# How good a predictor the *in vitro* antibiogram is, of *in vivo* antibiotic efficacy?

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## BACKGROUND

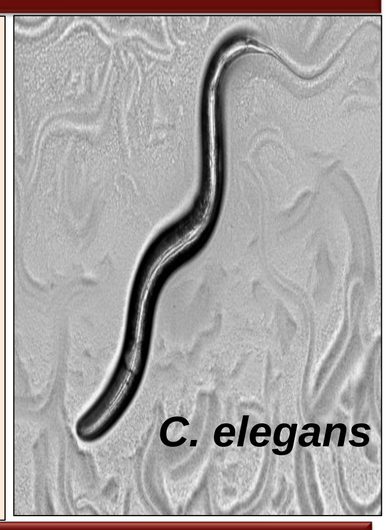
Antibiogram generated through disc diffusion assay (DDA) is widely used to assess antibiotic susceptibility of clinical isolates of pathogenic bacteria. However, given the vast difference between the matrix environment of this kind of *in vitro* assay and that present *in vivo*, it is worth investigating whether DDA is a useful predictor of *in vivo* antibiotic efficacy.

## OBJECTIVE

To investigate the correlation between antibiotic susceptibility observed in a DDA, and *in vivo* antibiotic efficacy observed in a worm model of infection.

## METHODS

We compared the *in vitro* antibiotic susceptibility profile of three antibiotic-resistant bacterial pathogens, *Pseudomonas aeruginosa*, *Clostridium difficile*, and methicillin-resistant *Staphylococcus aureus* (MRSA), with their susceptibility to antibiotics in an *in vivo* assay employing the nematode *Caenorhabditis elegans* as a model host. DDA used 39 antibiotics for each pathogen, while *in vivo* efficacy was checked for 74 antibiotics. We challenged the host worm (25 worms of L3-L4 stage per well) with either of these pathogens in presence or absence of antibiotics, and worm activity (a surrogate for their survival and healthspan) was quantified over a five-day period through an automated worm tracker. Correlation coefficient (r) between the diameter of zone of inhibition observed in disc diffusion assay, and *in vivo* antibiotic efficacy was determined for 26 antibiotics.




