Influential Quorum Sensing Proteins of Multidrug Resistant *Proteus mirabilis* Causing Urinary Tract Infections

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Catheter-associated urinary tract infections (CAUTI) has become an alarming hospital based disease with the increase of multidrug resistance (MDR) strains of Proteus mirabilis. High prevalence of long-term hospital based CAUTI for patients along with moderate percentage of morbidity due to ignorance, failure and MDR, necessitates an immediate intervention strategy to combat the deadly disease. Several reports and reviews focus on revealing the important genes and proteins essential to tackle CAUTI caused by P. mirabilis. Despite longitudinal studies and methodical strategies to circumvent the issues, effective means of unearthing the most influential proteins to target for therapeutic uses have been meagre. Here we have reported a strategic approach for identifying the most influential proteins from the complete set of proteins of the whole genome of P. mirabilis, besides comparing the interactomes comprising the autoinducer-2 (AI-2) biosynthetic pathway along with other proteins involved in biofilm formation and responsible for virulence. Essentially, we have adopted a computational network model based approach to construct a set of small protein interaction networks (SPIN) along with the whole genome (GPIN) to identify, albeit theoretically, the most significant proteins. These might actually be responsible for the phenomenon of quorum sensing (QS) and biofilm formation and thus, could be therapeutically targeted to fight out the

MDR threats to antibiotics of P. mirabilis. Our approach signifies the eigenvector centrality coupled with k-core analyses to be a better measure in addressing the pressing issues.

Introduction

Urinary tract infections (UTI) are the second most common infection prevalent amongst long-term hospital patients, second only to pneumonia. Failure to treat or a delay in treatment can result in systemic inflammatory response syndrome (SIRS), which carries a mortality rate of 20-50 percent [1-3]. While Escherichia coli remains the most often implicated cause of UTI in previously healthy outpatients, Proteus mirabilis take the lead for catheter-associated UTI (CAUTI), causing 10-44 percent of long-term CAUTIs [1, 3]. In comparison to normal cases, CAUTI is quite complicated and encountered by patients with multiple prior episodes of UTI, multiple antibiotic treatments, urinary tract obstruction and/or undergoing catheterization as also for those with spinal cord injury or anatomical abnormality [1-3]. Such complications of CAUTI caused by P. mirabilis arise from the usage of a diverse set of virulence factors by the organism to access and colonize the host urinary tract. These include, but not limited to, urease and stone formation, fimbriae and other adhesins, iron and zinc acquisition, proteases and toxins and biofilm formation [1]. Despite significant advances made for studying P. mirabilis pathogenesis, a meagre knowledge of its regulatory mechanism poses an urgent and pressing need to come up with unique health intervention processes for such patients. In attempts to provide such health interventions, longitudinal and epidemiological studies on P. mirabilis have been reported for extended-spectrum -lactamase (ESBL) and AmpC -lactamase .Furthermore, along with various other components of the membrane, several cytoplasmic factors interplay among themselves to regulate the cell-density dependent gene regulation. This enables the bacteria for cell-to-cell communication, a phenomenon known as quorum sensing (QS) [8]. Besides other phenotypic traits, QS controls the expression of the virulence factors responsible for pathogenesis of P. mirabilis [9]. Again, as per other reports, despite producing two cyclic dipeptides and encoding LuxS-dependent quorum sensing molecule, AI-2, during swarming, P. mirabilis has been reported to be have no strong evidence of QS (1,10-12). However, a highly ordered swarm cycle suggests an existing mechanism for multicellular coordination (13). Thus, the fact that MDR P. mirabilis are engaged in biofilm formation which is managed, albeit in parts, through quorum sensing brings out the complexity of CAUTI. To deal with such complexity, analyses of the mosaic mesh or networks of interacting proteins involved, commonly known as protein interaction networks (PINs), can provide sufficient insight to reveal the influential key role players of the phenomenon [14, 15]. The influential role players of phenomenon like QS can be determined by analysing the PIN involving the proteins in the pathway to produce the QS inducer. The essentiality of such small protein interactome (SPIN) can be brought about by an analysis for the most biologically relevant protein to target for inhibiting that phenomenon, also known as quorum quenching. Ideally, a determination of the number of interacting partners of a partic-

ular protein identifies its degree centrality (DC) which correlates with its essential nature in the biological scenario [16]. However, a deeper understanding of the essential nature of a particular protein comes upon analysing its interaction with other partners in the global network of all proteins. In this study, we have analysed the importance of other centrality measures like Closeness centrality (CC), Betweenness centrality (BC) and Eigenvector centrality (EC) [16] parameters for SPIN comprising the genes and proteins involved in quorum sensing. Again, analyses of a stipulated sets of QS proteins for a valuable knowledge about the most influential virulence proteins to render as drug targets for the QS phenomenon could be quite insufficient. Thus, we have carried out further analyses of the whole genome of P. mirabilis for a global analysis of the encoding proteins. This comprises the decomposition of the whole genome protein interactome (GPIN) to a core of highly interacting proteins through the k-core analysis approach [17]. Furthermore, to identify the functional modules in the global network [18], we have performed cartographic analyses and predicted the indispensability of certain sets of proteins which have been shown to be sharing similar functional modules empirically important for drug targets.

