b. **In order of importance, list of ten best papers of the candidate, highlighting the important discoveries/contributions described in them briefly (not to exceed 3000 words)**

1. Ali A, Kumar R, Khan A, **Khan AU**. "Interaction of LysM BON family protein domain with carbapenems: A putative mechanism of carbapenem resistance." ***Int J Biol Macromol. (Elsevier)*** 2020 May 25:S0141-8130(20)33329-8. doi: 10.1016/j.ijbiomac.2020.05.172. **(IF: 6.95)**

   Highlights: A novel mechansim of antibiotic resistance was discoved. *Lys*M domain, a membrane bound anchor protein was found to intervene with antibiotics being targeted to its site of action in bacterial cell. There is, LysM domain BON family protein, was found over 12-fold expressed under the induced state (***J Glob Antimicrob Resist.*** 2017 Mar;8:172-178). A hypothesis was proposed that LysM domain protein might have an affinity towards carbapenem antibiotics making them unavailable to bind with their target. Hence, we initiated a study to understand the binding mode of carbapenem with LysM domain protein. MICs of imipenem and meropenem against LysM clone were increased by several folds as compared to NP-6 clinical strain as well as DH5 α (PET-28a KPC-2) clone. This study further revealed a strong binding of both antibiotics to LysM domain protein. Molecular simulation studies of LysM domain protein with meropenem and imipenem for 80 ns has also showed stable structure. We concluded that overexpressed LysM domain under induced condition interacted with carbapenems, leading to enhanced resistance as proved by high MIC values. Hence, the study proved the proposed hypothesis that the **LysM domain** plays a significant role in the putative mechanism of antibiotics resistance.

2. He X, **Khan AU,** Cheng H, Pappas DL Jr, Hampsey M, Moore CL "Functional interactions between the transcription and mRNA 3' end processing machineries mediated by Ssu72 and Sub1. ***Genes Dev.*** (*CSH Press*) 2003 Apr 15;17(8):1030-42. doi: 10.1101/gad.1075203 **(IF: 11.36)**

Highlights: First time we have proposed that transcription and processing of pre-mRNA are coupled events. By using a combination of biochemical, molecular, and genetic methods, we have found that the phylogenetically conserved transcription factor Ssu72 is a component of the cleavage/polyadenylation factor (CPF) of Saccharomyces cerevisiae. Our results demonstrate that Ssu72 is required for 3' end cleavage of pre-mRNA but is dispensable for poly(A) addition and RNAP II termination. The in vitro cleavage defect caused by depletion of Ssu72 from cells can be rescued by addition of recombinant Ssu72. Ssu72 interacts physically and genetically with the Pta1 subunit of CPF. Overexpression of PTA1 causes synthetic lethality in an ssu72-3 mutant. Moreover, Sub1, which has been implicated in transcription initiation and termination, also interacts with Pta1, and overexpression of SUB1 suppresses the growth and processing defect of a pta1 mutation. Physical interactions of Ssu72 and Sub1 with Pta1 are mutually exclusive. Based on the interactions of Ssu72 and Sub1 with both the Pta1 of CPF and the TFIIB component of the initiation complex, we present a model describing how these novel connections between the transcription and 3' end processing machineries might facilitate transitions in the RNAP II transcription cycle.

3. Akhtar F, **Khan AU***, Misba L, Akhtar K, Ali A "Antimicrobial and antibiofilm photodynamic therapy against vancomycin resistant Staphylococcus aureus (VRSA) induced infection in vitro and in vivo. " ***Eur J Pharm Biopharm***. 2021 Mar; 160:65-76. doi: 10.1016/j.ejpb.2021.01.012 (**IF: 5.56**)

Highlights: In this article, antimicrobial photodynamic therapy (aPDT) has been initiated as an alternative therapy for bacterial infections. Here, we elucidated a possibility of its clinical application by reducing the treatment time and exposing curcumin to 20 J/cm^2 of blue laser light, which corresponds to only 52 s to counteract vancomycin resistant Staphylococcus aureus (VRSA) both in vitro and in vivo. To understand the mechanism of action, the generation of total reactive oxygen species (ROS) was quantified by 2'-7'-dichlorofluorescein diacetate (DCFH-DA) and the type of

phototoxicity was confirmed by fluorescence spectroscopic analysis. singlet oxygen was produced, indicating type-II phototoxicity. The therapeutic potential was validated in skin abrasion wistar rat model. The result showed significant inhibition of bacterial growth. Furthermore, immunomodulatory analysis with rat serum was performed. A significant reduction in expression of proinflammatory cytokines TNF-α and IL-6 were observed. Hence, the curcumin mediated aPDT with 20 J/cm^2 of blue laser treatment (for 52 s) could be used against multi-drug resistant bacterial infections and preformed biofilm formation as a potential therapeutic approach.

