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BRIEF REPORT

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Hand hygiene for prevention and control of viral infections: the role of Amuchina Gel Xgerm

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ABSTRACT

Introduction: Alcohol-based hand sanitizers are used in healthcare settings, but their efficacy against both enveloped and non-enveloped viruses requires further investigation.
Methods: In this study, viral suspensions composed by the test virus, an interfering substance, and the test product, were exposed to Amuchina Gel Xgerm (100 g of the product contains 74 g of ethanol 96%; Angelini Pharma S.p.A) at 80% dilution in water, according to the European Standard EN14476:2013+A2:2019 – quantitative suspension test for the evaluation of virucidal activity in the medical area – for chemical disinfectants and antiseptics, in the presence of bovine serum albumin 0.3 g/L. The residual viral titers were measured.
Results: Sixty second exposures to the product determined a ≥4 $\log_{10}$ reduction of virus titer in all cases. For adenovirus type 5, human coxsakievirus, herpesvirus 1 and influenza virus A H1N1, a ≥4 $\log_{10}$ reduction of virus titer was obtained already after 30 s. Amuchina Gel Xgerm demonstrated effectiveness against seven virus families, including both enveloped and non-enveloped viruses.
Conclusion: Amuchina Gel Xgerm is a preventive measure against infection spread in healthcare and community settings, contributing to improved healthcare management.

Keywords: Alcohol-based hand sanitizer, Enveloped virus, Infection prevention, Non-enveloped virus

Introduction

The transmission of infectious agents via contaminated objects (fomites) such as surfaces and hands is a significant concern in public health. Fomites play a well-documented role in the spread of viral infections in both healthcare settings and the community (1). Fomites contribute to the transmission of pathogens such as Enteroviruses, Rotaviruses, and Adenoviruses (2). Respiratory viruses, such as Influenza, Respiratory Syncytial Virus, and human Coronaviruses, can persist on fomites and the skin for periods of time up to a few days, facilitating transmission within the community (3).

Virus spreading is facilitated in contexts of overcrowding, social interaction, and close contact (2,3). Healthcare settings are central environments for viral transmission. Hospital common areas such as waiting rooms, restrooms, and elevators are particularly critical points, due to the large number of individuals passing through them daily; even when standard, methodical cleaning techniques are applied, it is challenging to keep such areas (especially door handles, surfaces, etc.) appropriately sanified and safe (4).

Cross-infections (such as patient-to-patient, personnel-to-patient, visitor-to-patient) co-acquired in healthcare environments can have serious consequences, given the high prevalence of vulnerable people, including elderly and immunocompromised subjects. In such environments, prevention of pathogen transmission remains the most efficient strategy, also considering the wide spread of (multi)drug resistant bacterial pathogens (5).

Viral pathogen transmission is highly likely in nurseries and schools due to close contact among children and inadequate hand and personal hygiene practices. The frequent social activities inside and outside schools may further favor overcrowding and microbial transmission (2,3). In addition, children are commonly a relevant unconscious vehicle of infectious agents toward vulnerable members of their families, further expanding the challenges in healthcare management. In these contexts, implementing effective infection prevention measures is crucial.

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Extensive literature documents that prevention of viral transmission is essential in all seasons. In temperate regions, the transmission of most respiratory viruses peaks during winter, when overcrowding and close contacts increase, given the time spent indoors. In contrast, some viruses, such as Adenoviruses, are transmitted throughout the year, whereas others, like non-rhinovirus Enteroviruses, may even peak during the summer months (6).

Emphasizing the central role of surface disinfection and proper hand hygiene is essential for significantly reducing the risk of viral infections and to control disease spreading (7). Indeed, it has been shown that children attending daycare centers experienced fewer respiratory and gastrointestinal issues when surfaces, such as the school buses and toys, are routinely disinfected (7). Accordingly, hygiene measures that are easy to implement, even in the absence of water and soap, are expected to significantly increase infection control with respect to viruses and other pathogens.

