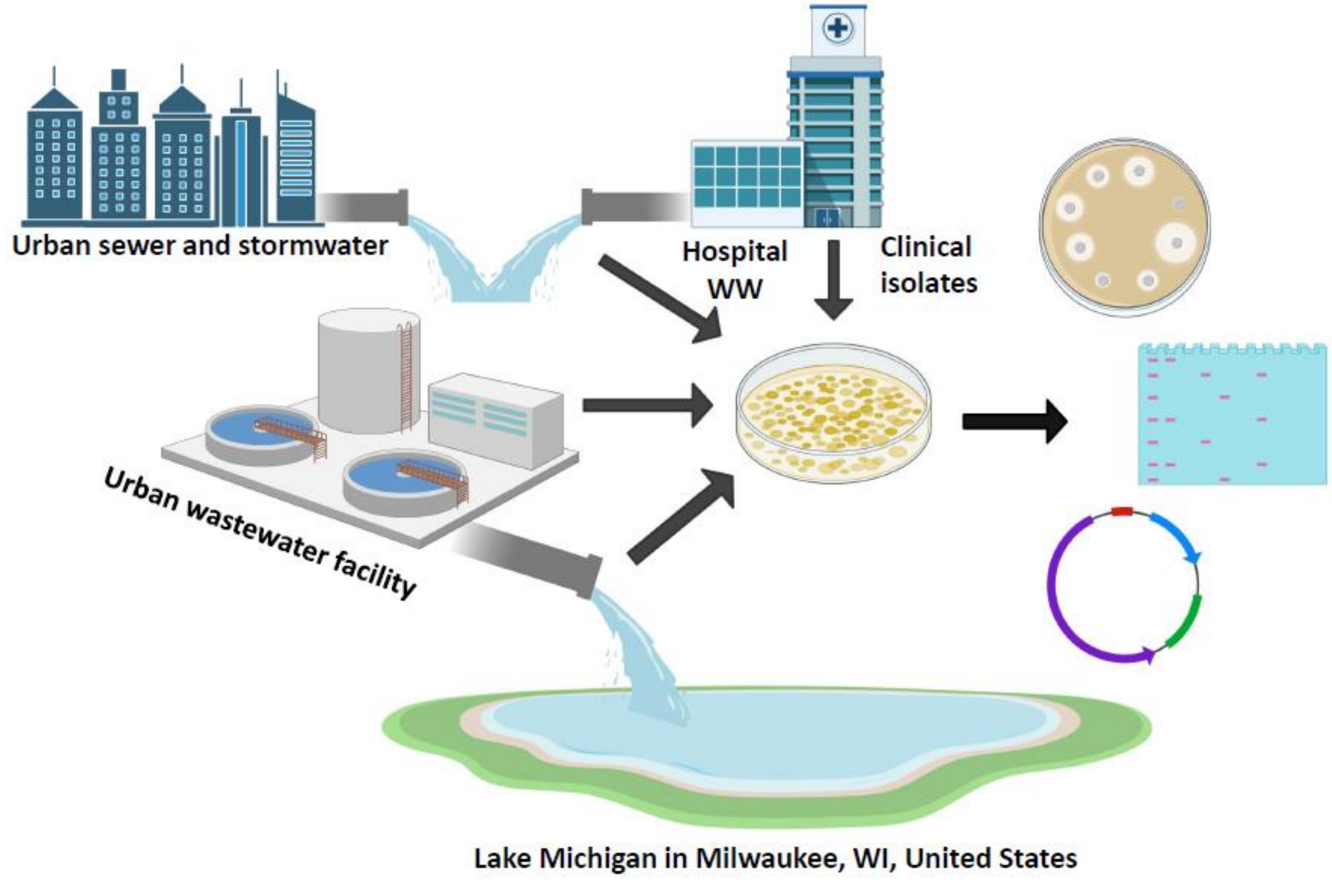
Name of the speaker: Troy Skwor, PhD

☑ **I have no link of interest.**

Antibiotic resistance patterns of *Escherichia coli* isolates from the clinic through the wastewater pathway

Troy Skwor, Ph.D.
Assistant Professor
Department of Biomedical Sciences
University of Wisconsin - Milwaukee
Skwor@uwm.edu



