Epidemiology and Antimicrobial Resistance of Pathogenic Enterobacteria in Ifanadiana District, Madagascar

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Abstract
Although most diarrheal diseases are self-resolving and easily treated with oral-rehydration therapy, it is common for antimicrobials to be used in isolated cases. Said agents will shorten the time of infection and thus limit the transmission of pathogens within the environment. Throughout the past decade, however, there have been numerous studies published that suggest antimicrobial resistance (AMR) within enteric bacteria is not only high but increasing, especially in developing regions of the world.

The objective of this study was to assess the prevalence of AMR within Ranomafana, Madagascar. We also evaluated behaviors potentially associated with the development of AMR using a comprehensive survey. We chose to place an emphasis on Salmonella spp., Shigella spp., and Vibrio cholera, as past studies have shown elevated prevalences. The results from this field season will help to support the existing health care framework, which Pivot ( pivotworks.org) has established in the region by offering updated information on the effectiveness of antibiotic treatment regimens.

Background
Diarrheal Disease
Madagascar is one of the poorest developing regions of the world. While efforts have been made to increase the living conditions of the poor, the population has access to clean water sources and less than 20% has access to proper sanitation facilities. As a result, diarrheal disease has become the leading cause of death in children under 5, with more than 1.5 billion cases a year, resulting in 3.3 million deaths per year. It is the second leading cause of mortality worldwide.

Study Site
Our study took place in and around Ranomafana National Park (RNP) a 43,500 hectare World Heritage Site, well known for its high levels of species diversity and endemism. Sixteen communities located around the parks edge were selected for inclusion in the study, which represent a gradient of developed environments from Urban (Ranomafana proper) to rural (Amphilavanana). The study populations included domestic livestock: bovine (Bos indicus), porcine (Sus domesticus), poultry (Gallus gallus), canine (Canis lupus familiaris), and humans from 80 randomly selected households.

Study Design / Field Collection

Objective 1: Assess the prevalence of Salmonella spp., Shigella spp., and Vibrio cholerae within the study populations in and around Ranomafana National Park.

Objective 2: Characterize the antimicrobial resistance profile within study populations through the use both antimicrobial susceptibility and genetic testing.

Objective 3: Examine and assess the behavioral aspects associated with antimicrobial use that could be related to the observed levels resistance through comprehensive surveys and interviews.

Study Populations

Humans: All individuals within 80 households across 16 villages in the RNP region (N=243).

Domestic Livestock: All bovine, porcine, and poultry from a given household included in the study (N=64).

Laboratory Methods

Antibiotic Susceptibility Testing
- Swabs from fecal samples were taken and preserved at -80°C in Cary-Blair transport medium until culturing.
- A single swab from the Cary-Blair medium was inoculated onto selective/differential media (MacConkey, XLD, TCBS) for the isolation of suspicious colonies of Vibrio cholerae, Salmonella spp., and Shigella spp.
- Additionally, a single fecal swab from the Cary-Blair was enriched for the isolation of Salmonella spp. in 10 mL of Selenite Cystine Broth and subsequently plated on XLD media.
- Suspect colonies were then tested in screening biochemicals and organism specific antisera to finalize the identity of isolated bacteria.

Molecular Analysis
- Total nucleic acid was extracted from all fecal specimens (N=309) preserved in RNAlater (Ambion) using the QIAamp DNA Stool Kit and subsequently stored at -20°C.
- Using conventional PCR we will screen the samples for St. enterica, Shigella spp., Vibrio cholerae, and E. coli.
- Various genes were then used to screen for SMR within the following antimicrobial classes: β-Lactams, Aminoglycosides, Tetracycline, Trimethoprim, Sulfamethoxazole, and Chloramphenicol.

Preliminary Results

In-depth genetic analysis via PCR of all collected fecal samples for genetic confirmation of pathogenic Shigella spp., Salmonella spp., and Vibrio cholerae.

Table 1: Prevalence of Shigella spp. relative to taxa

<table>
<thead>
<tr>
<th>Sample</th>
<th>Shigella flexneri</th>
<th>Shigella boydii</th>
<th>Shigella dysenteriae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>16</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Domestic Livestock</td>
<td>16</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: Diversity of Shigella spp. isolates

<table>
<thead>
<tr>
<th>Isolated Shigella spp. Number of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. flexneri</td>
</tr>
<tr>
<td>S. boydii</td>
</tr>
<tr>
<td>S. dysenteriae</td>
</tr>
</tbody>
</table>

Table 3: Antimicrobial resistance profile of Shigella spp. isolates from Ranomafana, Madagascar

<table>
<thead>
<tr>
<th>Antimicrobial Susceptibility Zone Diameter (mm)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>8 (Resistant)</td>
<td>42 (Susceptible)</td>
<td>44 (Susceptible)</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>8 (Resistant)</td>
<td>42 (Susceptible)</td>
<td>44 (Susceptible)</td>
<td></td>
</tr>
<tr>
<td>Polymyxin</td>
<td>40 (Susceptible)</td>
<td>40 (Susceptible)</td>
<td>40 (Susceptible)</td>
<td></td>
</tr>
</tbody>
</table>

Next Steps

- In-depth genetic analysis via PCR of all collected fecal samples for genetic confirmation of pathogenic Shigella spp., Salmonella spp., and Vibrio cholerae.
- Study Design: All collected fecal samples for genetic markers associated with resistance to β-Lactams, Aminoglycosides, Tetracycline, Trimethoprim, Sulfamethoxazole, and Chloramphenicol antimicrobial classes.
- Analysis of survey results.
- Report findings to all communities involved in study.
- Final report for masters thesis submission.
- Submit results for publication.

Acknowledgments and Contact Information
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