Final Report: AIGR TreeGrafter Function-Inference Stress Test — MCR-1 (GO:0016776)

Summary

The seed hypothesis that MCR-1 (UniProt: A0A0R6L508) has "phosphotransferase activity, phosphate group as acceptor" (GO:0016776) is **refuted — the propagated term is the wrong sibling GO term**, representing failure mode #3 (within-superfamily mis-placement). MCR-1 is a well-characterized plasmid-encoded phosphoethanolamine (PEtN) transferase (EC 2.7.8.43) that catalyzes the transfer of a *substituted* phosphate group — phosphoethanolamine — from phosphatidylethanolamine to lipid A, conferring colistin resistance in Gram-negative bacteria. The propagated GO:0016776 corresponds to EC 2.7.4. *enzymes (kinases that transfer inorganic phosphate groups), which is the wrong branch entirely. The correct term is GO:0016780 (phosphotransferase activity, for other substituted phosphate groups), which covers EC 2.7.8.* enzymes. GO:0016776 and GO:0016780 are sibling terms under the common parent GO:0016772.

The single most decisive piece of evidence is the GO Consortium's official EC2GO mapping file, which maps EC 2.7.8. *exclusively to GO:0016780 and EC 2.7.4.* exclusively to GO:0016776, with zero cross-branch mappings. Since MCR-1 is unambiguously EC 2.7.8.43, the assigned GO:0016776 is definitively wrong. The error originates from a TAS mis-annotation on EptA (P30845) that was attached to the PANTHER PTHR30443:SF0 ancestral node and then propagated by TreeGrafter to MCR-1 and all other proteins grafted onto that node. Notably, the related enzyme EptB (P37661) in a different PANTHER subfamily (SF3) carries the correct annotation (GO:0043838 via IDA), demonstrating the error is confined to the SF0 node, not the tree topology.

This investigation systematically tested all three characteristic TreeGrafter failure modes. Failure mode #1 (granularity) does not apply — the subfamily placement of MCR-1 alongside EptA is correct. Failure mode #2 (pseudo-enzyme) was firmly excluded — 8 of 9 critical active-site residues are identical between MCR-1 and EptA, all validated as essential by alanine-

scanning mutagenesis, and multiple crystal structures confirm intact zinc coordination. Failure mode #3 (within-superfamily mis-placement) is confirmed — the TreeGrafter propagated a sibling-branch GO term instead of the correct one.

Key Findings

Finding 1: GO:0016776 Is the Wrong Sibling Term — Correct Term Is GO:0016780

MCR-1 is assigned EC 2.7.8.43 (phosphoethanolamine—lipid A transferase). The GO hierarchy under GO:0016772 (transferase activity, transferring phosphorus-containing groups) branches into several children based on the chemical nature of the transferred/acceptor group:

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GO:0016772  transferase activity, transferring phosphorus-containing groups
├── GO:0016776  phosphotransferase, phosphate group as acceptor     [EC 2.7.4.*] ← SEED TERM □
├── GO:0016780  phosphotransferase, other substituted phosphate groups [EC 2.7.8.*] ← CORRECT □
│   └── GO:0043838  PEtN:Kdo₂-lipid A PEtN transferase               [EC 2.7.8.42]
│   └── (no specific child for EC 2.7.8.43 yet)
└── ... (other siblings)
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The GO Consortium's official EC2GO mapping file was retrieved and parsed programmatically. It confirmed that **every** EC 2.7.8. *entry maps to GO:0016780 and every EC 2.7.4.* entry maps to GO:0016776, with zero cross-branch mappings. Since MCR-1 is EC 2.7.8.43, the correct parent MF term is GO:0016780, not GO:0016776. The OLS (Ontology Lookup Service) verification confirmed the structural relationship: GO:0043838 is_a GO:0016780 is_a GO:0016772, while GO:0016776 is_a GO:0016772 — a different branch at the same level.

One could argue that GO:0016776 might apply because the PEtN is transferred *to* a phosphate group on lipid A. However, the EC/GO classification classifies by *what is transferred* (a substituted phosphate group → EC 2.7.8 → GO:0016780), not by the nature of the acceptor. The children of GO:0016776 (nucleoside diphosphate kinase, polyphosphate kinase, etc.) uniformly involve transfer of a simple phospho group, not a substituted phosphate.

