Received: 11 October 2023 | Revised: 7 November 2023 | Accepted: 10 November 2023

DOI: 10.1002/dta.3618

RESEARCH ARTICLE

WILEY

Substandard and falsified ivermectin tablets obtained for self-medication during the COVID-19 pandemic as a source of potential harm

Celine Vanhee[1] | Bram Jacobs[2] | Angélique Kamugisha[1] | Michael Canfyn[1] | Hans Van Der Meersch[3] | Bart Ceyssens[3] | Eric Deconinck[1] | Koenraad Van Hoorde[2] | Marie Willocx[1]

[1]Service Medicines and Health Products, Scientific Direction of Chemical and Physical Health Risks, Sciensano, Brussels, Belgium

[2]Service of Foodborne Pathogen, Scientific Direction of Infectious Diseases in Humans, Sciensano, Brussels, Belgium

[3]Federal Agency for Medicine and Health Care Products, Brussels, Belgium

Correspondence
Celine Vanhee, Service Medicines and Health Products, Scientific Direction of Chemical and Physical Health Risks, Sciensano, J. Wytsmanstraat 14, Brussels B-1050, Belgium.
Email: celine.vanhee@sciensano.be

Abstract

In 2019, a global viral pandemic, due to the SARS-CoV-2 virus, broke out. Soon after, the search for a vaccine and/or antiviral medicine began. One of the candidate antiviral medicines tested was ivermectin. Although several health authorities warned the public against the use of this medicine outside clinical trials, the drug was widely used at the end of 2020 and in 2021. Simultaneously, several reports started to emerge demonstrating serious adverse effects after self-medicating with ivermectin. It stands to reason that the self-administration of substandard or falsified (SF) medicines bearing harmful quality deficiencies have contributed to this phenomenon. In order to have a better view on the nature of these harmful quality deficiencies, SF ivermectin samples, intercepted in large quantities by the Belgian regulatory agencies during the period 2021–2022, were analyzed in our official medicines control laboratory. None of the samples ($n = 19$) were compliant to the quality criteria applicable to medicinal products. These SF products either suffered from a systematic underdosing of the active pharmaceutical ingredient or were severely contaminated with bacteria, two of which were contaminated with known pathogens that cause gastrointestinal illness upon oral intake. In addition to the direct risks of self-medicating with such a product, the improper usage and dosage of ivermectin medication might also facilitate ivermectin tolerance or resistance in parasites. This may have detrimental consequences on a global scale, certainly as the number of newly developed active pharmaceutical ingredients that can safely be used to combat parasites is rather scarce.

KEYWORDS
antiparasitic drugs, COVID-19, quality control, online medicines, substandard medicine

1 | INTRODUCTION

In December 2019, the first outbreak of the SARS-CoV-2 virus, responsible for causing the coronavirus disease 2019 (COVID-19), took place in the Wuhan province in China.[1,2] Soon after, this virus turned into a global pandemic and a global public health emergency.[3] Needless to say that since early 2020, the race for a vaccine and/or antiviral medicine was on. One of those candidate antiviral medicines tested was ivermectin, a broad spectrum antiparasitic drug, that had shown in vitro antiviral activity in the past.[4–8] Ivermectin has been used to treat humans for the past four decades, and through the years, more than 3 billion doses have been given to humans with a

high safety profile.[9] Consequently, the drug was added to the World Health Organization's List of Essential Medicines. Mid-2020, a study was released that demonstrated that ivermectin showed, at least in vitro, a highly significant reduction (99.8%) in viral SARS-COV-2 RNA after 48 h.[10] However, the dosage used was much higher in comparison with the standard dose for the treatment of parasitic infections such as onchocerciasis (river blindness) and strongyloidiasis or scabies.[11] Since the outcome of these results, multiple clinical trials were carried out to assess clinical outcomes,[12] with conflicting evidence. Moreover, some of these studies were withdrawn due to concerns about inconsistencies or even fraudulent practices.[12]

Consequently, the World Health Organization, the US Food and Drug Administration, and the European Medicines Agency are currently advising against the use and repurposing of this prescription medicine, against COVID-19, other than its use in clinical trials. Yet, the drug has been widely used and has even been called a "COVID-19 miracle drug," supported by vaccine opponents or even by health authorities in some countries.[13] However, several reports, originating from different countries, demonstrated that several people developed serious adverse effects after self-medicating and some of them were even hospitalized.[14–18] A large fraction of these cases were likely due to an ivermectin overdose, as a consequence of the self-administration of highly concentrated ivermectin medicines intended for livestock.[14–16] Indeed, an ivermectin overdose could result in disturbing gastrointestinal symptoms but it can also inflict hypotension and neurological effects such as decreased consciousness, confusion, hallucinations, seizures, coma, and even death.[16] However, not all cases could be attributed to an overdose with this veterinarian product. These undesired effects could also be due to self-medication and the possible interaction with other medication taken by the patient. Alternatively, the use of harmful substandard or falsified (SF) medicines, available through rogue online pharmacies, could contribute to the number of people with undesired side effects. A Hungarian study demonstrated that during the pandemic, Google users' queries regarding ivermectin were trending and peaked during the last week of November 2020 and March 2021. Consumers most likely found links leading directly or indirectly to illegal online retailers.[19] Evidently, law enforcement agencies, responsible to curtail the sales, purchasing, trafficking, and use of illegal medicines, encountered also more ivermectin tablets, intended for human use, during the pandemic.[20,21]

Based on the scientific literature, the major health risks associated with the use of these SF medicinal products are the fact that they are likely not produced under good manufacturing practices. Consequently, they may contain only inactive ingredients, wrong ingredients, the improper amount of active pharmaceutical ingredient (API), or potentially toxic impurities, or they can be contaminated with potentially pathogenic microorganisms.[22–31] Therefore, in order to assess the risks associated with the use of these substandard and falsified ivermectin products, we set out to perform a mapping of the potentially dangerous chemical and biological risks, other than the self-medication of prescription medicines and the usage of high doses of ivermectin intended for livestock. These samples were intercepted by Belgian regulatory agencies in the period 2021 and 2022.

2 | MATERIAL AND METHODS

2.1 | Solvents, reagents, and standard solutions

Mass spectrometry (MS)-grade acetonitrile and formic acid were purchased from Biosolve (Valkenswaard, the Netherlands). Water was generated using a milliQ-Gradient A10 system (Millipore, Billerica, USA). Reference standards utilized for the identification of ivermectin and the quantification of ivermectin and ivermectin impurity 8a-oxo-H_2B_{1a} were obtained from the US pharmacopoeia (USP). These reference standards corresponded to ivermectin (purity of 90.8%, mixture of 89.4% Ivermectin H_2B_{1a} and 1.4% Ivermectin H_2B_{1b}) and 3-*tert*-Butyl-4-hydroxyanisole (purity 99.5%), used to quantify 8a-oxo-H_2B_{1a}. Moreover, a reference standard to confirm the retention time of the 8a-oxo-H_2B_{1a} impurity (CAS number 102190-68-1) was purchased from Toronto research chemicals (Ontario, Canada) with a purity of 99.7%. A reference standard of cetirizine (cetirizine dihydrochloride, purity 100%) was obtained from Fagron (Waregem, Belgium).