Materials and Methods

Dataset Collection

The P. mirabilis QS pathways for autoinducer-2 (AI-2) biosynthesis were collected from curated reference databases of genomes and metabolic pathways like KEGG, MetaCyc and BioCyc [19, 20, 21]. The proteins involved in these pathways were extracted and fed as queries to the STRING 10.5 biological meta-database [22] to retrieve protein interaction datasets with at least 10 or 50 interactors having the default medium level confidence [period of access: January to February, 2018]. Detailed protein links file under the accession number 529507 in STRING was used to collect all the interactions of the whole genome proteins of P. mirabilis strain HI4320.

Interactome Construction

We have taken a stepwise approach to integrate and build the interactomes of the proteins. These are the small protein interactomes (SPIN) comprised of a) those involved in AI-2 biosynthetic pathway in the organism with small (10) and large (50) number of interactors (AIPS, AIPL, respectively), b) only QS genes found (QSPO), c) all QS genes reported as homologues (QSPH) present in P. mirabilis, d) all virulent genes reported (QSPV) and e) the whole genome of P. mirabilis (WGPM). The number of P. mirabilis proteins from the SPIN class of interactomes were 31 for AIPS, 102 for AIPL, 24 for QSPO, 42 for QSPH, 58 for QSPV and 3548 for GPIN. The individual protein interaction data, with medium confidence default values, were obtained from String 10.5. Interactions were 80 for AIPS, 435 for AIPL, 30 for QSPO, 129 for QSPH, 426 for QSPV and 358984 for GPIN, respectively. All individual interaction data obtained above were imported into Cytoscape version 3.6.0 [23] and Gephi 0.9.2 [24] to integrate, build

and analyse five SPIN namely AIPS, AIPL, QSPO, QSPH and QSPV and the GPIN. Interactomes were considered as undirected graphs represented by G = (V, E) consisting of a finite set of V vertices (or nodes) and E edges. An edge e = (u,v) connects two vertices (nodes) u and v. Each protein is represented as a vertex/node. The number of physical and functional connections/interactions/associations/links a node has with other nodes comprises its degree d (v) [25].

Network Analyses

SPIN

All the five SPIN were subsequently analyzed individually by utilizing the four important concepts of centrality applied to biological networks, namely, degree centrality (DC), closeness centrality (CC), betweenness centrality (BC) and eigenvector centrality (EC) [26-28]. This was done either via Gephi or the Cytoscape integrated java plugin CytoNCA [29]. The combined scores from different parameters considered in STRING were taken as edge weights for computing CytoNCA scores. Top 5 proteins for each of the centrality measures were taken for drawing venn diagrams through online tool Venny 2.1 [30] to find common proteins from each measures.

GPIN

Further analyses for GPIN were done by using MATLAB version 7.11, a programming language developed by MathWorks [31]. A primary understanding of the GPIN was obtained by plotting the distributions of network degree (k) against the Complementary Cumulative Distribution Function (CCDF). An idea of the core group of the very specific proteins was obtained from a K-core analysis of the proteins in the whole genome context. This essentially prunes the network to a k-core with nodes having degree at least equal to k and classifying them in k-shell (proteins, in our study) based on the variety of their interacting partners. This was done following the network decomposition (pruning) techniques to produce a sequence of subgraph of gradually increasing cohesion [17]. Further, a significant knowledge of the functional connectivity and participation of each protein was derived from the cartographic representation of the within-module degree zscore of the protein versus its participation coefficient, P, as per the methodology described by Guimera, et al. [32]. The z-score measures how 'well connected' a node i is to other nodes in the module while P measures how the node i is positioned in its own module and with respect to other modules. Participation of each protein reflected its intra- and inter-modular positioning, where functional modules were calculated based on Rosvall method [33]. Such analyses demanded the proteins to be mainly divided into two major categories namely the hub nodes and the non-hub nodes where the former is a connection point of many nodes. The category of latter has been assigned roles of ultra-peripheral nodes (R1), peripheral nodes (R2), non-hub connector nodes (R3) and the non-hub kinless nodes (R4). Likewise, the

hub nodes have been assigned as provincial hubs (R5), connector hubs (R6) and kinless hubs (R7) [32].

Results

The individual five SPIN

To have an understanding of the important protein(s) of QS in P. mirabilis, we have taken a stepwise approach of building five SPIN, with an ultimate goal to identify the influential virulent proteins to serve as potential candidates for therapeutic targets. A comparative picture of the parametric values of the top five rank holders in their descending order has been delineated in a tabular form (Table 1).