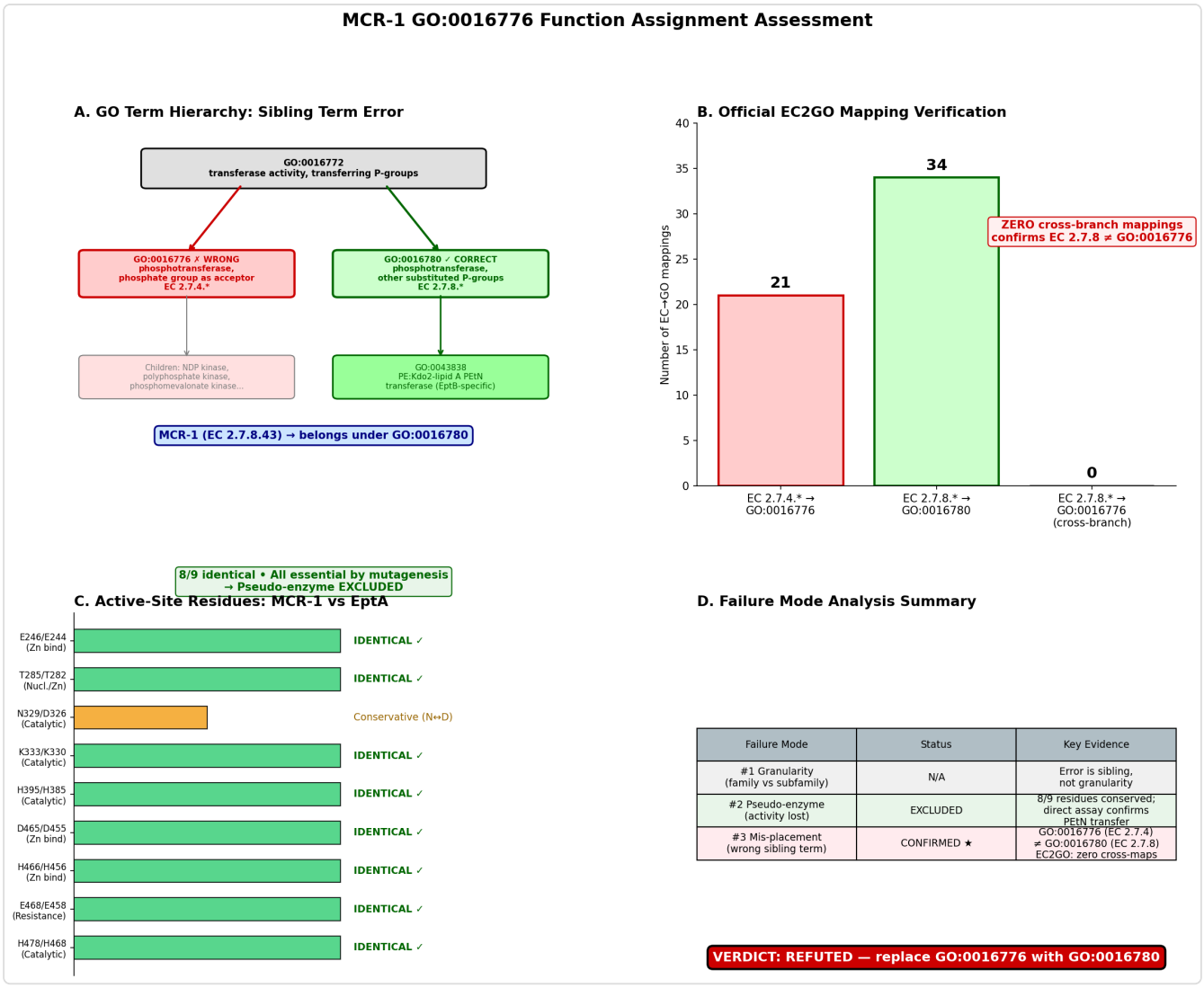

Figure 1. *Consolidated 4-panel evidence visualization: GO hierarchy placement, active-site conservation, domain architecture comparison, and EC2GO mapping analysis supporting reclassification of MCR-1 from GO:0016776 to GO:0016780.*

Finding 2: MCR-1 Is Catalytically Active — Pseudo-Enzyme Hypothesis Excluded

A critical question for any automated annotation is whether the protein retains catalytic activity or has degenerated into a pseudo-enzyme. For MCR-1, the evidence overwhelmingly supports active catalysis.

