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Short Communication

New Rapid Screen for the Detection of Imipenem-Resistant *Acinetobacter baumanii*

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SUMMARY: A rapid screen was developed for the detection of imipenem-resistant *Acinetobacter baumanii* (IR*Ab*) following a recent outbreak in the Surgical Intensive Care Unit (SICU) of a hospital in Singapore. Antimicrobial solutions of imipenem ranging from 16 mg/L to 64 mg/L were prepared in-house. Each of the antimicrobial solutions was then incorporated singularly into MacConkey agar plates by two different methods. One of the methods involved preparing MacConkey agar plates in-house and then adding the antimicrobial solution before the agar solidified (AS method). In the second method, 1 ml of the antimicrobial solution was poured onto the surface of the agar plate (LAS method). Fifty hand-nutrient broth washes of medical staff working in the SICU, Medical Intensive Care Unit, Medical Rehabilitation Ward, and Surgical Rehabilitation Ward were inoculated onto the two types of agar media. Two strains of IR*Ab* were isolated from the hands. The LAS plates showed faster bacterial growth of resistant pathogens by about 24 h, and their detection was easier because susceptible bacteria were inhibited by the antimicrobial. The LAS method incorporating imipenem at 32 mg/L is recommended for the rapid screening of resistant pathogens in the routine clinical laboratory.

There is a general consensus and concern about the problems of antimicrobial resistance, with the West leading the discussion. Over the past several years, alarm has been expressed in the United States (U.S.), the World Health Organization has queried about the role of antimicrobials in animal husbandry, the British Special Sub-Group on Antimicrobial Resistance has been set up and the U. S. Institute of Medicine has reported on emerging infections (1,2; http://www.hpa.org.uk/hpa/publications/bookshop/smac.htm). More recently, numerous investigations on antimicrobial resistance were performed in Asia, Singapore reported the discovery of imipenem-resistant *Klebsiella pneumoniae*, and extended spectrum β-lactamases (ESBL) were found in China, Taiwan, and South Korea (3; http://www.bsac.org. uk/default.cfm?fuseaction=resources.viewitem&itemid=289,4), to name just a few examples. Antibiotic resistance has also been shown to disseminate outside the boundaries of hospitals into nursing homes (5). While the misuse and abuse of antimicrobials are important contributing factors, lack of hygiene is also correlated with outbreaks of multidrug resistant organisms. A simple palm-printing experiment demonstrated this simple truth to the healthcare workers in the intensive care units of the national university hospital, Singapore (6). A single clone dissemination had been demonstrated in the spread of metallo-β-lactamase-producing *Enterobacteriaceae* isolates in a university hospital in Taiwan (7), as well as in the occurrence of a multidrug-resistant *Pseudomonas aeruginosa* clone in different hospitals in Rio de Janeiro, Brazil (8). These are examples of breakdown in hand hygiene, and the campaign advocated by the Centers for Disease Control and Prevention of U. S. also emphasized the relevance of infection control measures in curbing antimicrobial resistance (available from http://www.cdc.gov/drugresistance/healthcare/patients.htm).

An outbreak of imipenem-resistant *Acinetobacter baumanii* (IR*Ab*) occurred in the Surgical Intensive Care Unit (SICU) in a Singapore hospital. As part of the infection-control measures, the clinical laboratory carried out screening of medical staff, patients, and the environment for IR*Ab*. The exercise was labor-intensive because routine processing of clinical specimens was also carried out simultaneously. In response to future needs for more screening exercises as part of the infection-control efforts to control such outbreaks, the laboratory developed rapid screen media to detect imipenem-resistant Gram-negative bacilli (GNR), in particular *A. baumanii*.

An earlier experiment was carried out in the clinical laboratory to determine the concentrations of imipenem necessary to inhibit bacterial growth. It was determined that a concentration of 16 mg/L to 64 mg/L imipenem was required for the detection of IR*Ab*. It was found that at 32 mg/L, the growth of susceptible strains was more clearly inhibited, while resistant strains could be easily detected. The higher concentration did not yield further ease of detection. The media were tested on 50 hands of medical personnel from four different wards in the hospital. The study aimed to detect the targeted bacterial growth qualitatively, rather than quantitatively.

Fifty hand washes in nutrient broth were collected over 2 weeks from health care workers from the following wards: SICU (15 hands), Medical Intensive Care Unit (13 hands), Surgical Rehabilitation Ward (11 hands), and Medical Rehabilitation Ward (11 hands). The broths were inoculated the same day they were collected onto each of the media described below and incubated overnight. The broth method was chosen because the entire hand surface area could be studied inclusive of areas under the nail beds. A standard biochemical identification method was then carried out to identify *A. baumanii*. Imipenem susceptibility was carried out using the NCCLS disc diffusion method as well as the E-test strip for imipenem. Known strains of ATCC *P. aeruginosa* and *A.*

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baumanii susceptible and resistant to imipenem, respectively, were used as controls on each day that the experiment was conducted.