Table 1. The top 5 rank holders of P. mirabilis SPIN and GPIN. The bold cased proteins are present in the innermost 154th k- core.

Network	EC	BC	DC	CC
AIPS	MetG, LuxS, GcvP, Hpt, PMI3524	MtnN, CysK, LuxS, MetB, MnmC	MtnN, MnmC, CysK, LuxS, MetB	MtnN, CysK, LuxS, MetB, MnmC
AIPL	LuxS, ThrA MetH, MetL, PMI0028	MnmC, MtnN, LuxS, PMI3678, TrmA	MnmC, LuxS, MtnN, ThrA MetH	PMI3678, ThrA, MetH, PMI0028, PMI0626
QSPO	PMI1345, PMI1344, TrpE, PabA, PabB	YajC, PMI1345, GadC, RibD, PMI2708	PMI1345, PMI1344, TrpE, PabA, PabB	KdpE, Hfq, FlhD, FlhC, PMI1345
QSPH	PMI1345, GadC, TrpE, FlhD, FlhC	KdpE, Ffh, KdpD, LepB, FtsY	OppA, MppA, OppA2, OppD, OppC	FlhD, FlhC, PMI1423, AroF, AroG
QSPV	FliF,FliK,FlgG, FlgC, FlgI	RpoS, Eno, Irp, Pgm, PMI3678	CheY, PykA, PykF, Tal, FliN	RpoS, Eno, PMI3678, FliC, CsrA
GPIN	PolA, GuaA, DnaK, MetG, RecA	PolA, PMI3678, PMI1033 PMI2007, RpoS	PolA, PMI3678, RcsC, DnaK, GuaA	PMI2375 PMI2723, PMI0739, PMI3495, PMI2629

In most of the cases, at least three or two of the centrality measures unanimously brings out the same protein. These proteins are the ones reflected to be important through each SPIN analysis. For instance, AIPS has MetG and MtnN as the top rankers while LuxS and MnmC turns out to be important for AIPL. Others like QSPO, QSPH and QSPV have YajC, PMI1345, Hfq, RpoS,

flagellar proteins of the flh and fli operon and some other two-component systems proteins like CheY and KdpE as important rankers. The functions of these proteins are mentioned in Table 2. The top ranking proteins for each of these five SPINs have been reflected in Fig. 1 with Venn diagrams along with the common topmost rankers across all the five SPINs. It is worthwhile to note that the QSPO had a tightly packed network being formed with every protein almost connected to every other, a phenomenon related as high clustering coefficient. With respect to the above analyses of the individual interactomes of the SPIN, an idea about the importance of these proteins in their individual SPIN and finally across all SPIN could be obtained. However, for a drug to be effective globally, the indispensability issue of these proteins needs to be taken care of to tackle the MDR P. mirabilis. Thus, a broader picture with respect to the whole genome proteins of P. mirabilis was then examined to address the concern.