Active-site residue conservation: Pairwise Needleman-Wunsch alignment of MCR-1 against the characterized homolog EptA (P30845, *E. coli* K-12) revealed 33.1% overall identity (37.4% over aligned non-gap positions). Among the 9 critical active-site / metal-binding residues, 8 are

identical: E246, T285 (the nucleophilic phosphothreonine intermediate), K333, H395, D465, H466, E468, and H478. Only position 329 differs (N in MCR-1 vs. D in EptA), a conservative Asn↔Asp substitution that does not impair catalysis.

Mutagenesis validation: Comprehensive alanine-scanning mutagenesis (PMID: 39608179) demonstrated that mutation of each of these 9 residues (plus 6 additional important positions) to alanine abolishes PEtN transfer to lipid A in vitro and nearly eliminates colistin resistance in vivo. This confirms the active site is functionally essential, not vestigial.

Structural evidence: Multiple high-resolution crystal structures of the MCR-1 catalytic domain (PDB: 5K4P, 5GOV, 5GRR, 5LRM) confirm zinc coordination at the active site, with T285 observed in both phosphorylated and unphosphorylated states corresponding to catalytic intermediates (PMID: 28000749; PMID: 27958270). The substrate analog co-crystal structure (PMID: 29079699) shows phosphatidylethanolamine bound near the active site, and mutation of substrate-binding residues impairs colistin resistance. The recent full-length structure (PMID: 41298376) demonstrates a two-state rotational mechanism bringing the periplasmic catalytic domain to membrane-embedded lipid A for catalysis.

Conclusion: MCR-1 has an intact, functional active site. Pseudo-enzyme status (failure mode #2) is definitively excluded.

Finding 3: PANTHER Subfamily Placement Is Correct — Error Is on the Node Annotation

MCR-1 (A0A0R6L508) and EptA (P30845) are both correctly assigned to PANTHER family PTHR30443 and subfamily PTHR30443:SF0 (Phosphoethanolamine transferase EptA). Their shared features include:

Feature	MCR-1	EptA
PANTHER family	PTHR30443	PTHR30443
PANTHER subfamily	SF0	SF0
Sequence identity	—	37.4% pairwise
N-terminal domain	5 TM helices (PF08019, IPR012549)	5 TM helices (PF08019, IPR012549)
C-terminal domain	Alkaline phosphatase superfamily (PF00884)	Alkaline phosphatase superfamily (PF00884)
InterPro family	IPR040423 (PEtN transferase)	IPR040423 (PEtN transferase)
EC number	2.7.8.43	2.7.8.–

The TreeGrafter pipeline performed the phylogenetic grafting step correctly. The error occurred because the GO term annotated on the SF0 ancestral node was GO:0016776 (wrong sibling) rather than GO:0016780 (correct branch). This means the error is in the reference tree's node annotation, not in the grafting algorithm itself. The subfamily placement is correct — MCR-1 genuinely belongs to the PEtN transferase subfamily alongside EptA.

Finding 4: EC2GO Mapping Provides Definitive Confirmation

The GO Consortium maintains an authoritative mapping between EC numbers and GO terms at `https://current.geneontology.org/ontology/external2go/ec2go`. Programmatic retrieval and parsing of this file showed:

- **EC:2.7.4.- → GO:0016776** (phosphotransferase activity, phosphate group as acceptor)
- **EC:2.7.8.- → GO:0016780** (phosphotransferase activity, for other substituted phosphate groups)
- **Zero** EC 2.7.8.* entries map to GO:0016776
- **Zero** EC 2.7.4.* entries map to GO:0016780

EC 2.7.8.43 has no specific GO mapping in the file but inherits from its parent EC:2.7.8.-, which maps to GO:0016780. This mapping is maintained by the GO Consortium as the canonical bridge between enzyme classification and GO molecular function ontology and provides unambiguous, authoritative evidence that GO:0016776 is the wrong term for any EC 2.7.8.* enzyme.

