Chapter

Neisseria gonorrhoeae Ketol-Acid Reductoisomerase Is a Potential Therapeutic Target

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Abstract

The host-adapted human pathogen *Neisseria gonorrhoeae* is the causative agent of sexually transmitted infection gonorrhea. The increased emergence of gonorrhea infections worldwide, associated with the surging resistance to antimicrobial treatments is alarming. Antimicrobial resistance (AMR) is a global threat to human health and occur through various molecular mechanisms. This research aims to identify molecular therapeutic targets in *N. gonorhoeae* as a potential antibiotic adjuvant. This work is focused on ketol acid reductor-isomerase enzyme (KARI), an enzyme involved in the branched-chain amino acids biosynthesis. A BLASTp analysis revealed that KARI enzyme is highly conserved in *N. gonorrhoeae* strains and present in important bacterial pathogens including ESKAPE. Sequence alignment of different KARI proteins from various human bacterial pathogens and gut microbiota demonstrate that residues forming the active site and cofactors binding sites are conserved among all tested KARIs. A 3D homology-based model for gonococcal KARI was generated using Swiss model server and the KARI template from *S. aureus*. The generated 3D KARI model shows that this enzyme adapts a different conformation upon binding of cofactors, allowing the substrate binding and catalysis, while the active site adapts a closed state.

Keywords: *N. Gonorhoeae*, KARI- enzyme, therapeutic target, ESKAPE pathogens, antimicrobial resistance

1. Introduction

Sexually transmitted infections (STIs) are a major public health problem worldwide, affecting the quality of life and causing serious morbidity and mortality. Indeed, *Neisseria gonorrhoeae* (also known as the gonococcus) is the etiological agent of gonorrhea, the second most frequently reported sexually transmitted infection (STI) in the world [1] after *Chlamydia trachomatis*. This bacterium is a Gram-negative diplococcus that can affect both men and women, causing the infection of the urogenital, rectal, and pharyngeal sites [2].

Clinically, gonorrhea may be asymptomatic in many cases. However, clinical manifestations in men include dysuria, pain in the testicles, and purulent urethral

discharge with mucoid secretion from the penis. For women, painful urination, itching, or vaginal discharge might be noticed. Gonorrhea can also infect the rectum inducing pain with bowel movements, constipation or rectal discharge [2], and other sites such as the oropharyngeal mucosa, ocular, and anal mucosa.

Gonococcal infection can induce serious complications, ranging from salpingitis and epididymitis to pelvic inflammatory disease, ectopic pregnancy, and infertility. Gonococcal infection during pregnancy can cause various complications since the infection could be transmitted to newborns via vaginal delivery, which may cause neonatal ophthalmia. Untreated *N. gonorrhoeae*, and other STIs, were shown to facilitate the transmission and acquisition of the human immunodeficiency virus [2]. The control of gonorrhea relies on prevention, appropriate diagnostics, and effective antimicrobial treatment [3].

1.1 Pathogenesis of *N. gonorrhoeae*

In order to establish infection, *N. gonorrhea* first establishes colonization of the mucosal epithelium by adherence and attachment to various epithelial surfaces. This is the first step in pathogenesis, which is mediated by specific bacterial surface structures, including pili type IV (retraction) and opacity (Opa) proteins. The pili type IV retraction brings the gonococci to the cell surface and enable interactions with other surface structures [4].

The next step following adherence is replication of *N. gonorrhoeae,* colonies formation and possibly biofilms. An invasion and transcytosis occur with possible competition with the resident microbiota. During these initial stages in gonococcal infection, *N. gonorrhoeae* produces or sheds lipopolysaccharides, fragments of peptidoglycan, and outer membrane vesicles. The latter could activate Toll-like receptors signaling in epithelial cells, dendritic cells, and macrophages, leading to the release of cytokines and chemokines and then activation of the inflammatory transcription factor. These innate immune signaling pathways allow the recruitment a large number of polymorphonuclear leukocytes to the site of infection where they interact with and phagocytose *N. gonorrhoeae*. The gonococcal colonization could result in symptomatic or asymptomatic infection. In case of sufficient neutrophil influx into the site of infection, a symptomatic infection may occur [4].

Furthermore, *N. gonorrhoeae* avoids clearance by the host immune system in a process known as antigenic variation, due to pili type IV. During this process, gonorrhea alters its cell surface antigens by replacing portions of the expressed pilin gene (*pilE*) with segments of the silent pilin gene (*pilS*) through homologous recombination [5]. *N. gonorrhoeae* could also modulate the host iron innate immune defenses to survive intracellularly under limited bioavailability of iron. This bacterium can survive in association with monocytes and macrophages. Gonococcal stimulation of macrophages influences the pro-inflammatory response, leading to damage during natural infection [6].

1.2 Signs and symptoms

The gonorrhea infection is asymptomatic in more than 70% of infections, especially among females [7]. However, some symptoms may occur such as dysuria (frequent/painful urination), vaginal discharge (watery, creamy, or slightly green), itching/burning in the vaginal area, and bleeding from the vagina between periods.

Affected women can also have purulent or mucopurulent endocervical, commonly referred to as mucopurulent cervicitis [8].

Infection in the uterus and the fallopian tubes were also reported, leading to a painful infection of the pelvis, known as pelvic inflammatory disease (PID). As a result, a tubal pregnancy will occur and can lead to miscarriage and even death of the mother [9]. Pelvic infection causes fever, pain during intercourse, and pelvic pain. In case of severe infection, a tubo-ovarian abscess can be formed and can be fatal, requiring major surgery [9]. Other symptoms of gonorrhea in women include also lower stomach aches. Gonorrhea infection in men can affect the genital tract, leading to burning and painful urination, a pus-like discharge from the tip of the penis (white, green, or yellow), and pain or swelling in one of the testicle (less common) [10]. A throat infection and pain can also occur after a gonorrhea infection in men [11]. Gonorrhea can also affect the rectum, causing pain with bowel movements, rectal discharge, constipation, soreness, itching, bleeding, and discharge. The presence of gonorrhea is also considered as a co-factor in human immunodeficiency virus (HIV) transmission [12].

Accordingly, diagnosis requires appropriate laboratory tests for confirmation, case finding, and antimicrobial testing. Gonorrhea diagnosis is performed through the detection of the bacterium or its genetic material in the human body (genital or extragenital specimens) using culture test, microscopy, or nucleic acids amplification tests. Antimicrobial resistance (AMR) testing of gonococcal isolates should be a crucial part of laboratory diagnosis [13].