Dimethyl sulfoxide (DMSO), used as solvent for the residual solvent analysis of the different samples, was purchased from Merck (Darmstadt, Germany). High performance liquid chromatography (HPLC)-grade 2-propanol, acetone, dichloromethane, and pesti-S-grade ethylacetate, used to quantify the detected residual solvents, were also purchased from Biosolve (Valkenswaard, The Netherlands). Toluene and diethyl ether were, respectively, purchased from VWR prolabo (Fontenay-Sous-Bois, France) and Merck.

2.2 | Buffers and media for bioburden testing

Sabouraud dextrose agar with neutralizers (lecithin, tween, and histidine), trypto-casein-soy agar with neutralizers (lecithin, tween, histidine, and thiosulfate), violet red bile glucose agar, and buffered sodium chloride peptone with neutralizers (lecithin, tween, histidine, and thiosulfate) were purchased from Merck (Darmstadt, Germany).

Sterile analytical filter units 0.45 µm Nalgene™, used for the membrane filtration during the bioburden testing, were purchased from Thermo-Fisher Scientific (Rochester, NY, USA).

2.3 | Sample set

Nineteen samples were seized by the Belgian Federal Agency for Medicine and Health products and subsequently sent to our Official Medicines Control Laboratory where pictures were taken upon arrival and the information available on the packaging or blister was recorded (see Data S1). All seized samples were in the form of tablets.

2.4 | Screening for APIs

All samples were screened for the presence of synthetic drugs or medicines by both liquid chromatography coupled to tandem mass

spectrometry (LC–MS^2) and gas chromatography couppled to mass spectrometry (GC–MS) according to the methodology described previously.[31] Briefly, the screening of all samples was performed by ultra high performance liquid chromatogrpahy (UHPLC) coupled to tandem MS (UPLCMS^n) on a Dionex UltiMate 3000 Rapid Separation LC system (Thermo Scientific, Sunnyvale, CA, USA) connected to an amaZon™ speed ETD mass spectrometer (Bruker Daltonics, Bremen, Germany). The generated LC and MS data were analyzed by Compass Data Analysis 4.2 (Bruker Daltonics, Bremen, Germany), and the LC–MS^n spectra were compared with different libraries, including a homemade library, enclosing a total of about 5000 MS and MS^2 spectra. A compound was considered present if the shift of the retention time is less than or equal to 0.5 min (compared with the reference standard), *m/z* of the precursor ion is equal to the one present in the in-house library (error tolerated: 0.3 Da), and the MS^2 spectrum matches at least 85% with the reference spectrum (fragment ions and their relative intensities) of the library.[31,32]

The GC–MS analyses were performed on an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass detector (Agilent, Santa Clara, CA, USA). The MS data were analyzed by MassHunter, and spectra were compared with different libraries, including NIST20 mass spectral library (NIST, Gaithersburg, MD, USA) and the Cayman spectral library (Cayman chemical, Ann Arbor, Michigan, USA). A 0.30 Da precursor tolerance for MS spectra was allowed, and at least a matching score of 85% was required to be considered a possible hit.[31,32]

2.5 | Quantification of APIs

The total amount of ivermectin was determined on a Waters Acquity UPLC™ system (Waters Corp., Milford, MA, USA) including a binary solvent manager, sample manager flow through needle, column heater, and photodiode array detector connected to Waters Empower 3.7.0 data station. The chromatographic separation was performed at 40°C on an Acquity™ UPLC BEH RP18 Column (100 × 2.1 mm, 1.7 µm particle size) (Waters, Milford, MA, USA) with a mobile phase consisting of 0.1% formic acid in water (A) and acetonitrile (B) and flow rate of 0.5 mL/min. An isocratic run was utilized at 20% A and 80% B for 5 min. Ivermectin was monitored at a wavelength of 245 nm. Prior to the quantification of the real-life samples, the newly developed method was validated based on guidelines put forward by the international council for harmonisation of technical requirements for pharmaceuticals for human use (ICH)[33] and the total error approach[34–36] as previously done before by our research unit.[31,37]

2.5.1 | Validation of the quantification methodology

The selectivity and the specificity were assessed by comparing the three blank matrixes consisting of excipients often used to generate tablets (matrix 1: starch; matrix 2: magnesium stearate; and matrix 3: cellulose). Peak identity for ivermectin was assigned by analysis of a refence standard, comparing the retention time and ultraviolet absorbance spectra. The purity angle and purity threshold were checked for all spiked matrices and were found to be less than the purity threshold, indicating a good purity of the chromatographic peak. Next, also the lower limit of quantification and the upper limit of quantification were determined. The lower limit of quantification was defined as the lowest concentration with a signal to noise greater than 10 and the highest concentration for which accuracy and precision could be demonstrated and corresponded to a 5 µg/mL. The upper limit of quantification was determined as the highest concentration for which accuracy and precision could be demonstrated and corresponded to 500 µg/mL. Additionally, linearity was assessed for concentrations ranging from 5 to 500 µg/mL by applying least squares regression analysis. Adequate linearity was achieved as the regression coefficient (r) ≥ 0.99. Subsequently, matrix effects were evaluated by performing a *t*-test on the slope of the calibration curves obtained with and without one of the three matrices. All injections were performed in triplicate, and no significant differences were observed.

Next, the trueness, precision, and accuracy were determined by means of the total error approach. Hereto, ivermectin-spiked blank samples were made daily in triplicate at five concentrations in the linear range (5, 50, 100, 200, and 500 µg/mL) and analyzed for four consecutive days. The corresponding concentrations were back-calculated using the calibration lines generated on the same day. These calculated concentrations were then used to calculate the trueness and precision (repeatability and intermediated precision) and to generate accuracy profiles (see Data S2 and S3) for the developed method. Indeed, the calculation of the β-expectation tolerance limits, calculated at each concentration level, can be used as a predictive tool which guarantees that 95% of future results, given by the analytical method, will fall inside the predefined acceptance limits [−λ;λ]. The predefined acceptance limits were set to ±10% given that USP tolerates an error of 10% of labeled content of ivermectin in ivermectin tablets.[38] As can be seen in Data S2–S6, the obtained β-expectation tolerance limits did not exceed the acceptance limits, rendering the method suitable for quantification purposes.

2.5.2 | Quantification of the real-life samples

The quantification of the samples was performed on two separate units and a mixture of different units (minimal three units present in the mixture). All preparations ($n = 3$) were injected four times, and these 12 injections were used to generate the mean amount of ivermectin present in the sample. The measurement uncertainty was estimated as confidence interval using the standard deviations obtained for these preparations.

2.6 | Quantification of ivermectin impurity 8a-oxo-H_2B_{1a}

The quantity of ivermectin impurity 8a-oxo-H_2B_{1a} was determined on a Waters HPLC™ system (Waters Corp., Milford, MA, USA) connected

to a photodiode array detector. The chromatographic separation was performed at 30°C with the use of a Hypersil ODS C18 column (150 × 4.6 mm, 5 µm particle size) (Thermo Scientific™, Waltham, MA, USA) with a mobile phase consisting of 53:35:12 mixture of acetonitrile, methanol, and water, conform the methodology described in the USP.[38] The amount of the impurity was determined based on the area obtained for a known amount of 3-*tert*-Butyl-4-hydroxyanisole and the application of the correction factor (=0.98) mentioned in the USP. The reference standard of the impurity 8a-oxo-H_2B_{1a} was solely used to unambiguously confirm the retention time. The quantification was performed on a mixture of different units. The measurement uncertainty was estimated as confidence interval using the obtained standard deviations.

