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ARTICLE



Microbial contamination of main contact surfaces of Automated Teller Machines from Metropolitan Area of Porto

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ABSTRACT

Automated Teller Machines (ATM) are likely to be contaminated with various microorganisms because of their contact with the hands of many users daily. The main objective of this study was to investigate ATMs as a potential source of bacterial contamination. This study was conducted in the Metropolitan Area of Porto, in which 50 swab samples were cultured on selective media. Some isolates were identified based on colonial, morphological and biochemical characteristics. Susceptibility to several antibiotics was also evaluated for each isolated organism. Most contaminated ATMs had high numbers of Enterobacteriaceae and Enterococci. Staphylococci and Enterobacteriaceae were the most prevalent bacteria found. The recovered bacterial isolates varied in their antibiotic resistance pattern; Staphylococcus spp. demonstrated the most resistant profiles. With this pioneering study in Portugal, it was demonstrated that although ATMs in metropolitan area of Porto were not highly contaminated, some potentially pathogenic bacteria were present and resistant to some commonly used antibiotics.

KEYWORDS

Antibiotic resistance; ATM; Microbial contamination

Introduction

Automated Teller machines (ATM), or cash machines, are electronic devices that enable the clients of a financial institution to perform financial transactions without the need for a human response. ATMs are the most widely used form of computerised public technology since their invention in the late 1960s [1]. According to the ATM Industry Association, there are close to 3 million cash machines installed worldwide [2].

ATMs are used daily by hundreds of people with different socio-economic status and hygiene levels. The point of contact is the customer's hands to the surfaces of keypad and/or screen of these devices. Human beings have a marked tendency to pick up microorganisms from environmental objects, and hands have been shown to play an important role in their transmission [3]. Microorganisms can persist on environmental surfaces from hours to months [4] and cross infection of microorganisms between environmental surfaces and

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a host has been reported [3]. It has also been shown that microbes once attached to hands and some surfaces may be difficult to remove [5]. Thus, ATMs have a high possibility of being contaminated with different types of microorganisms through contact with the multiple users. Bik et al. [6] demonstrated that DNA signatures of microorganisms associated with human skin communities, including potential pathogens, were present in ATM keypads. Some authors demonstrated contamination of ATM machines, in particular in underdeveloped countries [3,5,7–9]. As far as we know, in Europe, there are few studies on this subject. Therefore, an investigation of the bacterial load of these devices may be useful to increase our awareness about possible transmission mechanisms of potential pathogens.

This study aimed to detect and/or enumerate microorganisms, especially pathogens, on the frequently touched metallic keypads and/or metallic buttons of ATMs. Identification of some pathogens and their antibiotic resistance were also investigated.

Materials and methods

Sampling

This study was carried out in the Metropolitan Area of Porto between October and December 2015. Samples were collected from 50 ATMs (32 in the interior of a bank, 18 outside), all sited in cities (ATMs numbers 1–25 from Gaia; 26–30 from Maia and 31–50 from Porto) and with many users per day. Analyses were performed using one cotton swab moistened in 1 mL sterile quarter strength Ringer's solution (Lab M, Bury, United Kingdom), which was scrubbed on all metallic contact surfaces used by operators (metallic keypads and/or metallic buttons) and re-suspended in 10 mL of Buffered Peptone Water (BPW, Merck, Darmstadt, Germany).

All samples were transported to the laboratory in a refrigerated box and analysed as soon as they arrived (within 24 h).

Microbiological analyses

Appropriate decimal dilutions were prepared for microbial enumeration: total viable microorganisms at 30°C [10], *Enterobacteriaceae* [11] and Enterococci on bile aesculin azide agar (BEAA, Biokar Diagnostics, Beauvais, France) [12].

