Correlates of Antimicrobial Resistance in Soil Bacteria: Insights from Healthcare Settings Across Different Communities

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ABSTRACT

Antimicrobial resistance (AMR) is a problem presenting serious challenges to human health such as uncontrolled infectious states. Causes of antimicrobial resistance have been studied and include misuse and overuse of antibiotics in health and agricultural settings. This study was conducted to better understand correlates of antimicrobial resistance within human health settings such as hospitals. The effect of population and proximity to hospitals on AMR were examined, as well as the propensity of lactose fermenting bacteria to develop resistance. Culture based methods using soil samples were utilized on MacConkey agar, a medium used to isolate gram-negative bacteria and test for lactose fermentation. Cultures sampled near health care settings in medium to large cities showed significantly higher frequencies of resistance to tetracycline, with almost all of the resistant species testing positive for lactose fermentation. These results implicate potential roles of urbanization as well as mechanisms of lactose fermenting bacteria in the emergence of antimicrobial resistant species. Increased understanding of AMR will aid the development of more effective protocols to address this issue and curb its proliferation. Better understanding of resistance mechanisms are also valuable for new target treatments to combat this problem.

METHODS

- Soil samples were obtained from locations in North Texas with varying proximity to hospitals in both large and small cities (Table 1).
- Serial dilutions of soil samples were made; 100 ug of each dilution was spread on MacConkey agar plates prepared with cycloheximide antifungal and tetracycline. Plate types included no antibiotic (NA), low dose 3ug/ml tetracycline (T3) and high dose 30 ug/ml tetracycline (T30).[4]
- Countable colony forming units (CFUs) were tallied after 36 hours of incubation at 35° C. NA plate CFUs were used along with plated volumes and dilution factors to determine the number of CFUs per gram of soil. The percentage of tetracycline-resistant (Tet^R) CFUs on antibiotic plates were calculated using values for CFUs per gram of soil along with antibiotic plate data (countable CFUs on T3 and T30 plates, dilution factors and volumes plated).
- Tet^R percentages were analyzed with respect to sample location population, distance from hospitals, and degree of lactose fermentation seen as red or pink colored CFUs.

RESULTS

- Samples which showed resistance (Tet^R) to either low dose (3 ug/ml) or high dose (30 ug/ml) tetracycline included those in a small, medium, and large cities with approximate populations of 100,000, 300,000-400,000, and over 1.3 million, respectively (Table 1).
- Medium sized cities were correlated with the highest level of low dose Tet^R, having overall 6 times greater Tet^R frequency compared to small or large cities. Medium and large cities showed high levels of high dose Tet^R, and small cities showed the lowest levels of Tet^R, irrespective of dosage (Table 1, Figs. 1 & 2).
- An inverse relationship was observed between Tet^R and proximity of sample collection site to hospitals. This relationship was markedly stronger in low dose Tet^R (Table 1, Fig. 3)
- The vast majority of Tet^R cells were lactose fermenters, irrespective of low/high dose resistance (Fig. 4 A-C).

Table 1. TetR Cells, City Population, and Hospital Proximity

Sample#	City Population	Miles to Nearest Hospital	% TetR 3ug/ml	% TetR 30 ug/ml
#1	102691	0.05	2.6215	0.0512
#2	102691	0.4	1.432	0
#3	102691	1.4	-	-
#4	102691	2.9	-	-
#5	1302868	1.6	2.7165	0.671
#6	1302868	0.3	3	0.108
#7	1302868	1.6	-	0.0704
#8	290190	0.3	-	0.725
#9	290190	4	-	-
#10	398431	0.1	16.667	-
#11	398431	0.1	22.967	1.209
#12	398431	2	10.299	0.163

Figure 4. Lactose fermenting bacterial cells on MacConkey agar. (A) Variation of lactose fermenting (pink) vs. non-fermenting (white) cells. (B) Typical Tet^R lactose fermenters observed. (C) Bright red/pink cells indicative of strong lactose fermenters

