data selected from other surgical approaches with high SSI risks as well. Along with the lower sample size, the power of the study is decreased by excluding other high infection cases namely gynaecological procedures also yielding many surgical site infections [8]. Furthermore, timetables in clinical trials involving surgeries can extend the time between randomization and intervention because the surgical schedule may not always coincide with the study's randomization times [9]. After their surgical procedure, patients were followed prospectively for 30 days and wound assessments were conducted according to Centers for Disease Control and Prevention (CDC) standards. Surgical Site Infections (SSI) is defined as wound infection within 30 days of operative procedure. The Pearson and Wilcoxon univariate test was used to analyse the discrete and continuous variables while multivariate analysis was used to analyse operative and preoperative variables.

The average postoperative hospital stays of 2 days with a mortality rate of 0.7% for uninfected patients was significantly less than the hospital stays of 13 days and mortality rate for patients who contracted an SSI after the 30-day follow up period of 3.6% respectively. 50 of the 55 SSI patients had microbiology cultures available for analysis: 26 patients (52%), of whom 16 had extended-spectrum β-lactamase producing *E.coli*, followed by gram-positive bacteria (19 patients (38%) and a considerable number of *Acinetobacter baumannii and Pseudomonas*. The most pertinent risk factors for SSIs during abdominal surgery were open surgical approach, emergency surgery, length of operation, and male sex also documented in [10-11].

Bellusse conducted a prospective cohort study on abdominal surgery patients from July 2016 to May 2017 [12]. Patients were progressively followed for 30 days after their procedure to see if the incision site became infected corresponding to the CDC's definition of what a surgical site infection is. Data was collected through patient assessments and at the 30 days follow up appointment in the surgical outpatient clinic. 484 patients aged 18 years or older took part in the cohort producing an incidence rate of 20.25% (98 patients contracted a surgical site infection). Greater than 60% of the patients who contracted infections were hyperglycemic making it more likely for a patient to contract an incision site infection. Those who were predisposed to preoperative hyperglycemia had a higher risk of infection than those not predisposed to preoperative hyperglycemia at the three glucose checks during the study (in the operating room, at the end of the surgical procedure, and 12 hours after surgery). SSI incidence rate in operating room (hyperglycemic: 4.20, not hyperglycemic: 0.70, relative risk: 5.98, 95% CI: 1.59-15.84), end of surgery (hyperglycemic: 1.52, not hyperglycemic: 0.56, relative risk: 2.67, 95% CI: 1.71-4.12), and 12 hours after surgery (hyperglycemic: 1.68, not hyperglycemic: 0.55, relative risk: 3.03, 95% CI: 1.94-4.66). The mean glucose level preoperative in the operating room was 97.04 mg/dL, 136.54 mg/dL at the end of surgical procedures, and 132.90 mg/dL 12 hours after surgery was completed. Preoperative hyperglycemia is an independent risk factor for surgical site infections ($p<0.5$) and patients with an elevated glycemic index have a higher risk for contracting SSI from abdominal surgery ($p \leq 0.5$).

Historical background of the problem

The gold standard for microorganism identification is culture methods (plate, liquid, broth, etc.) and serology; however, these methods take an extended amount of time and can be unreliable against many viral and bacterial strains. Valledor conducted a study on different diagnostic methods ability to detect bacteria that cause gastroenteritis (Salmonella species, Campylobacter species, and Yersinia enterocolitica) [13]. 400 stool samples were analysed of which 98 samples (24.5%) were positive for some form of bacteria. Each method tested performed well; however, real-time PCR positively predicted bacteria in the study samples between 95.8% to 100% of the time, the highest amongst all methods tested including culture method and serology. Popova conducted a study to validate real-time PCR (qPCR) against bacterial culture method in the detection of pathogenic bacteria causing periodontitis. Microbiology culture method is widely used because it can discover new species of bacteria and test their susceptibility to antibiotics which make it the "gold standard" [14]. Quantitative PCR (qPCR or Real-Time PCR) detected more cases of periodontitis quicker and more accurately than bacterial culture method in the study validating it is a viable diagnostic method.

