Chapter

Computational Studies of Drug Repurposing Targeting P-Glycoprotein-Mediated Multidrug Resistance Phenotypes in Priority Infectious Agents

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Abstract

ABCB1 P-glycoprotein (P-gp) is an ATP-dependent efflux pump with broad substrate specificity associated with cellular drug resistance. Homologous to role in mammalian biology, P-glycoproteins of bacterial and fungal pathogens mediate the emergence of multidrug resistance phenotypes, with widespread clinical/socioeconomic implications. This work aims to characterize P-gp homologues in certain WHO-prioritized infectious agents, namely (1) bacteria: *Acinetobacter baumannii* and *Staphylococcus aureus* and (2) fungi: *Aspergillus fumigatus*, *Candida albicans*, and *Cryptococcus neoformans*. PSI-BLAST searches against the genome of each of these organisms confirmed the presence of P-gp homologues. Each homologue was aligned against five known P-gp structures, for structural modeling. FDA-approved antibiotics used in the current line of therapy were retrieved from PubChem, and potential antibiotics were identified based on similarity and repurposing of the existing drugs. The most tenable target-ligand conformations from docking studies of the respective modeled P-gp structures and the antibiotic ligands were assessed for interacting residues within 4.5 Å of the ligand, probable binding pockets and relative efficacies of the new drugs. Our studies could lay the foundation for the development of effective synergistic or new therapies against these pathogens.

Keywords: P-glycoprotein, priority pathogen list, nosocomial infection, multidrug resistance, homology modeling, receptor-ligand docking, differential ligand affinity, synergistic effects

1. Introduction

1.1 Multidrug resistance (MDR)

Bacterial evolution tends to respond to the selection constraint of reckless antibiotic use, which has led to the emergence of drug-resistant strains mediated by

varied defense mechanisms. The main mechanisms whereby infectious agents develop resistance to antimicrobial chemotherapy include enzymatic inactivation, modification of the drug target(s), and reduction of intracellular drug concentration by changes in membrane permeability or by the overexpression of efflux pumps [1]. Multidrug resistance efflux pumps are recognized as an important component of resistance in both Gram-positive and Gram-negative bacteria [2]. Some bacterial efflux pumps may be selective for one substrate or transport antibiotics of different classes, conferring a multidrug resistance phenotype. With respect to efflux pumps, they provide a self-defense mechanism whereby antibiotics are extruded from the cell interior to the external environment. This results in sublethal drug concentrations at the active site that in turn may predispose the organism to the development of high-level target-based resistance [3]. Therefore, efflux pumps are viable antibacterial targets and the development of potent efflux pump inhibitors is a promising and valid strategy to rejuvenate the activity of antibiotics that are no longer effective against bacterial pathogens. The world is searching for new tools to combat multidrug resistance.

1.2 P-glycoprotein (P-gp)

ATP-binding cassette (ABC) transporters are found in all phyla and constitute one of the largest protein superfamilies. ABC transporters such as ABCB1 (P-glycoprotein/P-gp), ABCG2, and ABCC1 are well known for their association with multidrug resistance, effluxing structurally diverse compounds, powered by the hydrolysis of ATP [4]. P-gp also plays an important role in the pharmacokinetics of many drugs, altering their absorption, distribution, and excretion. P-gp has been extensively studied since 1976, when it was identified as the multidrug efflux pump in Chinese hamster ovary cells that had been selected for resistance to colchicine [3].

In eukaryotes, it takes the form of a single polypeptide chain consisting of two transmembrane domains (TMDs) that are usually arranged into six transmembrane-spanning α-helices that form the pathway through which substrate crosses the membrane. These domains also form the substrate-binding site (or sites) which contribute to transport specificity. The two nucleotide-binding domains (NBDs) couple the energy of ATP catalysis to transport [5]. In some prokaryotes, however, the P-gp structure comprises a monomeric assembly, namely, a single TMD and a single NTD. The various domains can comprise one, two, or four polypeptide chains, encoded by the same or different genes, which assemble into monomers, homo- or heterodimers, or tetramers.

Prokaryotes harbor both importers for nutrient uptake (including amino acids, sugars, and metal ions) and exporters (drugs, toxins, polysaccharides, lipids, and proteins), whereas eukaryotes harbor only exporters [6]. It is believed that this transporter functions through an alternate access mechanism involving two different conformations. Drug binding occurs to the inward-facing from the cytoplasm or the inner leaflet of the bilayer. After binding two molecules of MgATP, the nucleotide-binding domains (NBDs) dimerize and switch the transmembrane domain (TMDs) from the inward- to the outward-facing conformation, followed by the release of the drug to the extracellular milieu. ATP hydrolysis, ADP/Pi release, and NBD dissociation reset the transporter to the inward-facing conformation. The switch from inward to outward form certainly requires a highly flexible structure [4, 7, 8].

Substrate “promiscuity” or polyspecificity is a well-known characteristic of P-gp and the subject of much research. Attempts have been made to understand the ability of P-gp to recognize various chemically and structurally diverse substrates

through biochemical investigations and structural studies. Despite all these studies, the molecular basis of this unusual property still remains poorly understood and is a matter of intense debate [9].

2. Prioritizing pathogenic agents

Opportunistic pathogens with a response profile of drug resistance to antibiotic treatment are good candidates for study. The organisms chosen here included bacteria and fungi identified by the WHO as priority pathogens [10] as well as other nosocomial pathogens that pose an elevated threat level due to acquisition of MDR over the recent years. Nosocomial pathogens are subject to the evolutionary pressure exerted by constant exposure to antibiotics in hospitals that could accelerate the emergence of pathogenicity-related mutations.

2.1 *Acinetobacter baumannii*

Multidrug-resistant *Acinetobacter baumannii* strains are opportunistic bacterial pathogens primarily associated with nosocomial infections worldwide [11]. Due to the remarkable ability of *A. baumannii* to gain resistance to antibiotics, this bacterium is now considered to be a "superbug." *Acinetobacter baumannii* strains resistant to all clinically relevant antibiotics known have also been isolated. Although MDR *A. baumannii* (MDR-Ab) continues to disseminate globally, very little is known about its pathogenesis mechanisms. Once detected within specific areas of the hospital, various levels of intervention have been attempted to reduce the incidence and prevalence of infection due to MDR-Ab [12].

Acinetobacter baumannii and its close relatives belonging to genomic species 3 (*Acinetobacter pittii*) and 13TU (*Acinetobacter nosocomialis*) are important nosocomial pathogens, often associated with epidemic outbreaks of infection, that are only rarely found outside of a clinical setting. These organisms are frequently pandrug-resistant and are capable of causing substantial morbidity and mortality in patients with severe underlying disease, both in the hospital and in the community [13]. Several epidemic clonal lineages of *A. baumannii* have disseminated worldwide and seem to have a selective advantage over non-epidemic strains. Physicians are also facing challenging therapeutic quandaries when treating patients infected with MDR-Ab, because the increasing prevalence of resistance continues to restrict their treatment options [14].

Urban et al. [12] gave us a look into the MDR in *Acinetobacter baumannii*, discussing its medical relevance and treatment options. They sought to control infection due to MDR-Ab by identifying isolates as clonally related, leading to enhanced infection-control measures, including cohorting, surveillance, contact precaution, initial therapy with ampicillin/sulbactam and local polymyxin B, and, more recently, therapy with synergistic antibiotic combinations. Gupta et al. [15] demonstrated the existence of MDR-Ab and its significance. Park et al. [16] determined the complete genome sequence of *A. baumannii* strain 1656-2 to study biofilm formation. This strain is significant to the project due to its use in target selection.

