

# Antimicrobial Activity of Ceftazidime/NXL-104 Tested Against Gram-Negative Organisms, Including Multidrug-Resistant Subsets, Causing Infections in USA and European Medical Centers

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## Amended Abstract

**Background:** NXL-104 (NXL) is a novel non-β-lactam β-lactamase (BL) inhibitor of Ambler class A, C, and some D enzymes. Ceftazidime (CAZ) combined with NXL is currently being evaluated in clinical trials for the treatment of serious hospital infections, including those caused by antimicrobial-resistant (R) Gram-negative (GN) pathogens.

**Methods:** CAZ/NXL (NXL 4 μg/mL) and comparators were susceptibility (S) tested by CLSI broth microdilution methods against 5990 GN isolates cultured from 55 medical centers in the USA (27) and Europe (EU; 28) in 2009. The collection included: *Escherichia coli* (2170; 11.8% ESBL phenotype), *Klebsiella* spp. (KSP; 1156; 17.6% ESBL phenotype and 5.3% decreased S to carbapenems), *Pseudomonas aeruginosa* (PSA; 952; 24.6% imipenem [IMI]-R), *Enterobacter* spp. (ESP; 618; 23.4% CAZ-R), *Acinetobacter* spp. (ASP; 311; 47.9% IMI-R), *Serratia* spp. (272), *Proteus mirabilis* (234), *Citrobacter* spp. (120), indole-positive *Proteae* (109), and *Salmonella* spp. (48).

**Results:** CAZ/NXL was highly active against all Enterobacteriaceae (ENT) species, with MIC<sub>50</sub> of 0.06–0.25 μg/mL and MIC<sub>90</sub> of 0.5–1 μg/mL (main species listed in Table). ESBL phenotype and CAZ-R were more frequent among ENT from the EU compared with the USA, but CAZ/NXL was equally active against ENT from both regions. KSP with decreased S to carbapenems (5.0–5.9% resistance) were S to CAZ/NXL (96.8% inhibited at ≤4 μg/mL). NXL improved CAZ activity against PSA and CAZ/NXL inhibited >50% of IMI-R PSA at MIC of ≤4 μg/mL. All β-lactams tested had limited activity against ASP.

Cumulative % inhibited at CAZ/NXL MIC (μg/mL) of:

| Organism (no. tested)            | Cumulative % inhibited at CAZ/NXL MIC (μg/mL) of: |      |      |      |      |       |      |      |
|----------------------------------|---|------|------|------|------|-------|------|------|
|                                  | ≤0.06   | 0.12 | 0.25 | 0.5  | 1    | 2     | 4    | 8    |
| <i>E. coli</i> (2170)            | 32.4  | 82.4 | 95.4 | 99.5 | 99.9 | 100.0 | –    | –    |
| ESBL phenotype (256)             | 10.6  | 51.6 | 80.9 | 96.5 | 99.6 | 100.0 | –    | –    |
| <i>Klebsiella</i> spp. (1156)    | 17.5  | 65.6 | 84.1 | 93.7 | 97.4 | 99.4  | 99.8 | 99.9 |
| ESBL phenotype (204)             | 2.5   | 17.7 | 35.8 | 66.2 | 85.3 | 96.6  | 98.5 | 99.5 |
| Decreased S to carbapenems (62)  | 8.1   | 14.5 | 25.8 | 54.8 | 72.6 | 91.9  | 96.8 | 98.4 |
| <i>Enterobacter</i> spp. (618)   | 5.8   | 37.7 | 72.0 | 89.5 | 96.4 | 98.9  | 99.5 | 99.5 |
| Ceftazidime-R (143) <sup>2</sup> | 0.7   | 4.2  | 20.3 | 62.2 | 87.4 | 96.5  | 97.9 | 97.9 |
| <i>P. aeruginosa</i> (952)       | 0.0   | 0.1  | 0.7  | 3.0  | 17.2 | 68.6  | 83.7 | 93.4 |
| Imipenem-R (234) <sup>2</sup>    | 0.0   | 0.4  | 1.7  | 9.4  | 33.3 | 55.6  | 78.2 | –    |

**Conclusions:** CAZ/NXL was very active against ENT, including ESBL, KPC, and AmpC producers. CAZ/NXL was also active against PSA, including many IMI-R strains, but had limited potency against ASP.

