Surveillance Cultures and Duration of Carriage of Multi-Drug-Resistant

*Acinetobacter baumannii*

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**Running title**: Prolonged duration of carriage of MDR-AB

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Abstract

**Background:** Isolating carriers of multi-drug-resistant *Acinetobacter baumannii* (MDR-AB) is the main measure to prevent its spread. Identification of carriers accompanied by contact precautions is essential. We aimed to determine the appropriate surveillance sampling sites and the duration of carriage of MDR-AB.

**Methods:** We studied prospectively two groups of patients from whom MDR-AB was previously isolated: (1) those with recent clinical isolation (\(\leq 10\) days); (2) those with remote clinical isolation (\(\geq 6\) months). Screening for carriage was conducted from six sites: nostrils, pharynx, skin, rectum, wounds, and endotracheal aspirates. Strains recovered concurrently from different sites were genotyped using pulsed-field gel electrophoresis.

**Results:** Twelve of 22 with recent clinical isolation of MDR-AB had \(\geq 1\) positive screening culture, resulting in a sensitivity of 55% when 6 body sites were sampled. Sensitivities of single sites ranged from 13.5% to 29%. Among 30 patients with remote clinical isolation, screening culture was positive in five (17%), with a mean duration of 17.5 months from last clinical culture. Remote carriers had positive screening cultures from the skin and pharynx, but not from nose, rectum, wound, or endotracheal aspirates. Eleven strains from 5 patients were genotyped. In all but one case, isolates from different sites in a given patient were clonal.

**Conclusions:** Current methodology is suboptimal to detect MDR-AB carriage. Sensitivity of surveillance cultures is low, even when 6 different body sites are sampled. The portion of individuals with previous MDR-AB isolation who remain carriers for prolonged periods is substantial. These data should be considered when designing measures to limit the spread of MDR-AB.
In the past decade, multi-drug resistant *Acinetobacter baumannii* (MDR-AB) has emerged as a major nosocomial pathogen in many parts of the world, resulting in devastating outcomes in terms of morbidity, mortality, and costs (3, 4, 12, 14, 18, 26). Therapeutic options are often scarce, and in many instances there is not a single drug to administer against this pathogen (3, 12-14, 17-20). The International Network for the Study and Prevention of Emerging Antimicrobial Resistance defined the emergence of carbapenem resistance in *Acinetobacter baumannii* (AB) infections as a ‘global sentinel event,’ warranting prompt epidemiological and microbiological interventions (30).

The most effective measures reported thus far to reduce the burden of MDR-AB infections in hospitals are strict contact precautions, cohorting, applying a routine surveillance program in order to identify silent carriers, intense cleansing of the carrier's environment, managing infections appropriately, and attempts at decolonization (of questionable value) (4, 14, 23). These interventions, though cost-effective in the long run, pose a vast burden on hospital resources and personnel (2, 4, 10, 11, 13, 17, 23, 25, 26, 28).

*Acinetobacter* species are widely distributed in the environment and may be common commensals in humans (14). Rates of skin colonization as high as 25-40% in healthy ambulatory volunteers, and up to 75% in hospitalized patients, are reported (3-5, 26). The bacteria colonize the pharynx intermittently in 7% of the general population, and in addition can be isolated from sputum, urine, stool, and vaginal discharges (4, 14, 26). In contrast, MDR-AB is primarily a nosocomial pathogen, acquired in the healthcare setting, and its carriage and natural history have not been well studied (14).

This study had two principal aims: (1) to examine the sensitivity of culturing various body sites to detect carriage of MDR-AB among patients with recent MDR-AB clinical isolation, assuming that this group of patients continues to carry the organism at time of surveillance;
(2) to evaluate long-term carriage of MDR-AB among patients with a remote MDR-AB clinical isolation who are readmitted.
Materials and Methods

Setting

Tel-Aviv Sourasky Medical Center (TASMC) is a 1,200-bed tertiary care teaching hospital, comprising 45 wards, with 84,000 admissions and over 87,500 clinical cultures processed annually. Hospital computerized databases record patients with previously isolated MDR-AB presenting to the emergency rooms, and 'flagging' of the patient in the system results in immediate application of contact precautions. Israeli hospital over the past decade have been considered a hyper-endemic environment for MDR-AB, with a rate of detection of 1 per 140 hospital admissions of medical or surgical patients (3, 23).

