EVALUATION OF *IN VITRO* ACTIVITY OF DOUBLE BETA-LACTAM THERAPY AND RELATIONSHIP WITH PENICILLIN BINDING PROTEINS ACTIVITY IN *ESCHERICHIA COLI* ISOLATES

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INTRODUCTION

Penicillin binding proteins (PBPs) are involved in the construction of peptidoglycan, which is the major constituent of bacterial cell walls, and the target of β-lactam antibiotics.[1] There is little published research analysing the relationship between β-lactams with differing bacterial PBP targets, and how they can be manipulated in combinations with respect to clinical or microbiological outcomes (i.e. does expanded PBP activity via a combination lead to better *in-vitro*/*in-vivo* outcomes).

MATERIALS/METHODS

We systematically explored the relationship between double β-lactam therapy (with/without at least one partner being a β-lactamase inhibitor (βLI) antibiotic, e.g. ceftazidime/avibactam) against *Escherichia coli* (*E. coli*) strains of variable resistance *in-vitro* (*Table 1*). This included fully sensitive isolates, extended-spectrum β-lactamase producers (ESBLs) and a carbapenemase producer (CPE).

Table 1: Conceptual matrix of antibiotics (see below for Key) and the associated PBPs covered as monotherapy and combination therapy

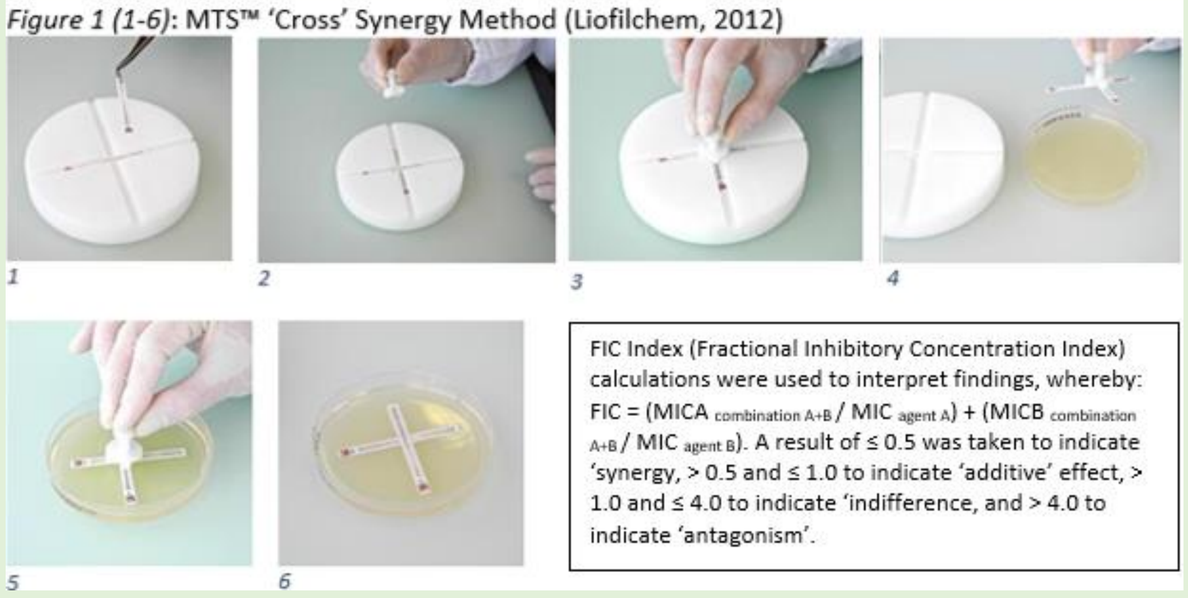
	PBP	1 AX	2. AZ	3. CFD	4. C/A	5. ME	6. P	7. PV	8. TE	9. P/T	10. COA
PBP		4 (7,8)	3	13	123	24	3	2	1a3	3	4
1 AX	4 (7,8)		34(7,8)	134(7,8)	1234(7,8)	24(7,8)	34(7,8)	24(7,8)	1a34(7,8)	34(7,8)	4(7,8)
2. AZ	3	34(7,8)		13	123	234	3	23	1a3	3	34
3. CFD	13	134(7,8)	13		123	1234	13	123	1a3	13	134
4. C/A	123	1234(7,8)	123	123		1234	123	123	123	123	1234
5. ME	24	24(7,8)	234	1234	1234		234	24	1a234	234	24
6. P	3	34(7,8)	3	13	123	234		23	1a3	3	34
7. PV	2	24(7,8)	23	123	123	24	23		1a23	23	24
8. TE	1a3	1a34(7,8)	1a3	1a3	123	1a234	1a3	1a23		1a3	1a34
9. P/T	3	34(7,8)	3	13	123	234	3	23	1a3		34
10. COA	4	4 (7,8)	34	134	1234	24	34	24	1a34	34	

Key:

	Combo = identical	1. Amoxicillin	6. Piperacillin
	Combo = no additonal target	2. Aztreonam	7. (Piv)mecillinam
	Combo = additional targets	3. Ceftazidime	8. Temocillin
	Not for combo	4. Ceftazidime/Avibactam	9. Piperacillin/Tazobactam
		5. Meropenem	10. Co-amoxiclav

For each of 10 antibiotics, the minimum inhibitory concentration (MIC) was determined individually (*Fig. 2*), and subsequently in combination with 9 further antibiotics, using the MTS™ 'cross' synergy method.[2] See *Fig. 1 (1-6)*.

