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aBiofilm: a resource of anti-biofilm agents and their potential implications in targeting antibiotic drug resistance

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ABSTRACT

Biofilms play an important role in the antibiotic drug resistance, which is threatening public health globally. Almost, all microbes mimic multicellular lifestyle to form biofilm by undergoing phenotypic changes to adapt adverse environmental conditions. Many anti-biofilm agents have been experimentally validated to disrupt the biofilms during last three decades. To organize this data, we developed the '*aBiofilm*' resource (http://bioinfo.imtech.res.in/manojk/abiofilm/) that harbors a database, a predictor, and the data visualization modules. The database contains biological, chemical, and structural details of 5027 anti-biofilm agents (1720 unique) reported from 1988–2017. These agents target over 140 organisms including Gram-negative, Gram-positive bacteria, and fungus. They are mainly chemicals, peptides, phages, secondary metabolites, antibodies, nanoparticles and extracts. They show the diverse mode of actions by attacking mainly signaling molecules, biofilm matrix, genes, extracellular polymeric substances, and many more. The QSAR based predictor identifies the anti-biofilm potential of an unknown chemical with an accuracy of ∼80.00%. The data visualization section summarized the biofilm stages targeted (*Circos plot*); interaction maps (*Cytoscape*) and chemicals diversification (*CheS-Mapper*) of the agents. This comprehensive platform would help the researchers to understand the multilevel communication in the microbial consortium. It may aid in developing anti-biofilm therapeutics to deal with antibiotic drug resistance menace.

INTRODUCTION

Biofilms are the assembled colony of microbes encapsulated with self-secreted cocoon (1,2). These are the major survival strategy adapted by almost all bacterial species against severe environments (3). In biofilms, unicellular bacteria mimic multicellular behavior by division of labor and cell-to-cell communication (4). Historically, Van Leeuwenhoek first detected microbial colonies from tooth surface using a simple microscope in 1676 (5). Later, Costerton *et al.* in 1978 coined the term '*biofilm*' for the colonial form of bacteria (6). The biofilms formed on living or nonliving like human body, plants, medical devices, rocks, etc. (7–9). The main constituent of a biofilm is the extracellular polymeric substances (EPS). This EPS matrix comprised of polysaccharides, proteins, extracellular DNA, lipids etc and its composition varies according to growth conditions, substrates, medium, and species (10).

The microbial colonization is either mono or polyspecies and driven through signaling molecules (11,12). For example, autoinducer-2 helps to develop the oral biofilms formed by *Streptococcus gordonii*, *Porphyromonas gingivalis* (11); and acyl homoserine lactones assist the growth of *Pseudomonas aeruginosa* and *Burkholderia cepacia* microcolonies (13). Similarly, competence stimulating peptides contribute in the biofilm formation of *Streptococcus mutans* (14); and tyrosol aids colonization among *Candida albicans* (15). Simultaneously, these microcolonies are difficult targets for the antibiotics to inhibit that pathogen.

Currently, drug resistance is the major crisis before scientific community in infectious diseases according to World Health Organization (WHO) report (http://www.who.int/mediacentre/factsheets/antibiotic-resistance/en/) (16). In biofilms, the bacteria display 10–1000-fold increase in antibiotic resistance. It happens because of the specialized features like efflux pumps, modifying enzymes, evading the immune system and target mutations (17–20). Sakhtah *et al.* in 2016 proved the role of multidrug efflux pump (MexGHI-OpmD) through a natural phenazine in the biofilm development of *P. aeruginosa*, an opportunistic

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pathogen (21). The pathogenicity of the facultative anaerobe *Vibrio cholerae* increased due the biofilm formation in the environment as well as during outbreaks (22). Similarly, switching to biofilm form increases the persistence of *C. albicans* during mucosal to systemic infection (23). The exoproteins of the biofilm EPS matrix corroborated as multivalent vaccine candidate against biofilm-associated chronic infections of *Staphylococcus aureus* (24). However, stubborn nature of biofilms helps the pathogens to overcome the host innate response as well as antibiotics (25).

