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Original Article

PROTEOMIC STUDY OF CARBAPENEM-RESISTANT *K. PNEUMONIAE* CLINICAL ISOLATES

VETRISELVI SUBRAMANIYAN[1], SURESH DHANARAJ[2]

[1]Department of Microbiology, School of Life Sciences, Vels Institute of Science, Technology, and Advanced Studies (VISTAS), Pallavaram, Chennai, 600117 Tamil Nadu, India
*Email: sureshdhanaraj. sls@velsuniv.ac.in

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ABSTRACT

Objective: Now it's a worldwide issue that carbapenem resistance is spreading. This has made *K. pneumoniae* infections more difficult to treat. All *Klebsiella pneumonia'*s proteins were examined in this study, which focused on the carbapenem-resistant bacteria's response to meropenem.

Methods: Proteomics (MALDI-TOF) and bioinformatics methods were combined to answer the new enigma of resistance. Functional annotation, pathway enrichment and protein–protein interaction were some of the uses of this data. Both KEGG and STRING played an important role (PPI).

Results: Proteins that help synthesise DNA and RNA, proteins that aid in carbapenem degradation, and proteins that aid energy and intermediate metabolism are all subdivided into two classes.007A

Conclusion: Bacterial survival and meropenem resistance may have been aided by four overexpressed proteins and their partners. A new anti-resistance medication based on these proteins could help restrict the growth of "bad bugs."

Keywords: *K. pneumoniae,* Carbapenem resistance, Uropathogens, Proteomics

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INTRODUCTION

An important public health issue is the proliferation of Enterobacteriaceae, the organisms responsible for KPC (*Klebsiella pneumoniae* carbapenemase) [1]. KPC-type-lactamases are responsible for resistance to nearly all-lactam antibiotics, including cephalosporins and carbapenems [2]. Low porin levels and increased synthesis of-lactamases have been associated to resistance in *K. pneumoniae* [3, 4]. In contrast, little attention has been made to the involvement of other proteins in the body in the establishment of resistance. When the environment changes, such as antibiotic stress, proteomes can adapt swiftly. As a result of antimicrobials disrupting bacteria's internal balance, cell activities accelerate to restore the damage. Proteome analysis of drug-resistant bacteria that have been exposed to antimicrobials could lead to the discovery of new methods bacteria to counteract the effects of antimicrobial agents [5]. Many biological mysteries can now be solved because to recent developments in proteomics and bioinformatics. Bacillus subtilis, Escherichia coli, and Mycobacterium TB have all been studied in this manner [6–9]. Other infections' reactions to antimicrobial medications have been studied with it, too.

The proteome of a multidrug-resistant clinical *K. pneumoniae* strain that carried blaKPC-2 was analysed after it was exposed to meropenem, despite the fact that proteins are the cellular building blocks. This study was not focused on KPC-2 carbapenemase. Rather, the primary goal was to identify proteins that changed in response to antibiotic stress and may play a role in bacterial defence and survival. With and without meropenem, the *K. pneumoniae* soluble whole cell proteomes were compared. This was accomplished by the use of mass spectrometry and two-dimensional gel electrophoresis. To our knowledge, this is the first study to examine how meropenem therapy affects the protein expression of *K. pneumoniae* KPC-2-lactamase producers. Studying the proteins produced in cells that are exposed to Meropenem helps us better understand how antibiotic therapy affects cells.

MATERIALS AND METHODS

Sample collection

It was the purpose of this investigation to discover how uropathogens propagated among patients with UTIs and which antimicrobials they were resistant to in the laboratory. In Chennai, Tamil Nadu, India, 745 people over the age of 18 who suspected they had a urinary tract infection were examined in outpatient settings. A total of 745 samples of urine were collected and analysed. They found that 340 of them had a lot of bacteria growing inside of them. It's important to note that this study was supported by medical documentation known as a "Performa." The patient's age, gender, antimicrobial sensitivity, and symptoms were all documented on this form. Urine samples from UTI patients were taken aseptically and stored in the refrigerator in sterile, screw-top marked urine containers (100 ml). Detection of the microorganisms required the use of biochemical techniques.

