

Purification, characterization and *in vitro* evaluation of polymyxin A from *Paenibacillus dendritiformis*: An underexplored member of the polymyxin family

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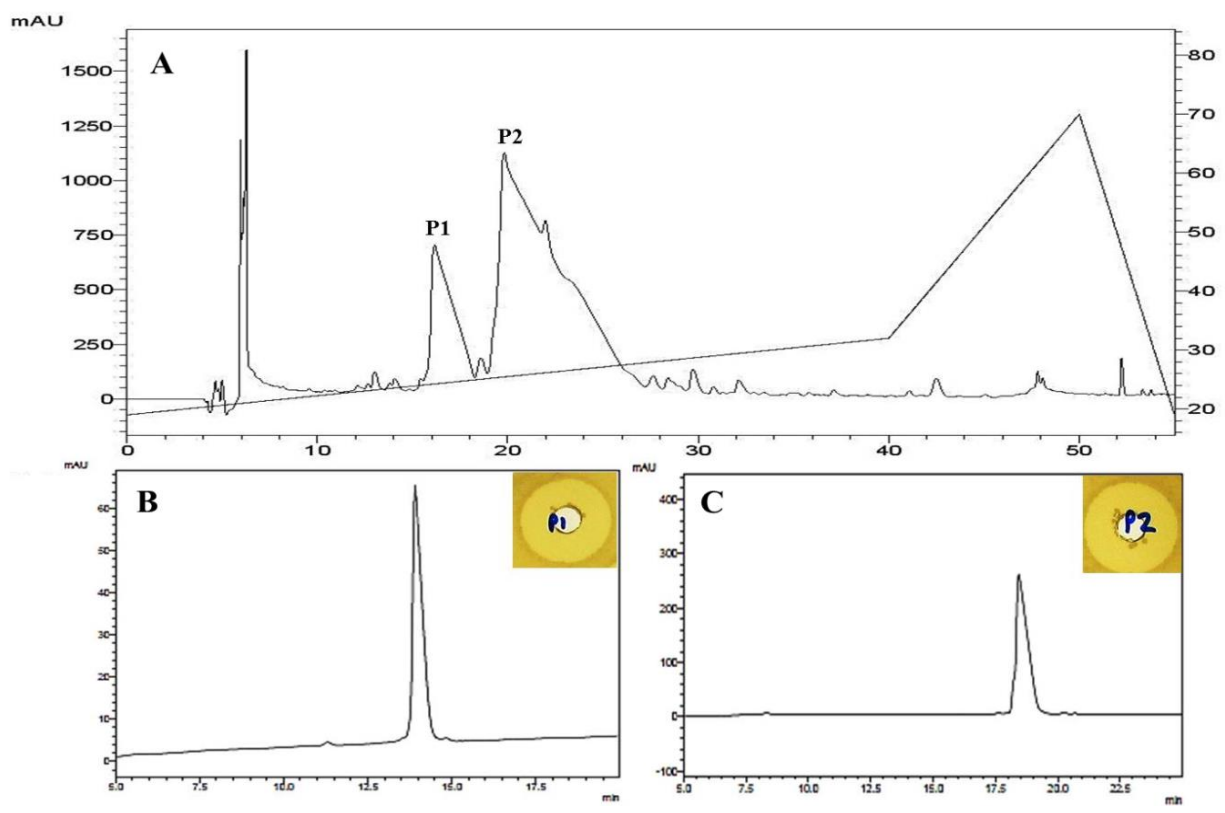