2.2 *Staphylococcus aureus*

Staphylococcus aureus is a major human pathogen that causes a wide range of clinical infections. Approximately 30% of the human population is colonized with

S. aureus; however, it is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections [17]. The WHO has categorized *Staphylococcus aureus* as a high-priority pathogen that possesses MDR, as a consequence of its acquisition of methicillin and vancomycin resistance.

Hiramatsu et al. [18] described the genetic basis for the remarkable ability of *S. aureus* to acquire multi-antibiotic resistance and proposed a novel paradigm for future chemotherapy against the multiresistant pathogens. The evolution of *Staphylococcus* or for that matter any bacterium does not halt. Lemaire et al. [19] examined the effect of P-gp on the modulation of the intracellular accumulation and activity of daptomycin towards phagocytosed *Staphylococcus aureus* in human THP-1 macrophages, in comparison with MDCK epithelial cells. Handzlik et al. [2] delineated recent achievements in the search for new chemical compounds able to inhibit multidrug resistance mechanisms in Gram-positive pathogens.

2.3 *Aspergillus fumigatus*

Aspergillus fumigatus is a saprophytic fungus that plays an essential role in recycling environmental carbon and nitrogen. Its natural ecological niche is the soil, wherein it survives and grows on organic debris. *Aspergillus fumigatus* is of the more prevalent opportunistic pathogens involved in human aspergillosis in which, though a minor disease, because of the increase in the number of immunosuppressed patients and the degree of severity of modern immunosuppressive therapies, the situation has changed dramatically in recent years. The diversity of patients and risk factors complicates diagnostic and therapeutic decision-making [20]. Invasive procedures are often precluded by host status; noninvasive diagnostic tests vary in their sensitivity and specificity. The ability of *Aspergillus* species to withstand antifungal treatment may be due in part to the presence of the MDR mechanism of drug efflux.

Latge [20] reviewed taxonomy of aspergillosis, its symptoms, diagnosis, virulence factors, defense mechanisms, epidemiology, and treatment. Little is known of the cellular and humoral defense mechanisms which are essential for the killing of *A. fumigatus* conidia and hyphae in the immunocompetent host. Tobin et al. [21] identified genes encoding proteins of the ATP-binding cassette superfamily in *Aspergillus fumigatus* and *Aspergillus flavus.* In *A. fumigatus*, two genes (AfuMDR1 and AfuMDR2) encoding proteins of the ATP-binding cassette superfamily were identified, which are the probable homologue of human P-gp.

2.4 *Candida albicans*

Candida species have emerged among the top three causes of microbial nosocomial infectious diseases in humans, resulting in 46–75% mortality. The incidence of candidiasis has increased sharply over the past few decades, primarily due to hospital interventions such as cancer chemotherapy, surgery, organ/bone marrow transplantation, and indwelling devices [22]. Of note, recently, the incidences of *albicans* and non-*albicans* species of *Candida* acquiring resistance to antifungals (particularly to azoles) have increased considerably which poses problems towards its successful chemotherapy [23]. Drug transporters, such as the ATP-binding cassette transporters encoded by *CDR1* and *CDR2* (*Candida* drug resistance), and a major facilitator superfamily (MFS) transporter encoded by*MDR1*, play key roles in azole resistance as deduced by their high level of expression in the majority of azole-resistant clinical *Candida albicans* isolates [22].

Schubert et al. [24] stated that constitutive overexpression of the Mdr1 efflux pump was an important mechanism of acquired drug resistance *C. albicans*. The Mdr1 efflux pump is a P-gp homologue and is hence significant to this project. Sun et al. [22] highlighted an extensive upregulation of *MDR1* as well as polyamine transporter genes in a fluconazole-resistant strain, going further to correlate the presence of MDR1 in *C. albicans* and its role in fluconazole resistance.

2.5 *Cryptococcus neoformans*

Cryptococcus neoformans is an encapsulated fungal pathogen that is remarkable for its tendency to cause meningoencephalitis, especially in patients with AIDS. While the disease is less common in children than adults, it remains an important cause of morbidity and mortality among HIV-infected children without access to antiretroviral therapy [25]. *Cryptococcus neoformans* is a basidiomycetous yeast ubiquitous in the environment and a model for fungal pathogenesis. *CneMDR1*, a gene encoding a protein related to several eukaryotic multidrug resistance proteins, was identified, cloned, and characterized from a clinical isolate of *Cryptococcus neoformans* [26].

Kao and Goldman [25] reviewed recent insights into both the biology and treatment of cryptococcosis with a special emphasis on the pediatric literature. Thornewell et al. [26] characterized the *CneMDR1* gene. Protein structure predictions suggested the presence of two putative 6-transmembrane (TM) domains as well as two ATP-binding domains, structural characteristics typical of ATP-binding cassette (ABC) proteins, including P-glycoprotein.

3. Sequence and structure analyses

3.1 Bacterial P-glycoprotein efflux pumps

Bacterial P-glycoproteins were identified based on homology to the mammalian P-gp in the following manner. The position-specific iterated BLAST (PSI-BLAST) was performed against a search set of nonredundant protein sequences in the organism of interest, using hP-GP as the query (hP-gp; UniProt P08183). Through a PSI-BLAST search, a large set of related proteins are compiled. It is used to identify distant evolutionary relationships between protein sequences. The algorithm parameters were set with an E-value of 0.001, and the scoring matrix BLOSUM62 was used. This step was performed on all four organisms of interest (*Aspergillus fumigatus*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Candida albicans*, *Cryptococcus neoformans*). Hundreds of hits were obtained for P-glycoprotein, and these results were prioritized according to predetermined parameters such as medical relevance, annotation status, and the presence of conserved regions. The results were analyzed, and the P-glycoprotein sequence of each organism was finalized and recorded as in Appendix A. The results were filtered for the organisms of interest and shown in **Table 1**.

Hundreds of hits are obtained for P-glycoprotein, and these results were prioritized according to medical relevance and sequence identity. The significance of the sequence identity is that, with a higher sequence identity, there is a higher similarity between the query sequence and the aligned sequence. This project will focus on nosocomial bacterial and fungal strains. The chosen sequences would have conserved regions determined through multiple sequence alignment with the ClustalX2

Organism	Max score	Total score	Query cover (%)	Ident (%)	Length
Aspergillus fumigatus	966	1389	97.00	42.00	1349
Acinetobacter baumannii	256	498	82.00	32.00	555
Staphylococcus aureus	268	504	58.00	34.00	578
Candida albicans	183	352	88.00	23.00	1606
Cryptococcus neoformans	931	931	97.00	42.00	1408

Table 1.
Summary of BLAST results.

software, the most widely used multiple alignment programs. The guide trees in Clustal were calculated using the neighbor-joining (NJ) method [27].