Detection of *Listeria* spp. was performed as described in ISO 11290-1 standard [13]. Coagulase-positive and – negative *Staphylococcus* were detected according to a Portuguese Standard [14] with some modifications. From each sample in BPW solution (*Sampling* section), 1 mL was transferred to simple Chapman broth (tryptone 5 g/L; meat extract 6 g/L; peptone protease 5 g/L; NaCl 75 g/L; lactose 7.5 g/L; agar 0.5 g/L) and incubated at 37°C for 24 and 48 h. Cultures were then transferred to Baird Parker Agar with egg yolk tellurite (BPA, Biokar Diagnostics) and plates incubated for 24 to 48 h at 37°C. Characteristic colonies on BPA were confirmed by coagulase test with rabbit plasma (bioMérieux, Marcy l'Etoile, France). Coliforms at 30°C and *Escherichia coli* were detected according to [15] and [16], respectively. After incubation in simple lactose broth (Lab M) at 30°C for 48 h, coliforms at 30°C were detected by growth and gas production in brilliant green broth (Oxoid, Basingstoke,

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United Kingdom) incubated at 30°C for 48 h and *E. coli* also by growth and gas production in brilliant green broth and by indole production on peptone water, both incubated at 44.5 °C for 48 h.

Characterisation and phenotypic identification of isolates

About 10% of colonies on each selective culture media were randomly selected and isolates were characterised using their colonial and cellular morphology, Gram reaction and conventional biochemical tests: Enterococci (n = 22) were tested for different growth conditions and acid production from several sugars [17]; *Listeria* isolate was identified according to the ISO 11290-1 standard [13]; Staphylococci (n = 66) were tested for colonial pigment, acetoin production, presence of haemolysis, DNase, coagulase, urease, acid production from fructose, D-galactose, D-mannitol, D-mannose, D-trehalose and D-xylose [18] and susceptibility to novobiocin (5 µg) and polymyxin B (300 IU) according to Iorio et al. [19]; when inhibition zones were ≥16 mm, isolates were classified as sensitive.

Classification of each group of isolates was based on Bergey's Manual of Determinative Bacteriology [18].

Antibiotic susceptibility testing

Antibiotics were chosen on the basis of their diverse representation of different classes of antimicrobial agents. Minimum inhibitory concentrations (MIC; μ g/mL) were determined, in duplicate, by ϵ -test for trimethoprim/sulphamethoxazole (SXT, AB Biodisk, Solna, Sweden) and by the agar dilution method for penicillin G, oxacillin, ceftazidime, chloramphenicol, nalidixic acid, nitrofurantoin (Sigma, Steinheim, Germany), ampicillin, vancomycin (Fluka, Steinheim, Germany), ciprofloxacin, erythromycin, gentamicin, tetracycline and rifampicin (kindly supplied by Labesfal, Portugal), according to the Clinical and Laboratory Standards Institute (CLSI) [20].

Each isolate was classified as sensitive, intermediate or resistant according to CLSI [20] and *Listeria* spp. isolate as described by Barbosa et al. [21].

Isolates exhibiting resistance to, at least, two of the antimicrobial agents of different classes were considered as multi-resistant strains.

Results

Table 1 shows results obtained after enumeration of indicator organisms. Tables 2 and 3, respectively, show the identification of isolated enterococci and staphylococci obtained by physiological and biochemical tests. Figure 1 shows the prevalence of antibiotic resistances found. Enterococci isolates showed higher resistance to tetracycline (36%) followed by ciprofloxacin (23%), rifampicin (18%) and erythromycin (14%); 31.8% were resistant to at least two antimicrobial agents of different classes, i.e. multi-resistant (Figure 1(a)). All *E. cecorum* species were resistant to erythromycin, tetracycline and ciprofloxacin. Remaining multi-resistant isolates were resistant to tetracycline and rifampicin (*E. casseliflavus, E. mundtii* and *E. faecalis*).

Listeria innocua isolate was sensitive to all antibiotics tested (data not shown).