Acinetobacter isolates were identified to the species level using the Vitek-2 system (bioMérieux, Hazelwood, MO). In order to differentiate the genomic pattern of the Acinetobacter species identified, PCR of the 16S-23S rRNA intergenic spacer (ITS) was carried out for the study isolates, according to an established protocol (9). Antimicrobial susceptibility testing was performed using the Vitek 2 AST GN09 card, and susceptibilities to imipenem and meropenem were confirmed by disc diffusion or E-test (AB Biodisk, Solna, Sweden). An AB isolate was defined as MDR if it was resistant to at least three classes of antibiotics (including penicillins, cephalosporins, monobactams, β-lactamase-inhibitor combinations, aminoglycosides and fluoroquinolones), while susceptibility to amikacin, ampicillin/sulbactam, imipenem, meropenem, and minocycline, were allowed. All isolates were processed according to the Clinical and Laboratory Standards Institute (CLSI) criteria (1).

Infection control practices during the study period

Throughout the study period, contact precautions for the duration of the hospital stay were advised for patients from whom MDR-AB was isolated. Upon admission of a previously identified carrier, or within 24 hours of initial culture of MDR-AB in a...
hospitalized patient, ward personnel received daily e-mail list of patients need to be under contact precautions, and were called by infection control practitioners to confirm adherence to contact precautions. In most cases, patients were in a multi-patient room and their bed was marked with a sign bearing the words, “contact precautions,” gowns and gloves for patient contact, and alcohol-based hand disinfectant. Periodic surveys revealed that the material required for contact precautions was present over 90% of the time; however, compliance with use was not systematically recorded.

Study design

Prospective surveillance was conducted, from June 1, 2006, to August 31, 2006. Hospitalized adults (>18 years of age) who had positive clinical cultures of MDR-AB isolated between December 1, 2002, and August 31, 2006, were considered for inclusion. Two groups of patients were included: (1) patients from whom MDR-AB was isolated from a clinical specimen in the preceding 10 days. We considered these patients with a recent positive clinical isolation to be carriers by definition. This group was studied to determine and compare the sensitivities of surveillance from six different body locations: (2) patients with a positive clinical isolation during previous admissions, ≥6 months, to study long-term carriage.

Each patient was approached by infection-control personnel, who after obtaining consent cultured the patient from four different surveillance sites: the nose (nostrils, bilaterally), the pharynx, the skin (the swab was pre-moistened in the transport media; the same swab was then used to culture the axillae, the antecubital fossae, and the groin bilaterally, in that order), and the rectum. Two additional sites were sampled in subsets of patients: wounds, if a draining ulcer was present and endotracheal aspirates, if the patient was intubated.
Swabs (CE0373®; MEUS; Piove di Sacco; Italy) were inoculated within 1 hour in enriched Brain Heart Infusion (BHI) broth, and incubated for 24 hours at 35°C. Samples were then streaked on selective MacConkey Agar® plates (Novamed Ltd; Jerusalem, Israel), containing 2 µg/ml amphotericin B and 8 µg/ml ceftazidime. Quality control of the selective plates was performed on a regular basis, using Escherichia coli ATCC strain 25922 as a susceptible strain, and a ceftazidime-resistant E. coli clinical isolate as a resistant strain. In a preliminary study, the enrichment method showed superior sensitivity compared to direct plating of swabs onto selective media, when performed in parallel (unpublished data). Representative colonies of each morphotype were picked in duplicates and transferred to Enterotubes (Enterotest®; Hy laboratories Ltd; Rehovot, Israel). Oxidase-negative non-fermentors, were further identified by the Vitek 2 system to the species level, and the antimicrobial susceptibility profile was determined. Isolates were stored at -70°C for further work-up.

**Epidemiologic data collection:**

Epidemiologic data were collected via patient interviews and chart review. Parameters assessed included demographics (age, sex), microbiologic parameters of previous clinical isolations, medical diagnosis at the current admission, long-term care facility residency, functional status, level of consciousness, co-morbidities (including calculation of the Charlson Comorbidity Index (22)), severity of illness (by using the McCabe score (6)), use of chronic invasive devices, recent invasive procedures, recent use of antibiotics, chronic medications, tobacco or alcohol use, recent immunosuppressive treatment (glucocorticoids or oncologic chemotherapy), malignant diseases, renal function, nutritional status and time intervals from most recent hospitalization and/or ICU stay.

