# Essential Metabolic Routes as a Way to ESKAPE from Antibiotic Resistance

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**Keywords: ESKAPE pathogens, thiamine, pyridoxal 5'-phosphate, antibiotic resistance.**

## Abstract

The antibiotic resistance is a worldwide concern and that requires a concerted action from physicians, patients, governmental agencies and academia to prevent infections and the spread of resistance, to track resistant bacteria, to improve the use of current antibiotics and to develop new antibiotics. Despite the efforts spent so far, the current antibiotics in the market are restricted to only five general targets/pathways highlighting the need for basic research focusing on the discovery and evaluation of new potential targets. Here we interrogate two biosynthetic pathways as potentially druggable pathways in bacteria. The biosynthesis pathway for thiamine (vitamin B1), absent in humans, but found in many bacteria, including pathogenic organism in the group of the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa* and *Enterobacter* species) and the biosynthesis pathway for pyridoxal 5'-phosphate and its vitamers (vitamin B6), found in S. aureus. Using current genomic data, we discuss the possibilities of inhibition of enzymes in the pathway and review the current state of the art in the scientific literature.

## 1 Introduction

Antibiotic resistance is an urgent threat to human health and requires urgent actions from physicians, patients, industries, governmental agencies and the academic community worldwide. According to the last document from the CDC regarding antibiotic resistance in the United States, from 2013, the number of people with serious infections caused by resistant bacteria reaches two million every year, with at least 23,000 deaths per year directly caused by these infections (1). The situation is similarly warning in Europe, where 25,100 deaths were reported from the European Centre for Disease Prevention and Control in 2007 (2). Globally, 700,000 deaths are estimated every year as a consequence of antibiotic resistance (3). The same CDC document lists four general action lines to address antibiotic resistance: (i) preventing infections and the spread of resistance; (ii) tracking resistant bacteria; (iii) improving the use of current antibiotics; (iv) development of new antibiotics (1).

Since bacteria have a short doubling time and efficient mechanisms for plasmid sharing, the development of antibiotic resistance is a very feasible weapon. So, as previously said by Walsh and Wencewicz, the development of resistance is not a matter of *if*, but rather a matter of *when* (4) and, despite the title of this paper (which is rather provocative), there is no way we can escape from it (5). On the contrary, the development/discovery of new antibiotics will tend to be a continuous goal to be achieved in drug discovery pipelines.

Interestingly, even after what has been named as the 'golden age' of antibiotics development, the drugs currently in the market are restricted to only five molecular targets and/or pathways (4): (*i*) the peptidoglycan/cell wall biosynthesis, site of action of beta-lactam antibiotics, for example; (*ii*) the protein biosynthesis, where the ribosome is an important target; (*iii*) DNA replication and RNA transcription; (*iv*) the folate biosynthesis pathway, and (*v*) the disruption of the bacterial membrane. Given the relevance of the antibiotic therapy and the emergence of antibiotic resistance, the available choices of current drugs are very narrow in the context of the mechanism of action restricted to only five molecular/pathway targets.

Antibiotic resistance, as a threat to human health, should be addressed in multiple, and simultaneous ways. For academia, an interesting way to address antibiotic resistance is the discovery and validation of new targets/pathways that could be specifically targeted by new antibiotic candidates. It is worthy of note that, according to Kelly and Davies (3), no new class of antibiotics was discovered and released for routine treatment since the 1980s, highlighting the outstanding role that academia can have in the preliminary research for discovery and validation of druggable targets/pathways.

About 7% of the *E. coli* genome, 303 genes, were shown to be composed of essential genes (5,6). Under stress conditions caused by a limited medium, additional 119 genes show some condition-dependent essentiality, including several genes related to the metabolism and synthesis of essential molecules such as amino acids, and nucleotides (5) and, obviously, some of these targets/pathways can be an interesting choices for the development of new antibiotic candidates. A promising approach to screen compounds for their activity in metabolic pathways was shown by Zlitni and coworkers (7). The authors searched for antibacterial compounds under poor nutrient media and found three potential compounds in this screening strategy (7). Worth of note, the metabolic profile of existing antibiotics showed that the supplementation of vitamins B5, B6, B1, and B2 did not significantly reverse the antimicrobial effect of any of the 24 inhibitors assayed (7), suggesting that the current antibiotics do not explore these essential pathways.

