An Outbreak of Acute Respiratory Disease Caused by *Mycoplasma pneumoniae* Onboard a Deployed US Navy Ship

Running Title: Shipboard Outbreak of *M. pneumoniae*

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ABSTRACT

We identified 179 cases of acute respiratory illness including 50 cases of radiographically-confirmed pneumonia over the course of four months on a deployed US Navy vessel. Laboratory tests showed *Mycoplasma pneumoniae* to be the etiological agent. This report represents the first published description of a shipboard outbreak of this pathogen.
Ships are notorious vectors for respiratory and gastrointestinal pathogens, which can spread readily in crowded onboard populations (1, 13, 20). Despite this, most ships remain isolated from the collection and diagnostic resources needed to identify outbreak pathogens. Military populations, which tend to be of higher density than civilian counterparts, are particularly susceptible to agents of acute respiratory disease (ARD), and pneumonia (21). A few respiratory disease outbreak investigations with diagnostic laboratory support have been conducted on military ships (6, 13, 24) and cruise ships (2, 3, 4, 19), usually in response to sharp outbreaks with high attack rates, and these have generally identified influenza virus as the disease agent.

Naval Environmental and Preventive Medicine Units (NEPMUs) can field mobile epidemiological response and investigation teams in response to outbreaks on US Navy vessels. To provide continuous surveillance capability for respiratory pathogens, the Naval Health Research Center (NHRC) began placing diagnostic specimen collection and storage equipment onboard vessels in 2002, and offers diagnostically accredited reference laboratory support for identification of etiological agents in collected specimens. NHRC also supports some ships in maintaining specific PCR capability onboard.

*Mycoplasma pneumoniae* is commonly associated with ARD and pneumonia outbreaks among civilians, usually children and young adults (5), and has been identified in pneumonia outbreaks among the crowded military recruit populations (8). *M pneumoniae* spreads through close contact with expired respiratory secretions (droplets), like influenza, but has a long incubation period [6-32 days (10)] and a resulting tendency to generate long, slow-spreading epidemics (7, 22) which may be difficult to recognize and control. *M pneumoniae* is challenging and slow to culture (16), and emerging...
molecular methods such as PCR offer increased sensitivity and more rapid identification from uncultured throat-swab specimens or sputum (23). These technologies greatly increase the functional value of testing for M. pneumoniae, as the resulting data can be obtained quickly enough to inform treatment and outbreak response decisions. In the outbreak described here, laboratory-supported identification of the responsible pathogen played a role in guiding the effective treatment of patients and limitation of further transmission.

Based upon review of routine syndromic surveillance data, a Navy Environmental Preventive Medicine epidemiological investigation team embarked aboard USS Boxer from 20-30 May, 2007 to confirm and characterize a suspected respiratory disease outbreak occurring while the ship was at sea. This team conducted chart reviews (the source of the data in Figure 1) and broad respiratory specimen collection (the source of the samples from 24-26 May). Samples spanning the breadth of the outbreak had been collected prior to the investigation by crewmembers, using collection and storage equipment provided by the Naval Health Research Center (NHRC) as part of ongoing shipboard respiratory disease surveillance (IRB research protocol #NHRC.2003.0002).

Oropharyngeal (throat) swab samples were collected from patients presenting with respiratory disease. Samples were stored in Viral Transport Media (Remel, Lemexa, KS) and cold chain was maintained at -80°C or below from sample collection until extraction.

All samples were shipped by air on dry ice to NHRC for laboratory analysis. Samples were extracted using the QIAamp 96 DNA Blood and Body Fluid Kit (Qiagen, Valencia, CA) according to manufacturer’s instructions, and tested for common
respiratory pathogens by PCR as listed in Table 1. Following PCR analysis, specimens were also tested by a College of American Pathologists (CAP)-accredited diagnostic protocol to confirm the presence of *M pneumoniae* by standard culture methods. A subset of 15 samples were also tested using the TessArray Resequencing Pathogen Microarray Kit (RPM v.1) (TessArae, LLC, Potomac Falls, VA), a functionally independent method that uses multiplexed PCR and resequencing microarrays to screen for diverse respiratory pathogens (14, 15).

