

Use of NXL104, a β -lactamase Inhibitor, to Detect *Klebsiella pneumoniae* Carbapenemase (KPC) in *Enterobacteriaceae*

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ABSTRACT

Background: NXL104 is a non- β -lactam covalent inhibitor of a broad spectrum of serine β -lactamases of classes A and C, including ESBLs (Extended Spectrum β -Lactamases) and KPC carbapenemases. The KPCs are of greater concern, conferring resistance to all β -lactams including the currently marketed inhibitors and the carbapenems. KPC enzymes have been reported in a variety of *Enterobacteriaceae* but remain difficult for clinical microbiology laboratories to detect. Ceftazidime (CAZ) + NXL104 disks are being developed to support the clinical development of CAZ/NXL104. We have invented a novel use for NXL104 disks as a diagnostic reagent for the detection of KPC β -lactamases expressed by strains of *Enterobacteriaceae*.

Methods: Bacterial isolates: Six clinical isolates known to harbour KPC-2 or KPC-3 (2 *K. pneumoniae*, 2 *Escherichia coli* and 2 *Enterobacter cloacae*) and five without KPCs but with ESBLs, including *K. pneumoniae* ATCC 700603 (SHV-18) were tested. MICs were determined as per CLSI microbroth dilution technique with CAZ and imipenem (IPM) alone or combined with NXL104 at a fixed concentration of 4 μ g/mL. Double disk synergy test for detection of KPC: Mueller-Hinton agar plates were inoculated with a lawn of $\sim 10^8$ cfu/mL adjusted test strains. A 10 μ g IPM disk was placed at a distance of ~ 20 -22 mm apart from a disk of 30 μ g CAZ+ 60 μ g NXL104. Plates were inverted and incubated at 37°C overnight.

Results: Synergy, indicating the presence of carbapenemase, was seen as an expansion of the IPM zone adjacent to the CAZ/NXL104 disk only for strains known to carry a KPC enzyme. These results correlated with the decrease in IPM MICs in the presence of NXL104 (≤ 0.125 – 2 μ g/mL) compared to its absence (8 – 64 μ g/mL) for the same strains.

Conclusion: These results demonstrate CAZ/NXL104 double disk synergy test as a potentially useful method for detecting KPCs in *Enterobacteriaceae* species in clinical microbiology laboratories.

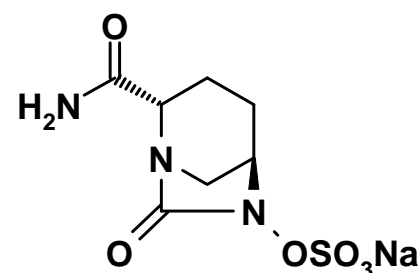
BACKGROUND

NXL104 is a novel non β -lactam β -lactamase inhibitor that has been shown *in vitro* and *in vivo* to inhibit both class A and class C β -lactamases, including ESBLs and KPC carbapenemases (1, 2, 3, 4). Amongst class A enzymes, KPCs represent a new family having potential for wide dissemination and its production confers resistance to all β -lactams including carbapenems. Several outbreaks of KPC-producing bacteria have occurred and identification of these clinical isolates will be critical for limiting the spread of this resistance mechanism. NXL104 in combination with ceftazidime is currently under clinical development and NXL104+CAZ disks have been developed to support the clinical microbiology program. We have invented a novel use of NXL104 disks for detecting KPC β -lactamases. The aim of the present study is to demonstrate the use of NXL104 disks as a diagnostic reagent for the detection of KPC β -lactamases expressed by strains of *Enterobacteriaceae*.

References

1. Livermore, D *et al.* J Antimicrob Chemother (2008) in press.
2. Stachyra, T *et al.* 47th ICAAC, Chicago, USA (2007), Poster # F1-320.
3. Miossec, C *et al.* 47th ICAAC, Chicago, USA (2007), Poster # F1-318.
4. Levasseur, P *et al.* 45th ICAAC, Washington, USA (2005), Communication, # F-1164.