In this context, the enzymatic routes for the biosynthesis of vitamins are very interesting pathways to be explored as potential targets for the discovery of new antibiotic candidates. Vitamin B1, for example, cannot be synthesized by humans, although several microorganisms can synthesize this vitamin, including pathogens. In the absence of thiamin (vitamin B1), the activity of several carbohydrate metabolism enzymes is impaired, including pyruvate dehydrogenase, which connects glycolysis and the citric acid cycle (8). Other thiamin dependent enzymes are transketolase, α-ketoacid decarboxylase, α-ketoacid dehydrogenase and acetolactate synthase (9). A very similar scenario was observed for pyridoxal 5'-phosphate (vitamin B6) in the model Gram-positive organism *Bacillus subtilis* (10).

The pathway involved in the synthesis of vitamins, in particular, vitamin B1 (thiamin) and B6 (pyridoxal), seem to be of great relevance, since they are involved in central processes in the metabolism of carbohydrates and amino acids and the enzymes are found in most bacteria, fungi and plants but not in humans (8), favoring the development of specific drugs with minimal side effects due to the interaction with the host with the same mechanism of action. However, a few questions still stand: (i) how feasible are the targets involved in the pathways for the biosynthesis of thiamin and pyridoxal for the microorganisms with a higher emergency in terms of resistance, or ESKAPE: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa* and *Enterobacter* species? (ii) What enzymes are present and what do we know about them?

Here we used the available genomic and proteomic data to address these questions focusing on the ESKAPE pathogens.

## 2 Methods

The analyses provided here are the result of the interrogation of the enzymes in the biosynthesis pathway for thiamin and pyridoxine/pyridoxal using the KEGG database (11,12). For this purpose, the KEGG pathway for thiamin metabolism (map 00730) was listed for humans as well as for the ESKAPE pathogens using, whenever possible, commercial strains rather than specific or antibiotic-resistant strains. In each analysis, the strain used for the analysis is cited. For the sake of comparison, some additional resistant strains were listed, with no differences in the observed genes for the pathways under study. A similar search was carried out for the vitamin B6 metabolism (map 00750) in KEGG, interrogating the existing enzymes for the ESKAPE pathogens in comparison with humans.

When necessary, the sequence of an enzyme of the pathway was used to search for homologues using BLAST (13) searches against the PDB (14) or the non-redundant database using BLAST default parameters.

## 3 Results and Discussion

### 3.1 Thiamin Biosynthesis

The biosynthesis pathway for thiamin involves two branches, as shown in Figure 1. In summary, thiamin is synthesized from hydroxymethyl (HMP, superior branch in Figure 1) and hydroxyethyl methyazole (THZ, inferior branch). Both compounds are phosphorylated by ThiD and ThiM, respectively. Finally, the enzyme ThiE, central to the pathway, is responsible to synthesize

thiamine phosphate by merging the two branches (15). Thiamin phosphate can be dephosphorylated to thiamine and then pyrophosphorylated to thiamin diphosphate by TPK. Alternatively, thiamin can be degraded to HMP and THZ by TenA, a thiaminase II enzyme.

According to the KEGG pathway database, humans lack many of the enzymes of this pathway, but not all of them. In humans, a TPK (TPK1) enzyme is found, with UNIPROT ID Q9H3S4. Other than that, the remaining enzymes in the biosynthetic pathway for thiamin are missing in humans, making them very attractive for the design of chemical probes that could be used as proof-of-concept compounds.

The analysis of the thiamin for *Enterococcus faecium* (ATCC 8459) in the KEGG database did not identify genes for ThiE, ThiM and TenA. For this species, only ThiD and TPK were positively identified. However, a BLAST search for TenA homologous within the non-redundant database restricted to *Enterococcus faecium* (TAXID 1352) identified a single result for thiaminase II (GenBank: SAM74984.1), four results for ThiE (SAZ10134.1, WP_086323306.1, WP_010732763.1 and WP_072538983.1) and two results for ThiM (SAZ10238.1, EJY48288.1). The small number of hits in the BLAST search may suggest some issues in the annotation.

Interestingly, when the *Enterococcus faecalis* thiamin pathway is compared with the pathway observed for *E. faecium*, many differences are found. The KEGG pathway for *E. faecalis* (ATCC 29212) shows that the entire pathway, as described in Figure 1 is found: ThiD, ThiM, ThiE, TPK and TenA, in contrast with *E. faecium*. Additionally, there is no change in the repertoire of enzymes in the pathway for the Vancomycin-resistant strain V583 as compared to the ATCC 29121 strain.