Figure 1 (1-6): MTS™ 'Cross' Synergy Method (Liofilchem, 2012)



FIC Index (Fractional Inhibitory Concentration Index) calculations were used to interpret findings, whereby: FIC = (MICA $_{\text{combination A+B}}$ / MIC $_{\text{agent A}}$) + (MICB $_{\text{combination A+B}}$ / MIC $_{\text{agent B}}$). A result of ≤ 0.5 was taken to indicate 'synergy, > 0.5 and ≤ 1.0 to indicate 'additive' effect, > 1.0 and ≤ 4.0 to indicate 'indifference, and > 4.0 to indicate 'antagonism'.

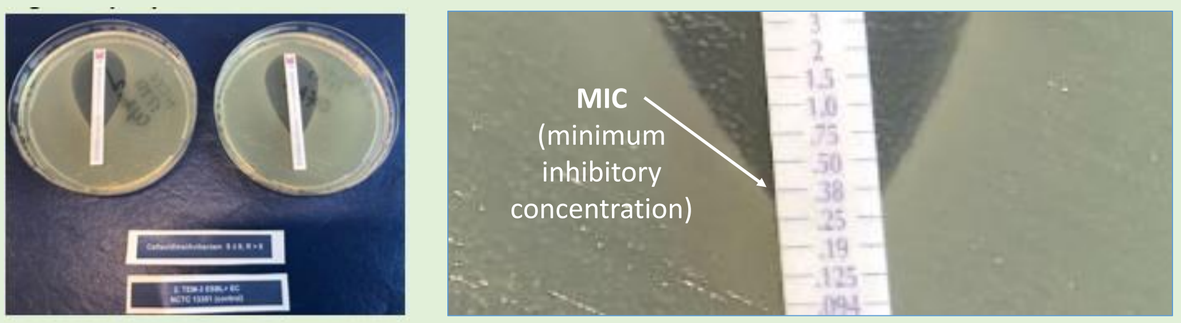


***Figure 2*: MIC of Ceftazidime/Avibactam vs. ESBL+ control strain (2) individually (in duplicate)**

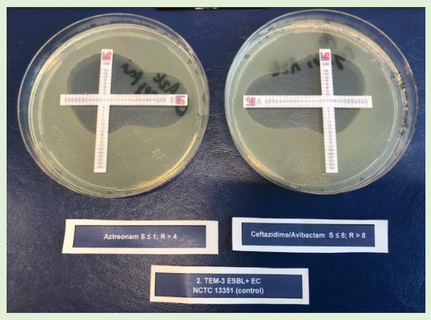
RESULTS

Overall, 86/630 (14%) of all combinations tested showed synergy (*Fig. 3.1*) and 408/630 (65%) were additive (*Fig. 3.2*). 136/630 (21%) combinations showed indifference (*Fig. 3.3*). See also *Fig. 4*. *Tables 2.1-2* show full results for isolates 1 & 2.

Of the 86 'bug-drug' combinations that showed synergy, 42/86 (49%) included Ceftazidime/Avibactam, representing 42/126 (33%) of all Ceftazidime/Avibactam based combinations tested. Synergy was most commonly detected in ESBL producers (58/86; 67% of synergistic combinations) and less frequently in the CPE (2/86; 2% of synergistic combinations) and fully sensitive isolates (8/86; 9% of synergistic combinations).

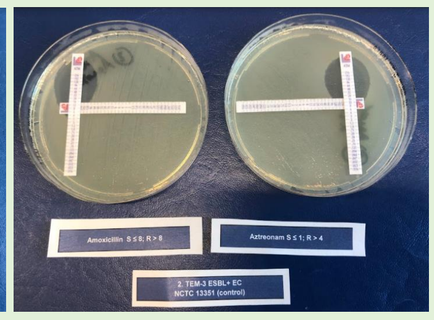
Additive effects were seen in 92/180 (51%) combinations versus ESBLs, compared to 18/90 (20%) in CPEs and 154/180 (86%) in fully sensitive isolates. No antagonism was identified with any antibiotic combination.

