Wastewater based surveillance of SARS-CoV-2 Omicron variants circulating in Charlotte, North Carolina

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Introduction

- The World Health Organization (WHO) declared Omicron (B.1.1.529) as SARS-CoV-2 Variant of Concern (VOC) on 26th November 2021[1].
- 1st clinical case caused by the Omicron variant was identified in USA on 1st December 2021. Later it rapidly spread all over the country[2].
- This variants require comparatively shorter incubation time and reproduces 3.19 times faster than the Delta variant[3]
- Because of the structural differences in the spike protein, Omicron variants can evade the naturally or vaccine induced immunity and cause infection.
- Wastewater based SARS-CoV-2 surveillance has already gained popularity as the alternative COVID-19 infection monitoring.
- Wastewater testing can also be useful for tracking SARS-CoV-2 VOCs as it can be representative of a large number of population by a single test.
- Rare mutation detection is always a challenge because of difficulties to differentiate between two highly similar sequences, one of which is significantly more abundant than other.

Objectives

- To detect and quantify Omicron variants from wastewater using digital droplet PCR targeting the mutation in the amino acid spike protein 764 and 856 position (N764K and N856K)[4]
- To determine the transmission dynamics of the Omicron variants by assessing the relative proportion of the strains circulating in Charlotte.

Experimental method

- Wastewater samples were collected from Sugar Creek and Mallard Creek wastewater Treatment Plant (WWTP) on a weekly basis, as well as from UNC Charlotte dormitories on thrice weekly from November 15, 2021, to January 31, 2022.
- 16 samples for the WWTP and 70 samples from UNC Charlotte campus were processed. All those samples were detected SARS-CoV-2 positive with RT-qPCR (N1 gene)