Isolation and Identification of *K. pneumoniae*

Culture observation

The uropathogens were cultured in the laboratory on a variety of media, including blood agar, MacConkey agar, and others. During incubation, you could observe the plates to see what type of colony had formed. When they were incubated on the plates, they exhibited various colony morphologies and sizes, as well as shapes and colours.

Microscopic examination

Gram staining was done on microscopic slides made from each of the colonies found on the plates.

Biochemical examination

Isolates exhibiting the morphology of *K. pneumoniae* were analysed using Indole, Methyl-red, Voges–Proskauer, and Citrate biochemical assays.

Identification of uropathogens

Ureters excreted 139 E. coli and Klebsiella spp germs from patients undergoing surgery (25.2 %) Proteus species were responsible for one in five of bacterial illnesses. CNS produced 12 infections, while Pseudomonas species caused 24 infections, both of which were caused by CNS, both of which were caused by CNS (12 %). So far, scientists have discovered 11 distinct types of S. aureus (3.2 %). Proteomics study was conducted on four strains of *Klebsiella pneumoniae* that were resistant to carbapenem.

Out of 86 Klebsiella spp 4 Carbapenem-resistant, *K. pneumoniae* isolates were chosen for Proteomics analysis

Antimicrobial susceptibility testing

A patient's carbapenem-resistant Klebsiella pneumonia strain was employed in the study. The Clinical and Laboratory Standards Institute (CLSI) recommended microdilution testing for meropenem susceptibility testing (DST) against a carbapenem-resistant Klebsiella pneumonia clinical isolate [10].

Culture and drug induction

Klebsiella pneumoniae subsp. pneumoniae (ATCC 700603) was used as a reference strain in this study's functional annotation studies. *K. pneumoniae* was cultivated in Luria Bertani broth at 37 degrees Celsius and 220 revolutions per minute (LB) from a clinical isolate (LB). We discovered that meropenem was present in one of the flasks that contained a MIC lower than 32 mg/ml. The OD600 of the bacteria was measured at 0.8 at this time in the experiment. Centrifugation at 8000 rpm for 8 min at 4 C collected them. Until the job was finished, the cell pellet was maintained at-80 C to ensure its safety. The identical method has been used in all of the physiological studies. Making protein samples is as simple as following the steps outlined here [11-12].

Protein sample preparation

The cells were resuspended in 50 mg Tris-HCL, 10 mg MgCl2, 0.1 % sodium azide (NaA), 1 mg Phenylmethylsulfonylfluoride (PMSF), and 1 mg EGTA at 1 g wet weight per 5 ml after being washed away with normal saline. We then heated the homogenate to 80 °C after it had been centrifuged at 12,000 g for 20 min at 4 C. The supernatant was frozen in a bottle of cold acetone overnight at-20o C. (1,4). The precipitated protein was collected by centrifugation at 12,000 rpm for 20 min. After allowing the protein to air dry, it was mixed with an acceptable amount of protein dissolving solution. We employed the Bradford method [13] to ascertain the protein concentration. The technological aspects of the project have been put to the test again.

2D SDS page

The pH range of the 13 cm IPG strips (GE Healthcare, Uppsala, Sweden) was 3–10. A total of 75 ug of processed proteins were electrophoresed in two separate gels for the second dimension of the experiment. 50,000 Vhs of proteins were focused on the IPG at a constant temperature of 20oC with the following IEF conditions: 100 V stepnhold for 1 h, 1000 V gradient for 1 h, 5000 Volts for 5 h, and 5000 Volts for 7 h. afterward, the IPG was rehydrated. The IEF was conducted under these conditions. Iodoacetamide, 2.5 %, and 2 % DTT were added to the IPG strips after they had been incubated in the IEF solution. One hour of 1W/gel and three hours of 13W/gel were used for PAGE in the second dimension at GE Healthcare, Uppsala, Sweden's SE600 (GE Healthcare, Uppsala, Sweden). The SE600 was used to accomplish this.