2.7 | Residual solvent analysis

The samples were analyzed for the occurrence of residual solvents, and the encountered solvents were quantified according to the methodology previously described.[31] Briefly, for the identification of the solvents present in the samples, the mass spectrometer was operated in full-scan mode, while for quantification, the mass spectrometer was operated in selected ion monitoring (SIM) mode. Starting from the stock solutions, dilutions were prepared in DMSO, brought into vials, and automatically sealed. DMSO was used as blank. For the analysis of the samples, 1 mL of DMSO was added to the tablets. Samples were prepared in duplicate and also injected in duplicate. A one-point calibration was performed in the linear range established for the encountered residual solvents.

2.8 | Bioburden analysis and identification of the encountered microorganisms

Bioburden testing was performed based on USP and European pharmacopoeia by utilizing the membrane filtration methodology.[38,39] Next, the encountered bacteria were isolated on trypto-casein-soy agar and subsequently analyzed by means of the MALDI Biotyper® (Bruker Daltonics, Bremen, Germany). The data generated by the matrix-assisted laser desorption ionization–time-of-flight mass spectrometry were processed by the MALDI Biotyper 3 software (Bruker Daltonics, Bremen, Germany).[31] Only hits with log (score) values equal to or higher than 2.00 were considered as a high confidence identification at species level. In case of hits with a lower log (score) value between 1.70 and 2.00, identifications were reported as an identification at the genus level. Log (score) values below 1.70 were considered as nonidentified.

In case of a high confidence identification of a member of the *Bacillus cereus* sensu lato (s.l.) clade, DNA extraction was performed and subjected to whole genome sequencing. Strain identification was done by using the *B. cereus* s.l. multilocus sequence typing scheme from PubMLST.org[40] and the BTyper3 software[41] as described previously for *B. cereus* s.l. strains encountered in substandard and falsified phosphodiesterase type 5 (PDE-5) inhibitors and in vitamin B2 products.[31,42]

3 | RESULTS

Ivermectin is a semisynthetic mixture of 22,23-dihydroavermectin B_{1a} (Ivermectin H_2B_{1a}) and 22,23-dihydroavermectin B_{1b} (Ivermectin H_2B_{1b}). These compounds are derived from the macrocyclic lactones avermectin B_{1a} and avermectin B_{1b}, products that are formed during the fermentation process of *Streptomyces avermitilis*, upon selective catalytic hydrogenation of the cis-22,23-double bonds of these avermectins (see Figure 1).[43] In order to use the generated ivermectin as raw material for medicines, the product has to comply with the requirements described in the European pharmacopoeia or USP, provided it has been produced under good manufacturing practice conditions.[38,39] Once the raw material is shown to be compliant, it can be used to generate a finished product, such as ivermectin tablets, ivermectin paste, ivermectin solutions for oral intake (veterinary medicine) or topical application, or ivermectin for injection purposes. Once again, these finished products are subjected to quality control testing,

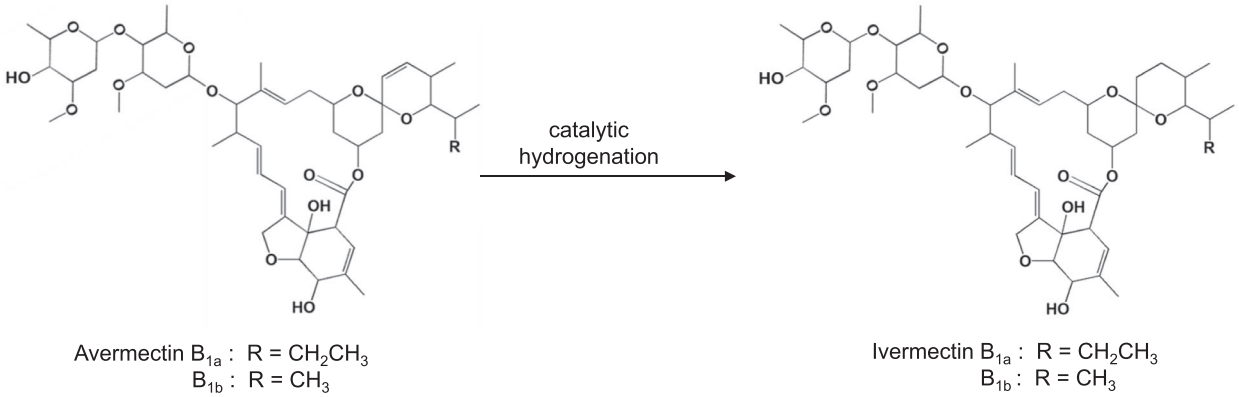

FIGURE 1 Chemical structures of the macrocyclic lactones avermectin B_{1a} and avermectin B_{1b}, precursors for the selective catalytic hydrogenation of the cis-22,23-double bonds to generate Ivermectin H_2B_{1a} and Ivermectin H_2B_{1b}, respectively.

generally according to the tests described in the ivermectin monographs present in the USP.[38] This ensures that the medicines that are available for the public contain the proper API, in the correct dosage, and do not contain potentially toxic levels of impurities or contaminants. Our 19 seized SF-medicinal products might not have been subjected to all these required quality control tests. All the analyzed samples did declare to be for human use and claimed to contain concentrations from 3 to 12 mg per tablet, corresponding to real-life concentrations used as antiparasitic medication for humans.

3.1 | Chemical quality control

The samples were screened for the presence of the API, the dosage of total ivermectin (Ivermectin H_2B_{1a} and Ivermectin H_2B_{1b}), and the amount of the impurity Ivermectin 8a-oxo-H_2B_{1a} as is described in the USP monography for ivermectin tablets.[38] The USP indicates that 90–110% of the API should be present and tolerates up to 2.0% of this impurity. In addition to the API and API-related impurities, processing impurities such as residual solvents or the presence of nonrelated APIs were assessed as they have been shown to be occasionally present in this type of samples.[25,27] These tests are not standard requirements for finished products as they should have been conducted on the raw material. As the used raw material was not available to us, the tests were performed on the samples (see Table 1). From Table 1, it can be noted that all samples contained the correct API (Table 1) but they suffered from systematic underdosing of the API as the majority contained 80–90% of the labeled amount and only 32% (6/19) of the samples contained 90–110% of the declared dosage (see Figure 2). One sample displayed a large variation in the amount of API present in the different pills originating from the same blister and thus resulted in a larger measurement uncertainty (Table 1).

Next, we also checked the amount of API autooxidation product 8-a-oxo-H_2B_{1a} as a marker of ivermectin degradation,[44] the presence

TABLE 1 Summary of the results obtained from the chemical quality control tests of the samples.