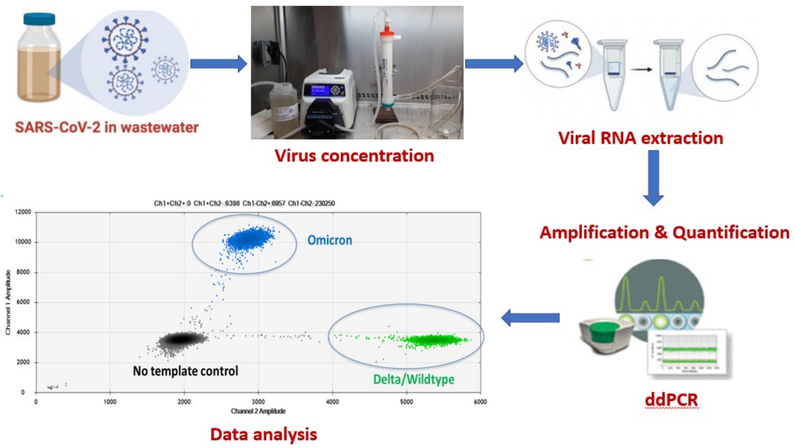

Fig 1: Experimental workflow for detecting variants from wastewater

Results

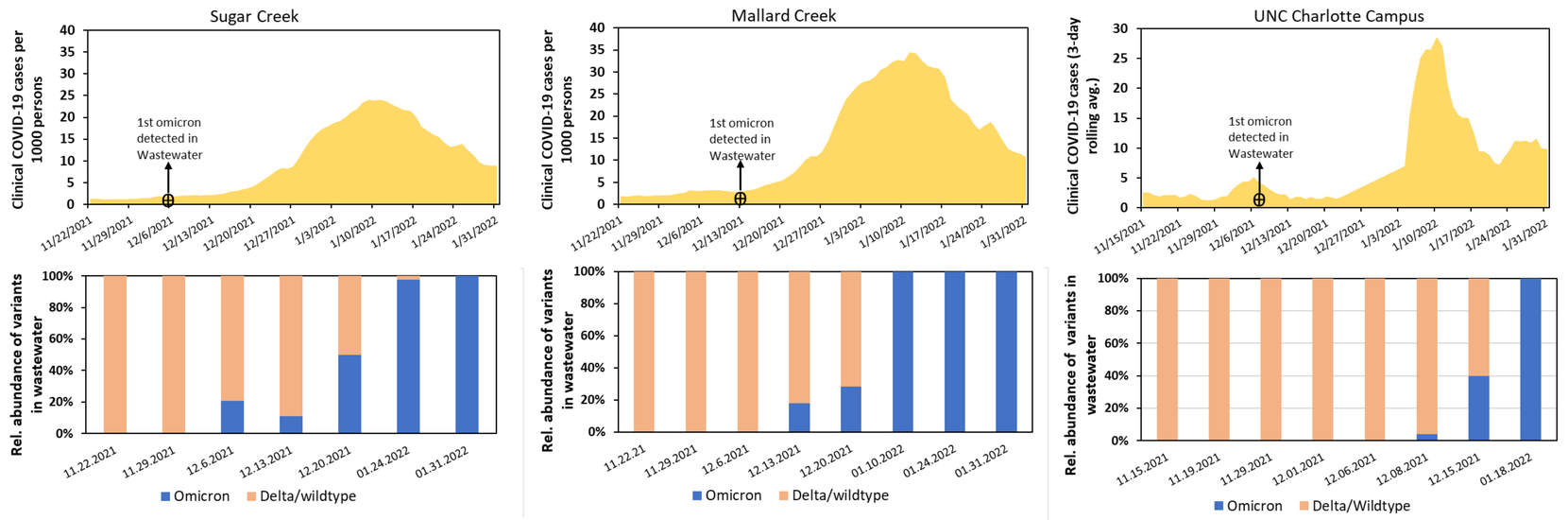

Fig 2: Emergences of the Omicron variants by displacing Delta variants circulating in wastewater that represent Mecklenburg County and UNC Charlotte campus. COVID-19 case counts were adjusted based on the boundary of the sewersheds that belong to a WWTP.

Interpretation

- Both N764K and N856K assay were highly specific to amplifying Omicron and Delta variant from wastewater (Table 1).
- Omicron variants were first detected in Sugar Creek WWTP sample which represent uptown Charlotte and CLT International airport that suggest to a link with national or international travelers (Fig 2).
- Mallard Creek WWTP showed 100% Omicron circulation in the early January
- COVID-19 cases started rising after 1-2 week of the first Omicron variants detected in wastewater corresponding to each areas.

Table1: Determining the assay specificity in discriminating Omicron and Delta

Controls Name	Omicron (Copies/rxn)		Delta (Copies/rxn)	
	N764K	N856K	N764K	N856K
Omicron_Posi-1	1223	1075	Negative	Negative
Omicron_Posi-2	8844	7634	Negative	Negative
Delta_Positive-1	Negative	Negative	9614	8932
Delta_Positive-2	Negative	Negative	1245	1155

Table2: Target sequences and mutation assay characteristics

Assay name	Reference genome	Mutant/Wild type Allele	Amplicon length
N764K[4]	GTGATTCAACTGAATGCAGCAATCTTTTGTTGCAATATGGCAGTTTTTGTACACAATTAAA[C/A]CGTGCTTTAACTGGAATAGCTGTTGAACAAGACAAAAACACCCAAGAAGTTTTTGCACAA	A/C	70
N856K[4]	AATATGGTGATTGCCTTGGTGATATTGCTGCTAGAGACCTCATTTGTGCACAAAAGTTTAA[C/A]GGCCTTACTGTTTTGCCACCTTTGCTCACAGATGAAATGATTGCTCAATACACTTCTGCAC	A/C	60

Conclusions

- Omicron variants were started circulating in the Mecklenburg County wastewater samples from the 2nd week of December 2021.
- Omicron variants were dominant over all other SARS-CoV-2 strains from early January 2022 and are responsible for the 4th wave of COVID-19 cases in the Mecklenburg County.
- This technique can give a nearly real-time transmission dynamic of the Omicron variant which can help the administration to take quick necessary public interventions such as awareness, preparedness, and control measures.
- This technique can be applied for tracking other SARS-CoV-2 VOCs by designing a new assay.

References

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Acknowledgements

Authors are thankful to the UNC Charlotte wastewater surveillance team for the sampling support. Also, Authors acknowledge Nita khanal and Samuel for helping in the RNA extraction process.