4. Ali, A, Kumar , R, Iquebal , MA, Jaiswal , S, Kumar D, and **Khan AU,** "Role of conserved residues in catalytic activity of NDM-1: an approach of site directed mutagenesis and molecular dynamics" ***Physical Chemistry Chemical Physics (RSC)*** , 2019, 21, 17821 **(IF: 3.9).**

Highlights: NDM-1 ia metallloprotein/ enzyme, hydrolyses carbapenems and having this marker in bacterial plasmid/genome, resistance against almost all antibiotics is observed. To understand the structure and function of NDM-1, we have generated novel mutations (N193A, S217A, G219A and T262A) near active sites and an omega-like loop to study the role of conserved residues of NDM-1. The affinity as well as the catalysis properties of these mutants were reduced considerably for imipenem, meropenem, cefotaxime, cefoxitin, and ceftazidimem compared to wild type, hence the catalytic efficiencies (Kcat/Km) of all mutant enzymes were reduced owing to the poor affinity of the enzyme. The IC50 values of these mutants with respect to each drug were reduced compared to wild type NDM-1. MD simulations and docking results from the mutant protein models, along with the wild type example, showed stable and consistent RMSD, RMSF and Rg behavior. The α-helix content values of all mutant proteins were reduced by 13%, 6%, 14% and 9% compared to NDM-1. This study revealed the impact role of active sites near residues on the enzyme catalytic activity of NDM-1.

5. Farhat N, Ali A, Bonomo, RA and **Khan AU*** "Efflux pump as an intervention to control infection cause by drug resistance bacteria " ***Drug Dis Today (Elsevier)*** 2020, 25(12):2307-2316. doi: 10.1016/j.drudis.2020.09.028. **(IF: 7.85)**

   Highlights: Antibiotic resistance has become a global concern for healthcare workers and physicians. Efflux pumps are one of the major mechanisms of resistance causing major problem of antibiotic resistance. In this article we describe several natural efflux pump inhibitors as intervention to combat antibiotic resistance. It was an invited review article.

6. Ali, A and **Khan AU** "Non-Active site mutation (Q123A) in New Delhi metalo-β lactamase (NDM-1) enhanced its enzyme activity" ***Int J Biological Macromolecule (Elsevier)*** 2018 112:1272-1277 (**IF: 6.95)**

   Highlights: We have initiated to further comprehend structure and function relation of NDM-1 marker by mutating some of its non-active site residues. A laboratory mutant of NDM-1 was generated by PCR-based site-directed mutagenesis, replacing Q to A at 123 position. The MICs of imipenem and meropenem for NDM-1^{Q123A} were found increased by 2 fold as compare to wild type and so the hydrolytic activity was enhanced (Kcat/Km) as compared to NDM-1 wild type. GOLD fitness scores were also found in favour of kinetics data. Secondary structure for α-helical content was determined by Far-UV circular dichroism (CD), which showed significant conformational changes. We conclude a noteworthy role of non-active-site amino acid residues in the catalytic activity of NDM-1. This study also provides an insight of emergence of new variants through natural evolution.

7. Zuberi A, Ahmad N and **Khan AU** " CRISPRi induced suppression of fimbriae gene (fimH) of a Uropathogenic Escherichia coli: a novel therapeutic approach against the battle between microbial biofilms and host immunity"

Front. Immunology 2017, 13;8:1552. doi: 10.3389/fimmu.2017.01552 (IF: **7.5).**

Highlights: Urinary tract infection (UTI) is one the common infections caused by the recalcitrant nature of biofilms, developed after the pathogen has adhered to the inner lining of the urinary tract. Although significant research has been made in recent years to control these types of infection, but as of yet, no approach has sufficiently been able to reduce the prevalence of UTIs. The main objective of this study was to prevent UTIs through targeting the fimH gene, which is the major virulent factor responsible for biofilm formation. The novelty of this work lies in the use of CRISPRi, a gene specific editing tool to control such types of infections. Accordingly, the system was designed to target *fimH* gene, responsible for bacterial adherence and this approach was successfully validated by performing microscopic, biofilm and adherence assays along with transfer of CRISPRi mediated gene editing system among the different species of bacterial population.