Ethanol at a concentration of 70–80% (v/v) is effective against various enveloped viruses, including SARS and MERS Coronaviruses, human and avian Influenza viruses, Human Herpesvirus 1 and 2, and Respiratory Syncytial Viruses (8). Ethanol is essential in hand sanitizers (rubs, gels, foams) in healthcare settings, and is listed by the WHO as an essential medicine, recommended at 80% (v/v) concentration. Due to its activity also against non-enveloped viruses, ethanol is a critical virucidal agent in hand antisepsis (9). Experimental studies using contaminated thumb- and fingertip pads demonstrated that samples exposed to ethanol gel (60%) showed significantly lower levels of virus infectivity compared to control samples. As a matter of fact, the infectivity titers were reduced by 3 to >4 $\log_{10}$ when compared to a reduction of ≤1 $\log_{10}$ for the hard-water rinse (10).

Alcohol-based hand sanitizers are commonly used in hospital settings for hand hygiene, ensuring rapid, consistent, and effective disinfection. Their portability, ease of use, and widespread availability have made them popular among the general population. One of these products, the gel rub Amuchina Gel Xgerm (100 g of the product contains 74 g of ethanol 96%; Angelini Pharma S.p.A), is already certified as a disinfectant and antiseptic agent. This sanitizer has been shown to efficiently reduce SARS-CoV-2 virus titers below detectable limits (≥4 log10 reduction of viral recovery) after 30 and 60 seconds exposures, as assessed by standard suspension tests (11). Given the widespread use of hand sanitizers and their significant impact on public health for prevention of viral spreading, an in-depth knowledge about the spectrum of their virucidal efficacy is crucial to implement their usage in a variety of settings, particularly those housing vulnerable people or where viral agents transmission is more likely to occur.

The present study aimed to establish *in vitro* the virucidal activity of Amuchina Gel Xgerm against a panel of enveloped and non-enveloped human viruses.

Materials and methods

The virucidal activity tests were performed according to the European Standard EN14476:2013+A2:2019 (12); the product was assessed at a final concentration of 80% (8ml of the product + 1 ml of viral suspension + 1 ml of interfering substance), in the presence of 0.3 g/L of bovine serum albumin as interfering substance – an organic material that might be present in environments where the virucidal agent is used, mimicking real-world conditions – (12), for either 30 or 60 s, at 20°C ±1°C. The non-enveloped viruses tested included Adenovirus type 5, Rotavirus A, and human Coxsakievirus B5. The enveloped viruses included Influenza A (H3N2 and H1N1), Respiratory Syncytial Virus, and human Herpesvirus 1. After exposure time, the test mixture was filtered using MicrospinTM S 400 HR columns and then subjected to the following procedures: a) eight 10-fold serial dilutions with a cold Minimum Essential Medium Eagle (MEM) + 2% fetal calf serum (FCS) were set up, each incubated with cells at 37°C for 5-7 days to quantify the cytopathic effect; b) if the product showed residual cytotoxicity on the cell line at the tested concentrations, then the large volume plating (LVP) method was used instead, at 80% concentration. The 50% tissue culture infectious dose ($TCID_{50}$) per ml value was calculated by the Spearman–Karber method and converted to $\log_{10}$ of $TCID_{50}$ (11). All the tests were performed with the appropriate controls in accordance with the European Standard EN14476:2013+A2:2019 (12).

Results

The viral strains tested, the $TCID_{50}$ viral load recovery values, and the corresponding reduction after exposure to Amuchina Gel Xgerm are shown in Table I. As established by the European Protocol (12), viral titer reduction values ≥4 $\log_{10}$ denote a complete inactivation of the virus. For all the tested viral pathogens, a 60 s treatment with the sanitizer reduced viral titers to undetectable levels, achieving a ≥4 $\log_{10}$ reduction in viral recovery (Table I). In particular, a ≥4 $\log_{10}$ reduction was observed already after 30 s of exposure when the product was tested against Coxsakievirus B5, Influenza virus (H1N1), Adenovirus type 5, and human Herpesvirus 1. The data from this study that the Amuchina Gel Xgerm passed the virucidal efficacy suspension test, for both enveloped and non-enveloped pathogens. These findings are in line with recent results observed against SARS-CoV-2 (11). The virucidal effects of Amuchina Gel Xgerm against non-enveloped viruses deserve special consideration, since such viruses are known to be significantly less susceptible to ethanol than the enveloped ones (13). The control test results are shown in Table II: all the controls resulted valid.

