Medical School, Aristotle University of Thessaloniki: common use objects and their bacterial burden.

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ABSTRACT: The aim of this study was to determine the microbial burden on common use objects in the Medical School of Aristotle University of Thessaloniki.

The research took place in the seven departments of the central building of the Medical School, from January to February 2010. Fifty samples (29 doorknobs, 21 faucet handles) were collected from various places of the departments using swabs moistened by nutrient broth. The samples were inoculated into nutrient broth, MacConkey agar and blood agar. Isolates were identified and typed by conventional procedures.

From the samples collected, 1-5 types of bacteria were isolated per object, presenting a growth scale from negligible to high. The most common isolate was coagulase negative *Staphylococcus* (27), followed by *Staphylococcus aureus* (17), *Bacillus spp* (16), *Enterobacteriaceae* (10), *Diphtheroid spp* (8), *Pseudomonas spp* (2) and the least occurring microorganism was *β-haemolytic Streptococcus* (1). The members of the Enterobacteriaceae isolated were: *Escherichia spp* (5), *Enterobacter spp* (3), *Pantoea spp* (1) and *Klebsiella spp* (1).

In conclusion, doorknobs and faucet handles in the Medical School bare a sufficient microbial population, which however mostly belong to the normal flora or to potential pathogens. The level of hygiene, at least regarding the cleaning of hands and objects of common use, appears satisfying.

Key Words: Bacteria, Doorknobs, Faucet handles, Medical School.

INTRODUCTION

Since the microscope discovery in the 17th century, microorganisms are known to be totally widespread in the environment, either natural or artificial. Most of them are symbiotic, few are pathogenic, while others may be opportunistic pathogens.

Public places and objects of common use naturally bear plenty of microbes, the quantity and the variety of which are connected to human touch, area cleanliness and hygiene conditions. 104 bacterial genera were detected in domestic toilettes in Düsseldorf Germany, 2009[1], 11 species of microbes, mostly staphylococcus, on telephone receivers in Lagos Nigeria, 2009[2], while similar surveys performed in Arizona, USA, and Turkey, reported about various objects in school classrooms and public telephone surfaces respectively[3,4].

Moreover, due to its nature it would be expected a Medical School to be a contaminated area since live microbes are treated and biological materials, such as blood, respiratory excretions, cerebrospinal fluid etc, are being transferred. Specimens' transportation to the laboratories follows the international safety standards and is performed by hospital nursing staff or rarely by patients' escorts.

The building is regularly frequented by 1100 students attending the first six semesters of their studies and about 100 employees including teachers, researchers, technicians, secretaries etc. The 1/6 of these students (students of the 6th semester) at the same time

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have their practice in various University hospital clinics.

Nowadays in industrial societies people are generally quite informed about rules of personal and public hygiene. In addition, the prevention politic against the recent A(H1N1) influenza pandemic of 2009-2010, resulted in measures intending to sensitize more the population about microbe transmission.

Aiming to investigate whether the former rules are applied, the present work tries to check the bacterial burden on commonly contacted surfaces of the main building of the Medical School of Thessaloniki.

Regarding the fact that the study has been performed during the influenza pandemic period, the influenza prevention campaign effectiveness could be at the same time checked.

MATERIALS AND METHODS

This study, focusing on common bacteria, was accomplished in the seven departments located in the central building of the Medical School of the Aristotle University of Thessaloniki during January and February 2010. These departments are the ones of Biology, Histology, Physiology, Biochemistry, A´ Microbiology, B´ Microbiology and Hygiene. Except for A´ and B´ Microbiology, they are mentioned as D3, D4, D5, D6 and D7. The Microbiology Departments are named and particularly mentioned in the study since they are the ones with the greatest number of biological specimens and contaminating materials.

