TECHNICAL REPORT

SARS-CoV-2 variant mutations conferring reduced susceptibility to antiviral drugs and monoclonal antibodies: a non-systematic literature review for surveillance purposes

July 2023

Summary

Antiviral drugs and monoclonal antibodies (mAbs), administered either separately or as combination therapy 'cocktails', have provided a valuable tool for fighting COVID-19. Surveillance data, coupled with data on antiviral treatment susceptibility, can guide clinical decisions on selecting the best therapy for the patient.

The aim of this report is to provide a non-systematic literature review on the currently available data on SARS-CoV-2 therapeutic mAbs and antiviral drugs authorised for use in the European Union/European Economic Area (EU/EEA). In total, 258 publications on the four approved mAbs and 23 publications on antiviral drugs were analysed. According to these studies, the ORF1ab mutations nsp5:S144A, nsp5:Q189K, nsp5:H172Y, nsp5:E166A, and nsp5:F140A conferred moderate to high reduction in susceptibility to Paxlovid (nirmatrelvir/ritonavir). The data indicated a highly reduced neutralisation capacity of Ronapreve (casirivimab/imdevimab) for all Omicron sub-lineages included in this report. The sub-lineages BA.1, BA.2, and BA.5 showed high reduction in susceptibility to Regkirona (regdanvimab). Xevudy (sotrovimab) showed high neutralisation efficacy against most SARS-CoV-2 variants, but moderate reduction in neutralisation activity for BA.2, BA.4, and BQ.1.1. Highly reduced neutralisation activity against the BQ.1 and BQ.1.1 sub-lineages was observed for Evusheld (tixagevimab/cilgavimab).

Monitoring the resistance of circulating new variants to mAb-based antiviral treatments is important for making decisions on whether some of the developed mAbs should be discontinued or different combinations of mAbs should be used. Neutralising susceptibility data are quite variable, leading to discordant findings among investigations. Therefore, adopting a global external standard for calibration would improve concordance across various tests and results could be provided in global units.

Introduction

As of June 2023, the SARS-CoV-2 pandemic had resulted in more than 766 million confirmed cases of COVID-19 and over 6.9 million deaths globally [1]. During the pandemic, non-pharmaceutical interventions such as stay-at-home measures, contact tracing, and social distancing were implemented at different phases to contain the spread [2-4]. To mitigate the impact of the disease, vaccines, antiviral drugs, and monoclonal antibodies (mAbs) were also developed. The development and licensure of vaccines against COVID-19 became a global effort, pushed forward at an unprecedented speed. The successful implementation of vaccines against COVID-19 less than a year after

Suggested citation: European Centre for Disease Prevention and Control. SARS-CoV-2 variant mutations conferring reduced susceptibility to antiviral drugs and monoclonal antibodies: a non-systematic literature review for surveillance purposes – July 2023. Stockholm: ECDC; 2023.

Stockholm, July 2023

ISBN 978-92-9498-644-3
doi: 10.2900/192733
Catalogue number TQ-04-23-728-EN-N

the beginning of the pandemic significantly contributed to decreasing the burden of COVID-19, both in terms of disease severity and mortality. Medical treatment of COVID-19 is currently mostly supportive, including oxygen therapy for severely ill patients and patients at risk of developing severe disease, and ventilation for critically ill patients. Antiviral drugs and mAbs, administered either separately or as combination therapy 'cocktails', have been used as another tool for fighting COVID-19. Their administration should be considered in consultation with clinical specialists and based on clinical guidelines for the treatment of adults and adolescents at risk of developing severe disease. For example, they can be administered to moderately to severely immunocompromised patients who may have an inadequate immune response to COVID-19 vaccination.

Antiviral drugs are used to disrupt the viral life cycle by preventing virus attachment to host cells, blocking proteolytic cleavage of the viral Spike (S) protein and suppressing or inhibiting viral replication [5]. Antiviral drugs currently authorised for use by the European Medicines Agency (EMA) include Paxlovid (PF-07321332/ritonavir) and Veklury (remdesivir). Paxlovid is a SARS-CoV-2 protease inhibitor containing nirmatrelvir (NTV), a main protease (Mpro) inhibitor specifically designed to block the activity of the SARS-CoV-2 Mpro enzyme and therewith inhibiting viral replication. It also contains low-dose ritonavir (RTV), a HIV-1 protease inhibitor and a strong cytochrome P450 (CYP) 3A inhibitor, acting as pharmacokinetic booster of NTV [6]. Remdesivir is a nucleoside analogue that inhibits RNA-dependent RNA polymerase (RdRp) with a broad-spectrum activity against multiple RNA viruses interrupting viral replication [7]. EMA has not recommended the marketing authorisation for Lagevrio (Molnupiravir) as its human medicines committee (CHMP) concluded that the clinical benefit of Lagevrio in the treatment of adults could not be demonstrated [8].

