NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
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Evaluation of in vitro synergy testing of South African invasive *Salmonella* Typhi isolates using the Liofilchem® MTS application system

Arvinda Sooka[1], Karen H. Keddy[1, 2]
[1]Centre for Enteric Diseases (CED), National Institute for Communicable Diseases and
[2]Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Background

The recommended treatment in South Africa for invasive *Salmonella* enterica subspecies Typhi (*Salmonella* Typhi) infections is ciprofloxacin, or in those cases alternatively azithromycin or ceftriaxone or cefotaxime as per the national guidelines (Table 1). Combination therapy with an aminoglycoside and a cephalosporin was used before the introduction of the fluoroquinolones.

In South Africa fluoroquinolone resistance in *Salmonella* Typhi was first reported in 2010 (K. Keddy et al. 2010). In 2015, fluoroquinolone resistance in *Salmonella* Paratyphi strains was described by Smith et.al 2015.

Table 1: National typhoid and paratyphoid fever guidelines of South Africa

Severity of disease	Ciprofloxacin susceptibility pattern	Recommended treatment
Acute uncomplicated	Susceptible	Ciprofloxacin (for children and adults**)
	Intermediately resistant/resistant	Azithromycin OR Ceftriaxone OR Cefotaxime
Severe or complicated	Susceptible	Ciprofloxacin (for children and adults**)
	Intermediately resistant/resistant	Ceftriaxone OR Cefotaxime

**Pregnant women should be treated with ceftriaxone or cefotaxime as ciprofloxacin is regarded as a FDA-category C agent and is not advised for pregnant women.

Objective

The objective of this study was to explore a novel method to evaluate combination therapy in vitro to aid therapeutic options for typhoid fever. Synergy testing of current antibiotics for usage against typhoid fever was evaluated by in vitro testing of two antibiotics, by determining the cross gradient with minimum inhibitory concentration (MIC) Test Strips.

Method

Synergy testing of twenty-five clinical invasive *Salmonella* Typhi strains was undertaken using Liofilchem® MIC Test Strips (Liofilchem, Roseto degli Abruzzi, Italy). The selected 25 invasive strains were resistant or intermediately resistant to ciprofloxacin (M100-S25 CLSI guidelines).

Minimum inhibitory concentration (MIC) was initially determined against single antimicrobials listed above, with MIC test strips (Figure 2).

Isolates were sub-cultured onto 5% blood ager plates and incubated overnight at 37°C. A suspension equivalent to McFarland 0.5 standard was used to seed Mueller Hinton agar plates. Two MIC Test Strips was used per agar plate and incubated overnight. The MIC value of each antimicrobial in the combination was read, recorded and calculated.

Synergy testing

Antibiotic combinations included ciprofloxacin against ampicillin, amikacin, azithromycin, chloramphenicol, ceftriaxone and streptomycin.

Figure 3 indicates ciprofloxacin strips were aligned at 90 degrees to the antibiotics listed at the point of the respective MIC for each isolate against each antimicrobial using the MTS platform. A MTS applicator (Figure 1) was used to carefully transfer the two MIC Test Strip onto a swabbed Mueller Hinton agar plate containing test strain and incubated overnight at 37°C.

Synergy interpretation

The fractional inhibitory concentration index (FIC) was calculated for each antibiotic in each combination using the following formula according to the manufactures instructions (Table 2):

MIC_A Minimum inhibitory concentration of antibiotic A
MIC_B Minimum inhibitory concentration of antibiotic B
MIC_{AB} Minimum inhibitory concentration of antibiotic A and B
MICBA Minimum inhibitory concentration of antibiotic B and A

$$\text{FIC index} = MIC_{AB}/MIC_A + MIC_{BA}/MIC_B$$

Table 2: Synergy and FIC value interpretation

Interpretation	FIC
Synergy	≤ 0.5
Additive	>0.5 and ≤ 1.0
Indifference	>1 and ≤ 4.0
Antagonism	> 4.0

A fractional inhibitory concentration index (FIC) was calculated for each antibiotic combination to interpret synergistic, additive, indifference and antagonistic interactions.