***Figure 3 (1-3)*: In-vitro antibiotic activity in combination**



1. SYNERGY: MICs of Aztreonam and Ceftazidime/Avibactam vs. ESBL control strain (2) when in combination (in duplicate)

2. ADDITIVE: MICs of Amoxicillin and Ceftazidime vs. FS control strain (1) when in combination (in duplicate)



3. INDIFFERENCE: MICs of Amoxicillin and Aztreonam vs. ESBL control strain (2) when in combination (in duplicate)

Table 2.1: Final Results Isolate 1 (Fully Sensitive *E. coli* 25922 control)

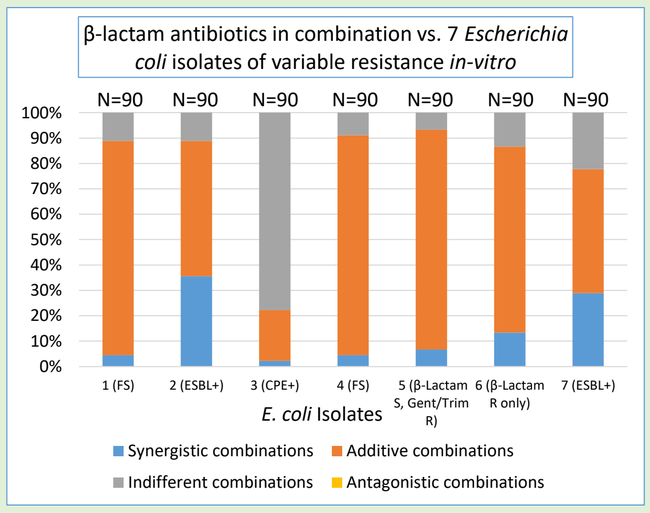
	PBP	1 AX	2 AZ	3 CFD	4 C/A	5 ME	6 P	7 PV	8 TE	9 P/T	10 COA
PBP		4(7,8)	3	13	123	24	3	2	1a3	3	4
1 AX	4(7,8)										
2 AZ	3										
3 CFD	13										
4 C/A	123										
5 ME	24										
6 P	3										
7 PV	2										
8 TE	1a3										
9 P/T	3										
10 COA	4										

Table 2.2: Final Results Isolate 2 (ESBL+ *E. coli* control)

	PBP	1 AX	2 AZ	3 CFD	4 C/A	5 ME	6 P	7 PV	8 TE	9 P/T	10 COA
PBP		4(7,8)	3	13	123	24	3	2	1a3	3	4
1 AX	4(7,8)										
2 AZ	3										
3 CFD	13										
4 C/A	123										
5 ME	24										
6 P	3										
7 PV	2										
8 TE	1a3										
9 P/T	3										
10 COA	4										

Key:

1. Amoxicillin	6. Piperacillin	Synergy
2. Aztreonam	7. Pivmecillinam	Additive
3. Ceftazidime	8. Temocillin	Indifference
4. Ceftazidime/Avibactam	9. Piperacillin/Tazobactam	Not tested
5. Meropenem	10. Co-amoxiclav	PBP = Penicillin binding protein targeted



Each isolate was exposed to 45 antibiotic combinations (n=90 in duplicate; n=630 across all 7 isolates). Of these combinations, 56% (25/45), 53% (24/45), 87% (39/45) and 53% (24/45) covered PBPs 1, 2, 3 and 4, respectively. 60% (27/45) provided expansive cover with 53% (24/45) including at least one βLI.

Of 86 combinations that gave rise to synergy, 60%, 65%, 93% and 56% targeted PBP1 to 4, respectively, with 58% providing expanded PBP activity and 88% a βLI. The presence of a βLI was statistically significantly more common in synergistic versus non-synergistic combinations (Chi-squared =11.6; p=0.0006 [336/544 vs. 76/86]). While PBP2 appeared to be more common in synergistic combinations, this was not statistically significant (Chi-squared = 2.8; p = 0.095 [280/544 versus 56/86]).

CONCLUSION

In the combinations tested, synergy or additive effects were common (78%); similar to our previous work with Fosfomycin/β-Lactam combinations (89%), but higher than with Fosfomycin/Non-β-Lactam combinations (28%).[3] The presence of PBP target expansion was similar in synergistic versus additive versus indifferent combinations (58%, 56% and 63%, respectively). Synergy was more common in ESBL-producing *E. coli* versus fully sensitive and CPE isolates.

Most of the synergistic 'bug-drug' combinations identified contained a βLI. This provisionally suggests βLI may play a key role in synergy. Confirmation using an alternative method and mechanistic elucidation is required. The clinical and microbiological importance of such effects remains unclear.

REFERENCES

1. Hellberg, M. (2007): Dimerization of the penicillin-binding proteins in *Escherichia coli*, *Examensarbete,* p20.
2. Liofilchem®, MTS™ Synergy Applicator System, (US patent US9365886B2): A device for standardising the *in-vitro* synergy testing of two antibiotics through the method of crossing the gradient strips. (Liofilchem, 2012). https://www.liofilchem.com/en/mts-en.html, accessed March 2022).
3. Suich J, Mawer D, Woude MVD, Wearmouth D, Eyre A, Burns P, Smeets T, Barlow G (2020): *In vitro* activity of fosfomycin, and synergy in combination, against Gram negative bloodstream infection isolates in a UK teaching hospital, *Access Microbiology, Microbiology Society*, DOI:10.1099/acmi.fis2019.po0187.

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