1.3 Diagnosis of gonorrhea

In case of symptomatic men infection with urethral discharge, diagnosis can be observed by microscopy, identifying gonococci as intracellular Gram-negative diplococci in polymorphonuclear leukocytes (magnification, ×1000). This cheap method is highly sensitive and specific, can provide rapid results and enables a complete AMR testing. Nonetheless, this method depends on the presence of discharge or secretions, and requires good optimization of many parameters, such as sample collection, storage and transport, culture methodology, as gonococci are fastidious i.e. highly sensitive to external environmental factors [13].

However, in the case of cervical, rectal, or pharyngeal gonorrhea, microscopy is not recommended, especially for asymptomatic patients; in fact that negative results do not exclude the presence of infection due to the low sensitivity of this method. Generally, the microscopy method does not provide any data on antimicrobial sensitivity. In settings with more resources, nucleic acids amplification tests could replace culture for the detection of gonococci. This method allows the detection of nonviable bacterium, with a higher sensitivity than other diagnostic methods, especially for rectal and pharyngeal samples. This method is also rapid, could be automated, and enable simultaneous detection of several pathogens, but it does not inform about antimicrobial resistance profile testing [13].

1.4 Treatment of *gonorrhea*

The empiric treatment of gonococcal infection recommended by the WHO was dual therapy (injectable ceftriaxone and azithromycin). However, some countries have transitioned to ceftriaxone monotherapy (increasing dose from 250 to 500 mg intramuscular injection). This is due to the increasing emergence of azithromycin

resistance and the treatment failure of dual therapy as it was reported in 2014 and 2018 in the United Kingdom (UK). This therapeutic strategy has been adapted by other countries, such as the UK, China, and Japan.

Pharyngeal infections are one of the typical treatment failure consequences as they are an important site of infection. Although they are predominantly asymptomatic. This is a warning that the era of untreatable gonorrhea is near, but new drugs that specifically target antibiotic-resistant *N. gonorrhoeae* is under current investigation. Currently, several promising agents are on the horizon for *N. gonorrhoeae*, including new antibiotics. Some new antibiotics target the GyrB subunit in DNA gyrase, such as zoliflodacin, and other target the topoisomerase IV, like the gepotidacin [14].

1.5 *Neisseria gonorrhoeae* antimicrobial resistance and epidemiology

N. gonorrhoeae has a great ability to develop resistance mechanism to available first-line antibiotics, such as penicillin, fluoroquinolones, and tetracyclines, increasing the burden of multidrug-resistant *N. gonorrhoeae* [15]. The study of the evolution of antimicrobial resistance (AMR) shows that the resistance of *N. gonorrhoeae* has been driven by the widespread use and misuse of antibiotics, in view of the natural absence of AMR elements in this bacterium [2]. With the introduction of each new antibiotic, resistance soon followed: penicillins (1943, resistance developed since 1989), fluoroquinolones (the 1980s, no longer recommended in 2007), tetracyclines (1962, high-level resistance noted in 1985), sulfonamides (1930s, up to 90% resistance reported in 1940), spectinomycin (1961, emergence of resistance in 1987), and azithromycin (1983, no longer recommended in 2007), cefixime (1983, clinical failures in Japan in 2010), and ceftriaxone (1980, first high-level resistance strain reported in 2009). Ceftriaxone is presently the last remaining empiric treatment option, highlighting the urgent need for research and development of new antibiotics and change in treatment regimens [2].

Indeed, the treatment failure, slow update of treatment guidelines in most countries, and the particular ability of the gonococci to develop and retain AMR make the global problem of gonococcal AMR worst in the foreseeable future. Consequently, severe complications of gonorrhea will emerge as a silent epidemic [13]. In fact, the WHO lists *N. gonorrhoeae* as a "priority pathogen", and reported over 78 million cases each year, with uncontrolled transmission and limited treatment options, untreatable gonorrhea will increase the incidence and complications from infections, like the infertility in women. Accordingly, the WHO established the Gonococcal Antimicrobial Surveillance Program (GASP) in 1992, to encourage countries to collect and report their AMR data for at least one antibiotic, in order to develop their own gonococcal AMR surveillance programs. Hence, the implementation of optimal surveillance programs is of utmost importance [2].

1.6 Antimicrobial resistance mechanisms in *N. gonorrhoeae*

N. gonorrhoeae is capable to damage its own genetic material because it is naturally competent for transformation during its life cycle and through different types of mutations [13]. This allows bacteria to survive and rapidly adapt to various environments (different sites in the human host). Gonococci develop all mechanisms of AMR to all antimicrobials used or recommended for treatment, e.g., (i) decreasing of the influx of antimicrobial and increasing of their efflux, (ii) modification of targets and reduction of affinity for antimicrobials, and (iii) enzymatic modification

or destruction of antimicrobials [13]. As an example, gonococcal resistance to penicillin and tetracycline is due to the mutation of *blaTEM* gene and the *tetM* [16], respectively, which are plasmid-borne and can be easily transferred.

AMR genetic determinants are chromosomally transcribed where some can provide high resistance levels *in vitro* and *in vivo* leading to treatment failure. The acquisition of a single AMR determinant could confer only a cumulative increase in AMR compared to the cumulative effect of certain AMR determinants. The interaction between them may result in a significant increase in AMR levels. For example, the development of several chromosomally inserted determinants results in the resistance of *N. gonorrhoeae* to penicillin.

This looming health threat has restimulated interest in the development of new antimicrobial therapies. Active efforts are being made by several pharmaceutical majors to identify the drug targets and develop new drugs to treat such diseases effectively [17]. These targets should be present in microbes and plants, but not in humans.

Based on previous studies, the branched-chain amino acid (BCAA) pathway has been considered an attractive target for antimicrobial drug discovery as a result of comparative pathway analysis between host and pathogen [17]. First, it has been shown that all enzymes in the pathway are essential for the growth of bacteria in culture. Second, this pathway is present only in bacteria, plants, and fungi but not in animals and humans. Hence, inhibitors that target these enzymes are likely to be nontoxic to humans [18].