In total 50 samples were collected from various areas of all 7 departments by the use of cotton swab sticks: 21 samples were obtained from faucet handles of toilets (7) and working areas (14), while 29 from doorknobs located in toilets (7), working areas (14) and front doors (8). One of the front doors is the main entrance of the building and the rest lead to the each of the seven departments of the building.

The samples were collected from the surfaces of the selected objects by swab sticks moistened in nutrient broth, inoculated each one separately onto MacConkey agar, blood agar and nutrient broth and incubated aerobically for 24h at 37^{o}C. After the 24h incubation the number and variety of the colonies developed on the Petri dishes were counted and the microbes were identified. The incubated nutrient broth was inoculated on MacConkey and blood agar and incubated aerobically at 37^{o}C for 24h more, in order give a second chance to the slightest microbial existence to appear; new isolates were treated as the original ones. Development of ≥100 colonies during the first inoculation on agars has been characterised as "high growth" and the number of colonies has been regarded as 100, while development only after the inoculation of the nutrient broth has been characterised as "insignificant growth" and the number of colonies has been neglected.

Regarding the identification, all isolates were treated by microscopy after Gram-staining. Gram positive cocci were catalase and coagulase tested while for Gram negative rods biochemical properties were checked by the use of automated systems (Vitek2, Biomerieux). Any other microbial species, if detected, would have been properly processed.

All results were finally summarized, evaluated and compared. The statistic analysis was performed by the use of SPSS programme (version 11.5).

RESULTS

The total numbers of bacterial colonies isolated were 370 from the 8 front door knobs (mean 46, range 0-100), 590 from the 28 working areas' doorknobs and faucet handles (mean 21, range 0-100) and 672 from the 14 toilets' ones (mean 48, range 0-100). (Table 1). Toilets and front doors seemed equally contaminated (p = 0.8767) while working areas were found bearing the least bacterial burden (p = 0.0003 and 0.0000 compared to front doors and toilets respectively).

On all doorknobs and faucet handles checked, bacteria were found as follows: coagulase negative *Staphylococcus* on 19 objects (13 doorknobs, 6 faucet handles), *Staphylococcus aureus* on 19 objects as well (10 doorknobs, 9 faucet handles), saprophytic bacilli on 15 objects (10 doorknobs, 5 faucet handles), *diphtheroids* on 8 objects (4 doorknobs, 4 faucet handles), Enterobacteriaceae on 10 objects (3 doorknobs, 7 faucet handles), *pseudomonas spp* on 2 faucet handles and *streptococcus spp* on 1 doorknob. The Enterobacteriaceae isolated were further identified as *Escherichia coli* on 1 doorknob and 3 faucet handles, *Escherichia vulneris* on 1 doorknob, *Enterobacter spp* on 3 faucet handles (*E. zergoviae* on two of them and *E.*

Table 1. Nº of colonies of bacteria isolated from doorknobs and faucet handles of the seven departments in total.

	Doorknobs	No of colonies isolated from doorknobs	Faucet handles	No of colonies isolated from faucet handles	Total (objects)	**Total (colonies)**
Front doors	8	370	-	-	8	**370**
Working areas	14	149	14	441	28	**590**
Toilets	7	353	7	319	14	**672**
Total	29	872	21	760	50	1632

Table 2. Nº of colonies of bacteria isolated from front doors' knobs.

	Coagulase negative S.	*S. aureus*	Bacilli saprofytic	Diphtheroids	Enterobacteriaceae
A΄ Microbiology		10	85		
B΄ Microbiology	1				
D3	11			30	Insignificant (*E.coli*)
D4		19	Insignificant growth		
D5					
D6	High growth			Insignificant growth	
D7			7		
Main door	High growth		7		Insignificant (*E.vulneris*)

sakazaki on the third), while *Klebsiella pneumoniae* and *Pantoea spp* on 1 doorknob and 1 faucet handle respectively. (Tables 2, 3, 4).

