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# Global Surveillance of the Activity of Sulbactam combined with the Novel β-lactamase Inhibitor ETX2514 against Clinical Isolates of *Acinetobacter baumannii* from 2014

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## Abstract

**Background**: Multidrug-resistant *Acinetobacter baumannii* infections are considered a serious threat by the Centers for Disease Control and other government agencies. They are among the most difficult to treat and are associated with high mortality rates due to the limited number of effective therapies available and inappropriate initial treatment choice. The novel diazabicyclooctenone β-lactamase inhibitor ETX2514 effectively restores the antibacterial activity of sulbactam against multidrug resistant (MDR) *A. baumannii* due to its potent inhibition of class A, C and D serine β-lactamases, which are commonly found in this organism.

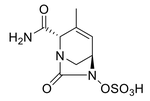
**Methods**: The *in vitro* activity of sulbactam-ETX2514 and comparator antibiotics was determined against clinical isolates of *A. baumannii* using the broth microdilution methodology recommended by the Clinical and Laboratory Standards Institute (CLSI). A total of 1,131 isolates were obtained from hospitalized patients in 151 medical centers within 38 countries during the year 2014.

**Results:** The activity of sulbactam-ETX2514 was 16-fold higher than that of sulbactam alone ($MIC_{50}$ values of 1 and 16 mg/L, respectively, and $MIC_{90}$ values of 4 and 64 mg/L, respectively). This increased activity was maintained against all subsets of resistant phenotypes including meropenem-resistant, colistin-resistant, multidrug-resistant, ESBL-positive, and non-MBL isolates, as well as isolates containing Ambler class D β-lactamases. There was a two-fold lower MIC for sulbactam-ETX2514 compared to sulbactam (32 vs 64 mg/L) alone against the one *A. baumannii* isolate carrying a gene encoding an MBL. $MIC_{50}$ and $MIC_{90}$ values were also remarkably consistent across subsets of isolates from different sources, from pediatric patients, and from the five geographic regions.

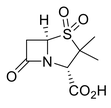
**Conclusion:** The activity of sulbactam-ETX2514 observed in this study suggests that further *in vitro* and clinical investigation is warranted to explore the utility of this agent against this difficult-to-treat nosocomial pathogen.

## Introduction

Infections caused by multidrug resistant bacterial pathogens are increasing dramatically via the spread of a number of different resistance mechanisms. One of the most remarkable is the rapid rise in the number and diversity of β-lactamases. Although several new β-lactamase inhibitors have recently been approved or are in clinical trials, their spectrum of activity does not address problematic multidrug resistant (MDR) pathogens such as *Acinetobacter baumannii*, a nosocomial pathogen which can cause severe infections with high mortality rates [1]. Even carbapenems have become ineffective against *A. baumannii*, due, in large part, to the emergence of class D β-lactamases [2].



**ETX2514**



**sulbactam**

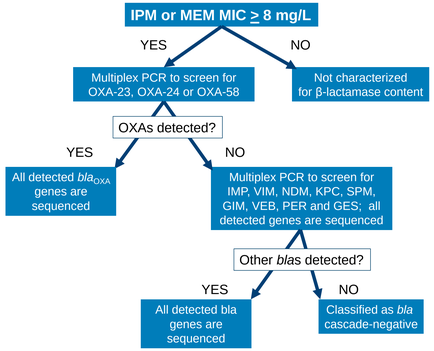
Sulbactam is a class A β-lactamase inhibitor with intrinsic antibacterial activity against *A. baumannii*; however, its therapeutic utility has been compromised by its susceptibility to recently acquired β-lactamases in this pathogen. ETX2514 is a novel diazabicyclooctenone BLI with broad-spectrum activity against class A, C and D β-lactamases, including those that degrade sulbactam. Therefore a combination of sulbactam and ETX2514 is currently being investigated for the treatment of drug-resistant *A. baumannii* infections. Accordingly, a surveillance study was conducted in 2014 to evaluate the *in vitro* activity of sulbactam-ETX2514 and comparator agents against contemporary clinical *A. baumannii* isolates collected from 151 medical centers around the world.

