

An insilico approach towards identification of virulence factors in *Eikenella corrodens* targeted by berberine

Contributors

Shebi S, Saveetha Dental College and Hospitals,
Chennai-600077, Phone number: 9790952889
E-mail address: shebisachu46@gmail.com

Arvina Rajasekar, Senior Lecturer,
Department of Periodontology,
Saveetha Dental College and Hospitals,
Chennai – 600077, Phone number: 09486442309
E-mail address: arvinar.sdc@saveetha.com

Corresponding Author

Arvina Rajasekar, Senior Lecturer,
Department of Periodontology, Saveetha Dental College and Hospitals,
Chennai – 600077 ,Phone number: 09486442309
E-mail address: arvinar.sdc@saveetha.com

ABSTRACT

Background: Berberine, a benzyloquinoline alkaloid found in a variety of medicinal plants, has a wide range of pharmacological characteristics. Berberine has diverse inhibitory effects on different bacteria, however one element of its selectivity by using insilico methods is that the impact is very good with the maximal inhibitory concentration for *Eikenella corrodens*.

Materials and Methods: The STITCHv5.0 pipeline was primarily used for identifying drug-protein interactions; VirulentPred and VICMPred were used for elucidating the virulence property and functional class of the proteins.

Results: Berberine interacts with proteins includes in cellular processes, metabolism, and pathogenicity, according to research. The pathogenic proteins attacked by the medications were found in the cytoplasm, which would increase the potency of drug as antibacterial activity

Conclusion: A thorough understanding of the interactions between these medications and their antibacterial agents would increase the list of advantages these treatments have in therapeutic situations. To validate the true interactions between medicines and pathogen protein repertoires, more in vitro study on a wide spectrum of infections is required.

Keywords: Berberine, Bacteria, Pathogenicity, Virulence

INTRODUCTION:

Due to the emergence of multidrug-resistant bacteria, the bulk of modern infectious illnesses are almost untreatable by standard antibiotic therapy. When germs develop biofilm, the severity and persistence of illnesses are exacerbated.(1) As a result, efforts are being made to create novel medications that are less susceptible to bacterial resistance mechanisms than existing drugs and can also target bacteria in biofilm. Natural products, particularly those derived from plants, have proven to be exceptional substances with distinctive features, preparing them ideal candidates for these more required medicines. *Eikenella corrodens* (previously *Bacteroides corrodens*) is a bacteria that can be found in the mouth,

gastrointestinal tract, and genitourinary tract.(2) It's a gram-negative bacillus that's fastidious, facultative, and anaerobic. Recently, Eikenella species have shown to cause effective illness in beings, while previously being thought to be non-pathogenic.(3) In adults, these wide variety of Eikenella species are related to neck infection, head infection, lung infection, arthritis, endocarditis, intra abdominal infection, pancreatic abscesses, skull infection, vertebral osteomyelitis, and infection after human bite wounds.(4) The infection caused by Eikenella in children are far less prevalent and have only been recorded on a few occasions.(5)

Eikenella corrodens is a gram-negative micro aerophilic bacteria which lives on human mucosal surfaces, particularly the oral cavity. Penicillin, tetracycline and chloramphenicol are generally effective antibiotics, although cephalexin, dicloxacillin are not. Eikenella is highly resistant to cefoxitin and third-generation cephalosporins. Antibiotics commonly used for empirical therapy of oropharyngeal infection may be less effective against Eikenella corrodens. Many medicines, including tetracycline, amoxicillin, streptomycin, erythromycin, sulfonamide, sulfa drug, penicillin G, ceftriaxone, and fluoroquinolones, are usually effective against this bacteria.(6) Clindamycin, cephalexin, erythromycin and metronidazole are usually resistant to it. The bacteriological and histological effects of Eikenella corrodens 1073-R (EcR) on ligature-induced periodontal abnormalities in immunosuppressed and non-immunosuppressed rats were investigated.(7)

Berberine, a benzyloquinoline alkaloid derived from the traditional medicinal plant *Coptis chinensis*, occurs as an active ingredient and has a variety of pharmacological effects. Its antibacterial, antiprotozoal, antidiarrheal, and antitrichoma properties have made it popular in Ayurvedic and Chinese medicine.(8) Berberine has varying inhibitory effects on different microorganisms, although in most of the study bacteria, the effect is relatively weak, with the lowest inhibitory concentration exceeding 64 $\mu\text{g mL}^{-1}$. Eikenella corrodens has the most inhibitory impact, which is one of its selectivity characteristics.(9) Berberine, given orally at a rate of 10 mg/kg/day for ten days, produced an excellent parasitological cure that was equivalent to that obtained with other well-known anti-giardial medications (quinacrine hydrochloride, furazolidone and metronidazole).(10) Therefore, the present study was done as an insilico approach towards identification of virulence factors in Eikenella corrodens targeted by berberine.