The BCAA pathway I is responsible for the synthesis of Leucine, valine, and isoleucine. However, this metabolic pathway is absent in humans and other animals, making them unable to synthesizee their own BCAAs and rely on obtaining these essential nutrients from their diet. Consequently, BCAA enzyme inhibitors are likely to be effective drugs, while not exerting any toxic effects in humans [1]. Ketol-acid reductor-isomerase (KARI) is the second enzyme in the branched-chain amino acid (BCAA) biosynthesis, which regulates many physiological activities in a variety of organisms from bacteria to fungi and plants. The conservation in fungi but absence in mammals of the BCAA biosynthetic pathway makes it the target for herbicides, fungicides, and antimicrobial compounds [19]. KARI catalyzes the conversion of 2-acetolactate and 2-aceto-2-hydroxybutyrate to 2,3-dihydroxyisoverate and 2,3-dihydroxy-3-methyl valerate, respectively. ILVC is a bifunctional enzyme that catalyzes two quite different reactions, but occurs at a common active site, acting both as an isomerase and as a reductase [19].

Previous studies showed the efficiency of some KARI inhibitors against *S. aureus*, *M. tuberculosis* with an inhibitory effect on bacterial growth leading to the killing of bacteria [1, 20]. By assessing the presence of the KARI enzyme in pathogens and understanding its structure and draggability, the design of novel antimicrobials to circumvent the resistance problems can be undertaken more rationally.

This review focuses on looking for the presence of the KARI enzyme among pathogens, bacteria, and fungi, the study of its expression and production during the host infection, and its common druggable site and susceptibility to previously recommended enzyme inhibitors.

2. Material and methods

2.1 Phylogenetic analysis

BLAST searches were carried out to identify different procaryotic ortholog of the *N. gonorrhoeae* (GenBank accession number EEZ44675.1) KARI protein

which is conserved in *Neisseria* (https://www.ncbi.nlm.nih.gov/ipg/EEZ44675.1). KARI sequences were aligned using ClustalW [21], and then a neighbor-joining tree was generated using MEGA software [22]. KARI sequences from *Escherichia coli* K12 (AKD89606.1) *Enterococcus faecium* (KXH23108), *Staphylococcus aureus* (MBU4945389.1), *Klebsiella pneumoniae* (MBC4258974.1), *Actinobacter baumanii* (EHU1490884.1), *Pseudomonas aeruginosa* (EJY59157.1), were used for phylogenetic analysis.

2.2 Sequence alignments and phylogenetic analysis

Multiple-sequence alignments were performed using the Clustal W webserver. The evolutionary relationship of *N. gonorrhoeae* to other pathogenic strains producing similar ketol-acid reductor-isomerase enzymes was examined using a phylogenetic analysis of the full-length KARI sequences with the MEGA software (version 11.0.10).

2.3 Homology modeling

The structural model of KARI was obtained from NCBI database http://www.blast.ncbi.nlm.nih.gov. Ketol acid reductor-isomerase (KARI) enzyme of *N. gonorrhoeae* was subjected for homology modeling using the Swiss model. The structural homolog, which was used as a template for this model, is ketol acid reducto-isomerase enzyme from *S. aureus (Sa* KARI*)* with PDB identifier 5w3k. The sequence similarity between the template and the model is about 33%. The KARI model and the template (5w3k) were superimposed using the PYMOL software (version 2.4.1) [23].

3. Results and discussion

3.1 Phylogenetic analysis of KARI from pathogens

According to the results of the BLAST search with the sequence of KARI protein from *Neisseria gonorrhoeae* FA19 (*Ng* KARI), KARI sequences for human pathogens were obtained. A phylogenetic analysis of the amino acid sequence with reported pathogens was conducted. As shown in the result, *Ng* KARI is closely related to the KARI from the ESKAPE pathogens. All of them are clustered in respective clades (**Figure 1a**).

The sequence alignment of KARI protein from other human pathogens is reported in **Figure 1b**. All tested pathogens share a common ancestor. However, KARI from *S. pneumonia*, *N. meningitidis*, *S. enterica*, *M. tuberculosis*, *B. cereus* and *B. anthracis* belong to a different clade than the other pathogens (*Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Yersinia pseudotuberculosis*, *Yersinia enterocoliticia*, and *Francisella pneumoniae*).

The presence of KARI was also assessed in various members of the gut microbiota, to understand if the latter will be inhibited by KARI inhibitors (**Figure 2**). KARI from *P. dentalis* and *B. fragilis* belong to the same clade, different from the other Gut bacteria (*F. nucleatum*, *B. bifidum*, *Lactobacillus* sp., and *A. muciniphila*).

3.2 KARI sequence alignment

The alignment of KARI enzyme was carried out using BLAST and Clustal W. Multiple-sequence alignment of *Ng* KARI and KARIs from human pathogens,