STRUCTURE OF NXL104



NXL104
(trans-7-oxo-6-(sulfooxy)-1,6-diazabicyclo[3.2.1]octan-2-carboxamide sodium salt)

METHODS

Disks:

Commercially available ceftazidime (CAZ 30 μ g) and imipenem (IPM 10 μ g) disks were obtained from Biorad (France). Ceftazidime/NXL104 disks were manufactured by MAST Diagnostics (UK). The charges on the disk were 30 μ gCAZ + 60 μ gNXL104 (CAZ/104).

Antibiotics and β -lactamase inhibitor:

Ceftazidime pentahydrate (Sandoz), Imipenem (USP), β -lactamase inhibitor NXL104 (Novexel).

Bacterial isolates:

Six clinical isolates known to harbour KPC-2 or KPC-3 (2 *Klebsiella pneumoniae*, 2 *Escherichia coli* and 2 *Enterobacter cloacae*) and five without KPCs but with ESBLs, including *K. pneumoniae* ATCC 700603 (SHV-18) were tested for the detection of the carbapenemase enzyme. Table 1 shows the phenotypes of the strains used in this study.

MIC determination:

MICs for CAZ, IPM, CAZ+NXL104, and IPM+NXL104 were determined using CLSI methods for antibiotic susceptibility testing with cation adjusted Mueller-Hinton (MH) broth. NXL104 was used at a constant concentration of 4 μ g/mL. MIC was defined as the lowest concentration that inhibited all visual growth. MICs of the CAZ+NXL104 and IPM+NXL104 combinations are expressed in terms of either CAZ or IPM concentrations.

Double disk synergy test for Detection of Carbapenemase:

Mueller-Hinton agar plates (150mm plastic Petri dishes) were inoculated with a lawn of 0.5 McFarland ($\sim 10^8$ cfu/mL) adjusted test strains. A 10 μ g IPM disk was placed at a distance of 20-22mm apart from a disk of 30 μ gCAZ + 60 μ g NXL104 (CAZ/NXL). Plates were inverted and incubated at 37°C overnight.

Interpretation of the synergy zone:

Synergy is seen as an expansion of the imipenem zone adjacent to the NXL104 containing disk. The following figures 1 – 4 demonstrate the absence or presence of the KPC carbapenemase in the above-mentioned strains.

RESULTS AND DISCUSSION

Table 1. MICs (μ g/mL) of CAZ and IMP alone and in combination with 4 μ g/mL of NXL104

ID	Organism	Mechanism	CAZ		IMP	
			alone	+NXL104	alone	+NXL104
2138	<i>E.coli</i>	KPC-2, TEM-1	128	0.5	16	0.25
J62	<i>E.coli</i>	KPC-3	64	2	8	≤ 0.125
7506	<i>E. cloacae</i>	KPC-2,TEM-1,KLUC-2	>128	4	128	0.25
MAC	<i>E. cloacae</i>	KPC-3, TEM-1, OXA-9	>128	4	64	2
VAKP	<i>K. pneumoniae</i>	KPC-2	128	1	32	2
YC	<i>K. pneumoniae</i>	KPC-2	>128	1	64	≤ 0.125
ATCC700603	<i>K. pneumoniae</i>	SHV-18	64	2	0.5	0.5
TN13	<i>E.coli</i>	CTX-M-14, CMY-2, TEM-1	128	0.5	0.5	0.125
TN58467	<i>K. pneumoniae</i>	DHA-1, SHV-2a, TEM-1	>128	1	0.5	0.125
Tunisie clone K4	<i>K. pneumoniae</i>	CTX-M-15, TEM-1, OXA-1	>128	1	0.25	0.25
Tunisie clone K1	<i>K. pneumoniae</i>	CTX-M-16, OXA-1	>128	2	1	1
KOL	<i>K. pneumoniae</i>	MOX-2, SHV-5, TEM-1	128	1	0.25	≤ 0.125