## Introduction

Bacterial isolates resistant to clinically available β-lactams present a challenge to successful treatment of serious infections. β-lactamase-mediated resistance, in particular, represents a significant clinical threat because of the mobile nature of the genes encoding these enzymes. Two strategies have been used to restore the utility of β-lactam compounds: (i) the design/discovery of novel β-lactam molecules that are refractory to enzymatic inactivation, and (ii) the inhibition of β-lactamases, thereby allowing the β-lactam to retain target concentrations.

NXL-104 (NXL) is a non-β-lactam β-lactamase inhibitor with promising activity against class A, C, and some D β-lactamases. β-lactamase enzymes are inactivated very efficiently by NXL, with low IC<sub>50</sub> (concentration resulting in 50% inhibition) values and low turnover numbers. NXL protects β-lactams from hydrolysis by a variety of enzymes.

In this study, we evaluated the activity of ceftazidime combined with NXL (CAZ/NXL) against a large collection of contemporary Gram-negative clinical isolates recovered in hospitals located in the USA and Europe during 2009.

## Material And Methods

**Bacterial isolates:** A total of 5,990 non-duplicate consecutive Gram-negative strains were collected during 2009 from medical centers located in the USA (27 sites) and Europe (28 sites). These isolates were collected from bloodstream, respiratory tract, or skin and soft tissue infections according to defined protocols. Only clinically significant isolates were included in the study (1 per patient episode). Species identification was confirmed by standard biochemical tests and/or use of the Vitek Systems (bioMérieux; Hazelwood, MO, USA), as necessary.

**Susceptibility testing:** Isolates were susceptibility tested by a reference broth microdilution procedure (Clinical Laboratory Standard Institute [CLSI]),<sup>1</sup> using validated microdilution panels manufactured by TREK Diagnostics (Cleveland, OH, USA). Susceptibility testing results were interpreted according to CLSI criteria.<sup>2</sup> *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were concurrently tested for quality assurance; all results were in the published ranges.

*E. coli* and *Klebsiella* spp. isolates for which the MICs of ceftazidime, ceftazidime, or aztreonam were ≥2 μg/mL were considered to be phenotype-positive for ESBL production.<sup>2</sup>

## Results

- All 2,170 *E. coli* strains were inhibited at ≤2 μg/mL of CAZ/NXL, and 100% were susceptible by CLSI breakpoint criteria for ceftazidime alone (Tables 1 and 2). Using the EUCAST<sup>3</sup> breakpoint for ceftazidime (≤1 μg/mL susceptible), CAZ/NXL susceptibility rates were 99.9%.
- Against *E. coli* strains displaying the ESBL phenotype, CAZ/NXL provided the best coverage (100% using ceftazidime CLSI breakpoint), followed by meropenem (99.2% susceptible; Table 2).
- Using the 4 μg/mL ceftazidime breakpoint established by the CLSI,<sup>2</sup> CAZ/NXL demonstrated the highest susceptibility rate among agents tested against *Klebsiella* spp. (99.8%; Table 2). Meropenem was also very active against this species (95.8–96.3% susceptible).
- Only 2 (0.1%) *Klebsiella* spp. strains had CAZ/NXL MIC >4 μg/mL: 1 KPC-producing *K. pneumoniae* from the USA (8 μg/mL), and a VIM-1-producing *K. pneumoniae* from Spain (32 μg/mL; Table 1). Both strains showed decreased susceptibility to carbapenems (meropenem MIC values of >8 and 2 μg/mL, respectively).

**Table 1. Frequency Distributions of Ceftazidime/NXL-104 When Tested Against Gram-Negative Isolates Collected in US and European Medical Centers During 2009**