Organizational context

Infections occurring approximately 30 days around the site or in a surgical incision are known as a surgical site infection (no implant) or within a year of an implant being placed and an infection resulting from the procedure [15]. Patients with a surgical site infection are 5 times more likely for hospital readmittance, and the chances the patient is sent to the ICU is 60% higher than those without surgical site infections [16]. Surgical Site Infections (SSI) happens more frequently during abdominal surgery and emergency trauma surgery than any other surgical procedures [17]. The overall incidence of surgical site infections from abdominal surgeries can reach 15%-25% considering the level of contamination. 3% of the patients usually perish from surgical site infections; however, infections from more complex abdominal surgical procedures like cardiac or gastrointestinal can increase the mortality rate to approximately 50% [18]. Diagnostic laboratories in hospitals must be able to quickly and accurately determine if the patient's surgical incision is infected so they can begin treatment. High sensitivity (detecting bacterial isolates) alone does not make a procedure effective; it also must have equally high specificity (precision, accuracy) in properly identifying the bacterial strain possibly present in surgical wounds.

Theoretical framework

The central dictum behind this project is detection of pathogenic bacteria can greatly be improved by PCR and DNA amplification. Investigations into outbreaks and infectious disease transmission in public health have utilized sub-typing methods in determining which pathogenic microorganisms has affected a community or group of patients [19]. While these techniques have been effective in microbiological identification, 3 problems present themselves: sub-typing only provides a portion of the genetic sequence of a pathogenic microbe, has limited success linking potential cases together in an outbreak, and has difficulty finding pathogens once they undergo any form of genetic mutation. Whole-genome sequencing provides a complete picture of a pathogen's genetic makeup allowing for comparative analyses for potential cases in an outbreak or public health emergency. Advances in sequencing platforms, especially RT-PCR (Real Time Polymerase Chain Reaction) devices, can easily differentiate between strains of bacteria with increased precision and in less time than conventional serotyping and bacterial plating methods. Furthermore, the upgraded platforms also allow for higher specificity and sensitivity and detection of multiple microbial targets at the same time known as multiplexing [20].

Diagnostic labs utilizing molecular genomic platforms are at an advantage when it comes to tracking and identifying rapidly mutating, lethal microorganisms. Itahashi conducted a study to identify and quantify pathogens causing corneal ulcers. Patients with confirmed corneal ulcers were enrolled in the study and their corneal scrapings were tested with 2 methods, real time PCR with cycling probe and bacterial culture method.

The RT-PCR assay used to test specimens including primers and probes were specifically created to test for *Staphylococcus aureus*, Staphylococcus pneumoniae, *Pseudomonas aeruginosa*, Methicillin-resistant S aureus, Candida spp., and Fusarium spp (key causes of corneal ulcers). 40 patients' eye samples were analysed in the study with 6 pair of eyes had negative response for both methods, 11 positive PCR tests, 2 positive culture results, 1 pair of eye tested positive for different pathogens, and 20 pair of eyes that had positive results detected by both methods. RT-PCR both detected and quantified bacterial (*Staphylococcus aureus*, Staphylococcus pneumoniae, *Pseudomonas aeruginosa*, Methicillin-resistant S aureus) and fungal (Candida sp and Fusarium sp) pathogens validating its worth as a diagnostic tool.

The current pandemic has caused a shift in diagnostic practices to include whole genomic sequencing and molecular techniques for information on an evolving threat. Coronaviruses have the insane ability to stockpile different evolutionary traits and nucleotide mutations as it adapts to a new environment or a new host organism. Gene regulation is important for viral cells because it allows for a certain degree of versatility, adaptability, and immunity to various external stimuli in different environments to ensure survival. Six different molecular diagnostic platforms (quantitative Reverse Transcription-PCR (RT-qPCR) Laboratory-Developed Test (LDT), a COBAS SARS-CoV-2 high-throughput system, 3 direct RT-qPCR kits, and Reverse Transcription-loop-mediated isothermal AMPlification (RT-LAMP)) plus rapid antigen test were evaluated for their diagnostic capacity to detect SARS-CoV-2 RNA by Nagura-Ikeda [20]. 103 SARS-CoV-2 positive patient samples were tested with these 7 methods and viral RNA being detected between 50.5%-81.6% of samples on molecular platforms. Antigens were detected only 11.7% of samples when tested by rapid antigen test. Despite varying sensitivities on the different platforms, each platform was verified as a reliable detection tool for the virus with rapid antigen testing being a less reliable option for detecting coronavirus RNA.