Risque infectieux et soin

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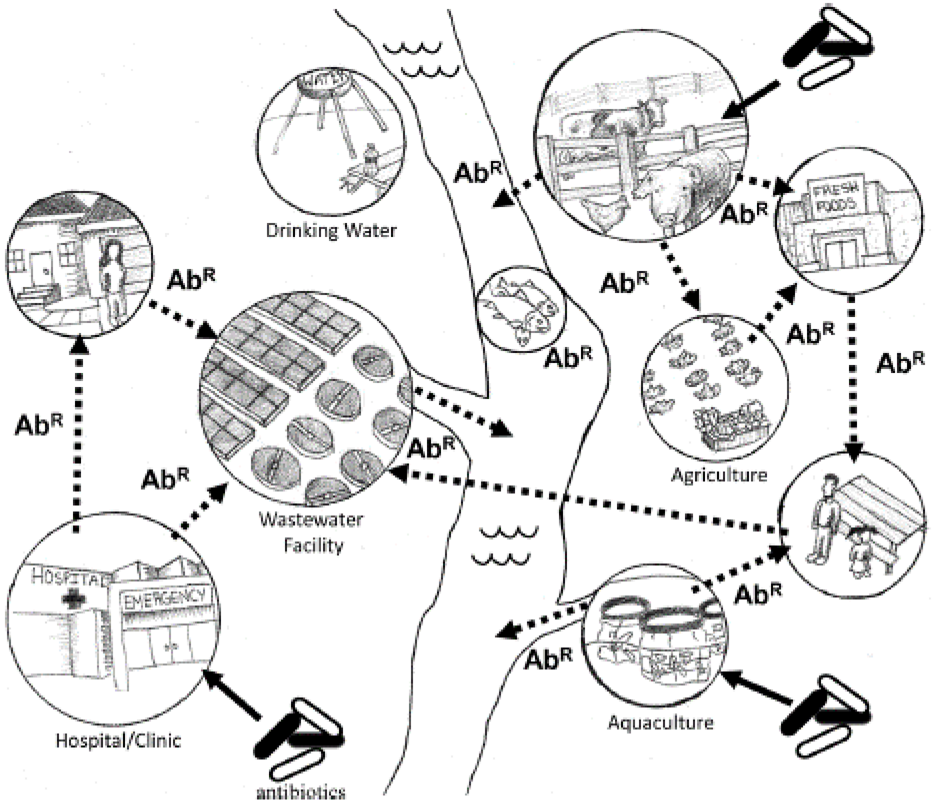
Bojar *et al.*, 2021; IJHEH, 238(113863)

BACKGROUND

- Antibiotic resistance - biggest global threat to health and food (World Health Organization)
- ESBL- and carbapenemase-producing *Enterobacterales* – **Priority Pathogen 1 CRITICAL (WHO)**
- *Escherichia coli* exists in the environment, foods, and as commensals and pathogens in mammals – One Health
- Swimming in recreational freshwater containing *E. coli* has been linked to acquiring *E. coli* infections (Soraas et al., 2013) and outbreaks (Graciaa et al., 2018b; Vanden Esschert et al., 2020)

One Health



Skwor and Kralova, 2019; ASM Press

Objective: To determine anthropogenic impact on antibiotic resistance entering the environment and determine their potential health risks

Antibiotic Resistance: Clinic through Wastewater

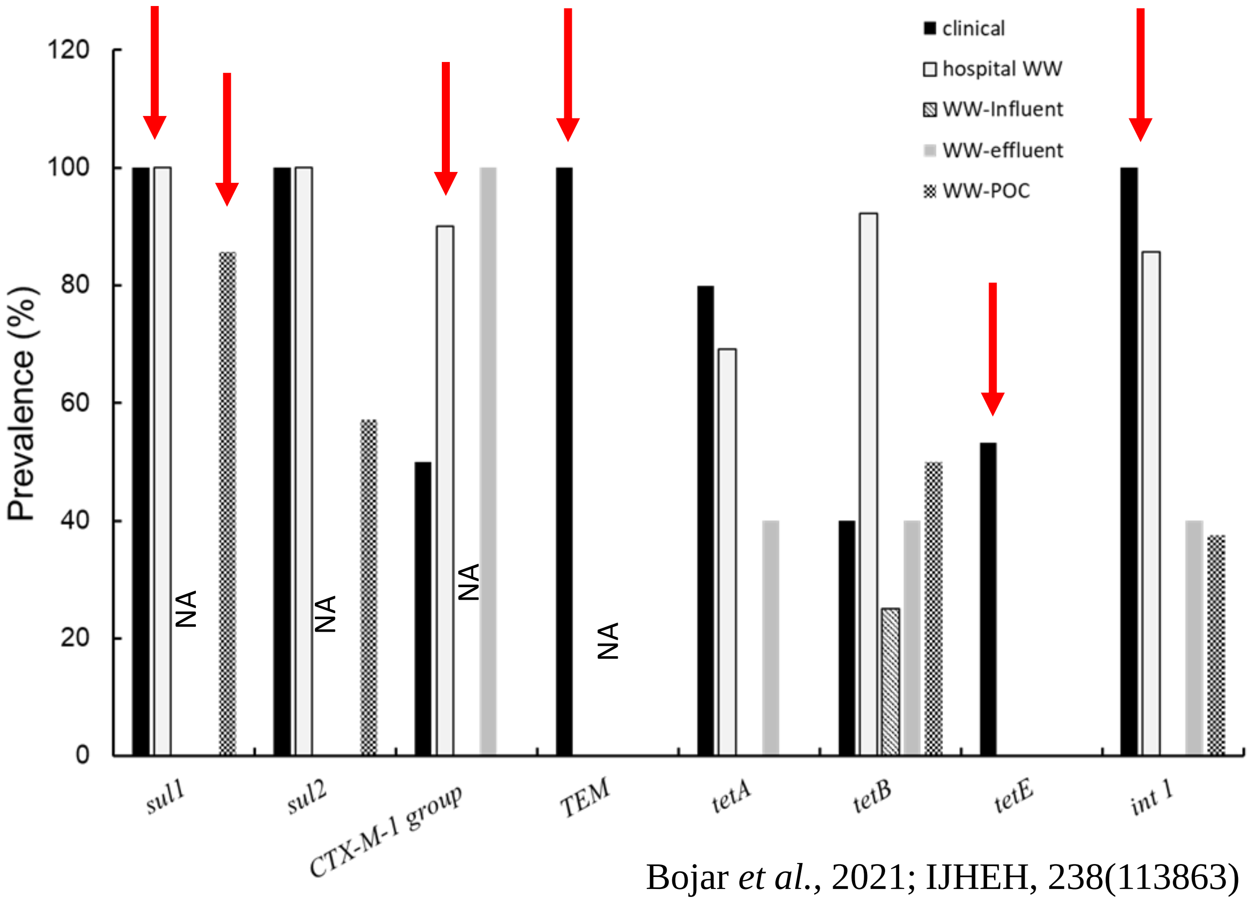
	Resistance % (n)		
Antibiotic	**UPEC (77)**	**Hospital WW (37)**	**WW Influent (30)**
CHL	N.D	2.7 (1)	6.7 (2)
SXT	15.6 (12)	35.1 (13)	0
GEN	5.2 (4)	29.7 (11)	0
CIP	26.0 (20)	29.7 (11)	0
TET	18.2 (14)	32.4 (12)	13.3 (4)
CRO	3.9 (3)	24.3 (9)	0
CAZ	1.3 (1)	16.2 (6)	0
ETP	1.3 (1)	0	0