| Organism group (no. tested) <sup>a</sup> | Cumulative % of strains inhibited at MIC (μg/mL): |      |                   |        |       |      |                   |        |       |      |                    |                    |     |
|--|---|------|-------------------|--------|-------|------|-------------------|--------|-------|------|--------------------|--------------------|-----|
|  | ≤0.03   |      | 0.06              | 0.12   | 0.25  | 0.5  | 1                 | 2      | 4     | 8    | 16                 | 32                 | >32 |
|  | 50%   | 90%  | CLSI <sup>2</sup> | EUCAST | 50%   | 90%  | CLSI <sup>2</sup> | EUCAST | 50%   | 90%  | CLSI <sup>2</sup>  | EUCAST             |     |
| <i>Escherichia coli</i> (2,170)          | 7.1   | 32.4 | 82.4              | 95.4   | 99.5  | 99.9 | 100.0             | –      | –     | –    | –                  | –                  |     |
| ESBL phenotype (256)                     | 3.5   | 10.6 | 51.6              | 80.9   | 96.5  | 99.6 | 100.0             | –      | –     | –    | –                  | –                  |     |
| <i>Klebsiella</i> spp. (1,156)           | 1.6   | 17.5 | 65.6              | 84.1   | 93.7  | 97.5 | 99.5              | 99.8   | 99.9  | 99.9 | 100.0 <sup>a</sup> | –                  |     |
| ESBL phenotype (204)                     | 2.0   | 2.5  | 17.7              | 35.8   | 66.2  | 85.3 | 96.6              | 98.5   | 99.0  | 99.5 | 99.5               | 100.0 <sup>a</sup> |     |
| Decreased carbapenem-S (62) <sup>b</sup> | 6.5   | 8.1  | 14.5              | 25.8   | 54.8  | 72.6 | 91.9              | 96.8   | 98.4  | 98.4 | 100.0 <sup>a</sup> | –                  |     |
| <i>Enterobacter</i> spp. (618)           | 1.3   | 5.8  | 37.7              | 72.0   | 89.5  | 96.4 | 98.9              | 99.5   | 99.5  | 99.5 | 99.5               | 100.0 <sup>b</sup> |     |
| Ceftazidime-R (143) <sup>2</sup>         | 0.0   | 0.7  | 4.2               | 20.3   | 62.2  | 87.4 | 96.5              | 97.9   | 97.9  | 97.9 | 97.9               | 100.0 <sup>b</sup> |     |
| <i>Serratia</i> spp. (272)               | 0.0   | 2.6  | 38.2              | 79.3   | 93.4  | 96.3 | 98.5              | 99.6   | 100.0 | –    | –                  | –                  |     |
| <i>Proteus mirabilis</i> (234)           | 19.2  | 86.3 | 98.7              | 100.0  | –     | –    | –                 | –      | –     | –    | –                  | –                  |     |
| Indole-positive Proteae (109)            | 11.0  | 67.9 | 88.1              | 95.4   | 97.2  | 99.1 | 100.0             | –      | –     | –    | –                  | –                  |     |
| <i>Citrobacter</i> spp. (120)            | 0.8   | 20.0 | 58.3              | 85.0   | 95.8  | 98.3 | 100.0             | –      | –     | –    | –                  | –                  |     |
| <i>Salmonella</i> spp. (48)              | 2.1   | 6.3  | 25.0              | 87.5   | 100.0 | –    | –                 | –      | –     | –    | –                  | –                  |     |
| <i>Pseudomonas aeruginosa</i> (952)      | 0.0   | 0.0  | 0.1               | 0.7    | 3.0   | 17.2 | 68.6              | 83.7   | 93.4  | 96.3 | 98.4               | 100.0              |     |
| Imipenem-R (234) <sup>2</sup>            | 0.0   | 0.0  | 0.4               | 1.7    | 9.4   | 33.3 | 55.6              | 78.2   | 86.7  | 94.0 | 100.0              | –                  |     |
| <i>Acinetobacter</i> spp. (311)          | 0.3   | 0.6  | 1.0               | 1.6    | 2.2   | 2.6  | 5.5               | 11.9   | 30.5  | 50.2 | 70.1               | 100.0              |     |
| Imipenem-R (149) <sup>2</sup>            | 0.0   | 0.0  | 0.0               | 0.7    | 0.7   | 0.7  | 1.3               | 12.8   | 32.2  | 58.4 | 100.0              | –                  |     |

a. Single isolate from Spain (VIM-type metallo-β-lactamase).

b. Defined as MIC ≥2 μg/mL to imipenem and/or meropenem.

c. Defined as MIC ≥16 μg/mL to ceftazidime.