Virus-targeting monoclonal antibodies are designed to decrease the severity of COVID-19 by binding to the SARS-CoV-2 S protein and causing direct neutralisation, antibody-dependent cellular phagocytosis, antibody-dependent cell-mediated cytotoxicity and/or complement activation [9]. Monoclonal antibodies which are currently authorised for use by EMA include Evusheld (tixagevimab/cilgavimab), Regkirona (regdanvimab), Ronapreve (casirivimab/imdevimab) and Xevudy (sotrovimab). The emergence of new SARS-CoV-2 variants with specific amino acid substitutions that make the virus less sensitive to the existing therapies has put the efficacy of mAbs in question [10]. For instance, the observed reduced efficacy of Evusheld (tixagevimab/cilgavimab) against emerging resistant variants have limited its use [11].

In the first quarter of 2022, there were a series of outbreaks globally related to the emergence of the Omicron lineage, which included, among others, BA.4, BA.5, BA2.2.75 and XBB.1.5 [12,13]. Unfortunately, all the variants of concern (VOCs) mentioned above have demonstrated reduced sensitivity to neutralisation with existing mAb therapies *in vitro* [14,15].

The risk of developing drug resistance is increased for immunocompromised patients, who are prone to prolonged infection due to their reduced ability to control virus replication. Prolonged use of antiviral treatment may lead to the emergence of SARS-CoV-2 variants with reduced susceptibility to drugs and may, in certain circumstances, result in viral resistance being the cause of failure of clinical treatment.

Surveillance data, coupled with data on antiviral treatment susceptibility, can guide clinical decisions on selecting the best therapy for the patient. In addition, the use of appropriate antiviral therapy can reduce the development of drug resistance. Continuous monitoring of antiviral drug resistance of the different emerging SARS-CoV-2 variants is therefore important.

Numerous phenotypic and genotypic laboratory techniques have been developed to assess virus neutralisation capacities and susceptibility to antiviral drugs [16]. Examples include the plaque/focus reduction neutralisation tests (PRNT/FRNT), microneutralisation assay (MNA), and pseudovirus (PSV) neutralisation assays [17,18]. PRNT/FRNT or TCID50 (Median Tissue Culture Infectious Dose)-based and MNA assays typically use replication-competent SARS-CoV-2 clinical isolates. While these assays permit assessment against the fully antigenic content and molecular complexity of VOCs, the disadvantage of these assays is the requirement of a biosafety level (BSL)-3 laboratory and the associated labour-intensive requirements. PRNT have shown to be more sensitive compared to other approaches [19], while FRNT and MNA offer faster turn-around times than PRNT. Antiviral and therapeutic resistance can be assessed with Inhibitory Concentration (IC)50 assays, expressed as the concentration of drug that is required to inhibit the viral growth by 50%, also called 'half maximal inhibitory concentration'. Genetic characterisation using Whole Genome Sequencing (WGS) allows for screening and identification of mutations in key areas of the viral genome that confer reduced susceptibility to antiviral drugs or mAbs.

Below, we provide an overview of the currently available data on SARS-CoV-2 therapeutic monoclonal antibodies and antiviral drugs authorised for use in the EU/EEA.

Methods

We compiled an in-house database using several different data sources: the Stanford University coronavirus antiviral & resistance database [20], COG-UK mutation explorer [21], journal publications, European Medicines Agency (EMA) summaries of product characteristics and United States Food and Drug Administration (FDA) emergency use authorisation fact sheets. Publications included in the database were obtained and reviewed monthly from four literature sources: PubMed using the search terms 'SARS-CoV-2 combined with monoclonal antibodies/antiviral drugs', Science Direct using the search terms 'SARS-CoV-2 combined with monoclonal antibodies/antiviral drugs', LitCovid

using the search terms 'SARS-CoV-2 combined with monoclonal antibodies/antiviral drugs' and BioRxiv/MedRxiv COVID-19 SARS-CoV-2 preprint servers. According to the inclusion criteria, only studies on antiviral drugs and monoclonal antibodies approved by the EMA were considered. The collection of data began in April 2022 and was continued until 1 December 2022. The database was updated regularly, and new virus variants and results were included. The following data were extracted: the assays used for antiviral resistance testing, the IC50 value of the used control, fold-reductions in neutralisation titre associated with variants and mutations and corresponding IC50 values for the quantification of the observed reduction. Details on mutations in the open reading frame 1ab (ORF1ab) and spike genes were collected from in vitro and in vivo studies, as well as VOCs' data for which the collection of spike and genomic mutations was known. Neutralisation data from replication-competent SARS-CoV-2 isolates and non-replication-competent pseudotyped viruses using VSV (vesicular stomatitis virus), HIV (human immunodeficiency virus type 1), MLV (murine leukaemia virus), and a lentivirus containing a SARS-CoV-2 S protein with specific mutations were also included in the database.

