



# Viral, bacterial, and fungal contamination of Automated Teller Machines (ATMs)

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## Abstract

**Introduction and Objective.** While the qualitative information about bacterial and fungal pollution of automated teller machine (ATM) surfaces is available in the scientific literature, there are practically no studies precisely quantifying this type of contamination. Regarding viruses, such data in relation to ATM surfaces are not available at all.

**Materials and method.** The quantitative and qualitative control of adeno- and coronaviruses, including SARS-CoV-2 (based on qPCR/RT-qPCR and v-qPCR/v-RT-qPCR), bacterial and fungal contaminants (based on morphological and biochemical characteristics followed by PCR/RAPD typing) deposited on internal and external ATM surfaces (swab sampling), as well as present in the air of premises housing the ATM machines (inertial impaction sampling) belonging to the network of one of the largest Polish banks was performed.

**Results.** As the air of premises housing ATMs was relatively clean, the internal (i.e. safe boxes and cash dispenser tracks) and external (i.e. touch screens and keypads) ATM surfaces were heavily polluted, reaching 599 CFU/cm<sup>2</sup>, 522 CFU/cm<sup>2</sup>, 17288 gc/cm<sup>2</sup> and 2512 gc/cm<sup>2</sup> for bacterial, fungal, coronaviral and adenoviral contaminants, respectively. The application of propidium monoazide (PMA) dye pretreatment for v-qPCR/v-RT-qPCR allows detection of the potentially infectious SARS-CoV-2 and adenoviral particulates on ATM surfaces.

**Conclusions.** The packaged banknotes and people involved in their distribution, as well as general population using ATMs, can be the sources of this type of contamination and its potential victims. Highly efficient hygienic measures should be introduced to prevent unwanted pollution of both the distributed means of payment and ATM surfaces, and to avoid subsequent dissemination of microbial contaminants.

## Key words

fungi, bacteria, viruses, Automated Teller Machine, surface contamination, bioaerosol

## INTRODUCTION

An Automated Teller Machine (ATM) is a computerized device the appearance of which and subsequent popularization has revolutionized the field of banking and changed the way different financial institutions interact with their customers. The ATM idea was created in 1939 by the Turkish-born American inventor Luther G. Simjian as a ‘hole-in-the-wall machine’ that would allow bank customers to make financial transactions. The first ATM prototype called a ‘bankograph’ appeared 21 years later, and since that moment, the idea of such self-service banking has been constantly growing. According to Retail Banking Research, as of 2020 there were 3.9 million ATMs across the globe. In most established markets, the number of ATMs continues to increase, indicating that, despite the challenge posed by alternative payment methods, such as contactless cards and mobile payments, the use of cash remains resilient [1]. Today, ATMs allow bank customers to check their account balances, withdraw or deposit cash, move funds between accounts, exchange currency, and print a statement of account transactions, making such financial services available twenty-four hours a day, seven days a week.

Cash remains the preferred means of payment in number terms for day-to-day purchases [2]. If the means of payment are microbiologically contaminated, then these contaminants can also be deposited on banknote counting, sorting and paying machines, thus creating real health risks for people who professionally handle and refill the ATMs, or use them as regular customers.

The hitherto obtained data reveal that frequently touched ATM surfaces are microbiologically contaminated and the microbiota present can be qualitatively very diverse. Researchers from Europe [3, 4], North America [5], South America [6], Africa [7, 8], and Asia [9, 10, 11] most often isolated the following microorganisms from the ATM surfaces: from among bacteria – *Staphylococcus aureus*, *S. epidermidis*, *Bacillus subtilis*, *B. cereus*, *Acinetobacter junii*, *A. baumannii*, *Neisseria macacae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *K. edwardsii*, *Proteus vulgaris*, *P. mirabilis*, *Morganella morganii*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Serratia spp.*, *Micrococcus spp.*, *Enterococcus faecalis*, *E. cecorum*, *Streptococcus spp.*, *Enterobacter spp.*, *Citrobacter spp.*, *Listeria monocytogenes*, and *L. innocui*; out of fungi, the species from *Candida* (including *C. krusei*, *C. glabrata*, *C. tropicalis*, *C. albicans*), *Rhodotorula*, *Geotrichum*, *Aspergillus* (*A. niger*), *Penicillium*, *Mucor*, *Rhizopus*, *Fusarium*, *Acremonium*, *Alternaria*, *Cladophialophora*, *Cladosporium*, *Cunninghamella*, *Curvularia*, *Drechslera*, *Scopulariopsis*, and *Trichoderma* genera.

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In the scientific literature, there are numerous studies devoted to the quantitative and qualitative assessment of microbial contamination of banknotes and coins. Evidence of such pollution has already been presented by researchers from all continents. Microbial contamination may affect up to 100% of the means of payment in circulation [12, 13, 14]. In extreme cases, the degree of contamination may reach  $10^9$  colony-forming units (CFU) per one banknote [15]. While the qualitative information about currency pollution is available, knowledge about the hygienic conditions of its packaging or distribution is practically unavailable in the scientific literature [16]. With regard to the bacterial and fungal pollution of ATMs, there are practically no studies precisely quantifying this type of contamination. With reference to the qualitative and quantitative identification of health threats caused by viruses, such data in relation to ATM surfaces having contact with means of payment are not available at all in the global literature on the subject. Hence, the aim of this study was to fill this gap by assessing viral, bacterial, and fungal contaminations of internal and (the most frequently touched) external ATM surface, as well as the air pollution in premises with ATMs.

## MATERIALS AND METHODS

**Sampling sites and sample types.** The 28 studied ATMs belonged to branches of the network of one of the largest Polish banks. All of them were located in urban areas in four cities with a population of around 20,000 and two cities with a population of over 700,000 in two provinces (in southern and central Poland). Together these two provinces constitute 16% of the area of Poland, with urbanization rates of 48% and 64%, respectively, and the shares of agricultural land in relation to the entire area of the provinces – 45% and 55%, respectively. Regarding their demographics profiles, both provinces are ethnically homogeneous with a 96.7% ‘Caucasian’ population.

All studied rooms housing the ATM machines were naturally ventilated. Their cubic capacities ranged from  $15\text{ m}^3$  –  $40\text{ m}^3$ . In none of the examined premises, both the history and current visible signs of moisture damages indicating hidden microbial growth, were noted. During the measurements, only the person performing the sampling was present in the studied rooms and, due to COVID-19 pandemic restrictions, this person was wearing rubber gloves and a face mask to cover the nose and mouth. The control of microbiological pollution was performed through the quantitative and qualitative analyzes of viral (adenoviruses, AdVs, and coronaviruses, CoVs, including SARS-CoV-2), bacterial, and fungal contaminants present in dust settled on internal and external ATM surfaces, as well as suspended in the air of the premises housing the ATM machines. All 28 ATMs were tested for bacterial and fungal contamination. Among them, 14 machines allowed bank customers not only to withdraw but also to deposit money, and those were additionally tested for viral pollution.

**Surface sampling.** The surface samples were always collected by swabbing. The settled dust was gathered from internal (i.e. safe boxes and cash dispenser tracks having direct contact with banknotes), and external (the most frequently touched parts, i.e. touch screens and keypads) surfaces of

ATMs using a sterile square-shaped ( $10 \times 10\text{ cm}$ ) template (COPAN Diagnostics, Murrieta, USA). For quantitative and qualitative assessment of corona- and adenoviruses, the samples were taken with sterile polyester fibre-tipped swabs prewetted in universal ViCUM<sup>®</sup> liquid transport medium formulated with a solution of balanced salts, bovine serum albumin, HEPES buffer, antibiotics (vancomycin) and antifungals (amphotericin B) (Deltaswab PurFlock Ultra ViCUM; Deltalab, Barcelona, Spain), which ensures the most effective recovery of viruses from nonporous fomites [17]. After sampling, the swabs were placed in test tubes filled with ViCUM<sup>®</sup> medium and glass beads and immediately (i.e. on the same day) transported (in a vertical position in a thermo container at  $4^\circ\text{C}$ ) to the laboratory for further analysis. In total, 14 coronaviral and 14 adenoviral samples from ATM surfaces were collected. Each sample was analyzed in duplicate.

In turn, for quantitative and qualitative analyzes of bacteria and fungi, samples were collected using sterile nylon flocked applicator (eSwab<sup>®</sup>; Copan Italia S.p.A., Brescia, Italy), subsequently immersed in Amies liquid and immediately transported (as described above) to the laboratory for further analysis. In total, 28 bacterial and 28 fungal samples were collected from ATM surfaces. Also in this case, each sample was analyzed in duplicate.

Upon arrival at the laboratory, all samples were processed immediately or (if the return from field measurements occurred in the evening or at night) placed in a refrigerator at  $4^\circ\text{C}$  until the next day [18, 19]. The samples were always processed not later than 24 hours from the moment of their collection.

