Diversity and Resistance to Extended Spectrum β-Lactams in *E. coli* from Multiple Irrigation Water Sources: A Conserve Study

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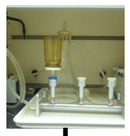
Background and Objective

E. coli **is a widely known water quality indicator for fecal contamination which sometimes causes common bacterial infections including gastroenteritis. However, due to extensive diversity in genetic substructure, Clermont et. al. (2000) divided this species into 4 different phylogroups: A, B1, B2 and D to place all *E. coli* with *a* similar genetic structure in one clade. Later it was reported that strains from two different phylogroups may have distinct phenotypic and genotypic characteristics. In addition, they differ in niches, host and mechanism of diseases. Some strains exhibit antimicrobial resistance (AMR) that can transfer to other bacterial pathogens and complicate disease outcomes.**
Fresh produce crops can be exposed to any of the pathogenic and antimicrobial resistant *E. coli* through irrigation water. Therefore, classification and investigation for resistance traits of *E. coli* isolated from irrigation water is an important step in ensuring food safety.

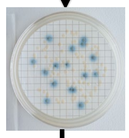
The purpose of this study was to determine the prevalence and diversity of different phylogroups (pathogenic and no-pathogenic), and β-lactam resistant *E. coli* isolated from surface and recycled wastewater in the Mid-Atlantic region of USA (Solaiman et al., 2000).

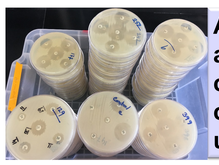
Methods

Water samples (N=333) were collected from 11 different sites (2 ponds (PW), 1 tidal brackish rivers (TB), 5 non-tidal freshwater rivers/creeks (NF), 3 reclaimed wastewater treatment plants(RW)) from eastern and western Maryland.



Phylogrouping by amplifying 3 virulence gene: *chuA, yjaA, TspE4C2*





Antimicrobial susceptibility against cefotaxime, cefoxitin, ceftriaxone, cefuroxime and ceftazidime using disk diffusion method.

Confirmation by amplifying *uidA* gene

Identification of extended spectrum β-lactam resistance gene: bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{CMY-1}, bla_{CMY-2}, bla_{OXA-1}, bla_{OXA-2}

Results and Discussion

E. coli **was isolated and identified from 288/333 (86.5%) water samples, 724 isolates were retrieved from all water samples.**

NF= Non-tidal freshwater river/creek, TB= Tidal brackish water, PW= pond water and RW= reclaimed or treated wastewater .

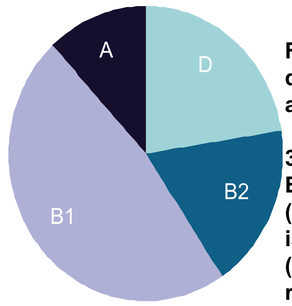


Figure 1: Prevalence of different *E. coli* phylogroups across all samples.

344 isolates were classified as B1 (47.5%) and 86 isolates as A (11.9%). Rest 131 and 163 isolates were classified as B2 (18.1%) and D (22.5%) respectively.

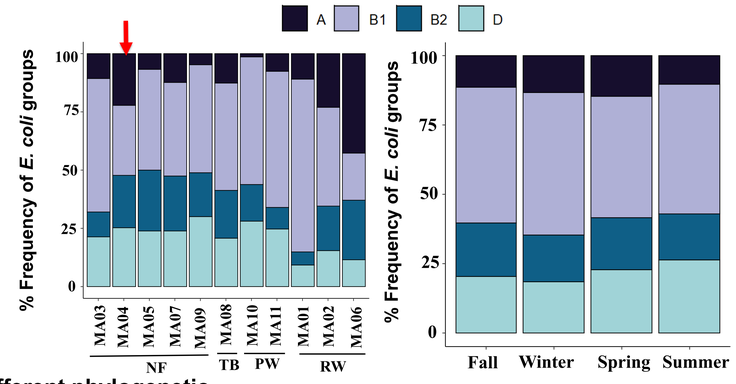


Figure 2: Distribution of different *E. coli* phylogroups in different sampling sites and seasons of the year.

Distribution of *E. coli* phylogroups varied by sites/ water types (Pearson's $\chi 2$ (n=724, df=9) = 36.899, p<0.001), but not by seasons (Pearson's Chi-squared test: χ-squared =5.517, df = 9, p-value = 0.787)

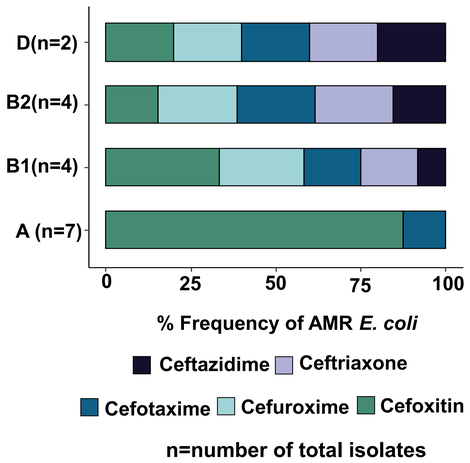
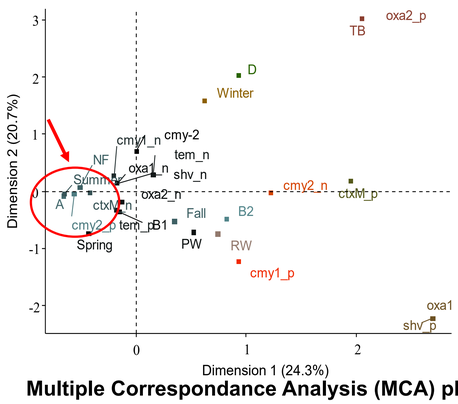


Figure 3: Different phylogenetic groups showing resistance against β-lactam antibiotics (AMR).

Only 17 (2.3%) isolates showed resistance to β-lactam antibiotics. Group A isolates were resistant to only cefoxitin and cefotaxime. D isolates exhibited resistance to all antimicrobials tested. B1 isolates predominantly showed resistance to cefoxitin.

Almost 75% of cefoxitin resistant isolates were in group A and B1.



Multiple Correspondance Analysis (MCA) plot

Figure 4: Association between phylogenetic groups of *E. coli* (n=17), season, water types and carriage of resistance genes.

Most prevalent gene was bla_{CMY-2} (70.6%) and least prevalent genes were , bla_{OXA-1}, bla_{OXA-2} and bla_{SHV} (5.9%). Nine (53%) carried multiple AMR genes. An association was detected among Group A, Summer, absence of *ctx-M*, and presence of *cmy-2*, supporting the data that 100% Group A isolates harboured bla_{CMY-2} and collected in summer.

References and Acknowledgement

Clermont et al. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl. Environ. Microbiol.* 66: 4555-4558.
Solaiman et al. 2020. A longitudinal assessment of Escherichia coli, total coliforms, Enterococcus and Aeromonas spp. dynamics in alternative irrigation water sources: A CONSERVE study
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Conclusions

Findings from this study indicated that water sources available for fresh crop irrigation contained a large percentage of potentially pathogenic *E. coli*, a small proportion of which exhibited resistance to β-lactam antibiotics that varied by phylogroups. Most of the isolates carried multiple AMR genes. β-lactam resistance gene profile also varied depending on phylogroups.
Future work should investigate the potential for resistance gene transmission to other pathogens and transmission of both pathogenic strains and AMR genes to fresh produce via irrigation water.