Results

We analysed 258 publications on the four approved mAbs and 23 results on antiviral drugs (Table 1). These publications covered data on 28 individual S protein mutations and 25 ORF1ab protein mutations. They also described the effect of 6 combinations of S protein mutations associated with reduced susceptibility to mAbs and antiviral drugs (Annex 1). These combinations generally consisted of sets of two, three, 15, 16, or 20 mutations that were present within the genome of a VOC. Two thirds of the results for mAbs were obtained using pseudotype neutralisation assays, while one third was obtained using assays with replication-competent SARS-CoV-2 isolates.

Table 1. Antiviral drugs and monoclonal antibodies approved by EMA and number of publications included in the database

Antiviral drugs	Number of publications	Monoclonal antibodies	Number of publications
Paxlovid (PF-07321332/ritonavir)	10	Evusheld (tixagevimab/cilgavimab)	71
Veklury (remdesivir)	13	Regkirona (regdanvimab)	34
		Ronapreve (casirivimab/imdevimab)	65
		Xevudy (sotrovimab)	88

The results in the data showed that the mutations E337/K/R/L and E340K/V/I/A occurring in the Delta variant conferred ≥100-fold reduction in susceptibility to Xevudy (sotrovimab). However, their global and EU/EEA frequency was quite low (<0.01%). The combination of mutations K417N+E484K+N501Y in the Beta and Gamma variants demonstrated a highly and moderately reduced neutralisation efficacy for Regkirona (regdanvimab) (≥100-fold and 25-99-fold), respectively. These mutations had a high global and EU/EEA frequency when occurring in Beta (79.5 and 83%, respectively). The mutations K417N+L452R+T478K present in the Delta variant conferred 25–99-fold reduction in susceptibility for Regkirona (regdanvimab). The set of mutations (G339D+S371L+S373P+S375F+K417N+ N440K+G446S+S477N+T478K+E484A+Q493R+G496S +Q489R+N501Y+Y505H) present in BA.1, BA.4, and BA.5 led to different fold-reductions in susceptibility to mAbs. When occurring in a BA.1 virus sub-lineage, these mutations conferred ≥100-fold reduction in susceptibility to Ronapreve, Sotrovimab, and Evusheld, with an EU/EEA frequency of 50%. On the other hand, the same set of mutations present in the virus sub-lineages BA.4 and BA.5 have led to moderately reduced neutralisation efficacy for Evusheld (tixagevimab/cilgavimab) (Table 2).

Table 2. Mutations and combinations of mutations in the S protein and their corresponding reductions in susceptibility to mAbs

Mutation (S protein)	Variant	mAbs				Global frequency**	EU/EEA frequency**
		Ronapreve (casirivimab/ imdevimab)	Xevudy (sotrovimab)	Evusheld (tixagevimab/ cilgavimab)	Regkirona (regdanvimab)		
P337K	Delta		304			0.00	0.00
P337R	Delta		192			0.00	0.00
P337L	Delta		192			0.01	0.01
P337T	Delta		10.62			0.00	0.00
P337H	Delta		5.13			0.00	0.00
G339D	BA.1	1.8	2			93.04	91.01
E340K	Delta		297			0.01	0.01
E340I	Delta		190			0.00	0.00
E340V	Delta		200			0.00	0.00
E340A	Delta		100			0.00	0.00
E340Q	Delta		50			0.00	0.00
E340G	Delta		18.2			0.00	0.00
S371L	BA.1	2.8	90.1			85.50	81.03
S373P	BA.1	3	4.9			86.22	83.08
S375F	BA.1	0.8	1.4			86.15	83.75
K417N	Beta	2.5				93.24	91.28
K417N	BA.1	2.5				66.26	72.04
N440K	BA.1	2.2	1.7			69.14	76.79
G446S	BA.1	3.3				70.44	78.85
L452R	Delta	5.4				96.53	97.35
S477N	BA.1		1.6			88.27	84.95
T478K	Delta	1.9				96.67	97.74
T478K	BA.1		2.3			88.47	85.26
E484A	BA.1	4.3	0.8			88.26	84.02
E484K	Beta	1.8				85.80	91.81
E484K	Gamma	1.8			8.7	94.62	96.40
Q493R	BA.1		1.3			88.37	82.35
G496S	BA.1	1.5	2.9			85.78	80.97
Q498R	BA.1		0.8			85.56	80.03
N501Y	Alpha	<2		5		97.34	98.29
N501Y	Beta		1.4			86.74	92.16
N501Y	Gamma		1.4		5.5	94.85	96.46
N501Y	BA.1		1.4			86.07	81.91
Y505H	BA.1		1.2			85.99	83.89
N501Y+P681H	Alpha				<5	96.62	97.28
K417N+E484K+N501Y	Beta	<2		<5	102	79.46	83.03
K417T+E484K+N501Y	Gamma	<2		<5	99.6	0.00	0.01
L452R+T478K	Delta	<2		<5		95.95	96.81
K417N+L452R+T478K	Delta				105.3	0.17	0.04
G339D+S371L+S373P +S375F+K417N+N440 K+G446S+S477N+T47 8K+E484A+Q493R+G 496S+ Q489R+N501Y+ Y505H	BA.1	1013	106.5	106		51.40	47.98
G339D+S371L+S373P +S375F+K417N+N440 K+G446S+S477N+T47 8K+E484A+Q493R+G 496S+ Q489R+N501Y+ Y505H	BA.2			5.4		0.07	0.01
G339D+S371L+S373P +S375F+K417N+N440 K+G446S+S477N+T47 8K+E484A+Q493R+G 496S+ Q489R+N501Y+ Y505H	BA.4			65		0.00	0.00
G339D+S371L+S373P +S375F+K417N+N440 K+G446S+S477N+T47 8K+E484A+Q493R+G 496S+	BA.5			40.5		0.00	0.00

