

CLAIMS

1. A 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 to simultaneously identify and determine the antimicrobial susceptibility of a target group of bacteria or fungi that have similar or identical clinical breakpoints for at least one antimicrobial or one antimicrobial class.
2. The method of claim 1, wherein the identification of the target group of bacteria or fungi is by detecting a signal that is specific to the target group of bacteria or fungi.
3. The method of claim 2, wherein the specific signal is detected by 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.
4. The method of claim 3, wherein the target gene is selected from *rplP*, *ompA*, *tuf*, *rpoB*, *ddl*, *ddlA*, *fdnG*, *sodA*, *gyrB*, O-antigen acetylase, *ecfX*, *tusA*, CPE, *sip*, and *muc*.
5. The method of any one of claims 1 to 4, wherein the target group of bacteria or fungi comprises a taxonomic order.
6. The method of claim 5, wherein the taxonomic order is the order Enterobacterales
7. The method of any one of claims 1 to 4, wherein the target group of bacteria or fungi represents a taxonomic family.
8. The method of claim 7, wherein the taxonomic family is selected from Enterobacteriaceae, Yersiniaceae, Morganellaceae, or a combination of said families.
9. The method of any one of claims 1 to 4, wherein the target group of bacteria or fungi comprises a taxonomic genus.
10. The method of claim 9, wherein the taxonomic genus is selected from *Enterococcus*, *Candida*, *Pseudomonas*, *Acinetobacter*, *Staphylococcus*, *Stenotrophomonas*, *Streptococcus*, and *Escherichia*, *Klebsiella*, *Enterobacter*, *Salmonella*, *Citrobacter*, *Serratia*, *Shigella*, *Corynebacterium*, *Micrococcus*, *Bacillus*, *Haemophilus*, *Propionibacterium*, *Bacteroides*, *Clostridium*, *Peptostreptococcus*, *Fusobacterium*, *Pasteurella*, *Lactobacillus*, *Aerococcus*, *Prevotella*, *Burkholderia*, *Moraxella*, *Vibrio*, *Listeria*, *Plesiomonas*, *Yersinia*, *Morganella*, *Providencia*, or *Proteus*.
11. The method of any one of claims 1 to 4, wherein the target group of bacteria or fungi represents a taxonomic species.
12. The method of claim 11, wherein the taxonomic species is selected from *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella*

- oxytoca*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Citrobacter koseri*, *Morganella morganii*, *Providencia stuartii*, *Proteus mirabilis*, *Proteus vulgaris*, *Candida albicans*, *Candida auris*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Acinetobacter pittii*, *Acinetobacter nosocomialis*,
5 *Haemophilus influenza*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Staphylococcus lugdunensis*, *Staphylococcus epidermidis*, *Stenotrophomonas maltophilia*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, or *Streptococcus pyogenes*, *Plesiomonas shigelloides*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio cholerae*.
13. The method of any one of claims 1 to 12 further comprising a step selected from
10 - 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
15 or virulence phenotype; or
both verifying and determining steps.
14. The method of any one of claims 1 to 13 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
20 clinical breakpoints for at least one antimicrobial or one antimicrobial classes.
15. The method of any one of claims 1 to 14, 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.
16. The method of claim 6, wherein the target gene is selected from *rplP*, *gyrB*, and *rpoB*
- 25 17. The method of claim 6, wherein the primer and probe oligonucleotides that hybridize more 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.
18. The method of claim 17, wherein the primer and probe oligonucleotides that hybridize
30 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.
19. A method comprising performing a single multiplexed quantitative real-time PCR assay as a reporter in the presence of at least one concentration of at least one antimicrobial or

antimicrobial class to simultaneously identify and determine the antimicrobial susceptibility of a plurality of bacterial or fungal strains from a biological sample.

20. The method of claim 19, wherein the 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.
21. The method of claim 20, wherein the biological sample is whole blood, plasma, serum, or a combination thereof.
22. The method of any one of claims 20 and 21, wherein the biological sample is cultured prior to performing the PCR assay.
23. The method of any one of claims 19 to 22, wherein the biological sample is a bacterial or fungal isolate.
24. The method of any one of claims 19 to 23, 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 classes.
25. The method of claim 24, 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 classes.
26. The method of any one of claims 19 to 25, 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.
27. The method of any one of claims 19 to 26, wherein the identification of the plurality of bacterial or fungal strains utilizes the TAGS (Temperature Assisted Generation of Signal) technology.
28. The method of any one of claims 19 to 27 further comprising 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; orboth verifying and determining steps.
29. A method comprising performing a single quantitative real-time PCR assay in the presence of at least one concentration of at least one antimicrobial or class of antimicrobials 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 the antimicrobial or the 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.

- 5 30. The method of claim 29, wherein the mathematical relationship is selected from Threshold Cycle (Ct), Slope, Inflection of a sigmoid curve fit, Absolute Fluorescence Intensity (AFI), or Endpoint Relative Intensity (ERI).
31. The method of claim 29, wherein the mathematical relationship is a relative expression between different antimicrobial concentrations or different antimicrobials and is selected
10 from ΔCt , $2^{-(\Delta Ct)}$, Δ Inflection, ΔAFI , or ΔERI , or a combination thereof.
32. The method of claim 30 wherein the mathematical relationship is a combination of Threshold Cycle (Ct), Slope, Inflection of a sigmoid curve fit, Absolute Fluorescence Intensity (AFI), or Endpoint Relative Intensity (ERI), or a combination thereof.
33. The method of claim 31 wherein the mathematical relationship is a combination of
15 relative expressions between different antimicrobial concentrations or different antimicrobials and is selected from ΔCt , $2^{-(\Delta Ct)}$, Δ Inflection, ΔAFI , or ΔERI , or a combination thereof.
34. The method of any one of claims 29 to 33 further comprising a step of selected from
20 - verifying the identification of the target bacterial or fungal strains or target group of bacteria or fungi; or
- determining a mechanism for an antimicrobial susceptibility phenotype or for a toxin or virulence phenotype; or
both verifying and determining steps.
35. The method of any one of claims 29 to 34 further comprising identifying and determining
25 the antimicrobial susceptibility of more than one target bacteria or fungi strains or more than one target groups of bacteria or fungi wherein each target strain or target group has similar or identical clinical breakpoints for at least one antimicrobial or one antimicrobial classes.
36. The method of any one of claims 29 to 35, wherein the PCR assay is a real-time PCR
30 assay.