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BACKGROUND

- CTX-M enzymes are now the predominant ESBLs, except in the US (1).
- Their spread accounts for most of the rise in oxyimino-cephalosporin resistant *E. coli* in Europe since 2001 (figure 1) (1).
- Many isolates with CTX-M ESBLs are multi-resistant, and treatment of severe infections largely depends on carbapenems, **BUT**:
 - Carbapenem resistance arises if an ESBL producer loses porins (2);
 - This mode of resistance particularly affects ertapenem and may be selected during therapy (3).
 - There is more general concern about carbapenem over-use and carbapenemase selection.
- CTX-M enzymes are inhibited by clavulanate and penicillanic acid sulfones, but many producers are resistant to penicillin + β -lactamase inhibitor combinations.
 - This is partly because *bla*_{CTX-M-15} –the commonest CTX-type- is often co-produced with OXA-1, an inhibitor-resistant penicillinase (4).
- Combinations of a β -lactamase inhibitor with an oxyimino-cephalosporin might be an alternative to carbapenems in infections due to ESBL producers, even when these co-produce OXA-1 or other inhibitor resistant penicillinases.
- We therefore evaluated NXL104 (figure 2) with ceftazidime and cefotaxime vs. reference and clinical strains with CTX-M enzymes.

Figure 1: Rising resistance to oxyimino-cephalosporins among *E. coli* in Europe, 2001 and 2006 compared (from <http://www.earss.rivm.nl>)

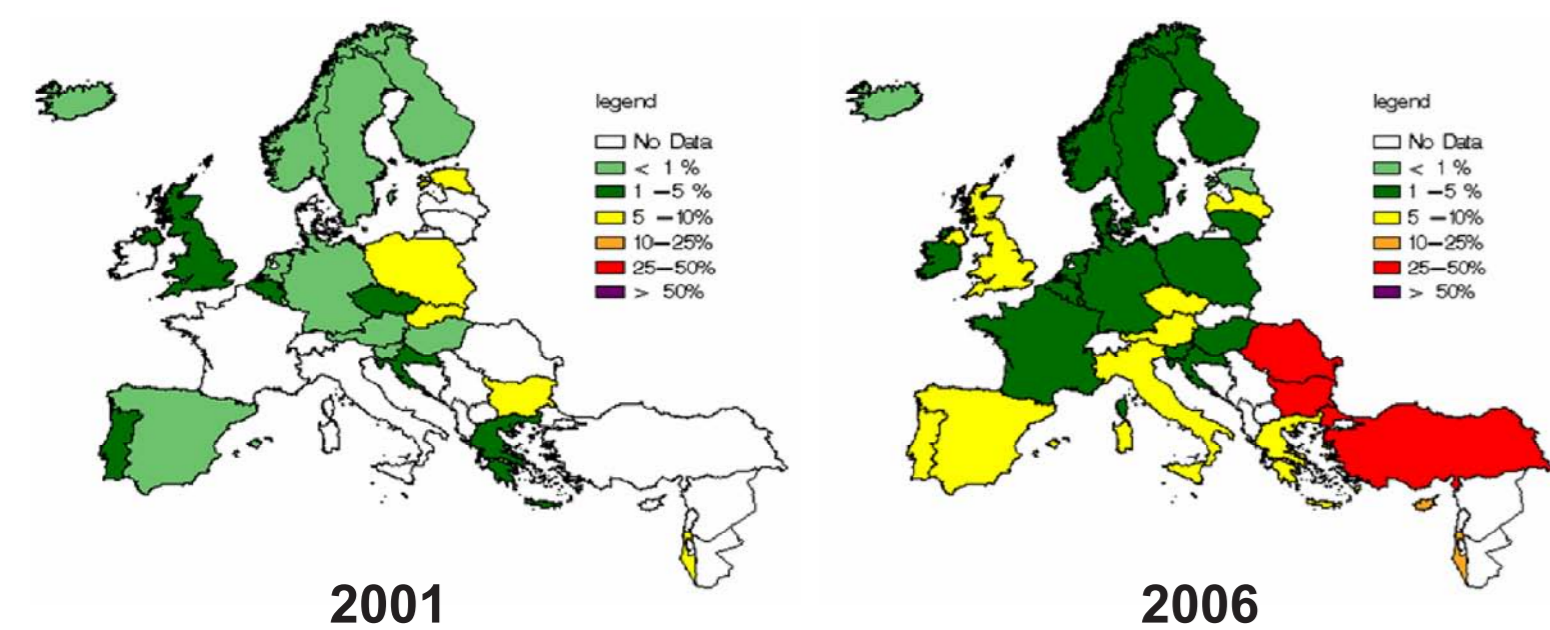
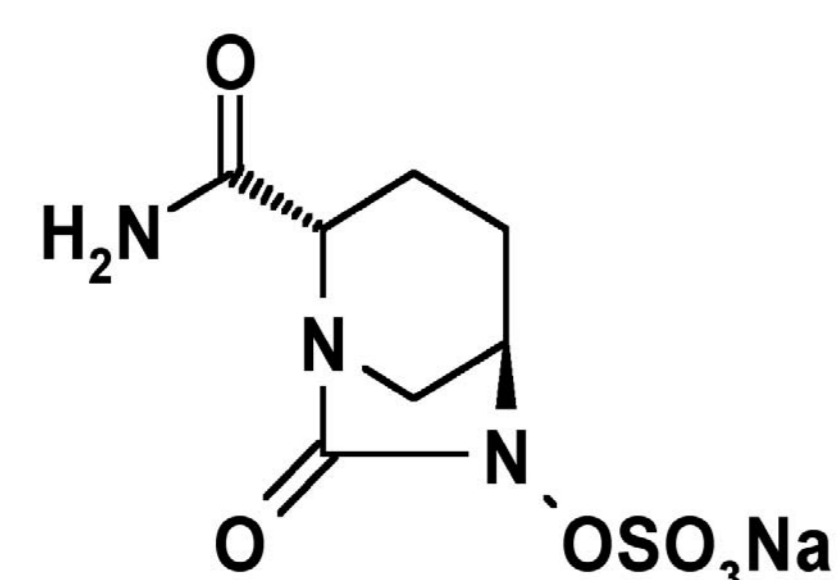