Many studies (1988-till date) reported the experimental testing of anti-biofilm agents against different microorganisms. These anti-biofilm agents have varied actions like targeting the quorum sensing (QS) signaling molecules (26), EPS matrix (27,28), genes involved in biofilm formation and adhesion (29) etc. The anti-biofilm agents verified as adjuvants or alternatives to conventional antibiotic treatments. Simultaneously, they also act synergistically with antibiotics and improve their inhibitory action against chronic infections (30).

The biofilm's universal distribution, varied composition, virulence and resistance towards antibiotics make them among one of the highly explored field. Since, there is no platform available to understand the diversified anti-biofilm agents; therefore, we developed the first comprehensive resource '***aBiofilm'*** in this concern. It harbors biological, chemical and structural details of 5027 entries belonged to varied anti-biofilm agents. It contains a database, a predictor (qualitative), and the data visualization modules.

MATERIALS AND METHODS

aBiofilm resource structure

The aBiofilm has three sub-categories namely a database, a predictor, and the data visualization. The database covers the chemical, biological, and structural information of anti-biofilm agents. Using curated anti-biofilm chemicals, we developed a Quantitative structure–activity relationship (QSAR) based predictor employing the Support Vector Machine (SVM), a machine learning technique. Further, we also carried out diverse analyses to better understand the anti-biofilm agents. The 'aBiofilm' architecture is available in the Supplementary Figure S1.

Database

Data acquisition and curation. We performed an intensive literature search to extract the relevant information about anti-biofilm agents for developing a database. We scanned the PubMed using a query (by combining suitable keywords and Boolean operators) to fetch the relevant articles:

$$((biofilm)\ AND\ (inhibit*\ OR antagonist\ OR interference\ OR modulator\ OR quench*))$$

The query resulted in ∼7500 articles till 15 September 2017 spread over last three decades. After the early screening, we filtered ∼3500 articles that may contain the data of biofilm inhibitors (excluding reviews and non-potential articles). Finally, the aBiofilm database composes the manually curated agents from 526 articles having the needed information. Finally, 5027 entries belonged to 1720 unique anti-biofilm agents targeting as many as 140 microorganisms with an average discovery rate of ∼167 entries and ∼57 unique anti-biofilm agents yearly.

Database organization. The records in the aBiofilm database include the biological, chemical and structural information. The biological details include the fields like anti-biofilm agents, type of anti-biofilm agents, organism/strain targeted, concentration of agent, percentage (%) inhibition. In addition, it also has the stage of biofilm targeted, mechanism of action, media and apparatus used to detect the biofilm inhibition, etc. Since the percentage inhibition was not directly provided in the maximum articles, therefore we calculated it using the absorbance readings by the following formula (31,32):

$$\%\,\text{Inhibition} = \left(\text{OD}_{(\text{control})} - \text{OD}_{(\text{experimental})}\right)/\text{OD}_{(\text{control})}$$

where OD (control) is the optical density of a control and OD (experimental) is the optical density of an experimental setup.

We manually extracted the chemical information from various chemical repositories like PubChem (https://pubchem.ncbi.nlm.nih.gov/), ChemSpider (http://www.chemspider.com/) and chemicalize (https://chemicalize.com/) (33,34). The chemical details include International Union of Pure and Applied Chemistry (IUPAC name), simplified molecular-input line-entry system (SMILES), Molecular formula, Molecular weight, IUPAC International Chemical Identifier (InCHl) and Lipinski rule for five. The structural details include the 2-dimensional and 3-dimensional view of each chemical. We manually sketched the compounds using chemicalize module available in ChemAxon package. Further, we calculated the chemical properties and the structure (*.sdf* format) of each chemical. Last, displaying the agents on the web server was accomplished through the mol2ps (http://merian.pch.univie.ac.at/~nhaider/cheminf/mol2ps.html) and JavaScript-Based Molecular Viewer (JSmol) from Jmol (http://jmol.sourceforge.net/).