d. According to document M100-S20-U.<sup>2</sup>

**Table 2. Activity of Ceftazidime/NXL-104 and Comparator Agents Tested Against 5,990 Gram-Negative Clinical Isolates Collected in US and European Medical Centers During 2009**

| Organism (no. tested)/ Antimicrobial agent | USA   |       |                   |        | EUROPE |       |                   |        | OVERALL |       |                   |        |
|--|-------|-------|-------------------|--------|--------|-------|-------------------|--------|---------|-------|-------------------|--------|
|  | MIC   |       | % Susceptibility  |        | MIC    |       | % Susceptibility  |        | MIC     |       | % Susceptibility  |        |
|  | 50%   | 90%   | CLSI <sup>2</sup> | EUCAST | 50%    | 90%   | CLSI <sup>2</sup> | EUCAST | 50%     | 90%   | CLSI <sup>2</sup> | EUCAST |
| <i>Escherichia coli</i> (2,170)            | 0.12  | 0.25  | 100.0             | 99.9   | 0.12   | 0.25  | 100.0             | 99.9   | 0.12    | 0.25  | 100.0             | 99.9   |
| Ceftazidime/NXL-104 <sup>a</sup>           | 0.12  | 0.25  | 100.0             | 99.9   | 0.12   | 0.25  | 100.0             | 99.9   | 0.12    | 0.25  | 100.0             | 99.9   |
| Ceftazidime                                | 0.25  | 1     | 93.4              | 90.9   | 0.25   | 2     | 93.2              | 87.2   | 0.25    | 2     | 93.3              | 88.8   |
| Cefepime                                   | ≤0.12 | 0.5   | 92.1              | 92.1   | ≤0.12  | 2     | 93.8              | 88.7   | ≤0.12   | 1     | 94.6              | 90.9   |
| Meropenem                                  | ≤0.12 | ≤0.12 | 99.9              | 99.9   | ≤0.12  | ≤0.12 | 99.8              | 99.9   | ≤0.12   | ≤0.12 | 99.9              | 99.9   |
| Piperacillin/tazobactam                    | 2     | 8     | 94.0              | 91.2   | 2      | 16    | 91.1              | 87.8   | 2       | 16    | 92.4              | 89.3   |
| ESBL-producers (256)                       | 0.25  | 0.5   | 100.0             | 100.0  | 0.12   | 0.5   | 100.0             | 99.4   | 0.12    | 0.5   | 100.0             | 99.6   |
| Ceftazidime/NXL-104 <sup>a</sup>           | 16    | >32   | 31.5              | 10.9   | 8      | 32    | 49.4              | 8.5    | 8       | 32    | 43.0              | 9.4    |
| Ceftazidime                                | 8     | >16   | 53.3              | 22.8   | 8      | >16   | 54.3              | 20.7   | 8       | >16   | 53.9              | 21.5   |
| Cefepime                                   | ≤0.12 | ≤0.12 | 98.9              | 98.9   | ≤0.12  | ≤0.12 | 99.4              | 100.0  | ≤0.12   | ≤0.12 | 99.2              | 99.6   |
| Meropenem                                  | 8     | 64    | 72.8              | 55.4   | 8      | >64   | 66.5              | 50.6   | 8       | >64   | 68.8              | 52.3   |
| Piperacillin/tazobactam                    | 8     | 64    | 72.8              | 55.4   | 8      | >64   | 66.5              | 50.6   | 8       | >64   | 68.8              | 52.3   |
| <i>Klebsiella</i> spp. (1,156)             | 0.12  | 0.5   | 99.7              | 98.1   | 0.12   | 0.5   | 99.8              | 96.4   | 0.12    | 0.5   | 99.8              | 97.4   |
| Ceftazidime/NXL-104 <sup>a</sup>           | 0.12  | 0.5   | 99.7              | 98.1   | 0.12   | 0.5   | 99.8              | 96.4   | 0.12    | 0.5   | 99.8              | 97.4   |
| Ceftazidime                                | 0.12  | 16    | 88.8              | 88.1   | 0.25   | >32   | 79.2              | 75.4   | 0.12    | 32    | 84.9              | 82.9   |
| Cefepime                                   | ≤0.12 | 2     | 92.4              | 89.9   | ≤0.12  | >16   | 84.2              | 80.3   | ≤0.12   | 16    | 89.0              | 85.9   |
| Meropenem                                  | ≤0.12 | ≤0.12 | 95.1              | 95.3   | ≤0.12  | ≤0.12 | 96.6              | 97.7   | ≤0.12   | ≤0.12 | 95.8              | 96.3   |
| Piperacillin/tazobactam                    | 4     | 32    | 88.4              | 82.6   | 4      | >64   | 77.3              | 73.3   | 4       | >64   | 83.8              | 78.8   |