**Laboratory elaboration of viral surface samples.** The shafts were cut off from the swabs and the remaining polyester fibre-tips with collected dust were vortexed thoroughly using a programmable rotator-mixer (Multi RS-60, Biosan, Riga, Latvia) at 800 rpm for 30 min at room temperature. The obtained suspensions were then concentrated using Amicon<sup>®</sup> Ultra-15 with 30 KDa cutoffs (Merck Millipore Ltd., Livingstone, UK) at  $3,200 \times g$  for 20 min at  $4^\circ\text{C}$  to the final volume of  $400\ \mu\text{l}$ . All processed samples were subsequently divided into two equal aliquots ( $200\ \mu\text{l}$ ). The first was intended for direct viral DNA/RNA extraction, the second for PMA dye pretreatment allowing detection of potentially infectious viral particles. According to Hong et al. [20], the PMA pretreatment combined with RT-PCR can discriminate between intact and damaged SARS-CoV-2 viral particles; this method is comparable to the gold standard plaque assay. Therefore, the samples were treated with PMAxx<sup>™</sup> Dye (20 mM in  $\text{H}_2\text{O}$ ; Biotium, Inc., Hayward, USA) for a final concentration of  $60\ \mu\text{M}$  [21]. Tubes were gently mixed by inverting several times and then incubated in the dark for 15 min at room temperature, rotated at 200 rpm. The treated samples were exposed to 40 W LED light with a wavelength of 460 nm for 15 min using a photo-activation system (PMA-Lite<sup>™</sup> LED Photolysis Device; Biotium, Inc.).

Extraction of viral DNA/RNA from both the treated ( $200\ \mu\text{l}$ ) and untreated ( $200\ \mu\text{l}$ ) aliquots was carried out with Kogene Power Prep Viral DNA/RNA Extraction Kit CE-IVD (Kogene Biotech, Seoul, South Korea), according to the manufacturer’s instructions, to produce a final volume of  $30\ \mu\text{l}$ . Obtained RNA/DNA samples were stored at  $-80^\circ\text{C}$  until further analysis.

Quantitative PCR/Reverse-Transcription quantitative PCR (qPCR/RT-qPCR) and viability quantitative PCR/viability Reverse-Transcription quantitative PCR (v-qPCR/v-RT-qPCR) assays were applied to confirm the presence of and quantify the abundance of viruses in the surface samples [22, 23]. Both qPCR/v-qPCR (for DNA viruses, i.e. AdVs) and RT-qPCR/v-RT-qPCR (for RNA viruses, i.e. CoVs), were performed using CFX96 real-time PCR thermocycler (Bio-Rad, Hercules, USA). The detection of AdVs and CoVs (SARS-CoV-2 and/or presumptive SARS-CoV-2, or other coronaviruses) were carried out with Adenovirus and SARS-CoV-2 VIASURE Real Time PCR Detection Kits (both: CerTest Biotec S.L., Zaragoza, Spain), respectively, according to procedures recommended by the manufacturer. The target genes employed for PCR-based detection and identification of viruses represent conserved regions with the hexon gene for AdVs and the ORF1ab and N genes for SARS-CoV-2. The VIASURE Real Time PCR Detection Kits utilize 8-well strips containing all the components necessary for the assay. In line with the EMMI guidelines [24], the key analytical data on the methods used and their control are given below. Each reaction mixture (20 µl) contained 5 µl of DNA/RNA sample, specific primers/probes, dNTPs, polymerase, and -in case of CoVs- reverse transcriptase, in a stabilized format, as well as an internal control to monitor PCR inhibition (IC), all reconstituted in 15 µl rehydration buffer. Reverse transcription for CoVs was carried out in a one-step procedure. AdV DNA target (hexon gene) was amplified and detected in the FAM channel and the internal control (IC) in HEX channel. In the case of CoV, the ORF1ab gene was amplified and detected in the FAM channel, N gene in ROX channel, and internal control (IC) in the HEX channel.

The cycling conditions for AdVs were as follows: polymerase activation at 95 °C for 2 min, then 45 cycles of denaturation at 95 °C for 10 s, and annealing at 60 °C for 50 s. In the case of CoVs, the reverse transcription at 45 °C for 15 min was followed by initial denaturation at 95 °C for 2 min, then 45 cycles of denaturation at 95 °C for 10 s, and annealing at 60 °C for 50 s. Both negative and positive controls, purchased from CerTest Biotec S.L., were included in each run. According to the manufacturer, the applied PCR kits have a detection limit of  $\geq 10$  RNA/DNA copies per reaction. In total, 14 treated and 14 untreated adenoviral, as well as 14 treated and 14 untreated coronaviral samples, were analyzed in duplicates. No inhibited samples were found.

All qPCR/RT-qPCR and v-qPCR/v-RT-qPCR data were collected and quantification cycles (Cq) were calculated using CFX96 manager software (Bio-Rad). A sample was considered positive when the obtained Cq value was below 40; internal (IC) and positive controls showed that the amplification signals and negative control did not reveal such a signal. In the case of SARS-CoV-2, if only the N gene target was positive, the interpretation was 'presumably positive for SARS-CoV-2', and the differentiation of SARS-CoV-2 from other coronaviruses, including animal varieties, requires further analysis. Quantification analyses were performed based on standard curves, obtained by amplification of positive control 10-fold dilutions (standard from  $1 \times 10^0$  to  $1 \times 10^5$  gene copies/µl), and log RNA/DNA copies were plotted against the Cq value. All tested samples showed amplification signal for internal control (IC). Based on each standard curve, the amplification efficiencies,  $E = 10^{-1/S} - 1$  (where

S is the slope of linear regression curve) were as follows:  $E_{\text{AdV hexon}} = 1.03$  ( $S = -3.26$ ,  $r^2 = 0.994$ ,  $y - \text{int} = 42.63$ ),  $E_{\text{CoV ORF1ab}} = 0.90$  ( $S = -3.58$ ,  $r^2 = 0.994$ ,  $y - \text{int} = 38.17$ ), and  $E_{\text{CoV N}} = 1.03$  ( $S = -3.25$ ,  $r^2 = 0.993$ ,  $y - \text{int} = 40.82$ ).

To minimize potential contamination, all analytical steps were performed in separate rooms, including RNA/DNA isolation, preparation of reagents, sample preparation, and amplification. All analyzes were carried out using only sterile RNase/DNase-free filter pipette tips. The obtained results were expressed as the number of viral genome copies per 100 cm<sup>2</sup> of tested surfaces (gc/100 cm<sup>2</sup>).

**Air sampling.** Bacterial and fungal particulates were gathered by applying inertial impaction. This technique uses a rapid change of air direction and the principle of inertia to separate solid particles from the air stream. Its most important advantage is the ability to simultaneously determine two key parameters of the aerosol, i.e. size and composition. Viable (understood in this study as culturable) bioaerosol samples were taken using a six-stage Andersen impactor (model WES-710, Westech Instrument, Upper Stondon, UK) at a flow rate of 28.3 l/min and at a height of 1–1.5 m above the floor level to simulate aspiration from the human breathing zone. At the beginning of each measurement cycle, bacterial aerosol was collected on blood trypticase soy agar (TSA 43001, bioMérieux, Marcy l'Etoile, France) and, after impactor reloading, fungi were aspirated on malt extract agar (MEA, Oxoid Ltd., Basingstoke, UK). Taking into account the limitations of Andersen impactor sampling (e.g. desiccation of agar surface, particle bounce and subsequent reaerosolization, friction during the passage of particles through the sampler, etc.) [25] and the expected bioaerosol concentrations in the tested premises [16], the sampling time was 5 min for both bacterial and fungal aerosols. Their concentrations were expressed as colony forming units in 1 m<sup>3</sup> of sampled air (CFU/m<sup>3</sup>).

**Laboratory elaboration of bacterial and fungal surface and air samples.** After surface sampling, in order to extract the collected microorganisms, the nylon flocked swabs were vortexed for 10 min using a programmable rotator-mixer (model Multi RS-60, Biosan, Riga, Latvia) in 5 ml distilled water. The spread plate method was applied, where 0.2 ml of the resulting suspension was spread evenly over the same media as used for bioaerosol sampling.

All impactor and settled dust samples were then incubated at the temperature of: bacteria – one day at 37 °C, followed by three days at 22 °C and three days at 4 °C (the extended incubation for bacteria allowed development of slow-growing strains at low temperatures); fungi – four days at 30 °C followed by four days at 22 °C [26]. After incubation, the viable microbial concentrations in the air and dust were calculated as colony forming units per 1 m<sup>3</sup> (CFU/m<sup>3</sup>) and per 1 cm<sup>2</sup> (CFU/cm<sup>2</sup>), respectively. Bacterial and yeast strains were identified by Gram staining (111885 Gram-color stain set, Merck KGaA, Darmstadt, Germany) [27], their morphology, and finally, by the biochemical API tests (bioMérieux). Filamentous fungi were identified according to their morphology using several identification keys [28, 29, 30, 31, 32]. The pathogens and species less frequently isolated in a non-occupational indoor environment were additionally analyzed by molecular methods (polymerase chain reaction (PCR) followed by random amplification of



polymorphic DNA-RAPD typing). In total, 8 (including one pathogenic) bacterial and nine (including five pathogenic) fungal species were analyzed in that way. DNA was isolated from pure bacterial/fungal cultures grown on TSA/MEA plates using QIAmp DNA (Qiagen, Hilden, Germany) or Fungi DNA (Syngen Biotech, Wrocław, Poland) Mini Kits. The isolated bacterial DNA was used as a template in PCR with BAK11w (5'-AGTTTGATCMTGGCTCAG-3') and BAK2 (5'-GGACTACHAGGGTATCTAAT-3') primer sets, which allow amplification of bacterial 16S rRNA gene fragments corresponding to *Escherichia coli* 16S rRNA gene positions 10 – 806. The isolated fungal DNA was used as a template in PCR with ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primer sets, which allow amplification of fungal genome fragment located between 18S and 28S rRNA genes, covering ITS1, 5.8S rRNA, and ITS2 fragments. The amplified PCR products were purified, sequenced using DNA analyzer (model 3730; Applied Biosystems, Waltham, USA), and compared with the GenBank database (National Center for Biotechnology Information, US National Library of Medicine, USA) using the BLAST (Basic Local Alignment Search Tool) algorithm [33].