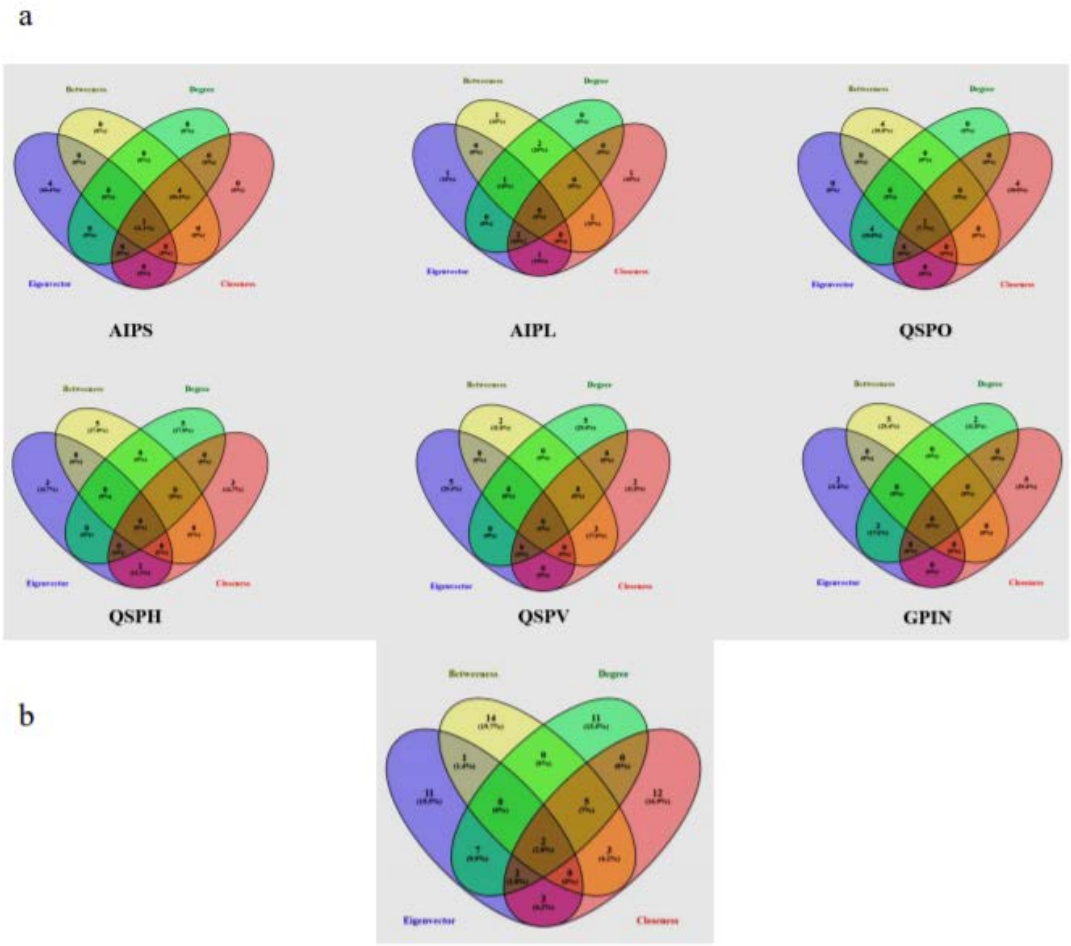

Figure 1: Venn diagram representation for P. mirabilis. a] The five top rankers of DC, CC, BC and EC parametric analyses of five individual SPIN and GPIN and b] The common centrality measures of all interactomes.

The complete GPIN

In an attempt to analyse the type of network being constructed from the empirical and theoretical results of physical and functional interactions amongst proteins laid down in STRING, we have observed the degree distribution of GPIN to be exponential showing a non-linear preferential attachment nature (Fig. 2a) [34]. Hereafter, in order to get an idea of the influential ones from the barrage of proteins involved in the five individual SPIN, we have performed a k-core analysis for them (Fig. 2b). Notably, the innermost core was 154th shell and had genes like thrA, cysK, metG, metL, trpE, rpoS, eno, etc. which have already been reflected from the four network centrality analyses of the SPINs (Table 1). Additionally, it is to be noted that top 5 EC and DC measures of the GPIN also had their position in the innermost 154th core, thereby indicating their importance in the global scenario. Other important genes e.g. luxS, PMI1345 from the k-core analyses were found in the 139th shell. The latter category was found to have direct involvement in QS. For the purpose of classification of the proteins based on their functional role and region in the network space of P. mirabilis, we have performed a cartographic analysis for the GPIN. Noticeably, the R6 quadrant had the top 5 proteins belonging to either the innermost 154th core or almost close to the 139th core containing most of the proteins related to QS. These are GltB and PMI3678 for the former and PMI3348, PMI0587 and PMI3517 for the latter.