## RESULTS

**Table 1. Correlation (r) between *in vitro* and *in vivo* effect of antibiotics against three pathogenic bacteria**

| Sr.No. | Class [1] | Antibiotic (µg/disc) | *Clostridioides difficile* (ATCC 9689) | | | | | Methicillin Resistant *Staphylococcus aureus* (ATCC 43300) | | | | | *Pseudomonas aeruginosa* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | A | | *In vivo* efficacy (worm activity count) | | Correlation (r) between 'A' and 'B' | A | | *In vivo* efficacy (worm activity count) | | Correlation (r) between 'A' and 'B' | A | | *In vivo* efficacy (worm activity count) | | Correlation (r) between 'A' and 'B' |
| | | | Zone of inhibition (mm) (Mean±SD) | Interpretation[(-)] | B 24 h end-point | 48 h end-point | | Zone of inhibition (mm) (Mean±SD) | Interpretation[2] | B 24 h end-point (Mean±SD) | 48 h end-point (Mean±SD) | | Zone of inhibition (mm) (Mean±SD) | Interpretation[2] | B 24 h end-point (Mean±SD) | 48 h end-point (Mean±SD) | |
| NA | | Health control | NA | | 34.75±3.88 | 19.33±7.30 | NA | NA | | 28.83±6.88 | 28.16±6.61 | NA | NA | | 39.1±15.84 | 49.3±9.66 | NA |
| | | Infection control | | | 2.41±1.06 | 0±0 | | | | 4.83±4.30 | 0±0 | | | | 3.2±4.21 | 1.6±2.06 | |
| | | Vehicle control | | | 4±0.47 | 0±0 | | | | 4.5±3.78 | 0±0 | | | | 2.4±3.09 | 2.7±3.71 | |
| 1 | Aminoglycoside | Streptomycin (25) | 26 ± 1.17 | S | 0.00 ± 0.00 | 0.00 ± 0.00 | -0.49 | 29 ± 0.89 | S | 22.67 ± 6.11 | 0.00 ± 0.00 | 0.47 | 23 ± 0.98 | - | 6.00 ± 5.20 | 1.50 ± 1.91 | -0.4 |
| 2 | | Tobramycin (10) | 26 ± 1.38 | S | 48.00 ± 16.97 | 0.00 ± 0.00 | | 15 ± 3.24 | I or R | 20.67 ± 10.95 | 0.00 ± 0.00 | | 29 ± 3.28 | S | 1.33 ± 2.31 | 50.33 ± 10.07 | |
| 3 | | Amikacin (30) | 29 ± 1.06 | S | 0.00 ± 0.00 | 0.00 ± 0.00 | | 25 ± 2.09 | S | 19.83 ± 8.50 | 0.00 ± 0.00 | | 28 ± 2.84 | S | 2.00 ± 3.46 | 3.20 ± 4.60 | |
| 4 | | Kanamycin (30) | 28 ± 0.41 | S | 0.00 ± 0.00 | 0.00 ± 0.00 | | 21 ± 0.75 | S | 21.17 ± 8.82 | 0.00 ± 0.00 | | 24 ± 1.60 | - | 0.00 ± 0.00 | 2.80 ± 4.38 | |
| 5 | Fluoroquinolone | Sparfloxacin (5) | 30 ± 1.03 | S | 41.33 ± 12.06 | 5.00 ± 7.07 | -0.55 | 40 ± 0.52 | S | 34.67 ± 15.44 | 34.50 ± 16.37 | 0.39 | 34 ± 1.32 | S | 27.25 ± 14.82 | 63.80 ± 31.06 | -0.21 |
| 6 | | Gatifloxacin (5) | 31 ± 0.82 | S | 45.67 ± 14.01 | 70.00 ± 18.38 | | 38 ± 0.41 | S | 34.00 ± 13.17 | 31.40 ± 19.15 | | 35 ± 1.21 | S | 13.00 ± 1.73 | 56.25 ± 13.87 | |
| 7 | | Ofloxacin (5) | 27 ± 1.22 | S | 44.00 ± 15.62 | 117.50 ± 20.51 | | 32 ± 1.61 | S | 38.00 ± 17.41 | 16.17 ± 12.97 | | 25 ± 1.47 | S | 28.00 ± 11.97 | 70.50 ± 15.95 | |
| 8 | | Norfloxacin (10) | 34 ± 1.78 | S | 34.33 ± 4.62 | 120.00 ± 35.36 | | 42 ± 1.56 | S | 31.83 ± 16.96 | 3.67 ± 4.23 | | 43 ± 2.23 | S | 23.00 ± 17.44 | 0.00 ± 0.00 | |
| 9 | | Ciprofloxacin (5) | 29 ± 1.10 | S | 57.00 ± 1.41 | 106.50 ± 7.78 | | 34 ± 0.63 | S | 7.20 ± 4.15 | 3.50 ± 5.43 | | 35 ± 0.75 | S | 20.00 ± 7.07 | 26.00 ± 16.52 | |
| 10 | | Levofloxacin (5) | 31 ± 1.16 | S | 38.33 ± 10.97 | 52.00 ± 73.54 | | 38 ± 1.82 | S | 34.25 ± 3.30 | 23.40 ± 8.88 | | 34 ± 1.22 | S | 20.25 ± 16.80 | 30.80 ± 15.01 | |
