Prevalence of antibiotic-resistant bacteria at a local daycare center in coastal North Carolina

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Abstract
Due to our society’s previous carelessness with antibiotic overprescribing and the relatively recent anti-vaccination trends amongst uneducated parents, I have become increasingly concerned with the possible threat of antibiotic resistant bacteria in our daycare centers. I swabbed the Toddler 2 Room, frequented by approximately ten 18 to 24-month old children, of a daycare center located in the coastal Carolina region during the months of October and February as a seasonal comparison. Swabs of high-traffic areas were transferred directly onto nutrient agar containing penicillin. Five bacterial species (n = 12) found in the October swabbing and fourteen bacterial species (n = 19) found in the February swabbing exhibited multiple antibiotic resistances. These results illustrate that the threat of multiple antibiotic drug resistance at daycare centers is an important issue that warrants further study.

Figure 1. Surface swabs from the fall sampling on three plates (from the left): plain nutrient agar, nutrient agar plus penicillin (to remove penicillin-resistant bacteria), and MacConkey agar (to determine if there was a significant presence of Enterobacteriaceae.)

Table 1. Antibiotic Resistance Profile for Fall 2013 Surface Samples

<table>
<thead>
<tr>
<th>Resistance Profile</th>
<th>Surface</th>
<th>Bacteria</th>
<th>PCN</th>
<th>DOK</th>
<th>CIP</th>
<th>AMC</th>
<th>SXT</th>
<th>CLN</th>
<th>RAM</th>
<th>PMP</th>
<th>AMP</th>
<th>ERY</th>
<th>TET</th>
<th>STP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Back Door</td>
<td>Staphylococcus aureus</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Toilet Floor</td>
<td>Staphylococcus aureus</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td></td>
</tr>
<tr>
<td>Toddler Sink</td>
<td>Staphylococcus aureus</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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Table 2. Antibiotic Resistance Profile for Winter 2014 Surface Samples

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<th>Resistance Profile</th>
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<th>DOK</th>
<th>CIP</th>
<th>AMC</th>
<th>SXT</th>
<th>CLN</th>
<th>RAM</th>
<th>PMP</th>
<th>AMP</th>
<th>ERY</th>
<th>TET</th>
<th>STP</th>
</tr>
</thead>
<tbody>
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<td>R</td>
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<td>R</td>
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<td>R</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Toilet Floor</td>
<td>Acinetobacter radioresistens</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Toddler Sink</td>
<td>Acinetobacter radioresistens</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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</tbody>
</table>

Methods

Introduction

Although the discovery of antibiotics was a major success in the medical field, the overuse and misuse of such valuable medications has led to the evolution of increasingly resistant strains of both bacterial and laboratory-stable antibiotic (LSA). If this bacterium were to be eradicated, the cost to public health was first recognized in the 1980’s, but only recently has attention been given to the presence of these bacteria on surfaces outside of hospitals or other healthcare-centered facilities (1). While many government agencies understand the gravity of the situation, the typical US adult is not aware that microbes are increasingly present on everyday surfaces (4). Unfortunately, the hygiene habits that we assume keep these dangerous microbes at bay could in fact be contributing to the development of their resistance. Using antibacterial such as trichlozan or pine oils to clean surfaces will release residues that can cause microbes to evolve over time and develop mechanisms of resistance (5). However, the situation is much more complex than the simple overuse of household and industrial cleaners.

Another major cause of multiple antibiotic resistance is the overprescribing and inappropriate use of antibiotics. The CDC recognizes this issue several years ago and organized the national Get Smart campaign, which focuses on improving the appropriate use of antibiotics (3). In this manner, both the prescribers and the patients can be working in tandem to contribute to the issue. These risk factors culminate in daycare center environments, where employees frequently use agents such as Pine-Sol® to clean the floors, and children are frequently being treated with antibiotics or common aliments such as sunburns and strep throat. This study focused on the identification of multiple antibiotic-resistant bacteria in a local daycare center near the University of North Carolina Wilmington. I swabbed the Toddler 2 Room during the months of October 2013 and February 2014, where I found five and fourteen multiple antibiotic-resistant species, respectively. After a species was identified as having multiple antibiotic resistances, a mixture of microscopy, biochemical testing and DNA sequencing were used to identify the bacteria.