Molecular diagnostics have become especially useful in the detection of pathogenic bacteria with the development of newer, more accurate techniques and equipment. Gel electrophoresis is an established method of separating nucleic acid and proteins by size and charge to measure length of DNA or RNA strands that range from 100 base pairs to 25 kilo-base pairs [21]. However, patient samples sometimes contain small amounts of genetic material and may not be sufficient for gel electrophoresis that works well with large strands of nucleic acid and macromolecules. PCR allows for DNA amplifications in small amounts which make it useful in detecting bacteria because plasmids in bacterial genetics aren't large enough for gel electrophoresis so the process of DNA amplification (RNA isolation/characterization, complementary DNA or cDNA synthesis, real time PCR data acquisition, normalizing factor generation, data normalization/analysis) allows for rapid identification of bacterial isolates [22]. Furthermore, real-time PCR techniques like GeneXpert, BD-Max, and TaqMan assays amplified DNA is measured at each step in the reaction cycle (real-time) as opposed to conventional PCR which accumulates amplified DNA called "amplicons" then measures them producing an end-point analysis.

Scholarly literature

The strategy for the search in this review was to find articles exploring molecular detection of multi-drug resistant organisms that cause various types of hospital-acquired infections. Furthermore, epidemiology and genetics of bacterial pathogens causing SSI infections to identify nucleotide pairings bestow multidrug antibiotic resistance and overall colonization of surgical incisions. Databases searched for literature review were Scopus, NCBI/PubMed, and DDBJ/EMBL/GenBank looking at antibiotic resistance genetics, epidemiology of common microbes found in surgical cultures, and infection prevention protocols used in the controlling of surgical site infections. Search terms used in the literature search: hospital-acquired infections, pneumonia, Enterobacteriacae, klebsiella genome, klebsiella antibiotic resistance, abdominal surgery SSI prevention, MRSA, mecA/spa/SCCmec, post-operative wound care, surgical wound culture, synovial fluid culture, GeneXpert, Acinetobacter baumannii genome, blaOXA23, blaOXA58, PCR, electrophoresis.

Ananthi and the team conducted a study to observe what types of aerobic bacteria colonize surgical wounds which leads to surgical site infections [23]. 102 patients took part in the study and pus was collected from their surgical procedure for microbiological analysis. 59% of the pus samples collected (61 samples) had bacteria proliferating in them with 51 of the total samples having only one bacterial strain identified with the remaining 10 having more than 1 bacterial strain identified. 73 bacterial isolates were extracted from the pus samples of the patients with 33 being Gram-positive cocci (sphere-shaped bacteria) and the remaining 40 being Gram-negative bacilli (rod-shaped bacteria) when identified through gram staining. *Staphylococcus aureus* was the most common bacteria identified (19 isolates) with *E. coli* and coagulase negative Staphylococcus strains the next most identified bacteria (18 and 11 isolates respectively). This proves that a vast number of microorganisms, viruses and fungi included, can cause surgical site infections from many forms of surgery plus much study into these organisms is warranted to better combat them.

Many microbiological studies into deep and superficial wound infections from abdominal surgeries will confirm that the main two causes of surgical site infections are *Staphylococcus aureus* and *E. coli*. These 2 bacterial strains colonize the skin close to incisions so when the epidermis breaks the bacteria infiltrates the wound if proper barrier precautions are not taken. However, 2 other microbial-resistant bacteria can also SSI with less ways to treat them: *Klebsiella pneumoniae and Acinetobacter baumannii*. Liu Li and Hua Chen each sequenced a strain of *Klebsiella pneumoniae* (HS11286 and XH209 respectively) which contained about 6 chromosomes per strain each coding for 5,316 genes (HS11286) and 5,023 genes (XH209) looking for the source of antibiotic resistance [24-25]. The blaTEM-1 and blaKPC-2 genes were identified on the pKHS2 plasmid while the adjacent pKHS3 plasmid has 13 defined resistant genes (tetG, cat, sul1, dfra12, aac (3)-Ia, etc.) in the HS11286 strain. Similar genes were found in XH209 strain of K, pneumoniae that code for enzymes known as carbapenemases and beta-lactamases that hydrolyze beta-lactam antibiotics and other microbials used to treat it.