3.2 Homology modeling

The target sequences and the suitable templates were chosen and aligned using ClustalX2. Multiple sequence alignment was performed between the targets and the templates so that the homology and evolutionary relationship between the sequences of the biological data set can be inferred [27]. This information was considered in the structure validation. The templates chosen are:

- 4M1M—*Mus musculus*
- 2HYD—*Staphylococcus aureus*
- 3B5Z—*Salmonella enterica*
- 3WME—*Cyanidioschyzon merolae*
- 4F4C—*Caenorhabditis elegans*

The p-glycoprotein sequences would be used as target sequences for structure modeling with SWISS-MODEL [28]. SWISS-MODEL is an open-source, structural bioinformatics tool used for the automated comparative modeling of three-dimensional protein structures. Several P-glycoprotein structures were modeled for each organism, using multiple templates. The templates having high sequence similarity with the target sequences were given preference. The objective of homology modeling is to identify the best template and build the PDB model of the macromolecule to be used in docking. Modeling of the predetermined templates was accepted if they resulted in high modeling (GMQE) scores. Each modeled structure was saved as a PDB file. The results are summarized in **Table 3**.

The validity was checked using the Ramachandran plot with tools such as Procheck. The structures were refined using energy minimization protocols, and the least energetic structure corresponding to each efflux pump protein was chosen for docking studies.

In summary, the FASTA sequences of the BLAST results were obtained and fed into the SWISS-MODEL to build homology models with the above set of templates. The SWISS-MODEL provided us with the top 100 templates that can be used to generate a homology model. To generate the best possible homology model, the templates were aligned with the target organisms using the multiple alignment tool Clustalx2, and a phylogenetic analysis is subsequently conducted.

Templates	Phylogenetic distance				
	A. fumigatus	***A. baumannii***	***C. albicans***	***S. aureus***	***C. neoformans***
3wme	0.635	0.701	0.844	0.707	0.632
4f4c	0.641	0.703	0.831	0.716	0.637
4m1m	**0.584**	0.707	0.842	0.711	**0.577**
2hyd	0.728	0.623	**0.81**	**0.602**	0.711
3b5z	0.678	**0.592**	0.827	0.661	0.694

Bold values indicate the phylogenetically nearest structure.

Table 2.
Phylogenetic distance between templates and the target sequence of each organism.

From **Table 2**, it could be inferred that in the cases of *Aspergillus fumigatus* and *Cryptococcus neoformans*, 4m1m was the most phylogenetically favored templates. *Candida albicans* and *Staphylococcus aureus* are phylogenetically favored to the 2hyd template, and *Acinetobacter baumannii* is phylogenetically closer to 3b5z.

The validity of the homology models was further checked with Phi-Psi graphs and Chi1-Chi2 plots for each residue type. The template comparison is done based on:

- Taxonomy of the target organism with respect to the templates
- Distance analysis

Subsequent to the Ramachandran plot validation, from **Table 3**, we can infer that 4m1m is preferred in *Aspergillus fumigatus, Aspergillus nidulans, Acinetobacter*

	Total residues	**Query cover**	**(%) Sequence identity**
Organism: *Aspergillus fumigatus*			
3wme.1.a	565	0.43	37.8
4f4c.1.a	1241	0.91	37.48
4m1m.2.a	**1251**	**0.91**	**42.15**
Organism: *Acinetobacter baumannii*			
3wme.1.a	**550**	**0.99**	30.29
4f4c.1.a	537	**0.99**	28.88
4m1m.2.a	545	0.97	**32.22**
Organism: *Candida albicans*			
4f4c.1.a	912	0.52	17
4m1m.2.a	**1272**	**0.7**	**18.83**
Organism: *Staphylococcus aureus*			
3wme	**575**	**0.98**	**29.75**
Organism: *Cryptococcus neoformans*			
3wme.1.a	608	0.41	**37.89**
4f4c.1.a	**1259**	**0.88**	37.74
4m1m.1.a	582	0.41	38.5

Bold values indicate optimal parameter values for each organism.

Table 3.
Results of template parameter comparison—homology results.

Antibiotic	PubChemID	SMILES format
Amikacin	37768	C1C(C(C(C(C1NC(=O)C(CCN)O)OC2C(C(C(C(O2)CO)O)N)O)O)OC3C(C(C(C(O3)CN)O)O)O)N
Colistin	5311054	CCC(C)CCCC(=O)NC(CCN)C(=O)NC(C(C)O)C(=O)NC(CCN)C(=O)NC1CCNC(=O)C(NC(=O)C(NC(=O)C(NC(=O)C(NC(=O)C(NC(=O)C(NC1=O)CCN)CC(C)C)CC(C)C)CCN)CCN)C(C)O
Kanamycin	6032	C1C(C(C(C(C1N)OC2C(C(C(C(O2)CN)O)O)O)O)OC3C(C(C(C(O3)CO)O)N)O)N
Netilmicin	90658113	CCNC1CC(C(C(C1OC2C(C(C(CO2)O)NC)(C)O)O)OC3C(CC=C(O3)CN)N)N
Sulbactam	130313	CC1(C(N2C(S1(=O)=O)CC2=O)C(=O)O)C
Amphotericin B	5280965	CC1C=CC=CC=CC=CC=CC=CC=CC(CC2C(C(CC(O2)(CC(CC(C(CCC(CC(CC(=O)OC(C(C1O)C)C)O)O)O)O)O)O)O)O)C(=O)O)OC3C(C(C(C(O3)C)O)N)O
Anidulafungin	166548	CCCCCOC1=CC=C(C=C1)C2=CC=C(C=C2)C3=CC=C(C=C3)C(=O)NC4CC(C(NC(=O)C5C(C(CN5C(=O)C(NC(=O)C(NC(=O)C6CC(CN6C(=O)C(NC4=O)C(C)O)O)C(C(C7=CC=C(C=C7)O)O)O)C(C)O)C)O)O)O
Isavuconazonium	6918606	CC(C1=NC(=CS1)C2=CC=C(C=C2)C#N)C(CN3C=[N+](C=N3)C(C)OC(=O)N(C)C4=C(C=CC=N4)COC(=O)CNC)(C5=C(C=CC(=C5)F)F)O
Itraconazole	55283	CCC(C)N1C(=O)N(C=N1)C2=CC=C(C=C2)N3CCN(CC3)C4=CC=C(C=C4)OC[C@H]5CO[C@](O5)(CN6C=NC=N6)C7=C(C=C(C=C7)Cl)Cl
Micafungin	477468	CCCCCOC1=CC=C(C=C1)C2=CC(=NO2)C3=CC=C(C=C3)C(=O)NC4CC(C(NC(=O)C5C(C(CN5C(=O)C(NC(=O)C(NC(=O)C6CC(CN6C(=O)C(NC4=O)C(C)O)O)C(C(C7=CC(=C(C=C7)O)OS(=O)(=O)O)O)O)C(CC(=O)N)O)C)O)O)O
Porfimer	57166	CC1=C(C2=CC3=NC(=CC4=NC(=CC5=C(C(=C(N5)C=C1N2)C(C)OC(C)C6=C(C7=CC8=C(C(=C(N8)C=C9C(=C(C(=N9)C=C1C(=C(C(=N1)C=C6N7)C)CCC(=O)O)CCC(=O)O)C)C)C(C)O)C)C)C(=C4CCC(=O)O)C)C(=C3C)CCC(=O)O)C(C)O
Voriconazole	71616	CC(C1=NC=NC=C1F)C(CN2C=NC=N2)(C3=C(C=C(C=C3)F)F)O
Fluconazole	3365	C1=CC(=C(C=C1F)F)C(CN2C=NC=N2)(CN3C=NC=N3)O
Clotrimazole	2812	C1=CC=C(C=C1)C(C2=CC=CC=C2)(C3=CC=CC=C3Cl)N4C=CN=C4
Nystop	11953884	CC1C=CC=CCCC=CC=CC=CC=CC(CC(C(C(CC(=O)CC(C(CCC(CC(CC(CC(=O)OC(C(C1O)C)C)O)O)O)O)O)O)O)C(=O)O)O)OC2C(C(C(C(O2)C)O)N)O
Clindamycin	29029	CCCC1CC(N(C1)C)C(=O)NC(C2C(C(C(C(O2)SC)O)O)O)C(C)Cl
Finafloxacin	11567473	C1CC1N2C=C(C(=O)C3=CC(=C(C(=C32)C#N)N4CC5C(C4)OCCN5)F)C(=O)O
Vancomycin	14969	CC1C(C(CC(O1)OC2C(C(C(OC2OC3=C4C=C5C=C3OC6=C(C=C(C=C6)C(C(C(=O)NC(C(=O)NC5C(=O)NC7C8=CC(=C(C=C8)O)C9=C(C=C(C=C9C(NC(=O)C(C(C1=CC(=C(O4)C=C1)Cl)O)NC7=O)C(=O)O)O)O)CC(=O)N)NC(=O)C(CC(C)C)NC)O)Cl)CO)O)O)(C)N)O