**Control of microclimate parameters.** The measurements were carried out in the 'summer season', defined as the period from May – September, when the average outdoor air temperature was above 10°C for at least seven consecutive days. During the collection of microbial samples, the air temperature and relative humidity, as major microclimate parameters influencing the growth of studied microbiota, were measured using a hytherograph (model Omniport 20, E+E Elektronik GmbH, Engerwitzdorf, Austria). All microclimate parameter measurements were performed in triplicate.

**Statistical analysis.** Due to the normal distribution of the vast majority of the collected data (analyzed by Shapiro-Wilk test), the data were statistically elaborated by analysis of variance (ANOVA – comparison of percentage contributions of microbial groups to the total microbiota on surfaces and in the air), *t*-test (comparison of microbial concentrations on ATM internal and external surfaces), and Pearson's correlation (between microbial concentrations and microclimate parameters) analysis using Statistica (data analysis software system) version 10 (StatSoft, Inc., Tulsa, OK, USA). Probability values were treated as statistically significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

**Microbiological contamination of ATM internal and external surfaces.** Concentrations of the studied microbial groups in swab samples from internal and external surfaces of ATMs and in the air samples from ATM rooms are presented in Table 1. In the case of bacteria and fungi, their concentrations on external ATM surfaces (maxima reached 599 CFU/cm<sup>2</sup> and 522 CFU/cm<sup>2</sup>, respectively) were higher than those collected from internal surfaces (maxima – 383 CFU/cm<sup>2</sup> and 86 CFU/cm<sup>2</sup>, respectively); however, due to the range of observed values, statistical analysis confirmed the significance of such relationship for bacterial levels only (*t*-test:  $P < 0.05$ ). External ATM surfaces are openly exposed, much more frequently touched and, if not housed, are freely open to microbial contaminants brought by customers on their hands, emitted with exhaled air, or originated from the surrounding environment.

In turn, in the case of SARS-CoV-2 (and/or presumptive SARS-CoV-2 or other coronaviruses), as well as adenoviruses, their concentrations on the external ATM surfaces were lower than those noted on internal surfaces. The higher persistence of viruses in the confined space of the ATMs is likely due to the fact that shielded surfaces are not exposed to direct UV-C light and disinfectants that can, if not destroy, then at least effectively deactivate these viruses. Recent studies devoted to checking the ability of SARS-CoV-2 survival on inanimate surfaces, such as plastic, stainless steel and glass, revealed that UV-C irradiation is highly effective in inactivating SARS-CoV-2 replication, and thus can provide rapid, efficient, and sustainable sanitization of these surfaces [34, 35, 36, 37].

Interpretation of the results of microbial concentration measurements obtained by the surface swab method is difficult due to the lack of widely recognized and accepted threshold limit values (TLVs) for this type of method and pollutants. Nevertheless, in the scientific literature, there are proposals defining the hygienic conditions of surfaces contaminated with fungal conidia in public utility premises (Tab. 2) [38]. Taking into account the above-mentioned degrees of mycological cleanliness, it can be concluded that all examined external and internal ATM surfaces were extremely contaminated.

**Microbiological contamination of the air in premises housing ATMs.** Simultaneously with the assessment of microbial surface contamination, bioaerosol measurements in the premises, in which the ATMs were located, were also carried out. In this case, due to COVID-19 pandemic restrictions on access to these rooms, the measurements were

**Table 1.** Bacterial, fungal, and viral concentrations on ATM internal and external surfaces, as well as in the air of premises with ATMs

Microorganisms	ATM - internal surfaces		ATM - external surfaces		Premises with ATMs - air			
	Mean	SD	Mean	SD	Mean	SD		
Bacteria	134 CFU/cm <sup>2</sup>	123	203 CFU/cm <sup>2</sup>	222	276 CFU/cm <sup>3</sup>	111		
Fungi	37 CFU/cm <sup>2</sup>	24	142 CFU/cm <sup>2</sup>	201	24 CFU/cm <sup>3</sup>	16		
Viruses	SARS-CoV-2 (and/or presumptive SARS-CoV-2 or other coronaviruses)	Total count	13.3 gc/cm <sup>2</sup>	13.7	1.2 gc/cm <sup>2</sup>	0.4	NS	–
		Potentially infectious	6.8 gc/cm <sup>2</sup>	7.9	BDL	–	NS	–
	Adenoviruses	Total count	11.3 gc/cm <sup>2</sup>	40.6	3.5 gc/cm <sup>2</sup>	5.7	NS	–
		Potentially infectious	2.3 gc/cm <sup>2</sup>	7.2	0.4 gc/cm <sup>2</sup>	1.2	NS	–

CFU - colony forming unit; gc - genome copy; SD - standard deviation; NS - not studied; BDL - below detection limit.

**Table 2.** Fungal hygiene guide for indoor surfaces [38]

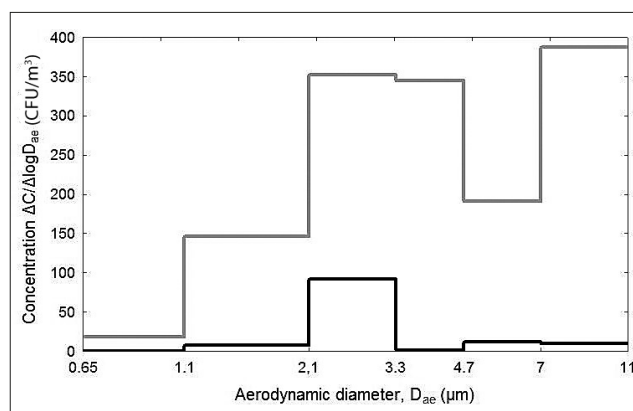
Concentration of fungal conidia on surface (CFU/cm <sup>2</sup> )	Hygiene rating
<0.5	Low
0.5 – 1	Normal
>1 – 2.5 + prevailing species	Elevated
>2.5 – 12.5 + dominant species	Contaminated
>12.5 + dominant species + confluent growth	Extreme contamination

limited to bacterial and fungal airborne contaminants only. As in the case of surfaces, the interpretation of the results of bioaerosol measurements in the indoor environment is limited by the lack of generally recognized TLVs. Determination of the degree of microbial air pollution expressed by the number of colony forming units (CFU) in 1 m<sup>3</sup> of the air is, so far, the best known and the most frequently used measure of exposure to harmful biological agents. In the hygienic assessment of the studied premises, the threshold limit values elaborated based on volumetric bioaerosol measurements and proposed by the Expert Group on Biological Agents (EGoBiA) at the Polish Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment, were applied (Tab. 3) [39]. Based on the TLVs proposed by EGoBiA, it can be concluded that the bioaerosol concentrations in rooms with ATMs were lower than the permissible values. Taking into account the behaviour of microbial particles in turbulent air characterized by their half-life times (that for the dominant species ranged from several dozen minutes to several hours – see below) [40], such picture may suggest that the air pollution and subsequent particulate deposition are not the major driving forces responsible for microbial contamination of ATMs. It is probable that most of the microorganisms settled on ATM surfaces may be provided by the people who collect or deposit cash, or use other services offered by ATMs. Also, microbiologically-contaminated banknotes withdrawn from ATMs may be responsible for the observed surface pollution.

**Table 3.** Recommended threshold limit values for microbial agents in residential and public utility premises [39]

Microbial agent	Residential and public utility premises
Mesophilic bacteria	5 × 10 <sup>3</sup> CFU/m <sup>3</sup>
Gram-negative bacteria	2 × 10 <sup>2</sup> CFU/m <sup>3</sup>
Thermophilic actinomycetes	2 × 10 <sup>2</sup> CFU/m <sup>3</sup>
Fungi	5 × 10 <sup>3</sup> CFU/m <sup>3</sup>

The use of a six-stage Andersen impactor allowed obtaining data on particle size distribution of microbial aerosols in premises housing the ATMs (Fig. 1). Taking into account the aerodynamic diameters ( $d_{ae}$ ) of dominant species (i.e. having the highest percentage contributions to the total microbiota – see below), i.e. from bacteria – staphylococci and micrococci with  $d_{ae}$  from ~0.75 μm to ~1 μm and from fungi – *Penicillium* and *Aspergillus* species with  $d_{ae}$  from ~2.3 μm to ~3 μm and from ~2.1 μm to ~3.6 μm, respectively [41, 42, 43], such analysis revealed that bacteria appeared in the air mainly in the form of fine and coarse particulates composed of cells/spores and/or their aggregates with dust particles, while fungi mainly as single conidia in the aerodynamic size range of 2.1–

**Figure 1.** Size distribution of bacterial (grey line) and fungal (black line) aerosols in the premises with ATMs

3.3 μm. If inhaled, bioaerosol composed of such particulates may reach and be deposited in case of bacteria within nasal and oral cavities, trachea, primary and secondary bronchi, in case of fungi within secondary bronchi. As interactions between airborne particulates and respiratory system cells largely depends on the place of aerosol deposition, both these groups of microorganisms may be responsible for the occurrence of adverse health effects in exposed individuals in the form of mucous membrane irritations, asthmatic reactions, and allergic inflammations.