For *Staphylococcus aureus* (NCTC 8325) and *Klebsiella pneumoniae* (subsp. pneumoniae ATCC 43816 KPPR1), the KEGG pathway indicates that all the enzymes in the pathway are observed, with no changes to an *S. aureus* resistant strain such as COL (MRSA) as compared to the NCTC 8325. For *Acinetobacter baumannii* (ATCC 14978), all enzymes are observed but TPK. Instead, thiamin phosphate may be converted directly to thiamin diphosphate by a thiamin-monophosphate kinase, and then converted to thiamin triphosphate by an adenylate kinase. In the case of *Pseudomonas aeruginosa* (NCGM 1900), some enzymes are missing: ThiD, TenA, TPK. Finally, *Enterobacter sp.* (638) has ThiD, ThiE, ThiM and misses TenA and TPK.

The overall panel of enzymes for the thiamin pathway for the ESKAPE pathogens is summarized in Table 1.

TenA was shown to play a dual role in thiamine synthesis and salvage (16): beyond its function to hydrolyze thiamin, TenA also deaminates aminopyrimidine to form 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP), with the latter activity being about 100 times faster than the former (16,17). In some organisms, such as *Bacillus subtilis*, the deaminase activity is somewhat redundant with the ThiC (ThiA) activity, since both activities provide HMP or HMP phosphate, that can be further phosphorylated by ThiD (9).

In the other branch of the thiamin biosynthesis pathway, thiazole phosphate can be generated from thiazole alcohol incorporated from the medium and phosphorylated by ThiM or it can be synthesized in a series of enzymatically catalyzed reactions using amino acids such as glycine or tyrosine as substrates (9). In this context, according to Begley and coworkers (9), lesions in the ThiM gene were observed to be prototrophic. In contrast, selective mutations of ThiD and ThiE led to the requirement of externally provided thiamin (9).

The ThiE is central to the pathway since the enzyme merges the two branches to finally synthesize thiamin phosphate (Figure 1). It is important to highlight that many of the mutational analysis was carried out in *E. coli* and, as Table 1 shows, there is significant variance among bacterial species.

Taking together, the analysis of the literature suggests that ThiD and ThiE may represent interesting targets for the development of chemical probes to further evaluate their function. TPK is expressed in humans and the inhibition of this enzyme could lead to harmful effects on the human host. TenA inhibition could be overcome by the somewhat functional redundancy with thiC (thiA) and ThiM function can be dispensable since thiazole phosphate can be synthesized *de novo*.

ThiD and ThiE are found in most ESKAPE organisms. Exceptions are *P. aeruginosa*, which lacks ThiD and *E. faecium* that lacks ThiE, according to KEGG data. For ThiE, in particular, it was reported that 4-amino-2-trifluoromethyl-5-hydromethylpyrimidine ($CF_3$-HMP), an HMP analogue, can be converted by ThiD to $CF_3$-HMP pyrophosphate, which in turn inhibits ThiE (8,18). This enzymatic inhibition culminated with the inhibition of *E. coli* growth (8), suggesting that this strategy might be promising for the development of new inhibitors.

In terms of the structural biology, some crystal structures of ThiD are available by the time of the writing of this paper, including the enzymes from *Salmonella enterica* (PDB ID 1JXH (19)), *Clostridioides difficile* (PDB ID 4JJP), *B. subtilis* (PDB ID 2I5B (20)), *Thermus thermophilus* (PDB ID 1UB0), *S. aureus* (PDB ID 4C5J (21)), *A. baumannii* (PDB ID 4YL5), *Bacteroides thetaiotaomicron* (PDB ID 3MBH) and the bifunctional enzyme from *Saccharomyces cerevisiae* (PDB ID 3RM5).

Interestingly, the *S. aureus* ThiD enzyme (*Sa*ThiD) has a dual role, phosphorylating HMP in the thiamin biosynthesis pathway as well as pyridoxal and pyridoxine in the pyridoxal biosynthesis pathway, with a $K_M$ almost 20 times greater for HMP than for pyridoxal and $k_{cat}$ values 3 times faster for pyridoxal (21), suggesting that *Sa*ThiD is more efficient as a pyridoxal kinase than as an HMP kinase. *Sa*ThiD was shown to be inhibited by Rugulactone, a natural product and, in the absence of thiamine in the medium, the MIC observed for Rugulactone was four times lower than in the presence of thiamine (22), suggesting that the inhibitory effect of Rugulactone is due to ThiD inhibition, although Rugulactone also inhibits other kinases.