**Pulsed-Field Gel Electrophoresis:**
Patients with ≥2 positive cultures from different body sites were genotyped and classified into genetic clusters using pulsed-field gel electrophoresis (PFGE). Since the initial isolates from previous admissions of the remote carriers were not available, we could not compare the PFGE patterns between previous and current isolations. The MDR-AB was cultured on MacConkey Agar®, and afterwards in BHI broth for 18 hours. Agarose discs of genomic DNA were prepared as previously described (21, 24). DNA was then cleaved using 20 U of the restriction enzyme Apal endonuclease (New England Biolabs, Beverly, MA) for 3 hours at 25°C (15, 21, 24). Electrophoresis was performed in a 1% agarose gel (BMA Products) prepared and run in 0.5 x Tris-borate-EDTA buffer on a CHEF-DR III apparatus (Bio-Rad Laboratories, Richmond, CA). The initial switch time was 5 seconds, the final switch time was 35 seconds, and the run time was 23 hours at 6 V/cm. Gels were stained in ethidium bromide, de-stained in distilled water, and photographed by using a Bio-Rad GelDoc 2000 camera. DNA patterns were analyzed visually and by using Diversity software (Bio-Rad). PFGE DNA patterns were compared and interpreted according to an established protocol (8, 24, 27).

Statistical analysis

Continuous variables were compared between groups using an unpaired t-test and a paired t-test within each group. Categorical variables were compared by the Pearson χ² test. For small samples, Anova and the Fisher exact test were used to analyze continuous and categorical variables, respectively. Statistical analyses were conducted using SPSS (version 13.0, SPSS Inc., Chicago, IL) and Stata (version 9.0, Stata Corp., College Station, Tex.). P values ≤ 0.05 were considered significant.
Results

Surveillance sensitivity study

Twenty-two patients with a recent clinical isolation of MDR-AB (≤10 days) were considered 'carriers' by definition. These patients were surveyed. Sixteen of the patients were men (73%), the mean age was 68 years (range 29-88), and the mean number of days since the last positive MDR-AB isolation was 6 (range 3-10). The index clinical culture of MDR-AB was obtained from endotracheal aspirates in 7 patients, from a wound in 6 patients, from the urine in 3 patients, from an intra-vascular catheter tip in 3 patients, and from the blood in 3 patients.

Of the 22 patients studied, 12 had at least one positive surveillance isolation (range 1-4). Therefore, the overall sensitivity of the multi-site surveillance approach was 55%. The surveillance-sites and clinical-sites cultures are depicted in Table I. No statistical difference between the various sites in terms of yield was found (p=0.36). The clinical syndrome and the number of days since the previous MDR-AB isolation were not significantly associated with surveillance culture results.

The clinical culture location most correlated with positive surveillance isolation was the endotracheal aspirate, followed by wounds: five of seven patients (71%) with a positive clinical culture from the endotracheal aspirate and four of six patients (67%) with a positive clinical culture from a wound had positive surveillance isolation at any site. Other sites of positive clinical culture – urine, intra-vascular catheter tip, and blood - were less predictive of positive surveillance isolation, with a correlation of 1 of 3 (33%) for each site.

Duration of carriage study

During the 3-month study period, 30 of 36 patients who had a previous remote (≥6 months) clinical isolation of MDR-AB and were readmitted to TASM agreed to participate in the study. One hundred and forty samples were obtained from these patients. In five
patients (17%), at least one surveillance isolation yielded MDR-AB. The mean duration from the first MDR-AB isolation from a clinical culture was 20 months (range 8-42), and from the last isolation 16 months (range 1-39). The duration from the last clinical isolation was similar between those found to be long-term carriers and those with negative surveillance cultures (Table II). Risk factors for prolonged carriage were a bed-ridden functional state, disorientation at admission, and status post coronary bypass surgery, as depicted in Table II. Other risk factors, including recent use of immunosuppressants, did not reach significance. The source of the previous positive isolation, and the current admission diagnosis did not affect the duration of carriage.

Among the remote clinical MDR-AB isolations cohort, 7 of 140 sites sampled yielded the organism, i.e., three patients had one site positive and two patients had two sites positive for MDR-AB. The skin was the source for 4 of the isolates, and the pharynx for 3. No MDR-AB were isolated from the nostrils or rectum (30 samples obtained from each site). The clinical-sites that were surveyed, wounds (17 samples) and endotracheal aspirates (3 samples), had no positive isolations as well.