Mutation (S protein)	Variant	mAbs				Global frequency**	EU/EEA frequency**
		Ronapreve (casirivimab/ imdevimab)	Xevudy (sotrovimab)	Evusheld (tixagevimab/ cilgavimab)	Regkirona (regdanvimab)		
Q489R+N501Y+ Y505H							
G339D+S371L+S373P +S375F+K417N+N440 K+G446S+S477N+T47 8K+E484A+Q493R+G 496S+ Q489R+N501Y+ Y505H	BA.2.75			15		0.00	0.00
G339D+S371F+S373P +S375F+ T376A+D405N+ R408S+K417N+N440K + S477N+ T478K+E484A+ Q493R+ Q498R+ N501Y+Y505H+H655Y + N679K+ P681H+ N764K	BA.2			<5		68.68	64.15

**A red cell indicates ≥100-fold reduction in susceptibility; an orange cell indicates a 25-99-fold reduction in susceptibility; a yellow cell indicates 5-25-fold reduction in susceptibility and a green cell indicates <5 -fold reduction in susceptibility. An empty cell indicates no neutralisation data.*
*** Global and EU/EEA frequency among sequenced isolates deployed to GISAID.*

The mutations found in ORF1ab, namely nsp5:S144A, nsp5:Q189K, nsp5:H172Y, nsp5:E166A, and nsp5:F140A conferred moderate to high reduction in susceptibility to Paxlovid (nirmatrelvir/ritonavir), while nsp12:S861G conferred 25–99-fold reduction in susceptibility to Veklury (remdesivir) (Table 3).

Table 4 summarises the neutralisation efficacy of the four EMA approved mAbs against 11 SARS-CoV-2 variants. The median fold-reduction values extracted from the data indicated a highly reduced neutralisation capacity of Ronapreve (casirivimab/imdevimab) for all Omicron sub-lineages, especially for BA.1 and BA.2.75 (>1000 fold-reduction). Similar results have been obtained for Regkirona (regdanvimab), to which the sub-lineages BA.1, BA.2, and BA.5 showed high reduction in susceptibility (>1 000-fold reduction). The extracted results have shown high neutralisation efficacy of Xevudy (sotrovimab) against most SARS-CoV-2 sub-lineages, but moderate reduction in neutralisation activity was noticed for BA.2, BA.4, and BQ.1.1. Evusheld (tixagevimab/cilgavimab) has been shown to have highly reduced neutralisation activity against the BQ.1 and BQ.1.1 sub-lineages (median value fold-reduction of 476).

Table 3. Mutations in the ORF1ab protein and their corresponding reductions in susceptibility to antiviral drugs

Mutation (ORF1ab protein)	Antiviral drugs		Global frequency**	EU/EEA frequency**
	Veklury (remdesivir)	Paxlovid (Nirmatrelvir/ritonavir)		
nsp5:G15S		4.4	0.2%	0.13%
nsp5:Y54A		23.6	0.0%	0.0%
nsp5:T135S		3.5	0.0%	0.0%
nsp5:F140A		39	0.0%	0.0%
nsp5:S144A		91.9	0.0%	0.0%
nsp5:H164N		6.4	0.03%	0.0%
nsp5:E166A		33.4	0.0%	0.0%
nsp12:V166A	10.4		0.0%	0.0%
nsp5:H172Y		233	0.0%	0.0%
nsp5:Q189K		65.4	0.0%	0.0%
nsp12:N198S	10.4		0.01%	0.01%
nsp5:D248E		3.7	0.01%	0.0%
nsp12:F480L	3.8		0.0%	0.0%
nsp12:D484Y	3.1		0.0%	0.0%
nsp12:V557L	5.7		0.0%	0.0%
nsp12:V792I	8		0.0%	0.0%
nsp12:E796G	2.6		0.0%	0.0%
nsp12:C799F	11.5		0.0%	0.0%
nsp12:C799R	2.7		0.0%	0.0%
nsp12:E802D	7.3		0.0%	0.0%
nsp12:E802A	3.9		0.0%	0.0%
nsp12:S861G	29.7		0.0%	0.0%
nsp12:S861A	2.8		0.0%	0.0%

**A red cell indicates ≥100-fold reduction in susceptibility; an orange cell indicates a 25–99-fold reduction in susceptibility; a yellow cell indicates 5–25-fold reduction in susceptibility and a green cell indicates <5-fold reduction in susceptibility. An empty cell indicates no neutralisation data.*