The comparison of microbial particle concentrations and size distributions from premises housing the ATMs with those obtained in money sorting facilities, revealed a better air quality in ATM premises (on average, one order of magnitude lower concentrations than e.g. in banknote sorting rooms) [16]. The size distributions of fungal particles in both these environments were almost identical in terms of courses of the distribution step plots, whereas for bacterial aerosol, the influence of external pollution sources was much more pronounced in the case of premises housing the ATMs, where higher concentrations of coarse particulate aggregates were noted.

**Percentage contribution and qualitative analysis of surface and airborne microbiota.** The results of percentage contributions of microbial groups to the total microbiota collected from ATM surfaces and from the air of premises housing the ATMs, together with their qualitative characteristics, are presented in Table 4 and Figure 2. In the ATM surface samples, 30 bacterial species belonging to 12 genera and 32 fungal species belonging to 13 genera were identified. Microorganisms from both these groups had already been found on the surfaces of different currencies [e.g. 44, 45, 46, 47], and also identified by other researchers on ATM surfaces [e.g. 3, 4, 5, 6, 7, 8, 9, 10, 11]. Analysis of percentage contribution showed that the dominant groups of microorganisms, both on the examined surfaces and in the air, were Gram-positive cocci and filamentous fungi. Despite a fairly uniform trend in this respect (ANOVA:  $P > 0.05$ ), the individual elements of the studied environment differed from each other. Fungal conidia and (almost in the same proportion to each other) Gram-positive cocci and Gram-negative rods predominated on the internal surfaces of ATMs. There may be several reasons for the dominance of these microbial groups. Among them there are microorganisms commonly found in the soil as well as on plants and animals. Since

**Table 4.** Microbiota isolated from ATM internal and external surfaces, as well as from the air of premises with ATMs

Microorganisms	ATM surface		Premises with ATMs	Microorganisms	ATM surface		Premises with ATMs		
	Internal	External	Air		Internal	External	Air		
Gram-positive cocci	<i>Jeotgalicoccus</i> spp. ^	×	×	Filamentous fungi	<i>Alternaria</i> spp. #	×	×		
	<i>Micrococcus luteus</i>	×	×		<i>Aspergillus flavus</i> # * ^		×	×	
	<i>Micrococcus</i> spp. #				<i>Aspergillus montevideensis</i> * ^	×	×		
	<i>Staphylococcus chromogenes</i>				<i>Aspergillus nidulans</i> # * ^		×		
	<i>Staphylococcus cohnii</i> subsp. <i>cohnii</i>				<i>Aspergillus niger</i> # * ^	×	×		
	<i>Staphylococcus epidermidis</i> #	×	×		<i>Cephalosporium charticola</i> #			×	
	<i>Staphylococcus hominis</i>	×	×		<i>Cladosporium cladosporioides</i> #	×	×		
	<i>Staphylococcus saprophyticus</i>	×	×		<i>Heterocephalum auranticum</i>			×	
	<i>Staphylococcus sciuri</i> #	×	×		<i>Mucor</i> spp. #	×	×		
	<i>Staphylococcus simulans</i>	×	×		<i>Oidiodendron citrinum</i>	×	×	×	
	<i>Staphylococcus succinus</i>	×	×		<i>Penicillium aurantiogriseum</i> #	×	×		
	<i>Staphylococcus vitulinus</i>	×	×		<i>Penicillium brevicompactum</i> #	×	×	×	
	<i>Staphylococcus xylosum</i>				<i>Penicillium chrysogenum</i> #	×	×		
	<i>Streptococcus</i> spp.				<i>Penicillium citrinum</i> #	×	×		
Nonsporing Gram-positive rods	<i>Brevibacterium</i> spp. #		×	<i>Penicillium commune</i> #	×	×	×		
	<i>Carnobacterium maltaromaticum</i> ^	×	×	<i>Prophythroma</i> spp. ^			×		
	<i>Cellulomonas</i> spp. #		×	<i>Scopulariopsis brevicaulis</i> #	×	×			
	<i>Corynebacterium</i> spp.		×	<i>Candida ciferrii</i>	×	×			
	<i>Exiguobacterium</i> spp. # ^	×	×	<i>Candida glabrata</i> * ^	×	×			
	Bacteria	<i>Glutamicibacter arilaitensis</i> ^	×	×	<i>Candida guilliermondii</i>	×	×		
		<i>Microbacterium</i> spp.		×	<i>Candida kefyr</i>	×	×		
		<i>Paenarthrobacter aurescens</i> ^	×	×	<i>Candida spherica</i>	×	×		
		Endospore-forming Gram-positive rods	<i>Bacillus amyloliquefaciens</i> # ^	×	×	<i>Candida zeylanoides</i>	×	×	
			<i>Bacillus cereus</i> #	×	×	<i>Cryptococcus albidus</i>	×	×	
<i>Bacillus licheniformis</i> #			×	×	<i>Cryptococcus humicola</i>	×	×		
<i>Bacillus megaterium</i>			×	×	<i>Cryptococcus laurentii</i>	×			
<i>Bacillus mycoides</i>			×	×	<i>Cryptococcus lutei</i>	×	×		
<i>Bacillus pumilus</i> #			×	×	<i>Cryptococcus terreus</i>	×	×		
Gram-negative rods		<i>Bacillus subtilis</i> #	×		<i>Cryptococcus uniguttulatus</i> ^	×	×		
	<i>Acinetobacter lwoffii</i>	×	×	<i>Geotrichum klebahnii</i> ^	×	×			
	<i>Pantoea agglomerans</i> * ^	×	×	<i>Rhodotorula glutinis</i>		×			
	<i>Pseudomonas cedrina</i>	×		<i>Rhodotorula minuta</i>	×				
	<i>Pseudomonas corrugata</i>	×		<i>Rhodotorula mucilaginosa</i>	×	×			
	<i>Pseudomonas grimontii</i>	×	×	<i>Saccharomyces cerevisiae</i>	×	×			
	<i>Pseudomonas koreensis</i>	×	×	<i>Trichosporon asahii</i> ^	×	×			
	<i>Pseudomonas lundensis</i>	×		Viruses	<i>SARS-CoV-2</i> * ^	×	×	NS	
	<i>Pseudomonas tolaasii</i>		×		<i>Adenoviruses</i> ^	×	×	NS	
	Mesophilic actinomycetes	<i>Psychrobacter sanguinis</i> ^	×						
<i>Psychrobacter</i> spp.		×	×						
<i>Sphingomonas paucimobilis</i>									
	<i>Nocardia</i> spp. #		×						

\* pathogens from risk groups 2 and 3 according to Commission Directives (UE) 2019/1833 [56] and 2020/739 [57], as well as Ordinance of Polish Minister of Health [58].

# cellulolytic microorganisms isolated from paper materials [59, 60, 61, 62, 63, 64, 65].

^ microorganisms identified using molecular methods.

NS - not studied.