In-gel digestion and MALDI-TOF/MS

2-DE gels stained with silver had protein spots removed. The peptides were broken down with the aid of trypsin, as has previously been done (Cho *et al.*, 2007; Perkins *et al.*, 1999). Trifluoroacetic acid (TFA) was utilized to re-dissolve the broken down peptides (TFA). In order to reduce the chemical background noise for MALDI-TOF, Zip-tip C18 pipette tips were employed (Millipore, Bedford, MA, USA). The MALDI plate was washed with a chromatin matrix solution (10 mg/ml CHCA in 0.5 % TFA/50 % acetonitrile, 1:1). The 4700 Proteomics Analyzer was used to capture all of the mass spectra in reflection mode (Applied Biosystems, Framingham, MA, USA). The NCBI database and MALDI fingerprint data were then consulted to find proteins using this method. After that, we turned to MASCOT (http://www.matrixscience.com) to conduct our protein research (Kurupati *et al.*, 2006; Perkins *et al.*, 1999).

Integration of a protein–protein interaction (PPI) network

For the purpose of finding persons to communicate to, the STRING database v10.0 (http://www.stringdb.org) was used [14–15]. STRING-based PPIs were discovered as a result of numerous experiments and genetic studies. Co-expression studies, domain fusions, high-throughput phylogenetic profiles, and gene neighborhood analyses uncovered the PPIs.

RESULTS

Carbapenem-resistant isolate was grown in this investigation under meropenem drug stress. Carbapenem-resistant *K. pneumoniae* with and without meropenem was the primary focus of this investigation. 2DE profiles of *K. pneumoniae* growing in the absence and presence of meropenem are shown in fig. 1. A total of four locations had a 1.5-to-1 ratio of drug-treated to untreated patients. A technique called MALDI-TOF/MS was utilised to study these protein spots in greater detail. Using MALDI-TOF, we identified proteins that were more abundant than usual in table 1. These proteins include catabolite repressor-activator, catabolite repressor, acetate kinase and beta-lactamase. How many times an enzyme is overproduced is determined by the densitometric ratio.

KEGG pathway analysis

The pathways that the overexpressed proteins are a part of are shown in fig. 2 and table 2. KEGG was able to identify the pathways that had a large number of abnormally expressed proteins. In ribosomes, the number of up-regulated proteins increased, as did the metabolism of microorganisms in various environments, betalactam resistance, secondary metabolite biosynthesis, propanoate metabolism, amino- and nucleotide-based sugar metabolism, carbon metabolism, and pyruvate metabolism, to name just a few. Information regarding how proteins interact with each other is depicted in fig. 3 (PPIs).

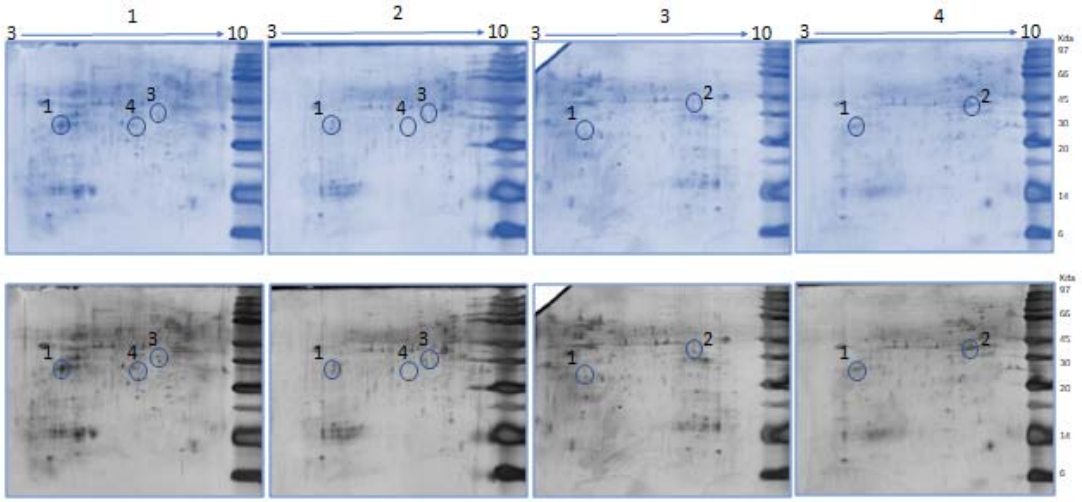

Fig. 1: Representative images of two-dimensional gel electrophoresis profile of the whole-cell lysate of *Klebsiella pneumoniae*. Arrows show proteins identified by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF)