## Methods

**Bacterial strains**: All isolates were obtained from specimens collected from patients with documented intra-abdominal infections (IAI), urinary tract infections (UTI), skin and soft tissue infections (SSTI), blood cultures, or lower respiratory tract infections (LRTI). Only one strain per patient infection episode was included in the surveillance program. Figure 1 shows the participating medical centers by region and country. Each site sent 7 - 8 isolates per medical center to a central laboratory, Laboratories International for Microbiology Studies (LIMS) a subsidiary of International Health Management Associates, Inc. (IHMA) in Schaumburg, IL, where the isolates were further evaluated and stored. Organism transport, confirmation of organism identification, quality assurance of data, and development and management of a centralized database were conducted by IHMA's central laboratory in the United States.

**Susceptibility Testing**: Minimum inhibitory concentrations (MICs) were determined using frozen broth microdilution panels prepared at IHMA. All broth microdilution testing, including panel manufacture, inoculation, incubation, inclusion/testing of quality control strains and data interpretation, was conducted following current CLSI guidelines [3]. Where indicated, ETX2514 was added at a concentration of 4 mg/L.

**Determination of β-lactamase content**: Any *A. baumannii* isolate which was resistant to meropenem or imipenem (MIC ≥ 8 mg/ L) was molecularly characterized according to the flowchart below. Genomic DNA was extracted from overnight colonies grown on blood agar using the QIAamp DNAMini Kit and the QIAcube instrument according to the manufacturer's instructions. *A. baumannii* isolates were first screened for the presence of $bla_{OXA-23}$, $bla_{OXA-24}$, and $bla_{OXA-58}$. If no OXA-type *bla* genes were detected, screening for $bla_{VEB}$, $bla_{PER}$, $bla_{GES}$, $bla_{SPM}$, $bla_{IMP}$, $bla_{VIM}$, $bla_{NDM}$, and $bla_{KPC}$ was carried out in a single multiplex PCR reaction and $bla_{GIM}$ was screened separately in a singleplex PCR reaction. Multiplex PCR reactions to detect all genes but $bla_{GIM}$ were performed using the Multiplex PCR kit (Qiagen), whereas the GIM reaction was performed using the Fast Cycling PCR kit (Qiagen). All genes were amplified for sequencing using the Fast Cycling PCR kit (Qiagen) per the manufacturer's instructions. Sanger sequencing of both strands of each PCR amplicon was performed at GENEWIZ, Inc. (South Plainfield, NJ). Sequence data was analyzed using SeqScape v.2.7 (Applied Biosystems) and compared to sequences available from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) and the Lahey Clinic (www.lahey.org).




**Notes on genotypes and phenotypes:**

- Based on the above testing scheme, any isolate defined as "ESBL" or "MBL" may also contain genes encoding other β-lactamases.
- "Non-MBL" was defined as any isolate for which an MBL gene was not detected by PCR. Note that this group may contain isolates with an MBL gene, but these went undetected if the isolates also contained a gene encoding an OXA β-lactamase.
- "MDR" was defined as any isolate that was resistant to three or more drug classes by CLSI interpretive criteria [4].

## Demographics

**Figure 1. Origins of clinical isolates by region (inner circle) and country (outer circle). The number of isolates from a given country is indicated in parentheses.**