*** Global and EU/EEA frequency among sequenced isolates deployed to GISAID.*

Table 4. Median fold-reduction values for neutralisation susceptibility of four mAbs to 11 variants and sub-lineages

	Ronapreve (casirivimab / imdevimab)	Xevudy (sotrovimab)	Evusheld (tixagevimab / cilgavimab)	Regkirona (regdanvimab)
Alpha	0.9	1.8	0.8	1.2
Beta	1.6	1	1.7	39
Gamma	0.85	1.35	0.75	99.5
Delta	2.2	1.5	1.05	8.6
BA.1	>1 000	4.8	81	>1 000
BA.2	164	29	7.75	>1 000
BA.4	135	27	6	
BA.5	432	19	20	>1 000
BA.2.75	>1 000	19	19.5	25
BQ.1	100	14	476	
BQ.1.1	100	41	476	

A red cell indicates ≥100-fold reduction in susceptibility; an orange cell indicates a 25–99-fold reduction in susceptibility; a yellow cell indicates 5–25-fold reduction in susceptibility and a green cell indicates <5-fold reduction in susceptibility. An empty cell indicates no neutralisation data.

Discussion

SARS-CoV-2 has infected millions of people worldwide since the beginning of the pandemic, and new variants are likely to continue to appear and have an impact on the regional and global epidemiological situation. Newly emerging variants, some of which have altered antigenic characteristics, may develop resistance to existing antiviral drugs and/or mAbs and may evade natural or vaccine-induced immunity. This could have clinical and public health implications, so efforts to detect new emerging virus variants and to develop effective drugs and treatment early should continue. Monitoring viral load and antiviral susceptibility, especially in immunocompromised patients, will continue to be important in order to quickly identify, assess, and counteract emerging resistance to available antiviral and/or therapeutic treatments. In particular, specimens from immunocompromised patients under treatment should be selected for antigenic characterisation, given the higher probability of acquiring mutations associated with resistance and antigenic drift due to prolonged viral shedding. The 'fitness' and the clinical and public health importance of any emerging virus variant should be swiftly evaluated for its resistance pattern using established methods and techniques.

The mutations S144A, Q189K, H172Y, E166A, and F140A in ORF1ab lead to partial or complete resistance to Paxlovid (nirmatrelvir/ritonavir), causing concerns regarding the decreased effectiveness of this drug against SARS-CoV-2 [22]. Ronapreve (casirivimab/imdevimab) showed complete loss of activity against all Omicron sub-lineages in vitro [23-25], while it effectively neutralised Alpha, Beta, and Delta variants [23,26,27]. Clinical studies have demonstrated statistically significant risk reduction (81%) in the development of symptomatic SARS-CoV-2 infections with casirivimab and imdevimab treatment versus placebo [28,29]. Regkirona (regdanvimab) showed highly reduced neutralising activity against Omicron sub-lineages BA.1, BA.2, and BA.5, leading to complete resistance in in vitro conditions [30]. Clinical studies conducted before the emergence of the Omicron variant have shown that Regkirona (regdanvimab) significantly reduced the proportion of patients progressing to severe/critical COVID-19 condition and that it shortened the time to clinical recovery [31,32]. Xevudy (sotrovimab) maintained complete neutralising activity against most SARS-CoV-2 variants. However, it demonstrated partial loss of activity against Omicron sub-lineages BA.2, BA.4, and BQ.1.1 in cell culture neutralisation assays but not in hamsters [33,34]. This discrepancy needs further investigation and may require the development of alternative methods to those currently available. Evusheld (tixagevimab/cilgavimab) showed highly reduced neutralising activity against the BQ.1 and BQ.1.1 sub-lineages. The United States Food and Drug Administration (FDA) issued a statement of a potential risk of treatment failure due to the development of viral variants that are resistant to Evusheld [11]. Virus-like particles (VLPs) pseudotyped with the S protein of Omicron sub-lineages BQ.1 or BQ.1.1 showed >2 000-fold reductions in neutralising activity [11]. However, it is not known how neutralisation data with pseudotyped VLPs or virus isolates correlate with clinical outcome in patients. Collection of clinical data (e.g. comorbidities, disease duration, clinical outcome) for patients under treatment, for which further genetic and/or antigenic virus characterisation is conducted, will enable the identification of amino acid substitutions associated with laboratory resistance that are clinically relevant.

Monitoring the resistance of circulating new variants to mAb-based antiviral treatments is important for making decisions on whether some of the developed mAbs should be discontinued or different combinations of mAbs should be used. Neutralising susceptibility data appeared quite variable, leading to discordant findings among investigations [35,36]. This variability comes from the fact that there are various neutralising assays, including those carried out in cell culture using clinical isolates, chimeric, recombinant, and pseudotyped viruses. There may be the need to develop new tools for drug and therapeutic assessment if traditional assays do not have the scope to evaluate changes that are outside of the spike region and/or require Fc mediated or cellular immunity elements to be present for full therapeutic effect to be modelled. Due to variations in the quantity of the virus inoculum and the cells used for culture, results for the same specimen against a given virus variant might vary even among laboratories employing the same type of assay [35,36]. It is anticipated that reproducibility between studies will increase as neutralising assays become more standardised, and as external controls like those offered by the WHO [37] are utilised more frequently.