The population size of each bacterium varied from insignificant growth (colonies appearing only after recultivating on agars cultures already incubated in nutrient broth) to high growth (≥100 colonies since the first incubation on agars). The largest populations were noted for coagulase negative *Staphylococcus*, often in high growth. Apart from Enterobacteriaceae, *Pseudomonas spp* and *Streptococcus spp*, which were only sporadically and rarely isolated, the least developed group of bacteria was *diphtheroids* presenting populations of <10 colonies except for the main doorknob of one department from which 30 *diphtheroid* colonies were developed (Figure 1).

The departments were classified according to the total number of colonies of each as follows: D6 404, D7 378, A΄ Department of Microbiology 193, D5 192, D3 97, D4 49, B΄ Department of Microbiology 27 colonies.

DISCUSSION

Undoubtedly, the search of microorganisms on objects presents a high interest due to the numerous diseases that may be caused by them. Detecting common infec-

Table 3. N° of colonies of bacteria isolated from working areas' doorknobs (Dk) and faucet handles (Fh).

		Coagulase negative S.	*S. aureus*	Bacilli saprofytic	Diphtheroids	Enterobacteriaceae	Other
A΄ Micro-biology	Dk	11					
	Fh		13	1			
B΄ Micro-biology	Dk	7					
	Fh	6	1		2		
D3	Dk	14			5	Insignificant (*E.sakazaki*)	
	Fh	8			3		
D4	Dk	4	Insignificant		Insignificant	Insignificant (*E.coli*)	
	Fh			4			
D5	Dk		2	1		Insignificant (*E.coli*)	Insignificant (*P.fluoresense*)
	Fh	High	1	1			
D6	Dk		1	3		Insignificant (*E.coli*)	
	Fh	High					
D7	Dk	High				1 (*E.coli*)	
	Fh	High	High	1			

Table 4. N° of colonies of bacteria isolated from toilets' doorknobs (Dk) and faucet handles (Fh).

		Coagulase negative S.	*S. aureus*	Bacilli saprofytic	Diphtheroids	Enterobacteriaceae	Other
A΄ Micro-biology	Dk	29	21	2			
	Fh		21				
B΄ Micro-biology	Dk	7	3				Insignificant (*Streptococcus*)
	Fh						
D3	Dk	16	1	4	3		
	Fh			1	2		
D4	Dk		Insignificant	22			
	Fh						
D5	Dk		41	4		Insignificant (*E.zegoviae*)	Insignificant (*Pseudomonas*)
	Fh		42				
D6	Dk	High				Insignificant (*Klebsiella*)	
	Fh	High	High				
D7	Dk		High			Insignificant (*Pantoea*)	
	Fh		53				