Table 1. Re	sults of enumeration (CFU/swab) and detectior	ו (presence or ab	sence/swab) of seve	eral microbic	logical agents for	50 ATMs studied.	
	Ш	numeration (CFU/swab)			Dete	ction (presence or abs	ence/swab)	
-	Total microorganisms		L		L	Staphylococcus		Listeria
Sample	at 30°C	Enterobacteriaceae	Enterococci	Colitorms 30°C	E. COli	coagulase +	Listeria spp.	monocytogenes
ATM1*	4.4x10 ²	<1.0x10 ¹	<1.0x10 ²	ı	ı	+	ı	ı
ATM2*	4.2x10 ²	1.0×10 ¹	<1.0x10 ²	ı	·		,	
ATM3*	5.0×10 ¹	<1.0x10 ¹	<1.0x10 ²		'			
$ATM4^{\dagger}$	5.6x10 ²	<1.0x10 ¹	<1.0x10 ²					
$ATM5^{\dagger}$	8.1x10 ²	3.0x10 ¹	<1.0x10 ²					
ATM6*	1.5×10 ²	<1.0x10 ¹	<1.0x10 ²		,			
$ATM7^{+}$	4.2x10 ²	1.0×10 ¹	<1.0x10 ²		,			
ATM8*	3.1x10 ³	<1.0x10 ¹	<1.0x10 ²	+	,			
ATM9*	1.3x10 ²	<1.0x10 ¹	<1.0x10 ²					
ATM10*	1.5x10 ²	<1.0x10 ¹	<1.0x10 ²		,			
$ATM11^{+}$	3.5x10 ²	1.0×10 ¹	<1.0x10 ²					
$ATM12^{+}$	2.8x10 ³	2.4x10 ²	<1.0x10 ²					
$ATM13^{+}$	2.0x10 ²	2.3x10 ²	<1.0x10 ²					
$ATM14^{\dagger}$	3.0x10 ²	<1.0x10 ¹	<1.0x10 ²				+	
$ATM15^{\dagger}$	7.4x10 ²	<1.0x10 ¹	<1.0x10 ²					
$ATM16^{+}$	2.7×10 ²	<1.0x10 ¹	<1.0x10 ²		'			
$ATM17^{+}$	1.9x10 ³	<1.0x10 ¹	<1.0x10 ²					
$ATM18^{\dagger}$	1.2x10 ³	1.1×10 ²	<1.0x10 ²		'			
$ATM19^{\dagger}$	4.5x10°	<1.0x10 ¹	<1.0x10 ²		'			
$ATM20^{\dagger}$	2.1x10°	<1.0x10 ¹	<1.0x10 ²		·	,		
ATM21*	1.1×10 ¹	6.0x10 ²	<1.0x10 ²		'		+	
ATM22*	3.9x10 ¹	<1.0x10 ¹	<1.0x10 ²		'			
$ATM23^{\dagger}$	5.0x10°	<1.0x10 ¹	<1.0x10 ²		·	,		
$ATM24^{\dagger}$	1.1x10 ¹	<1.0x10 ¹	<1.0x10 ²				+	
ATM25 [†]	1.5x10 ¹	<1.0×10 ¹	<1.0x10 ²	,	ı		,	,
$ATM26^{\dagger}$	>1.5x10 ¹	2.5x10 ³	3.7x10 ⁴	ı	·		,	
$ATM27^{\dagger}$	>1.0x10 ¹	<1.0x10 ¹	7.0x10 ²		,		,	
$ATM28^{\dagger}$	>1.6x10 ²	1.2x10 ⁵	<1.0x10 ²					
$ATM29^{\dagger}$	>1.6x10 ²	<1.0x10 ¹	1.0x10 ⁴		·	+		
$ATM30^{\dagger}$	8.1x10°	<1.0x10 ¹	<1.0x10 ²		,			
$ATM31^{\dagger}$	7.9x10 ²	<1.0x10 ¹	5.0x10 ²		,	+		
ATM32 [†]	1.6x10 ²	<1.0×10 ¹	<1.0x10 ²	,	ı	,	,	,
ATM33 [‡]	5.4×10^{2}	<1.0×10 ¹	2.0x10 ²	ı	ı		·	
ATM34*	8.5×10 ²	9.1×10 ¹	<1.0x10 ²	ı	ı		·	
								(Continued)