Acinetobacter baumannii causes many types of nosocomial infections; however, it is a commonly diagnosed as pathogen in blood infections much like Central-Line Associated Blood Stream Infections (CLABSI). A. baumannii can typically be isolated in surgical wards, Intensive Care Units (ICUs), and burn units where it causes a gambit of illnesses: system-wide bacterial infections (septicemia), urinary tract infections, and pneumonia [26]. Fang sequenced a strain of carbapenem-resistant Acinetobacter baumannii (XH386) which contains a Sequence Type (ST) linked to clonal complex 92 (CC92) and the pan-European clonal lineage II (EUII) which promotes antibiotic resistance [27]. The blaOXA-23 gene provides A. baumannii with protection against carbapenem by coding for oxacillinases, cloxacillinases, and carbapenemases to hydrolyze many forms of antimicrobials used to treat it.

Acinetobacter baumannii isolates collected from patients during a 5 year period (June 2009-November 2014) were analysed for general antibody susceptibility. All the isolates (101) were resistant to imipenem and carbapenem, 87 isolates contained sequence types linked to CC92 (ST191 and ST195 were common), and 95 isolates contained the blaOXA-23 gene or a similar carbapenemase (blaOXA-40, blaOXA-51, blaOXA-58, blaOXA-143). Somily and team conducted a comparison study with the GeneXpert MTB/RIF, an assay to detect mycobacterium (MTB) who is Resistant to Rifampin (RIF), against standard culture methods [28]. In the retrospective analysis, 103 respiratory and 137 non-respiratory samples underwent smear culture microscopy, mycobacterial culture, and the GeneXpert MTB/RIF assay for tuberculosis. 15 of the respiratory samples and 9 of the non-respiratory samples were positive in smear culture with 8 out of 9 also tested positive when cultured for mycobacterium tuberculosis. GeneXpert assay identified 15 positive respiratory cultures and 8 positive non-respiratory cultures with 100% sensitivity and an 88.8% positive

predictive value. There was a false positive when analysing 88 negative respiratory cultures generating a negative predictive value of 100% and a 98.9% sensitivity grade. 125 non-respiratory cultures tested negative for mycobacteria in smear culture, bacterial culture, and GeneXpert MTB assay which detected it with 100% sensitivity, specificity, negative predictive value, and positive predictive value. This proved that RT-PCR like GeneXpert could be just an effective culture method.

Massi evaluated the use of the GeneXpert MTB/RIF against the MGIT 960 liquid culture method on specimens of patients who were suspected to have spondylitis [29]. Spondylitis is an inflammation in the spinal vertebrae that can cause them to fuse over time reducing overall flexibility in the back and causing severe pain. Mycobacterium tuberculosis infections of the vertebrae are known as Pott's disease and usually occur in the upper thoracic and lower lumbar regions. Vertebrae samples with body tissue and fluid were crushed, centrifuged, mixed into an elution buffer, and 2 ml of the solution was pipetted into the testing cartridge. The process took 2 hours to run, and the results were read automatically then compared to the liquid culture run on the same specimens. Liquid culture only identified 31.42% positive specimens out of the 70 tested in the study. However, the GeneXpert assay detected bacterial isolate in 88.57% of specimens with validity testing calculating a sensitivity of 100%, specificity of 16.6%, positive/negative predictive values of 35.48% and 100% respectively. These statistics mean that for this study the GeneXpert MTB/RIF could detect bacteria in samples accurately, but it was not accurate at identifying mycobacterium in the study.

Wolk and team did a multicenter study to evaluate the effectiveness of the GeneXpert MRSA/SA assay at detecting these 2 strains from wound cultures [30]. Traditional methods to study wound and blood cultures would require incubation and plating which take considerable amounts of time slowing therapeutic intervention. Molecular diagnostics like real-time PCR allow for direct testing from patient specimens in a shorter period with just as much accuracy as culture method. 114 wound cultures and 406 blood cultures were collected for the study between the United States and Europe. Specimens collected from patients who were 18 years old and older were handled following federal medical privacy standards that govern human subject protection. 100% Specificity, 97.1% sensitivity (wound culture), and 98.3% sensitivity (blood culture) for Methicillin-Resistant *Staphylococcus Aureus* (MRSA) and 100% specificity with 100% sensitivity in both wound and blood culture for *Staphylococcus aureus*. This assay is accurate in detecting Staphylococci species which causes many skin and soft tissue infections in patients. When measuring genetic signatures from only one gene false positives are expected; however, real-time PCR searches for multiple genes and complexes which lowers the possibility of false positives.

