OMICS RESEARCH SYMPOSIUM

DRUG TARGET EXPLORATION IN HYPERVIRULENT *KLEBSIELLA PNEUMONIAE* STRAINS.

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Introduction

Hypervirulent *K. pneumoniae* is an emerging subtype of *K. pneumonia* defined by the presence of many biomarkers that have close association with a large virulence plasmid that contains genes responsible for aerobactin and salmochelin biosynthesis as well as *rmpA* and *rmpA2* which regulate the capsule mucoid. The confluence of hypervirulence and the propensity to become multi-, extreme- or pandrug-resistant and also the acquisition of extended-spectrum beta-lactamases and carbapenemases has the potential to make this pathogen one of the most feared "superbugs" in the history and evolution of pathogenic microorganisms.

The molecular functions of over 30% proteins (hypothetical proteins HPs) in many organisms are unknown. Functionally annotating HPs can aid the understanding of their roles and functions in metabolisms and the identification of previously unexplored drug targets in a particular organism. In the face of increasing resistance to conventional antibiotics, this experiment represents the initial phase of a study which aims to use a series of in silico analyses to explore and identify drug/vaccine candidates from the hypothetical proteins of four hypervirulent *K. pneumoniae* strains.

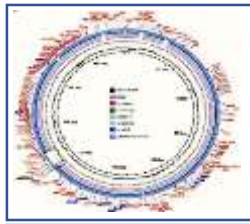

Figure 1: Structure of the hybrid drug-resistant virulent plasmid p11492-vir-CTXM and corresponding novel transposon Tn*6501* with *bla*CTX-M-24.

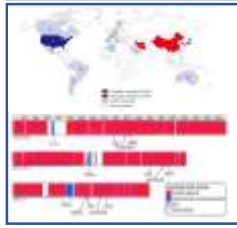

Figure 2A: Global distribution of hvKp

Figure 2B: Map of hypervirulence and carbapenem resistance hybrid plasmids

Methods

Sequence Retrieval
The whole proteome of hypervirulent *Klebsiella pneumoniae* (hvKp) TK421 (UP000338730) was retrieved from The Universal Protein Resource (UniProt), a database of protein sequence and functional information. The hypothetical proteins (HPs) were obtained using a Perl script.

The obtained HPs were subjected to a similarity search using protein BLAST (BLASTp) against the essential proteins of bacteria in the database of essential genes (DEG 15.2). The HPs that showed a similarity with proteins of DEG were considered essential proteins. A similarity search using BLASTp was carried out between the detected essential proteins and the human proteome. Essential proteins that demonstrated no hit with the human proteome were considered to be non-homologous to human proteins.

Function Prediction
Function prediction was carried out using InterPro, Pfam, and NCBI-BLASTp. The predicted functions were further verified using a protein-protein interaction (PPI) analysis and gene ontology (GO) analysis. Furthermore, the ENH proteins were subjected to STRING analysis to predict protein functions on the basis of the homolog hits and protein interactions.

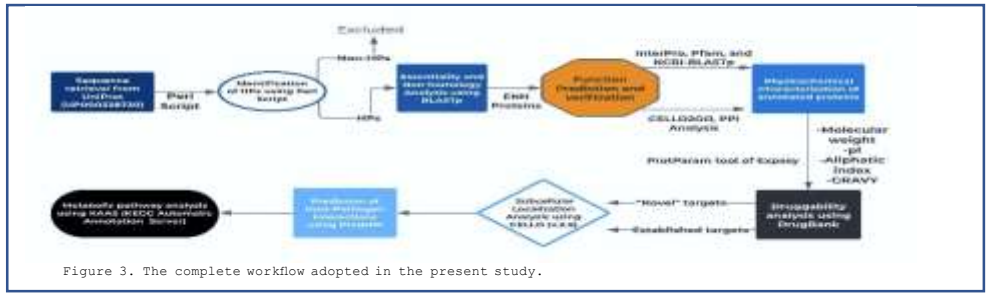

Figure 3. The complete workflow adopted in the present study.

Druggability and Subcellular Localization Analysis
A similarity search was performed between the annotated ENH proteins and known targets of DrugBank, a database that provides information about drugs and the corresponding targets. CELLO (v.2.5), a classification server that uses features of amino acid sequence to predict subcellular localization was used.

Prediction of Host-Pathogen Interactions
The host-pathogen interactions of the annotated ENH proteins were predicted using the interlog method against the full database of HPIDB. This method relies on a homology search of the query sequence against the known host- pathogen interaction data.