Predictor. In our database, there are 896 unique anti-biofilm agents (chemicals) with SMILES and defined inhibition efficiencies either low to high or 0–100%. We need a positive and a negative data sets to develop a classifier for predicting the biofilm inhibition efficiency of any compound. We found that in the literature >60% and <20% biofilm inhibition were considered 'high' and 'low' respectively for these extracted chemicals. Therefore, we have selected experimentally confirmed 492 chemicals with high (>60%) and low (<20%) inhibition efficiency as positive (271) and negative (221) datasets respectively for the classification based SVM model development. For a better classifier, we have not included the chemicals having moderate efficiency (35). We subdivided the 492 chemicals into T^{450} training/testing and V^{42} independent/validation datasets using a randomization method. This procedure was repeated in triplicates and all datasets performed equally well. Therefore, for the model development, we opted one of these random datasets.

First, we fetched the descriptors of all the chemicals using PaDEL software (36,37) that led to the 15352 sized vec-

tor. Further, the most contributing 40 features were selected using two filters, i.e. 'Remove Useless' available in Weka package (38) followed by minimum redundancy and maximum relevance (mRMR) algorithm (39). The SVM technique was used for the model development employing 10-fold cross validation (40). We evaluated the developed models using sensitivity, specificity, accuracy, Matthews correlation coefficient (MCC) and receiver operating characteristic (ROC) (35,40). Similarly, the robustness of the developed model was checked through independent/validation dataset. We provided the flowchart depicting the methodology of the predictor development in Supplementary Figure S2.

Analyses and data visualization. In the aBiofilm, we did three types of analyses to explore the anti-biofilm agents. First, we analyzed the data for highlighting the biofilm stages of the microbes targeted by different agents using CIRCOS software (41,42). Second, the interrelationship between four important fields of the database was highlighted i.e. the anti-biofilm agent acting on the particular stage of the biofilm of a microorganism through interaction network by Cytoscape software (43). Third, we evaluated the diversity of anti-biofilm chemicals using chemical clustering through *k-means* clustering method available in ChesMapper (44). We provided these analyses on the webserver under '*analyses*' menu.

Web server implementation. The aBiofilm web server was designed using LAMP software bundle (35,45,46). It uses ***L****inux* as the operating system; ***A****pache* for the web server; ***M****y**SQL* as the relational database management system and ***P****HP* (occasionally Perl or Python) as an object-oriented scripting language. The *front-end* of the web server was developed using scripting languages like HTML, CSS, XML, Javascript, etc. Similarly, we used PHP, MySQL, Perl, Python, in the *back-end* to make the server functional.

RESULT AND DISCUSSION

Database

Database statistics. Currently, the aBiofilm database harbors 5027 anti-biofilm agents (1720 unique) from 526 articles. Among the topmost anti-biofilm agents, we found 3-(4-fluorophenyl)-5-(3-ethoxy-4-hydroxybenzylidene)-2-thioxothiazolidin-4-one has 86 entries. Followed by 3-(pyridin-3-yl)-5-(3-ethoxy-4-hydroxybenzylidene)-2-thioxothiazolidin-4-one; 3-(4-fluorophenyl)-5-(3-(allyloxy)-4-hydroxybenzylidene)-2-thioxothiazolidin-4-one; 3-(3-fluorophenyl)-5-(3-ethoxy-4-hydroxybenzylidene)-2-thioxothiazolidin-4-one; 5-Fluorouracil; AHL lactonases; Norspermidine with 79; 79; 79; 58; 46 and 41 records (Figure 1A). Database has maximum anti-biofilm agents checked against *Staphylococcus aureus* i.e. 1044, followed by *Pseudomonas aeruginosa, Candida albicans*, *Escherichia coli*, *Staphylococcus epidermidis*, *Streptococcus mutans* with 1026, 380, 321, 263, and 239 hits (Figure 1B).