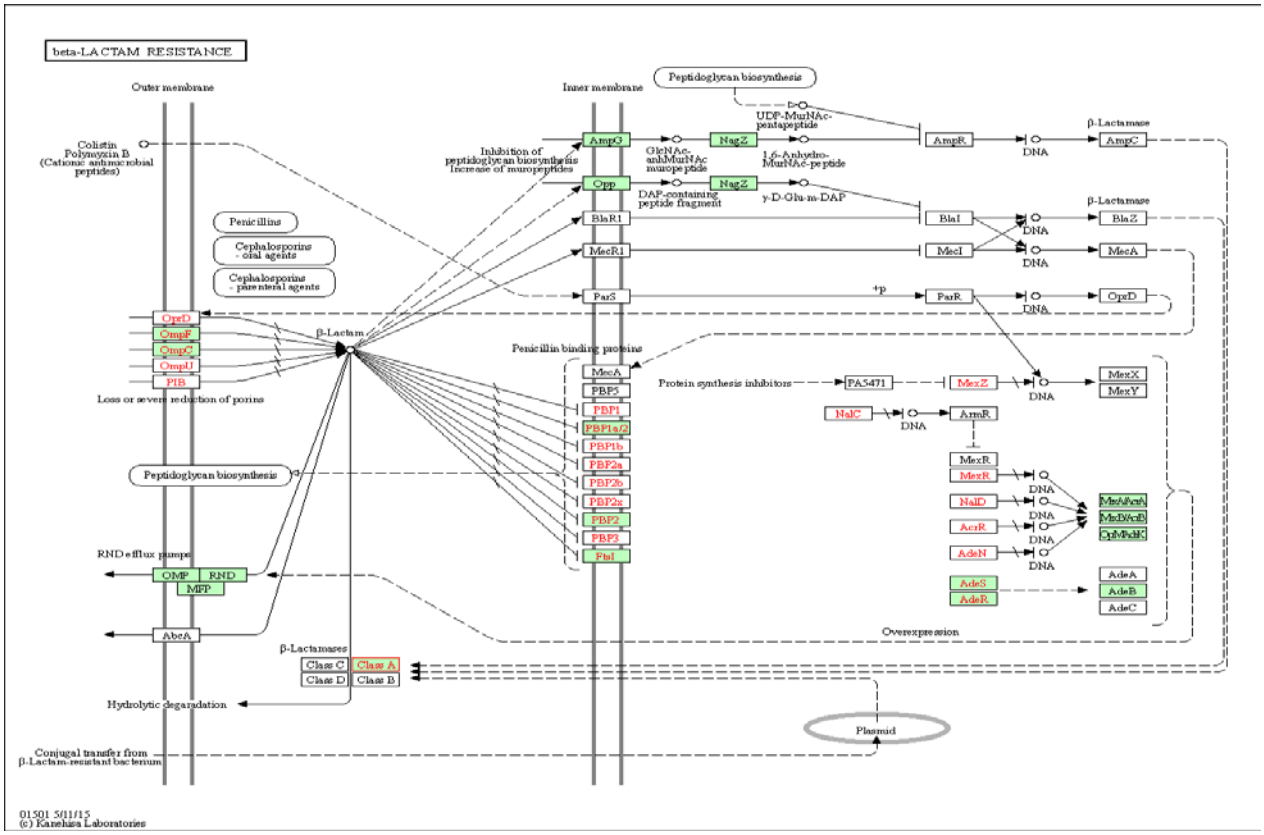

Fig. 2: Beta-lactam resistance pathway manually curated using KEGG. Overexpressed genes in bla (yellow) was successfully mapped on to this pathway

Table 1: Details of the over expressed proteome under meropenem stress in Klebsiella pneumonia clinical isolates