Sample no.	Quantity ivermectin on the label (mg/unit)	Measured amount of ivermectin (mg/unit)[a]	% ivermectin found compared with the amount declared[a]	Amount of 8-a-oxo-H_2B_{1a} found exceeding the tolerated limit[c]	Presence of nondeclared declared API	Amount of residual solvent exceeding the tolerated limits[d]	Compliant to performed quality control tests
1	6	5.5 (±0.1)	92.3 (±0.9)	–	–	–	Y
2	12	9.7 (±0.2)	80.8 (±1.8)	–	–	15,096 ppm 2-propanol	N
3	12	8.9 (±0.2)	73.8 (±1.8)	–	–	–	N
4	6	4.8 (±0.2)	79.9 (±2.2)	–	–	–	N
5	12	10.0 (±0.1)	83.9 (±1.3)	–	–	–	N
6	3	2.9 (±0.1)	95.8 (±1.9)	–	–	–	Y
7	6	5.0 (±0.1)	83.4 (±2.4)	–	Cetirizine (0.1 mg/unit)	–	N
8	12	11.7 (±0.1)	97.6 (±0.8)	–	–	11,494 ppm 2-propanol	N
9	6	5.0 (±0.1)	83.3 (±1.5)	–	–	–	N
10	12	10.3 (±0.1)	86.2 (±0.5)	–	–	–	N
11	12	9.7 (±0.1)	80.7 (±0.7)	–	–	–	N
12	3	2.4 (±0.02)	79.3 (±1.0)	–	–	–	N
13	6	4.4 (±0.3)	73.4 (±6.3)[b]	–	–	–	N
14	12	8.9 (±0.2)	73.8 (±1.8)	3.7 (±0.2)	–	–	N
15	12	9.2 (±0.4)	76.8 (±3.9)	–	–	–	N
16	6	3.3 (±0.03)	55.3 (±1.0)	–	–	–	N
17	3	2.6 (±0.1)	87.5 (±2.1)	–	–	–	Y
18	3	2.8 (±0.1)	92.8 (±4.2)	–	–	–	Y
19	12	11.3 (±0.1)	93.7 (±1.2)	–	–	–	Y

Abbreviation: API, active pharmaceutical ingredient.

[a]The values between brackets represent the uncertainty of the measurement and is expressed as confidence interval using the standard deviation of the generated quantification results.

[b]Occurrence of a large variation (>5% relative standard deviation) between the different tablets of the same blister.

[c]According to the US pharmacopoeia, the tolerance limits for the impurity 8-a-oxo-H_2B_{1a} for ivermectin tablets correspond to 2.0%.

[d]Only those residual solvents are listed where there was an exceedance in the tolerated amount. The amount of 2-propanol that is tolerated for tablets is set at 5000 ppm.

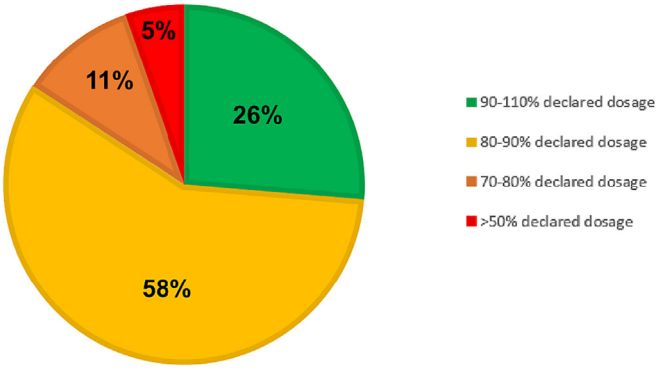

FIGURE 2 Results of the quantitative analysis of the 19 samples for label accuracy expressed as percent deviation of the claimed concentration.

of nondeclared APIs, and the amount of residual solvents (see Table 1). Sample 14 exceeded the tolerated limit for the API impurity, indicating that the ivermectin in these samples was degraded to a level exceeding the tolerated limit put forward by the USP. Interestingly, Sample 14, containing 3.7% of 8-a-oxo-H_2B_{1a}, corresponded to the sample that consisted of two morphologically different types of tablets, white tablets and beige-colored tablets with superficial cracks, which were much more fragile (see Data S1). Based on this observation, it might be possible that the blister consisted of older tablets and newer ones. Unfortunately, due to sample limitation, it was impossible to verify this hypothesis. Sample 7, on the other hand, contained a trace of cetirizine (mean dosage: 0.1 mg per tablet). Allergic reactions to cetirizine are extremely rare but there have been reports describing an anaphylactic shock upon oral intake of 10 mg of this molecule.[45] However, in this case, as the amount is 100 times less, the occurrence of this nonrelated API does very likely not pose any additional risk. Next, the amount of residual solvents was determined. As can be seen in Table 1, at least twice the tolerated amount of the residual solvent 2-propanol was detected in Samples 2 and 8. This solvent is considered a class 3 solvent and is limited to 5000 ppm per tablet.[46] These limits are based on acute or short-term genotoxicity studies, and thus, exceedance of these limits represents a higher risk. The detected levels of quantifiable amounts of residual solvents and the amount of the API impurity for all samples are available in Data S4.

3.2 | Biological quality control

In addition to the chemical quality control, the biological contamination, including bacteria and fungi, is checked for nonsterile products and is tolerated to a level that is consistent with patient safety. Whenever pharmacopeial limits are exceeded, adverse effects on patient health cannot be excluded. According to the USP, the acceptance criteria for ivermectin tablets would be 10^3 colony-forming units (CFU)/g in the total aerobic microbial count test (mainly bacteria) and 10^2 CFU/g in the total yeast and mold count test, corresponding to a maximum acceptable count of, respectively, 2000 and 200 CFU/g.[33] Moreover, the absence of *Escherichia coli*, a bile-tolerant gram-negative bacteria, has to be demonstrated. According to the US and European pharmacopoeias, the significance of recovered microorganisms must be evaluated and the absence of specific pathogens demonstrated, depending on the route of administration.[38,39]

Therefore, the total aerobic microbial count, total yeast and mold count, and the growth of bile-tolerant gram-negative bacteria were assessed in the seized SF ivermectin tablets. Next, the encountered bacteria were isolated and subsequently by means of matrix assisted laser desorption ionisatio-ntime of flight (MALD-ITOF), see Data S5. Our results, represented in Table 2, show that the majority of the samples was severely contaminated with bacteria that belonged to the *Bacillus* genus or closely related species that also produce endospores.[47]

Further genetic analysis of the *B. cereus* s.l. strains demonstrated that the strain encountered in Sample 1 matched a *B. cereus* s.s. strain with sequence type ST2302. The strain isolated in Sample 6 did not match a previously described sequence type (ST), but resembled ST201 as six of the seven loci were in common (see Data S6).

Next, the presence of several toxins, associated with the disease symptoms of a gastrointestinal *B. cereus* s.l. infection, were assessed. As can be seen in Data S6, the two strains contained genes that encode for enterotoxins (*nhe*, *hbl*, and *cytK2*) associated with gastrointestinal illness.[48]

Moreover, as it has been shown that *B. cereus* s.s. is typically resistant to penicillin and other β-lactam antibiotics[49] and can serve as a source for antibiotic-resistance gene transfer,[50] the presence of antibiotic-resistance genes in the encountered strains was also determined. As expected, both strains harbored the *bla* or *bla2* gene conferring resistance to β-lactam antibiotics.[50–52] The two strains were also predicted to be resistant to fosfomycin, as they carried either the *fosB* or *fosBx* gene, and to streptothricin, as the *satA* gene was encountered.[45–47]

In contrast with the recently performed PDE-5 inhibitors study,[31] no *Enterobacteriaceae* nor bile-tolerant fungi were detected. Moreover, none of the samples exceeded the threshold limits for fungal load.