8. Khan S, Khan SN, Meena R, Dar AM, Pal R and **Khan AU** "Photoinactivation of multidrug resistant bacteria by monomeric methylene blue conjugated gold nanoparticles" ***Journal of Photochemistry & Photobiology, B (Elsevier) : 2017,*** 174:150-161. **(IF: 6.2)**

   Highlights: In this study we have shown Concanavalin-A (ConA) directed dextran capped gold nanoparticles (GNP_{DEX}-ConA) enhanced the efficacy and selectivity of methylene blue (MB) induced killing of multidrug resistant clinical isolates. Here, we show that our complex MB@GNP_{DEX}-ConA is effective against range of MDR clinical isolates, including Escherichia coli, Klebsiella pneumoniae and Enterobacter cloacae. In our treatment modality negligible dark toxicity suggests photochemically driven process with 97% killing of MDR bacteria. GNP_{DEX}-ConA with monomeric form of MB departs maximum fluorescence decay time (τf: 1.7ns in HSA) and singlet oxygen (ΔΦ; 0.84) for improved activity in albumin rich infection sites. Further, the complex show least toxicity when tested against HEK293 mammalian cells. The principle component analysis (PCA) and confocal microscopy illustrates

cytosolic $^1O_2$ mediated type-II PDT as mechanism of action. Hence, MB@GNP_{DEX}-ConA mediated PDT is potential therapeutic approach against MDR infections and can be tailored to fight other infectious diseases.

9. **Khan AU,** Ali, A, Danishuddin, Srivastava, G and Sharma A " Potential inhibitors designed against NDM-1 type metallo B-lactamases: an attempt to enhance efficacies of antibiotics against multi-drug-resistant bacteria" ***Scientific Report*** 7(1):9207.

   Highlights: NDM-1 and its variants are the most prevalent types of metallo-β-lactamases, hydrolyze almost all antibiotics of β-lactam group leading to multiple-drug resistance in bacteria. No inhibitor has yet been obtained for NDM-1 or other class of metallo-β-lactamases. Therefore, strategies to identify novel anti-β-lactamase agents with specific mechanisms of action are the need of an hour. In this study, we have reported the discovery of novel non-β-lactam inhibitors against NDM-1 by multi-step virtual screening approach. The potential for virtually screened drugs was estimated through in vitro cell assays. Five chemical compounds were finally purchased and evaluated experimentally for their efficacies to inhibit NDM-1 producing bacterial cells, in vitro. The dissociation constants (Kd), association constant (Ka), stoichiometry (n) and binding energies (ΔG) of compounds with the respective targets were determined using isothermal titration calorimetry (ITC). Molecular dynamic simulation carried out for 25 ns revealed that these complexes were stable throughout the simulation with relative RMSD in acceptable range. Moreover, Microbiological and kinetic studies further confirmed high efficacies of these inhibitors by reducing the minimum inhibitory concentration (MIC) and catalysis of antibiotics by β-lactamases in the presence of inhibitors. Therefore, we conclude that these potential inhibitors may be used as lead molecules for future drug candidates.

10. **Khan AU** and Rehman MT “Role of non-active site residue Trp-93 in the function and stability of New Delhi Metallo-β-Lactamase-1 (NDM-1)”

Antimicrobial Agent Chemotherapy (ASM) 2015 Nov 2;60(1):356-60. **(IF: 4.7).**

Highlights: In this study, we investigated the significance of non-active-site residue Trp-93 in the structure and function of NDM-1. We cloned blaNDM-1 from an Enterobacter cloacae clinical strain (EC-15) and introduced the mutation of Trp-93 to Ala (yielding the Trp93Ala mutant) by PCR-based site-directed mutagenesis. Proteins were expressed and purified to homogeneity by affinity chromatography. The MICs of the Trp93Ala mutant were reduced 4- to 8-fold for ampicillin, cefotaxime, ceftazidime, cefoxitin, imipenem, and meropenem. The poor hydrolytic activity of the Trp93Ala mutant was also reflected by its reduced catalytic efficiency. The overall catalytic efficiency of the Trp93Ala mutant was reduced by 40 to 55% (the Km was reduced, while the kcat was similar to that of wild-type NDM-1 [wtNDM-1]). Heat-induced denaturation showed that the ΔGD (o) and Tm of Trp93Ala mutant were reduced by 1.8 kcal/mol and 4.8°C, respectively. Far-UV circular dichroism (CD) analysis showed that the α-helical content of the Trp93Ala mutant was reduced by 2.9%. The decrease in stability and catalytic efficiency of the Trp93Ala mutant was due to the loss of two hydrogen bonds with Ser-63 and Val-73 and hydrophobic interactions with Leu-65, Val-73, Gln-123, and Asp-124. The study provided insight into the role of non-active-site amino acid residues in the hydrolytic mechanism of NDM-1.