Table 4.
List of known FDA approved antibiotics.

baumannii, and *Candida albicans*; 4m1m is the best template. In *Cryptococcus neoformans, 4*f4c is preferred, and 3wme is preferred in *Staphylococcus aureus.*

4. Antibiotics of interest

A set of antibiotics were identified for the purposes of investigation and included known FDA-approved antibiotics (**Table 4**) against each of the target organisms as well as promising antibiotics that ranged from repurposed to investigational (**Table 5**).

For each structure, we surveyed the literature to determine the known antibiotics that are effective against it and against which the pathogenic strain might have

Compound	PubChemID	SMILES format
Levofloxacin	149096	CC1COC2=C3N1C=C(C(=O)C3=CC(=C2N4CCN(CC4)C)F)C(=O)O
Moxifloxacin	152946	COC1=C2C(=CC(=C1N3CC4CCCNC4C3)F)C(=O)C(=CN2C5CC5)C(=O)O
Tigecycline	54686904	CC(C)(C)NCC(=O)NC1=C(C2=C(CC3CC4C(C(=O)C(=C(C4(C(=O)C3=C2O)O)O)C(=O)N)N(C)C)C(=C1)N(C)C)O
Trovafloxacin	62959	C1C2C(C2N)CN1C3=C(C=C4C(=O)C(=CN(C4=N3)C5=C(C=C(C=C5)F)F)C(=O)O)F
Echinocandin	71723607	CC1CN2C(C1O)C(=O)NCC(CC(C(=O)NC(C(=O)N3CC(CC3C(=O)NC(C(=O)NC(C2=O)C(CCNC(CO)CO)O)C(CC4=CC(=C(C=C4)O)OC)O)O)C(C)O)NCC5CCC(CC5)C(=N)SC(=N)C6=CC=C(C=C6)N7CCC(CC7)(C8CCCCC8)OC)O
Terbinafine	1549008	CC(C)(C)C#CC=CCN(C)CC1=CC=CC2=CC=CC=C21
VL-2397	77843968	CC(C)CC1C(=O)NC(C(=O)NC(C(=O)NC(C(=O)NC(C(=O)NC(C(=O)N1)CC(=O)N)CCCN(C(=O)C)[O-])CCCN(C(=O)C)[O-])CCCN(C(=O)C)[O-])CC2=CC=CC=C2.[Al+3]
Bithionol	2406	C1=C(C=C(C(=C1Cl)O)SC2=CC(=CC(=C2O)Cl)Cl)Cl
Carvacrol	10364	CC1=C(C=C(C=C1)C(C)C)O
VT-1129	91886002	C1=CC(=CC=C1C2=CN=C(C=C2)C(C(CN3C=NN=N3)(C4=C(C=C(C=C4)F)F)O)(F)F)OC(F)(F)F
Aminocandin	16072305	CCCCCCCCOC1=CC=C(C=C1)C2=CC=C(C=C2)C(=O)NC3CC(CNC(=O)C4C(C(CN4C(=O)C(NC(=O)C(NC(=O)C5CC(CN5C(=O)C(NC3=O)C(C)O)O)C(CC6=CC=CC=C6)O)CO)C)O)NCCN
Caspofungin	16119814	C1CC(C(C1)N)C(=O)O
E1210	16719049	C1=CC=NC(=C1)OCC2=CC=C(C=C2)CC3=NOC(=C3)C4=C(N=CC=C4)N
Ceftobiprole	6918430	Nc1nc(ns1)\C(=N\O)\C(=O)N[C@H]2[C@H]3SCC(=C(N3C2=O)C(=O)O)\C=C\4/CCN([C@@H]5CCNC5)C4=O
Brilacidin	25023695	C1CNCC1OC2=C(C=C(C=C2NC(=O)CCCCN=C(N)N)C(F)(F)F)NC(=O)C3=CC(=NC=N3)C(=O)NC4=C(C(=CC(=C4)C(F)(F)F)NC(=O)CCCCN=C(N)N)OC5CCNC5
Radezolid	11224409	CC(=O)NCC1CN(C(=O)O1)C2=CC(=C(C=C2)C3=CC=C(C=C3)CNCC4=NNN=C4)F

Table 5.
List of repurposed and investigational antibiotics.

developed resistance via efflux pump activity. A set of ligands is created for each efflux pump, comprising of known and potential antibiotics. The PDB model of each antibiotic is generated using MarvinView by converting the canonical SMILES. This PDB model will act as the ligand during the docking process.

Open Babel is a file conversion software that provides a wide variety of options [29]. We use it to convert the canonical SMILES of the ligand set into a .pdb file in order to perform docking. However, we use this software again during visualization to convert the docked complex from .pdb to .pdbqt format in order for it to be recognized by RasMol.

5. Docking studies of pump-drug combinations

5.1 Docking of the bacterial efflux pumps with known and potential antibiotics

Computational docking is widely used for the study of protein-ligand interactions and for drug discovery and development. The methods are fast enough to allow virtual screening of ligand libraries containing tens of thousands of compounds. Typically, the process starts with a target of known structure, such as a crystallographic structure of an enzyme of medicinal interest. Docking is then used to predict the bound conformation and binding free energy of small molecules to the target. Single docking experiments are useful for exploring the function of the target, and virtual screening, in which a large library of compounds are docked and ranked, may be used to identify new inhibitors for drug development. With AutoDock, it is possible to accomplish the following: basic docking of a drug molecule with an anticancer target, a virtual screen of this target with a small ligand library, docking with selective receptor flexibility, active site prediction, and docking with explicit hydration.

The molecular docking was carried out using the AutoDock suite of tools [30]. The search algorithm used was the Lamarckian genetic algorithm (LGA), which could handle ligands with more degrees of freedom than the simulated annealing method used in earlier versions of AUTODOCK. LGA is the most efficient, reliable, and successful search algorithm and mimics a heuristic Lamarckian evolution, a controversial hypothesis proposed by Jean Batiste de Lamarck that phenotypic characteristics acquired during an individual's lifetime could become heritable traits. The affinity maps were used to compute for each ligand-target pair. The docking parameters were set to 10 runs per receptor-ligand complex yielding 10 poses per each docked complex. Based on the interaction energies, the pose with the smallest free energy of binding was identified as the best pose of the drug and the target.