Staph infections are the main cause of surgical site infections in spinal surgeries increasing the chances of death from complex procedures in the back. SSI from spinal surgery can manifest anywhere from 2 weeks to 3 months post-presenting either as a superficial, excessively draining abscess or proliferating deeper in the incision as it heals [31]. Magnetic Resonance Imaging (MRI) is still the standard diagnostic platform to use when a patient suspects a potential SSI even though more difficult cases may require more specialized tomography. Blood cultures are usually collected on surgical patients who develop SSI, but the bacterial yield is too low for full genomic sequencing. Inflammatory markers like "serum amyloid A" can quicken the diagnosis of SSI so microbial detection begins which will be easier with DNA amplification. Polymerase chain reaction is a better diagnostic method than collecting blood cultures because cultures produce too little DNA fragments to completely analyse and provide an accurate identification. Gram staining provides basic morphology and primary identification of an organism, but agarose gel electrophoresis will accurately measure the DNA strand and allow better visualization through fluorescence while providing more of a target nucleic acid strand. Culture method is effective but, in this case, PCR is the better detection method.

A complication that can arise in the treatment and detection of surgical site infections is known as culture negative surgical site infection. A patient presents with all the clinical symptoms of an SSI; however, there is no bacterial growth in their culture they get misdiagnosed. Bacterial culturing remains the standard in identifying pathogens in patient samples, but the results are too reliant on the presence of viable microorganisms when the sample is processed after being collected. Bal Das conducted a study in which they took 97 patients who developed SSI after a surgical procedure, but their specimen did not yield any bacterial growth in aerobic bacterial culture. The wound aspirates of the study patients of each patient were then tested with 16S RNA specific PCR to determine if each sample indeed had pathogenic bacteria present. The 16S RNA gene resides in all bacterial genomes with each strain having a derivation of this plasmid depending on the type of bacteria it is. This assay can accurately detect and identify bacterial isolates by the genetic nuances in the 16S RNA gene in the microbes' genetic structure allowing for quick molecular diagnosis. 55% of the wound aspirates tested (53 samples) had pathogenic bacteria in them when aerobic bacterial culture failed to find any bacteria at all. Microbial analysis showed that the most common organisms isolated were Bacillus spp, Pseudomonas spp, and Enterococcus spp, respectively. PCR is more sensitive and specific than conventional culturing with a wider application of uses and more detailed assays to test for virulent microorganisms.

Material and Methods

Polymerase Chain Reaction (PCR) is a technique amplifying DNA by making multiple copies of target sequences in a short period of time [32]. Molecular techniques like PCR have become particularly useful in the detection of pathogenic bacteria and other microbial isolates. Certain genetic sequences act as biomarkers for specific bacterial strains which allows for identification in small amounts before the exponential growth phase. Cepheid GeneXpert is a Real-Time Polymerase Chain Reaction (qPCR or quantitative PCR) that amplifies nucleic acid of microbiology specimens which provides detailed identification with high sensitivity and specificity [33]. Additionally, the BD-MAX RT-PCR system is another molecular testing platform that has been used in healthcare settings for advanced microbial testing like RSV, influenza, gynecological microbiology, and other tests. Samples are collected directly from patients, transferred directly into cartridges filled with media, and then loaded into the modular testing unit. This hands-free approach allows for no specimen manipulation and little contamination with debris, artifacts, and other colonizing strains.

Project design/method

A comparative study looking at two molecular diagnostic platforms used to detect bacteria in clinical settings. Samples were collected from patients who underwent abdominal surgical procedures were used for analysis. Each specimen was first gram stained for morphology and primary identification then subjected to each method in the study: BD-Max System (Staph-SR, MRSA-XT, and CRE assays) and Cephid GeneXpert RT-PCR (Carba-R and MRSA/SA assays). Procedural statistics will then be collected for analysis through the databases that archives each specimen ran. The reports for each specimen generated by each detection method will then be compared to reference plate culture for overall accuracy and efficiency in bacterial isolate identification.