For ThiE, the crystal structures of a few enzymes are available, including the enzyme from *Pyrococcus furiosus* (PDB ID 1XI3), *B. subtilis* (PDB IDs 1G4T, 3O15, 3O16, 1G69, 1G4E, 1G67, 1G4P, 1YAD (23)) and for the bifunctional enzyme from *Candida glabrata* (PDB IDs 3NL2 and 3NM1 (24)). No crystal structure of an ESKAPE pathogen ThiE is available to date.

### 3.2 Pyridoxal Biosynthesis

Pyridoxal, pyridoxine and pyridoxamine, together with their respective phosphate esters compose the six vitamers for vitamin B6 and can be interconverted (25). There are two biosynthetic routes for pyridoxal 5'-phosphate: a deoxyxylulose 5-phosphate-dependent pathway found in some bacteria and a ribose 5-phosphate-dependent pathway found in all kingdoms (25,26). This second and widespread pathway, curiously, depends on only two enzymes, as shown in Figure 2.

In this pathway, the enzyme Pdx2 converts L-glutamine into L-glutamate and an ammonium molecule. The latter is thought to diffuse to Pdx1 active site, which also used glyceraldehyde 3-phosphate and D-ribose 5-phosphate to synthesize pyridoxal 5'-phosphate (25,27). Interestingly, in order to the properly synthesize pyridoxal 5'-phosphate, a complex involving twelve Pdx1 molecules and twelve Pdx2 molecules has to be assembled (28–30) and this assembly is further stabilized by the interaction with L-glutamine (31).

Similar to what is observed to the thiamine biosynthesis pathway, humans lack the genes for Pdx1 and Pdx2, making the development of specific antibiotic candidates an attracting strategy. In the same direction, for some pathogenic organisms such as *Helicobacter pylori* (32), *Mycobacterium tuberculosis* (33), and *Streptococcus pneumoniae* (34), the depletion in vitamin B6 resulted in reduced virulence (21), indicating that the biosynthesis of this vitamin may be a good strategy for the design of new antibiotic candidates.

Very interestingly and in contrast to what was observed for the thiamine pathways, from the ESKAPE pathogens, *S. aureus* is the only microorganism that has the ribose 5-phosphate dependent pathway for the biosynthesis of pyridoxal 5'-phosphate, as shown in Table 2. As expected, a single enzyme (Pdx1 or Pdx2 alone) is never observed for these organisms (Table 2).

In contrast to what was observed for the thiamin biosynthesis pathway, *E. faecalis*, misses the Pdx enzymes, similar to what was found to *E. faecium*. On the other hand, some other pathogenic organisms, including organisms with high antibiotic resistance rates, have already been identified as susceptible to modulations of the pyridoxal phosphate biosynthesis pathway. Some organisms which have been the focus of basic research include *Plasmodium falciparum* and *Plasmodium vivax*, the malaria pathogens (35–38).

In terms of the structural biology of the enzymes in the vitamin B6 biosynthesis pathway, the *S. aureus* Pdx1 enzyme (UNIPROT ID Q2G0Q1) is a close homologue of *Bacillus subtilis* Pdx1 (81% identity, PDB ID 2NV1 (30)), *Geobacillus stearothermophilus* Pdx1 (78% identity, PDB ID 1ZNN (39)) and *Thermus thermophilus* enzyme (67% identity, PDB ID 2ZBT). *S. aureus* Pdx2, has *Geobacillus kaustophilus* (PDB ID 4WXY (28)), *Geobacillus stearothermophilus* (PDB ID 1Q7R) and *B. subtillis* (PDB IDs 2NV0 and 2NV2 (30)) enzymes as its close homologues, with 60%, 60% and 58% identity in sequence similarity respectively.

Using a structural homology model, Reeksting and coworkers identified some ribose 5'-phosphate analogues with interesting *in vitro* inhibitory effects on the enzyme Pdx1 from *P. falciparum* (40). The compounds were identified in a structure-based computational screening campaign and were shown to have inhibitory effects *in vivo*, as well as the *in vitro* effect. The authors also showed that the *in vivo* effects could be at least partially suppressed in a mutant strain overexpressing Pdx1 and Pdx2, in a clear indication that the effect of the analogues was due their inhibition of the pyridoxal 5'-phosphate biosynthesis pathway (40).

The inhibition of the Pdx2 activity by the glutamine analogue acivicin was also demonstrated to be a feasible strategy for the inhibition of the vitamin B6 biosynthesis pathway (41). Raschle and coworkers showed that when inhibited by acivicin, Pdx2 is incapable of interacting with Pdx1, thus disrupting the pyridoxal 5'-phosphate synthase activity. Interestingly, acivicin is a covalent inhibitor, that binds to a cysteine residue. So, it seems that multiple strategies may be available for the design of new binders, including the inhibition of Pdx2 (covalent and non-covalent), the inhibition of Pdx1 and possibly the inhibition of the assembly of the 24-mer complex with Pdx1/Pdx2.

