Clinical Microbiology
NEWSLETTER

CMN
Stay Current...
Stay Informed.

CMN
Vol. 37, No. 21
November 1, 2015
www.cmnewsletter.com

IN THIS ISSUE

Rapid Molecular Screening for Gram-Negative Antimicrobial-Resistance Genes with Commercially Available Methods

G. Terrance Walker, Ph.D., Tony J. Rockweiler, B.S., Rossio K. Kersey, B.S., Kelly L. Frye, B.S., Susan R. Mitchner, B.S., Douglas R. Toal, B.S., and Julia Quan, B.S., OpGen, Gaithersburg, Maryland

Abstract

Antibiotic-resistant bacteria threaten the well-being of many hospitalized patients and cause an added financial burden on health care systems. There is a particular challenge in identification of the antimicrobial-resistance phenotype for Gram-negative microbes, which are emerging as a serious global public health threat. Infections by multidrug-resistant Gram-negative bacteria are defined as those caused by bacteria with resistance to at least one agent in three or more antimicrobial categories. Of particular importance, cases of infections due to extended-spectrum β-lactamase and/or carbapenemase producers are increasing. Although active surveillance for Gram-negative bacilli is not as common as that for Gram-positive microbes, rapid identification of Gram-negative pathogens via passive surveillance is very common. As with other drug-resistant microbes, rapid identification of antimicrobial resistance is key to proper treatment and infection control with carbapenem-resistant bacilli and extended spectrum beta-lactamase producers. Their presence increasingly drives the need to screen infected and colonized patients in an attempt to improve patient management and infection control. Here, we briefly review commercially available molecular detection methods with focus on a new multiplex microfluidic PCR test (Acuitas MDRO Gene Test) that detects the hundreds of gene subtypes from the carbapenemase and extended-spectrum β-lactamases families, as well as the vancomycin resistance gene, *vanA*, found in Gram-positive microbes. We also describe the new Acuitas Resistome Test, which is a multiplex PCR test for 44 families of antibiotic resistance genes from culture isolates of Gram-negative bacilli.

Introduction

Each year, more than 2 million Americans are infected with antibiotic-resistant bacteria, resulting in 23,000 deaths. The lack of therapeutic options to combat these bacteria is well known (1), and microbiology cultures identify only a fraction of all colonized patients. In the United States, annual direct health care costs of multidrug-resistant organisms (MDROs) exceed $20 billion (1). According to the Centers for Disease Control and Prevention (CDC), Gram-negative MDROs are especially troubling (2).

For example, carbapenemase-resistant *Enterobacteriaceae* (CRE) can be found in many medical facilities, and outbreaks have been documented across the U.S. and other parts of the world (3). Enterobacteriaceae that produce carbapenemases (enzymes that deactivate carbapenems and most other β-lactam antibiotics) are increasingly being reported worldwide. Their emergence is troubling, since carbapenems have historically been used as the last-resort option for treatment of infections caused by resistant Enterobacteriaceae, including those producing extended-spectrum β-lactamases (ESBLs). CRE infections are associated with poor patient outcomes and high mortality. Current treatment options include the use of older agents, such as polymyxins, fosfomycin, and aminoglycosides, which are difficult to use due to efficacy and/or toxicity concerns (4).

Corresponding author: G. Terrance Walker, OpGen, 708 Quince Orchard Rd., Gaithersburg, MD, 20878. Tel.: 617-583-3197. Fax: 301-869-9684. E-mail: twalker@opgen.com

The CDC recommends a range of CRE screening measures to identify colonized patients and prevent transmission of infection, including, active surveillance testing of pre-specified high-risk patients (e.g., those in intensive care and long-term care) using primarily perianal or perirectal swabs, epidemiological linkage of colonized carriers and infected patients, and point prevalence surveys of patients and health care providers in hospitals and long-term care facilities (2). Like those colonized with Gram-positive MDROs, patients colonized with Gram-negative MDROs are reservoirs for transmission and self-infection (5). During one outbreak, a carbapenem-resistant *Klebsiella pneumoniae* isolate was undetected for 3 weeks and impacted 18 patients, 11 of whom died between patient transmissions (6).