MATERIALS AND METHODS:

Study design: Our current research uses an empirical study design to look for a nature of proteins or category of virulency in Eikenella corrodens that may interact with berberine. The STITCH v.5 pipeline was used to evaluate drug interactions with the Eikennella corodens proteome, and VICMPred and VirulentPred softwares were used to identify the virulence features of the targeted proteins.

Prediction of protein-drug interactions: The STITCH database (Version 5; 2016) claims a comprehensive programme for known and projected chemical-protein interconnection. The interactions are from computational divination and interactions collected from other (primary) datasets, and they

can be direct or indirect or functional. The protein repertoire that interacts with *Eikenella corrodens* was also utilised to predict virulence.

Virulence prediction: The pipelines VICMpred and VirulentPred were used to find virulence factors targeted by berberine in *Eikennella corrodens*. To validate the results, these tools used a support vector machine (SVM)-based five-fold cross-validation method. The VirulentPred tool was used to screen virulence factors based on amino acid composition, which is divided into two groups: virulent and avirulent. Proteins important in cellular processes, metabolism, information storage and pathogenicity are divided into four categories by VICMpred. The programme was conducted using the FASTA format of proteins acquired from the NCBI database as input.

Prediction of subcellular determination of proteins: Computational prognostications of protein subcellular limits aids in the development of new therapeutic targets or the validation of an antibacterial medication that targets the pathogenic protein. Because cell surface proteins can be utilised as vaccine targets, they are of tremendous interest. PSORTb V3.0 is an algorithm that assigns a protein's likely localization site based on its amino acid sequence.

Prediction of B-cell determinant in proteins: By using a Random Forest algorithm based on epitopes and non-epitope amino acids perceived from crystal structures, the BepiPred-2.0 server predicts B-cell antigenic determinant from a protein sequence. Residues with scores greater than 0.5 are projected to constitute epitopes and are highlighted in yellow on the graph.

RESULTS:

The STITCH pipeline was used to find out how red complex bacteria interact with the medications APAP and IB. (Figure 1) VirulentPred and VICMPred were also used to assess the virulence of each of the proteins interacting with the medicines. These algorithms produced ratings that confirmed the nature of the proteins and divided them into two groups: virulent and avirulent.

Proteins interconnecting with Berberine were initially related to metabolism, followed by cellular processes and information storage. Interestingly, the predicted epitomes and the scores from VirulentPred marked Transcriptional regulator, MerR family and TetR family as a virulence factor. (Figure 2) Berberine was found to react with proteins associated with both cellular processes and metabolism. Protein EIKCOROL_02509, EIKCOROL_01773 and EIKCOROL_00292 are found to be virulent with VirulentPred score 0.559, 0.959, 0.962 respectively and all the other proteins namely EIKCOROL_01725, EIKCOROL_02102, EIKCOROL_00541, EIKCOROL_00546, EIKCOROL_00198, EIKCOROL_00590, EIKCOROL_01573 are found to avirulent. (Table 1)

Figure 1: Network interconnection of berberine with proteins of *Eikenella corrodens*

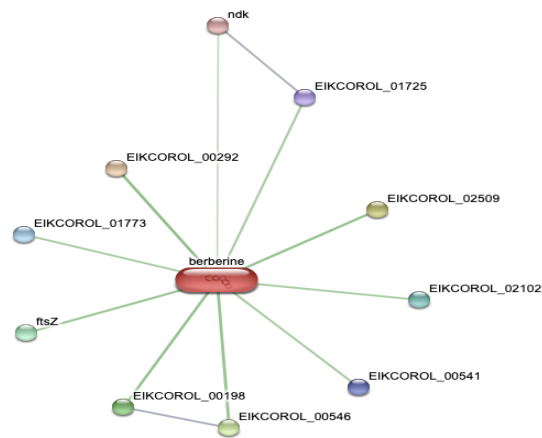


Figure 2: Predicted epitopes on virulent proteins (a) transcriptional regulator, MerR family [EIKCOROL_02509], (b) transcriptional regulator, TetR family [EIKCOROL_01773] and (c) transcriptional regulator, TetR family [EIKCOROL_00292]

