DIFFERENTIAL ASSOCIATION OF COLICIN V WITH ANTIBIOTIC EXPOSURE IN Klebsiella pneumoniae REVEALED BY DEEP LEARNING ANALYSIS

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INTRODUCTION: *Klebsiella pneumoniae* is an opportunistic hospital-associated pathogen that causes a wide range of infections. This pathogen is one of these multidrug-resistant organisms identified as an important threat to human health according to the World Health Organization. The specific factors that *K. pneumoniae* employ to escape the immune defenses are not fully elucidated. Presently, there are some characterized virulence factors, such as the capsule. The hypermucoviscous (HM) phenotype, appears to enhance the virulence of hypervirulent *K. pneumoniae* (hvKp) strains and is often considered a substitute marker for improved virulence. Colicins are a group of high molecular weight protein toxins produced primarily by *Escherichia coli*. Colicin V, however, stands out from most colicins due to its genetic

organization and mechanism of action. Its activity involves the formation of pores that cause membrane depolarization, interrupt ATP synthesis, and lead to rapid cell death. More recently, detecting Colicin V associated genes in *Klebsiella pneumoniae* has been linked to hypervirulence.

OBJECTIVE: Our aim was to identify the Colicin V in *K. pneumoniae* strains when exposed to ceftazidime, cefepime, meropenem and gentamicin.

MATERIAL AND METHODS: DNA of 138 *K. pneumoniae* isolates collected during Sars-CoV-2 pandemic were extracted using the QIAamp DNA Mini kit, sequencing was performed using NextSeq (Illumina), and the sequences were analyzed using the CABGen v1.09 online pipeline. Using deep learning models, we analyzed the proteins expressed by the 138 strains under each of the four antibiotics. It was observed a significant drop in attention coefficients for expressed proteins starting from the fifth protein. Therefore, we analysed the five most expressed proteins for each antibiotic.

RESULTS: Surprisingly, Colicin V was not among the top five for cefepime, gentamicin, or meropenem. However, for ceftazidime, Colicin V appeared in the top five 68 times. Ceftazidime resistance was observed in 65.7% of the samples distributed across both resistance and susceptible phenotypes.

DISCUSSION: Given this, we can raise some explanations for this observation, such as 1- the fact that ceftazidime (a 3rd-gen cephalosporin) is a potent inducer of AmpC β-lactamases in Enterobacteriaceae, including *K. pneumoniae*, and Colicin V production is often linked to SOS response activation under β-lactam stress. Cefepime (4th-gen cephalosporin) is more stable against AmpC and may not trigger the same stress response. 2- Ceftazidime disrupts outer membrane proteins (OMPs) like OmpK35/36 in *K. pneumoniae*, potentially activating colicin export systems. Meropenem (a carbapenem) primarily targets PBP2/3 with less OMP disruption, while gentamicin (aminoglycoside) acts via ribosomes, neither of which may trigger colicin regulators.

CONCLUSION: Our findings suggest a potential association between ceftazidime exposure and the induction or selection of Colicin V-related proteins in *K. pneumoniae*. Although the mechanisms underlying this association remain unclear, the data indicate that ceftazidime-induced stress responses may play an important role in regulating Colicin V expression. Further experimental and functional studies are necessary to validate these hypotheses and to better understand the contribution of Colicin V to antimicrobial resistance and hypervirulence in *K. pneumoniae*.

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