Double Disk Synergy Test for Detection of KPC β -lactamases

Figure 1. KPC negative strain

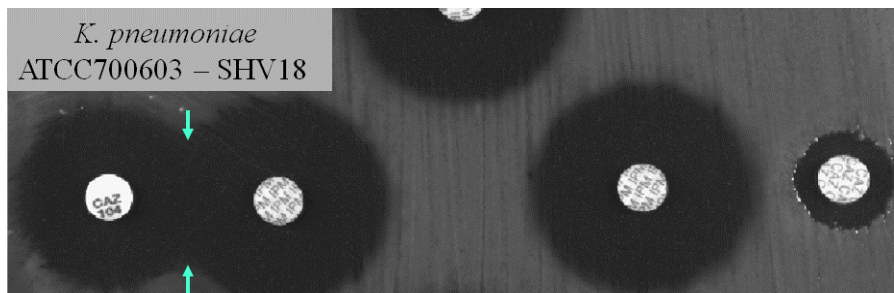


Figure 3. KPC 2 - producing strain

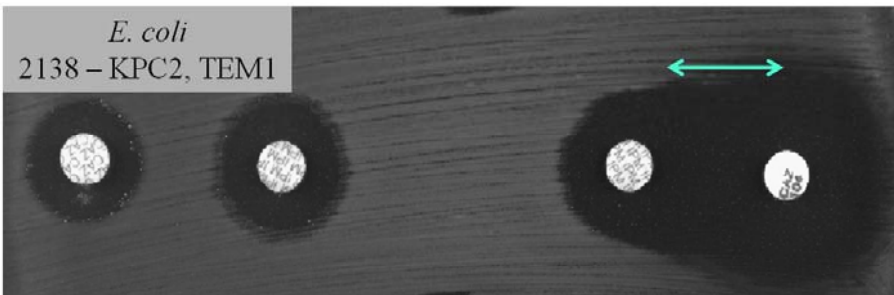


Figure 2. KPC 2 - producing strain

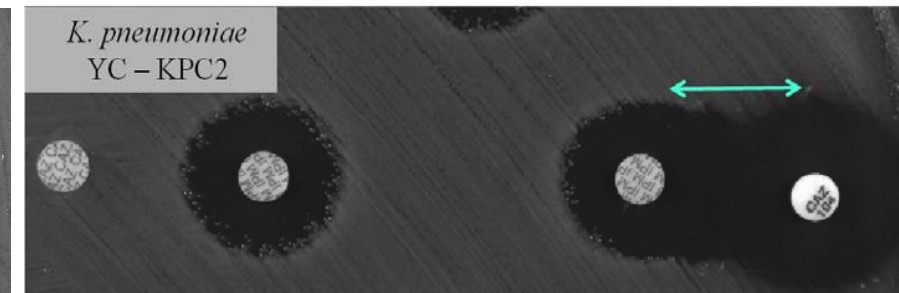
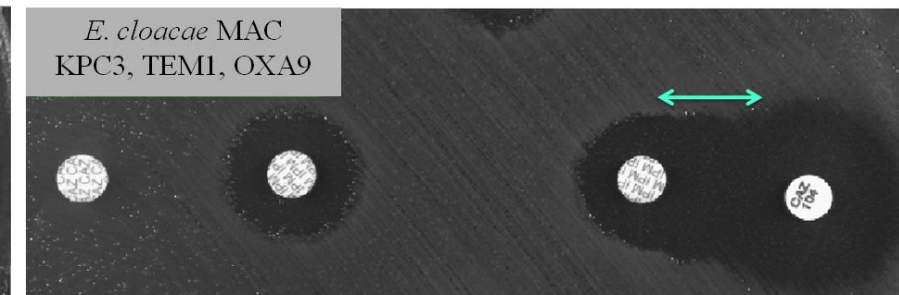


Figure 4. KPC 3 - producing strain



Figures 2 – 4: Presence of KPC enzyme shown by horizontal green arrows as expansion of the synergistic zone

CONCLUSION

❖ Synergy, indicating the presence of carbapenemase, was seen as an expansion of the imipenem zone adjacent to the CAZ/NXL104 disk only for strains known to carry this enzyme. Therefore, the CAZ/NXL104 disks could be a useful tool for detection of KPC producing strains in the clinical microbiology laboratories.

❖ The above results are in accordance with the decrease in imipenem MIC in the presence of NXL104 compared to its absence.