Spot ID	Gene ID	Accession No	Protein name	Score	Mol weight (KDa)	Calc pI
1	metK	A6TDV1	S-adenosylmethionine synthase	85	41.90	5.10
2	fruR	A6T4M3	Catabolite repressor-activator	61	38.09	6.73
3	ackA	A6TBY3	Acetate kinase	76	43.27	5.89
4	bla	A6TIL8	Beta-lactamase	59	31.15	5.69

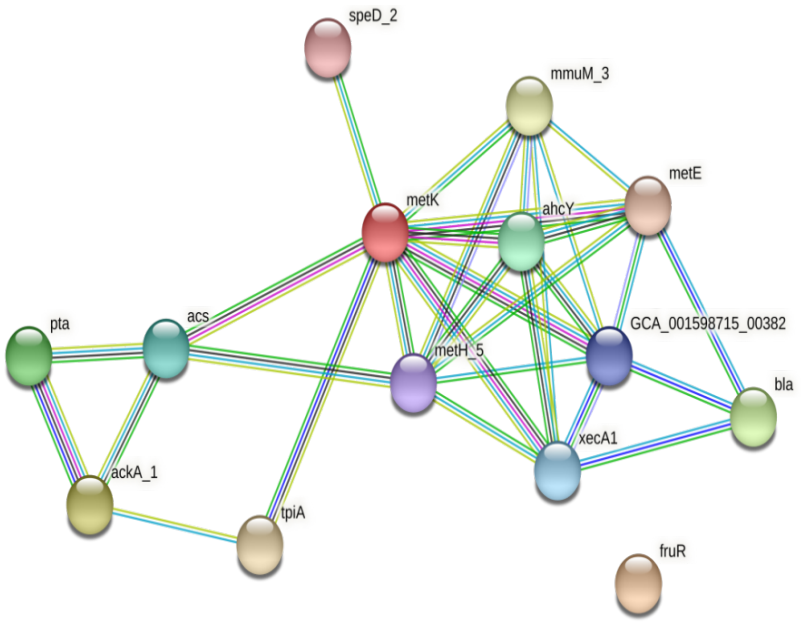

Fig. 3: Protein-protein interaction analysis

Table 2: Up-regulated proteins

#Clustering method	Cluster number	Cluster color	Gene count	Protein name	Protein identifier	Protein description
kmeans	1	Red	2	bla	1218098.GCA_001598715_00372	Beta-lactamase SHV-2; This enzyme hydrolyzes cefotaxime, ceftazidime and other broad-spectrum cephalosporins; Belongs to the class-A beta-lactamase family.
kmeans	1	Red	2	fruR	1218098.GCA_001598715_03002	Fructose repressor FruR; Unannotated protein
kmeans	2	Green	9	GCA_001598715_00382	1218098.GCA_001598715_00382	Methionine synthase II; Unannotated protein
kmeans	2	Green	9	ahcY	1218098.GCA_001598715_04751	Adenosylhomocysteinase; Unannotated protein
kmeans	2	Green	9	metE	1218098.GCA_001598715_02848	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase; Catalyzes the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine resulting in methionine formation; Belongs to the vitamin-B12 independent methionine synthase family.
kmeans	2	Green	9	metH_5	1218098.GCA_001598715_03235	B12-dependent methionine synthase; Unannotated protein
kmeans	2	Green	9	metK	1218098.GCA_001598715_03011	S-adenosylmethionine synthase; Catalyzes the formation of S-adenosylmethionine (AdoMet) from methionine and ATP. The overall synthetic reaction is composed of two sequential steps, AdoMet formation and the subsequent tripolyphosphate hydrolysis, which occurs prior to the release of AdoMet from the enzyme.
kmeans	2	Green	9	mmuM_3	1218098.GCA_001598715_00929	Homocysteine S-methyltransferase; Unannotated protein
kmeans	2	Green	9	speD_2	1218098.GCA_001598715_00728	S-adenosylmethionine decarboxylase alpha chain; Catalyzes the decarboxylation of S-adenosylmethionine to S-adenosylmethioninamine (dcAdoMet), the propylamine donor required for the synthesis of the polyamines spermine and spermidine from the diamine putrescine; Belongs to the prokaryotic AdoMetDC family. Type 2 subfamily.
kmeans	2	Green	9	tpiA	1218098.GCA_001598715_00277	Triosephosphate isomerase; Involved in the gluconeogenesis. Catalyzes stereospecifically the conversion of dihydroxyacetone phosphate (DHAP) to D-glyceraldehyde-3-phosphate (G3P); Belongs to the triosephosphate isomerase family.
kmeans	2	Green	9	xecA1	1218098.GCA_001598715_03411	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase; Unannotated protein
kmeans	3	Blue	3	ackA_1	1218098.GCA_001598715_02480	Acetate kinase; Catalyzes the formation of acetyl phosphate from acetate and ATP. Can also catalyze the reverse reaction; Belongs to the acetokinase family. Rule: MF_00020, ECO: 0000256|RuleBase: RU003835,
kmeans	3	Blue	3	acs	1218098.GCA_001598715_01908	Acetyl-coenzyme A synthetase; Catalyzes the conversion of acetate into acetyl-CoA (AcCoA), an essential intermediate at the junction of anabolic and catabolic pathways. Acs undergoes a two-step reaction. In the first half reaction, Acs combines acetate with ATP to form an acetyl-adenylate (AcAMP) intermediate. In the second half reaction, it can then transfer the acetyl group from AcAMP to the sulfhydryl group of CoA, forming the product AcCoA.
kmeans	3	Blue	3	pta	1218098.GCA_001598715_02481	Phosphate acetyltransferase; Involved in acetate metabolism; In the N-terminal section; belongs to the CobB/CobQ family.