Bojar *et al.*, 2021; IJHEH, 238(113863)

What are the ARGs responsible for these phenotypes?

Presence of Clinical ARGs in Wastewater

- Clinically common *sul1/sul2* elevated among POC
- *CTX-M-1* subgroup common in hospital WW
- *bla*$_{\text{TEM}}$ and *tetE* only evident in clinical strains
- *Int1* strains common in clinical strains and evident in POC



Bojar *et al.*, 2021; IJHEH, 238(113863)

Let's take a deeper look into ESBL-producing strains

ESBL-Prevalence among Various Sources

- **Cefotaxime**-resistant populations

Table 1. Prevalence of β-lactamases and *int1* genes among *E. coli* populations.

Source	N	Prevalence (%)							
		CTX-M-1 Subgroup	CTX-M-2 Subgroup	CTX-M-9 Subgroup [a]	TEM	OXA	SHV	KPC [b]	*int1*
Clinical	11	7 (64)	0	1 (9)	11 (100)	5 (45)	0	0	7 (64)
Hospital WW	22	21 (95)	0	0	13 (59)	19 (86)	0	9 (41)	17 (77)
Urban Influent	15	11 (73)	0	4 (27)	11 (73)	10 (67)	0	0	8 (53)
Treated effluent	16	13 (81)	0	5 (31)	12 (75)	13 (81)	0	0	9 (56)
Total	64	52 (81)	0	10 (16)	47 (73)	47 (73)	0	9 (14)	41 (64)

[a] Statical difference between sources $p = 0.005$; [b] $p < 0.001$.

Liedhegner et al.; 2022, *Antibiotics*, 11(2): 260-277

What is the correlation of ESBL with resistant phenotypes?

ESBL Correlation with Resistance Phenotypes

Table 2. Correlation of antibiotic resistance phenotypes and genotypes.

	CAZ
CAZ	1
CRO	0.1176 0.3548
CTX	0.1176 0.3548
ERT	**0.2925 *** **0.0190**
ESBL	**−0.3945 *** **0.0013**
$bla_{CTX\text{-}M\text{-}1}$	0.1732 0.1711
$bla_{CTX\text{-}M\text{-}9}$	**−0.4326 *** **0.0004**
bla_{OXA}	0.1508 0.2342
bla_{TEM}	−0.2093 0.0969
bla_{KPC}	**0.3346 *** **0.0069**

