potentially directly from patient serum. This assay is indicated as an aid in diagnosing and identifying antimicrobial susceptibility of specific pathogens that can cause bacteremia. This panel is designed to analyze the most common BSI Gram-negative and Gram-positive pathogens, with the potential for Fungi. The TaqMan 5`nuclease real-time PCR assay configuration coupled with the ID strategy should provide the capability to provide Minimum Inhibitory Concentration (MIC) and Susceptible, Intermediate and Resistant (SIR) information for polymicrobial samples, monomicrobial samples, and isolate testing as needed. These results should be used in conjunction with other clinical and laboratory findings. Standard laboratory protocols for processing positive blood cultures should be followed to ensure availability of isolates for supplemental testing as needed.

The present disclosure also relates to PCR-based rapid ID/AST phenotypic screening tests that are *in vitro* diagnostic tests utilizing PCR technology for the rapid identification and phenotypic susceptibility testing of a target pathogen or group of pathogens for a single drug, class of drugs, or combination of drugs. The PCR-based rapid ID/AST screening assays are performed either directly from clinical samples or from bacterial/fungal isolates. These assays will indicate the presence of problematic drug resistant pathogens to aid in patient and hospital safety. Examples of these types of tests are for Methicillin-Resistant *Staphylococcus aureus* (MRSA), Vancomycin-Resistant *Staphylococcus aureus* (VRSA), Vancomycin-resistant Enterococci (VRE), Carbapenem-Resistant Enterobacteriaceae (CRE), *Candida auris*, and MDR *Neisseria gonorrhoeae.*

In one aspect, the present disclosure refers to a method to simultaneously identify and determine the antimicrobial susceptibility of a target group of bacteria or fungi from a polymicrobial biological sample that have similar or identical clinical breakpoints for at least one antimicrobial or one antimicrobial class, the method comprising - performing a single quantitative real-time PCR assay as a reporter in the presence of at least one concentration of at least one antimicrobial or antimicrobial class using primer and probe oligonucleotides that hybridize more selectively to a target gene that is from the target group of bacteria or fungi compared to the target gene that is not from the target group of bacteria or fungi, and - identifying the target group of bacteria or fungi by detecting a signal generated during the single quantitative real-time PCR assay that is specific to the target group of bacteria or fungi, wherein the target group of bacteria or fungi comprises a taxonomic order, a taxonomic family, a taxonomic genus, or a taxonomic species; and wherein the target gene for determining the Enterobacterales order is *rplP*, *ompA, tuf, gyrB,* or *rpoB*; the target gene for determining the Enterobacteriaceae family is *rplP*, *ompA*, *tuf, gyrB,* or *rpoB*; the target gene for determining the Enterococcus genus is *tuf, rpoB, sodA, ddl,* or *gyrB*; the target gene for determining the Pseudomonas genus is *gyrB,* O-antigen acetylase, *rpoB, ecfX,* or *tuf*; the target gene for determining the Acinetobacter genus is *ompA*, *tusA*, *rpoB,* or *gyrB*; the target gene for determining *Strenotrophomonas maltophilia is fdnG, gyrB,* or *tuf*; the target gene for determining *Staphylococcus aureus* is *CPE, gyrB, nuc, rpoB, tuf,* or *ddlA*; the target gene for determining the *Staphylococcus epidermidis* is *altE,* or *femA*; the target gene for determining Coagulase-negative Staphylococci is *rpoB, tuf,* or *sodA*; the target gene for determining the Streptococcus genus is *tuf, gyrB, sip,* or *ddlA*; the target gene for determining *Streptococcus pneumonia* is *lytA, SP2020,* or *piaB*; the target gene for determining *Proteus mirabilis* is *UreR,* or *UreC*; and/or the target gene for determining *Candida albicans* is *ACT, RPB-1,* 5.8s ribosomal RNA, or 18s ribosomal RNA.

One example of the present disclosure relates to a method of using a quantitative real-time PCR assay as a reporter to simultaneously identify and determine the antimicrobial susceptibility of a group of bacteria or fungi that have similar or identical clinical breakpoints for at least one antimicrobial or one antimicrobial class. In one example, the target group of bacteria or fungi are present in bloodstream infections (BSI), gastrointestinal infections, respiratory infections, urinary infections, nasal infections, rectal infections or wound infections. In one example, identification of the group of bacteria or fungi is by detecting a signal that is specific to the group of bacteria or fungi. In one example, the specific signal is detected by using primer and probe oligonucleotides that hybridize more selectively to a target gene that is from the group of bacteria or fungi than to the target gene that is not from the group of bacteria or fungi. In one example, the target gene is selected from *rplP, ompA, tuf, rpoB, ddl, ddlA, fdnG sodA, gyrB,* O-antigen acetylase, *ecfX, tus*A, CPE, *sip*, and *nuc.*

In another example, the group of bacteria or fungi represents a taxonomic order. In one example, the taxonomic order is the order Enterobacterales. In another example, the

selectively to the target gene that is in the Enterobacterales taxonomic order than to the target gene that is not in the Enterobacterales taxonomic order comprise the nucleotide sequences comprising SEQ ID NOs: 1-16. In one example, the primer and probe oligonucleotides that hybridize more selectively to *gyrB* in the Enterobacterales order than to *gyrB* in the non-Enterobacterales order comprise the nucleotide sequences comprising SEQ ID NOs: 8-10.

In another example, the present disclosure relates to a method of using a multiplexed quantitative real-time PCR assay as a reporter to simultaneously identify and determine the antimicrobial susceptibility of a plurality of bacterial or fungal strains from a biological sample, i.e. from a polymicrobial biological sample. In one example, the biological sample is selected from whole blood, plasma, serum, red blood cell fraction, saliva, cerebrospinal fluid, semen, stool, urine, nasal swab, wound swab, dermal swab, rectal swab, bile, lymph, sputum, lavage fluid, or a combination thereof. In one example, the biological sample is whole blood, plasma, serum or a combination thereof. In one example, the biological sample is cultured prior to performing the PCR assay. In another example, the biological sample is a bacterial or fungal isolate. In another example, the plurality of bacterial or fungal strains are grouped into at least one group of bacteria or fungi that have similar or identical clinical breakpoints for at least one antimicrobial or antimicrobial class. In one example, the plurality of bacterial or fungal strains are grouped into more than one groups of bacteria or fungi wherein each group of bacteria or fungi has similar or identical clinical breakpoints for at least one antimicrobial or one antimicrobial class.

In another example, the identification of the plurality of bacterial strains utilizes a plurality of strain-specific 5` nuclease (TaqMan®) oligonucleotide probes, each labeled with fluorescent dyes that have different emission wavelengths. In one example, the identification of the plurality of bacterial strains utilizes the TAGS (Temperature Assisted Generation of Signal) technology. In another example, the method further comprises a step selected from verifying the identification of the plurality of bacterial or fungal strains, or determining a mechanism for an antimicrobial susceptibility phenotype or for a toxin or virulence phenotype, or both verifying and determining steps.

In another example, the present disclosure relates to a method of using a quantitative PCR assay to simultaneously identify and determine Susceptible, Intermediate and Resistant (SIR) information for a target bacterial or fungal strain or for a target group of bacteria or fungi to an antimicrobial or a class of antimicrobials wherein the identification of the target strain or target group and the determination of SIR information are derived from one or more mathematical relationships associated with PCR data.. In one example, the mathematical relationship is