DISCUSSION

The number of carbapenem-resistant *Klebsiella pneumoniae* is rapidly increasing, which is a major challenge for the world's health care infrastructure. Two well-known indications of carbapenem resistance, Carbapenemase overproduction and a deficiency of porin, are still in their infancy.

Several proteins were more active in the meropenem-treated culture than in the control group. Carbagenem resistance in *K. pneumoniae* may be a result of this mutation. Some of the groups of these four proteins include enzymes that alter DNA or RNA, energy and intermediate metabolism proteins, and carbapenem-disrupting proteins. Proteins other than KPC-type lactamase that play a significant role in *K. pneumoniae* resistance were a primary focus of our investigation.

In order to avoid overdosing the bacteria, it was administered at a concentration below the MIC (0.5 MIC). Overproduction of enzymes that aid in energy production, such as phosphoglycerate kinase, fructose-bisphosphate aldolase class II, phosphoglyceromutase, alcohol dehydrogenase, and malate dehydrogenase, was discovered in the treated bacterial culture. In the presence of stress, these enzymes may indicate that bacteria are consuming more energy. Despite the fact that this is not an antibiotic-mediated cell death process, we assumed that overproducing proteins would primarily influence metabolic pathways connected to antibiotic resistance. A

PPI network was also created using four proteins that were over expressed in the study population. Proteins with higher levels in our study were hub proteins, which have a role in translation, metabolism, and resistance to beta-lactam antibiotics [16]. They may need extra energy for protein and DNA repair, or they may need to expel the antibiotic from their cell, which would render it useless. To overcome their anxiety and return to the cell cycle, they may use a lot of energy. Proteins associated to stress are more likely to be expressed, according to recent studies [17].

Once anhydromuropeptides are degraded by AmpD, the ampC gene is unable to be expressed. A lot of muropep tides are produced and accumulate in the cytoplasm during pharmacological stress or beta lactamase-inducing medications (such meropenem), which activates the production of ampC through AmpR [18].

The Cpx envelope stress response was assumed to affect peptidoglycan (PG) molecule structure via the LysM domain/BON superfamily protein. E. coli would have a tough time adapting to envelope stress if this were the case. Cell wall remodelling and folding periplasmic proteins are two of Cpx's functions. ygaU is one of them. The Cpx envelope stress response can aid in PG homeostasis. When the cell wall undergoes a change, this reaction activates these genes to correct it. An enzyme termed L,D-transpeptidase and a LysM domain/BON superfamily member affected the PG crosslinking pattern in tests [19]. The Cpx envelope stress response was activated when E. coli cells were subjected to antibiotics such-lactams, which inhibit the cell wall division or elongation machinery [20].