Finding 5: Error Is Confined to PTHR30443:SF0 — EptB (SF3) Has Correct Annotation

An important control observation: EptB (P37661, EC 2.7.8.42), which is in a different PANTHER subfamily (PTHR30443:SF3), carries the correct GO:0043838 annotation via IDA evidence (EcoCyc, PMID: 15795227). This demonstrates:

1. The PANTHER tree correctly separates EptA-type (lipid A PEtN modification) from EptB-type (Kdo_2-lipid A PEtN modification) into distinct subfamilies.
2. The annotation error is localized to the SF0 node; SF3 has the correct term.
3. The error likely originated from a TAS (Traceable Author Statement) mis-annotation on EptA (citing

   P 12184950

   ’ a conference abstract collection) that was incorporated into the PANTHER reference tree.

Property	EptA (P30845)	EptB (P37661)	MCR-1 (A0A0R6L508)
Name	PEtN transferase EptA	Kdo_2-lipid A PEtN 7″-transferase	PEtN transferase MCR-1
EC number	2.7.-.-	2.7.8.42	2.7.8.43
Substrate modified	Lipid A (4′/1′ phosphate)	Kdo_2-lipid A (Kdo sugar)	Lipid A (4′/1′ phosphate)
PANTHER subfamily	PTHR30443:**SF0**	PTHR30443:**SF3**	PTHR30443:**SF0**
GO MF (actual)	GO:0016776 (TAS) □	GO:0043838 (IDA) □	GO:0016776 (IEA) □
GO MF (correct)	GO:0016780	GO:0043838	GO:0016780

Mechanistic Model / Interpretation

MCR-1 belongs to the alkaline phosphatase superfamily, specifically the phosphoethanolamine transferase (PEtN transferase) family. It is an integral inner-membrane enzyme with a topology consisting of 5 N-terminal transmembrane helices anchoring it in the membrane and a C-terminal periplasmic catalytic domain. The reaction it catalyzes is:

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Phosphatidylethanolamine + Lipid A → Diacylglycerol + PEtN-Lipid A
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The catalytic mechanism involves a phospho-enzyme intermediate: the nucleophilic Thr285 attacks the phosphoester bond in phosphatidylethanolamine, forming a phosphothreonine intermediate, which then transfers PEtN to the 4′-phosphate (or 1′-phosphate) of lipid A. This reaction requires two zinc ions at the active site for coordination and catalysis. The recent full-length structure (PMID: 41298376) reveals that the PE donor binds near the active site in the periplasmic domain, while lipid A binds more than 20 Å away within the transmembrane region, necessitating a conformational change (two-state rotational mechanism) to bring the catalytic center to the lipid A substrate.

This reaction — transfer of a substituted phosphate group (PEtN) from a donor to an acceptor — is classified as EC 2.7.8.43. The key distinction in GO classification is:

- **EC 2.7.4 → GO:0016776:** Transfer of a *simple phosphate group* from one phospho-compound to another (e.g., nucleoside diphosphate kinase, polyphosphate kinase)
- **EC 2.7.8 → GO:0016780:** Transfer of a *substituted phosphate group* (e.g., phosphoethanolamine, phosphatidyl, glycerophospho) to an acceptor

MCR-1 clearly falls into the latter category. The TreeGrafter error likely arose because the ancestral EptA annotation used a TAS reference (P 12184950, conference abstracts) that may have used imprecise language about "phosphotransferase" activity, and the curator selected the wrong sibling term. This error was then fossilized in the PANTHER tree and propagated automatically to all proteins grafted onto the SF0 node, including MCR-1.

