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Correlation of *in vitro* antibiogram with *in vivo* anti-pathogenic efficacy of different antibiotics against three gram-negative bacterial pathogens in the *Caenorhabditis elegans* infection model

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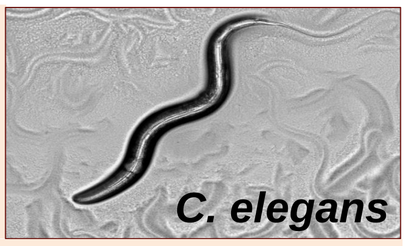
INTRODUCTION

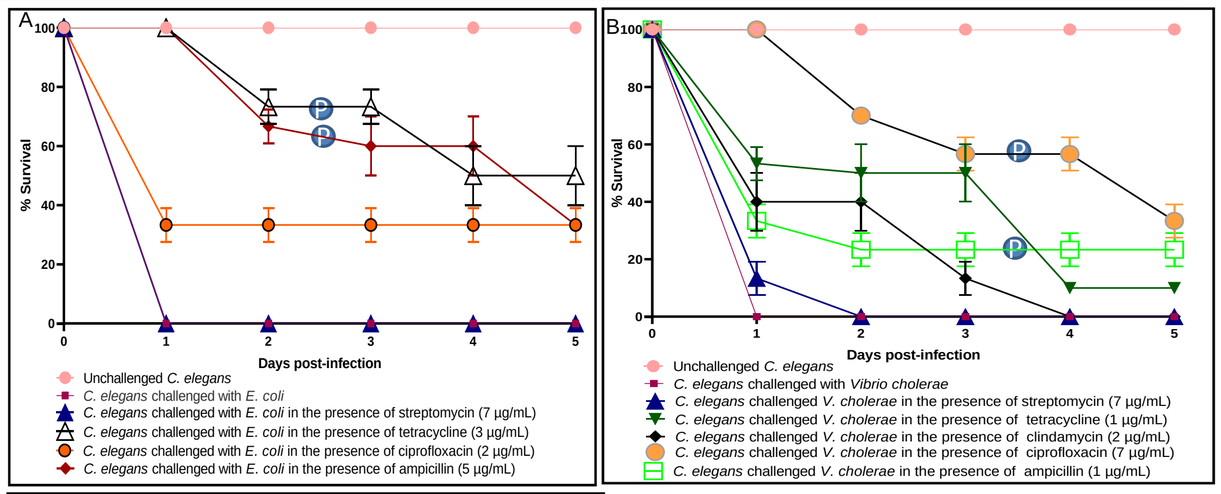
Though antibiogram generated through disc diffusion assay is a widely used method to assist the clinician in selecting appropriate antibiotics for patient treatment, correlation of *in vitro* efficacy of antibiotics with their *in vivo* efficacy needs deeper investigations.

METHODS

Antibiotic susceptibility assay: Antibiogram was generated by disc diffusion assay on cation-adjusted Mueller-Hinton agar, in line with CLSI guidelines[1]. MIC was determined through broth dilution assay in respective growth medium for each pathogen (Luria-Bertani broth for *V. cholerae*; Pseudomonas broth for *P. aeruginosa*; Nutrient broth for *E. coli*).



Anti-pathogenic assay[2]**:** The model host *Caenorhabditis elegans*, kept in M9 buffer, was challenged with three different antibiotic-resistant gram-negative bacterial pathogens (*Pseudomonas aeruginosa*, *Vibrio cholerae*, or *Escherichia coli*) in absence or presence of the MIC-levels of those antibiotics belonging to different classes, to whom these pathogens were shown to be sensitive in disc diffusion assay. Worm survival was quantified over a period of five days through microscopic live-dead count.