Liedhegner et al.; 2022, *Antibiotics*, 11(2): 260-277

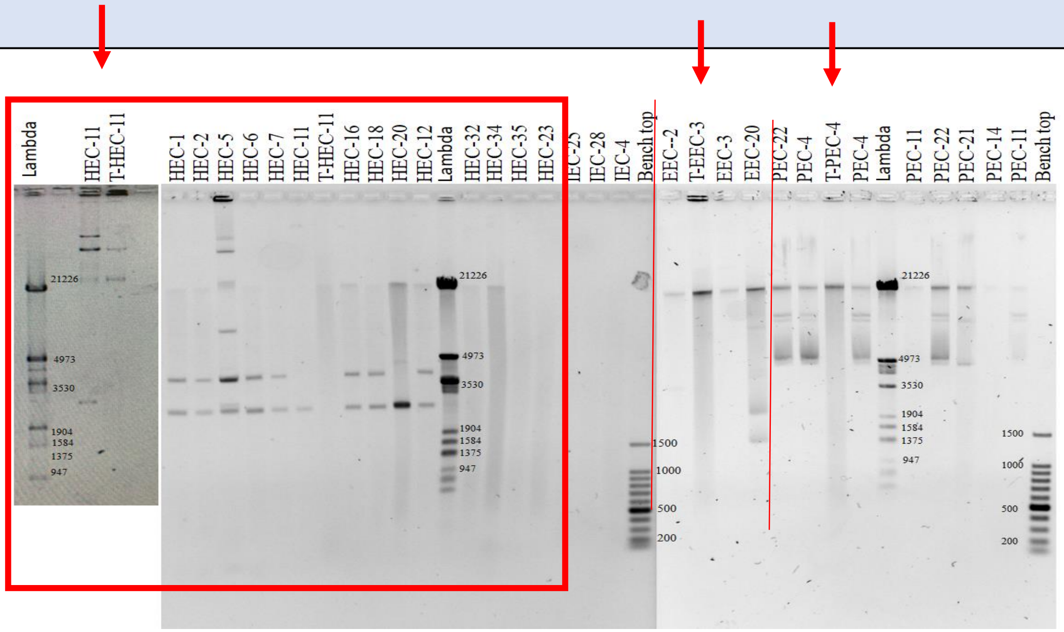
Are these ESBLs plasmid-mediated?

Plasmid-mediated Resistance

Table 2
Resistant profiles associated with plasmids.

Strain	CHL	SXT	GEN	CIP	TET	CRO	CAZ
Hospital Effluent							
HEC-11	S	R	R	R	R	R	R
T-HEC-11	S	S	S	S	S	R	R
WW Pre-chlorinated Effluent							
EEC-3	S	S	S	S	R	S	S
T-EEC-3	S	S	S	S	R	S	S
EEC-27	S	S	S	S	R	S	S
T-EEC-27	S	S	S	S	R	S	S
WW Post-chlorinated effluent							
PEC-4	S	R	S	S	R	S	S
T-PEC-4	S	R	S	S	R	S	S

Bojar *et al.*, 2021; *IJHEH*, 238(113863)



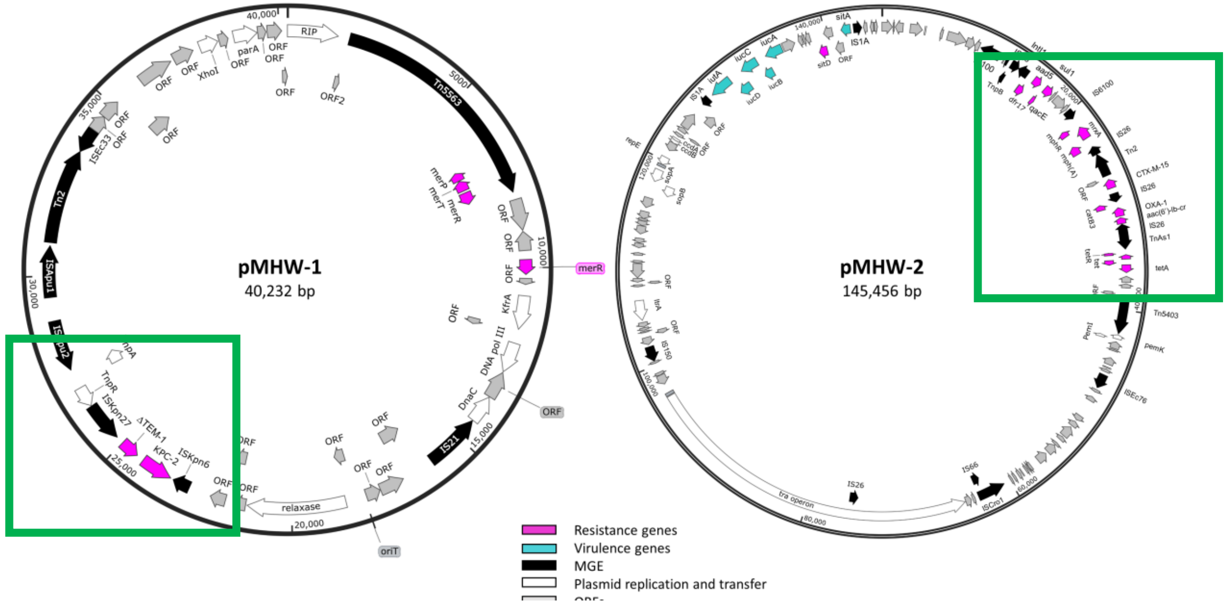
Strain	CHL	SXT	GEN	CIP	TET	CRO	CTX	CAZ	MEM
	Antibiotic susceptibility of transformed plasmids								
Hospital WW									
1000C-C3	S	S	S	S	S	R	R	R	R
1000C-C4	S	S	S	S	S	R	R	R	R
1000D-C3	S	S	S	S	S	R	R	R	S
1000D-C4	S	S	S	S	S	R	R	R	R
WW influent									
I-C3	S	S	S	S	S	R	R	R	S
I-C10	S	S	S	S	S	R	R	R	S
WW effluent									
E-C3	S	S	S	S	S	R	R	R	S
E-C4	S	S	S	S	S	R	R	R	S
E-C5	S	S	S	S	S	R	R	R	S
E-C9	S	S	S	S	S	R	R	R	S

S: susceptible; R: resistant

What ARGs are responsible for these phenotypes?