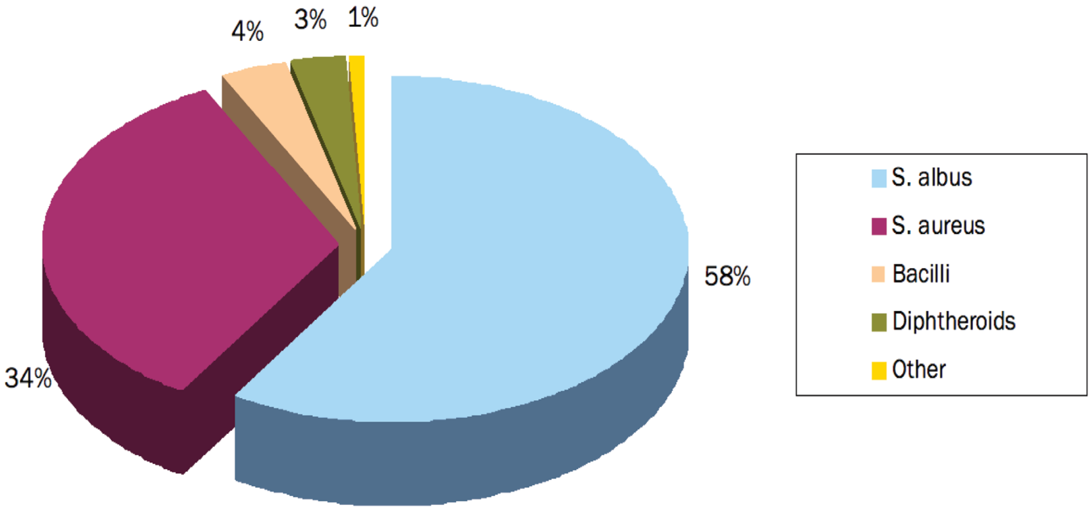

Figure 1. Percentage of bacteria isolated from all specimens examined.

tious agents, which exist mostly under low hygiene conditions, makes elimination much more effective regarding quantity as well as kind of microbes.

Plenty of findings stand in comparison to our Medical School's results in terms of indicating the microbial existence on various objects and areas examined.

Regarding places of medical interest, many researches have been carried out all over the world e.g. London, UK[5,6], Japan[7,8], Karlsruhe, Germany[9], Boston[10], referring to *staphylococcus aureus*. A study in Oman has proved the existence of at least 9 microbial species on objects of common use, such as doorknobs, in a University hospital with most frequent Enterobacteriaceae, *S. aureus* and coagulase negative *Staphylococcus*[11], while in the present study Enterobacteriaceae are found only in small numbers. A similar research in a hospital at Tel-Aviv, Israel, has shown that potential infectious agents may survive on doorknobs in lower numbers than on common use religious items[12].

From educational aspect, *Staphylococci aureus* as well as coagulase negative ones have been identified on computer keybords used by the students of the University of Toledo, USA[13]. Furthermore a primary school has been checked in Arizona, USA and a variety of heterotrophic bacteria was detected on objects like keyboards or faucet handles, as mentioned above[3].

From the aspect of highly frequented places, bacteria isolated from toilets in Düsseldorf, Germany(1) and Memphis, USA[14], or telephone devises in Lagos, Nigeria[2] and Sakarya, Turkey[4] confirm the wide presence of microorganisms not only in areas of medical or educational interest, but in the community in general.

All the above-discussed functional parameters characterise the largest Medical school of the country: a) place of medical interest, b) educational institution, c) place frequented by numerous people every day. A variety of common bacteria were isolated as in the researches mentioned above, though in the present study the microbial burden for each kind of bacteria was generally not very big. Fortunately no highly pathogenic bacteria (e.g. Salmonella) have been detected. On the contrary to the results of the present research, numerous bacteria of faecal origin were found in schools, hospital and other public places in Sussex, UK, in 1976[15]. The hypothesis that by the time passing hygiene measures have ameliorated does not totally agree with the recent isolation of *Salmonella spp* from toilet seats, flush handles and door handles in Birmingham, UK[16], neither with the detection of *Norovirus* on cupboard doorknobs, bathrooms etch in the Netherlands[17]. As for the significantly smaller bacterial burden found in working areas in comparison to front doors and toilets, this could be reasonably explained by the fact that each working area is used by much fewer people than the commonly used front doors and toilets.

The present research revealed that coagulase negative *Staphylococcus*, *S. aureus*, saprophytic Bacilli and *Diphtheroids* were the most frequently and abundantly encountered microbes. Enterobacteriaceae, *Pseudomonas spp* and *Streptococcus spp* were also isolated but less often and in much smaller quantities. Coagulase negative *Staphylococcus* and *Diphtheroids* constitute part of the normal human flora while bacilli are normally found in the environment as no disease causes. Thus it can be concluded that there is no particular risk regarding neither the health status of the individuals frequenting the target building nor a probable transmission to the related community.