We noted that maximum anti-biofilm agents belonged to a chemical category with 2445 entries. Further, Phytochemical, Peptide, Amino Acids, Bacterial extract, Enzymes, Nanoparticles have 1109, 619, 236, 227, 84, and 67

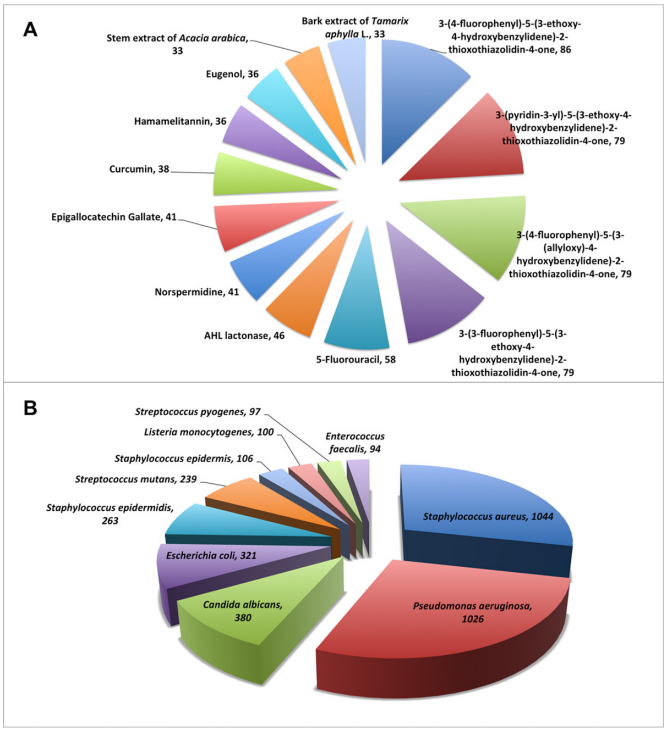

Figure 1. Diagrammatic representation of (**A**) topmost anti-biofilm agents in aBiofilm resource, (**B**) top-10 organism targeted with anti-biofilm agents available in aBiofilm resource.

records (Supplementary Figure S3A). Similarly, the stages of the biofilm targeted with anti-biofilm agents scanned and depicted in the Supplementary Figure S3B. The formation stage of biofilms targeted in 3940 instances, followed by adhesion, and pre-formed stages with 502 and 235 entries respectively.

Data retrieval. We have integrated the 'Browse' to explore and retrieve the overall data through six major fields, i.e. anti-biofilm agents, types of anti-biofilm agents, targeted organism, types of organism, preliminary assay, and journal names. User can click any of the six choices to display list of unique entries and the frequency. User will get an interactive tabular output of any sub categories displaying the important fields. It includes anti-biofilm ID hyperlinked to details of individual entry and anti-biofilm agent linked to an external chemical repository. Other fields are molecular formula of anti-biofilm agents, agent type, targeted organism (linked to NCBI-taxonomy browser), strain, inhibition efficiency, biofilm stages, growth media and reference PMID (hyperlinked to PubMed). Each anti-biofilm ID linked to the detailed biological, chemical, and structural information. The Supplementary Figure S4 has depicted the use of 'browse'.

We have also included a 'Search' tool to fetch the needed information according to user's requirement. User can enter the query for searching in all the fields, or any specific fields like anti-biofilm ID, anti-biofilm agent, SMILES, anti-biofilm agent type, targeted organism, preliminary assay, and PubMed ID. The search will display ten fields as provided in the browse choice. Further, detailed individual