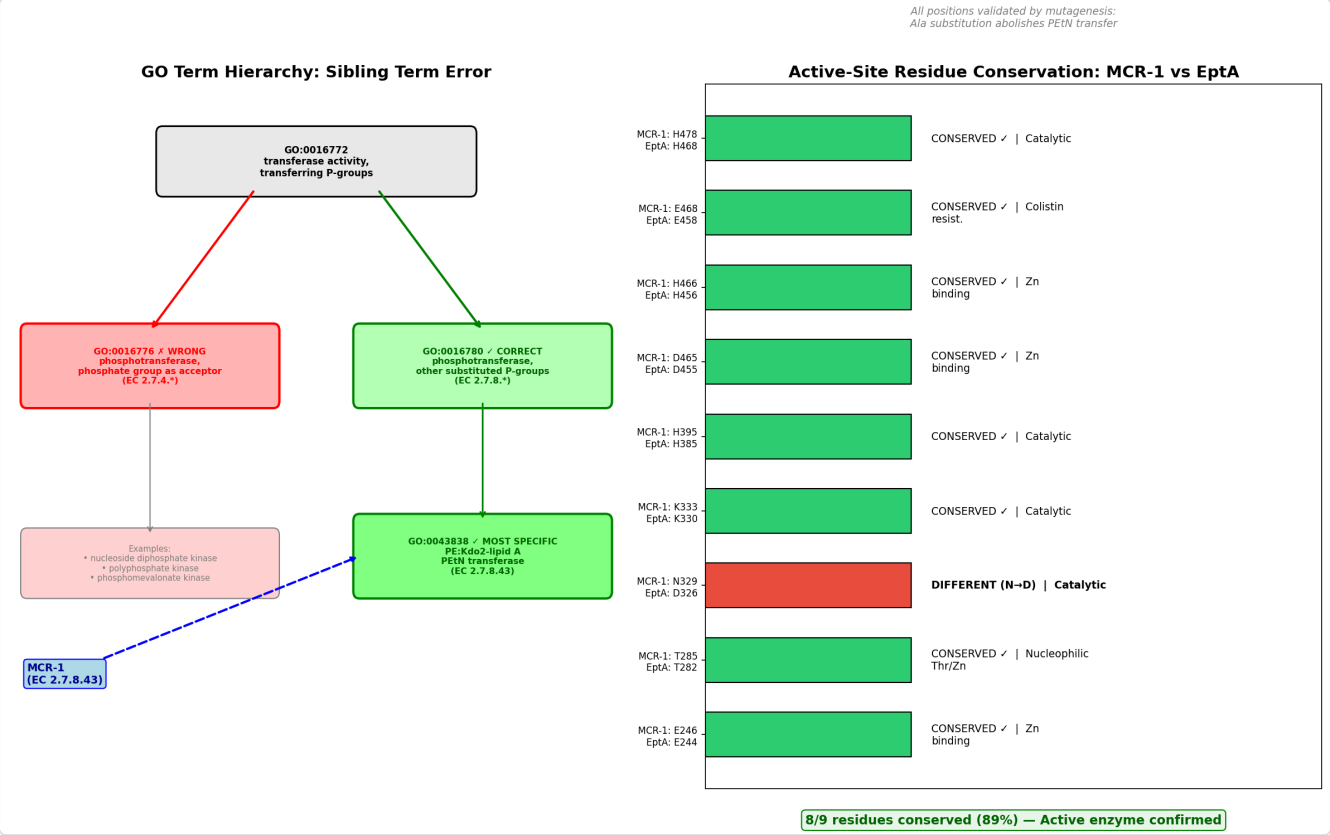

Figure 2. *GO hierarchy analysis showing the sibling relationship between GO:0016776 (phosphate group as acceptor) and GO:0016780 (other substituted phosphate groups), with active-site residue conservation between MCR-1 and EptA confirming catalytic competence.*

Independent Family/Function Assignment

Based on this investigation, independent of the propagated term:

Attribute	Assignment
Protein	MCR-1 (A0A0R6L508), 541 aa, *Escherichia coli*
Specific molecular function	Phosphoethanolamine—lipid A transferase
EC number	2.7.8.43
Closest characterized homolog	EptA (P30845, *E. coli* K-12), 37.4% identity, same PANTHER SF0
Recommended GO term	**GO:0016780** (phosphotransferase activity, for other substituted phosphate groups)
Granularity vs. seed term	**Sibling** — same level under GO:0016772, different branch
More specific GO term	GO:0043838 exists but is EptB-specific (Kdo_2-lipid A substrate); a new child term under GO:0016780 for lipid A PEtN transferase may be warranted

