Brief report

Longitudinal characterization of Acinetobacter baumannii-calcoaceticus complex, Klebsiella pneumoniae, and methicillin-resistant Staphylococcus aureus colonizing and infecting combat casualties

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Key Words:
Colonization
Drug-resistant Acinetobacter baumannii-calcoaceticus complex
Klebsiella pneumoniae
Methicillin-resistant Staphylococcus aureus MRSA

Over the last decade, a substantial increase in infections caused by multidrug-resistant organisms (MDROs) has occurred worldwide, especially among US casualties from Iraq and Afghanistan.\textsuperscript{1-3} Multidrug-resistant (MDR) Acinetobacter baumannii-calcoaceticus complex (ABC), extended-spectrum \textit{\textgamma}-lactamase (ESBL)-producing Klebsiella pneumoniae, and methicillin-resistant \textit{Staphylococcus aureus} (MRSA) have been identified as the most commonly colonizing and infecting MDRO pathogens from combat casualty at our facility.\textsuperscript{4} The high prevalence of MDRO infections in casualties from combat operations in Iraq and Afghanistan provided a unique opportunity to study the relationship between colonizing and serial infecting isolates.

MATERIALS AND METHODS

Patient population and study design

Combat casualties admitted to Brooke Army Medical Center (BAMC) and the US Army Institute of Surgical Research burn center between January 2006 and September 2008 were screened (nares for MRSA, axillae and groin for gram-negative organisms) for the presence of ABC, ESBL-producing \textit{K pneumoniae}, and MRSA.

Screening isolates were defined as organisms recovered from the nares, axillae, or groin from cultures obtained within 24 hours of admission. Infecting isolates were defined as those recovered from culture at any site during hospitalization that resulted in a clinical diagnosis of infection by the patient’s physician and antibiotic treatment (excluded perioperative prophylaxis). Two separate subgroups for comparison were planned: patients with positive surveillance cultures and infecting cultures for the same organism at any site during hospitalization that resulted in a clinical diagnosis of infection by the patient’s physician and antibiotic treatment (excluded perioperative prophylaxis). Two separate subgroups for comparison were planned: patients with positive surveillance cultures and infecting cultures for the same organism during their hospitalization and patients with serial infecting isolates separated by ≥7 days. The electronic medical record was reviewed for presence of bacteria, type of injury, and...
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Bacterial genotypic and phenotypic testing

Review Board. This study was approved by the BAMC Institutional
where applicable) was performed using SPSS 16.0 (SPSS, Inc, Chi-
cago, IL). This study was approved by the BAMC Institutional

Table 1
Source of infecting isolates of Acinetobacter baumannii-calcoaceticus complex, Klebsiella pneumoniae, and methicillin-resistant Staphylococcus aureus

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>Blood</th>
<th>Sputum</th>
<th>Wound</th>
<th>CSF</th>
<th>Urine</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screening and infecting isolates (n = 133)</td>
<td>7 (21)</td>
<td>12 (36)</td>
<td>12 (36)</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Serial infecting isolates (n = 134)</td>
<td>35 (26)</td>
<td>42 (31)</td>
<td>49 (37)</td>
<td>1 (1)</td>
<td>3 (2)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Klebsiella pneumoniae, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screening and infecting isolates (n = 12)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>11 (99)</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Serial infecting isolates (n = 92)</td>
<td>30 (33)</td>
<td>18 (20)</td>
<td>39 (42)</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>MRSA, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serial infecting isolates (n = 46)</td>
<td>30 (65)</td>
<td>6 (13)</td>
<td>9 (20)</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

ABC, Acinetobacter baumannii-calcoaceticus complex; CSF, cerebrospinal fluid; MRSA, methicillin-resistant Staphylococcus aureus.

Table 2
Number of patients with genotypic and phenotypic changes of Acinetobacter baumannii-calcoaceticus complex and Klebsiella pneumoniae between initial screening and subsequent infecting isolates

<table>
<thead>
<tr>
<th>Genotypic change only</th>
<th>Genotypic + phenotypic change</th>
<th>Phenotypic change only</th>
<th>No change</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC (n = 15)</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Antibiotic exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae (n = 3)</td>
<td>0</td>
<td>3*</td>
<td>3</td>
</tr>
<tr>
<td>Antibiotic exposure</td>
<td></td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

ABC, Acinetobacter baumannii-calcoaceticus complex.