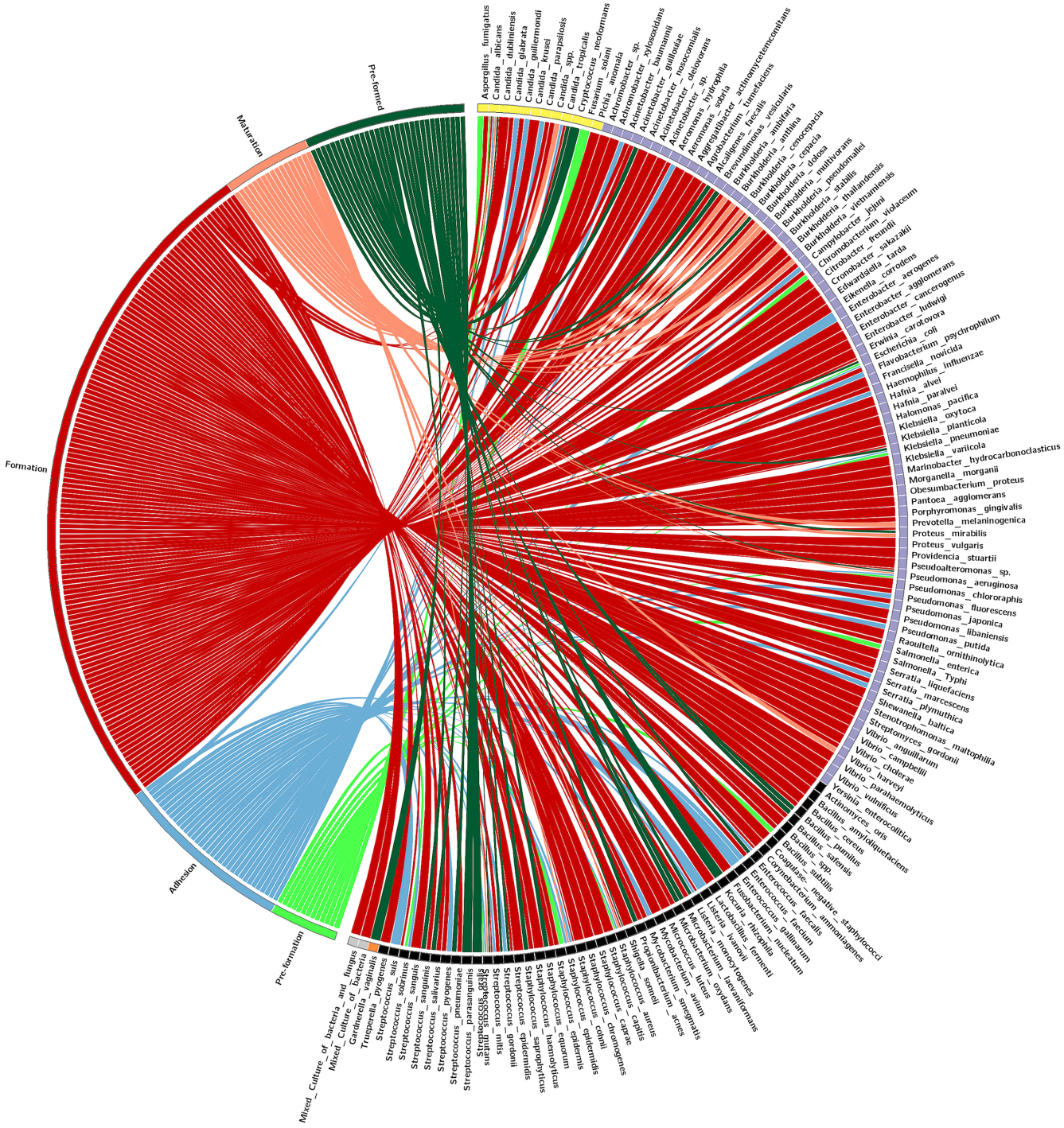

Figure 2. CIRCOS plot showing the information of stages of the biofilm among the organism targeted with anti-biofilm agents. CIRCOS plot divided into two parts. Rightmost part showed the organisms targeted through anti-biofilm agents while leftmost part represented the stages of the organism affected with anti-biofilm agents. The band color of the rightmost part is depicted in five different colors viz. yellow (Fungus), purple (Gram-negative bacteria), black (Gram-positive bacteria), orange (Gram variable bacteria), and dark gray (a mixed culture of bacteria and/or fungus). Whereas, leftmost part and rays (links) of circos is divided into five different colors according to the stages like light green (Pre-formation), blue (Adhesion), red (Formation), orange (Maturation), and green (Pre-formed).

web page of the entry is available by clicking on any anti-biofilm ID.

