Exploring the Relationship between Transport, Resistance, and Virulence Factors of Escherichia coli Collected from Swine Manure

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ABSTRACT

Broad spectrum antibiotics, such as Tylosin (naturally synthesized by Streptomyces fradiae) are used to treat infections in farm animals and are often administered in sub-therapeutic levels along with feed rations. The presence of bacteria resistant to antimicrobials in animal waste has raised concern related to their transport to surface and groundwater. Previous studies have shown that cells preferentially attach to sediments affecting their transport in overland flow; however, a lack of quantitative understanding of attachment mechanisms such as extracellular organelles and the relationship between these factors and virulence factors in humans is known. In a preliminary study, the presence of Tylosin resistant bacteria have been enumerated in the drainage samples collected beneath feedlot plots. This study shows the transport potential of resistant bacteria, the method of transport, the relationships between transport, resistance, and attachment and virulence factors is unknown. The objective of this research is to study these relationships in Escherichia coli collected from swine manure. Cultures will be screened for antibiotic resistance, and multiple antibiotic resistances. Resistant and non-resistant cultures will be grown in a chemostat to determine if a low nutrient environment affects the attachment and virulence factor expression. Further characterization of the phenotypic and genotypic attachment and virulence factors is needed.

INTRODUCTION

In swine production, the development of a large number of antibiotics and other additives has helped in the control of infections during growth, breeding, and finishing. These antibiotics are administered in subtherapeutic doses (0.4 grams of sand particles with an average diameter of 125 μm) to the animals to prevent diseases in animals at economically efficient rates. Tylosin, one of the antibiotics commonly used in the treatment of infections in farm animals and are often administered in sub-therapeutic levels along with feed rations. The presence of bacteria resistant to antimicrobials in animal waste has raised concern related to their transport to surface and groundwater. Previous studies have shown that cells preferentially attach to sediments affecting their transport in overland flow; however, a lack of quantitative understanding of attachment mechanisms such as extracellular organelles and the relationship between these factors and virulence factors in humans is unknown. In a preliminary study, the presence of Tylosin resistant bacteria have been enumerated in the drainage samples collected beneath feedlot plots. This study shows the transport potential of resistant bacteria, the method of transport, the relationships between transport, resistance, and attachment and virulence factors is unknown. The objective of this research is to study these relationships in Escherichia coli collected from swine manure. Cultures will be screened for antibiotic resistance, and multiple antibiotic resistances. Resistant and non-resistant cultures will be grown in a chemostat to determine if a low nutrient environment affects the attachment and virulence factor expression. Further characterization of the phenotypic and genotypic attachment and virulence factors is needed.

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LITERATURE CITED


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INTRODUCTION

In previous production, the development of a large number of antibiotics and other additives has helped increase gain and induce the feed required per unit of gain. At least 11 antibacterial and antifungal compounds or groups of compounds are widely used in each feed. These include various salts of bactrocin, chloromycetin, doxycycline, neomycin, streptomycin, specticin, penicillin, polymyxins, tetracycline, and tylosin. (Carbon and Lagrange, 1989). Veterinarians commonly use feed-grade antibiotics at therapeutic levels to treat acute disease outbreaks. This ensures that the bacterial pathogens causing the observed symptoms is treated with an antibiotic to which the pathogen has demonstrated susceptibility. However, in many instances, antibiotics are used to promote growth or weight gain and are fed at sub-therapeutic levels on a daily basis.

OBJECTIVE

To determine that select for antibiotic resistance by sub-therapeutic antibiotic use in agriculture co-varies for bacteria with increased attachment to sediment and increased frequency of virulence associated genes

PHENOTYPIC ATTACHMENT

ANALYSIS PREPARATION

Individual strains were grown for 12 hours in 10 ml of Mueller Hilton broth and transferred to a phosphate buffered water solution. Cultures were diluted to an optical density of 0.1 McFarland Standard. 408 strains from a library of 556 strains were characterized by enumeration of E. coli. 0.4 grams of sand particles with an average diameter of 125 μm were added to 45 ml of suspended cells.

TABLE 1

<table>
<thead>
<tr>
<th>Attachment Factor</th>
<th>Organic Sub-therapeutic</th>
<th>P pili</th>
<th>Type 1 pili</th>
<th>S fimbriae</th>
<th>Dr hemagluttinin</th>
<th>Attachment</th>
<th>Hemolysin A</th>
<th>Cytotoxic Necrotizing Factor</th>
<th>IOA S T A T E  U N I V E R S I T Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tylosin (MIC = 32 μg/ml)</td>
<td>Tylosin (MIC = 8 μg/ml)</td>
<td>Tylosin (MIC = 16 μg/ml)</td>
<td>Tylosin (MIC = 32 μg/ml)</td>
<td>Tylosin (MIC = 64 μg/ml)</td>
<td>Tylosin (MIC = 128 μg/ml)</td>
<td>Tylosin (MIC = 256 μg/ml)</td>
<td>Tylosin (MIC = 512 μg/ml)</td>
<td>Tylosin (MIC = 1024 μg/ml)</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2

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<tr>
<th>Isolates were queried by gene specific PCR for known virulence and attachment factors (n=43)</th>
</tr>
</thead>
</table>

FIGURE 1

Attachment is statistically significant among the treatments (p<0.001).

FIGURE 2

Attachment phenotype was calculated as the difference between total and unattached E. coli for each strain (Figure 2).

GENOTYPE ATTACHMENT AND VIRULENCE FACTORS

Isolates were queried by gene specific PCR for known attachment and virulence factors (Table 1)

Further Research

- Increased coding frequency for P pilus support our hypothesis

- Genes encoding P pilus have been previously reported as being associated with antibiotic resistance (Iturriaga et al., 2008; Arroyo et al., 2008)

ANTIBIOTIC RESISTANCE

Antibiotic Pre-screening

1) Tylosin resistant E. coli colonies were collected, m- plated on agar infused with

- Chloramphenicol (MIC = 2.5 μg/ml)

- Ampicillin (MIC = 5 μg/ml)

- Gentamycin (MIC = 4 μg/ml)

- Tetracycline (MIC = 50 μg/ml)

- Plates were incubated at 37°C for 24 hours

Further Research

- Continue cloning isolates by gene-specific PCR for known virulence and attachment factors

- Correlate attachment and antibiotic resistance phenotypes with virulence and attachment factor genotypes

Further Research

- Complete genotyping of P pilus and other attachment factors