Study Location

Surface sampling was conducted in the Toddler 2 Room at a daycare center in Jacksonville, NC. It is frequented by approximately ten 18 to 24-month-old children on a daily basis and is the first room in the daycare center where potty training is available.

Surface Sampling

All surface sampling was conducted on a random basis. Surface samples were collected with sterile cotton swabs and then transferred onto agar plates. For the sampling, three cotton swabs were used simultaneously on each surface to swab onto three different agar plates: nutrient agar, nutrient agar plus penicillin (10 units/ml), and MacConkey agar (Figure 1). After incubation, each unique colony on the NA + peni plates was transferred to a fresh nutrient agar (NA) plate for isolation and further analysis. For the back door, all swabs were transferred onto NA + peni plates only (Figure 2a). The same isolation and analysis procedures were used thereafter.

Resistant Profiling via Disc Diffusion Method

Several colonies of each isolate were suspended in sterile saline to match the turbidity of a 0.5 MacFarland standard. A 3 mm streak was performed on Mueller-Hinton II agar, and then the antibiotic discs were placed. After incubation, the zones of inhibition were measured and compared to cutoff values as set forth by the Clinical and Laboratory Standards Institute (See Figure 2b) [6]. See Tables 1 and 2 for the antibiotics tested.

Determinative Bacteriology

Each bacterial isolate was identified by Gram stain and brightfield microscopy at 1000x magnification (See Figure 2c). Based on preliminary identification, each isolate in the fall sampling was subjected to further biochemistry.

DNA Sequencing of 16S rRNA Gene

Several colonies of each isolate on nutrient agar were suspended in 50μL of nuclease-free water and DNA was obtained in the supernatant following boiling and centrifugation. The supernatant assayed by a PCR amplification using a 16S rRNA gene sequence. Each PCR reaction contained the following: 5μL of DNA (superior), 0.5 μM each primer (1509F and EC08), 1X GoTaq Green FlexiBuffer, 1.5 mM MgCl2, 0.2 mM dNTP (PerkinElmer), 1X GoTaq Flexi Buffer and 0.2 units of GoTaq Flexi DNA polymerase (PerkinElmer). Pairs of primers used for 16S rRNA gene sequence are: 1509F (5′-GTG TGA TCA GCA GAA TAT CCA) and EC08 (5′-GTC TGC GCT TCA GAT TGT TCT). PCRs were performed in 20μL reactions in thin-walled, 96-well reaction plates using the EcoBQ Primera (PerkinElmer). DNA sequences were submitted to BLAST (NCBI) to identify bacterial species.

Results

Due to the overuse of antibiotics, both the bacteria and the antibiotic itself evolve. The only way to combat this is to not use antibiotics (8). Unfortunately, the misuse of antibiotics is common, especially in daycare centers (9). This study focused on the identification of antibiotic-resistant bacteria in these centers, as well as the cases of antibiotic resistance in children.

Discussion

For the purposes of this discussion, all bacteria showing intermediate or resistant zones of inhibition for two or more antibiotics were defined as possessing multiple antibiotic resistances. It appears that there are more antibiotic-resistant bacteria present in this daycare center during the winter months, but there are several sources of potential error that could be contributing to these results. Most importantly, I had never performed the surface swabbing and experimental techniques that were required before collecting the bacteria in October 2013. Also, due to the low number of trials, I cannot claim that there is a statistically significant difference between the number of antibiotic-resistant bacteria found between the two collections.

What I found particularly worrisome about my results was the sheer number of antibiotics that some of the bacterial isolates were either resistant to or exhibited intermediate resistance to. Five of the isolates were resistant to five of the 12 antibiotics they were tested against, two were resistant to six antibiotics, and two were resistant to seven of the 12 antibiotics. Even more unsettling is the utility of these laboratory tests in clinical practice. For example, the Staphylococcus aureus bacterium that I isolated showed resistance to penicillin, erythromycin, and ampicillin. According to the Clinical and Laboratory Standards Institute, these antibiotics are recommended for the treatment of methicillin-resistant Staphylococcus aureus (MRSA) (9). In this case, one would hope that the physician prescribed one of the few appropriate options left, four of which are commonly stocked in pediatric suspensions. However, the results of this study do not offer any new information about the antibiotic resistance in daycare centers. It is hoped that the results of this study will be used to address the antibiotic resistance in daycare centers.

Acknowledgements

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References