*Patients with phenotypic change to an antibiotic who received that antibiotic in the interval from 7 days prior to the initial isolate through the phenotypic change were defined as having antibiotic exposure. Antibiotic received by patients who did not have phenotypic changes in their isolates between initial screening and subsequent infecting was not evaluated.

antibiotic usage. Antibiotic exposures occurring from 7 days prior to the original isolate collection through the time of recovery of subsequent isolates were considered as dichotomous variables. Statistical analysis ($\chi^2$ testing, Fisher exact test, Kruskal-Wallis where applicable) was performed using SPSS 16.0 (SPSS, Inc, Chicago, IL). This study was approved by the BAMC Institutional Review Board.

Bacterial genotypic and phenotypic testing

Isolates were cultured from frozen storage (−80°C) by 2 overnight passages on blood agar (Remel, Lenexa, KS) and characterized by pulsed-field gel electrophoresis (PFGE) as previously described. Genotypic change was defined as a change in 10% Dice coefficient between isolate’s PFGE type. Phenotypic change was defined as a change from susceptible to intermediate or resistant or a change from intermediate or resistant to susceptible.

RESULTS

During the study period, 1,676 patients were admitted to BAMC from overseas combat operations, with 1,084 patients having admission screening cultures performed. Burn patients contributed 75%, 71%, and 50%, respectively, of ABC, K pneumoniae, and MRSA screening isolates; the remainder was predominately orthopedic. Anatomical sources of infecting isolates are summarized in Table 1. Among all ABC and K pneumoniae isolates, 79% and 65% were MDR, respectively. In this cohort, 823 patients were screened for ABC specifically, and 73 (8.5%) of these were positive, of which 17 (23%) became subsequently infected, with a ratio of 4:1 colonized to infected. The evaluable screening and subsequently infecting patients/isolates, by organism, along with the changes in genotype and phenotype, are presented in Tables 2 and 3. High variability in PFGE type was demonstrated for all organisms with no apparent trends. The most frequent changes in antibiotic susceptibilities for ABC and K pneumoniae are summarized in Table 4. Duration of culture positivity did not correlate with either genotypic or phenotypic change of serial ABC isolates. There was no significant correlation ($P = .07$) between phenotypic change and genotypic change. Five patients had changes in antibiotic susceptibilities of the infecting MRSA isolates over time. Of these, changes from a susceptible to a resistant phenotype were seen for rifampin, fluoroquinolones, and tetracyclines.

DISCUSSION

Our study found that colonizing ABC isolates were unrelated to subsequent infecting isolates in over a quarter of instances, in contrast to previous reports noting that patients colonized with MDROs on admission subsequently develop infections with highly related strains. It is also surprising that a substantial proportion of those with serial infecting isolates from the same clinical specimen source undergo a change in genotype. The finding that over half of patients demonstrated either a genotypic or phenotypic change between colonizing and subsequent infecting isolates not fully explained by antibiotic pressure suggests a large role for nosocomial transmission. However, there was no apparent trend in subsequent infecting genotypes in patients who displayed a genotypic change between colonizing and infecting isolates. This may have been evident with a larger sample size. Another possible explanation is that some patients are colonized or infected with multiple different strains of ABC at any given time, and the characterization of one colony may not be representative of the entire population.

Despite the limitation of a small number of study patients, our results showed that patients’ K pneumoniae subsequent infecting isolates were the same strain as their original colonizing isolates, although approximately 20% did undergo genotypic change over the course of infection. However, changes in antibiotic susceptibility among isolates were normative and not explained fully by exposure to the specific antibiotic. It is likely that mechanisms effecting resistance to multiple antibiotics are partly to blame for this phenomenon, but the reversion to antibiotic susceptibility even under pressure by the antibiotic in question is
unusual and further supports the possibility of colonization/infection with multiple concurrent genotypes/phenotypes.

Our study has several limitations. Laboratory procedures for the storage of MDR isolates at our institution resulted in numerous isolates being unavailable for analysis, precluding inclusion in the study. A number of other factors, such as defined daily doses of antibiotics, device-days, and hospital length of stay may also have influenced strain variability and were not captured in this study. Finally, our definition of infection was clinical and not based on standardized criteria. However, this may serve to broaden applicability to the clinician considering whether to start therapy with the antimicrobial to which the patient's pathogen was susceptible last week.

Overall, these results demonstrate the ever-changing characteristics of resistant bacteria in individual patients but do not provide a clear etiology for these changes. These results demonstrate the limitations of antibiotic susceptibility patterns of a patient's prior isolate to guide subsequent empiric antibiotic therapy, given the frequent changes in antibiotic susceptibility over time.

**References**