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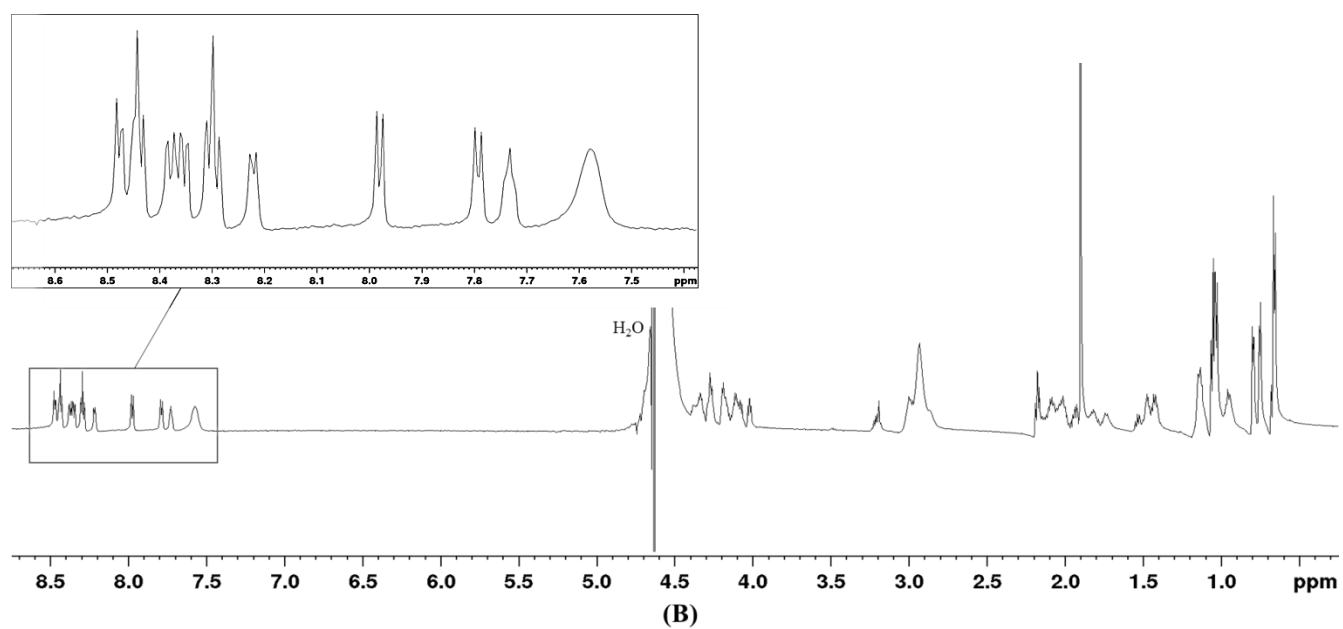
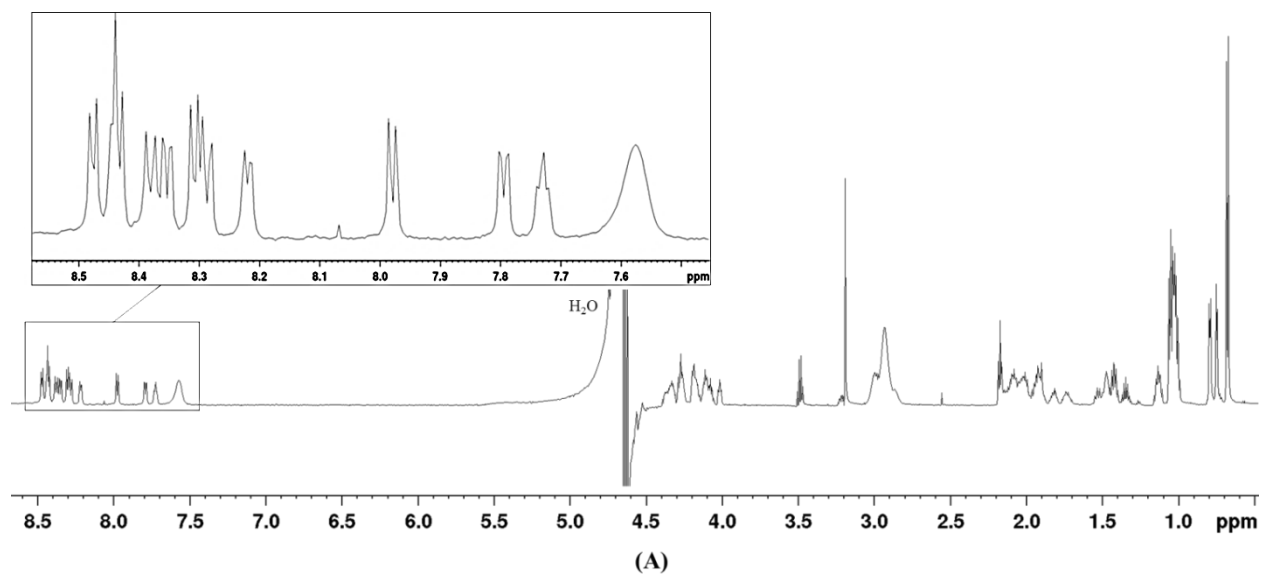
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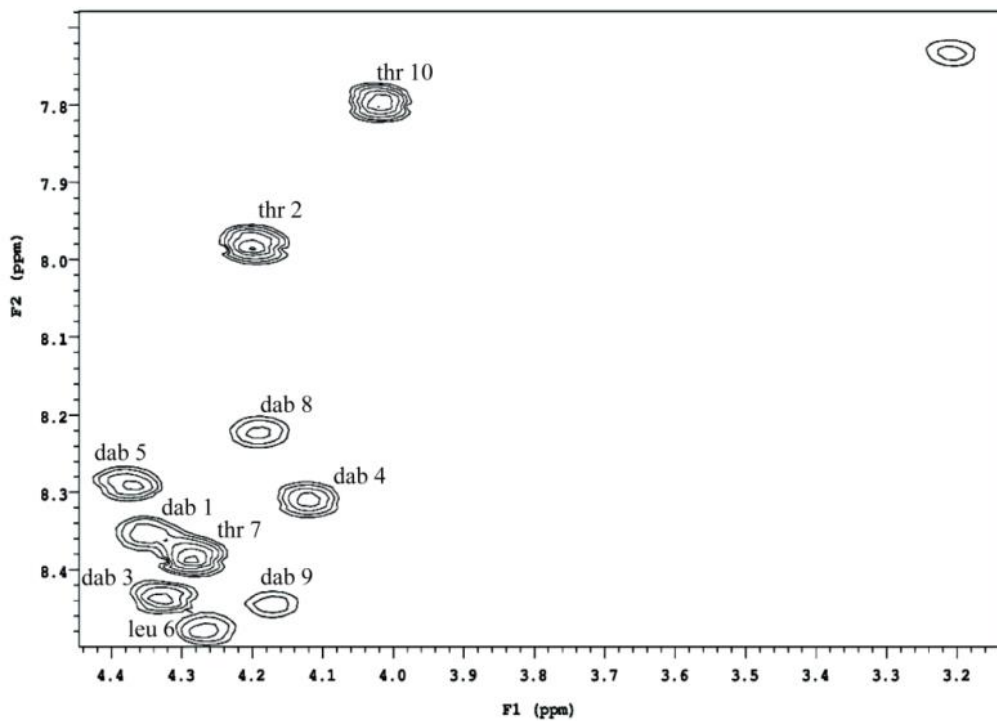