Project outcomes

- Exposing wound and body fluid cultures from abdominal surgical procedures to 2 different molecular detection platforms: GeneXpert RT-PCR and BD-Max RT-PCR. Examining for the presence of pathogenic bacterial isolates known to cause surgical site infections in a CLIA certified diagnostic lab
- Calculating the overall accuracy of each platform to determine which one is the best to detect SSI causing microorganisms in the wounds of abdominal surgical patients for better post-surgical therapy

Development process

GeneXpert RT-PCR: Wound culture swabs were placed in an elution

buffer to dislodge any isolates and then vortexed for 1 minute to 3 minutes. The solution was then placed in the testing cartridge which is filled with media for bacterial detection. For blood and body fluid specimens, 50 microliters of sample were pipetted into the testing cartridge in a similar manner. The cartridges were then placed into the modular testing hub and DNA amplification takes approximately 60 minutes for the Methicillin-Resistant *Staphylococcus Aureus*/*Staphylococcus Aureus* (MRSA/SA) assay and 48 minutes for the Carbapenem Resistant (Carba-R) assay. All cartridges were disposed in a specialized biohazard receptacle for all PCR cartridges run on the machine.

BD-Max RT-PCR System: The stems of wound culture swabs were either snapped or cut with a sterile instrument so the ends containing the specimen can be placed in the sample buffer tube. The tube was then vortexed for 1 minute. A reagent strip was then removed from whichever assay was run on the sample (Staph-SR, MRSA-XT, or CRE) and placed on the BD Max system rack. An Extraction tube and Master Mix tube was then added to the reagent strap then the strap was placed on the system rack. The specimen was then added to a BD Max PCR Cartridge (holds up to 24 samples) then placed into the BD Max System. The system racks were then loaded as well, and the specified assay was selected from a work list. The analysis process for the assays took approximately 2 hours to complete.

Evaluation plan

PCR statistics from each specimen run was collected and archived in the database of the central modular computing unit of the Cepheid GeneXpert and BD-Max system for 2 months (April 2021-May 2021). The reports were compared to the reference bacterial plate cultures grown to see how accurate each procedure was at identifying the bacterial strains in each sample. Sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) was calculated and charted using Excel statistical software. A comparison analysis was done once the values were calculated.

Results and Discussion

The key is the sensitivity and specificity of these new techniques which makes them more effective than standard methods. A sensitive test will reduce the number of false negatives because it will correctly detect samples that have pathogenic microorganisms in them. Furthermore, a test with good specificity will reduce the number of false positives because it will accurately weed out specimens that do not have any pathogens in them. Thus, the Positive Predictive Values (PPV; probability that a positive test has the disease) and Negative Predictive Value (NPV): probabilities that negative test really do not have the disease) is accurate reducing the chances of missed or incorrect diagnoses. Sensitivity, specificity, PPV, and NPV are important pillars when it comes to molecular detection which makes PCR such an important tool in rapidly diagnosing pathogens (Table 1).

62 samples were analyzed in the comparison study. 22 of the samples (35%) had bacteria in them when analyzed by both platforms. Many study specimens came from aparoscopic procedures (38 specimens) than laparotomy procedures (24 specimens). There were two false positives recorded during the study, both on the Gene Xpert, and 2 false negatives that were on both the BD Max and Gene Xpert. Both testing platforms had the same negative predictive value at 95%; however, the BD Max was more efficient when it came to detecting the isolates of pathogenic bacteria in the study specimens. It was superior to the Gene Xpert in positive predictive value and specificity (100% to 91%) and it was slightly more sensitive as well (96% to 95%) (Figure 1). The Gene Xpert could detect bacteria well but was not exactly accurate in identifying microbes with the 2 false positive and a false negative. An unpaired t-test was run to compare the efficacy of the BD-Max vs the Gene Xpert (p-value=0.0349) (Table 2). It confirmed the BD-Max was the better molecular testing platform for detecting pathogenic bacteria isolates.