| ESBL-producers (204)                       | 0.5   | 2     | 98.8              | 85.7   | 0.5    | 2     | 99.2              | 85.8   | 0.5     | 2     | 79.9              | 72.5   |
| Ceftazidime/NXL-104 <sup>a</sup>           | >32   | >32   | 9.5               | 7.1    | 32     | >32   | 17.5              | 9.2    | >32     | >32   | 14.2              | 8.3    |
| Ceftazidime                                | 16    | >16   | 38.1              | 19.0   | >16    | >16   | 37.5              | 21.7   | >16     | >16   | 37.7              | 20.6   |
| Cefepime                                   | ≤0.12 | >8    | 60.7              | 61.9   | ≤0.12  | 2     | 86.7              | 90.8   | ≤0.12   | >8    | 76.0              | 78.9   |
| Meropenem                                  | >64   | >64   | 23.8              | 19.0   | 64     | >64   | 25.8              | 18.3   | >64     | >64   | 25.0              | 18.6   |
| Piperacillin/tazobactam                    | 4     | 32    | 88.4              | 82.6   | 4      | >64   | 77.3              | 73.3   | 4       | >64   | 83.8              | 78.8   |
| Imipenem not-susceptible (62)              | 0.5   | 2     | 97.1              | 73.5   | 0.5    | 4     | 96.4              | 71.4   | 0.5     | 2     | 96.8              | 72.6   |
| Ceftazidime/NXL-104 <sup>a</sup>           | >32   | >32   | 5.9               | 5.9    | >32    | >32   | 14.3              | 14.3   | >32     | >32   | 9.7               | 9.7    |
| Ceftazidime                                | >16   | >16   | 14.7              | 5.9    | >16    | >16   | 25.0              | 17.9   | >16     | >16   | 19.4              | 11.3   |
| Cefepime                                   | >8    | >8    | 5.9               | 8.8    | 1      | >8    | 50.0              | 60.7   | >8      | >8    | 25.8              | 32.3   |
| Meropenem                                  | >64   | >64   | 5.9               | 5.9    | >64    | >64   | 3.6               | 3.6    | >64     | >64   | 4.8               | 4.8    |
| Piperacillin/tazobactam                    | >64   | >64   | 5.9               | 5.9    | >64    | >64   | 3.6               | 3.6    | >64     | >64   | 4.8               | 4.8    |
| <i>Enterobacter</i> spp. (618)             | 0.25  | 0.5   | 100.0             | 97.0   | 0.25   | 1     | 95.6              | 98.8   | 0.25    | 1     | 99.5              | 96.4   |
| Ceftazidime/NXL-104 <sup>a</sup>           | 0.25  | 0.5   | 100.0             | 97.0   | 0.25   | 1     | 95.6              | 98.8   | 0.25    | 1     | 99.5              | 96.4   |
| Ceftazidime                                | 0.25  | >32   | 78.9              | 74.8   | 0.25   | >32   | 70.8              | 65.1   | 0.25    | >32   | 75.4              | 70.8   |
| Cefepime                                   | ≤0.12 | 2     | 95.9              | 85.8   | ≤0.12  | 2     | 96.8              | 87.4   | ≤0.12   | 2     | 96.3              | 86.4   |
| Meropenem                                  | ≤0.12 | ≤0.12 | 97.8              | 97.8   | ≤0.12  | ≤0.12 | 98.4              | 98.4   | ≤0.12   | ≤0.12 | 98.1              | 98.1   |
| Piperacillin/tazobactam                    | 4     | 64    | 82.2              | 77.3   | 4      | >64   | 72.7              | 68.0   | 4       | 64    | 78.3              | 73.5   |
| Ceftazidime-resistant (143)                | 0.5   | 2     | 100.0             | 86.5   | 0.5    | 2     | 95.6              | 88.4   | 0.5     | 2     | 97.9              | 87.4   |
| Ceftazidime/NXL-104 <sup>a</sup>           | >32   | >32   | 0.0               | 0.0    | 32     | >32   | 0.0               | 0.0    | 32      | >32   | 0.0               | 0.0    |
| Ceftazidime                                | 2     | >16   | 81.1              | 31.1   | 1      | 16    | 88.4              | 53.6   | 2       | >16   | 84.6              | 42.0   |
| Cefepime                                   | ≤0.12 | 4     | 89.2              | 89.2   | ≤0.12  | 0.5   | 94.2              | 94.2   | ≤0.12   | 0.5   | 91.6              | 91.6   |