There are 2 types of carbapenem resistance. If the resistance results from bacterial cell wall impermeability or the presence of efflux pumps, then the mechanisms are not categorized as transmissible; therefore, the risk of outbreaks is low and isolation of patients is not performed. On the other hand, if carbapenem resistance is caused by transmissable genetic elements that promote carbapenemase enzyme production, there is an increased risk of health care-associated outbreaks, and patient isolation is recommended (2). Such carbapenemases are associated with a diverse variety of mobile and transmissible genetic elements, such as plasmids, transposons, and integrons. These enzymes carry multiple resistance genes and therefore confer resistance to several antibiotic classes, such as aminoglycosides, fluoroquinolones, tetracyclines, trimethoprim, sulfonamides, and phenicols (7). Carbapenem-resistant phenotypes may also be attributed to the hyper-production of ESBLs or AmpC β-lactamases (plasmids or chromosomally mediated class C β-lactamases) coupled with outer membrane impermeability due to efflux pump up-regulation and/or porin loss or mutation (7).

A report from the Agency for Healthcare Research and Quality suggests active screening programs can effectively control MDRO prevalence when they rapidly identify colonized patients and place them under contact isolation precautions (8). Furthermore, traditional infection control strategies that target only monitoring of clinical isolates as a trigger for initiating control interventions have not proved effective for *K. pneumoniae* carbapenemase (KPC) control and address only the tip of the iceberg, since there are thought to be approximately 100 colonized patients for every infected patient (8).

Clinical evidence suggests that asymptomatic individuals and patients colonized with MDROs are at risk for further infection. One study found that healthy patients who tested positive for carbapenem-resistant *Acinetobacter baumannii* cultures had 8.4 times the risk of developing a subsequent infection compared to patients who were not colonized (9). In another study, 32 out of 433 patients (7.4%) with carbapenem-resistant *K. pneumoniae* infections developed an infection within 2 to 40 days from colonization to infection (10). Rapid treatment is considered beneficial. In a study of 36 patients with CRE bloodstream infections, the reported time to treatment had a significant effect on the course of infection, with rectal screening leading to earlier recognition and prompt empirical treatment (11). The CDC suggests providers use a range of CRE screening measures to identify carriers and prevent the spread of infection (1). Current culture methods can take up to 4 days to confirm the bacteria and may identify only a fraction of all patients with CRE.

Gene Descriptions

Among Gram-negative MDROs, β-lactamases are the most common of all the resistance mechanisms. ESBLs and carbapenemases function by degrading one or more of the following antimicrobials: penicillins, cephalosporins, monobactams, and carbapenems. Genes for KPC, New Delhi metallo-β-lactamase (NDM), Verona integron-mediated metallo-β-lactamase (VIM), and inosine monophosphate (IMP) encode serine and metallo-β-lactamases with carbapenemase and broad-spectrum inhibition of antibiotics (12).

The presence of carbapenemases has been identified in Gram-negative bacilli and is becoming more prevalent. Carbapenemases have been identified in *Klebsiella* spp., *Escherichia coli*, *Enterobacter* spp., *Proteus mirabilis*, *Citrobacter freundii*, *Serratia marcescens*, *Salmonella enterica*, *Raoultella* spp., *Acinetobacter* spp., and *Pseudomonas aeruginosa* (12).

In the U.S., the highest Gram-negative MDRO mortality rates are associated with KPC and exceed 50% (12). Other mechanisms, like NDM, VIM, and IMP, are associated with mortality rates of 18 to 67% (6). The OXA gene family encodes a diverse set of β-lactamases effective against penicillins, cephalosporins, and carbapenems. Among Enterobacteriaceae worldwide, the CTX-M gene family represents the most common group of ESBLs, and a rapid emergence and spread of *K. pneumoniae* and *E. coli* expressing CTX-M is occurring (13).

Additionally, AmpC β-lactamases are also clinically relevant; they are chromosomally encoded cephalosporinases that are resistant to most β-lactam antibiotics and are often hard to detect by routine methods. AmpC enzymes can be inducible and overexpressed as a consequence of mutation. The occurrence of transmissible plasmids with acquired genes often results in increased β-lactamase production compared to chromosomally expressed *ampC* genes. Broad resistance, due to plasmid-mediated AmpC enzymes, can appear in organisms lacking or having low-level expression of a chromosomal *ampC* gene. Therefore, it may be useful to identify and discriminate between plasmid-mediated and chromosomally expressed AmpC β-lactamases (7).

Historic Methods

The modified Hodge test exploits the inactivation of a carbapenem by carbapenemase-producing strains. A carbapenem-susceptible indicator strain will extend growth toward a carbapenem-containing disc along the streak of inoculum of the tested strain (7). The modified Hodge test is limited by known false-positive reactions (especially among AmpC and CTX-M hyperproducers) in several studies and poor detection of NDM producers (7,14). Recently the RAPIDEC CARBA NP (bioMérieux, Marcy l'Étoile, France), has been added to the Modified Hodge Test as the carbapenemase detection method officially recommended by the Clinical