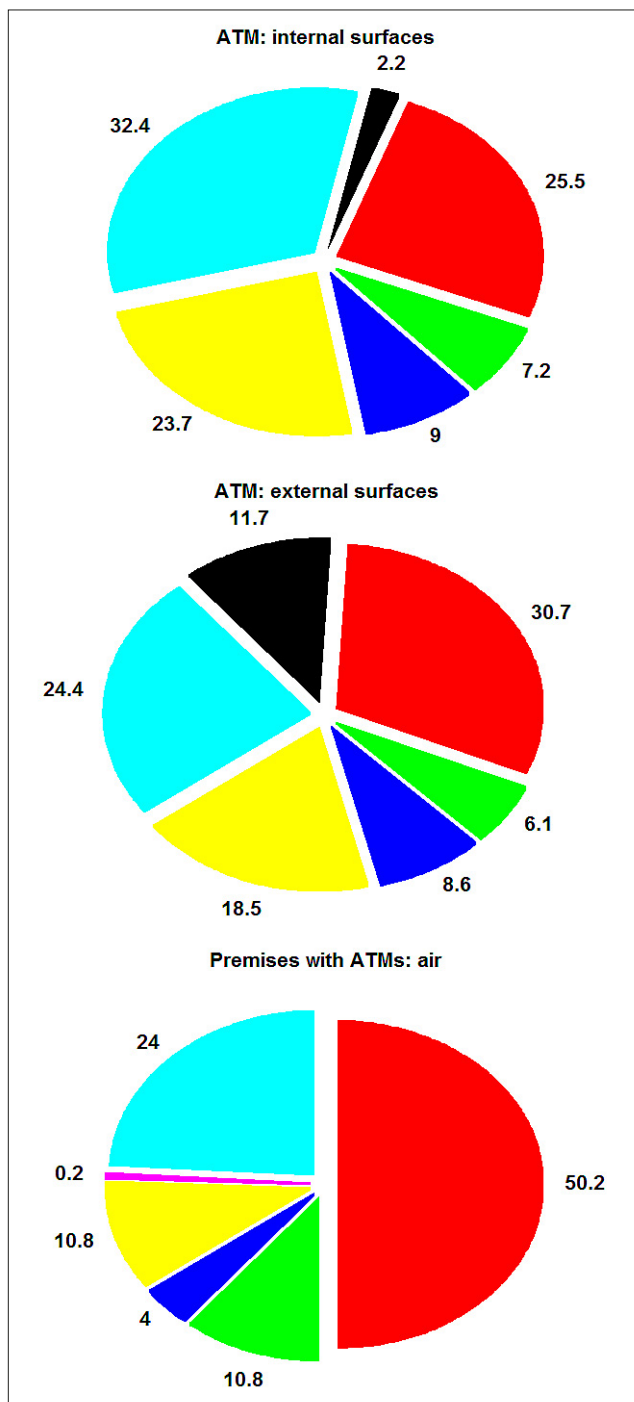


Fig. 2. Percentage contributions of microbial groups to the total microbiota isolated from ATM internal and external surfaces as well as from the air of premises with ATMs. The respective colors of slices represent: ▲ Gram-positive cocci, ▲ nonsporing Gram-positive rods, ▲ endospore forming Gram-positive rods, ▲ Gram-negative rods, ▲ mesophilic actinomycetes, ▲ filamentous fungi, and ▲ yeasts.

they are also quite common in indoor air [e.g. 48, 49], the process of their sedimentation removal from the air may have played a key role in contamination of the examined surfaces. Among isolated filamentous fungi and cocci, a number of microorganisms have cellulolytic properties (Tab. 4), and the potential contamination of banknotes could also be the reason for their domination on their internal ATM surfaces. It should also be emphasized that Gram-negative bacteria are a source of immunologically active endotoxins, which pose a serious threat to health. Their pathogenic effects are manifested mainly in the form of fever with chills and/

or inflammatory reactions of the respiratory system. Such adverse health effects can already be caused by even picogram amounts of these highly reactive particles [50].

On the external ATM surfaces, yeasts join the three above-mentioned dominant groups of microorganisms, constituting nearly 12% of the identified microbiota. While the presence of filamentous fungi, Gram-positive cocci and Gram-negative rods on these surfaces may be explained by their sedimentation (as carried by the ambient air or exhaled by people using ATMs) on exposed ATM elements, such a significant share of yeasts is probably the result of their direct transfer to keyboards and screens from the hands by people touching these elements while withdrawing cash. Although this was not formally tested, the way of yeasts being spread by hands is very likely, as in the case of, for example, mobile phones, computer touch screens or other mobile devices [51, 52]. Most of the yeasts are harmless human or environmental saprophytes, but in the case of impaired human immune mechanisms, they can become the cause of serious infections as opportunistic pathogens [53].

In the case of the remaining identified groups of microorganisms, i.e. Gram-positive endospore forming bacilli and nonsporing Gram-positive rods, their percentages on the internal and external surfaces of ATMs were similar to each other. Bacteria of the *Bacillus* genus most often inhabit soil and plants and, as environmental strains, they usually do not pose a threat to human health. They may probably be deposited on tested surfaces due to sedimentation from the atmospheric air or were directly transferred by people using ATMs (e.g. on their hands, hair or clothes). It should also be noted that these bacteria are able to form endospores, which enable them to survive in unfavourable environmental conditions for a long period of time. Their spores show greater resistance to disinfectants or detergents than the other bacterial groups. In turn, non-sporing Gram-positive rods in the human body most often colonize the skin, respiratory, gastrointestinal and urinary tracts, hence their presence on the surfaces of different objects is quite common. Nevertheless, their main habitat is the external environment (soil, plants), from which they can be transferred by humans to the surfaces they touch while withdrawing cash from ATMs.

The most prevalent in the air of premises housing ATMs were Gram-positive cocci and filamentous fungi, which together accounted for almost 85% of all identified microorganisms. Gram-positive cocci are described in the scientific literature as common both in outdoor (soil, water) and indoor environments, where their main source are people through emission from the respiratory tract and skin [54, 55]. The presence of both these reservoirs was the most likely cause of their significant quantitative advantage over other components of the air microbiota.

In turn, filamentous fungi, mainly of the *Aspergillus* and *Penicillium* genera, are the most common indoors, both in the air and on surfaces; however, as in the case of Gram-positive cocci, the outdoor environment with soil and vegetation is the abundant source from which their conidia can migrate to the premises on the body and clothes of people, and through any leaks in the building envelope [66]. In the indoor environment, fungal conidia can live for a long time on fomites, elements of heating and ventilation/air-conditioning systems, different building materials, maintaining their survival ability to survive even for several dozen years [67].



The scientific literature reveals that filamentous fungi, mainly from *Aspergillus*, *Cladosporium*, *Penicillium*, *Alternaria* or *Mucor* genera may pose a serious threat to human health, being the most common cause of allergy [68]. The occurrence and development of these fungi is also associated with the abundant release of allergens, mycotoxins, volatile organic compounds, and glucans into the environment. Fungal allergens are the main cause of atopic diseases. Contact with filamentous fungi and their bioproducts can cause allergic reactions, such as asthma, conjunctivitis, hay fever or allergic alveolitis. When mycotoxins enter the human body through the alimentary system, they can show toxic, carcinogenic, teratogenic, mutagenic, immunosuppressive, and immunotoxic effects; however, despite numerous studies, there is still insufficient information on the causal role of mycotoxins in respiratory diseases. In turn, volatile organic compounds produced by filamentous fungi may irritate the eyes, throat and nose, and may cause headaches, fatigue or nausea, and exacerbate the asthma symptoms. Glucans can also be responsible for eye and throat irritation, coughing, itchy skin, and various types of alveolitis [50, 55].

It is also worth noting that, unlike the external and internal ATM surfaces, the presence of mesophilic actinomycetes was found in the air of the studied premises. Their main reservoir is the outdoor environment as these bacteria can survive on plants, animals, rocks, and other uncovered surfaces. They are resistant to desiccation, and due to the fine (submicrometric) dimensions of their immunologically reactive spores, they can be airborne for a long period of time (i.e. more than 24 h in even gently turbulent air) and, if inhaled, responsible for allergic reactions [40]. On the other hand, no yeasts were identified in the indoor air. This fact can be explained by the coarse aerodynamic diameters of these fungal particulates; as their aerodynamic sizes exceed a few micrometers, their airborne half-lives decrease to several minutes, and it is highly probable that such large particles were not effectively captured by the impactor during the air sampling.

**Viral contamination of internal and external surfaces of ATMs.** The study of bacterial and fungal biota in surface swab samples was supplemented by the confirmation of the presence of viruses from *Coronaviridae* and *Adenoviridae* families representing RNA- and DNA-genome human pathogens, respectively (Tab. 1). Coronaviruses are enveloped viruses that contain a positive-sense, single-stranded RNA (+ssRNA) genome, whose virions are characterized by pleomorphism. Their diameter is usually around 125 nm, although it may vary from 60–220 nm. Their stability and preservation of the infectious properties of viruses in the environment are conditioned by many factors. Whereas in the indoor environment, both UV radiation and surface pH have limited effects on viral particles, the temperature and relative humidity may play a significant role in their stability, although the simultaneous influence of these factors is difficult to quantify. The hitherto obtained results show that on surfaces, infectious particles of *Betacoronavirus* genus (i.e. SARS-CoV-1, MERS-CoV) remain stable for up to 72 hours, while SARS-CoV-2 viral particulates maintain their stability usually from four to 72 hours – although on smooth surfaces at 20 °C and 50% relative air humidity, this time can be extended up to 28 days [69].

Depending on the age, health condition and genetic predisposition of infected people, coronaviruses may be

responsible for both mild symptoms and serious, multi-organ adverse health effects, often leading to the death of patients. Virus SARS-CoV-2 is the cause of the COVID-19 disease. The virus is transmitted from person-to-person through fine droplets with respiratory secretions during coughing, sneezing and/or talking, accounting for more than 97% of the total number of droplets per cough, droplets smaller than one micron are the most numerous of all [70]. The emitted droplets are quite heavy. The mass of the cough aerosol of the 'average' adult person amounts to 2.2 mg and the total mass of droplets released during 20 coughs may reach 154.5 mg and, as such, can be quickly deposited on surrounding surfaces [70, 71]. Therefore, infection can also occur indirectly, by touching contaminated objects or surfaces, and subsequently touching the eyes, nose or mouth with a contaminated hand [69, 72].

In the case of SARS-CoV-2 (and/or presumptive SARS-CoV-2 or other coronaviruses), in a total of 28 tested samples, 7% of them taken from internal and 4% collected from external ATM surfaces were found to be coronavirus positive, with maximum concentrations of 2512 gc/cm<sup>2</sup> and 116 gc/cm<sup>2</sup>, respectively. The applied analytical methodology made it possible to identify not only the total number of viral particles, but distinguish among them particles that retained their infectious potential. Regarding the samples taken from the surfaces of internal ATMs, potentially infectious SARS-CoV-2 particles were present in 4%, with a maximum concentrations of 1,360 gc/cm<sup>2</sup>. In the samples from the surfaces of external ATMs, no such potentially infectious particles were found – all concentrations were below detection limit, which may probably be the result of periodic disinfection treatments of the studied surfaces or the influence of environmental factors, e.g. exposure to UV radiation, changing temperature and humidity conditions, etc.