The available information on mAbs and antivirals neutralising efficacies against SARS-CoV-2 is constantly changing [38,39]. As a result, the data will need to be updated frequently when new assays and results are published, as well as when new variants emerge. Genotypic and phenotypic testing need to be implemented in the laboratories to monitor the emergence and spread of resistant virus strains. Neutralisation assays can be used to examine the neutralising capacity of therapeutic antibodies, while WGS data can be used in combination to assess the association of the different amino acid substitutions with resistance [16]. Adopting a global external standard for calibration would improve concordance across various tests and results would be provided in global units rather than IC50 values (for mAbs) or plasma dilution values [37,40]. Following the example of the existing influenza antiviral resistance surveillance system, establishment of a global surveillance system, criteria for evaluation of antiviral susceptibility and collection of clinical data to correlate with in vitro data for COVID-19 therapeutics could be considered.

Contributors

Contributing ECDC experts (alphabetical order): Eeva Broberg, Annette Kraus, Angeliki Melidou, Priyanka Nannapaneni, Maja Vukovikj.

Acknowledgements: Simon Funnell, UK Health Security Agency, United Kingdom.

References

1. World Health Organization (WHO) Coronavirus (COVID-19) Dashboard. Available at: https://covid19.who.int
2. Drożdżal S, Rosik J, Lechowicz K, Machaj F, Szostak B, Przybyciński J, et al. An update on drugs with therapeutic potential for SARS-CoV-2 (COVID-19) treatment. Drug Resistance Updates. 2021;59:100794.
3. Zhou L, Ayeh SK, Chidambaram V, Karakousis PC. Modes of transmission of SARS-CoV-2 and evidence for preventive behavioral interventions. BMC Infectious Diseases. 2021;21(1):1-9.
4. Uddin M, Mustafa F, Rizvi TA, Loney T, Al Suwaidi H, Al-Marzouqi AHH, et al. SARS-CoV-2/COVID-19: viral genomics, epidemiology, vaccines, and therapeutic interventions. Viruses. 2020;12(5):526.
5. Zhao L, Li S, Zhong W. Mechanism of Action of Small-Molecule Agents in Ongoing Clinical Trials for SARS-CoV-2: A Review. Frontiers in Pharmacology. 2022;13
6. Hashemian SMR, Sheida A, Taghizadieh M, Memar MY, Hamblin MR, Bannazadeh Baghi H, et al. Paxlovid (Nirmatrelvir/Ritonavir): A new approach to Covid-19 therapy? Biomed Pharmacother. 2023 Jun;162:114367.
7. Malin JJ, Suárez I, Priesner V, Fätkenheuer G, Rybniker J. Remdesivir against COVID-19 and Other Viral Diseases. Clinical Microbiology Reviews. 2020;34(1):e00162-20. Available at: https://journals.asm.org/doi/abs/10.1128/CMR.00162-20
8. European Medicines Agency (EMA). Refusal of the marketing authorisation for Lagevrio (molnupiravir). 2023 Available at: https://www.ema.europa.eu/en/documents/smop-initial/questions-answers-refusal-marketing-authorisation-lagevrio-molnupiravir_en.pdf
9. Taylor PC, Adams AC, Hufford MM, De La Torre I, Winthrop K, Gottlieb RL. Neutralizing monoclonal antibodies for treatment of COVID-19. Nature Reviews Immunology. 2021;21(6):382-93.
10. Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, et al. SARS-CoV-2 variants, spike mutations and immune escape. Nature Reviews Microbiology. 2021;19(7):409-24.
11. Food and Drug Administration (FDA). Fact Sheet for Healthcare Providers: Emergency Use Authorization for Evusheld™ (tixagevimab co-packaged with cilgavimab) 2023 Available at: https://www.fda.gov/media/154701/download
12. Tegally H, Moir M, Everatt J, Giovanetti M, Scheepers C, Wilkinson E, et al. Emergence of SARS-CoV-2 omicron lineages BA. 4 and BA. 5 in South Africa. Nature Medicine. 2022;28(9):1785-90.
13. Cao Y, Yisimayi A, Jian F, Song W, Xiao T, Wang L, et al. BA. 2.12. 1, BA. 4 and BA. 5 escape antibodies elicited by Omicron infection. Nature. 2022;608(7923):593-602.
14. Yamasoba D, Kosugi Y, Kimura I, Fujita S, Uriu K, Ito J, et al. Neutralisation sensitivity of SARS-CoV-2 omicron subvariants to therapeutic monoclonal antibodies. The Lancet Infectious Diseases. 2022
15. Mlcochova P, Kemp SA, Dhar MS, Papa G, Meng B, Ferreira IA, et al. SARS-CoV-2 B. 1.617. 2 Delta variant replication and immune evasion. Nature. 2021;599(7883):114-9.
16. European Centre for Disease Prevention and Control (ECDC). Technical guidance for antigenic SARS-CoV-2 monitoring. 2022 Available at: https://www.ecdc.europa.eu/sites/default/files/documents/Antigenic-SARS-CoV-2-monitoring-Joint-ECDC-WHO-report-June-2022.pdf
17. Perera R, Ko R, Tsang OTY, Hui DSC, Kwan MYM, Brackman CJ, et al. Evaluation of a SARS-CoV-2 Surrogate Virus Neutralization Test for Detection of Antibody in Human, Canine, Cat, and Hamster Sera. Journal of Clinical Microbiology. 2021 Jan 21;59(2)
18. Bewley KR, Coombes NS, Gagnon L, McInroy L, Baker N, Shaik I, et al. Quantification of SARS-CoV-2 neutralizing antibody by wild-type plaque reduction neutralization, microneutralization and pseudotyped virus neutralization assays. Nature Protocols. 2021 Jun;16(6):3114-40.
19. Perera RA, Mok CK, Tsang OT, Lv H, Ko RL, Wu NC, et al. Serological assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), March 2020. Eurosurveillance. 2020;25(16):2000421. Available at: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.16.2000421
20. Tzou PL, Tao K, Pond SLK, Shafer RW. Coronavirus Resistance Database (CoV-RDB): SARS-CoV-2 susceptibility to monoclonal antibodies, convalescent plasma, and plasma from vaccinated persons. PLOS ONE. 2022;17(3):e0261045. Available at: https://doi.org/10.1371/journal.pone.0261045
21. Wright DW, Harvey WT, Hughes J, Cox M, Peacock TP, Colquhoun R, et al. Tracking SARS-CoV-2 mutations and variants through the COG-UK-Mutation Explorer. Virus Evolution. 2022;8(1) Available at: https://doi.org/10.1093/ve/veac023
22. United States Food and Drug Administration (FDA) Fact Sheet for Healthcare Providers: Emergency Use Authorization for Paxlovidt. Rockville: FDA; 2023. Available at: https://www.fda.gov/media/155050/download