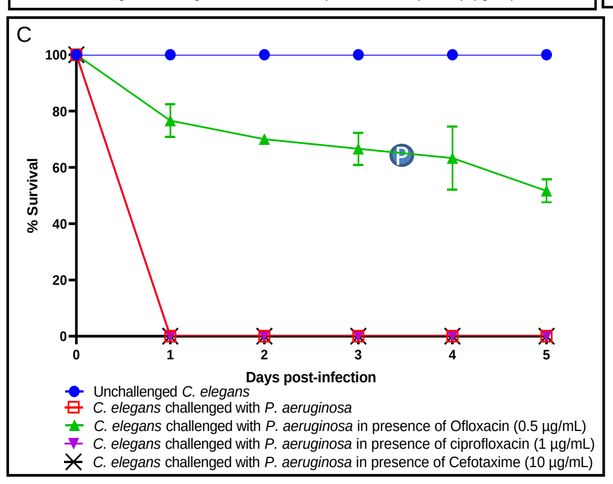


Figure 1. *In vivo* anti-pathogenic efficacy of various antibiotics against (A) *E. coli* (B) *V. cholerae and* (C) *P. aeruginosa*. 'P' indicates progenies i.e. worms were able to reproduce from that particular time-point onward. However progenies were ignored, while calculating survival percentage.

RESULTS

Table 1. Antibiogram of test pathogens

			E. coli		*V. cholerae*		*P. aeruginosa*	
Antibiotic Class	**Antibiotic**	**Conc. (µg/ disc)**	**Zone of Inhibition (mm)**	**Interpre-tation**	**Zone of Inhibition (mm)**	**Interpre-tation**	**Zone of inhibition (mm)**	**Interpre-tation**
Fluoro-quinolone	Ciprofloxacin(CIP)	5	51 ± 1.09	S	36 ± 0	S	46±2.82	S
	Ofloxacin	5	43 ± 2.28	S	30.1 ± 0.40	S	38±2.82	S
	Sparfloxacin	5	39.66 ± 3.07	S	23 ± 0	S	32±0	S
	Levofloxacin	5	45.16 ± 1.32	S	34.16 ± 0.40	S	40±0	S
	Nalidixic acid	30	28 ± 4.56	S	0	R	18±0	I
	Moxifloxacin	5	41.16 ± 1.32	S	33.83 ± 0.40	S	30±0	S
	Norfloxacin	10	39.83 ± 4.21	S	15 ± 0	I	36±6.36	S
	Gatifloxacin	5	44 ± 1.26	S	26 ± 0	S	36±0.70	S
Nitrofuran	Nitrofurantoin	300	20.5 ± 1.76	S	9 ± 0	R	0±0	R
Amino-glycoside	Tobramycin	10	28.83 ± 3.12	S	23 ± 0	S	26±5.65	S
	Kanamycin	30	27.83 ± 3.12	S	28.16 ± 0.40	S	14±2.82	R
	Gentamicin	10	32.5 ± 2.81	S	25 ± 0	S	24±0.70	S
	Amikacin	30	30.83 ± 3.12	S	35.16 ± 0.40	S	21±0.70	S
	Streptomycin	25	30.5 ± 0.83	S	25.83 ± 0.40	S	0±0	R
Sulfonamides	Co-Trimxazole	25	16.33 ± 2.94	S	34.66 ± 0.51	S	0±0	R
Polymyxin	Colistin	10	17.33 ± 2.58	S	-	-	18±0	S
β-lactam	Imipenem	10	22 ± 1.26	S	46 ± 0	S	28±3.53	S
	Augmentin	30	36.83 ± 1.32	S	32.16 ± 0.40	S	0±0	R
	Ampicillin	10	26.16 ± 2.40	S	37 ± 0	S	0±0	R
	Ticarcillin	10	26.16 ± 2.40	S	36 ± 0	S	-	-
	Cefepime	30	34.83 ± 1.32	S	32.83 ± 0.40	S	25±1.41	S
	Ceftriaxone	30	38.33 ± 2.65	S	40 ± 0	S	26±0.70	S
	Cefpodoxime	10	38 ± 2.19	S	19 ± 0	I	28±0	S
	Cefixime	5	35 ± 1.09	S	14.16 ± 0	R	0±0	R
	Cefotaxime	30	39.83 ± 1.16	S	38 ± 0	S	21±4.94	S
Amphenicol	Chloramphenicol	30	32.33 ± 2.58	S	37.5 ± 1.64	S	0±0	R
Rifamycin	Rifampicin	5	19.33 ± 2.58	I	35 ± 0	S	10±0	R
Glycopeptide	Vancomycin	30	15.66 ± 1.50	S	27 ± 0	S	0±0	R
Macrolide	Clindamycin	2	0	R	32.83 ± 0.40	S	0±0	R
Tetracyclines	Tetracycline	30	29.16 ± 0.98	S	34 ± 0	S	0±0	R
	Doxycycline HCl	30	27.33 ± 2.94	S	35 ± 0	S	0±0	R

R:Resistant; S:Sensitive; I:Intermediate.