**Genotyping**

Among the 52 patients studied (the two cohorts combined), 7 of the 17 patients with positive surveillance isolations had ≥2 positive sites, yielding a total of 16 strains. Eleven isolates from 5 patients were genotyped (figure 1); 3 distinct clones were detected, all known nosocomial clones, commonly associated with MDR-AB infections at TASMC (3). For each of the 5 patients in whom genotyping was performed, the same clone was found at 2 sites; in one patient an additional clone, at a third site, was also found. (Patient 517, figure 2).
Discussion

In this surveillance study we focused on two questions which are cardinal in limiting the spread of MDR-AB: which body site should be cultured in order to detect carriage of MDR-AB, and what is the duration of carriage? We found that culturing a single body site has very low sensitivity, not higher than 30%, and that even when multiple sites are sampled the sensitivity of detecting carriers of MDR-AB reaches only 55%. Sampling multiple body sites is time-consuming and costly, and not suitable for routine use by clinicians and clinical laboratories. The low sensitivity of single-site surveillance for MDR-AB is in contrast to much higher sensitivities of single-site screening for other MDR pathogens, e.g., rectal cultures for the detection of carriers of vancomycin-resistant enterococci, or nasal cultures to detect carriers of methicillin-resistant *Staphylococcus aureus* (16, 29). We also found that MDR-AB may be carried for long durations, up to 42 month, and that prolonged carriage affects at least 17% of patients with previous clinical isolations of MDR-AB. This proportion of long-term carriers is likely an underestimate due to the limited sensitivity of surveillance to detect carriers (55%), and implies that prolonged carriage of MDR-AB may affect 30% of patients with remote clinical isolations.

A combination of several factors may explain the low sensitivity of surveillance cultures. First, it was assumed that all patients from whom MDR-AB was isolated from clinical culture within the last 10 days are carriers, and this may not be true in all cases, i.e., certain patients may have MDR-AB at the infection site only. This is likely the explanation in very few cases, since both surveillance sites and clinically relevant sites (draining wounds and endotracheal aspirates from intubated patients) were sampled. Second, patients may have received an appropriate antimicrobial therapy against MDR-AB, which may have eradicated the pathogen prior to our sampling. However, among our cohort, only 2 have received such a regimen, as one patient received cefepime (in which the in-vitro results has a
questionable significance in the presence of an extended-spectrum β-lactamase producing organism), and the second patient received, only for one day prior to culture, colistin. Therefore, we believe that this factor did not considerably bias our results. Third, the sampling and microbiological methods used may not be sensitive enough to detect MDR-AB, particularly if they are present at the sampled body sites in low concentrations. Although an enrichment method was used, after confirming its superiority to direct culturing onto selective media, this method may still not be sensitive enough. Regarding the surveillance skin samples for example, we can only postulate that another method which samples larger skin surface area than swabs, would increase the yield of the surveillance cultures. Fourth, MDR-AB may occupy different body sites in different patients.

One might expect that the pharynx would be the best site to sample for carriage of MDR-AB for a number of reasons: *Acinetobacter* spp. were reported to be common commensals in the human pharynx, pneumonia is the most common clinical syndrome of AB infections, and the bacterium is known for its ability to rapidly colonize tracheotomies (4). In fact, we found that the pharynx had low sensitivity (23%) as a surveillance site. The skin was also suggested to be an appropriate site for surveillance of MDR-AB (16). In our cohort of patients with remote clinical isolation, 4 of 5 long-term carriers were identified by the skin samples. However, the skin had the lowest sensitivity as a surveillance site in patients with recent clinical isolation (13.5%), though as we previously mentioned, the appropriate methodology of obtaining skin samples is yet not well defined.

Isolating MDR-AB in hospitalized patients depends on external ecological variables and risk factors related to the patients themselves (7). Several reports have discussed in the past the risk factors associated with the development of MDR-AB infections in hospitalized patients (4, 14, 26). As far as we know, this is the first report that investigates the risk factors associated with prolonged MDR-AB carriage. Two of the risk factors identified, a bed-
ridden and a disoriented state, were both reported in the past as being associated with MDR-AB infections in hospitalized patients (4, 26). Small sample sizes limit the generalizability of these results, and do not allow for meaningful multivariate analysis. In addition, due to the low sensitivity of surveillance, and possible resultant misclassification of MDR-AB carriers as non-carriers, identifying these risk factors should be interpreted cautiously.

Another study limitation was that the previous MDR-AB strains of the remote carriers were not available for genotyping, and therefore we can only assume that remote carriers remained carriers of the same clone. However, when we genotyped isolates that were colonizing concurrently different body sites, we saw that a given clone can be isolated from multiple sites, and cause different clinical syndromes. In addition, the clones identified were all familiar, having been previously associated with MDR-AB outbreaks at TASMC (3), and therefore it is reasonable to assume that the long-term carriers acquired the strains in the nosocomial setting, continued to harbor them for several years, and probably spread them in their local outpatient environment as well.