Each drug is docked with each subsequent target using AutoDock 4.2. The results are analyzed to verify whether the pathogenic strain could develop resistance to known antibiotics using efflux pump activity and if the novel antibiotics could be effective against the development of such resistance.

5.2 Best pose analysis

The ligand pose with the least binding energy is defined as the best pose which was validated by clustering at 2.0 Å r.m.s. The clusterings signify the extent of difference between the various poses. Extremely similar poses will be clustered together, increasing the validity of that respective pose. Thus the best pose is selected based on the combination of the binding energy released and the clusterings of the pose. The output contains the docked structure between the

Organism	Known drug	ΔG kcal/mol
Aspergillus fumigatus	Amphotericin	−6.99
	Itraconazole	−3.71
	Anidulafungin	−3.06
	Micafungin	−2.97
	Voriconazole	−2.6
	Isavuconazonium	−1.1
	Porfimer	−0.31
Candida albicans	Amphotericin	−7
	Nystatin	−6.22
	Clotrimazole	−5.43
	Caspofungin	−3.57
Acinetobacter baumannii	Sulbactam	−4.86
	Kanamycin	−4.11
	Amikacin	−3.46
	Netilmicin	−1.75
	Colistin	4.54
Staphylococcus aureus	Finafloxacin	−5.9
	Cephalexin	−3.19
	Vancomycin	−3.18
Cryptococcus neoformans	Amphotericin	−6.7
	Fluconazole	−3.55
	Voriconazole	−2.3

Table 6.
Summary of the docking results of known antibiotics.

macromolecule and the best pose ligand. The output is a PDBQT file which is then converted to PDB format using Open Babel. **Tables 6** and 7 depict the best pose for every organism in a hierarchy, in the case of both known and investigational drugs, respectively.

5.3 Differential ligand binding affinity

The differential binding affinities of the repurposed ligands will be determined using the conventionally used drugs as a baseline. The differential binding affinity of a potential antibiotic with respect to a known antibiotic can be calculated by subtracting the binding energy value generated by the known antibiotic from that of the unknown antibiotic. A lower differential energy value is indicative of a more stable complex.

$$\Delta\Delta G_{potential} = \Delta G_{bind,potential} - \Delta G_{bind,known} \quad (1)$$

In the above formula, $\Delta\Delta G_{potential}$ is the differential binding affinity of the potential ligand, and ΔG_{bind} is the free energy released during docking. From **Table 8** it is evident that bithionol is the best investigational drug for the *Aspergillus*

Organism	Investigational drug	ΔG kcal/mol
Aspergillus fumigatus	Bithionol	−5.9
	Moxifloxacin	−4.76
	e1210	−4.12
	Terbinafine	−3.76
	Cresemba	−0.45
	Echinocandin	0.03
Candida albicans	e1210	−5.22
	Moxifloxacin	−4.76
	Levofloxacin	−4.66
	Aminocandin	−2.71
	Bithionol	−4.6
Acinetobacter baumannii	Levofloxacin	−6.34
	Gepotidacin	−5.58
	Tigecycline	−4.85
	Ceftobiprole	−4.72
	Moxifloxacin	−4.68
	Trovafloxacin	−4.03
	Tigecycline	−5.75
Staphylococcus aureus	Gepotidacin	−5.12
	Moxifloxacin	−4.92
	Ceftobiprole	−4.18
	934628_27_0	−3.89
	Radezolid	−3.63
	Brilacidin	0.82
Cryptococcus neoformans	Bithionol	−4.98
	e1210	−4.89
	Carvacrol	−4.23
	Moxifloxacin	−4.04
	vt-1129	−3.85

Table 7.
Summary of the docking results of investigational drugs.

fumigatus compared with other repurposed ligand used. From **Table 9** we can infer E1210 as a potential repurposed ligand for *Candida albicans*. **Table 10** depicts the differential binding abilities of repurposed ligand for *Acinetobacter baumannii* of which moxifloxacin is the best investigational drug. In **Tables 11** and **12**, tigecycline and bithionol were the most efficient potential antibiotics for the organisms *Staphylococcus aureus* and *Cryptococcus neoformans*, respectively.

5.4 Identification of interacting residues in each docked complex

The best pose of each docked complex is viewed using RasMol and Pymol v.1.3. All interacting residues within a radius of 4.5 Å of the ligand are restricted using

Aspergillus fumigatus	$\Delta\Delta G_{amphotericin}$	$\Delta\Delta G_{itraconazole}$	$\Delta\Delta G_{anidulafungin}$	$\Delta\Delta G_{micafungin}$	$\Delta\Delta G_{voriconazole}$	$\Delta\Delta G_{isavuconazonium}$	$\Delta\Delta G_{porfimer}$
Bithionol	1.09	**−2.19**	**−2.84**	**−2.93**	**−3.3**	**−4.8**	**−5.59**
Moxifloxacin	2.23	**−1.05**	**−1.7**	**−1.79**	**−2.16**	**−3.66**	**−4.45**
E1210	2.87	**−0.41**	**−1.06**	**−1.15**	**−1.52**	**−3.02**	**−3.81**
Terbinafine	3.23	**−0.05**	**−0.7**	**−0.79**	**−1.16**	**−2.66**	**−3.45**
Cresemba	6.54	3.26	2.61	2.52	2.15	0.65	**−0.14**
Echinocandin	7.02	3.74	3.09	3	2.63	1.13	0.34

Bold values indicate that the differential free energy of binding of the potential antibiotic is negative (i.e., stronger binding).

Table 8.
Differential binding affinities of the repurposed ligands for Aspergillus fumigatus.

Candida albicans	$\Delta\Delta G_{amphotericin}$	$\Delta\Delta G_{nystatin}$	$\Delta\Delta G_{clotrimazole}$	$\Delta\Delta G_{caspofungin}$
E1210	1.78	1	0.21	**−1.65**
Moxifloxacin	2.24	1.46	0.67	**−1.19**
Levofloxacin	2.34	1.56	0.77	**−1.09**
Bithionol	2.4	1.62	0.83	**−1.03**
Aminocandin	4.29	3.51	2.72	0.86

Bold values indicate that the differential free energy of binding of the potential antibiotic is negative (i.e., stronger binding).

Table 9.
Differential binding affinities of the repurposed ligands for Candida albicans.

Acinetobacter baumannii	$\Delta\Delta G_{sulbactam}$	$\Delta\Delta G_{kanamycin}$	$\Delta\Delta G_{amikacin}$	$\Delta\Delta G_{netilmicin}$
Moxifloxacin	**−1.48**	**−2.23**	**−2.88**	**−4.59**
Tigecycline	0.01	**−0.74**	**−1.39**	**−3.1**
Ceftobiprole	0.14	**−0.61**	**−1.26**	**−2.97**
Levofloxacin	0.18	**−0.57**	**−1.22**	**−2.93**

Bold values indicate that the differential free energy of binding of the potential antibiotic is negative (i.e., stronger binding).

Table 10.
Differential binding affinities of the repurposed ligands for Acinetobacter baumannii.