23. Takashita E, Kinoshita N, Yamayoshi S, Sakai-Tagawa Y, Fujisaki S, Ito M, et al. Efficacy of Antibodies and Antiviral Drugs against Covid-19 Omicron Variant. New England Journal of Medicine. 2022;386(10):995-8. Available at: https://www.nejm.org/doi/full/10.1056/NEJMc2119407
24. Meng B, Abdullahi A, Ferreira IATM, Goonawardane N, Saito A, Kimura I, et al. Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity. Nature. 2022/03/01;603(7902):706-14. Available at: https://doi.org/10.1038/s41586-022-04474-x
25. Fenwick C, Turelli P, Ni D, Perez L, Lau K, Lana E, et al. SARS-CoV-2 Omicron potently neutralized by a novel antibody with unique Spike binding properties. bioRxiv. 2022:2022.03.18.484873. Available at: https://www.biorxiv.org/content/biorxiv/early/2022/03/18/2022.03.18.484873.full.pdf
26. United States Food and Drug Administration (FDA). Fact Sheet for Health Care Providers Emergency Use Authorization (Eua) of REGEN-COV® (casirivimab and imdevimab). Rockville: FDA: 2021. Available at: https://www.fda.gov/media/145611/download
27. Boschi C, Colson P, Bancod A, Moal V, La Scola B. Omicron Variant Escapes Therapeutic Monoclonal Antibodies (mAbs) Including Recently Released Evusheld®, Contrary to 8 Prior Main Variant of Concern (VOC). Clinical Infectious Diseases. 2022;75(1):e534-e5. Available at: https://doi.org/10.1093/cid/ciac143
28. Herman GA, O'Brien MP, Forleo-Neto E, Sarkar N, Isa F, Hou P, et al. Efficacy and safety of a single dose of casirivimab and imdevimab for the prevention of COVID-19 over an 8-month period: a randomised, double-blind, placebo-controlled trial. The Lancet Infectious diseases. Oct;22(10):1444-54.
29. European Medicines Agency (EMA). Ronapreve Summary Of Product Characteristics. 2022 Available at: https://www.ema.europa.eu/en/documents/product-information/ronapreve-epar-product-information_en.pdf
30. VanBlargan LA, Errico JM, Halfmann PJ, Zost SJ, Crowe JE, Purcell LA, et al. An infectious SARS-CoV-2 B.1.1.529 Omicron virus escapes neutralization by therapeutic monoclonal antibodies. Nature Medicine. 2022 2022/03/01;28(3):490-5. Available at: https://doi.org/10.1038/s41591-021-01678-y
31. European Medicines Agency (EMA). Regkirona Summary Of Product Characteristics. 2022 Available at: https://www.ema.europa.eu/en/documents/product-information/regkirona-epar-product-information_en.pdf
32. Lee S, Lee SO, Lee JE, Kim K-H, Lee SH, Hwang S, et al. Regdanvimab in patients with mild-to-moderate SARS-CoV-2 infection: A propensity score–matched retrospective cohort study. International Immunopharmacology. 2022 2022/05/01/;106:108570. Available at: https://www.sciencedirect.com/science/article/pii/S1567576922000546
33. Takashita E, Yamayoshi S, Simon V, van Bakel H, Sordillo EM, Pekosz A, et al. Efficacy of Antibodies and Antiviral Drugs against Omicron BA.2.12.1, BA.4, and BA.5 Subvariants. New England Journal of Medicine. 2022;387(5):468-70. Available at: https://www.nejm.org/doi/full/10.1056/NEJMc2207519
34. Driouich JS, Bernadin O, Touret F, de Lamballerie X, Nougairède A. Activity of Sotrovimab against BQ.1.1 and XBB.1 Omicron sublineages in a hamster model. Antiviral Res. 2023 Jul;215:105638.
35. Huang Y, Borisov O, Kee JJ, Carpp LN, Wrin T, Cai S, et al. Calibration of two validated SARS-CoV-2 pseudovirus neutralization assays for COVID-19 vaccine evaluation. Scientific Reports. 2021;11(1):1-13.
36. Sholukh AM, Fiore-Gartland A, Ford ES, Miner MD, Hou YJ, Tse LV, et al. Evaluation of cell-based and surrogate SARS-CoV-2 neutralization assays. Journal of Clinical Microbiology. 2021;59(10):e00527-21.
37. Kristiansen PA, Page M, Bernasconi V, Mattiuzzo G, Dull P, Makar K, et al. WHO International Standard for anti-SARS-CoV-2 immunoglobulin. The Lancet. 2021;397(10282):1347-8.
38. Cromer D, Steain M, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralising antibody titres as predictors of protection against SARS-CoV-2 variants and the impact of boosting: a meta-analysis. The Lancet Microbe. 2022;3(1):e52-e61.
39. Earle KA, Ambrosino DM, Fiore-Gartland A, Goldblatt D, Gilbert PB, Siber GR, et al. Evidence for antibody as a protective correlate for COVID-19 vaccines. Vaccine. 2021;39(32):4423-8.
40. Kung Y-A, Huang C-G, Huang S-Y, Liu K-T, Huang P-N, Yu K-Y, et al. Antibody titers measured by commercial assays are correlated with neutralizing antibody titers calibrated by international standards. medRxiv. 2021 [preprint]. Available at: https://www.medrxiv.org/content/10.1101/2021.07.16.21260618v1