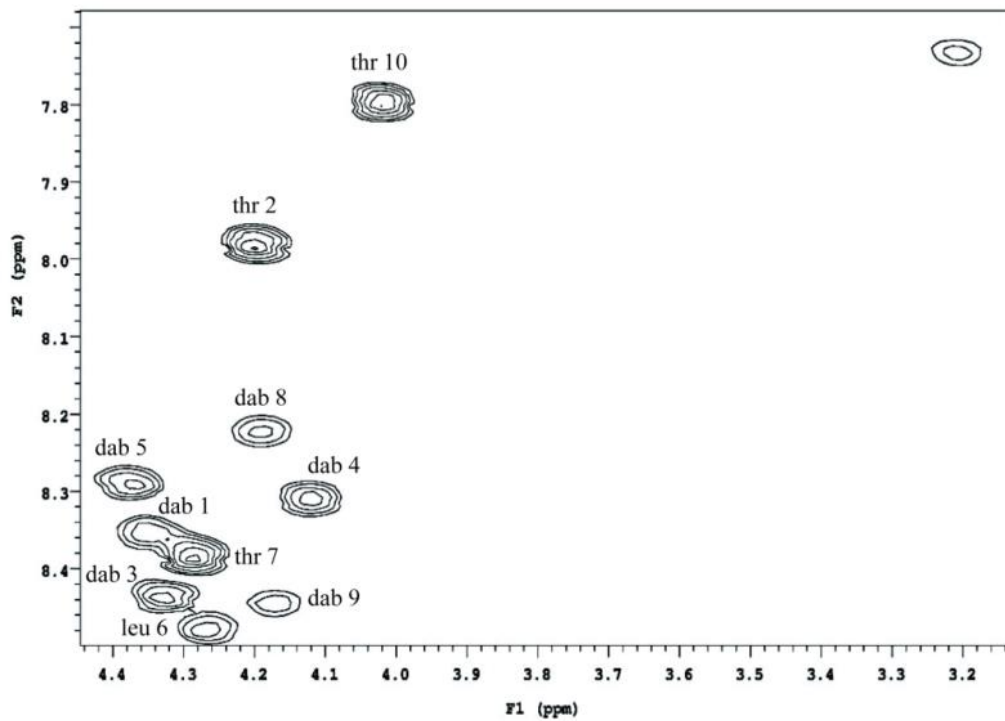
Supplementary Figure S1 (A) HPLC profile of cation dialysate. (B) Peak 1 and (C) Peak 2 purified. Insets show the antimicrobial activity of both compounds