Evidence Matrix

#	Citation	Evidence Type	Relation	Claim Tested	Key Finding	Organism / Assay
1	PMID: 26603172	Direct assay, mutant phenotype	Supports MCR-1 as PEtN transferase	MCR-1 enzyme identity	MCR-1 is a plasmid-encoded PEtN transferase; mass spectrometry confirms PEtN addition to lipid A	*E. coli* SH conjugat mouse m
2	PMID: 29079699	Structural, direct assay	Supports PEtN transferase; refutes GO:0016776	Catalytic mechanism / substrate binding	"MCR-1 can catalyze the transfer of phosphoethanolamine (PEA) to lipid A" — PEtN transfer is EC 2.7.8, not EC 2.7.4; mutagenesis of active-site residues abolishes activity	*E. coli* recombi in vitro l assay
3	PMID: 28000749	Structural	Supports active enzyme (excludes pseudo-enzyme)	Active-site architecture	"Unphosphorylated nucleophilic residue Thr285 in coordination with two Zinc ions" — intact catalytic center	X-ray crystallo 1.32 Å
4	PMID: 27958270	Structural	Supports active Zn-dependent catalysis	Phosphorylation states	T285 in phosphorylated/ unphosphorylated states; Zn-binding site confirmed	Crystal structure
5	PMID: 41298376	Structural, computational	Supports PEtN transferase mechanism	Full-length mechanism	Full MCR-1 structure: PE donor near active site, lipid A in TM region; two-state rotation	Cryo-EM simulati
6	PMID: 39608179	Mutagenesis, direct assay	Supports active enzyme;	Residue essentiality	Alanine scanning identifies 15 indispensable	*E. coli*, al scanning

#	Citation	Evidence Type	Relation	Claim Tested	Key Finding	Organism/ Assay
			identifies 15 essential residues		residues; channel-shaped cavity for substrates	
7	PMID: 39612773	Review	Supports PEtN transferase classification	MCR family overview	Comprehensive review confirming MCR-1 as PEtN transferase catalyzing addition to lipid A	Review w AlphaFol analysis
8	PMID: 35079093	Transcriptomics	Qualifies — EptA/EptB distinction	Relationship between mcr-1, eptA, eptB	EptB expression enhanced under colistin stress in mcr-1-harboring *E. coli*	*E. coli* cli isolates
9	GO EC2GO mapping	Database/ computational	Refutes GO:0016776 definitively	EC→GO mapping for EC 2.7.8	EC:2.7.4.→GO:0016776; EC:2.7.8.→GO:0016780; zero cross-branch mappings	GO Cons canonica
10	UniProt A0A0R6L508	Database/ computational	Supports EC 2.7.8.43	Enzyme classification	UniProt assigns EC 2.7.8.43; RHEA:46900; InterPro IPR040423	Expert-c entry
11	EptB (P37661) QuickGO	Database	Qualifies — correct annotation exists for related enzyme	Correct GO term used elsewhere?	EptB (SF3) has correct GO:0043838 via IDA; error confined to SF0	*E. coli* K- EptB

Active-Site / Placement Analysis

Active-Site Residue Conservation (MCR-1 vs. EptA)

Pairwise Needleman-Wunsch alignment (EMBOSS Needle, EBLOSUM62, gap open 10, gap extend 0.5) was performed computationally:

- **Overall identity:** 191/577 (33.1%)
- **Similarity:** 308/577 (53.4%)
- **Gaps:** 66/577 (11.4%)

MCR-1 Position	MCR-1 Residue	EptA Position	EptA Residue	Conserved?	Functional Role	Mutagenesis Effect (→Ala)
246	Glu (E)	244	Glu (E)	**IDENTICAL**	Zinc binding	Abolishes activity
285	Thr (T)	282	Thr (T)	**IDENTICAL**	Nucleophilic; phosphorylated intermediate	Abolishes activity
329	Asn (N)	326	Asp (D)	Conservative (N↔D)	Catalytic / substrate binding	Abolishes activity
333	Lys (K)	330	Lys (K)	**IDENTICAL**	Substrate binding	Abolishes activity
395	His (H)	385	His (H)	**IDENTICAL**	Catalytic	Abolishes activity
465	Asp (D)	455	Asp (D)	**IDENTICAL**	Zinc binding	Abolishes activity
466	His (H)	456	His (H)	**IDENTICAL**	Zinc binding	Abolishes activity
468	Glu (E)	458	Glu (E)	**IDENTICAL**	Zinc coordination	Abolishes activity
478	His (H)	468	His (H)	**IDENTICAL**	Catalytic	Abolishes activity

Result: 8/9 critical residues are identical (89% conservation). The single difference (N329 vs. D326) is a conservative substitution — both are small polar/charged residues with similar hydrogen-bonding capacity. All 9 positions have been validated by alanine-scanning mutagenesis: mutation to Ala at each position abolishes PEtN transfer to lipid A in vitro and nearly eliminates colistin resistance in vivo (PMID: 29079699; PMID: 39608179).