Liedhegner et al.; 2022, *Antibiotics*, 11(2): 260-277

WGS of Hospital WW Isolate 1000C-3



Liedhegner et al.; 2022, *Antibiotics*, 11(2): 260-277

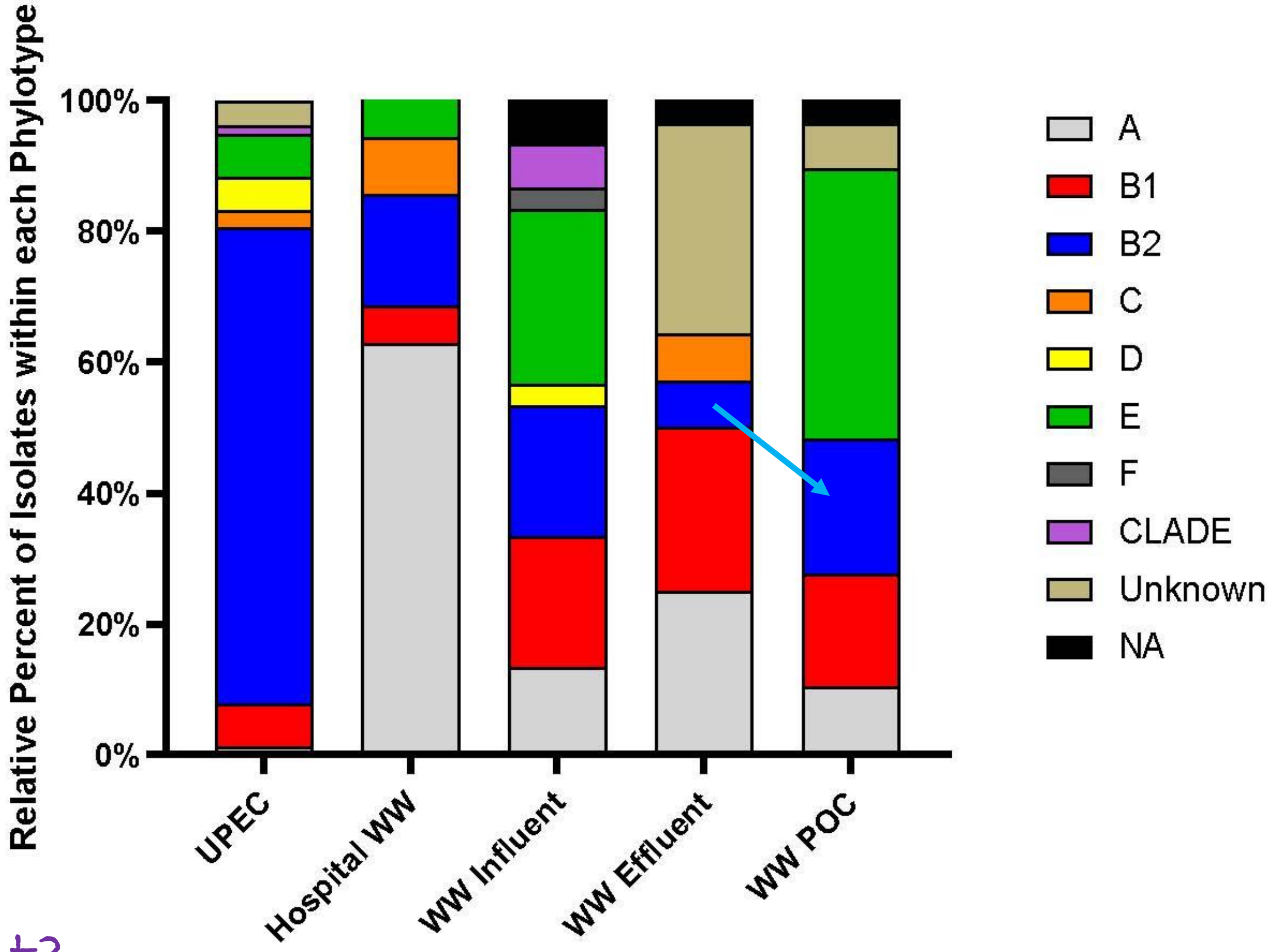
Are wastewater populations similar to clinical strains?

Table 3. Resistome of MDR hospital wastewater *E. Coli* 1000C-3 isolate.