Finally, a salvage pathway for vitamin B6 is found in many bacteria, as well as in humans. In *E. coli*, it involves two enzymes: PdxK and PdxY. These kinases act phosphorylating the vitamers pyridoxal, pyridoxine and pyridoxamine into their phosphorylated forms (20,25,42). Interestingly, in some organisms, including pathogenic microorganisms such as *S. aureus*, the PdxK activity is carried out by the HMP kinase ThiD, showing some functional convergence between the vitamin B6 and B1 pathways (21). Not surprisingly, this enzyme has also been shown to be validated druggable target for some pathogens, such as *Trypanosoma brucei* for example (43), although specificity may be an important issue, since humans also have genes for PdxK known to be inhibited by drugs like theophylline, for example, with known neurotoxic effects (44).

In conclusion, the biosynthesis pathways for vitamin B1 (thiamine) and B6 (pyridoxal 5'-phosphate) may be interesting molecular targets for the development of new chemical probes aiming to inhibit the synthesis of these essential cofactors in pathogenic organisms. Model compounds, mainly based on substrate analogues showed that inhibition of the vitamin B1 biosynthetic enzymes, ThiD and ThiE, as well as Pdx1, Pdx2 and PdxK in pyridoxal 5'-phosphate biosynthesis pathway have shown an initial proof of concept for the model and it is up the scientific community and/or researcher in industry to explore these target further.

## 4 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## 5 Author Contributions

CW, IP and ASN conceived the project. LGM and RFG analyzed the thiamine pathway, while ALCB and LOCD analyzed the pyridoxal pathway. All authors wrote and approved the manuscript.

## 6 Funding

Financial support was provided by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) through grants 2017/18173-0, 2015/26722-8 and 2015/13684-0 and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), through grants 303165/2018-9 and 406936/2017-0.

## 7 Acknowledgments

The authors thank the funding agencies FAPESP and CNPq. We are also indebted to Maria Auxiliadora M. Santos, Lívia Regina M. Margarido, Josimar Sartori and João Fernando Possatto for their technical support.

## 8 References


1. CDC. Antibiotic resistance threats in the USA. (2013)

2. WHO. Prioritization of Pathogens to Guide Discovery, Research and Development of New Antibiotics for Drug-Resistant Bacterial Infections, Including Tuberculosis. (2017) doi:10.1192/bjp.111.479.1009-a

3. Kelly R, Davies SC. Tackling antimicrobial resistance globally. *Med J Aust* (2017) **207**:371-373.e1. doi:10.5694/mja17.00865

4. Walsh CT, Wencewicz T a. Prospects for new antibiotics: a molecule-centered perspective. *J Antibiot (Tokyo)* (2013) **67**:7–22. doi:10.1038/ja.2013.49

5. Brown ED, Wright GD. Antibacterial drug discovery in the resistance era. *Nature* (2016) **529**:336–343. doi:10.1038/nature17042

6. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: The Keio collection. *Mol Syst Biol* (2006) doi:10.1038/msb4100050

7. Zlitni S, Ferruccio LF, Brown ED. Metabolic suppression identifies new antibacterial inhibitors under nutrient limitation. *Nat Chem Biol* (2013) **9**:796–804. doi:10.1038/nchembio.1361

8. Du Q, Wang H, Xie J. Thiamin (vitamin B1) biosynthesis and regulation: A rich source of antimicrobial drug targets? *Int J Biol Sci* (2011) **7**:41–52. doi:10.7150/ijbs.7.41

9. Begley TP, Downs DM, Ealick SE, McLafferty FW, Van Loon APGM, Taylor S, Campobasso N, Chiu HJ, Kinsland C, Reddick JJ, et al. Thiamin biosynthesis in prokaryotes. *Arch Microbiol* (1999) **171**:293–300. doi:10.1007/s002030050713

10. Richts B, Rosenberg J, Commichau FM. A survey of pyridoxal 5'-phosphate-dependent proteins in the gram-positive model bacterium Bacillus subtilis. *Front Mol Biosci* (2019) **6**: doi:10.3389/fmolb.2019.00032

11. Kanehisa M. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res* (2000) **28**:27–30. doi:10.1093/nar/28.1.27

12. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: New perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res* (2017) **45**:D353–D361. doi:10.1093/nar/gkw1092

13. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. BLAST+: Architecture and applications. *BMC Bioinformatics* (2009) **10**:1–9. doi:10.1186/1471-2105-10-421

14. Berman HM, Battistuz T, Bhat TN, Bluhm WF, Bourne PE, Burkhardt K, Iype L, Jain S, Fagan P, Marvin J, et al. The Protein Data Bank. *Acta Crystallogr Sect D-Biological*

*Crystallogr* (2002) **58**:899–907. doi:10.1107/S0907444902003451

15. Backstrom AD, McMordie RAS, Begley TP. Biosynthesis of Thiamin I: The Function of the thiE Gene Product. *J Am Chem Soc* (1995) **117**:2351–2352. doi:10.1021/ja00113a025

16. Begum A, Drebes J, Kikhney A, Müller IB, Perbandt M, Svergun D, Wrenger C, Betzel C. Staphylococcus aureus thiaminase II: Oligomerization warrants proteolytic protection against serine proteases. *Acta Crystallogr Sect D Biol Crystallogr* (2013) **69**:2320–2329. doi:10.1107/S0907444913021550

17. Jenkins AH, Schyns G, Potot S, Sun G, Begley TP. A new thiamin salvage pathway. *Nat Chem Biol* (2007) **3**:492–497. doi:10.1038/nchembio.2007.13

18. Reddick JJ, Nicewonger R, Begley TP. Mechanistic studies on thiamin phosphate synthase: Evidence for a dissociative mechanism. *Biochemistry* (2001) **40**:10095–10102. doi:10.1021/bi010267q

19. Cheng G, Bennett EM, Begley TP, Ealick SE. Crystal structure of 4-amino-5-hydroxymethyl-2-methylpyrimidine phosphate kinase from Salmonella typhimurium at 2.3 Å resolution. *Structure* (2002) **10**:225–235. doi:10.1016/S0969-2126(02)00708-6

20. Newman JA, Das SK, Sedelnikova SE, Rice DW. The Crystal Structure of an ADP Complex of Bacillus subtilis Pyridoxal Kinase Provides Evidence for the Parallel Emergence of Enzyme Activity During Evolution. *J Mol Biol* (2006) **363**:520–530. doi:10.1016/j.jmb.2006.08.013

21. Nodwell MB, Koch MF, Alte F, Schneider S, Sieber SA. A subfamily of bacterial ribokinases utilizes a hemithioacetal for pyridoxal phosphate salvage. *J Am Chem Soc* (2014) **136**:4992–4999. doi:10.1021/ja411785r

22. Nodwell MB, Menz H, Kirsch SF, Sieber SA. Rugulactone and its Analogues Exert Antibacterial Effects through Multiple Mechanisms Including Inhibition of Thiamine Biosynthesis. *ChemBioChem* (2012) **13**:1439–1446. doi:10.1002/cbic.201200265

23. Peapus DH, Chiu HJ, Campobasso N, Reddick JJ, Begley TP, Ealick SE. Structural characterization of the enzyme-substrate, enzyme-intermediate, and enzyme-product complexes of thiamin phosphate synthase. *Biochemistry* (2001) **40**:10103–10114. doi:10.1021/bi0104726

24. Paul D, Chatterjee A, Begley TP, Ealick SE. Domain organization in Candida glabrata THI6, a bifunctional enzyme required for thiamin biosynthesis in Eukaryotes. *Biochemistry* (2010) **49**:9922–9934. doi:10.1021/bi101008u

25. Mukherjee T, Hanes J, Tews I, Ealick SE, Begley TP. Pyridoxal phosphate: Biosynthesis and catabolism. *Biochim Biophys Acta - Proteins Proteomics* (2011) **1814**:1585–1596. doi:10.1016/j.bbapap.2011.06.018

26. Ehrenshaft M, Bilski P, Li M, Chignell CF, Daub ME. A highly conserved sequence is a novel gene involved in de novo vitamin B6 biosynthesis. *Proc Natl Acad Sci U S A* (1999) **96**:9374–9378. doi:10.1073/pnas.96.16.9374

27. Neuwirth M, Strohmeier M, Windeisen V, Wallner S, Deller S, Rippe K, Sinning I, Macheroux P, Tews I. X-ray crystal structure of Saccharomyces cerevisiae Pdx1 provides insights into the oligomeric nature of PLP synthases. *FEBS Lett* (2009) **583**:2179–2186. doi:10.1016/j.febslet.2009.06.009