Staphylococcus aureus	$\Delta\Delta G_{finafloxacin}$	$\Delta\Delta G_{cephalexin}$	$\Delta\Delta G_{vancomycin}$
Tigecycline	0.15	**−2.56**	**−2.57**
Gepotidacin	0.78	**−1.93**	**−1.94**
Moxifloxacin	0.98	**−1.73**	**−1.74**
Ceftobiprole	1.72	**−0.99**	**−1**
934628_27_0	2.01	**−0.7**	**−0.71**
Radezolid	2.27	**−0.44**	**−0.45**
Brilacidin	6.72	4.01	4

Bold values indicate that the differential free energy of binding of the potential antibiotic is negative (i.e., stronger binding).

Table 11.
Differential binding affinities of the repurposed ligands for Staphylococcus aureus.

Cryptococcus neoformans	$\Delta\Delta G_{amphotericin}$	$\Delta\Delta G_{fluconazole}$	$\Delta\Delta G_{voriconazole}$
Bithionol	1.72	**−1.43**	**−2.68**
E1210	1.81	**−1.34**	**−2.59**
Carvocrol	2.47	**−0.68**	**−1.93**
Moxifloxacin	2.66	**−0.49**	**−1.74**
VT-1129	2.85	**−0.3**	**−1.55**

Bold values indicate that the differential free energy of binding of the potential antibiotic is negative (i.e., stronger binding).

Table 12.
Differential binding affinities of the repurposed ligands for Cryptococcus neoformans.

Antibiotics	Interacting residues
Amikacin	Ala64, Ser67, Arg68, Arg75, Met103, Tyr104, Glu107, Thr110, Glu115
Colistin	Ala87, Tyr90, Leu91, Ser94, Ser95
Kanamycin	His290, Glu294, Leu295, Asp297, Leu298, Pro299
Netilmicin	Asp277, Val278, Asn279, Glu280, Lys281
Sulbactam	Ser160, Lys161, Arg164, Lys165, Met168, Gln283
Ceftobiprole	Ile120, Asp123, Gly153, Val156, Arg157, Ser160, Met163, Leu268, Lys273, Thr276
Gepotidacin	Gly153, Val156, Arg157, Ser160, Leu268, Lys273, Thr276, Asn279, Leu282, Gln283, Leu286
Levofloxacin	Val78, Tyr79, Ala80, Lys81, Leu82, Leu83, Arg84, Leu85, Tyr90, Asn93, Lys101, Ser291, Val292, Glu294, Leu295, Leu296
Moxifloxacin	Leu384, Ser385, Arg388, Met393, Tyr411, Gly412, Leu414, Arg465, Ala466, Lys469
Tigecycline	Tyr79, Leu83, Tyr90, Ser95, Ile98, Met393, Asn395, Gln397, Val398, Val399, Phe401, Tyr411, Arg465
Trovafloxacin	Ile391, Ala392, Met393, Asn395, Phe401, Tyr411, Gly412, Leu414, Arg465, Ala466, Lys469

Table 13.
Analysis of interacting residues for Acinetobacter baumannii.

Antibiotics	Interacting residues
Amphotericin	Thr221, Asn225, Phe756, Tyr759, Ser760, Gln953, Lys956, Ser957, Glu960, Ala963
Anidulafungin	Trp762, Thr763, Leu764, Phe942, Gly1058, Thr1059, Phe1061, Ser1062, Asp1066, Met1067, Gly1068, Lys1071, Asn1072
Isavuconazonium	Asn392, Leu938, Gly941, Phe942, Arg944, Phe945, Gln1055, Ala1057, Gly1058, Thr1059, Phe1061, Ser1062
Itraconazole	Phe388, Gly391, Asn392, Leu938, Gly941, Phe942, Phe945, Gln1055, Gly1058, Thr1059, Phe1061, Ser1062, Met1067
Micafungin	Trp762, Leu938, Gly941, Phe942, Phe945, Tyr946, Ala949, Gln950, Gln953, Gly1058, Phe1061, Gly1068, Lys1071, Asn1072
Porfimer	Lys210, Glu215, Arg219, Asp223, Ala405, Ala408, Lys409, Ser412, Arg416
Voriconazole	Ser760, Leu761, Trp762, Thr763, Leu764, Val765, Lys766, Gly1068, Lys1071, Asn1072
Bithionol	Phe348, Phe352, Tyr355, Ile385, Gln793, Gln796, Tyr800, Phe1052, Gln1055, Ser1056, Thr1059
Cresemba	The238, Phe242, Val393, Ala394, Gly397, Gln398, Phe400, Thr401
E1210	Glu186, Gln228, Arg954, Ser955, Ala958, Lys995, Gln996, Lys999, Ser1003
Echinocandin	Thr218, Thr221, Gln580, Arg581, Val752, Gln755, Phe756, Lys758, Tyr759, Glu876, Glu877, Lys956, Glu960, Ala963
Moxifloxacin	Arg307, Tyr1113, Thr1115, Arg1116, Glu1118, Gln1119, Val1121, Gly1142, Cys1143, Gly1144, Lys1145, Ser1146, Thr1147, Tyr1156
Terbinafine	Gln755, Phe756, Glu757, Lys758, Tyr759, Arg875, Glu877

Table 14.
Analysis of interacting residues for Aspergillus fumigatus.

RasMol. By studying the PDB file constituting the restricting structure, we can identify the atoms that are present within the interacting residues. These interacting residues are then analyzed for recurrences, which are found to be the most active

interactive residues within the respective macromolecule. An analysis of the interacting residues showed us that:

- (Leu268, Lys273, Thr276, Asn279) and (Gly153, Val156, Arg157, Ser160) are some recurring residues in *Acinetobacter baumannii* (**Table 13**).
- (Phe942, Gly1058, Thr1059, Phe1061, Ser1062) and (Asn392, Leu938, Gly941) are some recurring residues in *Aspergillus fumigates* (**Table 14**).
- (Phe1143, Thr1146, Phe1173, Asn1176, Tyr1177, Arg1179, Ile1180, Ile1317) and (Gly978, His1357, Leu1358) are some recurring residues in *Candida albicans* (**Table 15**).

Antibiotics	Interacting residues
Amphotericin	Phe1143, Thr1146, Leu1147, Phe1151, Val1152, Ser1170, Phe1173, Val1174, Asn1176, Tyr1177, Arg1179, Ile1180, Ile1313, Glu1314, Ile1318, Tyr1319
Caspofungin	Ser1104, Val1105, Leu1106, Arg1107, Ser1108, Phe1113, Ile1121, Phe1125, Asp1332
Clotrimazole	Tyr1175, Phe1178, Arg1179, Phe1182, Val1183, Arg1187, Thr1254, Ser1257, Ser1258, Phe1261, Phe1262
Nystatin	Arg1002, Thr1005, Val1006, Pro1007, Trp1008, Asp1009, Ile1010, Phe1011, Asn1135, Phe1143, Phe1173, Pro1323, Pro1327
Aminocandin	Asp836, Val953, Asp954, Met1110, Asp1114, Tyr1354, Asp1359, Pro1360, Val1361, Arg1380, Thr1381, Ala1383, Gly1384, Leu1390, Gln1422, Leu1573, Asp1574, Ser1575, Gly1576
Bithionol	Phe1143, Thr1146, Phe1173, Asn1176, Tyr1177, Arg1179, Ile1180, Ile1317, Glu1318, Tyr1319
E1210	Arg992, Lys993, Thr994, Arg995, His996, Glu997, Gln998, Glu999, Glu1000, Ser1001, Arg1002, Thr1116, Arg1120, Arg1124, Met1332, Lys1333
Levofloxacin	Val953, Val975, Gly978, His1357, Leu1358, Asp1359, Pro1360
Moxifloxacin	Tyr977, Gly978, Ser1111, Phe1112, Thr1115, Lys1333, Arg1355, Lys1356, His1357, Leu1358

Table 15.
Analysis of interacting residues for Candida albicans.