4 | DISCUSSION

The chemical and biological quality controls performed on these SF ivermectin tablets, procured for self-medication purposes during the COVID-19 pandemic, illustrate the risk to public health as more than half of these products were severely contaminated with bacteria. These findings are in agreement with the previous observations made for recently seized PDE-5 inhibitors,[31] suggesting that this issue is more a general hygiene issue and not only limited to a certain type of API. In this case, two samples were even severely contaminated (>10^3 CFU/g) with a potentially pathogenic endospore-forming bacteria, known to be able to cause gastrointestinal illness.[48] In the present study, the encountered *B. cereus* s.s. isolates did not contain the *ces* gene cluster encoding the emetic toxin cereulide that has been responsible for some lethal outcomes upon gastrointestinal infection in the past.[53–57] However, a gastrointestinal infection with

TABLE 2 Bioburden determination and identity of the bacterial species present in those samples that exceeded the US pharmacopoeia limits.

No.	Microbial load			Identified microorganism
1	TAMC	Not compliant	Biofilm	*Bacillus cereus* sensu stricto[a]
	TYMC	Compliant	—	
	VRBG	No growth	—	
2	TAMC	Complaint	—	n.a.
	TYMC	Compliant	—	
	VRBG	No growth	—	
3	TAMC	Compliant	—	n.a.
	TYMC	Compliant	—	
	VRBG	No growth	—	
4	TAMC	Compliant	≈60 CFU/g	*Metabacillus halosaccharovorans* and *Lysinibacillus xylanilyticus*
	TYMC	Compliant	≈40 CFU/g	
	VRBG	No growth	—	
5	TAMC	Not compliant	Biofilm	*Bacillus infantis*
	TYMC	Compliant	—	
	VRBG	No growth	—	
6	TAMC	Not compliant	Biofilm	*B. cereus* sensu stricto[a]
	TYMC	Compliant	≈25 CFU/g	
	VRBG	No growth	—	
7	TAMC	Not compliant	Biofilm	*Cytobacillus oceanisediminis and Robertmurraya* sp.
	TYMC	Compliant	—	
	VRBG	No growth	—	
8	TAMC	Compliant	—	n.a.
	TYMC	Compliant	—	
	VRBG	No growth	—	
9	TAMC	Compliant	—	n.a.
	TYMC	Compliant	—	
	VRBG	No growth	—	
10	TAMC	Compliant	—	n.a
	TYMC	Compliant	—	
	VRBG	No growth	—	
11	TAMC	Compliant	—	n.a
	TYMC	Compliant	≈25 CFU/g	
	VRBG	No growth	—	
12	TAMC	Not compliant	Biofilm	*Robertmurraya* sp.
	TYMC	Compliant	—	
	VRBG	No growth	—	
13	TAMC	Not compliant	Biofilm	*Bacillus* sp.
	TYMC	Compliant	—	
	VRBG	No growth	—	
14	TAMC	Not compliant	Biofilm	*Bacillus licheniformis*
	TYMC	Compliant	—	
	VRBG	No growth	—	
15	TAMC	Compliant	—	n.a
	TYMC	Compliant	—	
	VRBG	No growth	—	

(Continues)

TABLE 2 (Continued)

No.	Microbial load			Identified microorganism
16	TAMC			n.a.
	TYMC	Compliant	—	
	VRBG	No growth	—	
17	TAMC	Not compliant	Biofilm	*Mesobacillus campisaliis*
	TYMC	Compliant	—	
	VRBG	No growth	—	
18	TAMC	Not compliant	Biofilm	*Bacillus subtilis*
	TYMC	Compliant	—	
	VRBG	No growth	—	
19	TAMC	Not compliant	Biofilm	*B. subtilis*
	TYMC	Compliant	—	
	VRBG	No growth	—	

Abbreviations: CFU, colony-forming units; n.a., not applicable; TAMC, total aerobic microbial count; TYMC, total yeast and mold count; VRBG, violet red bile glucose.
[a]Species determined by whole genome sequencing.

non-emetic *B. cereus* s.l. strains also can result in uncomfortable symptoms such as diarrhea and abdominal pain.[58,59] These symptoms are due to the production of cytotoxin K, hemolysin BL, and nonhemolytic enterotoxin, for which all genes were accounted for in the isolated strains. These toxins are produced in the intestine by viable enteropathogenic *B. cereus* s.l. after the acid-resistant spores have passed the stomach.[60] Predicting the course of disease or evaluating the health risk associated with a contamination with enteropathogenic *B. cereus* s.l. is difficult as the actual toxin production is strain-specific and very variable.[58] Although the majority of the gastrointestinal infections with (non-emetic) *B. cereus* s.l. resolves relatively quicky, more severe complications might be occurring in people with a weak or compromised immune system[53] or when the bacteria end up in the blood stream or other organs. There, non-emetic *B. cereus* s.l. bacteria can cause serious adverse effects and even result in necrosis and death.[61] Additionally, the other samples were also contaminated with more "exotic" *Bacillus* species or species from related genera, for which little to no information is available on their pathogenicity. Interestingly, some of them are endemic to South and Southeast Asia, concurring the claimed provenance of some samples.

Two other samples also exceeded the tolerated amount of the residual solvent 2-propanol by at least a factor two, with a maximum value of 1.5 mg 2-propanol per tablet. It is unlikely that this will result in an acute intoxication as previous studies have shown that the oral intake of up to 6 mg 2-propanol per kg bodyweight for 6 weeks had no acute effect.[62] Furthermore, one sample contained an undeclared API (cetirizine), which could inflict an anaphylactic shock upon oral intake, although this severe reaction to cetirizine is rather rare.[45] Despite the fact that the number of samples analyzed was limited and was only seized in one European country during the COVID-19 pandemic, the findings illustrate to some extent the plethora of quality defects that can be encountered with SF medicines from rogue internet pharmacies. Additional and more dangerous quality defects might be a severe overdosing or the presence of a toxic contaminant at clinically relevant dosages. Nevertheless, all these seized products would have been taken without any medical supervision or therapeutic indication, while this prescription medicine was still undergoing clinical trials against COVID-19 and its effectivity or lack of it had yet to been proven. Although it is assumed that ivermectin is generally well tolerated, it can induce side effects such as diarrhea, nausea, skin rashes, and effects on the nervous system, including tremor, dizziness, confusion, and drowsiness.[63,64] In higher doses and overdose cases, these side effects can be more severe and it can even induce comas.[14–18,64,65] Therefore, it remains pivotal to warn patients of the possible direct risks associated with self-medication with prescription medicines, including the use and procurement of SF medicines.

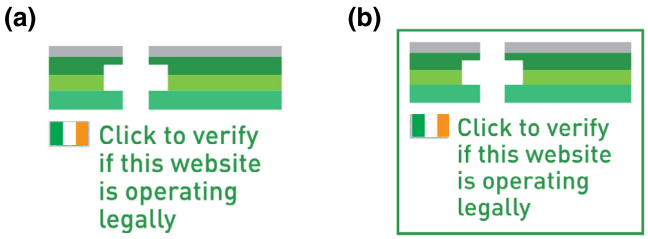

FIGURE 3 Examples of the European Union (EU) logo for either a colored background (a) or a white background (b). The logo consists of a typical pictogram of a white cross with four green lines, the national flag of the EU member state, and the textual part which, depending on the EU member state, can be in the different national languages. More detailed information can be found on the dedicated EU page.[48]

The European Union took a common initiative against SF medicines that was concretized in the falsified medicines directive 2011/62/EU. One of the mandatory actions put forward by this directive is the use of a specific logo (see Figure 3) for websites of legally operating online pharmacies/retailers since 2015.[66] Upon clicking on

the logo, a link is made to the website of the national competent authority listing all legally operating online pharmacies/retailers, which should list the pharmacy of choice.[67] If this redirection does not happen or no logo is present on the site, very likely the site corresponds to a rogue online pharmacy. Some European Union states, including Belgium, even go further and limit the online access to prescription medicines, still requiring a patient to get the desired prescription medicines at traditional brick-and-mortar pharmacies. Some efforts are still required to create awareness of the importance of this logo as people might still not be aware.