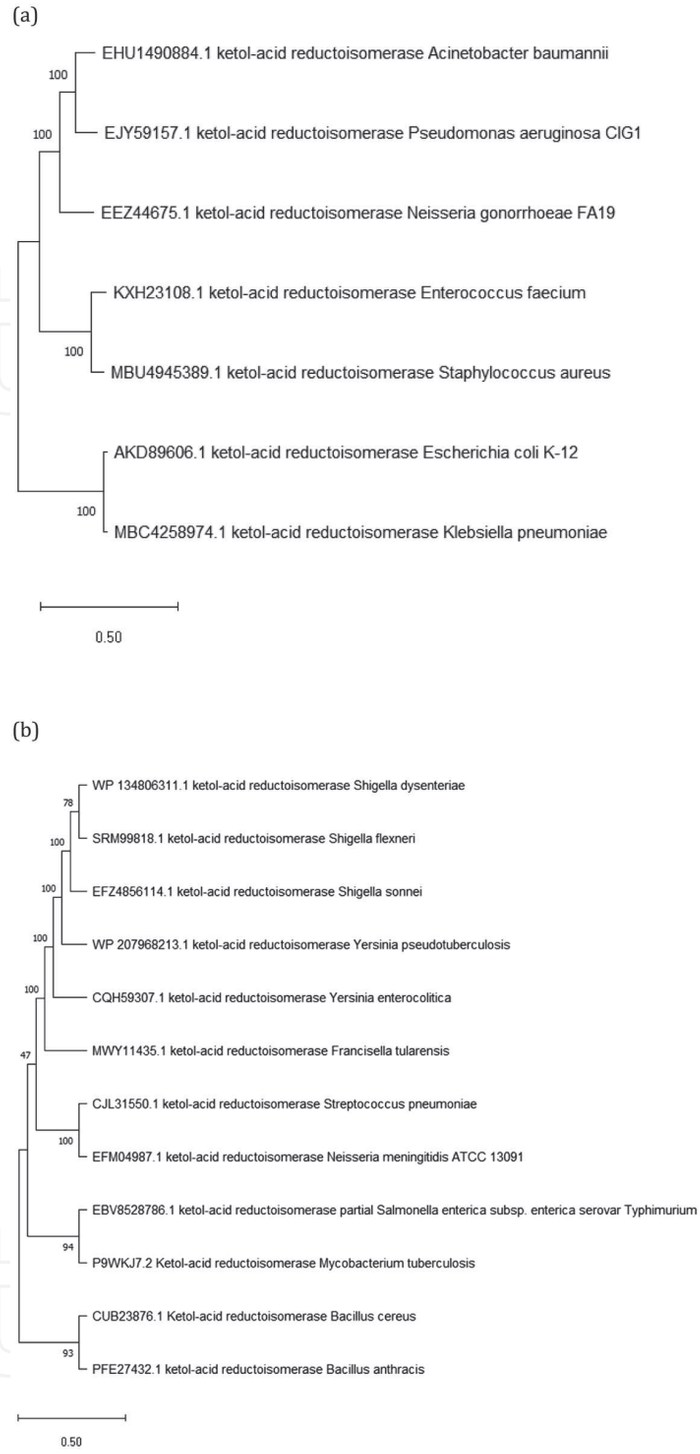

Figure 1.
Phylogenetic tree based on KARI sequence between N. gonorrhoeae and ESKAPE pathogens (a) and other human pathogens (b). Accession numbers are as follows: (a) A. baumannii (EHU1490884), P. aeruginosa CIG1 (EJY 59157), E. faecium (KXH23108), S. aureus (MBU4945389), E. coli K12 (AKD89606), and K. pneumoniae (MBC4258974). (b) S. dysenteriae (WP 134806311.1), S. flexneri (SRM99818.1), S. sonnei (SRM99818.1), Y. pseudotuberculosis (WP 207968213), Y. enterocolitica (CQH59307.1), F. tularensis (MWY11435.1), S. pneumoniae (CJL31550.1), N. meningitidis ATCC 13091 (EFM04987.1), S. enterica (EBV8528786.1), M. tuberculosis (9WKJ7.2), B. cereus (CUB23876.1), and B. anthracis (PFE27432.1).

revealed that residues constituting NADP(H) and Mg^{2+} binding sites are well conserved, while the overall length of each KARI is different. The tested bacteria share different residues, especially in the active pocket, and cofactors binding sites. Indeed, different KARIs have almost identical active site structures.

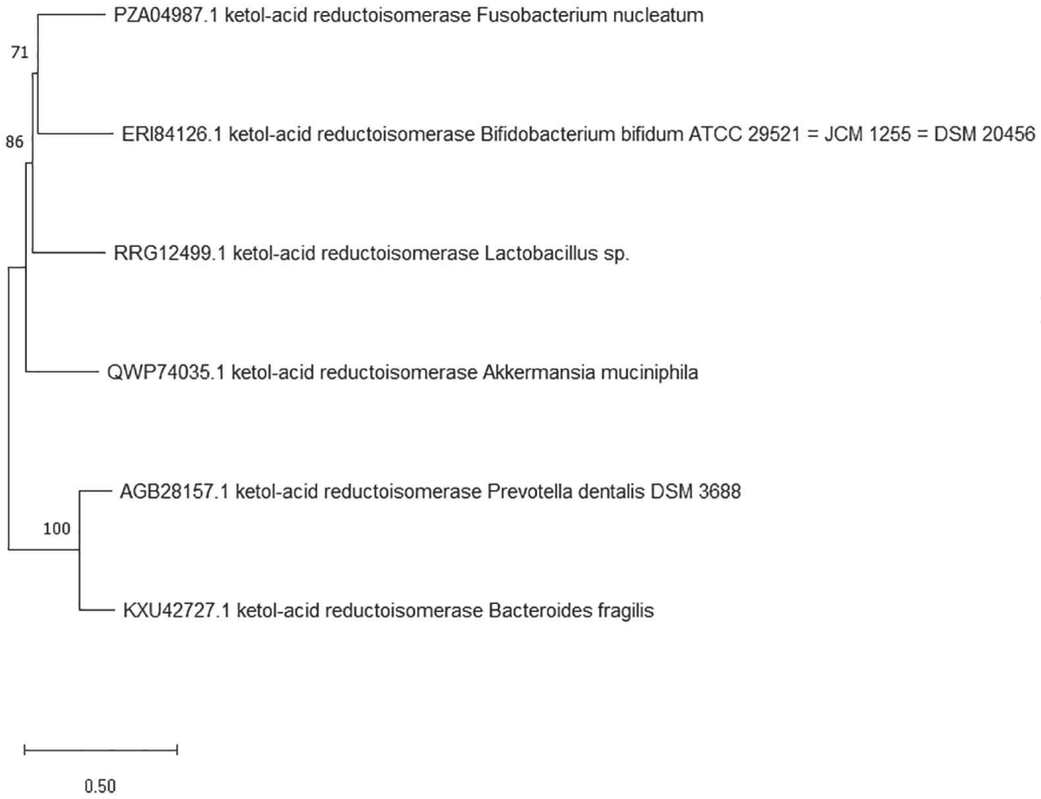

Figure 2.
Phylogenetic tree based on KARI sequence between N. gonorrhoeae and some members of the gut microbiota. Accession numbers are as follows: (a) F. nucleatum (PZA04987.1), B. bifidum (ERI84126), Lactobacillus (RRG12499), A. muciniphila (QWP74035), P. dentalis (AGB28157), and B. fragilis (KXU42727). The tree was constructed using neighbor-joining analysis based on KARI protein sequences. The scale bar represents 0.5 substitutions per nucleotide position.

Analysis of residues contacting NADP(H) and Mg^{2+} identified five amino acid residues, in *Sa* KARI, as contacting ones: Arg-47, Asp-81, Ser-51, Asp-189, and Glu-193 (**Figure 3b**). KARIs harbor a GxGxxG motif, which is part of the nucleotide-binding site by phosphate-bridging interaction (**Figure 3c**), and Mg^{2+} is required for NADP(H) binding. The study of residue mutations' effect on NADPH binding to the KARI's structure show that residues A71, R76, and S78 are in the loop connecting the β2 sheet and the αB helix, referred to as the β2αB loop. R76, and S78 establish direct contact with the 2′-phosphate of NADPH. Sequence alignment of KARI show a variable length of the β2αB loop among tested bacteria (**Figure 4**). This loop is crucial for the cofactor specificity [24].