In turn, adenoviruses are non-enveloped viruses. Their genome consists of linear double-stranded DNA and the virions are 70–90 nm in diameter. Adenoviruses cause respiratory infections, conjunctivitis, haemorrhagic cystitis, and gastroenteritis. These viruses spread as airborne droplets, by direct contact or through the faecal-oral route, allowing for throat infections. They infect the muco-epithelial cells of the respiratory and alimentary systems, conjunctiva and cornea, causing direct cell damage. In adults, they are responsible for approximately 15% of food infections requiring hospitalization [30]. Their virions are resistant to desiccation, detergents, gastrointestinal secretions (acids, proteases, bile), and even low concentrations of chlorine. On surfaces, infectious AdV particulates remain stable from seven days to three months [73]. People in crowded and frequented places (premises housing the ATMs should be treated as one of them) are particularly vulnerable to this type of viral infection.

In the case of adenoviruses, in a total of 28 tested samples, the presence of viruses from this group was found in 11% of samples taken from internal and in 14% of samples taken from external ATM surfaces, with maximum concentrations of 17,288 gc/cm<sup>2</sup> and 1,536 gc/cm<sup>2</sup>, respectively. Potentially infectious adenovirus particles were present in all 11% of samples (maximum concentrations – 2,992 gc/cm<sup>2</sup>) collected from the internal surfaces of ATMs. In the case of particles collected from external ATM surfaces, only 4% contained infectious AdV particulates (maximum concentration – 378 gc/cm<sup>2</sup>).



**Pathogens and biodeteriogens found on ATM surfaces as occupational and public health hazards.** Among the isolated microbiota, eight pathogens were found, including seven from Group 2 (*Pantoea agglomerans* bacterium; filamentous fungi of *Aspergillus* genus, including: *A. flavus*, *A. niger*, *A. montevidensis*, and *A. nidulans*; *Candida glabrata* yeast; viruses from the adenovirus group) and one from Group 3 (SARS-CoV-2 virus), according to their level of risk of infection as classified under Commission Directives 2019/1833 [56] and 2020/739 [57], as well as the Ordinance of Polish Minister of Health [58]. The *P. agglomerans* Gram-negative rod has strong allergenic and endotoxic properties and is one of the main causes of various forms of allergic alveolitis [74]. *A. flavus* is one of the most common causes of allergic bronchial aspergillosis, infection of the ear and eye, sinusitis, and less often of skin aspergillosis. This fungus has gained a bad reputation due to its ability to produce the aflatoxin B<sub>1</sub> – the most carcinogenic substance of biological origin. *A. fumigatus* is an infective microorganism causing systemic mycoses in humans, responsible for pulmonary, ocular, nasal, cerebral, bone, cardiovascular, skin, and organ infections, especially in immunocompromised patients. *A. fumigatus* has been reported as a cause of asthma, rhinitis, extrinsic allergic alveolitis (usually farmer's lung), allergic bronchopulmonary aspergillosis and aspergilloma. *A. nidulans* is a common opportunistic pathogen of humans and animals. Having pathogenic and allergenic potentials, it has been reported as the cause of aspergilloma in the lungs, or disseminated infection in immunodeficient patients. Finally, *A. niger* can infect the human ear and skin and be the cause of airway allergy. It is an opportunistic pathogen responsible for aspergillosis in immunocompromised patients, and infection of the central nervous system. In turn, *C. glabrata* yeast is an etiological factor of inflammations of the oral cavity, respiratory tract, and urogenital system, as well as multifocal candidiasis [30, 32, 53]. The health hazards caused by corona- and adenovirus have already been briefly characterized above.

Of the identified microbiota, 25 microorganisms represented the species with cellulolytic properties and had previously been identified on paper materials [59, 60, 61, 62, 63, 64, 65]. Their presence on the tested surfaces and in the air of the examined premises may be related to contaminated banknotes. Microbes with cellulolytic properties are able to hydrolyze cellulose and use the products of its decomposition as a source of carbon and energy. In Poland, banknotes are usually in use from one to even 12 years, depending on their denomination. During this time, they are passed from hand-to-hand and from wallet-to-wallet almost continuously. Banks, being aware of this, try to limit their microbial contamination. In the National Bank of Poland, paper money is subjected to high temperature (160 °C) and subsequently kept in isolation for a period of one to two weeks. Banks from other countries (e.g. China) also act in this way. Many companies also use special machines to disinfect banknotes and coins on a smaller scale. Such devices usually use a triple disinfection system. First, the money is irradiated with UV-C light, then it is ozonized, and finally subjected to hot air, which is distributed due to a special circulation mechanism based on infrared lamps. Such a disinfection system is completely safe for people, does not damage banknotes, and prevents the spread of pathogens responsible for infectious diseases.

**Influence of microclimate parameters on ATM surface and airborne microbiota.** The mean values (and ranges) of air temperature and relative humidity in premises with ATMs were as follows: 24.3 °C (22.8–25.3) and 55% (40–70). There was no statistically significant influence of either of these parameters on the concentrations of tested microbial groups present in the air and on the ATM surfaces (Pearson correlation:  $P > 0.05$ ).

**Limitations of the study.** The major limitations seem to be the location and the number of evaluated ATMs and, by that, the representativeness of the results related to the limited number of analyzed samples. All examined ATMs were located in urban areas in a Central European country with a temperate climate, which in some way determined the qualitative composition of the microbiota. The impact of confounding factors, such as rural or urban locations, on ATMs pollution cannot be excluded, but it is difficult to unambiguously estimate their significance. Therefore, all these limitations should be borne in mind as they may, to some extent, bias the described exposure. Moreover, the swab samples from the internal parts of ATMs required a synchronization of activities with the cash handling company. Cash turnover is a sensitive sphere and in practice no 'outsider' has access to ATM locations, which are interesting from the research point of view and, at the same time, are in direct contact with banknotes. Their loading requires the observance of special safety procedures and is limited in time, which is associated with a significant limitation of access to this type of ATM 'sensitive places'. Nevertheless, the results obtained during this study from the number of tested samples seem to be representative both for this type of site and for its microbiological hazards; however, this was not formally tested.

Another significant limitation was the inability to collect air samples for the quantitative and qualitative determination of viruses. These studies were conducted during the COVID-19 pandemic. Modern ATMs represent a critical service offering for both on-the-go customers who rely on quick access to cash and the financial institutions that manage them. A major component in keeping bank customers satisfied is having ATMs stocked and secure to meet customer demand. Optimized ATM management is vital to the overall cash supply chain of a bank. As a result, the bank's authorities agreed only to carry out research that would not require much time. Hence, it was only possible to take air samples (short term impactor sampling) for bacteria and fungi. Collecting air samples for virus analysis from both studied groups would require the use of a specific methodology (e.g. air sampling air on filters [23] or into liquids [75]), which would significantly extend the sampling time, and this, as understood by the bank's authorities, in the limited space of the interior in which ATM was located, would make it difficult for potential customers to use this type of service.

In the case of bacteria and fungi in this study, only their viable concentrations were taken into account for exposure assessment. From the point of view of adverse health effects, the presence of infectious particulates in the air and on surfaces is critical; however, the harmful influence of non-viable microorganisms and their immunologically reactive fragments can also affect the health status of people using ATMs. The lack of such information may also underestimate the real exposure.

The use of biochemical API (Analytical Profile Index) tests in the qualitative analysis of bacteria and yeasts also represented a significant cognitive limitation. For a long time, taxonomical identification of these microorganisms was based on their morphological and biochemical characteristics. Since the end of 1990s, this has tended to be replaced by molecular approaches which offer more exhaustive and sensitive tools. The API system has numerous clear drawbacks, one of which is that this method of testing can only identify known bacteria that are in the API databases. The results can be inconclusive (there is an approximately 5–10% misidentification rate while using API kits), and often cannot identify specific microbial strains. Nevertheless, the API system does have a few advantages. It does not involve a complex methodology, is standardized, rapid, safe and easy to perform, and is relatively inexpensive and widely used, and which still allows easy comparisons between the results of different studies. As such, it can be still used as an efficient tool for environmental microbial community screening [76]. In the present study, the API tests were used for that particular purpose, to screen the microbial community on ATMs, including infectious agents. To be infectious, microorganism must be viable. In this context, the application of both biochemical and molecular techniques to select and subsequently confirm the taxonomical origin of specific species seemed to be a reasonable scientific-economic compromise.