Similar to previous studies with antibiotics and antimalaria medicine, our data illustrate a systematic underdosing of the API in SF medicines (reviewed in the literature[68,69]). Although this underdosing of ivermectin does not represent an additional risk as those described above for those that self-administer this API to treat or prevent COVID-19, this could represent a high risk for those that need this treatment to combat parasitic infections. These patients will then be denied of the proper amount of essential medicine. Therefore, the use of these SF medicines in low- or middle-income countries, where the drug is used for the treatment of parasitic infections of humans, can be considered of higher risk because this drug is generally taken every 3 to 6 months for at least a decade.[70] Moreover, one sample displayed a large variation in the amount of API present in the different pills originating from the same blister. Furthermore, it might be possible that some samples have dissolution profiles with low equivalence, as often seen with substandard and falsified medicines.[71–73] This phenomenon may be the cause of insufficient drug efficacy and can result in the aggravation of illness or even the induction of resistance. Although this dissolution test is a mandatory test according to the USP monography for ivermectin tablets,[38] unfortunately, it was impossible to perform this assay on the samples as a large amount of material was required, which was not available. Taken together, the findings are quite worrisome as parallels can be drawn with the effect that improper usage and dosage had on antibiotics resistance. Indeed, recently emerging reports suggested that improper usage and dosage of ivermectin medication might also facilitate ivermectin resistance or tolerance in parasites.[74–77] Therefore, a continuous vigilance of international and national regulatory health agencies in low-, middle-, and high-income countries remains essential in order to curtail the trafficking and use of SF antiparasitic products and the consequential increase of parasite tolerance due to improper usage or dosage of these products. Certainly, bearing in mind that the number of newly developed APIs that can safely be used to combat parasites is rather scarce.[78]

ORCID

Celine Vanhee https://orcid.org/0000-0002-6634-3706
Bram Jacobs https://orcid.org/0000-0001-7307-9619

REFERENCES

1. Tan WJ, Zhao X, Ma XJ, et al. A novel coronavirus genome identified in a cluster of pneumonia cases—Wuhan, China 2019−2020. *China CDC Wkly*. 2020;2(4):61-62. doi:10.46234/ccdcw2020.017
2. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727-733. doi:10.1056/NEJMoa2001017
3. World Health Organization. Coronavirus disease (COVID-19) pandemic 2023. Accessed September 19, 2023. https://www.who.int/europe/emergencies/situations/covid-19
4. Caly L, Wagstaff KM, Jans DA. Nuclear trafficking of proteins from RNA viruses: potential target for anti-virals? *Antiviral Res*. 2012;95(3):202-206. doi:10.1016/j.antiviral.2012.06.008
5. Wagstaff KM, Sivakumaran H, Heaton SM, Harrich D, Jans DA. Ivermectin is a specific inhibitor of importin alpha/beta mediated nuclear import able to inhibit replication of HIV-1 and dengue virus. *Biochem J*. 2012;443(3):851-856. doi:10.1042/BJ20120150
6. Lundberg L, Pinkham C, Baer A, et al. Nuclear import and export inhibitors alter capsid protein distribution in mammalian cells and reduce Venezuelan equine encephalitis virus replication. *Antiviral Res*. 2013;100(3):662-672. doi:10.1016/j.antiviral.2013.10.004
7. Götz V, Magar L, Dornfeld D, et al. Influenza A viruses escape from MxA restriction at the expense of efficient nuclear vRNP import. *Sci Rep*. 2016;6(1):23138. doi:10.1038/srep23138
8. Jans DA, Martin AJ, Wagstaff KM. Inhibitors of nuclear transport. *Curr Opin Cell Biol*. 2019;58:50-60. doi:10.1016/j.ceb.2019.01.001
9. Laing R, Gillan V, Devaney E. Ivermectin—old drug, new tricks? *Trends Parasitol*. 2017;33(6):463-472. doi:10.1016/j.pt.2017.02.004
10. Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Res*. 2020;3:104787. doi:10.1016/j.antiviral.2020.104787
11. Bray M, Rayner C, Noël F, Jans D, Wagstaff K. Ivermectin and COVID-19: a report in antiviral research, widespread interest, an FDA warning, two letters to the editor and the authors' responses. *Antiviral Res*. 2020;78:104805. doi:10.1016/j.antiviral.2020.104805
12. Marcolino MS, Meira KC, Guimarães NS, et al. Systematic review and meta-analysis of ivermectin for treatment of COVID-19: evidence beyond the hype. *BMC Infect Dis*. 2022;22(1):639. doi:10.1186/s12879-022-0
13. Schellack N, Strydom M, Pepper MS, et al. Social media and COVID-19-perceptions and public deceptions of ivermectin, colchicine and hydroxychloroquine: lessons for future pandemics. *Antibiotics (Basel)*. 2022;11(4):445. doi:10.3390/antibiotics11040445
14. Temple C, Hoang R, Hendrickson RG. Toxic effects from ivermectin use associated with prevention and treatment of Covid-19. *N Engl J Med*. 2021;385(23):2197-2198. doi:10.1056/NEJMc2114907
15. United States Food and Drug Administration, Ivermectin intended for animals: letter to stakeholders—do not use in humans as a treatment for COVID-19 2020. Accessed September 19, 2023. https://www.fda.gov/safety/medical-product-safety-information/ivermectin-intended-animals-letter-stakeholders-do-not-use-humans-treatment-covid-19
16. Centers of Disease Control, Rapid increase in ivermectin prescriptions and reports of severe illness associated with use of products containing ivermectin to prevent or treat COVID-19 2021. Accessed September 19, 2023. https://emergency.cdc.gov/han/2021/pdf/CDC_HAN_449.pdf
17. Barac A, Bartoletti M, Azap O, et al. Inappropriate use of ivermectin during the COVID-19 pandemic: primum non nocere! *Clin Microbiol Infect*. 2022;28(7):908-910. doi:10.1016/j.cmi.2022.03.022
18. Campillo JT, Faillie JL. Adverse drug reactions associated with ivermectin use for COVID-19 reported in the World Health Organization's pharmacovigilance database. *Therapie*. 2022;77(6):747-749. doi:10.1016/j.therap.2022.03.002
19. Fittler A, Adeniye L, Katz Z, Bella R. Effect of infodemic regarding the illegal sale of medications on the internet: evaluation of demand and online availability of ivermectin during the COVID-19 pandemic. *Int J Environ Res Public Health*. 2021;18(14):7475. doi:10.3390/ijerph18147475