Upon the binding of cofactors, NADP(H) and Mg^{2+}, the N-terminal domain of KARI undergoes large local conformational changes, only in the NADP(H) binding site. Four Mg^{2+}-binding residues are also identified (D190, E194, E226, and E230) [19]. The side chains of these residues rotate upon metal ion binding. Previously, the mutation of R47 and D81 induce rotameric changes in other bulky residues (His-31, Lys-52, Phe-54, and His-135), resulting in the NADP(H) binding pocket broadening, and then a weak binding to the structure [25].

Other conserved residues are identified. According to available KARI's structure analysis, these amino acids interact with inhibitors. Notably, KARI binds different ligands other than metal ions and NADP(H), such as IpOHA, cyclopropane-1, 1 dicarboxylic acid (CPD) and 2-(dimethyl phosphoryl)-2-hydroxyacetic acid

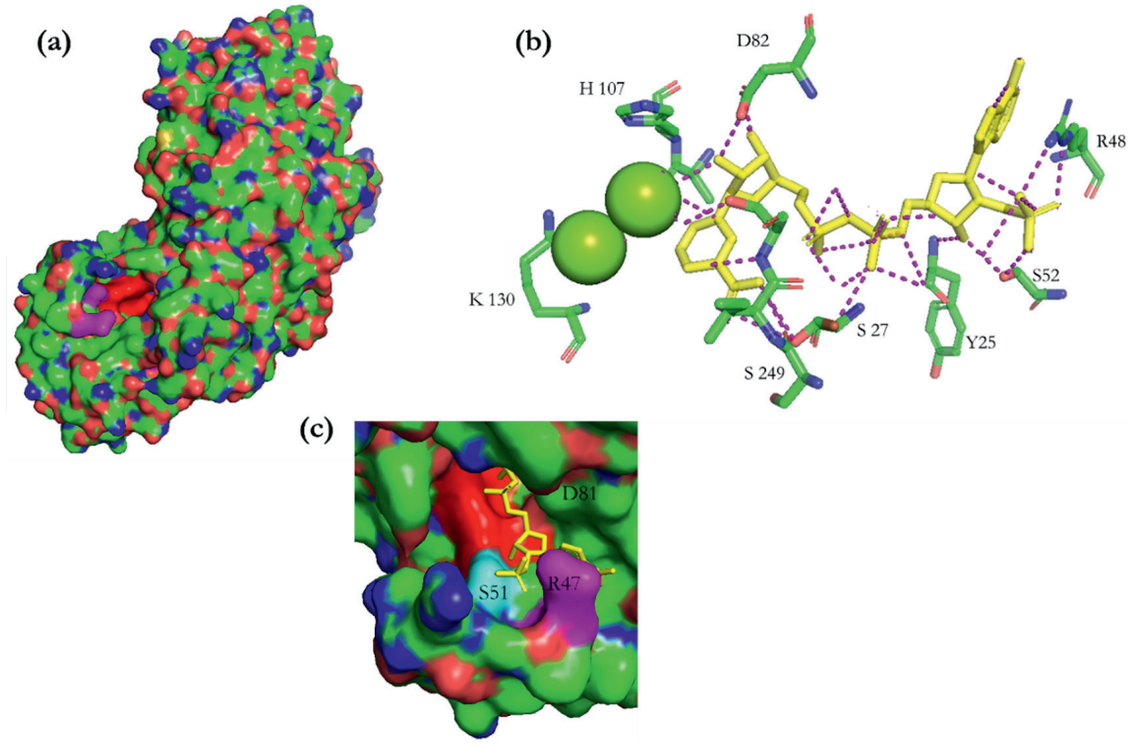

Figure 3.
Surface representation of the crystal structure of KARI from Staphylococcus aureus (Sa KARI) (PDBID: 5w3k) (Sa KARI), the NADP(H) binding site is shown as a red cavity. (b) Stereo view of the binding mode of NADP(H). NADP(H) is shown as yellow sticks, and metals are shown as green spheres. Polar contacts with residues within 5 of the NADPH are shown in magenta in dashed lines (c) surface representation of the NADP(H) binding site pocket of Sa KARI. NADP(H) is shown as yellow sticks.

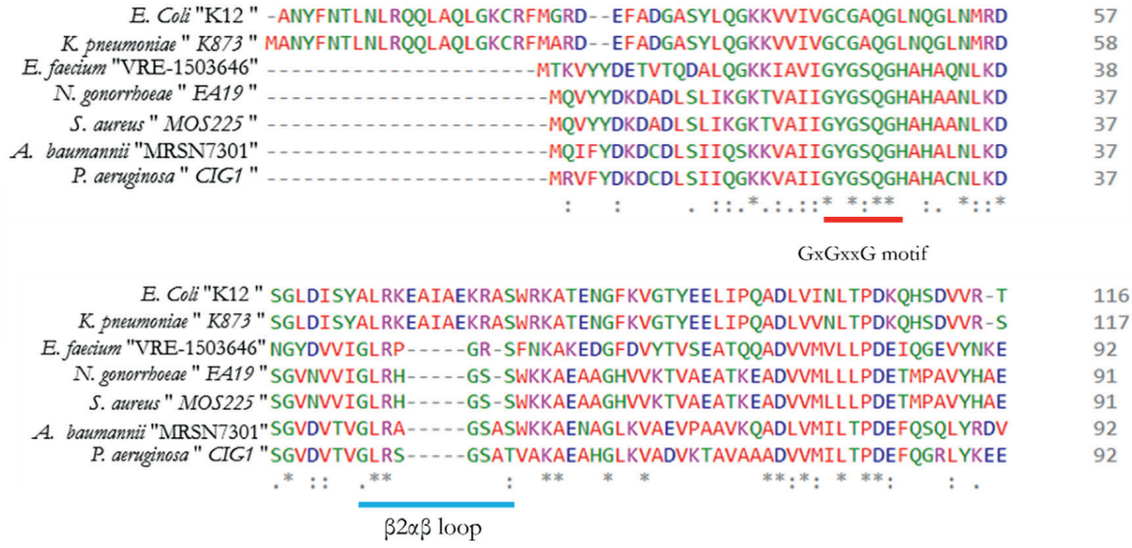

Figure 4.
Multiple alignments of KARI partial sequence from members of the ESKAPE pathogens groups. Clustal W was used to align KARI sequences from six members of the ESKAPE (Escherichia coli strain K12, Staphylococcus aureus strain MOS225, Klebsiella pneumoniae strain K783, Acinobacter baumannii strain MRSN7301, Pseudomonas aeruginosa strain CIG1, and Entercoccus faecium strain VRE-1503646) against the orthologue from N. gonorrhoeae. The alignments were used to identify regions possessing the greatest similarities. The conservation of residues is indicated above the alignments as follows: asterisk, complete identity; colon, conservation of a strong group; period, conservation of a weak group. GxGxxG motif is shown in red line, while β2αB loop is shown in blue line.