Figure 2. Structure of NXL104



ORGANISMS & METHODS

The organisms tested comprised:

- *E. coli* DH5 α transformants with sequenced CTX-M enzymes (n=3).
- Clinical *Enterobacteriaceae* with CTX-M enzymes, typed by sequencing or group-specific PCR (n = 70). These were recent reference or survey (5) submissions.
 - 15 had CTX-M-15 enzyme accompanied by OXA-1.
- Clinical *Enterobacteriaceae* resistant to ertapenem (MICs >2 mg/L) owing to combinations of CTX-M, or other ESBLs or AmpC, together with porin loss (n= 17). All were recent reference submissions (2).
 - MICs were determined by CLSI agar dilution.
 - NXL104 was used at 4 mg/L.

RESULTS

Enterobacteriaceae transformants – (Table 1)

- MICs of cefotaxime for the transformants were 8-32 mg/L and were reduced >512-fold by NXL104 4 mg/L; MICs of ceftazidime were 2 mg/L and were reduced 16-32 –fold.

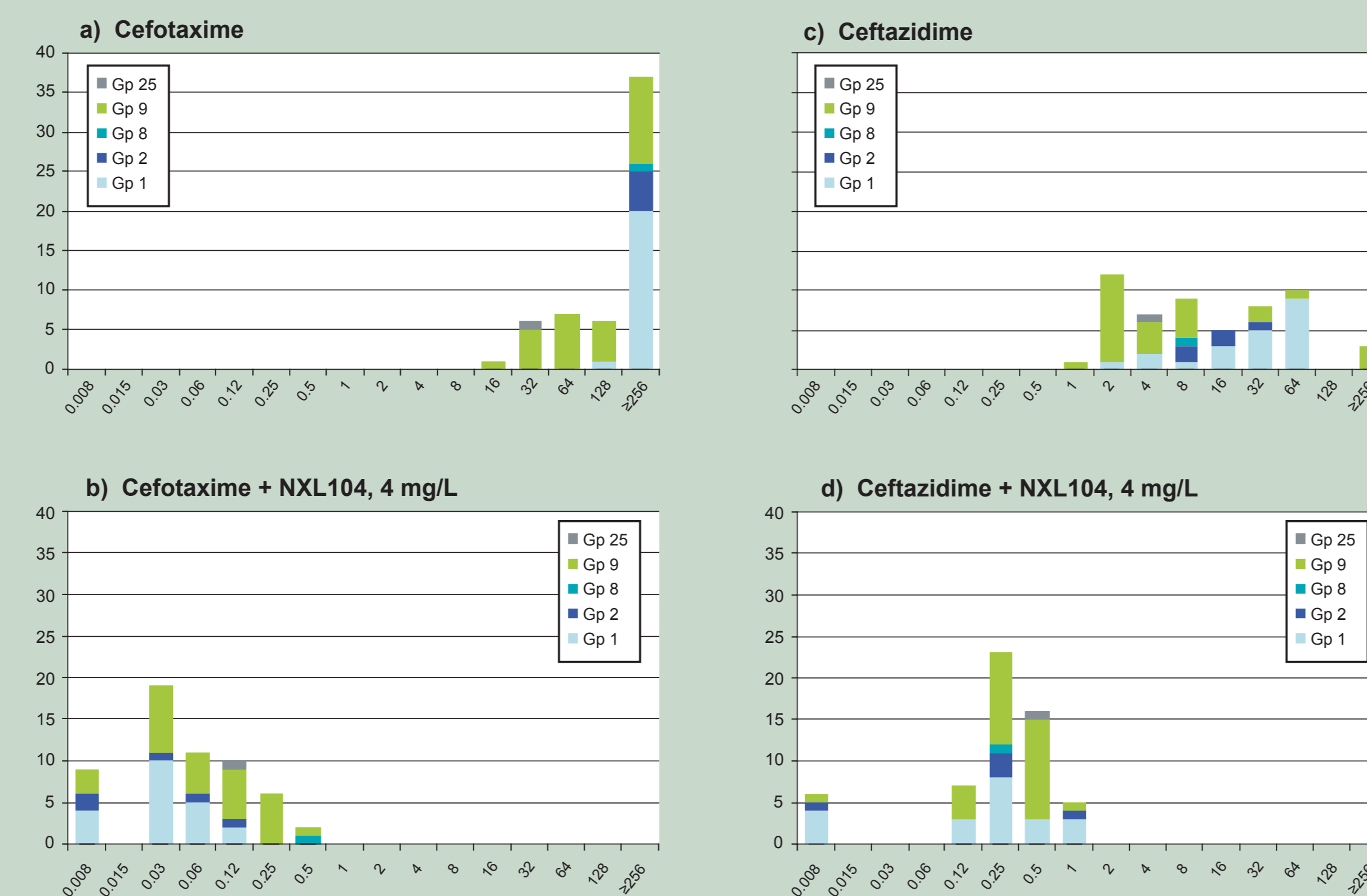
Table 1. MICs (mg/L) for *E. coli* DH5 α transformants with CTX-M enzymes

	Ceftazidime	Ceftazidime + NXL104	Cefotaxime	Cefotaxime + NXL104	Piperacillin / tazobactam	Imipenem
CTX-M-15	2	0.06	8	≤0.015	2	0.12
CTX-M-9	2	0.25	16	≤0.015	1	0.12
CTX-M-14	2	0.06	32	≤0.015	1	0.12
-R recipient	0.125	0.06	0.03	≤0.015	0.5	0.12

Ertapenem-susceptible *Enterobacteriaceae* isolates (figure 3)

- NXL104 achieved >1000-fold potentiation of cefotaxime for producers of CTX-M enzymes belonging to each of the 5 groups, with MICs reduced from ≥16 mg/L to 0.03 to 0.06 mg/L; full potentiation was maintained against isolates that co-produced OXA-1 enzyme.
- NXL104 reduced ceftazidime MICs for isolates with CTX-M-15 enzyme (± OXA-1) from >64 mg/L to 0.25-1 mg/L.
- NXL104 reduced ceftazidime MICs for isolates with other CTX-M types from 2-8 mg/L to 0.12-0.5 mg/L.