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Total microorganisms Staphylococcus Listeria $f = 310^{\circ}$ $Enterbacteriacea$ Enterobacteriacea Enterobacteriacea Listeria Listeria $f = 310^{\circ}$ $Enterobacteriacea$ Enterobacteriacea Enterobacteriacea Enterobacteriacea Enterobacteriacea Enterobacteriacea Enterobacteriacea Listeria P_{11} $f = 350^{\circ}$ $< 10010^{\circ}$ $< 10010^{\circ}$ $< 10010^{\circ}$ $< 10010^{\circ}$ $< 000000000000000000000000000000000000$			Enumeration (CFU/swab)			Deteo	ction (presence or abs	sence/swab)	
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		Total microorganisms					Staphylococcus		Listeria
	e	at 30°C	Enterobacteriaceae	Enterococci	Coliforms 30°C	E. coli	coagulase +	Listeria spp.	monocytogenes
	5 [‡]	4.6x10 ²	<1.0x10 ¹	1.0x10 ²	•				•
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	e [‡]	3.6x10 ¹	<1.0x10 ¹	<1.0x10 ²		,			
	7#	2.7x10 ¹	<1.0x10 ¹	<1.0x10 ²					
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	38 [‡]	9.1x10 ¹	<1.0x10 ¹	<1.0x10 ²					
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	39^{\dagger}	2.1x10 ²	<1.0x10 ¹	<1.0x10 ²					
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	±0†	1.1x10 ²	<1.0x10 ¹	<1.0x10 ²			+		
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	1 1*	3.2x10 ²	<1.0x10 ¹	<1.0x10 ²					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	1 2*	2.2x10 ³	<1.0x10 ¹	<1.0x10 ²					
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	1 3*	1.0x10 ¹	6.7x10 ²	<1.0x10 ²					
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	¥4	8.4x10 ³	1.0x10 ¹	<1.0x10 ²		,			
	5*	1.8x10 ³	2.8x10 ³	1.0x10 ²			+		
7^* 9.3×10^3 $< 1.0 \times 10^1$ 3.0×10^2 $ -$ <td>.6⁺</td> <td>3.1x10²</td> <td><1.0x10¹</td> <td>1.0x10²</td> <td></td> <td></td> <td>+</td> <td></td> <td></td>	.6 ⁺	3.1x10 ²	<1.0x10 ¹	1.0x10 ²			+		
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$:7*	9.3x10 ³	<1.0x10 ¹	3.0x10 ²					
9 [†] 1.8 × 10 ² <1.0×10 ¹ <1.0×10 ² - - </td <td>*81</td> <td>1.8 x10¹</td> <td><1.0x10¹</td> <td>1.0x10²</td> <td></td> <td></td> <td></td> <td></td> <td></td>	*81	1.8 x10 ¹	<1.0x10 ¹	1.0x10 ²					
0 [†] 2.2×10 ² <1.0×10 ¹ <1.0×10 ²	t9⁺	1.8 x10 ²	<1.0x10 ¹	<1.0x10 ²					
	0 ⁺	2.2x10 ²	<1.0x10 ¹	<1.0x10 ²		ı	ı	·	