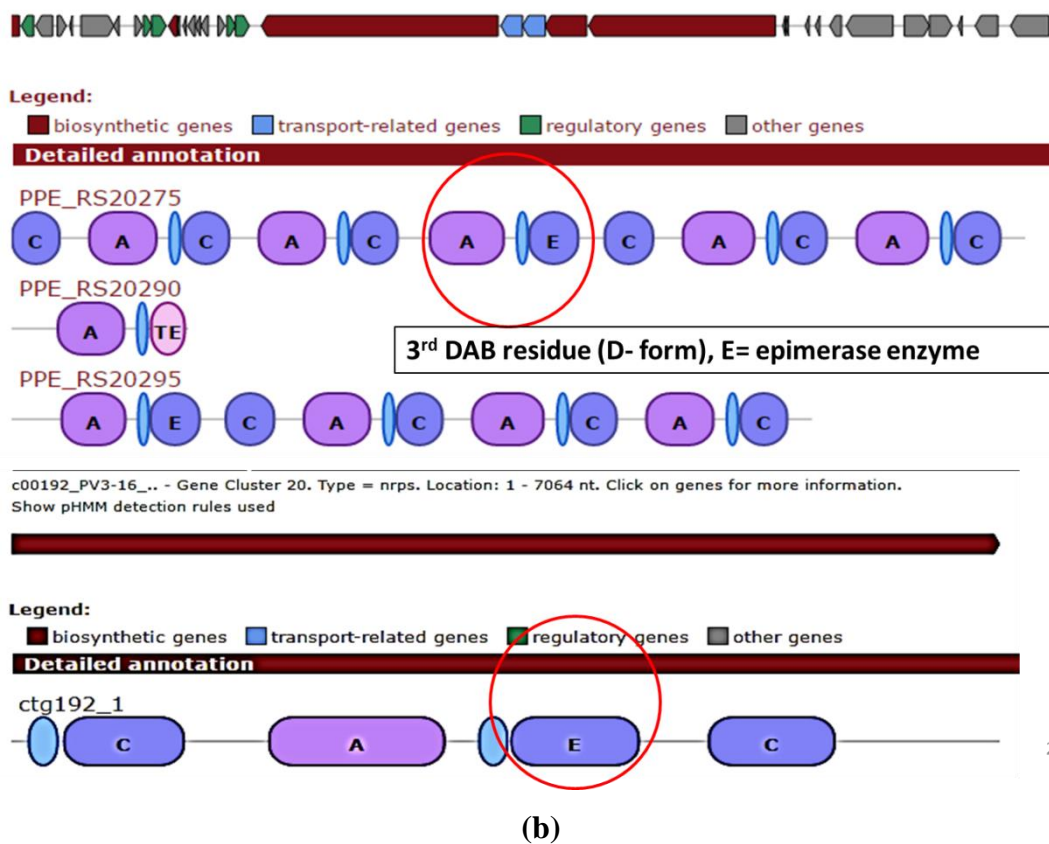
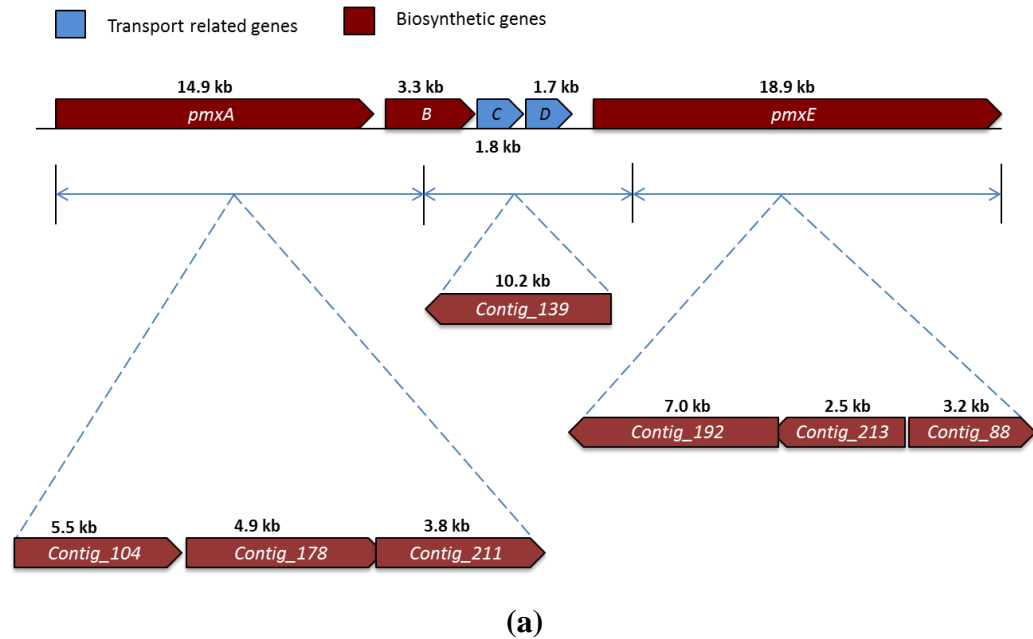
Supplementary Figure S2 Appearance of colony of PV3-16 strain in tip-splitting pattern on 1.5% agar medium containing 0.2% peptone. 5 μ l log-phase culture was spotted and incubated at 37 °C. The plate shown here is after 3-days incubation and then stained with crystal violet dye to visualize the patterns