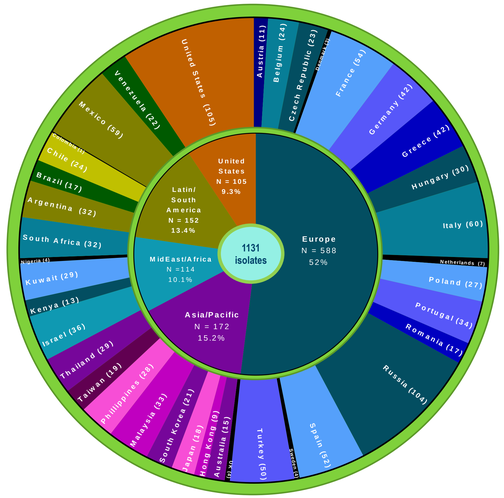



**Table 1. Resistant subsets listed by region**

| Genotype or subgroup | Asia/ Pacific | Europe | Latin/ South America | MidEast/ Africa | United States | Grand Total | Percent Total |
|---|---|---|---|---|---|---|---|
| *Acinetobacter baumannii* (all) | 172 | 588 | 152 | 114 | 105 | 1131 | -- |
| meropenem-resistant (MIC of ≥8 mg/L) | 109 | 362 | 117 | 84 | 59 | 731 | 64.6 |
| colistin-resistant (MIC of ≥4 mg/L) | 2 | 41 | 1 | 5 | 7 | 56 | 5 |
| MDR | 109 | 401 | 127 | 80 | 61 | 778 | 68.8 |
| ESBL (molecular) | | 6 | 4 | 1 | | 11 | 1 |
| MBL | | | 1 | | | 1 | 0.09 |
| % of Total | 15.2 | 52.0 | 13.4 | 10.1 | 9.3 | 100 | -- |

**Table 2. Resistant subsets stratified by infection type**

| Genotype or subgroup | IAI | UTI | LRTI ≥48 Hrs | LRTI <48 Hrs | SSTI | Blood |
|---|---|---|---|---|---|---|
| *Acinetobacter baumannii* (all) | 77 | 134 | 444 | 109 | 258 | 102 |
| meropenem-resistant (MIC of ≥8 mg/L) | 57 | 69 | 315 | 75 | 144 | 68 |
| colistin-resistant (MIC of ≥4 mg/L) | 1 | 4 | 23 | 7 | 17 | 4 |
| MDR | 58 | 74 | 326 | 77 | 168 | 72 |
| ESBL (molecular) | | | 8 | 1 | 2 | |
| MBL | | | | | | 1 |
| % of Total | 6.8 | 11.9 | 39.3 | 9.6 | 22.8 | 9.0 |

**IAI**, intra-abdominal infection; **UTI**, urinary tract infection; **LRTI ≥48 hrs**, hospital-associated lower respiratory tract infection; **LRTI <48 hrs**, non-hospital-associated lower respiratory tract infection; **SSTI**, skin and soft tissue infection; **Blood**, blood cultures.

## Results

**Table 3. Global MIC frequency distributions for sulbactam alone or in the presence of 4 mg/L ETX2514**

| | Drug (mg/L) | N | ≤0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | >128 | $MIC_{50}$ | $MIC_{90}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. baumannii* (all isolates) | Sulbactam | 1131 | | | 2 | 4 | 93 | 164 | 95 | 91 | 157 | 271 | 194 | 51 | 9 | | |
| | Cumulative % Sensitive | | | | 0.2 | 0.5 | 8.8 | 23.3 | 31.7 | 39.7 | 53.6 | 77.5 | 94.7 | 99.2 | 100 | 16 | 64 |
| | SUL-ETX2514 | 1131 | 11 | 7 | 70 | 227 | 405 | 285 | 122 | | 1 | 3 | | | | | |
| | Cumulative % Sensitive | | 1 | 1.6 | 7.8 | 27.9 | 63.7 | 88.9 | 99.6 | | 99.7 | 100 | | | | 1 | 4 |
| meropenem-resistant | Sulbactam | 731 | | | | | | 6 | 22 | 64 | 147 | 245 | 188 | 50 | 9 | | |
| | Cumulative % Sensitive | | | | | | | 0.8 | 3.8 | 12.6 | 32.7 | 66.2 | 91.9 | 98.8 | 100 | 32 | 64 |
| | SUL-ETX2514 | 731 | 3 | 2 | 12 | 81 | 283 | 230 | 116 | | 1 | 3 | | | | | |
| | Cumulative % Sensitive | | 0.4 | 0.7 | 2.3 | 13.4 | 52.1 | 83.6 | 99.5 | | 99.6 | 100 | | | | 1 | 4 |
| colistin-resistant | Sulbactam | 56 | | | | | | 4 | 3 | 5 | 6 | 21 | 9 | 7 | 1 | | |
| | Cumulative % Sensitive | | | | | | | 7.1 | 12.5 | 21.4 | 32.1 | 69.6 | 85.7 | 98.2 | 100 | 32 | 128 |
| | SUL-ETX2514 | 56 | | | 2 | 7 | 18 | 25 | 4 | | | | | | | | |
| | Cumulative % Sensitive | | | | 3.6 | 16.1 | 48.2 | 92.9 | 100 | | | | | | | 2 | 2 |
| MDR | Sulbactam | 778 | | | | | | 8 | 37 | 71 | 149 | 264 | 191 | 49 | 9 | | |
| | Cumulative % Sensitive | | | | | | | 1 | 5.8 | 14.9 | 34.1 | 68 | 92.5 | 98.8 | 100 | 32 | 64 |
| | SUL-ETX2514 | 778 | 2 | 2 | 16 | 91 | 290 | 253 | 120 | | 1 | 3 | | | | | |
| | Cumulative % Sensitive | | 0.3 | 0.5 | 2.6 | 14.3 | 51.5 | 84.1 | 99.5 | | 99.6 | 100 | | | | 1 | 4 |
| ESBL (molecular) | Sulbactam | 11 | | | | | | | | | 1 | 4 | 6 | | | | |
| | Cumulative % Sensitive | | | | | | | | | | 9.1 | 45.5 | 100 | | | 64 | 64 |
| | SUL-ETX2514 | 11 | | | | 4 | 3 | 3 | 1 | | | | | | | | |
| | Cumulative % Sensitive | | | | | 36.4 | 63.6 | 90.9 | 100 | | | | | | | 1 | 2 |
| MBL | Sulbactam | 1 | | | | | | | | | | | 1 | | | | |
| | SUL-ETX2514 | 1 | | | | | | | | | | 1 | | | | | |