| 11 | | Moxifloxacin (5) | 30 ± 0.98 | S | 37.67 ± 4.16 | 41.50 ± 36.06 | | 43 ± 0.98 | S | 40.50 ± 18.02 | 31.00 ± 11.58 | | 34 ± 3.01 | S | 9.50 ± 5.20 | 62.67 ± 20.84 | |
| 12 | Quinolone (1st generation) | Nalidixic acid (30) | 22 ± 0.55 | S | 4.00 ± 5.66 | 75.00 ± 106.07 | NA | 18 ± 0.52 | - | 6.80 ± 9.34 | 3.00 ± 7.35 | NA | 0 ± 0.00 | R | 43.00 ± 25.46 | 2.00 ± 4.00 | NA |
| 13 | Third generation cephalosporin | Ceftriaxone (30) | 15 ± 1.13 | S | 34.67 ± 12.50 | 0.00 ± 0.00 | 0.93 | 30 ± 1.87 | S | 22.83 ± 14.92 | 4.33 ± 4.04 | -0.32 | 24 ± 2.65 | S | 21.50 ± 2.12 | 29.50 ± 6.36 | -0.18 |
| 14 | | Cephotaxime (30) | 15 ± 0.75 | S | 22.00 ± 3.61 | 0.00 ± 0.00 | | 35 ± 0.55 | - | 17.75 ± 5.74 | 1.00 ± 2.00 | | 22 ± 1.47 | S | 18.75 ± 6.70 | 73.67 ± 27.93 | |
| 15 | | Cefixime (5) | 0 ± 0.00 | R | 0.00 ± 0.00 | 1.50 ± 2.12 | | 20 ± 0.52 | - | 20.50 ± 4.68 | 6.00 ± 5.83 | | 12 ± 0.55 | - | 21.00 ± 2.00 | 18.33 ± 9.61 | |
| 16 | Carbapenem | Imipenem (10) | 41 ± 0.89 | S | 0.00 ± 0.00 | 0.00 ± 0.00 | NA | 42 ± 2.61 | - | 7.80 ± 8.50 | 0.00 ± 0.00 | NA | 34 ± 1.03 | S | 69.00 ± 35.36 | 37.75 ± 12.12 | NA |
| 17 | Aminopenicillin | Ampicillin (10) | 12 ± 0.78 | S | 0.00 ± 0.00 | 0.00 ± 0.00 | | 31 ± 2.04 | S | 38.33 ± 21.20 | 0.67 ± 1.63 | | 19 ± 0.96 | - | 44.33 ± 10.69 | 60.67 ± 10.69 | |
| 18 | Glycopeptide | Teicoplanin (10) | 21 ± 0.82 | S | 24.67 ± 15.57 | 0.00 ± 0.00 | | 25 ± 0.52 | S | 31.40 ± 11.87 | 0.67 ± 1.63 | | 0 ± 0.00 | R | 2.75 ± 2.22 | 4.00 ± 5.66 | |
| 19 | Macrolide | Erythromycin (15) | 30 ± 2.17 | S | 30.00 ± 5.66 | 52.00 ± 9.90 | -0.92 | 0 ± 0.00 | R | 21.00 ± 13.08 | 2.00 ± 4.90 | 0.97 | 11 ± 1.21 | - | 25.50 ± 12.66 | 0.00 ± 0.00 | 0.44 |
| 20 | | Azithromycin (15) | 25 ± 1.21 | S | 43.00 ± 8.49 | 0.00 ± 0.00 | | 0 ± 0.00 | R | 22.50 ± 7.42 | 0.00 ± 0.00 | | 15 ± 0.55 | - | 48.50 ± 3.54 | 45.00 ± 20.66 | |
| 21 | | Clarithromycin (15) | 32 ± 1.03 | S | 31.00 ± 10.44 | 0.00 ± 0.00 | | 11 ± 1.15 | I or R | 28.00 ± 11.96 | 0.00 ± 0.00 | | 10 ± 0.89 | - | 46.67 ± 3.06 | 50.75 ± 18.73 | |
| 22 | Oxazolidinone | Linezolid (30) | 31 ± 0.82 | S | 29.50 ± 19.09 | 4.50 ± 6.36 | NA | 40 ± 0.63 | S | 37.25 ± 3.95 | 0.00 ± 0.00 | NA | 0 ± 0.00 | R | 28.50 ± 6.36 | 43.50 ± 3.54 | NA |
| 23 | Tetracycline | Doxycycline (hydrochloride) (30) | 32 ± 1.63 | S | 43.00 ± 31.19 | 58.00 ± 11.31 | | 40 ± 0.41 | - | 36.50 ± 11.29 | 35.50 ± 17.72 | | 16 ± 4.58 | - | 24.60 ± 7.70 | 92.00 ± 10.15 | |
| 24 | Amphenicol | Chloramphenicol (30) | 28 ± 1.41 | S | 37.50 ± 4.95 | 86.50 ± 2.12 | | 31 ± 1.67 | S | 56.00 ± 16.24 | 28.25 ± 8.42 | | 15 ± 3.33 | - | 53.00 ± 15.64 | 4.00 ± 6.73 | |
| 25 | Aminocoumarin | Novobiocin (5) | 19 ± 1.55 | S | 5.50 ± 0.71 | 0.00 ± 0.00 | | 38 ± 1.17 | S | 23.40 ± 10.95 | 0.00 ± 0.00 | | 11 ± 1.42 | - | 6.50 ± 5.97 | 33.00 ± 6.56 | |
| 26 | Rifamycin | Rifampin (5) | 17 ± 1.33 | S | 34.00 ± 10.44 | 67.50 ± 13.44 | | 41 ± 0.58 | - | 30.50 ± 11.47 | 27.80 ± 12.97 | | 10 ± 0.55 | - | 67.50 ± 7.55 | 159.00 ± 30.25 | |