Inhibitor	Ligands	PDBID	Reference
CPD	E230	5W3K	[12]
	S251		
	NDP		
	Me^{2+} ions		
IpOHA	D188,	4YPO	[12]
	E192		
	E224		
	E228		
	Mg^{2+} ions		
Tartaric acid	D190	4TSK	[13]
	E194		
	C199		
	G230		
	S251		
	NDP		
Cyclopentylamino(oxo) acetic acid	E193	6C5N	
	D189		
	E229		
	S250		
	Mg^{2+} ions		

Table 1.
Summarization of ligands residues for KARI's inhibitors.

(Hoe704). These compounds are the most extensively characterized KARI inhibitors investigated to date. They are transition state analogs. Residues involved in these inhibitors binding are conserved among bacteria, as described in **Table 1**. These inhibitors could be tested with KARI from *N. gonorrhoeae*, to assess its effect on bacterial growth rate, its viability, and antimicrobial resistance.

In the presence of Mg^{2+} ions, the active site of KARI becomes open and accessible to solvent, while the NADP(H) binding reduces the space between the domains, and the active site adopt a closed conformation. Hence, the active site changes its surface structure to become appropriate for substrate binding. The open-close transition state has been thought to facilitate substrate binding and catalysis. This feature provides then possibilities for the development of inhibitors able to bind to both of structural conformations of KARI (i.e. ± NADPH) [6].

3.3 KARI modeling

The modeling of KARI enzyme from *N. gonorrhoeae* was carried out using the Swiss-model webserver, using as a template the KARI enzyme from *Staphylococcus aureus* (PDBID 5w3k). As shown in **Figure 5**, the KARI model shows high similarity in the active pocket (AFAHGFNIH) and N-terminal and C-terminal domains. A

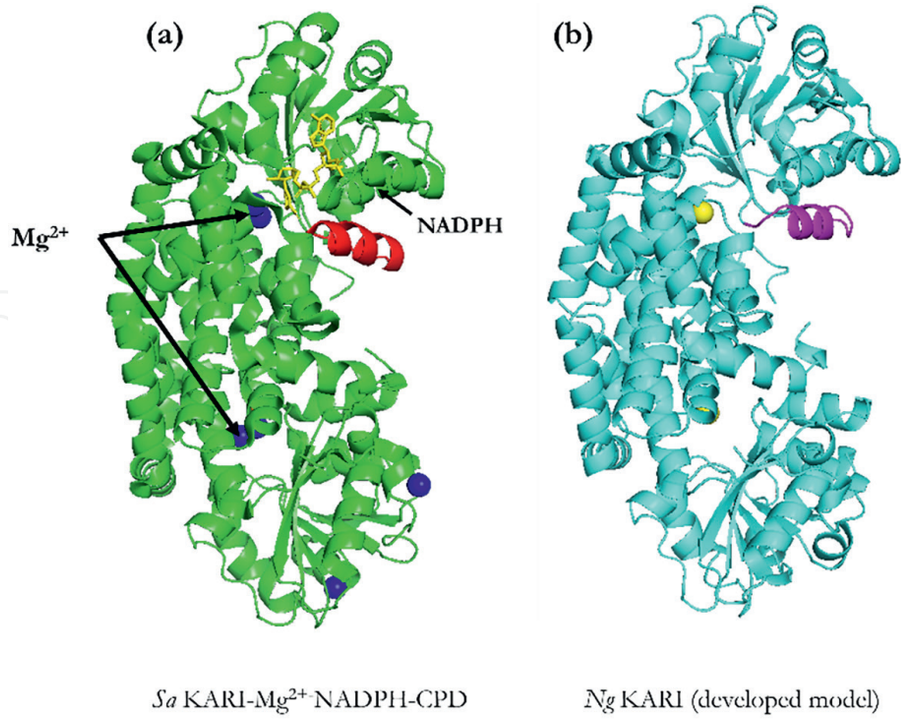

Figure 5.
Comparison of the crystal structure of the Ng KARI (model) with Sa KARI-Mg^{2+}-NADPH-CPD complex. (a) Mg^{2+} ions (blue), and NADPH (red) are shown, respectively, as spheres and sticks. In (b) the Mg^{2+} ions (yellow) are drawn as spheres. The residues colored in red in Sa KARI (i.e. residues 131–145) and magenta in Ng KARI (i.e. residues 130–144) have different orientations in the two enzymes, probably due to the binding of NADPH.

comparison of the N-terminal section of *Ng* KARI with the *Sa* KARI shows that they are some prominent structural differences, particularly in the region from *Ng* KARI as illustrated in **Figure 5**.

The overall fold of the *Ng* KARI resembles that of the *Sa* KARI-NADP(H) complex. No significant domain movements are observed between these two enzymes apart from a change in orientation of the polypeptide associated with the NADP(H) binding site. KARIs from class I differ in their quaternary structures by being dimeric like *Ng* KARI, *Sa* KARI, and KARI from *Mycobacterium tuberculosis* (*Mt* KARI), while others like KARI from *Campylobacter jejuni* (*Cj* KARI) are dodecameric. In their respective active sites, there are two differences between *Ng* KARI and *Sa* KARI, i.e., G100/A106 and L103/ F109. These differences are conserved between *Ng* KARI and *Mt* KARI, G100/G104, L103/L107, with G130/G129. The catalytic residue E230 and Mg^{2+} ligands are highly conserved between all tested pathogens.

3.4 KARI structure analysis

The KARI crystal structure is an asymmetric dimer. This latter is formed by one protomer in the holo-form due to the cofactors (Mg^{2+} and NADP(H)) binding, while the other is in the apo-form. As described previously for similar KARI enzymes, the dimerization is crucial for the construction of the active site, which is formed by some regions of the N-terminal domain and C-terminal domain of one subunit and the C-domain of the other subunit in the dimer. This arrangement is shared by other KARIs from other bacteria.