**Table 4. Global MIC frequency distributions for SUL-ETX2514 categorized by infection type**

| Infection Type | Drug (mg/L) | N | ≤0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | >128 | $MIC_{50}$ | $MIC_{90}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IAI | Sulbactam | 77 | | | | | 2 | 10 | 10 | 11 | 10 | 18 | 14 | 2 | | 16 | 64 |
| | SUL-ETX2514 | 77 | | | 6 | 9 | 25 | 23 | 12 | | 1 | 1 | | | | 1 | 4 |
| UTI | Sulbactam | 134 | | | | | 15 | 30 | 14 | 6 | 20 | 29 | 13 | 7 | | 16 | 64 |
| | SUL-ETX2514 | 134 | | 1 | 11 | 32 | 52 | 24 | 14 | | | | | | | 1 | 4 |
| LRTI ≥ 48hrs | Sulbactam | 444 | | | | 2 | 30 | 53 | 32 | 30 | 67 | 113 | 98 | 16 | 3 | 32 | 64 |
| | SUL-ETX2514 | 444 | 3 | 4 | 18 | 84 | 175 | 113 | 47 | | | | | | | 1 | 4 |
| Pediatric | Sulbactam | 37 | | | | 2 | 8 | 10 | 3 | 1 | 1 | 4 | 7 | 1 | | 2 | 64 |
| | SUL-ETX2514 | 37 | 1 | 1 | 5 | 9 | 11 | 8 | 2 | | | | | | | 1 | 2 |
| Total | | 692 | | | | | | | | | | | | | | | |

**Table 5. Global MIC frequency distributions for SUL-ETX2514 against meropenem-resistant isolates categorized by enzyme group**

| Enzyme Group | Drug (mg/L) | N | ≤0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | >128 | $MIC_{50}$ | $MIC_{90}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ESBL | Sulbactam | 5 | | | | | | | | | 1 | 3 | 1 | | | -- | -- |
| | SUL-ETX2514 | 5 | | | | | 2 | 3 | | | | | | | | -- | -- |
| GES ESBL-like | Sulbactam | 6 | | | | | | | | | | 1 | 5 | | | -- | -- |
| | SUL-ETX2514 | 6 | | | | 4 | 1 | | 1 | | | | | | | -- | -- |
| MBL | Sulbactam | 1 | | | | | | | | | | | 1 | | | -- | -- |
| | SUL-ETX2514 | 1 | | | | | | | | | | 1 | | | | -- | -- |
| OXA | Sulbactam | 668 | | | | | | 7 | 54 | 143 | 228 | 180 | 48 | 8 | | 32 | 64 |
| | SUL-ETX2514 | 668 | 3 | 2 | 9 | 72 | 263 | 208 | 108 | 0 | 1 | 2 | | | | 1 | 4 |
| *bla*-cascade negative | Sulbactam | 51 | | | | | | 6 | 15 | 10 | 3 | 13 | 1 | 2 | 1 | 8 | 32 |
| | SUL-ETX2514 | 51 | | | 3 | 5 | 17 | 19 | 7 | | | | | | | 2 | 4 |
| Total | | 731 | | | | | | | | | | | | | | | |