There were an equal number of both gram-positive and gram-negative bacteria in the study specimens (11 cases of each). *Staphylococcus aureus* (methicillin sensitive) was the most isolated bacterium in the study (10 isolates) followed by *Escherichia coli* (5 isolates) and three tied with 2 isolates (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumanii*) (Table 3). An antibiotic susceptibility test revealed that methicillin, oxacillin, and other beta lactam agents worked well against *Escherichia coli* and *Staphylococcus spp* (*Staphylococcus hominis* and *Staphylococcus aureus*). The strains of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumanii* found in the specimens possessed genes that code for proteins that make them resistant to common antibiotics (NDM, VIM, and OXA-48). They were resistant to many of the antibiotics used in the susceptibility test including vancomycin, carbapenems, various penicillins, and tigecycline. A cohort study conducted by Falcone and team showed that a combination treatment of ceftazidime and avibactam with aztreonam was effective against bacteria that had genes that produce proteins to hydrolyze carbapenems (KPC, NDM, VIM, OXA-48, IMP, etc.) [34].

Table 1. Molecular testing platforms efficiency.

Platform	Positive Predictive Value	Negative Predictive Value	Sensitivity	Specificity
BD Max	100%	95%	96%	100%
Gene Xpert	91%	95%	95%	91%

Table 2. Comparison analysis (Unpaired t-test).

Platform	Mean	Standard Deviation	SEM	N
BD Max	0.9775	0.0263	0.0131	4
Gene Xpert	0.93	0.0231	0.0115	4
P value	0.0349	-	-	-
Mean Difference	0.0475	-	-	-
t value	2.71	-	-	-
Standard Error	0.017	-	-	-

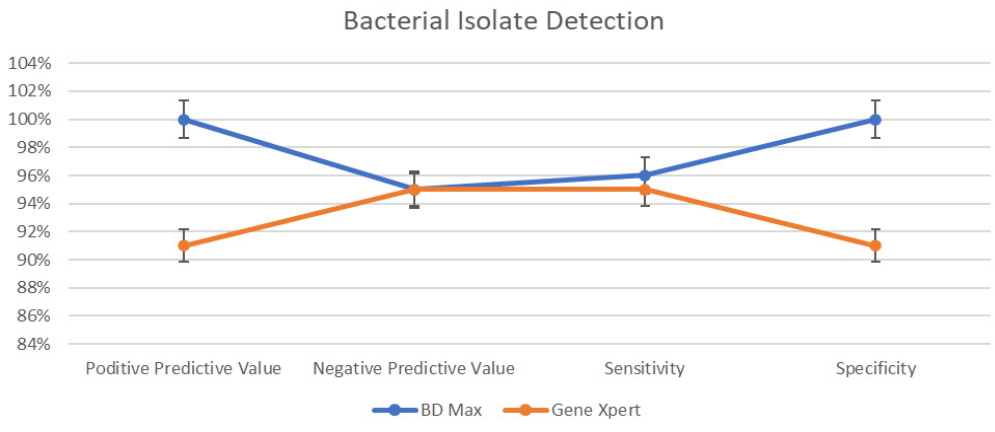

Figure 1. Bacterial detection efficiency.

The limitations of this study are mainly the power and the sample size. The COVID-19 pandemic was a major factor because it reduced the number of specimens collected for the study (Table 4). Protocols established because of the COVID-19 pandemic limited the numbers of inpatient and outpatient procedures being conducted in healthcare settings. Furthermore, limiting the type of surgery to just abdominal procedures handicapped the overall power of the study (Figure 2). Gynecologic surgeries like hysterectomies also produce a high number of surgical site infection cases that come from several independent risk factors. surgical patients for better post-surgical therapy

Application and benefits

Target audience: Healthcare networks and trauma centres were focus of this study. Surgical Site Infections are of the most common hospital-acquired infections that a patient can get in healthcare settings. Infection control practices must be followed to prevent cases of these infections during surgical procedures and while patients are recovering in surgical wards as well. If a patient unfortunately contracts a surgical site infection, rapid detection of SSI causing microorganisms is imperative so hopefully this project shows them the benefits of various types of molecular detection.

Beyond the local setting: Major public health organizations have championed the use of molecular testing to diagnose infectious diseases. During the pandemic, the combined INFLUENZA/Respiratory Viruses (RSV)/SARS-CoV-2 assay ran on the Cepheid Gene Xpert and the RSV/SARS-CoV-2 assay ran on the BD-Max were the preferred confirmatory test to determine if someone had the antigens for COVID-19. Many patients had taken rapid COVID-19 as a quick determination if the virus is in their system; however, the rapid test is not that accurate as many false positives were detected. PCR testing was the main diagnostic procedure that physicians, epidemiologist, and health department workers trusted for an accurate diagnosis.

