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SHORT COMMUNICATION

Epidemiology and detection of acinetobacter using conventional culture and in-house developed PCR based methods

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Summary Active surveillance cultures for multidrug-resistant (MDR) gram-negative bacteria is one strategy to control outbreaks. The objectives of the study are to evaluate the prevalence of *Acinetobacter* colonization and to compare conventional culture and in-house developed PCR based method. Swabs were collected from patients transferred from another organization or were admitted to the intensive care units. Swabs were cultured by conventional method and were tested using in-house LightCycler® 2.0 real-time PCR method. Of 449 tested samples, the majority came from cardiac step down unit (188, 42%), male medical floor (80; 18%), and coronary care unit (66; 13.4%). Of the total specimens, 14 (3%) were positive by PCR and 12 (2.6%) were positive by routine cultures. The positivity rates among wounds, respiratory, perineal, and nasal samples were 3.2%, 9.7%, 4.6% and 0.8% respectively. Two positive samples by PCR were negative by routine culture.

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The overall concordance rate was 99.5% and the positive concordance rate was 85.7%. The current study revealed a low prevalence of MDR *Acinetobacter* among the studied population. The LightCycler® 2.0 PCR produced comparable positive results to routine cultures.
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Introduction

Acinetobacter spp. in general and *Acinetobacter baumannii* in particular are increasingly recognized pathogens in the healthcare setting leading to healthcare associated infections [1—3]. The emergence of multi-drug resistant (MDR) *Acinetobacter* is of particular importance and necessitates the development of rapid and sensitive molecular detection methods to institute the appropriate therapeutic and infection control measures. Such methodology may rely on PCR or loop-mediated isothermal amplification (LAMP) assay [4—6]. In Saudi Arabia, many studies showed high percentage of carbapenem resistant *A. baumannii* [7—9]. Active surveillance cultures for multidrug-resistant (MDR) gram-negative bacteria is one strategy to control outbreaks among patients in intensive care units and patients transferred from facilities with high prevalence rates of MDR organisms [10,11]. The effectiveness of screening cultures for the detection of MDR *Acinetobacter* was 55% even if six samples were obtained [12].

We preemptively developed a screening program to screen patients admitted to the intensive care unit and those admitted from other hospitals. Those patients were put on contact isolations and screened for *Acinetobacter*. Since, routine cultures take longtime for the results; we sought to develop a rapid method for the detection of *Acinetobacter* spp. and to evaluate the prevalence of *Acinetobacter* among this cohort of patients.

Materials and methods

This is a prospective study in a general hospital with 350 beds. Swabs were obtained from the nose and wounds. A total of 449 different multi-site swabs were obtained between January and July 2014.

Laboratory processing and culture methods

The swabs were inoculated on Blood agar plate and CHROMACIN *Acinetobacter* selective agar (Saudi Prepared Media laboratory SPML). Identification and antibiotic resistance testing were done after 24 h of incubation at 37 °C and 5% CO_2 using Vitek2 system (BioMérieux). MDR was defined as resistance to more than two of the following classes: antipseudomonal cephalosporins, antipseudomonal carbapenems, ampicillin/sulbactam, fluoroquinolones, and aminoglycosides [13].

DNA extraction

DNA was extracted using the MagNA-Pure-Compact-System (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. The swabs were broken into 1.5 Eppendorf tube, and 1 ml of sterile 0.9% NaCl was added and the mixture was vortexed vigorously. A total of 400 μl of the sample suspension was pipetted into MagNa pure sample tube and DNA extraction was performed using DNA-bacteria protocol. The DNA sample was eluted in 50 μl total volume.

Primer design

The primers and probes targeting 16S RNA sequence of *Acinetobacter* spp. were designed by TIB Molbiol (Syntheselabor GmbH, Eresburgstr, Berlin). The forward primer was Acineto_S: 5-ACAgCgATgTgATgCTAA-3 and the reverse primer was: Acineto_B: 5-TATTCACCgCggCATT-3. To increase the specificity of the PCR, two internal oligonucleotide hybridization probes: Acineto_FLU probe: 5-TTgCAgACTCCAATCCgg—FL (18 bp, Tm = 56.3) and LCRED640-CTACgATCggCTTTTTgAgAT—PH (21 bp, Tm = 54.5) were added. The size of the amplicon was 115 bp.

PCR amplification

PCR amplifications were carried out in 10-μl volumes containing 2.5 μl of template DNA as shown in Table 1. The PCR conditions of the assay was as follows: after 10 min at 95 °C for FastStart Taq DNA polymerase activation, 30 amplification cycles were performed, each with 5 s denaturation at 95 °C,

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