	Phenotypic characterization ¹ (n)	E. casseliflavus (2)	E. cecorum (3)	E. faecalis (3)	E. faecium (1)	E. mundtii (3)	E. saccharolyticus	(2) E. solitarius (1)	Enterococcus spp.	(2)
	D-Xylose	t	nt	nt	,	nt	nt	nt	nt	
	Sucrose	+	+	+	+	+	+	+	+	
	D-Sorbitol		,	+	,	+	+	+	- (*3)	
E	D-Mannitol	+	,	+	+	+	+	+	+	
uction fro	Maltose	+	+	+	+	+	+	+	+	
Acid prod	Lactose	+	+	+	+	+	+	ī	+ (*2)	
	Glycerol	,	,	+	+	+ (*1)	ī	+	+ (*2)	
	Fructose	+	+	+	+	+	+	+	+	
	Arabitol						+	ı	- (*1)	
	D-Adonitol		,	,	,	ı	ı			
	Hemolysis	σ	β (*1 α)	β (*1 α)	٨	٨	β	>	β (*3 γ)	
	Growth in the presence of 6.5% NaCl	+	,	+	+	+	+	+	- (*2)	
	Growth at pH 9.6	+	+	+	+	+	+	+	+	
	Growth at 45°C	+	+	+	+	+	+	+	+ (*2)	
	Growth at 10°C	+	+	+	+	+	+	+	+	
	Aesculin hydrolysis	+	+	+	+	+	+	+	+	

Table 2. Characterisation of Enterococcus spp. isolates and their phenotypic identification.

Legend: ¹Identification according to Bergey's Manual of Determinative Bacteriology [18]; (n) – number of isolates; (*) – the number in parenthesis refer to the number of isolates with opposite results in each test; nt – not tested; β – total lysis of erythrocytes; α – partial lysis of erythrocytes; γ – no lysis of erythrocytes.

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				Acid produ	uction from							Suscep	tibility	
Colony	Acetoin										Deoxyribonuclease		Polymyxin	Phenotypic
pigment	production	Fructose	D-Galactose	D-Mannitol	D-Mannose	D-Trehalose	D-Xylose	Urease	Coagulase	Hemolysis	(Dnase agar)	Novobiocin	В	characterization ¹ (n)
+		+	+	+		+	ц	ī		>	•	Я	æ	S. arlettae (2)
+	+ (*3)	+	+	+	+	+	nt	+	+	g	+	S	ж	S. aureus (5)
	,	+	+	'	+	'	nt	+	'	α (*1γ)	ı	S	ж	S. epidermidis (6)
+	,	+	+	+	+	+	nt	+	'	>	ı	Я	ж	S. gallinarum (3)
+ (*1)	,	+	+	+ (*1)		+	nt		'	g		S	æ	S. haemolyticus (3)
	,	+	+	'	,	'	nt	+	'	7		S	S	S. hominis (1)
	,	+	+	'	+	+	nt	+	+	g	+	S	ж	S. intermedius (1)
+	,	+	+	+	,	+	nt	+	'	>		Я	ж	S. kloosii (1)
	,	+	+ (*1)	,	+	+	nt	+ (*1)	,	γ (*β)	- (*1)	S	ж	S. lugdunensis (3)
+	,	+	,	+	,	+	nt	+	,	7		Я	S	S. saprophyticus (4)
- (1*) -	,	+	+	+ (*3)	,	+	nt	+	,	Я		S	ж	S. warneri (5)
+	,	+	,	+	+	+	+	+	,	g		Я	ж	S. xylosus (1)
- (*7)	- (*4)	+ (*8)	+ (*13)	- (2*)	- (*5)	- (*8)	nt	+ (*4)		β (*5α,γ)	- (*2)	S (*5)	R (*4)	Staphylococcus spp. (31)
Legend: ¹ results in	ldentificatio 1 each test;	n accordir nt – not t	ng to Bergey's ested; β – tot	s Manual of C tal lysis of en	Jeterminative ythrocytes; α	e Bacteriology – partial lysis	. [18]; (n) – 5 of erythn	- number ocytes; y	of isolates; - no lysis	(*) – the nu of erythrocy	imber in parenthesis tes.	refer to the	number of i	solates with opposite
results h	n each test;	nt – not t	ested; b - toi	tal lysis of er	ythrocytes; α	– partial lysi	s ot erythr	ocytes; γ	- no lysis	ot erythrocy	tes.			