Figure 3. MIC distributions for cefotaxime and ceftazidime with and without NXL104 vs. producers of CTX-M ESBLs



The isolates comprised *Escherichia coli* (37), *Klebsiella* spp. (19) and *Enterobacter* spp. (10); most CTX-M group 1 enzymes were CTX-M-15; most group 9 enzymes were CTX-M-9 or 14. Fifteen of the isolates with the Group 1 enzyme CTX-M-15 additionally had OXA-1

Ertapenem-non-susceptible (MIC >2 mg/L) *Enterobacteriaceae* isolates (Table 2)

- These isolates were ertapenem non-susceptible (MIC >2 mg/L), but varied in imipenem susceptibility, as is typical for the phenotype (2).
- All proved susceptible to cefotaxime + NXL104 at ≤2 mg/L.
- All except *E. aerogenes* H043100370 were susceptible to ceftazidime + NXL104 at ≤4 mg/L.
 - On re-examination this isolate (MIC of ceftazidime + NXL104, 64 mg/L; cefotaxime + NXL104, at 1 mg/L) was found to also harbour KPC-3 enzyme.
 - The retained activity by cefotaxime + NXL104 vs. *Enterobacteriaceae* with KPC enzymes supports data in Poster FI-318

Table 3. MICs (mg/L) for clinical *Enterobacteriaceae* isolates with combinations of β -lactamase and impermeability, conferring resistance to ertapenem

Specimen ID	Genus/ species	Mechanism(s) in addition to impermeability	Ceftazidime	Ceftazidime + NXL104	Cefotaxime	Cefotaxime + NXL104	Piperacillin / tazobactam	Ertapenem	Imipenem
H04416107	<i>E. aerogenes</i>	AmpC	>128	0.5	128	0.12	128	>16	1
H043100370	<i>E. aerogenes</i>	AmpC + KPC-3	>128	64	>128	1	>128	>16	>32
H043100371	<i>Enterobacter</i> spp.	AmpC	32	1	64	0.5	16	>16	0.5
H060340523	<i>E. aerogenes</i>	AmpC	>128	2	>128	2	>128	>16	8
H053520202	<i>E. cloacae</i>	AmpC	>128	2	>128	2	>128	14	4
H045100290	<i>E. cloacae</i>	CTX-M 1 & 9 group	128	0.5	>128	0.25	64	4	1
H050980341	<i>E. cloacae</i>	ESBL	>128	2	128	0.25	128	>16	1
H051000441	<i>E. cloacae</i>	ESBL	>128	2	>128	1	>128	>16	8
H042640247	<i>E. aerogenes</i>	ESBL	>128	1	>128	1	128	>16	16
H053720086	<i>Klebsiella</i> spp.	CTX-M 1 group	>128	1	>128	0.25	>128	8	0.5
H054000396	<i>Klebsiella</i> spp.	CTX-M 1 group	128	1	>128	0.25	>128	>16	1
H054120535	<i>Klebsiella</i> spp.	CTX-M 1 group	>128	2	>128	0.25	>128	>16	2
H054120566	<i>K. pneumoniae</i>	CTX-M 1 group	>128	2	>128	0.5	>128	>16	1
H054200417	<i>Klebsiella</i> spp.	CTX-M 1 group	>128	0.5	>128	0.12	>128	8	0.5
H055120250	<i>Klebsiella</i> spp.	CTX-M 1 group	>128	4	>128	1	>128	>16	4
H061260284	<i>Klebsiella</i> spp.	CTX-M 1 group	>128	0.03	>128	0.03	>128	4	0.25
H051880451	<i>E. cloacae</i>	CTX-M 9 group	128	2	>128	1	128	4	2

CONCLUSIONS

- Combinations of NXL104 with ceftazidime or cefotaxime had MICs of ≤1 mg/L against typical *Enterobacteriaceae* isolates with CTX-M enzymes,
- Cephalosporin + NXL104 combinations were also active, at ≤4 mg/L, against *Enterobacter* and *Klebsiella* strains with ertapenem resistance arising via combinations of ESBLs or AmpC with impermeability.
- Cephalosporin + NXL104 combinations may be alternatives to carbapenems for treatment of infections due to *Enterobacteriaceae* with ESBLs.
- Combinations of NXL104 with cephalosporins that are active vs. MRSA (i.e. ceftaroline and ceftobiprole) deserve evaluation.

REFERENCES

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