## CONCLUSIONS

Automated Teller Machines may be the source of harmful microbiological agents. As shown in this study, as the air of the premises housing the ATMs was relatively clean (the average microbial concentrations were below the propose threshold limit values for bacterial and fungal airborne pollutants), the internal (i.e. safe boxes and cash dispenser tracks having direct contact with banknotes) and external (the most frequently touched parts, i.e. touch screens and keypads) surfaces of ATMs were heavily contaminated reaching 599 CFU/cm<sup>2</sup>, 522 CFU/cm<sup>2</sup>, 17288 gc/cm<sup>2</sup> and 2512 gc/cm<sup>2</sup> for bacterial, fungal, adenoviral and coronaviral contaminants, respectively. Among the pathogens isolated from the studied surfaces, representatives of all these microbial groups were detected, and all of them can be responsible for a broad spectrum of serious adverse health outcomes. The presence of potentially infectious corona- and adenoviral particulates on both the internal and external surfaces of ATMs is of particular concern. The application of PMA dye pretreatment and subsequent use of viability quantitative PCR/viability Reverse-Transcription quantitative PCR (v-qPCR/v-RT-qPCR) allow to detect among the viral pathogens isolated from the studied ATM surfaces the potentially infectious SARS-CoV-2 and adenoviral particulates. This clearly indicates that microbiologically contaminated ATMs may pose a serious health threat to the users thus exposed. Hence, highly efficient hygienic measures should be introduced to prevent unwanted pollution of both the distributed means of payment and ATM surfaces, and to avoid subsequent dissemination of microbial contaminants. In view of the currently observed health effects caused by the global COVID-19 pandemic, the introduction and compliance with the proper hygienic condition of ATMs is of particular social importance.

## REFERENCES

1. ATM Industry Association: Global ATM installed base to reach 4M by 2021. <https://www.atmia.com/news/global-atm-installed-base-to-reach-4m-by-2021/11086/> (access: 2022.07.20).
2. Banque de France Bulletin no. 232: Article 4. Despite the decline in the use of cash, it remains the most widely used means of payment in France. <https://publications.banque-france.fr/en/despite-decline-use-cash-it-remains-most-widely-used-means-payment-france> (access: 2022.07.20).
3. Barbosa JIB, Albano HDCP, Silva F, et al. Microbial contamination of main contact surfaces of Automated Teller Machines from Metropolitan Area of Porto. *Int J Environ Stud.* 2020;77:208–221. <https://doi.org/10.1080/00207233.2019.1674584>
4. Özkan VK. Determination of microfungus contamination on automated teller machines and bank cards in Marmaris, Turkey. *J Pharm Chem Biol Sci.* 2016;3:528–534.
5. Bik HM, Maritz JM, Luong A, et al. Microbial community patterns associated with automated teller machine keypads in New York City. *mSphere* 2016;1:e00226–16. <https://doi.org/10.1128/mSphere.00226-16>
6. Aquino S, de Lima JEA, da Silva MO, et al. Detection of pathogenic bacteria and fungi on biometric surface of Automated Teller Machines located in Brazilian public hospital. *Afr J Microbiol Res.* 2019;13:219–231. <https://doi.org/10.5897/AJMR2019.9055>
7. Dawodu OG, Akanbi RB. Isolation and identification of microorganisms associated with automated teller machines on Federal Polytechnic Ede campus. *PLoS ONE* 2021;16:e0254658. <https://doi.org/10.1371/journal.pone.0254658>
8. Okoro J, Oloninefa SD, Ojonigu AF, et al. Assessment of some selected automated teller machines in Kaduna metropolis for pathogenic bacteria contamination. *Br J Environ Stud.* 2018;6:19–35.
9. Allemailem K, Alrasheedi D, Joseph R, et al. A prevalence study of microbial contamination on the surfaces of Automated Teller Machines (ATMs) in Qassim region, Saudi Arabia. *J Infect Publ Health* 2020;13:338–339. <https://doi.org/10.1016/j.jiph.2020.01.089>
10. Mahmoudi H, Arabestani MR, Alikhani MY, et al. Antibigram of bacteria isolated from automated teller machines in Hamadan, West Iran. *GMS Hyg Infect Control* 2017;12:1–6. <https://doi.org/10.3205/dgkh000288>
11. Nagajothi J, Jeyakumari D, Vigneshwaran S, et al. Study of prevalence of microbial contamination with its antibiotic resistance pattern in automated teller machine in and around Puducherry, India. *Int J Earth Environ Health Sci.* 2015;1:27–31. <https://doi.org/10.4103/2423-7752.159924>
12. Hassan M, Honua M. The hygienic and microbial status of Sudanese banknotes, Khartoum state, Sudan. *Int J Community Med Pub Health* 2017;4:923–927. <https://doi.org/10.18203/2394-6040.ijcmph20171308>
13. Pereira de Fonseca T, Pessoa R, Sanabani SS. Molecular analysis of bacterial microbiota on Brazilian currency notes surfaces. *Int J Environ Res Pub Health* 2015;12:13276–13288. <https://doi.org/10.3390/ijerph121013276>
14. Sucilathangam G, Reventh AM, Valvizhi G, et al. Assessment of microbial contamination of paper currency notes in circulation. *Int J Current Microbiol Appl Sci.* 2016;5:735–741. <https://doi.org/10.20546/ijemas.2016.502.082>
15. Snehalatha V, Malashree R, Soni P. Isolation, enumeration and antimicrobial susceptibility of predominant microbes associated with currency notes. *Int J Curr Microbiol Appl Sci.* 2016;5:650–657. <https://doi.org/10.20546/ijemas.2016.508.073>
16. Górny RL, Gołofit-Szymczak M, Wójcik-Fatla A, et al. Microbial contamination of money sorting facilities. *Ann Agric Environ Med.* 2021;28(1):61–71. <https://doi.org/10.26444/aaem/132321>
17. Park GW, Chhabra P, Vinje J. Swab sampling method for the detection of human norovirus on surfaces. *J Visual Exp.* 2017;120:e55205. <https://doi.org/10.3791/55205>
18. Sánchez-Romero MI, García-Lechuz Moya JM, González López JJ, et al. Collection, transport and general processing of clinical specimens in microbiology laboratory. *Enferm Infecc Microbiol Clin.* 2019;37:127–134. <https://doi.org/10.1016/j.eimc.2017.12.002>
19. Silvestri E, Hall K, Chambers-Velarde Y, et al. Sampling, laboratory and data considerations for microbial data collected in the field. Cincinnati (OH), U.S. Environmental Protection Agency, 2018.
20. Hong W, Xiong J, Nyaruaba R, et al. Rapid determination of infectious SARS-CoV-2 in PCR-positive samples by SDS-PMA assisted RT-qPCR. *Sci Total Environ.* 2021;797:149085. <https://doi.org/10.1016/j.scitotenv.2021.149085>
21. Leifels M, Cheng D, Sozzi E, et al. Capsid integrity quantitative PCR to determine virus infectivity in environmental and food applications – A systematic review. *Water Res.* 2021;11:100080. <https://doi.org/10.1016/j.wroa.2020.100080>
22. Chung YS, Lee NJ, Woo SH, et al. Validation of real-time RT-PCR for detection of SARS-CoV-2 in the early stages of the COVID-19 outbreak in the Republic of Korea. *Sci Rep.* 2021;11:14817. <https://doi.org/10.1038/s41598-021-94196-3>
23. Moreno T, Pintó RM, Bosch A, et al. Tracing surface and airborne SARS-CoV-2 RNA inside public buses and subway trains. *Environ Int.* 2021;147:106326. <https://doi.org/10.1016/j.envint.2020.106326>