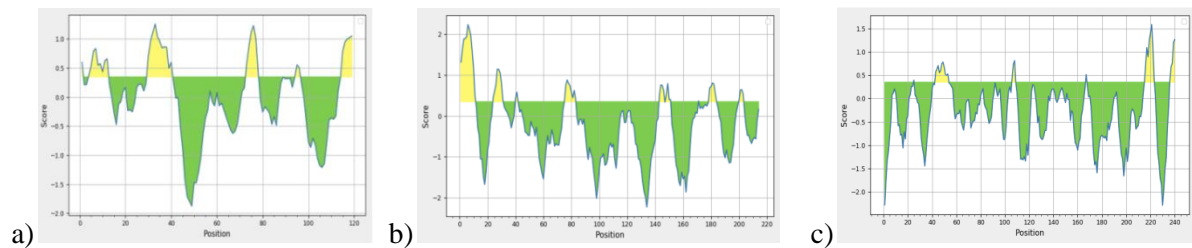


Table 1: Proteins of *Eikenella corrodens* interconnecting with berberine

Organism	Identifier	Proteins which interacts with berberine	VICMPred Functional Class	VirulentPred	VirulentPred Score
<i>Eikenella corrodens</i>	EIKCOROL_01725	Kinase domain protein	Virulence factors	Avirulent	-0.997
	EIKCOROL_02509	Transcriptional regulator, MerR family	Metabolism Molecule	Virulent	0.5559
	EIKCOROL_02102	HTH-type transcriptional regulator MtrR	Metabolism Molecule	Avirulent	-0.164
	EIKCOROL_00541	Transcriptional regulator, TetR family	Metabolism Molecule	Avirulent	-0.526
	EIKCOROL_00546	Cu(I)-responsive transcriptional regulator	Metabolism Molecule	Avirulent	-0.356
	EIKCOROL_00198	Cd(II)/Pb(II)-responsive transcriptional regulator	Cellular process	Avirulent	-0.846
	EIKCOROL_01773	Transcriptional regulator, TetR family	Cellular process	Virulent	0.9590
	EIKCOROL_00292	Transcriptional regulator, TetR family	Metabolism Molecule	Virulent	0.9624
	EIKCOROL_00590	Cell division protein FtsZ	Cellular process	Avirulent	-1.036
	EIKCOROL_01573	Nucleoside-2-P kinase	Metabolism Molecule	Avirulent	-1.000

DISCUSSION:

When selecting a molecule or medicine to be studied in vitro or in vivo, in silico validation is unavoidable.(11) In addition to lowering the expense of conducting studies, it also provides information on the exact mechanism or pathways that can be targeted during preliminary screening, perhaps shortening the process and making it more distinct.(12) In this state of affairs, the current work aims to discover potential relationships, particularly between *Eikennella corrodens* virulence factors.

One of the most important contributors to periodontal diseases is *Eikennella corrodens*. The removal of these organisms from the infection site remains a difficult task.(13) Berberine, a benzyloquinoline alkaloid extracted from the traditional medicinal plant *Coptis chinensis*, occurs as an active constituent and has a variety of pharmacological properties that are used in the treatment of periodontitis. It has anti-inflammatory, antiprotozoal, antidiarrheal, antitrichoma, and analgesic properties.(8) Berberine inhibits diverse bacteria in different ways, as evidenced by several in vitro investigations.(14) The current study advances our knowledge of medication antibacterial activity by triggering a molecular mechanism that could generate bactericidal or bacteriostatic activity.

Berberine is an isoquinoline-type alkaloid found in a variety of plants.(8) Berberine has been shown to have modest antimicrobial action against Gram-positive bacteria but high antimicrobial activity against Gram-negative bacteria, and we know that *Eikennella corrodens* is a Gram-negative bacteria, hence berberine has a stronger antibacterial impact. (15) Additionally our study showed that Berberine had the virulence factors, cellular factor and metabolic factors on some proteins of *Eikennella corrodens*. Most of the in vitro studies explained so far have focused the antibacterial activity of Berberine, but only a very less number of studies have reported the molecular mechanisms related to antimicrobial activity of the drug Berberine. Mechanisms reported generally includes obstruction of bacterial DNA replication, damage to cell membrane, activity against plasmids, down regulation of efflux pumps, less biofilm production.(16)

The current work identifies multiple important protein interactions of berberine in the fight against *Eikennella corrodens*. Berberine has been identified to target virulence factors as well as proteins involved in cellular processes, information storage, and metabolism.(17) However, in vitro investigations are needed to investigate the mechanisms that contribute to bacterial susceptibility in order to expand the use of such medications and justify their inclusion in the catalogue of prophylactic agents. Further study in this area could help uncover the correlative and antagonistic effects of these medications when used in amalgamation with standard antibiotics, perhaps opening up new ways to combat lethal infections in the antibiotic-resistant era.