Antibiotic Resistance Genes	Location	Accession #	Resistance Target
aac(6')-Ib-cr	Plasmid	DQ303918	Aminoglycoside and fluoroquinolone resistance
aadA5	Plasmid	AF137361	Aminoglycoside resistance
aph(3'')-Ib	Plasmid	AF321551	Aminoglycoside resistance
aph(6)-Id	Plasmid	M28829	Aminoglycoside resistance
blaCTX-M-15	Plasmid	AY044436	Beta-lactam resistance
blaOXA-1	Plasmid	HQ170510	Beta-lactam resistance
blaKPC-2	Plasmid	AY034847	Beta-lactam resistance
blaTEM-350	Plasmid	WP045286946	Beta-lactam resistance
mdf(A)	Chromosomal	Y08743	Macrolide resistance
mph(A)	Plasmid	D16251	Macrolide resistance
catB3	Plasmid	AJ009818	Phenicol resistance
sul1	Plasmid	U12338	Sulphonamide resistance
tet(A)	Plasmid	AJ517790	Tetracycline resistance
tet(B)	Chromosomal	AF32677	Tetracycline resistance
dfrA17	Plasmid	FJ460238	Trimethoprim resistance
sitABCD	Plasmid	AY598030	Disinfectant resistance
qacE	Plasmid	X68232	Disinfectant resistance
bacA	Chromosomal	3002986	Peptide antibiotic resistance
mdtG	Chromosomal	3001329	MFS antibiotic efflux pump
mdtH	Chromosomal	3001216	MFS antibiotic efflux pump
mdtP	Chromosomal	3003550	MFS antibiotic efflux pump
mdtN	Chromosomal	3003548	MFS antibiotic efflux pump
tolC	Chromosomal	3000237	MFS antibiotic efflux pump
emrA	Chromosomal	3000027	MFS antibiotic efflux pump
emrB	Chromosomal	3000074	MFS antibiotic efflux pump
emrK	Chromosomal	3000206	MFS antibiotic efflux pump
emrY	Chromosomal	3000254	MFS antibiotic efflux pump
evgA	Chromosomal	3000833	MFS antibiotic efflux pump
H-NS	Chromosomal	3000676	MFS antibiotic efflux pump
ampH	Chromosomal	3004612	ampC-type beta-lactamase
ampC1	Chromosomal	3004611	ampC-type beta-lactamase
cpxA	Chromosomal	3000830	RND Efflux pump
gadX	Chromosomal	3000508	RND Efflux pump
mdtE	Chromosomal	3000795	RND Efflux pump
mdtF	Chromosomal	3000796	RND efflux pump
acrA	Chromosomal	3004043	RND Efflux pump
acrB	Chromosomal	3000216	RND Efflux pump
acrD	Chromosomal	3000491	RND Efflux pump
baeS	Chromosomal	3000829	RND Efflux pump
baeR	Chromosomal	3000828	RND Efflux pump
marA	Chromosomal	3000263	RND Efflux pump
yojI	Chromosomal	3003952	Peptide antibiotic efflux pump
pmrF	Chromosomal	3003578	Phosphoethanolamine transferase
kdpE	Chromosomal	3003841	Aminoglycoside efflux pump
msbA	Chromosomal	3003950	Nitroimidazole efflux pump
Point mutations		PMID	
parC AGC-> ATC		8851598	Quinolone resistance
parE TCG -> GCG		28598203	Quinolone resistance
gyrA TCG -> TTG		8891148	Quinolone resistance
gyrA GAC-> AAC		12654733	Quinolone resistance

Phylotyping of *Escherichia coli*

- ➢UPEC predominately B2
- ➢Hospital WW most phylotype A
- ➢Elevated B2 in POC compared to WW effluent