Antibiotics	Interacting residues
Amphotericin	The280, Glu583, Pro584, Thr585, Leu586, Phe587, Gly641, Glu642, Leu646, Leu647, Gly649, Lys652, His1020, Ser1023, Glu1024, Gly1027, Ala1028
Fluconazole	Asp801, Ile802, Gln803, Ala804, Arg806, Ala807, Val810, Ala811, Gly812, Glu813, Asp814, Lys815, Gln946, Lys949
Voriconazole	SER818, Ser819, Phe820, Gly821, Arg825, Ala1131, Ser1132, Arg1135, Asp1138
Bithionol	Met343, Phe346, Gly347, Ala350, Leu351, Val394, Gly395, Gly398, Ser399, Glu402, Trp907, Gln948
Carvacrol	Lys1002, Val1003, Val1004, Leu1006, Lys1007, Asp1008, Met1011
E1210	Ile802, Gln803, Ala804, Arg806, Ala807, Val810, Ala811, Gly812, Lys815, His933, Ala938, Ser941, Asn942, Ser1132
Moxifloxacin	Lys296, Arg1000, Leu1001, Lys1002, Gly1114, Phe1117, Thr1118, Pro1121
VT1129	Ile802, Gln803, Ala804, Arg806, Ala807, Val810, Ala811, Gly812, Glu813, Lys815

Table 16.
Analysis of interacting residues for Cryptococcus neoformans.

Antibiotics	Interacting residues
Cephalexin	Arg186, Thr302, Thr305, Gln306, Phe308, Ala309
Finafloxacin	VAL178, Phe182, Ser247, Phe248, Ile251, Asn252, Gly292, Arg295, Arg296, Ala299
Vancomycin	Pro172, Leu1776, Thr177, Tyr179, Val180, Phe181, Gly183, Arg184, Lys187
9346	Ala106, Leu107, Ser108, Ala109, Tyr112, Tyr322, Ile324, Phe390, Thr410, Arg414
Brilacidin	Tyr94, Arg97, Lys98, Tyr101, Ile121, Val124, Ile425, Leu426, Phe427, Ser428, Glu473, Arg474
Ceftobiprole	Tyr112, Ala113, Asn115, Gln116, Val117, Gly118, Gln119, Val120, Ile121, Phe427
Gepotidacin	Gln105, Ala106, Leu107, Ser108, Ala109, Tyr112, Ile324, Arg389, Phe390, Arg414, Leu419
Moxifloxacin	Tyr12, Trp87, Asn90, Lys91, Tyr94, Asp95, Lys98
Radezolid	Arg97, Tyr101, Gln105, Tyr112, Gln116, Val117, Gly118, Gln11, Val120, Ile121, Val124, Ile125
Tigecycline	Gln105, Ala106, Leu107, Ser108, Ala109, Tyr322, Asp323, Ile324, Asn385, Arg389, Phe390, Arg414, Gln421, Ile425

Table 17.
Analysis of interacting residues for Staphylococcus aureus.

- (Ile802, Gln803, Ala804, Arg806, Ala807, Val810, Ala811, Gly812, Glu813, Lys815) are some recurring residues in *Cryptococcus neoformans* (**Table 16**).

- (Ala106, Leu107, Ser108, Ala109, Tyr112, Tyr322, Ile324, Phe390) and (Tyr112, Gln116, Val117, Gly118, Gln119, Val120) are some recurring residues in *Staphylococcus aureus* (**Table 17**).

6. Conclusion

The homology modeling was performed to determine the best template, from which we concluded that 4m1m is preferred in *Aspergillus fumigatus, Aspergillus nidulans, Acinetobacter baumannii*, and *Candida albicans*. In *Cryptococcus neoformans* 4f4c is preferred, and 3wme is preferred in *Staphylococcus aureus*.

The molecular docking led us to conclude that bithionol, levofloxacin, e1210, tigecycline, and bithionol were the most efficient potential antibiotics for the organisms *Aspergillus fumigatus, Acinetobacter baumannii, Candida Albicans, Staphylococcus aureus*, and *Cryptococcus neoformans*, respectively. Each of the potential antibiotics was found to be more effective than a number of the known antibiotics in the treatment of that respective organism.

An analysis of the interacting residues showed us that:

- (Leu268, Lys273, Thr276, Asn279) and (Gly153, Val156, Arg157, Ser160) are some recurring residues in *Acinetobacter baumannii.*

- *(*Phe942, Gly1058, Thr1059, Phe1061, Ser1062) and (Asn392, Leu938, Gly941) are some recurring residues in *Aspergillus fumigatus.*

- (Phe1143, Thr1146, Phe1173, Asn1176, Tyr1177, Arg1179, Ile1180, Ile1317) and (Gly978, His1357, Leu1358) are some recurring residues in *Candida albicans*.

- (Ile802, Gln803, Ala804, Arg806, Ala807, Val810, Ala811, Gly812, Glu813, Lys815) are some recurring residues in *Cryptococcus neoformans*.

- (Ala106, Leu107, Ser108, Ala109, Tyr112, Tyr322, Ile324, Phe390) and (Tyr112, Gln116, Val117, Gly118, Gln119, Val120) are some recurring residues in *Staphylococcus aureus*.

Appendix 1: FASTA sequences obatained from PSI – BLAST searches

>SST02482.1 lipid A export permease/ATP-binding protein MsbA [*Acinetobacter baumannii*]

MIDKDLSTRQTFRRLWPTISPFKAGLFVAAIALVINAAGDAFMISLLKPLL-
DEGFEKADNDVLKWLPLAMLGLIIVRGASSFV-
STYCVSWVSGQVVMSMRRKLFGHMMGMPVSFFDQQSTGTLLSRITYDSEQ-
VAASSSGALITIIREGAYIIGLFAMMFYYSWQLSLILIVIAPIVSITIRIVSKRFR-
KISKNMQTGMGHVTASAEQMLKGHKEVLIFGGQKVE-
TERFNKVSNNMRSQSMKMVTASAISDPIIQLIASFALAFVLYAASFPEIREQLSPG-
TIAVVFSSMFALMRPLKSLTNVNSQFQRGMAACQTLFSILDTEQEK-
DEGTKVLSNVKGDIEFENVTFTYATKEHPALDDISFTLPAGKSVALVGRSGSGK-
STIANLITRFYDIDKGSIRIDGHDIREYTLESLRNQVALVSQHVYLFNDTIAN-
NIAYATDGRFSREQIEKAAEMAYAMDFIAKLDKGLDTVIGEN-
GVMLSGGQRQRIAIARALLRDAPILILDEATSALDTESERAIQAALDELQKNRTSL-
VIAHRLSTIENADEILVVQDGRIIERGNHKALLALEGAYAQLHKIQFSQ

>WP_051575420.1 ABC transporter ATP-binding protein [*Staphylococcus aureus*]