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Figure 1. Percentage of isolates (%), belonging to different genera or family, that were sensitive (\Box), intermediate (\Box) or resistant (\blacksquare) to several antibiotics.

As observed in Figure 1(b), staphylococci were more resistant to β -lactams with resistances to penicillin G, ampicillin and oxacillin of 66%, 56% and 12%, respectively, followed by lower proportions of strains resistant to erythromycin (45%) and ceftazidime (21%).

No relevant resistances of *Enterobacteriaceae* were found, with exception of about 60% for nitrofurantoin (Figure 1(c)).

Discussion

Detection and enumeration of indicator organisms are often used, since their presence may indicate the possible presence of pathogens [22].

Total viable microorganisms ranged from 10^1 to 10^3 CFU/swab (Table 1). Aldosary [23] found values for total viable counts in ATMs, between 3.8×10^3 CFU and 1.3×10^5 CFU and Morioka et al. [24] showed that viable bacterial cells were detected in more than 90% of ATMs analysed.

For the majority of the machines, *Enterobacteriaceae* counts were below the detection limit of the enumeration technique. Nevertheless, counts of 1.2×10^5 CFU/swab and 2.8×10^3 CFU/swab were detected (Table 1). It is known that *Enterobacteriaceae* presence on hands is a good indicator of poor hygiene, since their number is reduced after handwashing [25].

Other indicator organisms group, as enterococci, were detected on 10 samples at levels close to detection limit (1.0 x 10^2 CFU/swab); ATMs 26 and 29 were exceptions with 10^4 CFU/swab (Table 1). Seven *Enterococcus* species were presumptively identified, *E. faecalis* and *E. cecorum* being the most prevalent (Table 2).

Besides the detection of indicator organisms as coliforms and E. coli, the presence of Listeria spp. and the pathogen L. monocytogenes was also evaluated. Listeria monocytogenes has been implicated in several outbreaks of foodborne listeriosis, which is an infection with high morbidity and mortality rates, largely confined to its risk groups of pregnant women, the elderly and immunocompromised individuals [26]. Many studies have shown the L. monocytogenes ability to colonise most surfaces and equipment in the food industry [27]. In this sense, it is relevant to evaluate the presence of this pathogen on the ATM surfaces, manipulated by so many users belonging to risk groups. Escherichia coli and L. monocytogenes were not detected on any sample (data not shown). Coliforms were detected on ATM number 8, Listeria spp. on ATM number 14 (identified as L. innocua; data not shown) and Staphylococcus coagulase positive on six ATMs (Numbers 1, 29, 31, 40, 45, 46). From 66 isolates of staphylococci, the most prevalent genus found, 12 different species were identified and 31 isolates were only characterised to the genus level (Table 3). From these, six isolates were Staphylococcus coagulase positive (five S. aureus and one S. intermedius) and 60 Staphylococcus coagulase negative (CNS).

These results showed the presence of CNS and *S. aureus*, as already described in other studies [3,5,8,28]. *Staphylococcus aureus*, in particular, is one of the major components of the skin and nose microbiota, which probably explains its prevalence as a contaminant. Transmission of *S. aureus*, including methicillin-resistant *Staphylococcus aureus* (MRSA), occurs mainly by direct contact between the skins of human beings, but *S. aureus* can survive long periods on inanimate objects. These may also represent an

important reservoir for dissemination [29]. Users' hands are probably an important source of contamination on the interfaces, since hands typically touch innumerable surfaces, objects and body fluids [30,31].