There are three genes that play an important role in antibiotic resistance and can be employed to circumvent its resistance mechanism: NagZ (nagZ), OmpC (ompC), and Bla. Based on these overexpressed proteins, we devised a theory about carbapenem resistance. Proteins overexpressed in the body may affect carbapenem cleavage, modification, and transport. This could lead to carbapenem resistance as a result [21, 22].

CONCLUSION

This work employed proteomics and bioinformatics to examine the whole proteome of a carbapenem-resistant *K. pneumoniae* clinical isolate under stress. They are classified into four categories: protein translational machinery complexes, enzymes or proteins involved in DNA/RNA modification, proteins involved in carbapenem cleavage, modification, transport, and energy metabolism, and proteins involved in intermediate metabolism. All of them have been connected in some manner to carbapenem resistance. Pathways and protein-protein interactions were discovered to be over-expressed in these four proteins, pathways, and proteins that interact with one another. Numerous factors aided bacteria and meropenem resistance. The targets and routes of these proteins may provide new avenues for studying drug resistance and developing novel anti-drug agents. As a consequence, malevolent defects may be prevented from being created.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Authors declare no conflict of interest.

REFERENCES

1. Endimiani A, Hujer AM, Perez F, Bethel CR, Hujer KM, Kroeger J. Characterization of blaKPC-containing *Klebsiella pneumoniae* isolates detected in different institutions in the Eastern USA. J Antimicrob Chemother. 2009 Mar;63(3):427-37. doi: 10.1093/jac/dkn547. PMID 19155227, PMCID PMC2640158.
2. Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: a new threat to our antibiotic armamentarium. Arch Intern Med. 2005 Jun 27;165(12):1430-5. doi: 10.1001/archinte.165.12.1430, PMID 15983294.
3. Martinez Martinez L, Pascual A, Hernandez Alles S, Alvarez Diaz D, Suarez AI, Tran J. Roles of beta-lactamases and porins in activities of carbapenems and cephalosporins against *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 1999 Jul;43(7):1669-73. doi: 10.1128/AAC.43.7.1669, PMID 10390220, PMC89341.
4. Jacoby GA, Mills DM, Chow N. Role of beta-lactamases and porins in resistance to ertapenem and other beta-lactams in *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2004 Aug;48(8):3203-6. doi: 10.1128/AAC.48.8.3203-3206.2004, PMID 15273152, PMC478483.
5. Freiberg C, Brotz Oesterhelt H, Labischinski H. The impact of transcriptome and proteome analyses on antibiotic drug discovery. Curr Opin Microbiol. 2004 Oct;7(5):451-9. doi: 10.1016/j.mib.2004.08.010. PMID 15451499.
6. Hessling B, Bonn F, Otto A, Herbst FA, Rappen GM, Bernhardt J. Global proteome analysis of vancomycin stress in staphylococcus aureus. Int J Med Microbiol. 2013 Dec;303(8):624-34. doi: 10.1016/j.ijmm.2013.08.014. PMID 24161710.
7. Dos Santos KV, Diniz CG, Veloso Lde C, de Andrade HM, Giusta Mda S, Pires Sda F, Santos AV, Apolonio AC, de Carvalho MA, Farias Lde M. Proteomic analysis of Escherichia coli with experimentally induced resistance to piperacillin/tazobactam. Res Microbiol. 2010 May;161(4):268-75. doi: 10.1016/j.resmic.2010.03.006. Epub 2010 Apr 8. PMID: 20381611.
8. Rangarajan N, Sangeetha R, Mohanasundaram S, Sampath, Porkodi K, Dass Prakash MV. Additive inhibitory effect of the peels of citrus limon and citrus sinensis against amylase and glucosidase activity. IJRPS 2020;11(4):6876-80. doi: 10.26452/ijrps.v11i4.3661.
9. Lata M, Sharma D, Deo N, Tiwari PK, Bisht D, Venkatesan K. Proteomic analysis of ofloxacin-mono resistant Mycobacterium tuberculosis isolates. J Proteomics. 2015 Sep 8;127(A):114-21. doi: 10.1016/j.jprot.2015.07.031. PMID 26238929.
10. Wayne PA. Performance standards for antimicrobial susceptibility testing: 24 informational supplement. CLSI. 2014;M100:S24.
11. Qayyum S, Sharma D, Bisht D, Khan AU. Protein translation machinery holds a key for transition of planktonic cells to biofilm state in Enterococcus faecalis: A proteomic approach. Biochem Biophys Res Commun. 2016 Jun 10;474(4):652-9. doi: 10.1016/j.bbrc.2016.04.145. PMID 27144316.
12. Mohanasundaram S, Doss VA, Maddisetty P, Magesh R, Sivakumar K, Subathra M. Pharmacological analysis of hydroethanolic extract of Senna alata (L.) for *in vitro* free radical scavenging and cytotoxic activities against Hep G2 cancer cell line. Pak J Pharm Sci. 2019;32(3):931-4.
13. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976 May 7;72:248-54. doi: 10.1006/abio.1976.9999, PMID 942051.
14. Sharma D, Lata M, Faheem M, Khan AU, Joshi B, Venkatesan KM. Tuberculosis ferritin (Rv3841): potential involvement in amikacin (AK) and kanamycin (KM) resistance. Biochem Biophys Res Commun. 2016 Sep 16;478(2):908-12. doi: 10.1016/j.bbrc.2016.08.049. PMID 27521892.
15. Sharma D, Khan AU. Role of cell division protein divIVA in Enterococcus faecalis pathogenesis, biofilm and drug resistance: A future perspective by in silico approaches. Microb Pathog. 2018 Dec;125:361-5. doi: 10.1016/j.micpath.2018.10.001. PMID 30290265.
16. Domenech Sanchez A, Benedi VJ, Martinez Martinez L, Alberti S. Evaluation of differential gene expression in susceptible and resistant clinical isolates of *Klebsiella pneumoniae* by DNA microarray analysis. Clin Microbiol Infect. 2006 Sep;12(9):936-40. doi: 10.1111/j.1469-0691..01470.x. PMID 16882305.
17. Susin MF, Baldini RL, Gueiros Filho F, Gomes SL. GroES/GroEL and DnaK/DnaJ have distinct roles in stress responses and during cell cycle progression in Caulobacter crescentus. J Bacteriol. 2006 Dec;188(23):8044-53. doi: 10.1128/JB.00824-06. PMID 16980445, PMCID PMC1698207.
18. Mohanasundaram S, Rangarajan N, Sampath V, Porkodi K, Pennarasi M. GC-MS and HPLC analysis of Antiglycogenolytic