In conclusion, our study demonstrates that the current methodology to detect MDR-AB carriage is suboptimal, and that persistent carriage of MDR-AB occurs in a substantial proportion of patients. Improved methods of surveillance are necessary and long-term contact precautions for MDR-AB carriers should be considered.
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References


Table I: Sensitivity of surveillance cultures from different body sites among patients with recent clinical culture of MDR-AB\(^a\) (≤10 days)

<table>
<thead>
<tr>
<th>Type of culture site</th>
<th>No. of patients sampled</th>
<th>No. with MDR-AB(^a)</th>
<th>Sensitivity rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surveillance sites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nostrils</td>
<td>22</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>Pharynx</td>
<td>22</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>Skin</td>
<td>22</td>
<td>3</td>
<td>13.5</td>
</tr>
<tr>
<td>Rectum</td>
<td>21</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td><strong>Clinical sites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound(^b)</td>
<td>9</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>Endotracheal aspirates(^c)</td>
<td>7</td>
<td>2</td>
<td>29</td>
</tr>
</tbody>
</table>

\(^a\) Multi-drug resistance *Acinetobacter baumannii*

\(^b\) Only discharging wounds were cultured

\(^c\) Endotracheal aspirates were obtained only from intubated patients
Table II: Several epidemiological parameters and their association with MDR-AB $^a$
prolonged carriage in univariate analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Carriers (n=5)</th>
<th>Non-carriers (n=25)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean) in years</td>
<td>78±12</td>
<td>69±18</td>
<td>0.34</td>
</tr>
<tr>
<td>Sex (men)</td>
<td>1</td>
<td>16</td>
<td>0.095</td>
</tr>
<tr>
<td>Months from last MDR-AB $^a$ isolation</td>
<td>17.5 ± 16</td>
<td>16 ± 8.5</td>
<td>0.73</td>
</tr>
<tr>
<td>Resident of LTCF $^b$</td>
<td>3</td>
<td>7</td>
<td>0.68</td>
</tr>
<tr>
<td>Bed-ridden</td>
<td>4</td>
<td>4</td>
<td>0.011</td>
</tr>
<tr>
<td>Disoriented consciousness</td>
<td>3</td>
<td>2</td>
<td>0.022</td>
</tr>
<tr>
<td>Charlson Comorbidity Index-mean (22)</td>
<td>4.6</td>
<td>4</td>
<td>0.24</td>
</tr>
<tr>
<td>McCabe severity of illness score-mean (6)</td>
<td>2</td>
<td>2.4</td>
<td>0.33</td>
</tr>
<tr>
<td>Recent use of immunosuppressant $^c$</td>
<td>2</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>Permanent invasive devices</td>
<td>3</td>
<td>7</td>
<td>0.68</td>
</tr>
<tr>
<td>Recent surgery $^d$</td>
<td>2</td>
<td>7</td>
<td>0.59</td>
</tr>
<tr>
<td>Recent invasive procedure $^d$</td>
<td>1</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Antibiotics on admission</td>
<td>2</td>
<td>7</td>
<td>0.59</td>
</tr>
<tr>
<td>s/p coronary bypass surgery</td>
<td>2</td>
<td>0</td>
<td>0.023</td>
</tr>
<tr>
<td>Chronic insulin injections</td>
<td>2</td>
<td>1</td>
<td>0.064</td>
</tr>
<tr>
<td>Chronic theophylline therapy</td>
<td>2</td>
<td>1</td>
<td>0.064</td>
</tr>
</tbody>
</table>

$^a$ Multi-drug resistance Acinetobacter baumannii

$^b$ Long-term care facility

$^c$ Including glucocorticoids and anti-cancerous chemotherapy in the previous 3 months

$^d$ Previous 6 months
Legend to figure

Distribution of pulsed-field gel electrophoresis clones among patients with ≥22 positive isolates. Lane 1, Lambda ladder molecular size marker; Lanes 2-3, clone H, patient 811, with isolates recovered from pharynx and skin, respectively; Lanes 4-5, clone D, patient 502, with isolates recovered from skin and rectum, respectively; Lanes 6-7, clone C, patient 515, with isolates recovered from skin and rectum, respectively; Lanes 8-10, patient 517, with isolates recovered from sputum (clone D), nose (clone C), and rectum (clone D), respectively; Lanes 11-12, clone C, patient 518, with isolates recovered from pharynx and nose, respectively.