With a few exceptions, the analysed ATMs were not highly contaminated, compared with some others studies. Tekerekoğlu et al. [32] found higher values of contamination on ATMs in Turkey with CNS between $1.5 \times 10^3 - 1.2 \times 10^4$ CFU/mL and *E. coli* between $10^3 - 10^4$ CFU/mL. Saroja et al. [9], in India, showed a total microbial load ranging from $4.0 \times 10^{\circ}$ to 1.9×10^5 CFU/mL and presence of *E. coli* and *Klebsiella* spp. The other studies reviewed are also outside Europe, mostly in developing countries [3,5,7,33]. On the other hand, it should be noted that ATMs keyboards in Portugal are smooth, easily cleanable and metallic; this may influence persistence of contaminants, since many metals have antimicrobial properties [34].

Some ATMs are usually located in open spaces, exposed to wind and rain, others inside bank buildings are more protected, and this could be a factor influencing the microbial load. But Table 1 shows that no trend was found between location and the contamination level, unlike studies that have shown that ATMs near hospitals are more contaminated [8,35]. Hundreds of different people use ATMs daily, so the contamination level is possibly a result of usage frequency, poor hygiene status of users and environmental conditions around the ATMs.

Multi-resistances found in *Enterococcus* isolates from the ATMs studied are pertinent since their clinical importance is directly related to the antibiotic resistance [36]. The possible presence of some virulence factors combined with antibiotic resistance causes concern because they enhance their role as effective opportunists in nosocomial infections [37].

Listeria innocua isolate, from ATM 14, was sensitive to all antibiotics tested (data not shown). Jarvis et al. [38] also demonstrated that most *Listeria* species were susceptible to all antibiotics.

Staphylococcus spp. showed the most resistant profiles, as already observed by others [3,5]. The resistance of *Staphylococcus* spp., especially *S. aureus*, is already known and their role in infections, particularly nosocomial infections, is widely reported [39]. None of the isolates identified as *S. aureus* were resistant to oxacillin. Zhang et al. [28] found 0.5% of MRSA isolates from the 15.5% of the ATMs that yielded *S. aureus*. The rankings of multi-resistance showed that 41.5% of *Staphylococcus* spp. isolates exhibited resistance to two or more classes of antibiotics; of these, 18.2% were *Staphylococcus* spp., but only 3% were resistant to oxacillin. Of the six *S. epidermidis* isolates, five showed multi-resistant profile (resistance to penicillin, ampicillin, erythromycin and some to gentamicin). Oxacillin resistance has been increasing in coagulase-negative *Staphylococcus* [40].

About 60% of *Enterobacteriaceae* were resistant to nitrofurantoin and just 3% to chloramphenicol (Figure 1(c)). This high percentage of resistant isolates to nitrofurantoin should be highlighted, because resistance to this drug has remained virtually unchanged since its discovery [41]. Nonetheless, some studies have already shown the emergence of resistant strains [42,43]. No relevant resistances to other antibiotics tested were found, even knowing the high ability of this family to acquire antibiotic resistances [39].

In this study, some bacterial species were found to coexist on an interface of the ATMs analysed, which have different characteristics, including resistance to some antibiotics. Occurrence of resistance in pathogens may reduce the effectiveness of previously useful

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antibiotics. The risk of spreading antibiotic-resistant bacteria through contact with ATM machines should not be neglected, in terms of impact on public health.

With this pioneering study on microbial contamination in Portugal, it was demonstrated that although ATMs in the metropolitan area of Porto were not highly contaminated, some potentially pathogenic bacteria were present and resistant to some commonly used antibiotics. Since many people may be in contact with these surfaces daily, there is a risk of possible contamination and transmission of pathogens in the community. This is a question for the maintenance of public health. Further studies should be carried out on hygiene measures to be implemented at these sites and/or the possible cost/benefit advantage of replacing the current material that constitutes ATMs with antimicrobial materials. Meanwhile, disinfection of the hands after use of such equipment is advisable. Retrofitting the sites of ATMs with bactericidal gel dispensers, as in hospitals, would not be a costly safeguard.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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