Cases occurred in the chronological sequence illustrated in Figure 1. 69/179 cases (38.5%) met the clinical case definition used for acute respiratory disease (including both a fever of $\geq 38^\circ$C and cough or a sore throat, or radiologically confirmed pneumonia). All samples collected before May 24 met this case definition, while the 12 collected during the investigation (May 24-26) were from personnel displaying any respiratory symptom. 159/179 (88.8%) patients self-presented for respiratory complaints. Of these, 55 (30.7%) had documented fever ($\geq 38^\circ$C). Sixty-six patients received a chest x-ray during their evaluation, with 50/66 (75.8%) positive for infiltrate by radiologist-confirmed reading. The average age for all patients was 27.0 years, with 141 (78.8%) males and 38 (21.2%) females. Of 179 patients, 70 (39%) gave a positive smoking history.

PCR results are shown in Table 1. A timecourse of *M pneumoniae* PCR and culture results is shown in Figure 2 (only a subset of cases were sampled, primarily on the basis of time available to ship's medical personnel). Neither of the two samples collected prior to Feb 2007 (Oct 2006 and Jan 2007) were positive for *M pneumoniae*
(omitted from Figure 2). PCR testing identified the presence of *M. pneumoniae* in 24/31 samples collected during the outbreak (1 Feb – 23 May).

*M. pneumoniae* was confirmed by tissue culture in 20/24 PCR-positive samples. All PCR-negative samples were also negative by culture. Analysis of 15 samples using the RPM v.1 microarray system also supported PCR results. 7 were positive for *M. pneumoniae* by both PCR and microarray analysis, 7 were negative by both methods, and one was positive by microarray and negative by PCR.

This investigation confirms an outbreak of respiratory illness aboard a US Navy ship during the period 1 Feb - 23 May, 2007. The ship experienced roughly 16 cases of respiratory illness per week during this period in a population of 1074 personnel. Additionally, USS Boxer experienced 48 cases of radiographically confirmed pneumonias over a period of nine weeks (5.4 cases per week).

The epidemiological and laboratory data implicate *M. pneumoniae* as the most likely causative organism. By late May disease transmission had experienced a decrease, resulting in a downslope in the disease curve as shown in Figure 1. Only one of the 12 oropharyngeal specimens collected from 24-26 May was positive for *M. pneumoniae* by PCR, as shown in Table 1 and Figure 2. It should be reiterated that these 12 samples were collected with a broader case definition (any respiratory symptom) than previous samples, as described above. In this investigation, the observed respiratory illness attack rate was approximately 17% (179 cases/1074 at-risk personnel).

This is the first documented shipboard outbreak of *M. pneumoniae*. The shape of the epidemiological curve (multiple peaks, propagative pattern) is inconsistent with transmission patterns of typical seasonal influenzas, which generally create a much
steeper singular curve. The high rate of pneumonias relative to ARD cases is also different than would be expected for influenza among healthy young adults.

Based on identification of the source of this outbreak, the ship's medical personnel implemented screening, case recognition, treatment, and isolation procedures that quickly brought the outbreak under control. While these measures are not the subject of this report, they were dependent on the investigation and laboratory findings reported here.
ACKNOWLEDGMENTS

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REFERENCES


Table 1. PCR results for potential pathogens.

<table>
<thead>
<tr>
<th>Pathogen (test method reference)</th>
<th>Number (percent) positive by PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period 1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(n=2)</td>
</tr>
<tr>
<td>Adenovirus (18)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Coronavirus (9, *)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Influenza Virus A (11)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Influenza Virus B (11)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>RSV (12)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Bordatella pertussis</em> (17)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Chlamydia pneumoniae</em> (17)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em> (17)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em> (17)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> samples collected before 31 Jan 2007  
<sup>b</sup> samples collected between 1 Feb 2007 and 23 May 2007  
<sup>c</sup> samples collected between 24 May 2007 and 26 May 2007  
*One of the type-specific coronavirus PCR tests was developed and validated by Kay Holmes and Mary Catherine Smith at Colorado Health Sciences Center, Denver, CO, and has not been published.
FIGURES

Figure 1. Respiratory disease and pneumonia cases on USS Boxer.

Figure 2. Laboratory results for oropharyngeal swab samples collected aboard USS Boxer.