MKFKKFISYYRPYKRIFGLTLICSLLVTVITLVIPLIIRYITENLIQHFSVAHV-
KEIYLLGAAMVLLILIQFLCHIFIDYYGHVMGAKMEKDMSEELYE-
HIQSQPHHFFDRNSTGGLMSRLTGDLENLSELYHHGPEDILMYIIRFIGAVVIL-
LYINVELTIVMMLFIPIMIVVYWYYIKKLSSIYEQDKATNAEIHGFLENTIS-
GIKVTKSFTNESFESNQYKSLNKKAIEIKKKVHKYEALYNEIIGSIIQAMPVIIIVL-
GALLIMKKEISIGDLLAFVLYVGNIATPIEVLVKLSVQYNE-
GISGFNRFFKLMQLKPDITSENTHQQQSPHSNGAIQFDHVYFQYDQEYIIHNLNL-
TIEPGAYIAIVGPSGSGKSTIANLLPRFYDVTSGSITINHQDIRTIPLEELRQKI-
GIVQQDVYIFSSTVYENIKYGNPEASMDEIIHASKLANAHEFIQQLPNGYHTQI-
GEKGAKLSGGQKQRLSIARMFLKNPEVVILDEATSALDNLSEKVVQQ-
SLEQLTLNRTTIVIAHRLSTIRNADNIYVLTKEGIIESGNHDTLIEKQG-
FYYRMYINEEN

>KHC36224.1 alpha-factor-transporting ATPase [*Candida albicans* P76067]

MFQEKSEKSSFPKRSSSLRSPSDSPAITSKNVFMFVNYSKDWPLILVGILLMGG-
SAIATPMNTYIYGEIMGKLSQFYLQDQSNHSFSQDIVKLCVGLIGIGCCK-
MILVWLGMFTWLKFGEIQQSRARMQIYNKIINESQSWYDSKQNLIGQLTQINR-
CIEELRSCNGEILASLMQTIVLILALLIMSFYQSWSTTLIIMASFPIMALCG-
WYFGKLTYKAQQDENEVTSKASKVFNWCYVNPEMVRFFNSKNIELTKFKQ-
LIEKSAQFYYKLSHAVAANTAVLKTLTLMMFVQGFWFGNYLLSHNTI-
TINQLFTCFSSCLMLGQAVSGITELLAILNTGHAAADKISGFLLQPPS-
KAKLLLLHSKYPPFEIGSIYFKNVWFESNSQNSVAILQDVSFGILQNQFNF-
VIGKSGSGKSTIAKLLMRLYSVSRGTIEIDTVSIDKLDPKYICQNIILLEQNP-
VIFDDKTIAENIAIAIVDDYDSLQAIPYYLIEQSAHFALLSDLDLNMKVNQLTLSG
GQQQRISIARAYLKNSPVLIMDESFSALDTETKQCLIEKVKKWRIGKTTIFITHEY-
KNILDDENVIILDQGMIKNQGQFKKMKNEEIVQNYKSQGIETS-
SYETTSQSFSDNTKLPDGDYNYKTNPYILKDLESQIKEDTDNEKLMGVLAIL-
RYCSSTINGKSLLGFGILLAIFQGGSSPVFSYCFSKLLSTSLDSSIGLNSTQKILQWS-
CISLSIAIFTGVTSYLSEFILNYCGENWIVSLRQLTFFKLNNQDLSFFTRFDTNWSS-

SEITALLMNDTRDLRNLISQFFPLLANLVSMTLIGIIWSIVSGWKLALV-
GISFVPLVLLVTVLYGKILESIENKYKCKVNNVELDLYRTITTIRTIKIFNIQQY-
FETVFKEDLKVLNSIGVYRALQTGIGFAISDLFSSIGQAIILFYGMKLISQFQY-
NYSQLLQVITLLSFTISNASILIHQLPEITRGQRAGTFIVKLLKDITST-
MEVNDSCGVSSVRKRNSKSGSDSIGTIGPVKDNQLFKKVTTDNDTLAISFNNVSF-
SYPNKLPYILQLKSISLDVKKFTTIGIVGQSGSGKSTILKILFRLYDIKISPDSNTTK-
KYHDQTVKIFNQNLYLINSGLLCQTIAIVPQFPKFFSGTIYDNLTYGINNTN-
SAGSNSSSSVSDSEIIKILKLVNLHQFIVSLPQGLLTIMNDSDNDNDNGNENENE-
NENGNTISTSSSTSFTFSGGQLQLLAIARALLRNPKILLLDECTSNLDPITTKIIIN-
VIKSLHGKLTILFVTHDKELMRIADNLIVMKDGQIVEQGDFQQLISND-
GEFTKITKTII

>OWZ59602.1 ATP-binding cassette, subfamily B (MDR/TAP), member 1 [*Cryptococcus neoformans* var. grubii c45]

MSASPGLTAAAAGPDHLQARRDEKVIDSEKDALAHDAHAVNSGIPYPTA-
TAPNVGAPTVPIIVGRVSSAPEGKISRSSIAASSDTLRNSPLEKPISNAVSKSH-
PYKKSKFDFLKSRKKKEEEERKNKEKEKEASVLPPVSFFALFRFAA-
PLEIIAMVLGLVLAVAAGSCQPLMTLIFGRLTTSFTNYAVIANQISQGGLTPET-
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KAGSLAEEVIGSIRTVQAFGKEKILGDKFADHIEQSKIVGRKGSIFEGFGLSIMFF-
VIYAAYALAFFYGGILVSNGQADSGIVINVFMSILIGSFSMAMLAPELAAVTKAR-
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SEGIVQDALDKASRGRTTITIAHRLSTIRDADRIYVMGGGEVLEQGSHNDLLA-
NENGPYAQLVNNQKLAQEAAAEALQVDDDIDDLDDAVFIGGSSPM-
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GAVTSNLADQPQKVQGLFGPTLGTVVQSCATLIGGCIIGLCYGPLLALIGIACI-
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FYTVLNSIVFASIQAGNVFTFVPDASKANSSAASIFRSIDNEPAINAES-
NEGKVLDHKHVVGHVRIEGVHFRYPTRPGVRVLRNLTIDVPAGTY-
VALVGPSGCGKSTTIQMLERFYDPLAGRVTLDGIDIKELNLASYRSQISLVSQEP-
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TEVGGKGSQLSGGQKQRIAIARALIRNPKVLLLDEATSALDSQSEKVVQEALD-
KAAKGRTTIAIAHRLSSIQHSDRIYYFSEGRVAEHGTHQELLAKKG-
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>KEY77376.1 ABC multidrug transporter Mdr1 [*Aspergillus fumigatus* var. RP-2014]

MPAPETGASSREKSLEDLQVATLEKGRSTSSFGADNEKPHDHHSLSDTI-
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DIQTLNLRWLRQQISLVSQEPVLFSTTIFRNIEHGLIGTKFEHESKDKIRELVE-
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DEATSALDTKSEGVVQAALDKAAEGRTTIVIAHRLSTIKTAHNIVAMVGG-
KIAEQGTHDELVDRKGTYYKLVEAQRINEEKEAEALEADADMDADDFGQEGV-
TRIKTAVSSSNSLDAVDEKARLEMKRTGTQKSVSSAVLSKKVPEQFE-
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SILRQDISFFDREENSTGALTSFLSTETKNLSGVSGVTLGTIIMTSTTLGAAMIIA-
LAIGWKLALVCISVVPILLACGFLRFYMLAQFQQRSKSAYEGSASYACEATSAIRT-
VASLTREQDVWGVYHDQLQKQGRKSLISVLRSSLLYASSQALVFFCVALGF-
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