Discussion

The present data support the notion that the use of hand sanitizers may effectively disrupt the virus transmission chain, thereby reducing the risk of potential disease spread in different contexts, including hospital and healthcare settings, public institutions such as schools, and social gatherings like parties, concerts and summer camps. Amuchina Gel Xgerm is easy to use, making it an effective alternative when soap and water are not immediately available, providing sanitization against a broad range of viral particles (6).

The preventive use of hand sanitizers not only represents a first line of defense against the initial infection but also

TABLE 1 - Viral load before and after Amuchina Gel Xgerm treatment

Type of infection caused	Virus [strain]	Exposure time (s)	Input load virus titer (log_{10} $TCID_{50}$)	Ouput load virus titer (log_{10} $TCID_{50}$)	Reduction (log_{10} $TCID_{50}$)
Non-enveloped					
Respiratory infections	Adenovirus type 5 [Adenoid, 75 ATCC VR-5]	30	6.63 ± 0.00	2.5 ± 0.00	> 4.00 ± 0.00
Other infections	Rotavirus A [HumanG8P10, RVB-1221 FLI]	60	6.17 ± 0.40	≤ 1.33 ± 0.00	≥ 5.50 ± 0.40
	Human Coxsackievirus B5 [ATCC VR-185]	30	5.50 ± 0.00	≤ 1.03 ± 0.00	≥ 5.47 ± 0.00
Enveloped					
Respiratory infections	Influenza A/swine/lowa/15/30 virus (H1N1) [ATCC-VR-1683]	30	6.63 ± 0.00	2.50 ± 0.00	> 4.00 ± 0.00
	Influenza A/Hong Kong/8/68 virus, (H3N2), TC adapted [ATCC VR-1679]	60	6.50 ± 0.00	≤ 1.03 ±0.00	≥ 5.47 ± 0.00
	Human Respiratory Syncytial Virus [ATCC VR-26]	60	4.50 ± 0.00	≤ 1.33 ± 0.00	≥ 4.17 ± 0.00
Other infections	Human Herpesvirus 1 [ATCC VR-733]	30	7.50 ± 0.00	≤ 3.50 ± 0.00	≥ 4.00 ± 0.00

$TDID_{50}$ was calculated using Spearman - Kaber method: TDI_{50} = V - [(S/100-0.5) x D], where: V = negative log of the highest virus concentration tested; S = sum of the % affected wells at each concentration; D = log of dilution.

The indicated values are the mean ± standard error of 6 replicate samples.

TABLE 2 - Results of the control assays, performed according to the EU protocol

						Cell susceptibility		Efficacy of suppression	
Type of infection	**Virus [strain]**	**Cel line [source]**	**Test method**	**Sample**	**Virus titer# log_{10} $TCID_{50}$**	**Virus titer# log_{10} $TCID_{50}$**	**Result***	**Virus titer# log_{10} $TCID_{50}$**	**Result***
Non-enveloped									
Respiratory infections	Adenovirus type 5 [Adenoid, 75 ATCC VR-5]	HeLa [ATCC CCL-2]	Filtration	80%	6.63 ± 0.00	6.89 ± 0.00	0.00	6.38 ± 0.00	0.13
				PBS		6.89 ± 0.00	–	–	–
Other infections	Rotavirus A [HumanG8P10, RVB-1221 FLI]	MA-104 [CCLV-RIE 142]	Filtration and LVP	80%	6.17 ± 0.40	6.00 ± 0.45	0.33	5.67 ± 0.35	0.50
				PBS		6.33 ± 0.35	–	–	
	Human Coxsackievirus B5 [ATCC VR-185]	LLC-MK2 [CCLV-RIE 1418]	Filtration and LVP	80%	5.50 ± 0.00	5.33 ± 0.35	0.33	5.33 ± 0.35	0.17
				PBS		5.67 ± 0.35	–	–	–
Enveloped									
Respiratory infections	Influenza A/swine/ lowa/15/30 virus (H1N1) [ATCC-VR-1683]	LLC-MK2 [ATCC CCL-7.1]	Filtration	80%	6.63 ± 0.00	6.00 ± 0.00	0.625	6.50 ± 0.00	0.00
				PBS		6.63 ± 0.00	–	–	–
	Influenza A/Hong Kong/8/68 virus (H3N2), TC adapted [ATCC VR-1679]	MDCK [ATCC CCL-34]	LVP	80%	6.50 ± 0.00	6.50 ± 0.00	0.83	6.00 ± 0.45	0.50
				PBS		7.33 ± 0.35	–	–	–
	Human Respiratory Syncytial Virus [ATCC VR-26]	Hep-2 [ATCC CCL-23]	Filtration and LVP	80%	4.50 ± 0.00	4.33 ± 0.35	0.17	4.17 ± 0.40	0.33
				PBS		4.50 ± 0.00	–	–	–
Other infections	Human Herpesvirus 1 [ATCC VR-733]	VERO [ATCC CCL-81]	Filtration	80%	7.50 ± 0.00	7.17 ± 0.40	0.50	7.33 ± 0.37	0.17
				PBS		7.67 ± 0.35	–	–	–