The KARI enzyme is composed of two distinct domains, the N-terminal (1–181) and C-terminal (182–327). The N-terminal domain, composed of alpha helix and beta

sheets, harbor the NADPH-binding domain ilvN (14–177), with the binding sites for NADP (R48 and S52), while the catalytic domain ilvC is present in the C-terminal domain (183–326). The metal binding sites are present in both N-terminal and C-terminal domains (N39, V70, K71, and A73), and (D190 and E194), respectively. The structure of KARI binds four Mg^{2+} ions in the active sites and five Mg^{2+} on the surface of the protein.

A high electron density is shown in two locations deep inside the active site of KARI. This density is ascribed to magnesium ions coordinated differently to the structure. The first Mg^{2+} ion (Mg^{2+} (I)) is coordinated by the side chains of two residues (D188 and E192), and four water molecules, while the second Mg^{2+}(II) is coordinated by the side chains of D188, E224 and E228, in addition to three water molecules. The metal ligands adopt an octahedron coordination geometry, with distances varying between 2.0 and 2.1 A between the metal and its ligand. The average B-factors for Mg^{2+}(I) and Mg^{2+}(II) are 11.5 Å2 and 10 Å2, respectively, supporting a highly ordered structure in this region of KARI [26].

Because of the absence of crystal structures of KARI from *N. gonorrhoeae*, the structure analysis is based on similar structure for close organisms having similar amino acid sequences. Based on the crystal structure of Mg KARI, the active site pocket and thus the magnesium ions are exposed to the solvent, allowing ready access to the substrates, the NADPH, or an inhibitor able to prevent their binding to the structure. The expected binding site for NADPH includes residues from Y22 to G26. All these residues are solvent accessible, hence the residue S24 seems to be the main entrance to the active site. Thus, the designed inhibitors should be designed that target this surface with metal coordination.

4. Conclusion

Increasing concerns associated with overusing antibiotics in animals and humans make it urgent to find new alternatives for treating bacterial infections and diseases. BCAA pathways enzymes are promising antimicrobial alternatives being developed as potential drug targets absent in animals and humans. Comparative studies of KARI from different bacteria bestows the idea that this essential enzyme can be targeted for anti-bacterial drug design. Therefore, KARI is considered a good target, due to a non-homologous protein in comparison with human proteins, and its targeting will be safe for humans. In this review, we assess the presence of KARI in most human pathogens, especially the ESKAPE group, due to its high antibiotic-resistance causing severe infections.

Since the 3D structure for KARI from *N. gonorrhoeae* was nonreported yet, a model of this enzyme was produced using the Swiss model. Indeed, the *Ng* KARI was modeled in silico based on X-ray crystallography structure for *Sa* KARI, used as a template. It was evaluated that cofactors ligands (Mg^{2+} and NADPH) were conserved among human bacterial pathogens. Upon the binding of these cofactors, KARI adapts a different conformation allowing the substrate binding and catalysis, while the active site adapt to a closed state.

Competitive inhibitors, targeting the active site are promoting drugs for the growth inhibition of pathogens. However, the NADPH binding site and the active site are highly conserved among bacteria, including the gut microbiome. In fact, a growing number of studies have shown that antibiotics can result in microbial dysbiosis, due to their broad-spectrum activities, when subsets of commensal

microbes will be indiscriminately killed or inhibited. Notably, different antibiotics or their combinations have different antimicrobial spectra and will result in different damages to the microbiome. The disruption of gut microbiota contributes to numerous diseases, including diabetes, obesity, autism, and superinfection in critically ill patients.

Under normal physiological conditions, the microbiota maintains a homeostatic state. It also plays a crucial role in many aspects of physiological processes, including maintaining the integrity of the gut mucosal barrier, promoting the development of the immune system, and protecting against enteric pathogens. Inappropriate antibiotics impact host immunity by altering the bacterial metabolites and the signals transmitted from gut microbiota to the host (intestinal epithelial cells and intestinal immune cells).

Because antibiotic administration elicits many side effects, restriction of the overuse of antibiotics is imperative. However, it is unrealistic to completely abandon antibiotics in clinical practice, especially for patients with severe infections. Therefore, safe strategies should be proposed to attenuate and/or avoid antibiotic-induced microbial dysbiosis and gut microbiome disruption. Firstly, intravenous administration of KARI's inhibitors drugs (or adjuvant) may avoid the gut flora alteration. The adjuvant administration can be followed by the administration of PRRs agonist, or probiotics. Indeed, the probiotic intervention is able to reduce the antibiotic-associated diarrhea [27]. A recent study suggested that co-administration of probiotics and antibiotics prevents *C. difficile* infection in patients receiving antibiotics [28]. On the other hand, gut microbiota transplantation is a new strategy able to control intestinal inflammation and restore the intestinal homeostasis.

KARI's inhibitors remain a good alternative for external treatment for dermatological infections like staphylococcal skin infections in deep wounds. The external application or the intravenous administration of the drug will avoid the alteration of gut flora. Even with need for an oral administration of these drugs, the disorder of gut flora will be partial, due to the high population density of a healthy microbiome, compared to pathogens. Therefore, probiotics can help to maintain the gut flora in sufficient density to maintain the gut mucosal barrier.

Moreover, KARI is an attractive target for the development of new biocides. Some tested KARI's inhibitors inhibit the enzyme in the nanomolar range [29]. Furthermore, the study of KARI-expression during bacterial host-infection may confer new insight on the importance of these targets in the pathogen metabolism and growth. The investigation of the effect of potent KARI's inhibitors on pathogen growth in the presence or not of appropriate antibiotic allow further progress toward the understanding of the inhibitor's effect on pathogen and host-homeostasis, and the development of new inhibitors that specifically target KARIs in pathogenic processes.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Wun SJ, Johnson LA, You L, McGeary RP, Brueck T, Schenk G, et al. Inhibition studies of ketol-acid reductoisomerase from pathogenic microorganisms. Archives of Biochemistry and Biophysics. 2020;**692**:108516

[2] Ey L, Pc A, Jd K. Epidemiology, treatments, and vaccine development for antimicrobial-resistant *Neisseria gonorrhoeae*: Current strategies and future directions. Drugs. Jul 2021;**81**:1153-1169. DOI: 10.1007/s40265-021-01530-0