20. Swiss medic, Illegal imports 2021: Swissmedic seizes significantly more packages than in 2020. 2022. Accessed September 19, 2023. https://www.swissmedic.ch/swissmedic/en/home/humanarzneimittel/market-surveillance/medicinal-products-from-the-internet/statistical-data/importe-illegaler-am-2021.html
21. Australian government, the therapeutic good administration (TGA). TGA warns against buying ivermectin online from unverified sources. *Reactions Weekly*. 2022;1887(1):7. doi:10.1007/s40278-022-07672-3
22. World Health Organization WHO global surveillance and monitoring system for substandard and falsified medical products. World Health Organization. 2017. Accessed September 19, 2023. https://apps.who.int/iris/handle/10665/326708
23. The organization for economic cooperation and development (OECD). *Trade in Counterfeit Pharmaceutical Products, Illicit Trade, OECD*. Vol. 2020. Publishing; 2020. doi:10.1787/a7c7e054-en
24. Fadeyi I, Lalani M, Mailk N, Van Wyk A, Kaur H. Quality of the antibiotics—amoxicillin and co-trimoxazole from Ghana, Nigeria, and the United Kingdom. *Am J Trop Med Hyg*. 2015;92(6 Suppl):87-94. doi:10.4269/ajtmh.14-0539
25. Kaur H, Allan EL, Mamadu I, et al. Prevalence of substandard and falsified artemisinin-based combination antimalarial medicines on Bioko Island. *Equatorial Guinea BMJ Glob Health*. 2017;2(4):e000409. doi:10.1136/bmjgh-2017-000409
26. Kao SL, Chan CL, Tan B, et al. An unusual outbreak of hypoglycemia. *N Engl J Med*. 2009;360(7):734-736. doi:10.1056/NEJMc0807678
27. Jackson G, Arver S, Banks I, Stecher VJ. Counterfeit phosphodiesterase type 5 inhibitors pose significant safety risks. *Int J Clin Pract*. 2010;64(4):497-504. doi:10.1111/j.1742-1241.2009.02328.x
28. Deconinck E, Canfyn M, Sacré PY, Courselle P, De Beer JO. Evaluation of the residual solvent content of counterfeit tablets and capsules. *J Pharm Biomed Anal*. 2013;81-82:80-88. doi:10.1016/j.jpba.2013.03.023
29. Pullirsch D, Bellemare J, Hackl A, et al. Microbiological contamination in counterfeit and unapproved drugs. *BMC Pharmacol Toxicol*. 2014;15(1):34. doi:10.1186/2050-6511-15-34
30. Tie Y, Adams E, Deconinck E, Vanhee C. Substandard and falsified antimicrobials: a potential biohazard in disguise? *Drug Test Anal*. 2020;12(2):285-291. doi:10.1002/dta.2740
31. Vanhee C, Jacobs B, Mori M, et al. Uncovering the quality deficiencies with potentially harmful effects in substandard and falsified PDE-5 inhibitors seized by Belgian controlling agencies. *Forensic Sciences*. 2023;3(3):426-451. doi:10.3390/forensicsci3030031
32. Tie Y, Vanhee C, Deconinck E, Adams E. Development and validation of chromatographic methods for screening and subsequent quantification of suspected illegal antimicrobial drugs encountered on the Belgian market. *Talanta*. 2019;194:876-887. doi:10.1016/j.talanta.2018.10.078
33. International Conference on Harmonisation (ICH) of technical requirements for registration of pharmaceuticals for human use—validation of analytical procedures. Accessed 30/05/2023. Available online: https://database.ich.org/sites/default/files/Q2(R1)Guideline.pdf
34. Feinberg M. Validation of analytical methods based on accuracy profiles. *J Chromatogr a*. 2007;1158(1-2):174-183. doi:10.1016/j.chroma.2007.02.021
35. Feinberg M, Laurentie M. A global approach to method validation and measurement uncertainty. *Accredit Qual Assur*. 2006;11(1-2):3-9. doi:10.1007/s00769-005-0081-9
36. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories. 2005.
37. Vanhee C, Barhdadi S, Kamugisha A, et al. The development and validation of a targeted LC-HRAM-MS/MS methodology to separate and quantify *p*-Synephrine and *m*-Synephrine in dietary supplements and herbal preparations. *Separations*. 2023;10(8):444. doi:10.3390/separations10080444
38. *United States Pharmacopoeia 42*. United States Pharmacopeial Convention, Inc.; 2022.
39. *European Pharmacopoeia 10.0*. Council of Europe, Strasbourg; 2022.
40. Jolley KA, Maiden MC. BIGSdb: scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics*. 2010;11(1):595. doi:10.1186/1471-2105-11-595
41. Carroll LM, Cheng RA, Kovac J. No assembly required: using BTyper3 to assess the congruency of a proposed taxonomic framework for the *Bacillus cereus* group with historical typing methods. *Front Microbiol*. 2020;11:580691. doi:10.3389/fmicb.2020.580691
42. Bogaerts B, Fraiture MA, Huwaert A, et al. Retrospective surveillance of viable *Bacillus cereus* group contaminations in commercial food and feed vitamin B2 products sold on the Belgian market using whole-genome sequencing. *Front Microbiol*. 2023;14:1173594. doi:10.3389/fmicb.2023.1173594
43. Kircik LH, Del Rosso JQ, Layton AM, Schauber J. Over 25 years of clinical experience with ivermectin: an overview of safety for an increasing number of indications. *J Drugs Dermatol*. 2016;15(7):325-3903. doi:10.1128/AAC.02412-14
44. Stong JD, Pivnichny JV, Mrozik H, Waksmunski FS. Radical-induced oxidation of oxahydrindene (hexahydrobenzofuran) portion of 22,23-dihydroavermectin B_{1a}: identification of autooxidation products. *J Pharm Sci*. 1992;81(10):1000-1003. doi:10.1002/jps.2600811008. PMID: 1432608
45. Afonso N, Shetgaonkar P, Dang A, Rataboli PV. Cetirizine-induced anaphylaxis: a rare adverse drug reaction. *Br J Clin Pharmacol*. 2009;67(5):577-578. doi:10.1111/j.1365-2125.2009.03394.x
46. International Conference on Harmonisation (ICH) of technical requirements for the registration of pharmaceuticals for human use, Q3C (R8): impurities: guidelines for residual solvents, Step 4 2021. Accessed September 19, 2023. https://database.ich.org/sites/default/files/ICH_Q3C-R8_Guideline_Step4_2021_0422_1.pdf
47. Patel S, Gupta RS. A phylogenomic and comparative genomic framework for resolving the polyphyly of the genus *Bacillus*: proposal for six new genera of *Bacillus* species, *Peribacillus* gen. nov., *Cytobacillus* gen. nov., *Mesobacillus* gen. nov., *Neobacillus* gen. nov., *Metabacillus* gen. nov. and *Alkalihalobacillus* gen. nov. *Int J Syst Evol Microbiol*. 2020;70(1):406-438. doi:10.1099/ijsem.0.003775
48. Ehling-Schulz M, Lereclus D, Koehler TM. The *Bacillus cereus* group: *Bacillus* species with pathogenic potential. *Microbiol Spectr*. 2019;7. doi:10.1128/microbiolspec.GPP3-0032-0018
49. Citron DM, Appleman MD. In vitro activities of daptomycin, ciprofloxacin, and other antimicrobial agents against the cells and spores of clinical isolates of *Bacillus* species. *J Clin Microbiol*. 2006;44(10):3814-3818. doi:10.1128/JCM.00881-06
50. Fiedler G, Schneider C, Igbinosa EO, et al. Antibiotics resistance and toxin profiles of *Bacillus cereus*-group isolates from fresh vegetables from German retail markets. *BMC Microbiol*. 2019;19(1):250. doi:10.1186/s12866-019-1632-2
51. Bianco A, Capozzi L, Monno MR, et al. Characterization of *Bacillus cereus* group isolates from human bacteremia by whole-genome sequencing. *Front Microbiol*. 2021;11:599524. doi:10.3389/fmicb.2020.599524
52. Fraccalvieri R, Bianco A, Difato LM, et al. Toxigenic genes, pathogenic potential and antimicrobial resistance of *Bacillus cereus* group isolated from ice cream and characterized by whole genome sequencing. *Foods*. 2022;11(16):2480. doi:10.3390/foods11162480
53. Orrick MJ, Bush AM, Junga Z. A cereus-ly rare cause of chronic diarrhea. *Am J Gastroenterol*. 2021;116(1):S1226. doi:10.14309/01.ajg.0000785384.55403.41
54. Lund T, De Buyser ML, Granum PE. A new cytotoxin from *Bacillus cereus* that may cause necrotic enteritis. *Mol Microbiol*. 2000;38(2):254-261. doi:10.1046/j.1365-2958.2000.02147.x