28. Smith AM, Brown WC, Harms E, Smith JL. Crystal structures capture three states in the catalytic cycle of a pyridoxal phosphate (PLP) Synthase. *J Biol Chem* (2015) **290**:5226–5239. doi:10.1074/jbc.M114.626382

29. Guédez G, Hipp K, Windeisen V, Derrer B, Gengenbacher M, Böttcher B, Sinning I, Kappes B, Tews I. Assembly of the eukaryotic PLP-synthase complex from Plasmodium and activation of the Pdx1 enzyme. *Structure* (2012) **20**:172–184. doi:10.1016/j.str.2011.11.015

30. Strohmeier M, Raschle T, Mazurkiewicz J, Rippe K, Sinning I, Fitzpatrick TB, Tews I. Structure of a bacterial pyridoxal 5'-phosphate synthase complex. *Proc Natl Acad Sci* (2006) **103**:19284–19289. doi:10.1073/pnas.0604950103

31. Neuwirth M, Flicker K, Strohmeier M, Tews I, Macheroux P. Thermodynamic characterization of the protein-protein interaction in the heteromeric Bacillus subtilis pyridoxalphosphate synthase. *Biochemistry* (2007) **46**:5131–5139. doi:10.1021/bi602602x

32. Grubman A, Phillips A, Thibonnier M, Kaparakis-Liaskos M, Johnson C, Thiberge JM, Radcliff FJ, Ecobichon C, Labigne A, de Reuse H, et al. Vitamin B6 is required for full motility and virulence in Helicobacter pylori. *MBio* (2010) **1**:1–9. doi:10.1128/mBio.00112-10

33. Dick T, Manjunatha U, Kappes B, Gengenbacher M. Vitamin B6 biosynthesis is essential for survival and virulence of Mycobacterium tuberculosis. *Mol Microbiol* (2010) **78**:980–988. doi:10.1111/j.1365-2958.2010.07381.x

34. Qaidi S El, Yang J, Zhang JR, Metzger DW, Bai G. The vitamin B6 biosynthesis pathway in streptococcus pneumoniae is controlled by pyridoxal 5'-phosphate and the transcription factor PdxR and has an impact on ear infection. *J Bacteriol* (2013) **195**:2187–2196. doi:10.1128/JB.00041-13

35. Wrenger C, Knöckel J, Walter RD, Müller IB. Vitamin B1 and B6 in the malaria parasite: Requisite or dispensable? *Brazilian J Med Biol Res* (2008) **41**:82–88. doi:10.1590/S0100-879X2008005000006

36. Kronenberger T, Lindner J, Meissner KA, Zimbres FM, Coronado MA, Sauer FM, Schettert I, Wrenger C. Vitamin B6-dependent enzymes in the human malaria parasite plasmodium falciparum: A druggable target? *Biomed Res Int* (2014) **2014**: doi:10.1155/2014/108516

37. Kronenberger T, Schettert I, Wrenger C. Targeting the vitamin biosynthesis pathways for the treatment of malaria. *Future Med Chem* (2013) **5**:769–779. doi:10.4155/fmc.13.43

38. Wrenger C, Eschbach ML, Müller IB, Warnecke D, Walter RD. Analysis of the vitamin B6 biosynthesis pathway in the human malaria parasite Plasmodium falciparum. *J Biol Chem* (2005) **280**:5242–5248. doi:10.1074/jbc.M412475200

39. Zhu J, Burgner JW, Harms E, Belitsky BR, Smith JL. A new arrangement of (β/α)8 barrels in

the synthase subunit of PLP synthase. *J Biol Chem* (2005) **280**:27914–27923. doi:10.1074/jbc.M503642200

40. Reeksting SB, Müller IB, Burger PB, Burgos ES, Salmon L, Louw AI, Birkholtz LM, Wrenger C. Exploring inhibition of Pdx1, a component of the PLP synthase complex of the human malaria parasite Plasmodium falciparum. *Biochem J* (2013) **449**:175–187. doi:10.1042/BJ20120925

41. Raschle T, Amrhein N, Fitzpatrick TB. On the two components of pyridoxal 5′-phosphate synthase from Bacillus subtilis. *J Biol Chem* (2005) **280**:32291–32300. doi:10.1074/jbc.M501356200

42. Newman JA, Das SK, Sedelnikova SE, Rice DW. Cloning, purification and preliminary crystallographic analysis of a putative pyridoxal kinase from Bacillus subtilis. *Acta Crystallogr Sect F Struct Biol Cryst Commun* (2006) **62**:1006–1009. doi:10.1107/S1744309106035779