Method

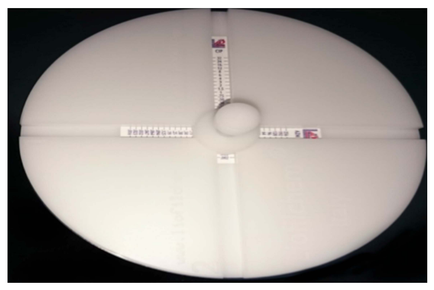

Figure 1: MST platform

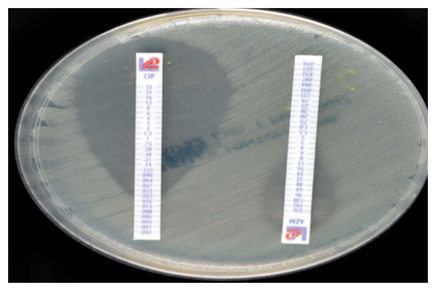

Figure 2: Single MIC Test Strip

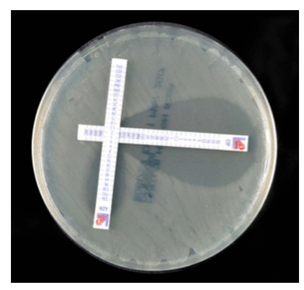

Figure 3: MIC Test Strips aligned at 90 degrees

Results

Of the 25 isolates, six FIC values were obtained for each isolate (150 in total). Overall, synergy was seen in 15.3% (23/150) of combinations, additive inhibitions in 20% (30/150), indifference in 63.4% (95/150) and antagonism 1.3% (2/150) (Figures 4-9).

Ciprofloxacin in combination with streptomycin and ciprofloxacin in combination with ceftriaxone were the most active combinations (Figure 7).