'-' Interpretation guide for Antimicrobial Susceptibility Test (AST) through DDA not available; NA: Not Applicable; *In vivo* efficacy: Red= No activity, Blue = statistically higher than Infection Control, or at par to the Health Control, Green= Statistically higher than the Health Control, Black= inconclusive; Values shown here are derived from two or more independent assays, and p-value cut-off for statistical significance was kept ≤ 0.05.

Shaded cells represent cases of mismatch between *in vitro* and *in vivo* antibiotic effect

## MAJOR FINDINGS

**Table 2. Comparative list of top effective antibiotics as per DDA or *in vivo* assay**

| *Cl. difficile* | | MRSA | | *P. aeruginosa* | |
|---|---|---|---|---|---|
| DDA[#] | *In vivo* efficacy[*] (24 h end-point) | DDA[#] | *In vivo* efficacy[*] (24 h end-point) | DDA[#] | *In vivo* efficacy[*] (24 h end-point) |
| Imipenem | Ciprofloxacin | Moxifloxacin | Chloramphenicol | Norfloxacin | Imipenem |
| Norfloxacin | Tobramycin | Imipenem | Moxifloxacin | Ciprofloxacin | Rifampin |
| Clarithromycin | Gatifloxacin | Norfloxacin | Ampicillin | Gatifloxacin | Chloramphenicol |
| Gatifloxacin | Ofloxacin, Azithromycin, Doxycycline (hydrochloride), Sparfloxacin, Levofloxacin, Moxifloxacin, Chloramphenicol, Ceftriaxone, Norfloxacin, Rifampin | Rifampin | Ofloxacin, Linezolid, Doxycycline (hydrochloride), Sparfloxacin, Levofloxacin, Gatifloxacin, Norfloxacin, Teicoplanin, Rifampin, Clarithromycin | Imipenem | Azithromycin, Clarithromycin, Ampicillin, Nalidixic acid |
| Levofloxacin | | Doxycycline (hydrochloride) | | Levofloxacin | |

[#]Antibiotics are arranged in decreasing order of diameter of zone of inhibition.

[*]Only those antibiotics are listed, which supported worm activity (in face of bacterial challenge) at par to or higher than the health control (worms not challenged with pathogen). All antibiotics put in a single cell are statistically identical in terms of their efficacy.

- Overall, for none of the three pathogens studied, antibiotic effect observed in DDA was found to have strong correlation with *in vivo* antibiotic efficacy.
- For *P. aeruginosa*, the r- value was -0.21, based on 24 h end-point; and -0.01, based on 48 h end-point.
- For *Cl. difficile*, the r- value based on 24 h end-point and 48 h end-point was 0.29 and 0.28 respectively.
- For MRSA, the r- value based on 24 h end-point and 48 h end-point was 0.31 and 0.49 respectively.
- Against *Cl. difficile*, a strong positive correlation (r=0.93) was found between results of DDA and *in vivo* assay for third-generation cephalosporin.
- In case of *P. aeruginosa*, *Cl. difficile*, and MRSA, mismatch between DDA and *in vivo* assay was found to be there for 4, 11, and 5 antibiotics respectively as per 24 h end-point.
- Most striking mismatch between DDA-antibiogram and *in vivo* antibiotic efficacy was observed in case of aminoglycosides against *Cl. difficile* and *P. aeruginosa*; macrolides against MRSA; oxazolidinone against *Cl. difficile* and *P. aeruginosa*; and nalidixic acid against all the three pathogenic bacteria.

## REFERENCES

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## CONCLUSION

No strong correlation between *in vitro* antibacterial susceptibility testing and *in vivo* efficacy assay was observed. We propose antibiotic susceptibility testing in a *C. elegans* platform worthy of further investigation as a potentially more robust predictor of antibiotic efficacy for therapeutic purpose. Investigations with more pathogens, and more number of antibiotics over wider concentration range are warranted.

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## LIMITATIONS

- All antibiotics were tested at a single concentration (9.6 ppm).
- Difference in composition of Muller-Hinton agar used for DDA, and M9 buffer used for *in vivo* assay might have contributed to relatively poor correlation between results of both assays.