Supplementary Figure S3 One dimensional ¹H NMR spectra of peak1 (A) and peak 2 (B) with the expansion of amide region



Supplementary Figure S4 HH-COSY showing the α -H/amide-H correlations. Upper panel (P 1) and lower panel (P 2)



Supplementary Figure S5 Biosynthetic gene cluster for polymyxin A. (a) Upper panel shows the polymyxin gene cluster reported in the literature by Choi et al. Gene cluster in PV3-16 strain is dispersed in seven contigs. (b) Detailed annotation of PmxE gene showing gene for epimerase enzyme responsible for incorporation of D-Dab at third position. 4

Supplementary Table-S1 Antibiotic-susceptibility pattern of clinical isolates

Clinical isolate	CAZ 30	TE 30	AK 30	PI 100	S 10	FO 200	NIT 300	CTX 30	CIP 5	NX 10	CXM 30	TR 5	CFM 5	AMC 30	PIT 100/10	A/S 10/10
3185	0 R	10 R	21 S	0 R	20 S	34 S	22 S	0 R	0 R	10 R	0 R	0 R	0 R	10 R	18 I	12 I
7932	13 R	9 R	20 S	9 R	22 S	31 S	19 S	10 R	8 R	9 R	0 R	0 R	0 R	10 R	26 S	14 I
14504	17 R	0 R	20 S	13 R	11 R	31 S	20 S	9 R	0 R	11 R	0 R	0 R	0 R	12 R	27 S	14 I
14363	0 R	10 R	27 S	7 R	15 S	34 S	23 S	0 R	13 R	11 R	0 R	0 R	0 R	10 R	24 S	13 I
9062	9 R	15 R	19 S	0 R	18 S	34 S	23 S	0 R	0 R	9 R	0 R	0 R	0 R	0 R	20 I	11 R
7210	16 R	0 R	21 S	12 R	18 R	36 S	22 S	11 R	0 R	5 R	0 R	0 R	0 R	12 R	27 S	16 S
7534	16 R	16 R	21 S	16 R	11 R	31 S	16 I	15 R	0 R	12 R	0 R	0 R	0 R	12 R	25 S	17 S
13425	0 R	15 R	27 S	8 R	0 R	37 S	23 S	10 R	13 R	11 R	0 R	31 S	21 S	11 R	23 S	13 I
14084	13 R	13 R	26 S	11 R	22 R	40 S	28 S	15 R	12 R	10 R	0 R	0 R	0 R	17 I	31 S	17 S
GMCH16	0 R	11 R	0 R	0 R	22 S	23 S	8 R	8 R	10 R	0 R	0 R	0 R	0 R	0 R	12 R	0 R
GMCH10	0 R	11 R	9 R	0 R	0 R	18 S	10 R	0 R	14 R	17 S	0 R	0 R	0 R	0 R	13 R	0 R
1573	0 R	22 S	0 R	0 R	19 S	24 S	11 R	0 R	9 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R
GMCH13	13 R	10 R	22 S	9 R	9 R	10 R	11 R	13 R	10 R	10 R	0 R	0 R	0 R	9 R	17 R	13 I