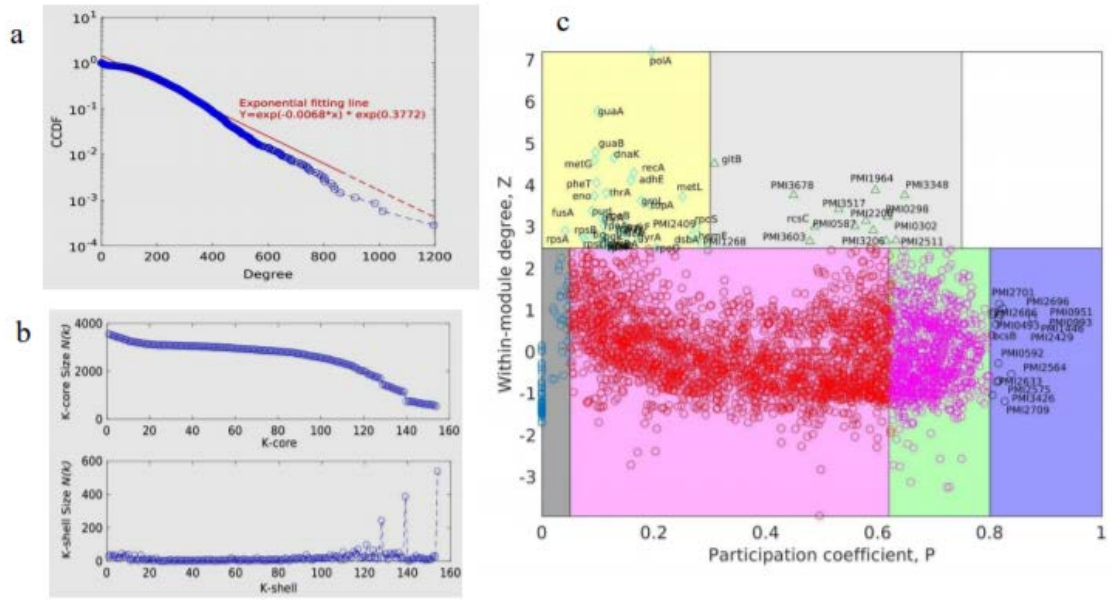

Figure 2: a] The degree distribution of the proteins from the GPIN of P. mirabilis b] Distribution of the kcore (top) and k-shell (bottom) sizes for the set of proteins from the GPIN of P. mirabilis. c] Cartographic representation for classification of proteins from the GPIN of P. mirabilis based on its role and region in network space

Moreover, upon looking deep into EC classification of R6 quadrants, all top 5 proteins, namely PolA, GuaA, DnaK, MetG and RecA were from the innermost 154th core. Furthermore, analysis after sorting of module followed by R quadrant, k-core followed by either module or EC measures, all revealed the proteins to be mostly belonging to the R6 or R5 categories, besides their 154th or 139th core classification (Data not shown). It is worthwhile to mention here that a similar sorting analyses of BC with respect to Quadrant and k-core had revealed proteins mostly from R2 or R3, none of them occupying the innermost 154th core, except RplP and RpoS. **Table 2: Functions of the topmost proteins of individual P. mirabilis networks with respect to network centrality measures.**

Protein name	Description of function
MetG	Is required not only for elongation of protein synthesis but also for the initiation of all mRNA translation through initiator tRNA(fMet) aminoacylation.
MtnN	Catalyzes the irreversible cleavage of the glycosidic bond in both 5'- methylthioadenosine (MTA) and S-adenosylhomocysteine (SAH/AdoHcy) to adenine and the corresponding thioribose, 5'-methylthioribose and Sribosylhomocysteine, respectively.
LuxS	Involved in the synthesis of autoinducer 2 (AI-2) which is secreted by bacteria and is used to communicate both the cell density and the metabolic potential of the environment. The regulation of gene expression in response to changes in cell density is called quorum sensing. Catalyzes the transformation of S-ribosylhomocysteine (RHC) to homocysteine (HC) and 4,5-dihydroxy-2,3-pentadione (DPD).
MnmC	Catalyzes the last two steps in the biosynthesis of 5-methylaminomethyl-2- thiouridine (mnm5s2U) at the wobble position (U34) in tRNA. Catalyzes the FAD-dependent demodification of cmnm5s2U34 to nm5s2U34, followed by the transfer of a methyl group from S-adenosyl-L-methionine to nm5s2U34, to form mnm5s2U34.
PMI3678	Catalytic activity
PMI1345	Catalyzes the transfer of the phosphoribosyl group of 5-phosphorylribose-1-pyrophosphate (PRPP) to anthranilate to yield N-(5'-phosphoribosyl)- anthranilate (PRA).
FlhD	Functions in complex with FlhC as a master transcriptional regulator that regulates transcription of several flagellar and non-flagellar operons by binding to their promoter region. Activates expression of class 2 flagellar genes, including fliA, which is a flagellum-specific sigma factor that turns on the class 3 genes. Also regulates genes whose products function in a variety of physiological pathways.
FliF	The M ring may be actively involved in energy transduction.
PolA	In addition to polymerase activity, this DNA polymerase exhibits 5'-3' exonuclease activity.
RplP	Binds 23S rRNA and is also seen to make contacts with the A and possibly P site tRNAs.