Predictor. We have developed a QSAR based predictor for identification of the inhibition efficiency of any chemicals against biofilm. During 10-fold cross-validation on T^{450} dataset, the predictive model achieved the sensitivity, specificity, accuracy, MCC, and ROC of 77.92%, 80.77%, 79.18%, 0.58 and 0.83 respectively. Similarly, the developed model performed equally well on the independent/validation dataset (V^{42}) with sensitivity, specificity, accuracy, MCC, and ROC of 86.36%, 75.00%, 80.95%, 0.62 and 0.88 correspondingly. We have also given the experimental and predicted anti-biofilm efficiency of the independent/validation dataset (V^{42}) for comparison in the Supplementary Table S1. It suggested good correlation between the experimental and the predicted anti-biofilm activity. A user can provide the input chemical in the form of SMILES/sdf/mol format for predicting the anti-biofilm potential. Further, a user can also draw the query chemical in the integrated JSME window (47). The output is displayed in a tabular format including query SMILES, predicted anti-biofilm efficacy (low or high) with a color gradient, 2D (with marked stereochemistry) and 3D structure, and a few drug-likeness properties. Beside, we are also checking the query compound in 'aBiofilm' database and displayed the available chemicals including experimental efficacy and other details.

Analyses and data visualization. We performed ***biofilm stages*** analysis using CIRCOS plot by highlighting three important fields of the database namely organism, its group and stages of the biofilm targeted (Figure 2). The CIRCOS plot has two parts namely organisms and stages of biofilms. One hundred forty unique organisms further classified into five different groups and connected to the stages targeted by the anti-biofilm agents. The plot showed biofilms of maximum microbes including bacteria (both Gram-positive and Gram-negative) and fungus targeted at the formation stage e.g. *Burkholderia* spp., *Candida* spp., *Staphylococcus* spp., and many more. Further, some of the pathogenic bacteria like *P. aeruginosa*, *C. albicans*, *E. coli*, *S. aureus,* etc. aimed for more than one stages like adhesion, formation, maturation and pre-formed.

We used cytoscape software based ***interaction maps*** to represent the four important fields of the aBiofilm database i.e. anti-biofilm agents, inhibition efficiency, stage of biofilm in the targeted organism. The interaction map displayed some important pathogens like ESKAPE (*Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *P. aeruginosa* and *Enterobacter* species) and non-ESKAPE (top-10). For example, the two stages of biofilm (formation and pre-formed) of the *K. pneumoniae* targeted by eighteen different anti-biofilm agents namely phenanthrene, norspermidine, myristic acid, cyanidine, etc. with high and medium inhibition efficiency (Figure 3). All the prokaryotic (Gram-positive and Gram-negative bacteria) and eukaryotic (fungus or yeast) microbes undergoes biofilm mode of growth. Remarkably, the biofilm development in the ESKAPE pathogens is the primary cause of nosocomial infections worldwide (48). Various anti-biofilm agents developed