43. Jones DC, Alphey MS, Wyllie S, Fairlamb AH. Chemical, genetic and structural assessment of pyridoxal kinase as a drug target in the African trypanosome. *Mol Microbiol* (2012) **86**:51–64. doi:10.1111/j.1365-2958.2012.08189.x

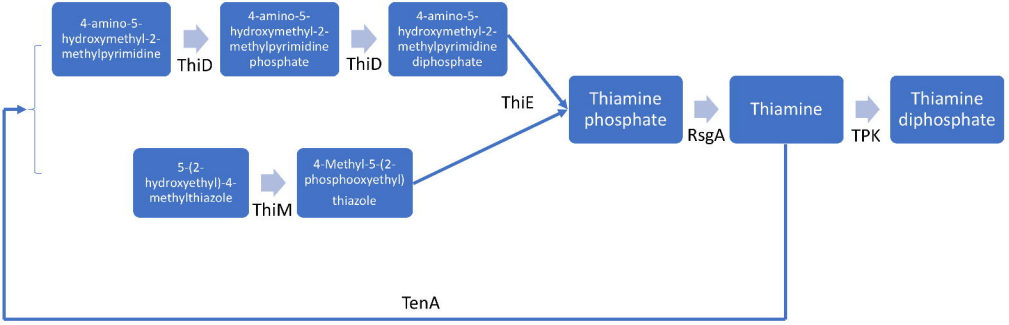
44. Gandhi AK, Desai J V., Ghatge MS, di Salvo ML, Di Biase S, Danso-Danquah R, Musayev FN, Contestabile R, Schirch V, Safo MK. Crystal structures of human pyridoxal kinase in complex with the neurotoxins, ginkgotoxin and theophylline: Insights into pyridoxal kinase inhibition. *PLoS One* (2012) **7**:1–11. doi:10.1371/journal.pone.0040954

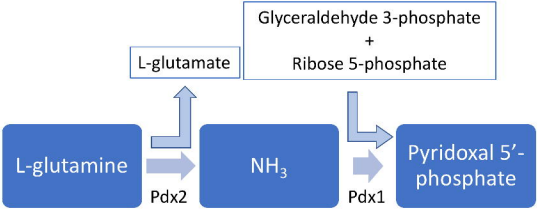
**Table 1. Panel of observed enzymes for the thiamine pathway in ESKAPE pathogens.**

| **Enzyme** | *Enterococcus faecium (ATCC 8459)* | *Staphylococcus aureus (NCTC 8325)* | *Klebsiella pneumoniae (subsp. Pneumoniae ATCC 43816 KPPR1)* | *Acinetobacter baumannii (ATCC 17978)* | *Pseudomonas aeruginosa (NCGM 1900)* | *Enterobacter sp. (638)* |
|---|---|---|---|---|---|---|
| **ThiD** | ✓ | ✓ | ✓ | ✓ | × | ✓ |
| **THiM** | × | ✓ | ✓ | ✓ | ✓ | ✓ |
| **ThiE** | × | ✓ | ✓ | ✓ | ✓ | ✓ |
| **TPK** | ✓ | ✓ | ✓ | × | × | × |
| **TenA** | × | ✓ | ✓ | ✓ | × | × |

**Table 2. Panel of observed enzymes for the pyridoxal 5'-phosphate pathway in ESKAPE pathogens.**

| **Enzyme** | *Enterococcus faecium (ATCC 8459)* | *Staphylococcus aureus (NCTC 8325)* | *Klebsiella pneumoniae (subsp. Pneumoniae ATCC 43816 KPPR1)* | *Acinetobacter baumannii (ATCC 17978)* | *Pseudomonas aeruginosa (NCGM 1900)* | *Enterobacter sp. (638)* |
|---|---|---|---|---|---|---|
| **Pdx1** | × | ✓ | × | × | × | × |
| **Pdx2** | × | ✓ | × | × | × | × |


4-amino-5-hydroxymethyl-2-methylpyrimidine
ThiD
4-amino-5-hydroxymethyl-2-methylpyrimidine phosphate
ThiD
4-amino-5-hydroxymethyl-2-methylpyrimidine diphosphate
ThiE
5-(2-hydroxyethyl)-4-methylthiazole
ThiM
4-Methyl-5-(2-phosphooxyethyl) thiazole
Thiamine phosphate
RsgA
Thiamine
TPK
Thiamine diphosphate
TenA


Glyceraldehyde 3-phosphate
+
Ribose 5-phosphate
L-glutamate
L-glutamine
Pdx2
$NH_3$
Pdx1
Pyridoxal 5'-phosphate