CLAIMS

1. 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.

2. The method of claim 1 further comprising a step selected from

- verifying the identification of the target group of bacteria or fungi with additional primer and probe oligonucleotides that hybridize more selectively to a second target gene that is from the target group of target bacteria or fungi compared to the second target gene that is not from the target group of bacteria or fungi; or

- determining a mechanism for an antimicrobial susceptibility phenotype or for a toxin or virulence phenotype; or

both the verifying and the determining steps.

3. The method of any one of claims 1 to 2 further comprising simultaneously identifying and determining the antimicrobial susceptibility of more than one target groups of bacteria or fungi, wherein each target group of bacteria or fungi has similar or identical clinical breakpoints for at least one antimicrobial or one antimicrobial class.

4. The method of any one of claims 1 to 3, wherein 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.

5. The method of any one of claims 1 to 4, wherein single quantitative real-time PCR assay is a single multiplexed quantitative real-time PCR assay to simultaneously identify and determine the antimicrobial susceptibility of a plurality of bacterial or fungal strains from the polymicrobial biological sample.

6. The method of claim 5, wherein the polymicrobial biological sample is selected from whole blood, plasma, serum, red blood cell fraction, saliva, cerebrospinal fluid, semen, urine, stool, rectal swab, nasal swab, wound swab, dermal swab, bile, lymph, sputum, lavage fluid, or a combination thereof.

7. The method of claim 6, wherein the polymicrobial biological sample is cultured prior to performing the PCR assay.

8. The method of claim 7, wherein the polymicrobial biological sample is a bacterial or fungal isolate.

9. The method of any one of claims 5 to 8, wherein 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 antimicrobials or antimicrobial class.

10. The method of claim 9, wherein 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.

11. The method of any one of claims 5 to 10, wherein the identification of the plurality of bacterial or fungal strains utilizes a plurality of strain-specific 5` nuclease (TaqMan) oligonucleotide probes, each probe labeled with a fluorescent dye and each fluorescent dye having a different emission wavelength from another fluorescent dye.

12. The method of any one of claims 1 to 11, wherein the primer and probe oligonucleotides that hybridize more selectively to the *rplP*, *gyrB*, or *rpoB* target gene that is in the Enterobacterales taxonomic order than to the target gene that is not in the Enterobacterales taxonomic order is selected from the group comprising the nucleotide sequences comprising SEQ ID NOs: 1-16; and/or

wherein the primer and probe oligonucleotides that hybridize more selectively to the *tuf*, *rpoB, ddl,* or *gyrB* target gene that is in the *Enterococcus* genus than to the target gene that is not in the *Enterococcus* genus is selected from the group comprising the nucleotide sequences comprising SEQ ID NOs: 50-66; and/or

wherein the primer and probe oligonucleotides that hybridize more selectively to the *tuf*, *ompA, rpoB,* or *gyrB* target gene that is in the *Acinetobacter* genus than to the target gene that is not in the *Acinetobacter* genus is selected from the group comprising the nucleotide sequences comprising SEQ ID NOs: 17-28; and/or

wherein the primer and probe oligonucleotides that hybridize more selectively to the *tuf*, *gyrB*, or *rpoB* target gene that is in the Pseudomonas genus than to the target gene that is not in the Pseudomonas genus is selected from the group comprising the nucleotide sequences comprising SEQ ID NOs: 32-40; and/or

wherein the primer and probe oligonucleotides that hybridize more selectively to the *fdnG, gyrB,* or *tuf* target gene that is in the *Strenotrophomonas maltophilia* species than to the target gene that is not in the *Strenotrophomonas maltophilia* species is selected from the group comprising the nucleotide sequences comprising SEQ ID NOs: 41-49; and/or

wherein the primer and probe oligonucleotides that hybridize more selectively to the *CPE*, *gyrB*, or *ddlA* target gene that is in the *Staphylococcus aureus* species than to the target gene that is not in the *Staphylococcus aureus* species is selected from the group comprising the nucleotide sequences comprising SEQ ID NOs: 67-69, and 72-78; and/or

wherein the primer and probe oligonucleotides that hybridize more selectively to the *CPE*, *gyrB*, or *ddlA* target gene that is in the *Staphylococcus aureus* species than to the target gene that is not in the *Staphylococcus aureus* species is selected from the group comprising the nucleotide sequences comprising SEQ ID NOs: 67-69, and 72-78; and/or

wherein the primer and probe oligonucleotides that hybridize more selectively to *tuf* target gene that is in the *Streptococcus* genus than to the target gene that is not in the *Streptococcus* genus species is selected from the group comprising the nucleotide sequences comprising SEQ ID NOs: 100-102.