**Table 5. Activity of sulbactam-ETX2514 vs. comparator agents**

| | mg/L | | | CLSI classification | | |
|---|---|---|---|---|---|---|
| Drug | Range | $MIC_{50}$ | $MIC_{90}$ | %Sus | %Int | %Res |
| Sulbactam | 0.25 - >128 | 16 | 64 | na | na | na |
| SUL-ETX2514 | ≤0.06 - 32 | 1 | 4 | na | na | na |
| Amikacin | ≤0.25 - >32 | >32 | >32 | 42.4 | 4.8 | 52.8 |
| Aztreonam | ≤0.06 - >128 | 64 | >128 | na | na | na |
| Ceftazidime | 0.12 - >128 | 64 | >128 | 32.5 | 2.6 | 64.9 |
| Colistin | ≤0.06 - >8 | 1 | 2 | 95.1 | 0.0 | 5.0 |
| Doripenem | 0.06 - >4 | >4 | >4 | 34.2 | 1.6 | 64.2 |
| Imipenem | ≤0.03 - >8 | >8 | >8 | 35.2 | 1.4 | 63.4 |
| Levofloxacin | ≤0.03 - >4 | >4 | >4 | 29.4 | 7.4 | 63.2 |
| Meropenem | ≤0.06 - >8 | >8 | >8 | 34.0 | 1.3 | 64.6 |
| Tigecycline | ≤0.015 - >8 | 1 | 2 | na | na | na |

CLSI susceptibilities defined by CLSI document M100-S25 [4]. where applicable; na= no breakpoint defined.

**Table 6. Regional counts and molecular characterization by enzyme groups of meropenem-resistant isolates**

| Enzyme Group | *bla* | Europe | Latin/South Amer | United States | Asia/Pacific | MidEast/Africa | N |
|---|---|---|---|---|---|---|---|
| ESBL | PER-1 | 1 | 4 | | | | 5 |
| GES ESBL-like | GES-11 | 5 | | | | 1 | 6 |
| MBL | NDM-1 | | 1 | | | | 1 |
| OXA Carbapenemase | OXA-23 | 231 | 54 | 18 | 101 | 65 | 469 |
| | OXA-239 | | 26 | | | | 26 |
| | OXA-24 | 18 | | 6 | | | 24 |
| | OXA-40 | | 1 | 4 | | | 5 |
| | OXA-58 | 9 | 6 | | 3 | 3 | 21 |
| | OXA-72 | 80 | 16 | 5 | 3 | 9 | 113 |
| OXA Carbapenemase + OXA Spectrum undefined | OXA-23; OXA-435 | 2 | | | | 1 | 3 |
| | OXA-398 | | 3 | | | | 3 |
| OXA Spectrum undefined | OXA-420 | 1 | | | | | 1 |
| | OXA-435 | 1 | | | | | 1 |
| | OXA-437 | 1 | | | | | 1 |
| | OXA-440 | | | | 1 | | 1 |
| *bla*-cascade negative | | 13 | 6 | 26 | 1 | 5 | 51 |
| Total | | 362 | 117 | 59 | 109 | 84 | 731 |

## Conclusions

- Sulbactam-ETX2514 demonstrated 16-fold increased activity against isolates of *A. baumannii* compared to sulbactam alone ($MIC_{90}$ of 4 mg/L versus 64 mg/L).
- This activity was maintained among meropenem-resistant, colistin-resistant, and MDR isolates.
- Sulbactam-ETX2514 showed potent activity against isolates producing class D (OXA-type) β-lactamases
- Sulbactam-ETX2514 was weakly active against one isolate containing a class B metallo-β-lactamase.
- The activity of sulbactam-ETX2514 was consistent across subsets of isolates from IAI, UTI, and HA-LRTI or from pediatric patients, as well as across geographic regions.
- The activity of sulbactam-ETX2514 observed in this study suggests that further *in vitro* characterization and clinical development is warranted to explore the utility of this agent.

## References

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