Implications for professional specialization: Molecular testing apparatus have a wide array of tests that can be run on them. Sexually transferred infections (Gonorrhea, Chlamydia, Syphilis, etc), Norovirus, Streptococcus, Clostridium difficile, and many more infectious agents. Most of the BD MAX Systems and other molecular platforms are installed in hospital laboratories, reducing the added time and complexity of needing to send samples to a reference lab. Whole genome sequencing can help improve treatment paradigms by providing a full picture of what medications a virus, bacterium, fungi, or protozoan could be resistant to. Continuing development of this technology could lead to breakthroughs in genetic counselling and epidemiology once a better understanding of microbial genetics is ascertained through more research.

Given the numerous verification studies on this matter, molecular testing platforms should be an integral part of any diagnostic practice. Healthcare networks should install some systems in all their hospitals and adjunct facilities to increase the menu of tests that can be ran. It will pay for itself by saving the money and time it takes to send samples out to be tested by a third party. Preferably, the BD-MAX RT-PCR system should be the platform of choice; however, there are other options that provide an accurate detection with a good level of sensitivity: Abbott ID Now, ARIES Molecular Diagnostics, NeuMoDX Molecular System, Taqman Diagnostic Platform, etc. In a nutshell, whole genomic sequencing has evolved to a place to where it is an asset to providing a deeper understanding to the microorganisms that cause various forms of illness in patients.

Table 3. Pathogenic bacteria isolated from surgical samples.

Organism	Number of cases
Staphylococcus aureus	10
Pseudomonas aeruginosa	2
Escherichia coli	5
Acinetobacter baumanii	2
Klebsiella pneumoniae	2
Staphylococcus hominis	1
Total of bacterial isolates	22

Table 4. Reference culture analysis.

Organism	% of Culture
Staphylococcus aureus	45%
Pseudomonas aeruginosa	10%
`Escherichia coli	23%
Acinetobacter baumanii	10%
Klebsiella pneumoniae	10%
Staphylococcus hominis	2%

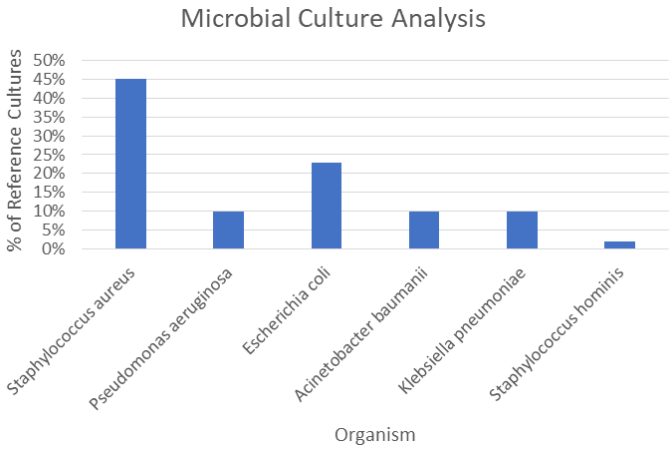

Figure 2. Bacterial plating culture etiology.

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Conclusion

The primary hypothesis and study assumptions were vindicated through this study. The Cepheid Gene Xpert completed analysis in an average time of 46 minutes which was considerably faster than the BD-Max (average analysis time: 1 hour 52 minutes). However, the BD-Max was more accurate, efficient, and sensitive when it came to isolating and identifying pathogenic bacteria in the study specimens. The BD-Max RT-PCR system looks at over 36 different genetic markers on multiple loci allowing for a clearer picture as to what makes these microorganisms so infectious. Many verification studies have been done on the validity of molecular testing and whole genome sequencing platforms in diagnostic laboratories with much

data supporting their use in hospitals. Improved accuracy and more detailed information in an accelerated time than plate culturing method helps physicians begin treatment sooner and provides a better understanding on the type of microbe their dealing with. The next steps would be to find ways to get more platforms like the BD-Max, Gene Xpert, etc into hospitals to help improve patient outcomes.

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