Discussion

We have started with the proteins involved in P. mirabilis AI-2 biosynthesis pathway (Data not shown) and derived the AIPS along with AIPL. While the former connects the proteins of the pathway as reported by default in STRING with only 10 interactors, the latter has been formed upon extending those to 50 interactors per protein query. The idea was to incorporate other related proteins having connectivity to the AI-2 whose analysis might give more insight about QS in P. mirabilis. Moreover, it was necessary to have an idea of the robustness of the proteins involved in QS pathways and thus, the tightly connected QSPO was constructed where proteins were found to have a clustering coefficient of almost 1. Again, with the homologous proteins reported to be involved in QS in other species from KEGG database, it was necessary to look into their association with acknowledged QS proteins of P. mirabilis. Thus, QSPH was constructed to take into consideration of this fact and analyse further. Furthermore, with multiple genes and proteins reviewed for the virulence of P. mirabilis [1], including those involved for QS phenomenon, it was necessary to have an interactome QSPV constructed to analyse their interactions and involvement. All these SPIN were constructed to have an understanding of the influential proteins responsible for QS in P. mirabilis. To confirm that the large network, is neither random like that proposed by Erdos and Renyi [39] nor a small-world type proposed by Watts and Strogatz [40], we have observed the connectivity distribution, P(k), of a particular node in the GPIN getting connected to k other nodes, for large values of k. The GPIN roughly followed the power-law and is free of a characteristic scale [41] with an exponential decay of the degree distribution. Initially, we have analysed the constructed GPIN with a k-core topological parameter. Proteins which belong to outer shell have lower k value and thus, reflect limited number of interacting partner proteins. Moreover, proteins which belong to inner k-core/shell are specific ones, highly interacting with each other and thus, can be considered to be the most important ones. Decomposition of this core, decomposes the network and thus, makes this the innermost core. We have found the 154th core as the innermost one having many proteins involved in the biosynthesis of amino acids, including cysteine and methionine, the amino acid precursor of the components of AI-2 biosynthetic pathway. These proteins rank top for most of the EC measures across the other five SPIN as well. Furthermore, the 139th core was on focus due to its nearby proximity to the innermost core and comprising most of the proteins directly involved in QS. Such inner core member proteins are highly robust, central and thus highly interactive in nature [42]. Our analyses till this far revealed LuxS and PMI1345 to be the prominent EC proteins in the 139th core of the genome. Interestingly, only PolA and RplP, top rankers of BC measures, made it to the innermost 154th core compared to the other topmost EC proteins in that core. This probably reflects the importance of EC measure to reveal the prominent stakeholders of the machinery responsible for the very survival and probably virulence of the organism. Any effective drug target should, thus be selected from this core group with high EC rank. A further delving deep into the functional connectivity of the modules formed in network topological space reinforced our findings this far. The non-hubs and the hubs formed the functional groups of R1-4 and R5-7, respectively. With high P values and z-

score, the kinless hubs nodes (R7) are supposed to be important in terms of functionality, which has high connection within module (P) as well as between modules (z). With the same logic, having the least P and z measures, the ultra-peripheral nodes (R1) occupy the least connecting position in the network followed by the peripheral nodes (R2). These nodes can be pruned easily without much affecting the whole network while decomposing it to reach the core. This is nothing but the outermost shells of the k-core measures (refer previous section). The non-hub connectors (R3) are expected to take part in only a small but fundamental sets of interactions. This is just opposite to those of the provincial hubs class (R5) which have many within-module connections. The non-hub kinless nodes (R4) are those with links homogeneously distributed among all modules. The most conserved in terms of decomposition as well as evolution would be however, those from the connector hubs (R6) with many links to most of the other modules. The system would try to retain these connections as essential ones for their very survival. We have observed mostly R5 and R6 classes of proteins occupying the innermost 154th and the QS-involved 139th cores. Furthermore, the EC measures brings out this importance when compared to other measures of centralities. With the highest number (17) of fimbrial operons reported in any sequenced bacterial species, four P. mirabilis fimbriae, namely, MR/P, UCA, ATF and PMF have shown prominent roles in biofilm formation [43]. The thickness, structure, and the amount of exopolysaccharides produced by some biofilms formed by P. mirabilis are influenced by important acylated homoserine lactones [9]. Moreover, some virulence factors are regulated by QS molecules like acylated homoserine lactones (acyl-HSLs) [44]. Of the two QS types, LuxS is an essential enzyme for AI-2 type which is coded by luxS gene having S-ribosylhomocysteine lyase activity [12]. Acetylated homoserine lactone derivatives modifies the expression of virulence factors of P. mirabilis strains [45]. The flhDC master operon is a key regulator in swarmer cell differentiation in Proteus mirabilis, it is known to cause an increased viscosity and intracellular signals [46]. Furthermore, the extracellular signals can be sensed by two-component regulators such as RcsCRcsB [46]. It is important to note that, we have observed many of these already known genes and proteins, viz LuxS, FlhDC to be reflected from our studies as well. To this end, other genes and proteins, e.g. PMI1345 not reported to have connections with the QS and virulence, have also been unearthed from our study. Thus, it is imperative that an in-depth analysis as that mentioned here would bring out the importance of the proteins unearthed through the process.

Conclusion

This work schematically delineates an approach of figuring out the most influential protein in a system of interacting proteins of P. mirabilis. It deals with the computational framework of building of the theoretical networks comprising the five individual SPIN followed by the network parametric analytical approach of identifying the most interacting protein connected to other important proteins in the concerned phenotypes. This is reinforced by the disintegration of the GPIN to the innermost core of the proteins, essential for virulence and survival followed by

analysis of centrality measurements. All these lead to the identification of LuxS and PMI1345 to be the most influential ones amongst a group of other proteins being benefitted through network centrality and decomposition analyses. A further investigation of the GPIN brought forth the proteins of important conserved class, potential enough to be the most important ones and thus, influential amongst the barrage of other proteins of the whole genome of P. mirabilis.