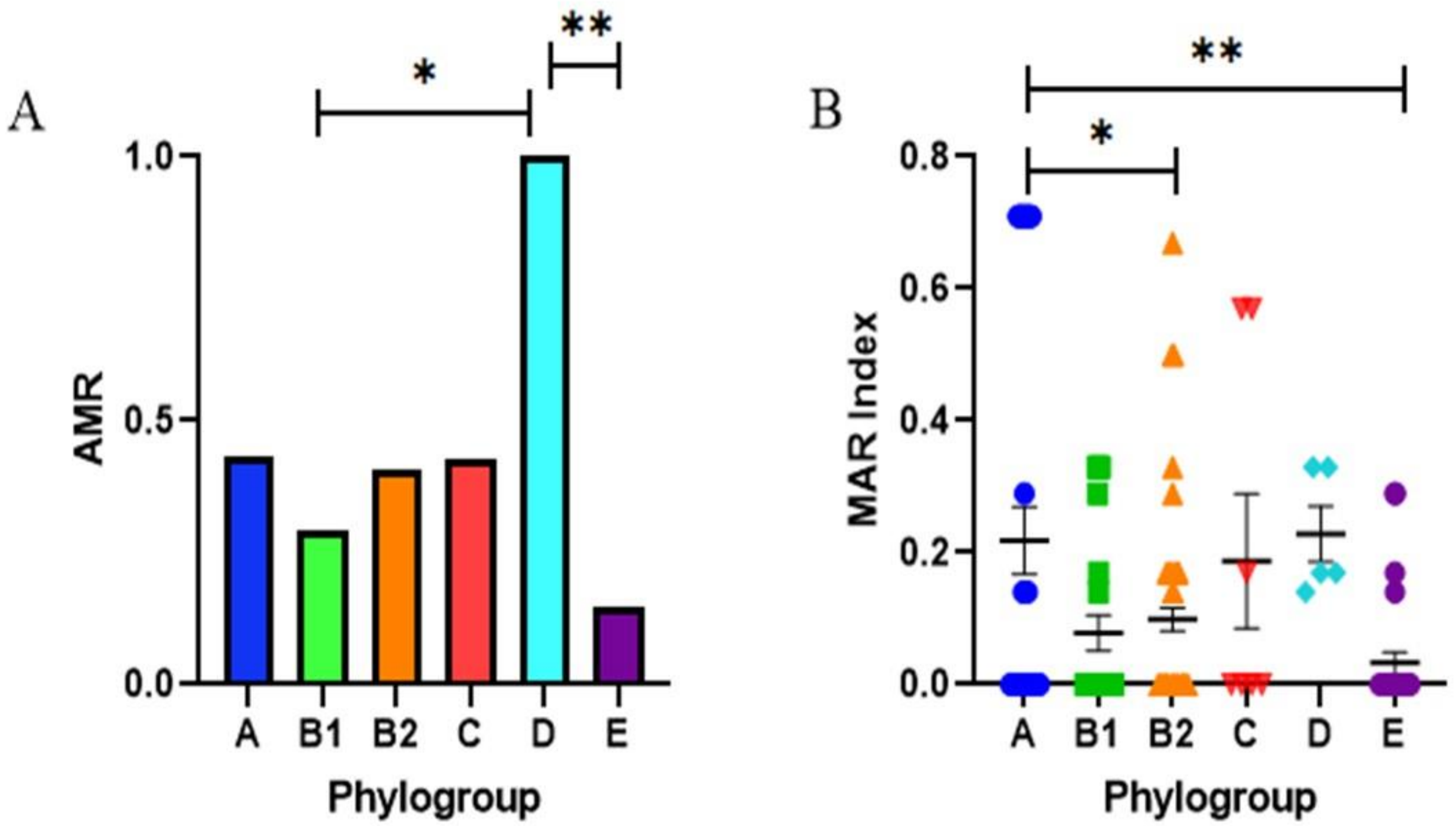
Bojar *et al.*, 2021; *IJHEH*, 238(113863)

What phylotypes are most resistant?

Phylogroups and Antibiotic Resistance

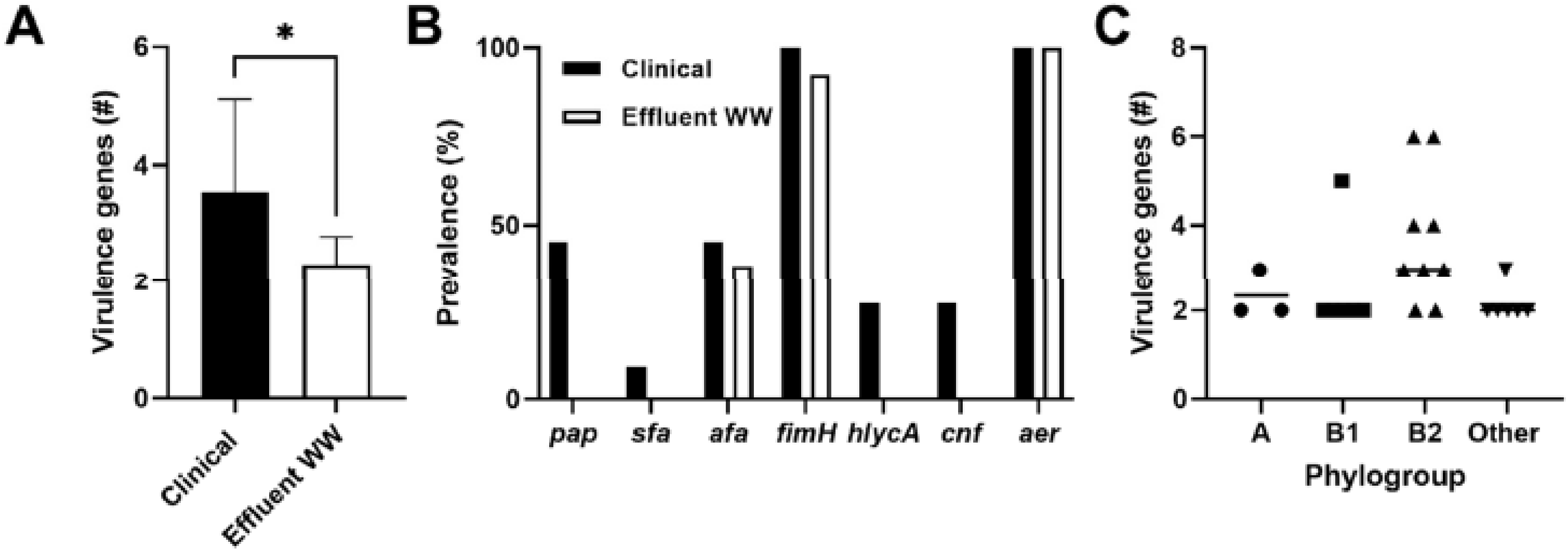
- Phylogroup D exhibits the highest AMR prevalence
- Highest MAR index – Phylotype A

Are WW effluents potentially pathogenic?