55. Dierick K, Van Coillie E, Swiecicka I, et al. Fatal family outbreak of *Bacillus cereus*-associated food poisoning. *J Clin Microbiol*. 2005;43(8):4277-4279. doi:10.1128/JCM.43.8.4277-4279.2005
56. Naranjo M, Denayer S, Botteldoorn N, et al. Sudden death of a young adult associated with *Bacillus cereus* food poisoning. *J Clin Microbiol*. 2011;49(12):4379-4381. doi:10.1128/JCM.05129-11
57. Colaco CMG, Basile K, Draper J, Ferguson PE. Fulminant *Bacillus cereus* food poisoning with fatal multi-organ failure. *BMJ Case Rep*. 2021;18(14):e238716. doi:10.1136/bcr-2020-238716
58. Schoeni JL, Wong AC. *Bacillus cereus* food poisoning and its toxins. *J Food Prot*. 2005;68(3):636-648. doi:10.4315/0362-028x-68.3.636
59. Jessberger N, Dietrich R, Granum PE, Märtlbauer E. The *Bacillus cereus* food infection as multifactorial process. *Toxins*. 2020;12(11):701. doi:10.3390/toxins12110701
60. Ceuppens S, Uyttendaele M, Drieskens K, Rajkovic A, Boon N, Wiele TV. Survival of *Bacillus cereus* vegetative cells and spores during in vitro simulation of gastric passage. *J Food Prot*. 2012;75(4):690-694. doi:10.4315/0362-028X.JFP-11-481
61. Bottone EJ. *Bacillus cereus*, a volatile human pathogen. *Clin Microbiol Rev*. 2010;23(2):382-398. doi:10.1128/CMR.00073-09
62. Wills JH, Jameson EM, Coulston F. Effects on man of daily ingestion of small doses of isopropyl alcohol. *Toxicol Appl Pharmacol*. 1969;15(3):560-565. doi:10.1016/0041-008x(69)90058-1
63. United States Food and Drug Administration, Leaflet Stromectol® tablets. 2009. Accessed September 23, 2023. https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/050742s026lbl.pdf
64. Chandler RE. Serious neurological adverse events after ivermectin—do they occur beyond the indication of onchocerciasis? *Am J Trop Med Hyg*. 2018;98(2):382-388. doi:10.4269/ajtmh.17-0042
65. Dy JSH, Juangco DN. Identifying the toxidrome of ivermectin toxicity. *Cureus*. 2023;15(7):e42603. doi:10.7759/cureus.42603
66. European commission. The falsified medicines directive 2011/62/EU. 2011. Accessed September 19, 2023. https://health.ec.europa.eu/system/files/2016-11/dir_2011_62_en_0.pdf
67. European commission. EU logo for online sale of medicines. 2017. Accessed September 19, 2023. https://health.ec.europa.eu/system/files/2016-11/logosancointernet_charte_v2_0.pdf
68. Johnston A, Holt DW. Substandard drugs: a potential crisis for public health. *Br J Clin Pharmacol*. 2014;78(2):218-243. doi:10.1111/bcp.12298
69. Chaccour C, Kaur H, Del Pozo JL. Falsified antimalarials: a minireview. *Expert Rev Anti Infect Ther*. 2015;13(4):505-509. doi:10.1586/14787210.2015.1015990
70. Crump A. Ivermectin: enigmatic multifaceted 'wonder' drug continues to surprise and exceed expectations. *J Antibiot (Tokyo)*. 2017;70(5):495-505. doi:10.1038/ja.2017.11
71. Deconinck E, Andriessens S, Bothy JL, Courselle P, De Beer JO. Comparative dissolution study on counterfeit medicines of PDE-5 inhibitors. *J Pharm Anal*. 2014;4(4):250-257. doi:10.1016/j.jpha.2014.03.002
72. Tie Y, van Loock K, Deconinck E, Adams E. Evaluation of impurities and dissolution profiles of illegal antimicrobial drugs encountered in Belgium. *Drug Test Anal*. 2020;12(1):53-66. doi:10.1002/dta.2690
73. Rahman MS, Yoshida N, Tsuboi H, et al. Patient safety and public health concerns: poor dissolution rate of pioglitazone tablets obtained from China, Myanmar and internet sites. *BMC Pharmacol Toxicol*. 2021;22(1):12. doi:10.1186/s40360-021-00478-x
74. Seyoum Z, Zewdu A, Dagnachew S, Bogale B. Anthelmintic resistance of strongyle nematodes to ivermectin and fenbendazole on cart horses in Gondar, Northwest Ethiopia. *Biomed Res Int*. 2017;2017:5163968. doi:10.1155/2017/5163968
75. Fissiha W, Kinde MZ. Anthelmintic resistance and its mechanism: a review. *Infect Drug Resist*. 2021;4:5403-5410. doi:10.2147/IDR.S332378
76. Flota-Burgos GJ, Rosado-Aguilar JA, Rojas-Becerril R, Rodríguez-Vivas RI, Trinidad-Martínez I. Evidence of resistance to ivermectin in the gastrointestinal nematodes of horses from Mexican southeast. *Vet Parasitol Reg Stud Reports*. 2023;44:100907. doi:10.1016/j.vprsr.2023.100907.223
77. Mbuagbaw L, Sadeghirad B, Morgan RL, et al. Failure of scabies treatment: a systematic review and meta-analysis. *Br J Dermatol*. 2023. doi:10.1093/bjd/ljad308
78. Selzer PM, Epe C. Antiparasitics in animal health: quo vadis? *Trends Parasitol*. 2021;37(1):77-89. doi:10.1016/j.pt.2020.09.004

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Vanhee C, Jacobs B, Kamugisha A, et al. Substandard and falsified ivermectin tablets obtained for self-medication during the COVID-19 pandemic as a source of potential harm. *Drug Test Anal*. 2023;1-11. doi:10.1002/dta.3618