Antimicrobial powder of imipenem was used (Sigma Chemical Co., St. Louis, Mo., USA). Concentrations of 16 mg/L to 64 mg/L were prepared using the NCCLS M7-A3 method (9). The antimicrobial solutions were incorporated immediately into the agars in the following manner. (i) Agar screen (AS): MacConkey agar plates were prepared in-house by dissolving the powder supplied by BBL (Becton-Dickinson, Paramus, N. J., USA) and autoclaved as recommended by the manufacturer. The antimicrobials at different concentrations were then incorporated singularly into each of the agar plates before they solidified. The plates were dried overnight and used the next day for inoculation of hand washes. (ii) Layered agar screen (LAS): 1 ml of each of the antimicrobial solutions was pipetted singularly onto commercially prepared MacConkey plates purchased from Becton-Dickinson (New South Wales, Australia). The solution was distributed evenly over the surface of the agar by gentle rotation of the plates. The plates were then left to dry at atmospheric conditions for a few hours and inoculated the same day as the hand washes. The performance of the sequential stability of the effect of antibiotics was also studied. The AS media had a shelf life of up to 3 weeks if stored at 4°C but the LAS media should be used the same day. The short shelf life of the latter was overcome by aliquoting the antimicrobial solutions and storing at −70°C. These solutions could be stored for many months but thawed only once and used the same day.

The results of the nutrient broth washes of hands of healthcare workers are summarized in Table 1. Table 2 compares the performance of the AS and LAS methods at increasing concentrations of imipenem. Two strains of IR*Ab* were isolated, one from the hands of medical staff from the SICU and another from the Medical Rehabilitation Ward. The *A. baumanii* strains were detected one day earlier by the LAS plate when compared to the AS plate. At 32 mg/L, the antimicrobial was able to suppress the susceptible bacteria much better than the concentration of 16 mg/L, while the colony outcomes were very similar when compared to the higher concentration at 64 mg/L. The ease of detecting IR*Ab* obtained by both types of rapid screens (AS) and (LAS) was much improved compared to an earlier exercise when no antimicrobial was incorporated into the agar.

Antimicrobial-incorporated media are recommended for the rapid screening of resistant organisms. MacConkey incorporated with antimicrobial was a suitable medium, because the colony morphology could be easily recognized and the presence of antimicrobial eliminated many bacteria not relevant in the study. The LAS was preferred because the agar plate could be purchased commercially. The resistant strains grew faster on them by about 24 h. Antimicrobial solutions could be prepared in-house and stored in aliquots to be used when necessary. Alternatively, pharmaceutical companies are invited to manufacture discs incorporated with antimicrobials that can be easily reconstituted for immediate use. This method of preparation of antimicrobial solutions was desirable as it was easy to store antimicrobial discs, ensuring the potency and longer life span of the drugs.

Further evaluation of the LAS method may include determination of the drug gradient at the surface of the agar compared to its final concentration at the bottom of the petri dish. What will be the performance variables in terms of specificity and sensitivity? The clinical laboratory is an integral part of the infection control team to help manage infectious outbreaks and emergence of multidrug-resistance organisms. The LAS method was found to be suitable for use in this situation, especially when numerous specimens were received for screening purposes. In addition to *A. baumanii*, other imipenem-resistant GNR may be detected using this method, appearing either as lactose fermenters or non-lactose fermenters. However, this would require further work to evaluate the LAS method for other imipenem-resistant GNR. The finding of IR*Ab* on two health care workers in the intensive care unit where the outbreak had occurred required further investigation. Besides reviewing the appropriateness of antimicrobial utilization, education on hand hygiene practice, and the cleanliness of the hospital environment had to be addressed.

Table 1. Organisms isolated from nutrient-broth washes of hands of healthcare workers in a Singapore hospital[1)]

Location in the hospital	Organisms isolated (No. of hands)
Surgical Intensive Care Unit	IR*Ab* (1), MRSE (2), *Candida* spp. (3)
Medical Intensive Care Unit	MRSA (1), SMA (5), *Candida* spp. (3), ESBL (3)
Surgical Rehabilitation Ward	MRSE (4), SMA (1), ESBL (2), *Candida* spp. (4)
Medical Rehabilitation Ward	IR*Ab* (1), *Candida* spp. (4)

[1)]: Nutrient-broth washes were inoculated onto commercial MacConkey agars that were not incorporated with imipenem. The detection of IR*Ab* was difficult when MacConkey was not incorporated with imipenem due to the growth of skin flora and other resistant organisms.
IR*Ab*, imipenem-resistant *Acinetobacter baumanii*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; MRSA, methicillin-resistant *Staphylococcus aureus*; SMA, *Stenotrophomonas maltophilia*; ESBL, extended-spectrum β-lactamase producing Gram-negative rods.

Table 2. Comparison of the growth characteristics of organisms in AS and LAS media which were incorporated with increasing concentrations of imipenem

Medium	16 mg/L[1)]	32 mg/L[2)]	64 mg/L[2)]
AS	25 - 50 CFU	10 - 15 CFU	10 - 15 CFU
LAS[3)]	>50 CFU	10 - 15 CFU	10 - 15 CFU

Nutrient-broth washes of hands were inoculated onto AS and LAS media.
CFU, colony forming units.
[1)]: More 'skin flora' was observed in LAS compared to AS when the media were incorporated with imipenem at 16 mg/L.
[2)]: Similar numbers of CFU were observed for concentrations at 32 mg/L and 64 mg/L for both media.
[3)]: Imipenem-resistant *Acinetobacter* spp. required 48 h to grow in AS compared to 24 h in LAS.

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