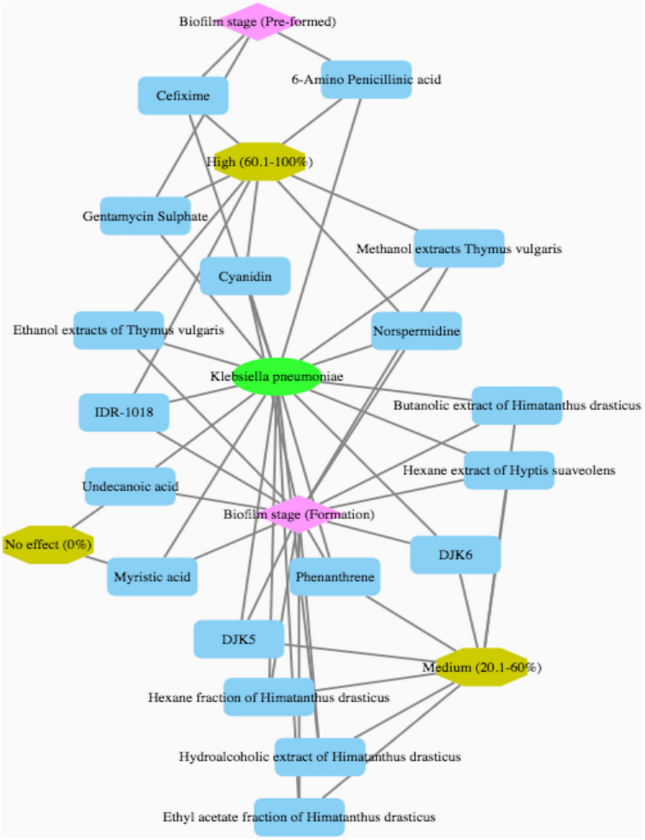

Figure 3. Interaction map of *Klebsiella pneumoniae* showing the interrelationship between anti-biofilm agents, inhibition efficacy and stage of organism targeted. Green colored box showing targeted organism, pink colored box means the stage of biofilm, blue boxes depicts anti-biofilm agents, and yellow box signifies the targeting efficiency.

against ESKAPE and non-ESKAPE pathogens like *S. aureus* (49), *P. aeruginosa* (50), *C. albicans* (51), *E. faecalis* (52) and many more included in our database. These pathogens targeted at single or multiple stages of biofilm formation like adhesion (49), maturation (53), pre-formed (54), etc.

We carried out ***Chemical Spacing*** analysis to check the chemical diversity of the anti-biofilm agents (Supplementary Figure S5). Clustering represented as a concentric circle in which, the core shows the embedded chemicals in 3D space and highlighting the superimposed structure of each cluster. While the outer circle showed the zoomed view of each cluster called the maximum common subgraph (MCS). The chemical clustering is providing the information of a total number of the chemicals in every cluster with Pearson's correlation coefficient (0.93) of the embedding. The anti-biofilm agents present wide-ranging chemical diversity and MCS. They consist of aliphatic core backbone namely propane, pentane, and 2-amino-1-(2-methylpyrrolidin-1-yl)propan-1-one which target important biofilm stages like formation, and pre-formed in the pathogens like *P. aeruginosa*, *C. albicans*, *S. aureus*.

CONCLUSION

Antibiotic drug resistance threat aggravated because of the faster rate of the resistance development in the microbial community. Relatively the discovery of novel antibiotics is slow (55). Intriguingly, biofilm adaptation among the microbes is one of the leading causes of antibiotic resistance (56,57). Therefore, aBiofilm would be very helpful to the researchers struggling to develop effective and broad-spectrum anti-biofilm agents. This is the first resource having all types of anti-biofilm agents reported in literature, i.e. chemicals, phytochemicals, amino acids, antibiotics, peptides, antibody, phages and many more. We are providing the platform for chemical, biological and structural information of anti-biofilm agent from three decades. The researchers can pick up the extensive information of single and multi-species biofilm targeting agents. Beside, a generalized predictor can identify the anti-biofilm activity of any chemical. However, this tool has limit in predicting organism/strain specific anti-biofilm activity. Varied data visualization support the users by exploring the mechanism and diversity of the anti-biofilm agents. Last, this would aid the researchers to understand, explore, and design novel and effective biofilm targeting approaches and would further intensify the research on biofilm specifically to tackle the menace of drug resistance.

AVAILABILITY

The aBiofilm resource is an open access web server and available at http://bioinfo.imtech.res.in/manojk/abiofilm/. We will update the resource regularly at half yearly or when enough data is available.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR online.

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