Mean ± standard error of 6 replicate samples.

*Viral interference control (log_{10} titer difference): test is acceptable if the titer difference between the neutralized test product-treated and PBS-treated cellular monolayers is ≤1.0 log_{10} in the viral interference control.

PBS: Phosphate-buffered saline; TCID50: 50% tissue culture infective dose; LVP: large volume plating

plays a crucial role in mitigating the risk of multiple infections (14). Vulnerable and immunocompromised individuals face an increased risk of severe outcomes, especially when concomitant infections may simultaneously occur, further complicating the initial illness (15,16). As a result, the clinical condition can deteriorate, making treatment and recovery more challenging.

Integrating hand hygiene practices into daily routines and public health strategies can be useful for protecting vulnerable populations and minimizing the overall impact of infectious diseases in our complex and dynamic society. Increasing awareness of the efficacy of the use of hand sanitizers through sensitizing campaigns will likely benefit the healthcare system, not only as reduced access of people to hospitals but also as overall saving costs for the healthcare system.

The hereabove demonstrated broad-spectrum virucidal activity of the alcohol-based hand sanitizer Amuchina Gel Xgerm, strongly supports its usage in environments where the risk of virus transmission is high, such as hospitals, schools, and public spaces. Different sets of investigations are needed to establish the efficacy of this gel against other pathogens, such as bacterial agents.

Amuchina Gel Xgerm has been assessed using an *in vitro* suspension assay, a standard method for testing virucidal activity, in accordance with European Standard EN14476:2013+A2:2019 criteria (12); interestingly, the products is effective, even in the presence of an interfering substance, such as bovine serum albumin, that, according to the standard European protocol (12), mimics the real conditions on the field. In fact, in the environment, viruses are typically agglomerated with proteins and other biomolecules that influence their interactions with host cells. The inclusion of BSA in viral suspensions helps create a real-world environment by providing a matrix that mimics the biological fluids encountered during actual infections. This can affect how viruses attach to and enter host cells, which is critical for understanding their pathogenicity. The observed wide spectrum efficacy underlines the relevance of hand sanitizer in many different situations when there is a risk of viral pathogens spreading via fomites.

Limitations

The main limitation of the present work is that being an *in vitro* study, it does not address the efficacy of the product in real-world settings; variability in user application techniques and adherence to hand hygiene recommendations, as well as environmental factors such as temperature and humidity, which can affect virus persistence on surfaces, have not been taken into account.

Conclusions

Amuchina Gel Xgerm hand sanitizer exerts a broad spectrum virucidal activity *in vitro*, being effective against both enveloped and non-enveloped viruses.

Promoting hand hygiene practices can limit the spreading of viral infections within the general population and enhance the protection of the most vulnerable individuals in clinical settings.

Given the epidemiological importance of the transmission of viral pathogens in hospitals and in the community, these findings underscore the potential impact of this alcohol-based formulation that will positively impact public health by reducing the transmission of infectious diseases.

Disclosures

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Author contribution: ACdJ, MIR, DF, and MP: conception of the work, acquisition, analysis, interpretation of data for the work; drafting the work and revising it critically. CC and EB: interpretation of data for the work; drafting the work and revising it critically for important intellectual content. All authors approved the final version of the manuscript submitted and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data availability statement: The data that support the findings of this study are available from the corresponding author, CC, upon reasonable request.

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