GMCH12	0 R	0 R	0 R	0 R	22 S	23 S	9 R	0 R	11 R	12 R	0 R	0 R	0 R	0 R	0 R	0 R
1428	0 R	24 S	0 R	9 R	0 R	26 S	14 R	0 R	12 R	13 I	0 R	0 R	0 R	0 R	13 R	0 R
GMCH04	0 R	17 S	0 R	0 R	12 I	16 S	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R
GMCH11	0 R	22 S	0 R	0 R	12 I	18 S	11 R	0 R	0 R	0 R	0 R	28 S	0 R	0 R	10 R	0 R
827	0 R	19 S	0 R	0 R	20 S	22 S	0 R	0 R	15 R	13 I	0 R	27 S	0 R	0 R	0 R	0 R
B8	0 R	25 S	0 R	0 R	12 I	23 S	13 R	0 R	11 R	12 R	0 R	0 R	0 R	0 R	0 R	0 R
GMCH18	0 R	36 S	13 R	0 R	0 R	24 S	0 R	0 R	0 R	12 R	0 R	18 S	0 R	10 R	13 R	18 S
GMCH05	9 R	0 R	13 R	0 R	0 R	19 S	0 R	0 R	14 R	17 S	0 R	0 R	0 R	0 R	12 R	15 S
GMCH 14	9 R	0 R	12 R	0 R	0 R	25 S	0 R	0 R	15 R	21 S	0 R	0 R	0 R	14 I	17 R	22 S
GMCH06	0 R	11 R	0 R	10 R	26 S	29 S	0 R	16 R	0 R	0 R	0 R	0 R	0 R	10 R	17 R	0 R

Clinical isolate	CAZ 30	TE 30	AK 30	PI 100	S 10	FO 200	NIT 300	CTX 30	CIP 5	NX 10	CXM 30	TR 5	CFM 5	AMC 30	PIT 100/10	A/S 10/10
PA1	0 R	ND	10 R	16 R	ND	ND	0 R	11 R	0 R	11 R	0 R	ND	ND	ND	17 R	ND
PA2	0 R	ND	10 S	16 R	ND	ND	0 R	22 S	0 R	10 R	0 R	ND	ND	ND	17 R	ND
PA3	31 S	ND	22 S	32 S	ND	ND	10 R	29 S	19 R	28 S	21 R	ND	ND	ND	36 S	ND
PA4	31 S	ND	13 R	28 S	ND	ND	0 R	26 S	11 R	0 R	20 S	ND	ND	ND	27 S	ND
PA5	0 R	ND	0 R	16 R	ND	ND	0 R	12 R	10 R	10 R	0 R	ND	ND	ND	17 R	ND
PA6	32 S	ND	33 S	30 S	ND	ND	0 R	30 S	20 S	32 S	24 S	ND	ND	ND	31 S	ND

The results were observed as zone of inhibition around the disc as denoted by the value in the boxes. R= Resistant; S= Sensitive; I= Intermediate; ND=not determined

CAZ 30 µg	Ceftazidime
TE 30 µg	Tetracycline
AK 30 µg	Amikacin
PI 100 µg	Piperacillin
S 10 µg	Streptomycin
FO 200 µg	Fosfomycin
NIT 300 µg	Nitrofurantoin
CTX 30 µg	Cefotaxime
CIP 5 µg	Ciprofloxacin
NX 10 µg	Norfloxacin
CXM 30 µg	Cefuroxime
TR 5 µg	Trimethoprim
CFM 5 µg	Cefixime
AMC 30 µg	Amoxyclav
PIT 100/10 µg	Piperacillin/Tazobactam
A/S 10/10 µg	Ampicillin/Sulbactam