There are some limitations that must be addressed: (a) the interactions driven by the compound towards proteins may be functional or just a physical interaction; (b) homology between the host and pathogen proteins must be validated to avoid any unwanted side effects; and (c) the drug protein interactions

recorded by in silico methods may not replicate in a biological environment. As a result, careful experimental designs must be built and tested utilising in vitro and in vivo models.

CONCLUSION:

A thorough understanding of the interactions between these medications and their antibacterial activity would add to the long list of advantages these treatments have in therapeutic situations. More in vitro research on a huge range of contagious microbes is needed to confirm the genuine interrelation between the medicines and pathogen protein repertoires.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

REFERENCES:

1. Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. Enterococcus faecalis an Emerging Microbial Menace in Dentistry-An Insight into the In-Silico Detection of Drug Resistant Genes and Its Protein Diversity. *J Clin Diag Res.* 2018;12(10):GC06- GC10.
2. Vijayashree PJ, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen *A.baumannii* and related species. *Arch Oral Biol.* 2018;94:93-98.
3. Suzuki N, Yoneda M, Hirofujii T. Mixed red-complex bacterial infection in periodontitis. *Int J Dent.* 2013;2:1-6.
4. Nizet V, Rubens CE. Pathogenic mechanisms and virulence factors of group B streptococci. In: Nizet V, Rubens CE, editors. *Gram-positive pathogens.* Washington: DC; 2000. pp. 125-136.
5. Farley MM. Group B streptococcal disease in non-pregnant adults. *Clin Infect Dis.* 2001;33:556-561.
6. Zewdu E, Cornelius P. Antimicrobial resistance pattern of *Salmonella* serotypes isolated from food items and personnel in Addis Ababa, Ethiopia. *Trop Anim Health Prod.* 2009;41:241-249
7. Hara Y. *Green tea: health benefits and applications.* New York, USA: Marcel Dekker; 2001.
8. Ushanthika T, Smiline Girija AS, Paramasivam A, Priyadharsini JV. An in silico approach towards identification of virulence factors in red complex pathogens targeted by reserpine. *Nat Prod Res* 2019;17:1-6.
9. Priyadharsini JV. In silico validation of non-antibiotic drugs, acetaminophen, and ibuprofen as antibacterial agents against red complex pathogens. *J Periodontol.* 2019;90(12):1441-8.
10. Bai L, Takagi S, Ando T, Yoneyama H, Ito K, Mizugai H, Isogai E. Antimicrobial activity of tea catechin against canine oral bacteria and the functional mechanisms. *J Vet Med Sci.* 2016;78(9):1439-1445.
11. Ramos MA, Da Silva PB, Spósito L, De Toledo LG, Bonifácio BV, Rodero CF, et al. Nanotechnology-based drug delivery systems for control of microbial biofilms: a review. *Int J Nanomedicine.* 2018;13:1179-1213.
12. Dube A, Nicolazzo JA, Larson I. Chitosan nanoparticles enhance the plasma exposure of (–)-epigallocatechin gallate in mice through an enhancement in intestinal stability. *Eur J Pharm Sci.* 2011;44(3):422-6.

13. Foster LJ, Ho S, Hook J, Basuki M, Marcal H. Chitosan as a biomaterial: Influence of degree of deacetylation on its physiochemical, material and biological properties. *PLoS One*. 2015;10(8):e0135153.
14. Gibbs F, Kermasha S, Alli I, Catherine N, Mulligan B. Encapsulation in the food industry: a review. *Int J Food Sci Nutr*. 1999;50(3):213-24.
15. Gopalakrishnan L, Ramana LN, Sethuraman S, Krishnan UM. Ellagic acid encapsulated chitosan nanoparticles as anti-hemorrhagic agent. *Carbohydr Polym*. 2014;111:215-21.
16. Gupta PD, Birdi TJ. Development of botanicals to combat antibiotic resistance. *J Ayurveda Integr Med*. 2017;8(4):266-75.
17. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. *Int J Antimicrob Agents*. 2010;35(4):322-32.