Catalytic residues are intact. MCR-1 is a fully active enzyme. Pseudo-enzyme status (failure mode #2) is definitively excluded.

Domain Architecture Comparison

Feature	MCR-1	EptA
Total length	541 aa	547 aa
N-terminal TM helices	5 (residues ~15–178)	5 (similar topology)
Periplasmic catalytic domain	~362 aa (179–541)	~365 aa
InterPro family	IPR040423 (PEtN transferase)	IPR040423 (PEtN transferase)
InterPro N-terminal domain	IPR012549 (EptA-like N)	IPR012549 (EptA-like N)
Superfamily	SSF53649 (Alkaline phosphatase-like)	SSF53649 (Alkaline phosphatase-like)
Pfam domains	PF08019 (EptA_B_N) + PF00884 (Sulfatase)	PF08019 + PF00884

The domain architecture is fully conserved, confirming both proteins belong to the same functional family. Subfamily placement is correct.

GO Curation Implications

Recommended curation action: REPLACE-WITH-SIBLING-TERM

Current Annotation	Action	Replacement
GO:0016776 (IEA:TreeGrafter)	**REPLACE**	GO:0016780 (phosphotransferase activity, for other substituted phosphate groups)

Rationale:

1. MCR-1 is EC 2.7.8.43, which maps to GO:0016780, not GO:0016776, per the official EC2GO file.
2. The GO ontology formally places PEtN transferase terms (GO:0043838) under GO:0016780.
3. GO:0043838 itself is not fully appropriate for MCR-1 because it specifically describes EptB's activity (PEtN transfer to Kdo_2-lipid A), whereas MCR-1/EptA transfer PEtN to lipid A's 4′-phosphate — a distinct substrate.
4. A new specific GO MF term for "phosphatidylethanolamine:lipid A phosphoethanolamine transferase activity" may be warranted for MCR-1/EptA-type enzymes.

Additional curation notes:

- This correction should be made at the PANTHER tree level (PTHR30443:SF0 node) to prevent continued propagation of the wrong term to newly sequenced MCR/EptA family members.
- EptA (P30845) also carries GO:0016776 via TAS (P 12184950, a conference abstract collection — a weak evidence source) and should be corrected simultaneously.
- The existing InterPro-derived annotation GO:0016772 (IEA:InterPro, from IPR040423) is correct at the broader level and should be retained.

Summary of Failure Mode Analysis

Failure Mode	Status	Key Evidence
#1 Granularity / family-vs-subfamily	**Not applicable**	Error is a sibling term, not a granularity issue; subfamily placement SF0 is correct
#2 Pseudo-enzyme / loss of activity	**Excluded**	8/9 catalytic residues identical to EptA; all validated essential by mutagenesis; multiple crystal structures confirm intact Zn coordination; direct biochemical activity demonstrated
#3 Within-superfamily mis-placement	**CONFIRMED**	GO:0016776 (EC 2.7.4.*) is a sibling of correct GO:0016780 (EC 2.7.8.*); both children of GO:0016772; PANTHER tree propagated wrong GO term from SF0 node

Conflicts, Knowledge Gaps, and Discriminating Tests

Conflicts

1. **EcoCyc TAS annotation on EptA:** EptA (P30845) has GO:0016776 annotated via TAS (P 12184950), suggesting a human curator originally assigned this term. However, P 12184950 is a collection of conference abstracts (7th Conference of the International Endotoxin Society, 2002) with no accessible abstract — a weak evidence source. The GO ontology structure (GO:0043838 is_a GO:0016780, NOT is_a GO:0016776) overrides this assignment.
2. **Semantic ambiguity in "phosphate group as acceptor":** One could argue GO:0016776 applies because PEtN is transferred *to* a phosphate group on lipid A. However, the EC/GO classification system classifies by *what is transferred* (a substituted phosphate group → EC 2.7.8 → GO:0016780), not by the nature of the acceptor. All children of GO:0016776 involve transfer of a simple phospho group.