Bojar *et al.*, 2021; *IJHEH*, 238(113863)

Potential pathogens among **ESBL**-producers



Liedhegner et al.; 2022, *Antibiotics*, 11(2): 260-277

Does Hospital WW populations survive WW treatment?

Hospital WW composition in WW effluents

Table 2
Contribution of Hospital Effluent and WWTP Combined Influent microbial community composition to WWTP Effluent microbial community composition based on FEAST molecular source tracking results.

Source	Sink	% Contribution[a]	Mean % contribution
Hospital Effluent	Pre-Chlorinated Effluent	1) 0.68 2) 5.17 3) 9.37	5.08
WWTP Combined Influent		1) 18.09 2) 4.07 3) 2.38	8.18
Hospital Effluent	Post-Chlorinated Effluent	1) 12.44 2) 13.13 3) 8.89	11.49
WWTP Combined Influent		1) 4.08 2) 5.61 3) 4.25	4.25

[a] Note that the % contribution is based upon three separate sampling timepoints. Hospital Effluent and Influent samples were used as sources for Pre- and Post- Chlorinated Effluent collected from the same sampling date.

Beattie *et al*., *2020; Science of the Total Environment* 740:140186

- Hospital WW is more resilient to wastewater treatment processes => ***enters into recipient environmental sources?***

Who are the cefotaxime- and meropenem-resistant bacterial populations entering the environment from wastewater?

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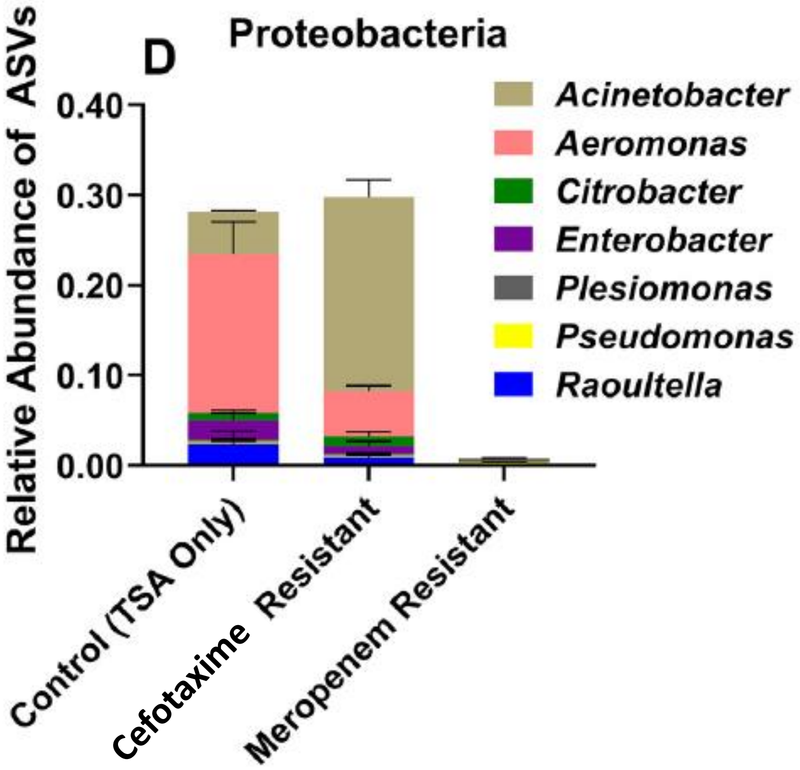
Cefotaxime and Meropenem-resistant populations

- Plate POC filters on antibiotic containing TSA plates
- 16h after incubation => isolate DNA and perform 16S library

➢ ***Aeromonas*** *and* ***Acinetobacter*** *species predominate among cefotaxime-resistant post-chlorinated effluents*



Beattie *et al., 2020; Science of the Total Environment* 740:140186

Conclusions

- Clinical *E. coli* populations exhibit higher AMR rates than urban WW populations
- Hospital WW are the most multi-drug resistant and comprised of phylotype A
- Treated WW effluents are a rich source of antibiotic resistant potentially-pathogenic bacteria
- Chlorination potentially kills weaker populations leaving more resistant ones
  - ***Supported by both culture and non-culture based techniques***
- ***Antimicrobial resistance is inversely correlated with country's GNI***

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