and Glycogenic compounds in kaempferol 3-O–gentiobioside containing Senna alata L. leaves in experimental rats. Translational Metab Syndr Research. 2021;4:10-7.

19. Bernal Cabas M, Ayala JA, Raivio TL. The Cpx envelope stress response modifies peptidoglycan cross-linking via the L, D-transpeptidase LdtD and the novel protein YgaU. J Bacteriol. 2015 Feb;197(3):603-14. doi: 10.1128/JB.02449-14. PMID 25422305, PMCID PMC4285979.
20. Delhaye A, Collet JF, Laloux G. Fine-tuning of the Cpx envelope stress response is required for cell wall homeostasis in Escherichia coli. mBio. 2016 Feb 23;7(1):e00047-16. doi: 10.1128/mBio.00047-16, PMID 26908573, PMCID PMC4791840.
21. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003 Nov;13(11):2498-504. doi: 10.1101/gr.1239303, PMID 14597658, PMCID PMC403769.
22. Sivakumar S, Mohanasundaram S, Rangarajan N, Sampath V, Velayutham Dass Prakash MV. *In silico* prediction of interactions and molecular dynamics simulation analysis of Mpro of a severe acute respiratory syndrome caused by novel coronavirus 2 with the FDA-approved nonprotein antiviral drugs. J Appl Pharm Sci. 2022;12(05):104-19. doi: 10.7324/JAPS.2022.120508.