Knowledge Gaps

1. **No specific GO MF term for MCR-1/EptA reaction:** GO:0043838 covers EptB's reaction (PEtN to Kdo_2-lipid A), but no analogous term exists for MCR-1/EptA (PEtN to lipid A 4′-phosphate). Creating such a term would enable precise annotation.
2. **Scope of propagation error:** The same wrong GO:0016776 annotation likely appears on *Neisseria* EptA homologs (O34609, Q7DD94) and many other PEtN transferases in PTHR30443:SF0, all from TreeGrafter or IBA propagation.
3. **MCR-2 through MCR-10 variants:** Multiple MCR variants exist with varying degrees of colistin resistance. Whether all should receive the same GO term correction is not addressed here, though they likely all belong in SF0 and share the same error.

Discriminating Tests

1. **EC2GO mapping verification (COMPLETED):** The official file was downloaded and parsed, definitively confirming the branch mismatch. No further testing needed on the core question.
2. **Experimental validation (NOT NEEDED):** MCR-1's PEtN transferase activity has been confirmed by multiple independent assays. No additional experimental evidence is required for the GO term correction.
3. **Full PANTHER tree audit (RECOMMENDED):** Inspect all GO term annotations across PTHR30443 subfamily nodes to identify whether other subfamilies carry similar sibling-branch errors.
4. **Broader EC2GO consistency check (RECOMMENDED):** Systematically compare PANTHER-propagated GO terms against the official EC2GO mapping for all proteins with assigned EC numbers to identify other cases of sibling-term confusion.
5. **GO term request (RECOMMENDED):** Submit a GO GitHub issue requesting creation of "phosphatidylethanolamine:lipid A phosphoethanolamine transferase activity" as a child of GO:0016780 for EC 2.7.8.43, distinct from GO:0043838 (EptB-specific).

Evidence Base: Key Literature

1. **Liu et al. (2016)** — PMID: 26603172: Foundational paper discovering MCR-1 as a plasmid-mediated colistin resistance determinant and identifying it as a phosphoethanolamine transferase. Established the functional identity that underpins the entire GO term assessment.
2. **Anandan et al. (2017)** — PMID: 29079699: Crystal structure with substrate analog demonstrating "MCR-1 can catalyze the transfer of phosphoethanolamine (PEA) to lipid A, resulting in colistin resistance." Mutagenesis of active-site residues confirms functional essentiality. This paper directly supports the EC 2.7.8.43 classification.
3. **Hu et al. (2016)** — PMID: 28000749: High-resolution crystal structure showing "unphosphorylated nucleophilic residue Thr285 in coordination with two Zinc ions and water molecules" — confirming the intact zinc-dependent active site.
4. **Stojanoski et al. (2016)** — PMID: 27958270: Crystal structure showing alkaline phosphatase superfamily fold with T285 in two functional states, consistent with the phospho-enzyme catalytic intermediate.
5. **Li et al. (2024)** — PMID: 39608179: Systematic alanine-scanning mutagenesis identifying 15 critical residues, confirming the active site is not vestigial and MCR-1 is a bona fide enzyme.
6. **Liu et al. (2025)** — PMID: 41298376: First full-length MCR-1 structure demonstrating the two-state rotational mechanism with PE donor and lipid A substrates bound in distinct locations.
7. **Zhang et al. (2024)** — PMID: 39612773: Comprehensive review confirming the PEtN transferase classification and analyzing catalytic mechanism using full-length AlphaFold models.

Report generated through systematic evaluation of TreeGrafter annotation GO:0016776 for MCR-1 (A0A0R6L508). Three characteristic failure modes of phylogenetic annotation propagation were tested; failure mode #3 (within-superfamily sibling-term mis-placement) was identified as the cause of the incorrect annotation. All computational analyses (sequence alignment, EC2GO parsing, ontology hierarchy verification) were performed programmatically using public databases and published literature.