Rapid Identification of Live Acinetobacter spp. in Bronchoalveolar Lavage Specimens by Automated Immunofluorescence Microscopy

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ABSTRACT

Acinetobacter spp. are responsible for up to 10% of cases of nosocomial pneumonia and present a global challenge. Clinicians need a rapid and reliable assay for identification of this diverse genus, which is important for treatment and for management of drug-resistant bacteria. We present a rapid and sensitive method that required species identification and enumeration of live bacteria in respiratory specimens.

Molecular methods shorten the identification, but cannot differentiate the many species, because they generally quantify some metabolic or genetic component. These are important criteria for many types of specimen, particularly in diagnosing pneumonia. In order to alleviate the delays required for culturing, it would be ideal to analyze live organisms extracted directly from a patient specimen. We present a method that meets this need by using a fluorescent polyclonal anti-chicken secondary antibody. Fluorescent polyclonal anti-chicken IgY was bound to 16 of 20 Acinetobacter spp. by a polyclonal antibody coupled with automated growth tracking of individual bacteria to determine viability. The experimental methods tested in this study were intended to become part of a new rapid diagnostic system using bacteria extracted directly from a patient specimen without prior enrichment cultured or colony isolation.

INTRODUCTION

Hospital acquired infections (HAI), and particularly nosocomial pneumonia, are leading causes of morbidity and mortality in critically ill patients. Acinetobacter spp. are highly capable of antibiotic resistance and are often found in respiratory specimens. Their antibiotic resistance can be plasmid-mediated, and they are commonly found in respiratory specimens. They are often highly drug resistant, their identity and phenotype vary, and current methods are inadequate for their identification.

Background: Bacteriological diagnostic methods for microbial identification are generally based on enrichment culturing and colony isolation. Culturing methods are often highly drug resistant, their identity and phenotype vary, and current methods are inadequate for their identification. In order to alleviate the delays required for culturing, it would be ideal to analyze live organisms extracted directly from a patient specimen.

Methods: Polyclonal antibodies specific for Acinetobacter spp. surface antigens were coupled with fluorescent polyclonal anti-chicken secondary antibody. Fresh bacterial cells were plated into the dark field of idle Vacutainer tubes and incubated in a 1:1 ratio (McFarland standard). After incubation, the tubes were used directly for analysis without enrichment culturing or colony isolation. Results: 19 of 20 Acinetobacter spp. were obtained from ATCC® and JM Laboratories (N. Liberty, IA). The collection included 19 A. baumannii and 1 Acinetobacter genomospecies-13, plus 28 non-Acinetobacter species of species often found in respiratory specimens.

RESULTS

Acinetobacter spp. and non-Acinetobacter species were obtained from ATCC® and JM Laboratories (N. Liberty, IA). The collection included 19 A. baumannii and 1 Acinetobacter genomospecies-13, plus 28 non-Acinetobacter species of species often found in respiratory specimens.

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