[3] Unemo M, Nicholas RA. Emergence of multidrug-resistant, extensively drug-resistant and untreatable gonorrhea. Future Microbiology. 2012;**7**:1401-1422

[4] Quillin SJ, Seifert HS. *Neisseria gonorrhoeae* host-adaptation and pathogenesis. Nature Reviews. Microbiology. 2018;**16**:226-240

[5] Voter AF, Callaghan MM, Tippana R, Myong S, Dillard JP, Keck JL. Antigenic variation in *Neisseria gonorrhoeae* occurs independently of RecQ-mediated unwinding of the pilE G quadruplex. Journal of Bacteriology. 2020;**202**:e00607-e00619

[6] Zughaier SM, Kandler JL, Shafer WM. *Neisseria gonorrhoeae* modulates iron-limiting innate immune defenses in macrophages. PLoS One. 2014;**9**:e87688

[7] Lutz AR. Screening forasymptomatic extragenital *Gonorrhoea* and *Chlamydia* in men who have sex with men: Significance, recommendations, and options for overcoming barriers to testing. LGBT Health. 2015;**2**:27-34

[8] Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, et al. Sexually transmitted infections treatment guidelines, 2021. MMWR - Recommendations and Reports. 2021;**70**:1-187

[9] Gonorrhea in women: symptoms, diagnosis, causes, and treatment. Flohealth - 1 Mob Prod Women's Health. 2022. Available from: https://flo.health/menstrual-cycle/sex/stis/gonorrhea-in-women [Accessed: September 6, 2022].

[10] Gonorrhoea - Symptoms. NhsUk 2018. 2022. Available from: https://www.nhs.uk/conditions/gonorrhoea/symptoms/ [Accessed: September 6, 2022]

[11] Gonorrhea: Causes & Symptoms. Clevel Clin. 2022. Available from: https://my.clevelandclinic.org/health/diseases/4217-gonorrhea [Accessed: September 6, 2022].

[12] Whelan J, Abbing-Karahagopian V, Serino L, Unemo M. Gonorrhoea: A systematic review of prevalence reporting globally. BMC Infectious Diseases. 2021;**21**:1152

[13] Unemo M, Shafer WM. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: Past, evolution, and future. Clinical Microbiology Reviews. 2014;**27**:587-613

[14] Huband MD, Bradford PA, Otterson LG, Basarab GS, Kutschke AC, Giacobbe RA, et al. In Vitro antibacterial activity of AZD0914, a new Spiropyrimidinetrione DNA Gyrase/Topoisomerase inhibitor with potent activity against Gram-Positive, fastidious Gram-Negative, and atypical bacteria | Antimicrobial Agents and Chemotherapy. Jan 2015;**59**(1):467-474

[15] Abrams AJ, Trees DL. Genomic sequencing of *Neisseria gonorrhoeae*

to respond to the urgent threat of antimicrobial-resistant gonorrhea. Pathogens and Disease. Jun 2017;**75**(4): ftx041. Available from: https:// DOI org/10.1093/femspd/ftx041

[16] Morse SA, Johnson SR, Biddle JW, Roberts MC. High-level tetracycline resistance in *Neisseria gonorrhoeae* is result of acquisition of streptococcal tetM determinant. Antimicrobial Agents and Chemotherapy. 1986;**30**:664-670

[17] Morya VK, Kumari S, Kim E. Virtual screening and evaluation of Ketol-acid Reducto-Isomerase (KARI) as a putative drug target for Aspergillosis. Clinical Proteomics. 2012;**9**:1

[18] Lin X, Kurz JL, Patel KM, Wun SJ, Hussein WM, Lonhienne T, et al. Discovery of a pyrimidinedione derivative with potent inhibitory activity against *mycobacterium tuberculosis* Ketol-acid Reductoisomerase. Angew Chem Weinheim Bergstr Ger. 2021;**27**:3130-3141

[19] Wang Y, Liu S, Yin X, Yu D, Xie X, Huang B. Functional analysis of Keto-acid Reductoisomerase ILVC in the entomopathogenic fungus *Metarhizium robertsii*. Journal of Fungi. 2021;**7**:737

[20] Krishna VS, Zheng S, Rekha EM, Nallangi R, Sai Prasad DV, George SE, et al. Design and development of ((4-methoxyphenyl)carbamoyl) (5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)amide analogues as *mycobacterium tuberculosis* ketol-acid reductoisomerase inhibitors. European Journal of Medicinal Chemistry. 2020;**193**:112178

[21] Clustal W, Clustal X version 2.0 | Bioinformatics | Oxford Academic. 2022. Available from: https:// academic.oup.com/bioinformatics/ article/23/21/2947/371686 [Accessed: August 21, 2022]

[22] Kumar S, Nei M, Dudley J, Tamura K. MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. Briefings in Bioinformatics. 2008;**9**:299-306

[23] DeLano WL. Pymol: An open-source molecular graphics tool. CCP4 Newsl Protein Crystallography. 2002;**40**:82-92

[24] Chen C-Y, Ko T-P, Lin K-F, Lin B-L, Huang C-H, Chiang C-H, et al. NADH/NADPH bi-cofactor-utilizing and thermoactive ketol-acid reductoisomerase from *Sulfolobus acidocaldarius*. Scientific Reports. 2018;**8**:7176

[25] Kim G, Shin D, Lee S, Yun J, Lee S. Crystal structure of IlvC, a Ketol-acid Reductoisomerase, from *streptococcus Pneumoniae*. Crystals. 2019;**9**:551

[26] Lv Y, Kandale A, Wun SJ, McGeary RP, Williams SJ, Kobe B, et al. Crystal structure of *mycobacterium tuberculosis* ketol-acid reductoisomerase at 1.0 Å resolution – A potential target for anti-tuberculosis drug discovery. The FEBS Journal. 2016;**283**:1184-1196

[27] Zhang S, Chen D-C. Facing a new challenge: The adverse effects of antibiotics on gut microbiota and host immunity. Chinese Medical Journal. 2019;**132**:1135-1138

[28] Goldenberg JZ, Mertz D, Johnston BC. Probiotics to prevent *Clostridium difficile* infection in patients receiving antibiotics. JAMA. 2018;**320**:499

[29] Bayaraa T, Kurz JL, Patel KM, Hussein WM, Bilyj JK, West NP, et al. Discovery, synthesis and evaluation of a novel ketol-acid reductoisomerase inhibitor. Angew Chem Weinheim Bergstr Ger. 2020;**26**(41):8958-8968