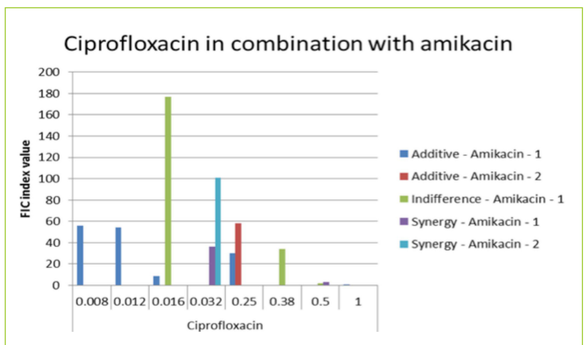

Figure 4: Synergy interpretation of amikacin

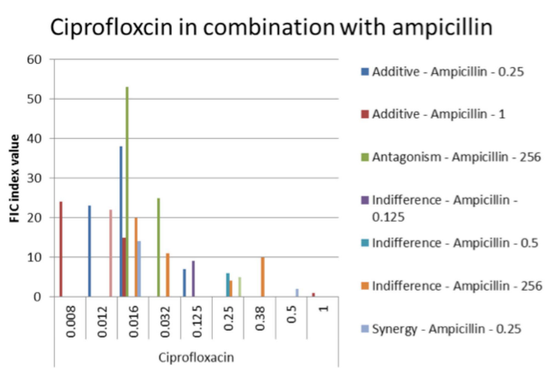

Figure 5: Synergy interpretation of ampicillin

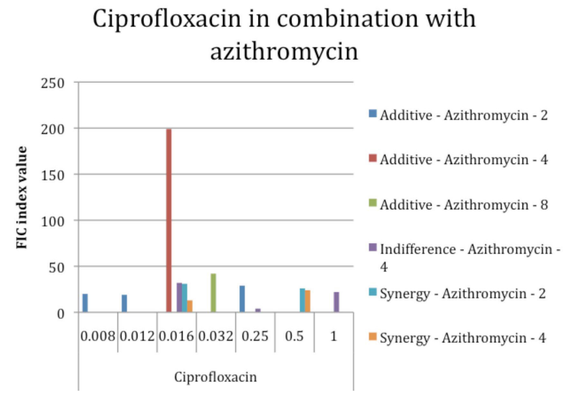

Figure 6: Synergy interpretation of azithromycin

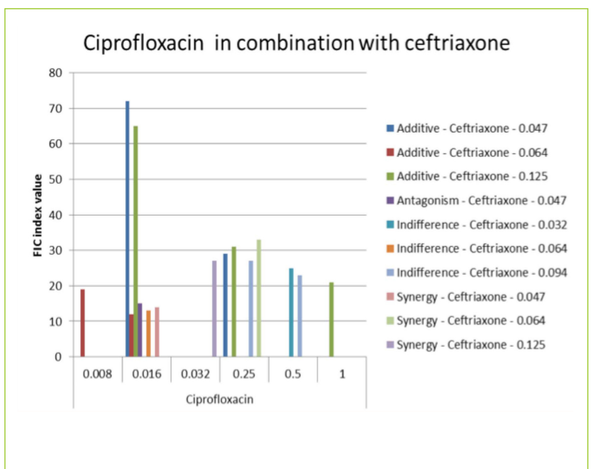

Figure 7: Synergy interpretation of ceftriaxone

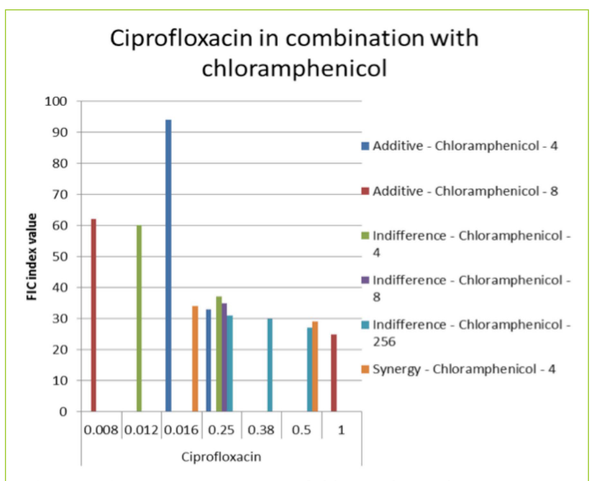

Figure 8: Synergy interpretation of chloramphenicol

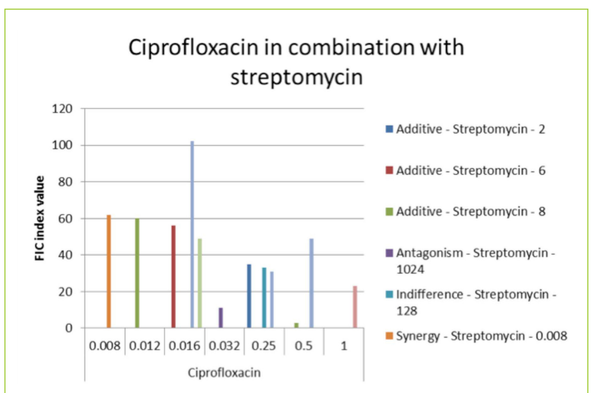

Figure 9: Synergy interpretation of streptomycin

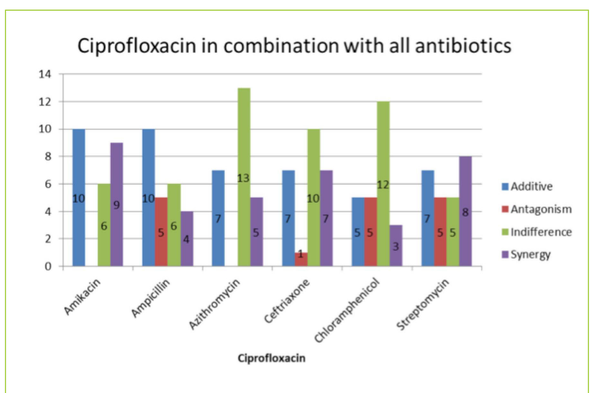

Figure 10: Synergy interpretation of ciprofloxacin in combination with the 6 antibiotics.

Conclusions

The MTS method proved to be useful in obtaining rapid results, simple to use and cost effective. Combination therapy including ciprofloxacin with an aminoglycoside may be an alternative for treatment of *Salmonella* Typhi infections intermediately resistant to one or more of the recommended antimicrobials in South Africa. These in vitro results would need to be confirmed in a clinical setting.

References:

1. Keddy KH, Smith AM, Sooka A, Ismail H, Oliver S. Fluoroquinolone-Resistant Typhoid, South Africa. Emerg Infect Dis. (2010);16(5):879-880
2. Smith A.M, Tau N, Sooka A, Keddy KH. Microbiological characterisation of Salmonella enterica serotype Paratyphi, South Africa, 2003-2014.
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3. Patel JB, Cockerill FR, Bradford PA et al. 2015. Clinical and Laboratory Standards Institute. M100-S25. Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement;35(3):1-236

Acknowledgments

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