Table 2. *In vivo* anti-pathogenic effect of antibiotics at MIC levels

Antibiotic	***E. coli***		***V. cholerae***		***P. aeruginosa***	
	MIC concentration (µg/mL)	**Anti-pathogenic assay**	**MIC concentration (µg/mL)**	**Anti-pathogenic assay**	**MIC concentration (µg/mL)**	**Anti-pathogenic assay**
		Survival benefit (%) on fifth day		**Survival benefit (%) on fifth day**		**Survival benefit (%) on fifth day**
Streptomycin	7	0	7	0	-	-
Clindamycin	-	-	2	0	-	-
Ampicillin	5	33.33%*** ± 5.77	1	23.33%*** ± 5.77	-	-
Tetracycline	3	50%*** ± 10	1	10%*** ± 0	-	-
Ciprofloxacin	2	33.33%*** ± 5.77	7	33.33%*** ± 5.77	1	0
Ofloxacin	-	-	-	-	0.5	51.66***± 4.08
Cefotaxime	-	-	-	-	10	0

***$p \leq 0.001$; '-' :Not applicable

Table 3. Correlation between *in vitro* and *in vivo* efficacy of antibiotics

	'r' between diameter of ZoI and *in vivo* efficacy		**'r' between MIC and *in vivo* efficacy**	
	1St day endpoint	**5th day endpoint**	**1St day endpoint**	**5th day endpoint**
E. coli	0.52	0.04	-0.97	-0.80
V. cholerae	0.62	0.75	0.23	0.14
P. aeruginosa	0.20	0.20	-0.53	-0.53

r: Correlation coefficient; ZOI: Zone of Inhibition; MIC: Minimum Inhibitory Concentration

DISCUSSION

- While ampicillin, ciprofloxacin, and tetracycline could offer significant protection to the worm population in face of *V. cholerae* challenge, streptomycin and clindamycin failed to do so. Streptomycin also could not rescue worms against *E. coli*. As ampicillin and ciprofloxacin were effective against *E. coli* too, antibiotic-response of both these pathogens in worm model can be said to have some commonality.
- While ciprofloxacin, a fluroquinolone antibiotic effective against remaining two pathogens, could not protect the worm population from *P. aeruginosa* attack, another quinolone antibiotic ofloxacin could do this.
- Cefotaxime, a third-generation cephalosporin also failed to confer any protection on worm population challenged with *P. aeruginosa*.
- The correlation between 'MIC' and 'in vivo efficacy (as per first-day endpoint)' was stronger in case of *E. coli* (r: -0.97) than *P. aeruginosa* (r: -0.53). In case of *V. cholerae* (r: 0.23), antibiotics with lower MIC exhibited lesser in vivo efficacy than those with higher MIC.
- Correlation between 'diameter of zone of inhibition' and '*in vivo* efficacy (as per first-day endpoint)' was found to be better for *V. cholerae* (r:0.62) than remaining two pathogens.
- The observed changes in antibiotic efficacy across the three assay formats may in part be due to differences in the composition of the media employed during each assay.

CONCLUSION

Further investigation with a broader range of antibiotics and pathogens should be conducted to check whether *in vivo* assays with simple model hosts can be a better predictor of clinical efficacy of antibiotics, than the conventional disc diffusion or broth dilution assays.

REFERENCES

1. Clinical and Laboratory Standards Institute (CLSI). (2024). Performance Standards for Antimicrobial Susceptibility Testing (CLSI Document M100, 34th edition).
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