24. Borchardt MA, Boehm AB, Salit M, et al. The Environmental Microbiology Minimum Information (EMMI) guidelines: qPCR and dPCR quality and reporting for environmental microbiology. *Environ Sci Technol.* 2021;55:10210–10223. <https://doi.org/10.1021/acs.est.1c01767>
25. Reponen T, Willeke K, Grinshpun SA, et al. Biological particle sampling. In: Kulkarni P, Baron PA, Willeke K, editors. *Aerosol Measurements: Principles, Techniques, and Applications*. Hoboken; 2011. p. 549–570.
26. Lagier J-C, Edouard S, Pagnier I, et al. Current and past strategies for bacterial culture in clinical microbiology. *Clin Microbiol Rev.* 2015;28:208–236. <https://doi.org/10.1128/CMR.00110-14>
27. Fischer G, Dott W. Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene. *Arch Microbiol.* 2003;179:750–782. <https://doi.org/10.1007/s00203-002-0495-2>
28. Domsch KH, Gams W, Anderson TH. *Compendium of soil fungi*. Eching, IHV Verlag; 1993.
29. Fisher F, Cook NB. *Fundamentals of diagnostic mycology*. Philadelphia: Saunders Company; 1998.
30. Murray PR, Rosenthal KS, Pfaller MA. *Medical microbiology*. 7th ed. Philadelphia: Elsevier Saunders; 2013.
31. Samson RA, Hoekstra ES, Frisvad JC. *Introduction to food- and airborne fungi*. 7th ed. Utrecht: Centraalbureau voor Schimmelcultures; 2004.
32. St-Germain G, Summerbell R. *Identifying fungi: a clinical laboratory handbook*. Belmont: Star Publishing; 2011.
33. Frank JA, Reich CI, Sharma S, et al. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol.* 2008;74:2461–2470. <https://doi.org/10.1128/aem.02272-07>
34. Biasin M, Bianco A, Pareschi G, et al. UV-C irradiation is highly effective in inactivating SARS-CoV-2 replication. *Sci Rep.* 2021;11:6260. <https://doi.org/10.1038/s41598-021-85425-w>
35. Freeman S, Kibler K, Lipsky Z, et al. Systematic evaluating and modeling of SARS-CoV-2 UVC disinfection. *Sci Rep.* 2022;12:5869. <https://doi.org/10.1038/s41598-022-09930-2>
36. Gidari A, Sabbatini S, Bastianelli S, et al. SARS-CoV-2 survival on surfaces and the effect of UV-C light. *Viruses* 2021;13:408. <https://doi.org/10.3390/v13030408>
37. Sobotka P, Przychodźki M, Uściło K, et al. Effect of ultraviolet light C (UV-C) radiation generated by semiconductor light sources on human beta-coronaviruses' inactivation. *Materials.* 2022;15:2302. <https://doi.org/10.3390/ma15062302>
38. Kemp P, Neumeister-Kemp H. *Australian mould guideline*. Osborne Park: Enviro Trust; 2010.
39. Pośniak M, Skowron J. *Harmful agents in working environment. Limit values*. Warsaw: CIOP; 2020.
40. Górny RL. Microbial aerosols: sources, properties, health effects, exposure assessment – a review. *KONA Powder Particle J.* 2020;37:64–84. <https://doi.org/10.14356/kona.2020005>
41. McCullough NV, Brosseau LM, Vesley D. Collection of three bacterial aerosols by respirator and surgical mask filters under varying conditions of flow and relative humidity. *Ann Occup Hyg.* 1997;41:6777–6790. [https://doi.org/10.1016/S0003-4878\(97\)00022-7](https://doi.org/10.1016/S0003-4878(97)00022-7)
42. Reponen T, Willeke K, Ulevičius V, et al. Effect of relative humidity on the aerodynamic diameter and respiratory deposition of fungal spores. *Atmos Environ.* 1996;30:3967–3974.
43. Simon X, Duquenne P. Feasibility of generating peaks of bioaerosols for laboratory experiments. *Aerosol Air Quality Res.* 2013;13:877–886. <https://doi.org/10.4209/aaq.2012.12.0340>
44. Abirami B, Kumar R, Saravanamuthu R. Studies on the fungal flora of Indian currency. *Asian J Res Pharm Sci.* 2012;2:33–36. <https://doi.org/10.52711/2231-5659>
45. Alwakeel SS, Naseer AL. Bacterial and fungal contamination of Saudi Arabian paper currency and cell phones. *Asian J Biol Sci.* 2011;4:556–562. <https://doi.org/10.3923/ajbs.2011.556.562>
46. Ayandele AA, Adeniyi SA. Prevalence and antimicrobial resistance pattern of microorganisms isolated from Naira notes in Ogbomosho North, Nigeria. *J Res Biol.* 2011;8:587–593.
47. Dehghani M, Dehghani V, Estakhri J. Survey of microbial contamination of Iranian currency papers. *Res J Pharm Biol Chem Sci.* 2011;2:242–248.
48. Flannigan B, Samson R, Miller JD, editors. *Microorganisms in home and indoor work environments: diversity, health impacts, investigation and control*. 2nd ed. Boca Raton: CRC Press – Taylor and Francis; 2011.
49. Kumar P, Singh AB, Singh R. Comprehensive health risk assessment of microbial indoor air quality in microenvironments. *PLoS ONE* 2022;17:e0264226. <https://doi.org/10.1371/journal.pone.0264226>
50. Ławniczek-Wałczyk A, Górny RL. Endotoxins and  $\beta$ -glucans as markers of microbiological contamination – characteristics, detection, and environmental exposure. *Ann Agric Environ Med.* 2010;17:193–208.
51. Amanah A, Apriyanto DR, Fitriani H. Isolation of surveillance pathogenic fungal microbial contaminant on mobile phone. *Open Access Maced J Med Sci.* 2019;7:3493–3496. <https://doi.org/10.3889/oamjms.2019.685>
52. Kordecka A, Krajewska-Kula E, Łukaszuk C, et al. Isolation frequency of *Candida* present on the surfaces of mobile phones and hands. *BMC Infect Dis.* 2016;16:238. <https://doi.org/10.1186/s12879-016-1577-0>
53. Krzyżciak P, Skóra M, Macura AB. *Atlas of human pathogenic fungi*. Wrocław: Medpharm; 2011.
54. Chiller K, Selkin BA, Murakawa GJ. Skin microflora and bacterial infections of the skin. *J Invest Dermatol Symp Proc.* 2001;6:170–174. <https://doi.org/10.1046/j.0022-202x.2001.00043.x>
55. Macher J, editor. *Bioaerosols: assessment and control*. Cincinnati: ACGIH; 1999.
56. Commission Directive (EU) 2019/1833 of 24 October 2019 amending Annexes I, III, V and VI to Directive 2000/54/EC of the European Parliament and of the Council as regards purely technical adjustments. *OJ.* 2019;L 279:54–79.
57. Commission Directive (EU) 2020/739 of 3 June 2020 amending Annex III to Directive 2000/54/EC of the European Parliament and of the Council as regards the inclusion of SARS-CoV-2 in the list of biological agents known to infect humans and amending Commission Directive (EU) 2019/1833. *OJ.* 2020;L 175:11–14.
58. Ordinance of the Minister of Health of 11 December 2020 amending ordinance on hazardous biological agents in the work environment and the protection of health of workers occupationally exposed to them. *Law Gazette* 2020, pos. 2234.
59. Di Bella M, Randazzo D, Di Carlo E, et al. Monitoring biological damage on paper-based documents in the historical archive of the Palermo astronomical observatory. In: *Conservation Science in Cultural Heritage*; 2015. p. 85–94.
60. Hagaggi NSA. Biodegradation of cellulosic raw materials by extracellular carboxymethyl cellulase produced by *Exiguobacterium aurantiacum*. *Egypt J Exp Biol (Bot).* 2018;14:11–18. <https://doi.org/10.5455/egyjebb.20170807070652>
61. Kwaśna H, Karbowska-Berent J, Behnke-Borowczyk J. Effect of fungi on the destruction of historical parchment and paper documents. *Pol J Environ Stud.* 2020;29:2679–2695. <https://doi.org/10.15244/pjoes/111236>
62. Sterflinger K. Fungi: their role in deterioration of cultural heritage. *Fungal Biol Rev.* 2010;24:47–55. <https://doi.org/10.1016/j.fbr.2010.03.003>
63. Strzelczyk-Brząszkiewicz AB. Biodeterioration of historic objects by microorganisms and insects. Toruń: Wydawnictwo Adam Marszałek; 2012.
64. Urzi C, de Leo F. Biodeterioration of Cultural Heritage in Italy: State of Art. Proceedings of ARIADNE 8 Workshop; 2001; Prague, Czech Republic. Prague: Academy of Sciences of the Czech Republic; 2001.
65. Zyska B. Fungi isolated from library materials: A review of the literature. *Int Biodeterior Biodegrad.* 1997;40:43–51. [https://doi.org/10.1016/S0964-8305\(97\)00061-9](https://doi.org/10.1016/S0964-8305(97)00061-9)
66. Samson RA. Ecology and general characteristics of indoor fungi. In: Adan OCG, Samson RA, editors. *Fundamentals of Mold Growth in Indoor Environments and Strategies for Healthy Living*. Wageningen; 2011. p. 101–116.
67. Mandrioli P, Caneva G, Sabbioni C. *Cultural heritage and aerobiology. Methods and measurement techniques for biodeterioration monitoring*. Dordrecht: Kluwer Academic Publishers; 2003.
68. Obtulowicz K, editor. *Practical allergology*. Warsaw: PZWL; 2001.
69. Riddell S, Goldie S, Hill A, et al. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. *Virol J.* 2020;17:145. <https://doi.org/10.1186/s12985-020-01418-7>
70. Zayas G, Chiang MC, Wong E, et al. Cough aerosol in healthy participants: fundamental knowledge to optimize droplet-spread infectious respiratory disease management. *BMC Pulm Med.* 2012;12:11. <https://doi.org/10.1186/1471-2466-12-11>
71. Li H, Leong FY, Xu G, et al. Airborne dispersion of droplets during coughing: a physical model of viral transmission. *Sci Rep.* 2021;11:4617. <https://doi.org/10.1038/s41598-021-84245-2>
72. Stobnicka-Kupiec A, Gołofit-Szymczak M, Górny RL, et al. Prevalence of Bovine Leukemia Virus (BLV) and Bovine Adenovirus (BAV) genomes among air and surface samples in dairy production. *J Occup Environ Hyg.* 2020;17:312–323. <https://doi.org/10.1080/15459624.2020.1742914>
73. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis.* 2006;6:130. <https://doi.org/10.1186/1471-2334-6-130>
74. Dutkiewicz J, Mackiewicz B, Lemieszek MK, et al. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part III. Deleterious effects: infections of humans, animals and plants. *Ann Agric Environ Med.* 2016;23:197–205. <https://doi.org/10.5604/12321966.1203878>
75. Brisebois E, Veillette M, Dion-Dupont V, et al. Human viral pathogens are pervasive in wastewater treatment center aerosols. *J Environ Sci.* 2018;67:45–53. <https://doi.org/10.1016/j.jes.2017.07.015>
76. Hung L-L, Miller JD, Dillon K, editors. *Field guide for the determination of biological contaminants in environmental samples*. 2nd ed. Fairfax: AIHA; 2005.