Multi-drug resistant organisms (MDRO) are a major and growing global concern. Every year, more than two million Americans are infected with antibiotic resistant bacteria, resulting in 23,000 deaths. The CDC recommends screening measures for carbapenem-resistant Enterobacteriaceae (CRE) including point prevalence surveys of patients and healthcare providers in hospital and long-term care facilities, active surveillance and testing of pre-specified high-risk patients, and epidemiological linkages of colonized and infected patients. Patients colonized with multidrug-resistant organisms (MDROs) pose a risk for active infection or transmission of MDROs to other patients. OpGen provides the Acuitas™ MDRO Gene Test from results from peri-anal swabs within 24 hours after sample receipt through CLIA lab. The test detects antibiotic resistance genes in actively infected patients or subjects colonized with MDROs as an aid to patient management and antibiotic stewardship for improved patient outcomes, transmission prevention and control, and better health economics.

Introduction

Test specificity was demonstrated across a variety of gene subtypes using 243 culture isolates with reported genotypes for KPC, NDM, VIM, IMP, OXA, CTX-M and other beta lactamase genes along with VanA. The specificity panel included several common enteric species with a variety of potential clinical isolates. Genotyping and susceptibility profiles including susceptible Gram-negative bacilli, carbapenem-resistant Gram-negative bacilli, carbapenem-resistant Enterobacteriaceae (CRE) and carbapenem-resistant Gram-negative bacilli (3rd and 4th generation) Gram-negative bacilli, Vancomycin resistant Enterococci and Vancomycin resistant enterococci.

Test specificity was 100% consistent with reported antibiotic resistance genotypes or resolved genotypes using independent PCR confirmation tests. The test did not cross react with other antibiotic resistance genes such as SHV, TEM, ACT/MIR, CMY-2, DHA-1, ACT-16 or VEB-1. The antibiotic resistance genotypes were consistent with the reported genotypes based on antibiotic susceptibility testing.

Reproducibility (Table 3)

We demonstrated analytical sensitivity (limit-of-detection, LOD) for each target antibiotic resistance gene in the test using negative control swabs that were sparsely quantitated with bacterial culture isolates harboring reported antibiotic resistant genotypes. Spiked culture levels were determined by parallel counting of colony forming units (CFUs) on culture plates.

LODs for the target antibiotic resistance genes in the test resulted from 13 to 250 CFUs per peri-anal swab.

Analytical Sensitivity (Table 4)

The gene test exhibited excellent reproducibility over three days, multiple laboratory operators and gene target levels with replicate PCR cycle threshold values (Ct) exhibiting intra- and inter-assay variation <12% CV.

Conclusions

1. The Acuitas™ MDRO Gene Test is a sensitive and specific test for direct detection of antibiotic resistance genes from CREs, ESBLs and VREs. The Acuitas™ MDRO Gene Test detects antibiotic resistance genes in actively infected patients or subjects colonized with MDROs as an aid to patient management and antibiotic stewardship for improved patient outcomes, transmission prevention and control, and better health economics.

2. Specificity results from the Acuitas™ MDRO Gene Test were 100% consistent with reported antibiotic resistance genotypes or resolved genotypes using independent PCR tests as confirmation.

3. Antibiotic resistance genotypes were consistent with reported antibiotic susceptibility profiles. The test’s limit-of-detection (LOD) ranged from 13 to 250 CFUs per peri-anal swab for the individual target antibiotic resistant genes.

4. The test exhibited excellent reproducibility over three days, multiple laboratory operators and gene target levels with replicate PCR cycle threshold values (Ct) exhibiting intra- and inter-assay variation <12% CV.

5. The Acuitas MDRO Gene Test is more sensitive (100%) than CRE culture (72%) for the detection of CREs.

Methods

The Acuitas™ MDRO Gene Test is a multiplex PCR test that detects the antibiotic resistance gene families KPC, NDM, VIM, IMP, OXA, CTX-M and VanA across 243 genotypes of gene subtypes. We evaluated the specificity of the test by genotyping 243 clinical isolates with reported antibiotic resistance genotypes and phenotypic antibiotic susceptibility profiles. Analytical sensitivity and reproducibility were determined using peri-anal swabs spiked with antibiotic resistant culture isolates.

Results

The gene test exhibited excellent reproducibility over three days, multiple laboratory operators and gene target levels with replicate PCR cycle threshold values (Ct) exhibiting intra- and inter-assay variation <12% CV.

Analytical Specificity (Tables 1 & 2)

Test specificity was demonstrated across a variety of gene subtypes using 243 culture isolates with reported genotypes for KPC, NDM, VIM, IMP, OXA, CTX-M and other beta lactamase genes along with VanA. The specificity panel included several common enteric species with a variety of potential clinical isolates. Genotyping and susceptibility profiles including susceptible Gram-negative bacilli, carbapenem-resistant Gram-negative bacilli, carbapenem-resistant Enterobacteriaceae (CRE) and carbapenem-resistant Gram-negative bacilli (3rd and 4th generation) Gram-negative bacilli, Vancomycin resistant Enterococci and Vancomycin resistant enterococci.

Test specificity was 100% consistent with reported antibiotic resistance genotypes or resolved genotypes using independent PCR confirmation tests. The test did not cross react with other antibiotic resistance genes such as SHV, TEM, ACT/MIR, CMY-2, DHA-1, ACT-16 or VEB-1. The antibiotic resistance genotypes were consistent with the reported genotypes based on antibiotic susceptibility testing.

Results from the Acuitas™ MDRO Gene Test were compared to results from a conventional CRE screening culture (Quest Diagnostics, Test Code 91669) using negative peri-anal swab specimens that were quantitatively spiked with clinical isolates that included Enterobacteriaceae and carbapenem-resistant Enterobacteriaceae (CRE) with a variety of phenotypic antibiotic susceptibility profiles. Each isolate was tested at approximately 200, 2000, 20,000 and 200,000 colony forming units (CFUs) per peri-anal swab.

The Acuitas™ MDRO Gene Test was more sensitive (99%) than the CRE screening culture with the gene test detecting 10 CRE samples that were negative by CRE culture including some or all CPU levels of the following organisms with the indicated carbapenemase gene families: K. pneumoniae (VIM), K. pneumoniae (IMP), E. coli (NDM and CTX-M-1) and S. marcescens (OXA-48).

Additionally, the Acuitas™ MDRO Gene Test detects other carbapenem-resistant Gram-negative bacilli (e.g. A. baumannii (OXA-23 and OXA-51) and Pseudomonas (VIM)) for which the CRE culture is not designed.

References

2. Guidance for Control of Carbapenem-resistant Enterobacteriaceae (CRE), CRE Toolkit from the CDC (2012).