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Author contributions

CL conceived the concepts, planned and designed the analyses. Data were generated by SP, MIA and SM and analyzed by CL. Artwork was done by MIA and SP and tabulation was done by SM. CL primarily wrote and edited the manuscript aided by additional help from SP.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Schaffer, J. N., Pearson, M. M. (2015). Proteus mirabilis and urinary tract infections. Microbiology spectrum, 3(5).

[2] Jacobsen, S. M., Shirtliff, M. E. (2011). Proteus mirabilis biofilms and catheter-associated urinary tract infections. Virulence, 2(5), 460-465.

[3] https://emedicine.medscape.com/article/226434-overview#a6

[4] Wang, J. T., Chen, P. C., Chang, S. C., Shiau, Y. R., Wang, H. Y., Lai, J. F., ... Lauderdale, T. L. Y. (2014). Antimicrobial susceptibilities of Proteus mirabilis: a longitudinal nationwide study from the Taiwan surveillance of antimicrobial resistance (TSAR) program. BMC infectious diseases, 14(1), 486.

[5] Luzzaro, F., Brigante, G., DAndrea, M. M., Pini, B., Giani, T., Mantengoli, E., ... Toniolo, A. (2009). Spread of multidrug-resistant Proteus mirabilis isolates producing an AmpC-

type -lactamase: epidemiology and clinical management. International journal of antimicrobial agents, 33(4), 328-333.

[6] Eliopoulos GM, Maragakis LL, Perl TM. Acinetobacter baumannii: epidemiology, antimicrobial resistance, and treatment options. Clinical Infectious Diseases. 2008; 46(8):1254-63.

[7] Czerwonka, G., Guzy, A., Kaua, K., Grosicka, M., Daczuk, M., Lechowicz, ., ... Kaca, W. (2016). The role of Proteus mirabilis cell wall features in biofilm formation. Archives of microbiology, 198(9), 877- 884.

[8] Rutherford, S. T., Bassler, B. L. (2012). Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harbor perspectives in medicine, 2(11), a012427.

[9] Stankowska, D., Czerwonka, G., Rozalska, S., Grosicka, M., Dziadek, J., Kaca, W. (2012). Influence of quorum sensing signal molecules on biofilm formation in Proteus mirabilis O18. Folia microbiologica, 57(1), 53-60.

[10] Holden, M. T., Ram Chhabra, S., De Nys, R., Stead, P., Bainton, N. J., Hill, P. J., ... Rice, S. (1999). Quorum sensing crosstalk: isolation and chemical characterization of cyclic dipeptides from Pseudomonas aeruginosa and other gram negative bacteria. Molecular microbiology, 33(6), 1254-1266.

[11] Campbell, J., Lin, Q., Geske, G. D., Blackwell, H. E. (2009). New and unexpected insights into the modulation of LuxR-type quorum sensing by cyclic dipeptides. ACS chemical biology, 4(12), 1051-1059.

[12] Schneider, R., Lockatell, C. V., Johnson, D., Belas, R. (2002). Detection and mutation of a luxSencoded autoinducer in Proteus mirabilis. Microbiology, 148(3), 773-782.

[13] Rauprich, O., Matsushita, M., Weijer, C. J., Siegert, F., Esipov, S. E., Shapiro, J. A. (1996). Periodic phenomena in Proteus mirabilis swarm colony development. Journal of bacteriology, 178(22), 6525-6538.

[14] Pan, A., Lahiri, C., Rajendiran, A., Shanmugham, B. (2015). Computational analysis of protein interaction networks for infectious diseases. Briefings in bioinformatics, 17(3), 517-526.

[15] Lahiri, C., Pawar, S., Sabarinathan, R., Ashraf, M. I., Chand, Y., Chakravortty, D. (2014). Interactome analyses of Salmonella pathogenicity islands reveal SicA influential for virulence. Journal of theoretical biology, 363, 188-197.

[16] Jeong, H., Mason, S. P., Barabsi, A. L., Oltvai, Z. N. (2001). Lethality and centrality in protein networks. Nature, 411(6833), 41.

[17] Seidman, S. B. (1983). Network structure and minimum degree. Social networks, 5(3), 269-287.

[18] Guimera, R., Amaral, L. A. N. (2005). Functional cartography of complex metabolic networks. nature, 433(7028), 895.

[19] Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., Morishima, K. (2016). KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic acids research, 45(D1), D353-D361

[20] Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M., Tanabe, M. (2015). KEGG

as a reference resource for gene and protein annotation. Nucleic acids research, 44(D1), D457-D462.

[21] Caspi, R., Billington, R., Ferrer, L., Foerster, H., Fulcher, C. A., Keseler, I. M., ... Ong, Q. (2015). The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. Nucleic acids research, 44(D1), D471-D480.

