Conclusion

The current investigation centered on the study of antibiotic resistance and virulence in *K. pneumoniae*, using a genomics method followed by a phenotypic assessment.

Genomics can be a valuable method for investigating antimicrobial resistance and virulence. It enables the surveillance and epidemiology of AMR and virulence genes, as well as the identification of the most prevalent sequence types, drug-resistance genes, and virulence genes. Additionally, genomics provides a comprehensive understanding of the total gene composition, including novel genes, multiple gene copies, and their genomic locations. This approach also provides a comprehensive understanding of the plasmid contents, prophage contents, and other mobile genetic components, which are essential for studying the dissemination of antimicrobial resistance and pathogenicity.

The research on antimicrobial resistance found that the frequency of extensively drug-resistant (XDR) isolates and the level of resistance among multidrug-resistant and XDR isolates had risen in newly collected isolates in 2020 compared to earlier isolates collected in 2016–17. Based on this work, we can infer that there is a scarcity of *K. pneumoniae* genomes from India, excluding South India. Particularly, there is a notable lack of genomic data from the western and northern regions of India. Therefore, it is imperative to conduct genomic surveillance in order to get a comprehensive understanding of the situation. Upon evaluating the available genomes, it was determined that the ST231 sequence type was the most prevalent in India, followed by ST147, which was subsequently surpassed by ST14. All three of these STs included carbapenemases in their genome, namely *bla*OXA-48-like (*bla*OXA-48, *bla*OXA-181, and *bla*OXA-232), with *bla*OXA-232 being the most prevalent. They also carried *bla*NDM, primarily *bla*NDM-5 and *bla*NDM-1. In addition, some strains from ST147, ST437, and ST2096 were found to have two types of carbapenemases, resulting in an increase in the minimum inhibitory concentration of carbapenem drugs to a level more than 512 ug/ml. This is an immediate cause of concern. Therefore, it is essential to conduct monitoring of these specific strains in order to effectively combat the emergence of antimicrobial resistance. Nevertheless, the targets of interest, including *bla*OXA-232 and *bla*NDM-5, in addition to the ESBL gene *bla*CTX-M-15, have the potential to serve as suitable candidates for diagnosis in *K. pneumoniae*. Consequently, these targets might potentially be used as diagnostic markers focused on India for antimicrobial resistance in *K. pneumoniae*. Further, it is feasible to address the difficulties posed by the antibiotic resistance and virulence of these deadly bugs by harnessing their own genetic material, referred to as prophages. By using lower quantities of Mitomycin C, it is possible to induce the lysogeny of prophages in the bacterial genome. This

process may effectively mitigate the challenges posed by antimicrobial resistance and biofilm-related issues.

In terms of pathogenicity, the most predominant capsular types detected were K51 and K64, while the most predominant O-serotypes detected were O1 and O3/O3a. Furthermore, the majority of the ST231, ST14, and ST147 strains exhibited these combinations, with ST231 (K51 + O1) being the most prevalent. Regrettably, ST231 has developed significant resistance to many drugs, including carbapenems. Additionally, it has several virulence genes, which makes this strain most concerning for India. Unfortunately, the ST231 has been found in the majority of Indian states. In addition, another worrisome strain that was identified was ST23 (K1 + O1), which exhibited the largest number of pathogenic genes in its genomes. Fortunately, the prevalence of this strain was rather low. However, we strongly recommend sequencing a larger number of genomes from various regions throughout India in order to have a more comprehensive understanding of the situation.

However, phenotypic investigations have verified that in half of the examined isolates, the lethal combination of K51 + O1 exhibited serum resistance and was also relatively less vulnerable to phagocytosis. Nevertheless, when considered separately, a significant association between the O1 serotype and serum resistance was seen. This is concerning since the O1 serotype was the most prevalent O-type, making up around 60% of the *K. pneumoniae* genomes detected in India.

Based on the available data, it can be concluded that Indian *K. pneumoniae* genomes exhibit multiple STs and K-types, which makes them challenging to target for therapy. However, the diversity in O-serotypes is relatively low, with O1 alone accounting for 60% of the cases. This percentage increases to 80% when combined with O3/O3a and O2a. Therefore, taking into account all of these factors by specifically targeting these three O-serotypes listed above as vaccine candidates for *K. pneumoniae*, that have the potential to achieve success in preventing many lives from being lost worldwide.

We also attempted to demonstrate if the use of incorrect antibiotics, resulting from a lack of antimicrobial resistance profile for the specific bacterium, might increase siderophore synthesis, a significant component of *K. pneumoniae*'s pathogenicity. Overall, it was seen that the usage of ciprofloxacin against the ciprofloxacin-resistant *K. pneumoniae* strain may significantly enhance the synthesis of siderophores. Overall, it was shown that the use of ciprofloxacin against the ciprofloxacin-resistant *K. pneumoniae* strain significantly enhances the synthesis of siderophores. This may exacerbate the patient's health by facilitating the

proliferation of pathogens that might potentially result in significant tissue damage or even death. Additionally, in this work, we also discovered that limiting the availability of free iron to these bacteria might serve as an alternative treatment. We observed that the use of DIP, an iron chelator, effectively inhibited the development of the bacterial isolates. However, it did lead to a minor rise in siderophore synthesis in some cases.

The rising prevalence of colistin and tigecycline resistance in India is alarming. This is due to the lack of effective methods to identify this resistance, attributed to factors such as the mcr gene and alterations in many genes including *mgrB, phoPQ, pmrAB*, and *ramR* responsible for colistin and tigecycline resistance respectively, which limit treatment efficacy and result in therapeutic delays. To overcome these challenges, the research suggests hybrid sequencing using long read sequencing technologies like PacBio and Nanopore and Illumina to address the issues of gene truncation, gene loss during trimming and assembly preparation. Furthermore, these methods provide the identification of important virulence genes, their genetic composition, plasmids, and spread of AMR and virulence, enabling accurate tracking of gene locations and precise estimation of gene copy numbers. In the context of vaccine development, the O1 antigen of *K. pneumoniae* can be targeted due to its abundance and significant pathogenicity properties found in this study.