Results

Table 1: Functionally Annotated HPs and their DrugBank Interactions

Protein ID	Predicted Function	Drugbank Interaction
[illegible]	DNA topoisomerase type I	1. Pefloxacin and Novobiocin (Both approved drugs with inhibitory actions); 2. Thymidine monophosphate, Adenosine 3',5'-diphosphate, and Thymidine-3',5'-Diphosphate (All in experimental stage)
[illegible]	Thiamine biosynthesis protein	-
[illegible]	Maltose isomerase	-
[illegible]	Chaperone lipoprotein	-
[illegible]	Putative integral membrane protein	-
[illegible]	DNA binding	-
[illegible]	TolB-like lipoprotein	-
[illegible]	Putative inner membrane protein YbhQ	-
[illegible]	Esterase YqiA	-
[illegible]	CTP synthase	1. L-Glutamine (Approved; can act as an antagonist and a substrate)
[illegible]	Baratar family protein (Barnase inhibitor)	-
[illegible]	transcriptional regulator	-

The retrieved proteome from UniProt contained a total of 5,057 protein sequences from which 401 HPs were identified and analyzed. Sixty-five percent (65%) of the annotated ENH HPs were localized in the cytoplasm including the two ENH HPs that showed interactions in DrugBank, thereby making them strong candidates as drug targets.

Host-pathogen interaction analysis revealed only 1 protein interacting with 15 human protein while others showed no interaction.

The same protein was revealed to be involved in biosynthesis of cofactors, pyrimidine metabolism and metabolic pathways including carbohydrate metabolism and amino acid metabolism.

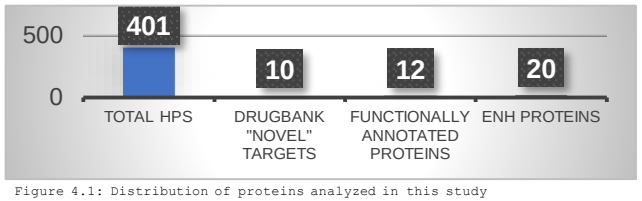

Figure 4.1: Distribution of proteins analyzed in this study

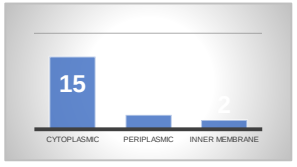

Figure 4.2: Subcellular localization of candidate targets

Figure 4.3: Host-pathogen interaction

Conclusions

In the majority, there is a wide knowledge gap about the occurrence, evolution and dissemination of hvKp, as well as the convergence of hvKp, multidrug resistance, and carbapenem resistance (CR-hvKp) in *K. pneumoniae*. While China has been a hotspot for these superbugs, there have also been widespread reports of infections caused by CR-hvKp in other countries including Canada, Germany, UK, Japan, India, Algeria, Saudi Arabia, Spain, France, Italy, Sweden, Australia, Singapore, and the US. Furthermore, the emergence of CR-hvKp strains have stiffened efforts at managing hvKP infections, and this difficulty could make this pathogen the next worldwide "superbug" in waiting. The current battle with failing antibiotic options thus calls for the development of effective alternative therapeutic options for the control of every strain of hvKp.

With the ongoing failure of conventional antibiotics, it becomes urgent to explore other options, bearing in mind the possibilities of bioinformatics, one of which this study has explored. Having a good understanding of the functions of HPs helps to further grasp what their role is in biochemical/physiological pathways. This could also lead to the identification of novel groups of therapeutic targets. This present study functionally annotated 12 out of 20 pathogen-specific essential proteins.

Ten (10) of the annotated proteins were considered as "novel" owing to their lack of interaction with any known drug in DrugBank database. The other 2 proteins showed interactions with different drugs, however, 3 of the drugs are still at the experimental stage, making the interacting target protein eligible for further analysis. While none of the novel candidate targets showed no interaction with any human protein, that does not rule them out as potential drug targets that can help in the designing of inhibitory therapeutic agents against hypervirulent strains of *K. pneumoniae*. The structural analyses of identified target proteins and the analysis of 3 other hypervirulent *K. pneumoniae* strains are ongoing. Completing this study could provide efficient and reliable drug target/vaccine candidate against the rising superbugs.

Selected References

Altschul, S. (1990). Basic local alignment search tool. J. Mol. Biol. 215, 403-410.

Shahbaaz, M., Bisetty, K., Ahmad, F., and Hassan, M. I. (2016). Current advances in the identification and characterization of putative drug and vaccine targets in the bacterial genomes. Curr. Top. Med. Chem. 16, 1040-1069.

Lee C-R, Lee JH, Park KS, Jeon JH, Kim YB, Cha C-J, Jeong BC and Lee SH (2017) Antimicrobial Resistance of Hypervirulent *Klebsiella pneumoniae*: Epidemiology, Hypervirulence-Associated Determinants, and Resistance Mechanisms. Front. Cell. Infect. Microbiol. 7:483.

Zhiyuan Yang, Xi Zeng, and Stephen Kwok-Wing Tsui. (2019) Investigating function roles of hypothetical proteins encoded by the *Mycobacterium tuberculosis* H37Rv genome. BMC Genomics, 20:394

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