Annex 1

Individual and combination of SARS-CoV-2 spike mutations for which data were available.

Gene	Mutation	Number of publications
ORF1ab	G15S	1
ORF1ab	Y54A	1
ORF1ab	T135S	1
ORF1ab	F140A	1
ORF1ab	S144A	1
ORF1ab	H164N	1
ORF1ab	E166A	1
ORF1ab	V166A	1
ORF1ab	H172Y	1
ORF1ab	Q189K	1
ORF1ab	N198S	1
ORF1ab	D248E	1
ORF1ab	F480L	2
ORF1ab	D484Y	2
ORF1ab	V557L	2
ORF1ab	S759A	1
ORF1ab	V792I	1
ORF1ab	E796G	1
ORF1ab	C799F	1
ORF1ab	C799R	1
ORF1ab	E802A	2
ORF1ab	E802D	2
ORF1ab	S861A	1
ORF1ab	S861G	1
S	P337H	1
S	P337K	1
S	P337L	1
S	P337R	1
S	P337T	1
S	G339D	2
S	E340Q	1
S	E340V	1
S	E340A	1
S	E340G	1
S	E340I	1
S	E340K	1
S	S371L	2
S	S373P	2
S	S375F	2
S	K417N	3
S	N440K	2
S	L452R	1
S	S477N	1
S	T478K	1
S	T478K	1
S	E484A	2
S	E484K	5
S	Q493R	1

Gene	Mutation	Number of publications
S	G496S	2
S	Q498R	1
S	N501Y	8
S	Y505H	1
S	L452R+T478K	5
S	K417N+E484K+N501Y	14
S	N501Y+P681H	2
S	G339D+S371F+S373P+ S375F+T376A+D405N+ R408S+K417N+N440K+ S477N+T478K+E484A+ Q493R+Q498R+N501Y+ Y505H	6
S	G339D+S371F+S373P+S375F+ T376A+D405N+R408S+K417N+ N440K+S477N+T478K+E484A+ Q493R+Q498R+N501Y+Y505H+ H655Y+ N679K+ P681H+ N764K	2
S	G339D+S371L+S373P+ S375F+K417N+N440K+ G446S+S477N+T478K+ E484A+Q493R+G496S+ Q489R+N501Y+Y505H	5