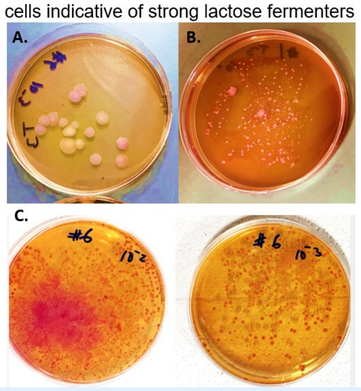


Figure 1. Low Dose (3 ug/ml) Tetracycine Resistance vs. Population

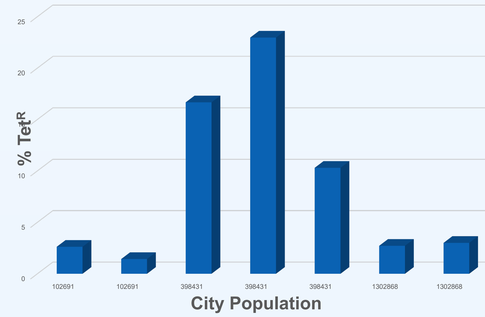


Figure 2. High Dose (30 ug/ml) Tetracycine Resistance vs. Population

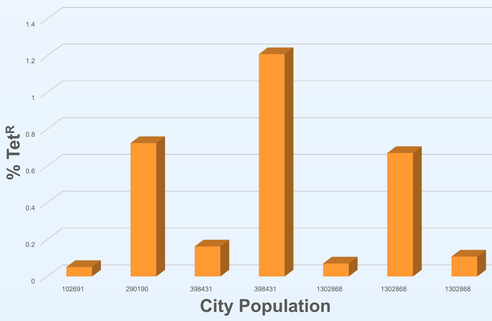
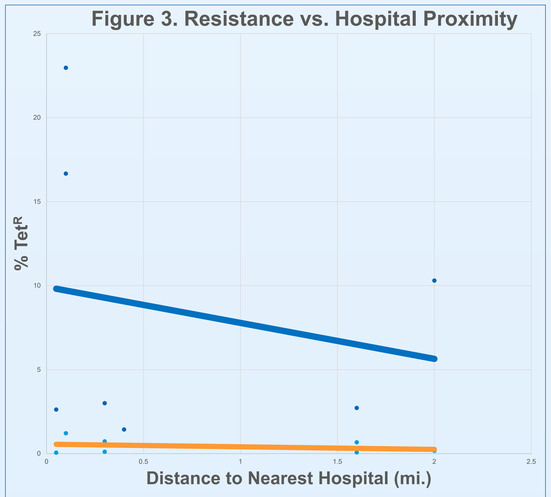


Figure 3. Resistance vs. Hospital Proximity



DISCUSSION

Isolating gram-negative bacteria to test for AMR is optimal as they are often more resistant due to their outer membrane.[1] MacConkey agar is optimal medium for this purpose as it selects for gram-negative bacteria as well as distinguishes lactose fermentation by way of acids resulting from fermentation which drops the pH of the media and affects the color indicator of its dye.[2] The majority of Tet^R cultures in this study were lactose fermenters. More research is warranted with respect to correlations between mechanisms involved in lactose fermentation and those leading to AMR.

It was not surprising to see a strong AMR correlation with proximity to hospitals due to more frequent antibiotic use in these locations. With respect to populations, this study showed that more populated areas increase the chance of AMR, a correlate that is not uncommon in AMR.[3] Studies have previously shown that due to many health and/or agricultural activities, microbes in urban soil tend to have higher resistance to different types of antibiotics.[5] These activities may even affect the metabolism and enzymatic activities of microbes.[6] However, it was surprising to see the highest resistance among samples from medium-sized cities. This is an interesting finding that warrants more research. Potential correlates could be particular microbial species outcompeting others, or different human activities contributing to higher AMR frequency. Given the increase in urbanization and healthcare activities are only likely to increase in the future, focusing on antibiotic resistance in soil bacteria is important for comparing the effect of different modern practices on AMR in communities.

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