Remarkable is the fact that from the seven departments studied these of Microbiology rank among the less risky on the basis of the microbial burden of all the common bacteria found. Such a result would be rather unexpected since these departments bear the heaviest transfer and processing of potentially contaminating materials. Although spread of bacteria when cleaning has been suggested by researchers in Denmark[18], our outcome confirms the general rule that the preventive hygiene measures are applied more strictly when the hazard of infection increases as in laboratories of Microbiology, where surfaces and objects are cleaned many times daily. Furthermore the A(H1N1) influenza pandemic, developing at the time of the research, must have motivated even more persistent cleaning and disinfecting practices since the B΄ Department of Microbiology includes the National Influenza Centre for Northern Greece, which has had the extreme burden of the pandemic influenza laboratory diagnosis and investigation for the region.

Whether the objects and places were perilously contaminated or not is a matter that concerns students as well as University workers. Nevertheless, the conclusion was reassuring. Although quite a big number of various bacteria exist, personal and public health related to the AUTH Medical School building seem not in risk. Thus, a proper hygiene level is there confirmed permitting all to work under appropriate conditions.

Ιατρική Σχολή ΑΠΘ: Κοινόχρηστα αντικείμενα και το βακτηριακό τους φορτίο.

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ΠΕΡΙΛΗΨΗ: Σκοπός της εργασίας ήταν η ανίχνευση μικροοργανισμών σε αντικείμενα κοινής χρήσης σε χώρους της Ιατρικής Σχολής του Αριστοτελείου Πανεπιστημίου Θεσσαλονίκης.

Η έρευνα πραγματοποιήθηκε στα επτά Εργαστήρια του κεντρικού κτιρίου της Ιατρικής Σχολής, κατά την περίοδο Ιανουαρίου-Φεβρουαρίου 2010. Με βαμβακοφόρους στυλεούς εμβαπτισμένους σε θρεπτικό ζωμό ελήφθησαν δείγματα από 50 αντικείμενα (29 πόμολα θυρών, 21 βάνες) από ποικίλους χώρους των Εργαστηρίων. Καλλιεργήθηκαν αεροβίως σε θρεπτικό ζωμό, αιματούχο άγαρ και άγαρ MacConkey. Τα μικρόβια, που αναπτύχθηκαν, ταυτοποιήθηκαν και τυποποιήθηκαν με μικροσκόπηση και βιοχημικές δοκιμασίες.

Βρέθηκαν 1-5 είδη μικροβίων ανά αντικείμενο με ανάπτυξη του κάθε είδους από αμελητέα έως άνω των 100 αποικιών. Όλα τα μικροβιακά είδη που ανιχνεύθηκαν ανήκουν είτε στα μη παθογόνα είτε στα ευκαιριακά παθογόνα. Συγκεκριμένα, κατά σειρά συχνότητας στα 50 ελεγχθέντα αντικείμενα απομονώθηκαν τα εξής: Σταφυλόκοκκοι πηκτάση αρνητικοί (27), σταφυλόκοκκος χρυσίζων (17), σαπροφυτικοί βάκιλλοι (16), εντεροβακτηριοειδή (10), σαπροφυτικά διφθεροειδή (8), ψευδομονάδα (2) και β-αιμολυτικός στρεπτόκοκκος (1). Τα γένη των εντεροβακτηριοειδών που απομονώθηκαν ήταν: *Escherichia spp* (5), *Enterobacter spp* (3), *Pantoea spp* (1) και *Klebsiella spp* (1).

Συμπερασματικά, οι βάνες και τα πόμολα θυρών του κτιρίου της Ιατρικής Σχολής φέρουν ικανό αριθμό μικροβίων ως προς τα είδη και το φορτίο τους, δεν φαίνεται όμως να αποτελούν μέσο διακίνησης βακτηρίων με μεγάλη λοιμογόνο δύναμη. Το επίπεδο υγιεινής, τουλάχιστον όσον αφορά τον καθαρισμό των χώρων και το πλύσιμο των χειρών, φαίνεται ικανοποιητικό.

Λέξεις Κλειδιά: Βακτήρια, Πόμολα, Βάνες, Ιατρική Σχολή.

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