[22] Szklarczyk, D., Morris, J. H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M., ... Jensen, L. J. (2016). The STRING database in 2017: quality-controlled proteinprotein association networks, made broadly accessible. Nucleic acids research, gkw937.

[23] Cline, M. S., Smoot, M., Cerami, E., Kuchinsky, A., Landys, N., Workman, C., ... Hanspers, K. (2007). Integration of biological networks and gene expression data using Cytoscape. Nature protocols, 2(10), 2366.

[24] Bastian, M., Heymann, S., Jacomy, M. (2009). Gephi: an open source software for exploring and manipulating networks. Icwsm, 8, 361-362.

[25] Diestel R. Graph theory. Springer-Verlag Berlin and Heidelberg, 2000.

[26] Koschtzki, D., Schreiber, F. (2004, October). Comparison of Centralities for Biological Networks. In German Conference on Bioinformatics (pp. 199-206).

[27] zgr, A., Vu, T., Erkan, G., Radev, D. R. (2008). Identifying gene-disease associations using centrality on a literature mined gene-interaction network. Bioinformatics, 24(13), i277-i285.

[28] Pavlopoulos, G. A., Secrier, M., Moschopoulos, C. N., Soldatos, T. G., Kossida, S., Aerts, J., ... Bagos, P. G. (2011). Using graph theory to analyze biological networks. BioData mining, 4(1), 10.

[29] Tang, Y., Li, M., Wang, J., Pan, Y., Wu, F. X. (2015). CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein interaction networks. Biosystems, 127, 67-72.

[30] Oliveros, J. C. An interactive tool for comparing lists with Venns diagrams (20072015).

[31] MATLAB and Statistics Toolbox Release 2010b, The MathWorks, Inc., Natick, Massachusetts, United States.

[32] Guimera, R., Amaral, L. A. N. (2005). Cartography of complex networks: modules and universal roles. Journal of Statistical Mechanics: Theory and Experiment, 2005(02), P02001.

[33] Rosvall, M., Bergstrom, C. T. (2011). Multilevel compression of random walks on networks reveals hierarchical organization in large integrated systems. PloS one, 6(4), e18209.

[34] Vzquez, A. (2003). Growing network with local rules: Preferential attachment, clustering hierarchy, and degree correlations. Physical Review E, 67(5), 056104.

[35] Claret, L., Hughes, C. (2000). Rapid Turnover of FlhD and FlhC, the Flagellar Regulon Transcriptional Activator Proteins, during Proteus Swarming. Journal of bacteriology, 182(3), 833-836.

[36] Wang, M. C., Chien, H. F., Tsai, Y. L., Liu, M. C., Liaw, S. J. (2014). The RNA chaperone Hfq is involved in stress tolerance and virulence in uropathogenic Proteus mirabilis. PLoS One, 9(1), e85626. https://doi.org/10.1371/journal.pone.0085626.

[37] Rhoads, D. B., Laimins, L., Epstein, W. O. L. F. G. A. N. G. (1978). Functional organization of the kdp genes of Escherichia coli K-12. Journal of bacteriology, 135(2), 445-

452.

[38] Pearson, M. M., Sebaihia, M., Churcher, C., Quail, M. A., Seshasayee, A. S., Luscombe, N. M., ... Hauser, H. (2008). Complete genome sequence of uropathogenic Proteus mirabilis, a master of both adherence and motility. Journal of bacteriology, 190(11), 4027-4037.

[39] Erds, P., Rnyi, A. (1960). On the evolution of random graphs. Publ. Math. Inst. Hung. Acad. Sci, 5, 17-61.

[40] Watts, D. J., Strogatz, S. H. (1998). Collective dynamics of small-world networks. Nature, 393(6684), 440.

[41] Albert, R., Jeong, H., Barabsi, A. L. (2000). Error and attack tolerance of complex networks. Nature, 406(6794), 378.

[42] Alvarez-Hamelin. I, Dall'Asta. L, Barrat. A. and Vespignani. A, Advances in Neural Information Processing Systems 18, MIT Press, Cambridge, MA, 2006; 41-50.

[43] Scavone, P., Iribarnegaray, V., Caetano, A. L., Schlapp, G., Hrtel, S., Zunino, P. (2016). Fimbriae have distinguishable roles in Proteus mirabilis biofilm formation. FEMS Pathogens and Disease, 74(5), ftw033.

[44] Henke, J. M., Bassler, B. L. (2004). Bacterial social engagements. Trends in cell biology, 14(11), 648-656.

[45] Stankowska, D., Kwinkowski, M., Kaca, W. (2008). Quantification of Proteus mirabilis virulence factors and modulation by acylated homoserine lactones. J Microbiol Immunol Infect, 41(3), 243-253.

[46] Fraser, G. M., Hughes, C. (1999). Swarming motility. Current opinion in microbiology, 2(6), 630- 635.