Original article

Microbial populations in the city buses of Thessaloniki, Greece

Antonios Morsi Yeroyannis, Anastasia Stoimeni, Konstantinos Tsaknakis, Maria Exindari
Laboratory of Microbiology, Faculty of Health Sciences, School of Medicine, Aristotle University of Thessaloniki

Summary

Objectives: Public places including public means of transport are biologically characterized by a variety of microorganisms with great diversity. The aim of this research is to investigate the bacteria which exist in the city buses of a million habitants city, Thessaloniki.
Materials - Methods: The samples came from the area of the stop buttons located on the bus handles of selected buses. The sampled buses were from lines No 2 and 3, which cross the entire city, from east to west. The samples were collected at the end of each route at peak hours (10 am to 2 pm) and cultured on McConkey and blood agar. The isolates were identified and typed.
Results: In 18/39 (46.2%) of the cultures, *Staphylococcus* strains were found, 50% of them being *Staphylococcus aureus*. Other isolates were: diphtheroids 5/39 (12.82%), Moraxella 3/39 (7.7%), Gram (-) rods 2/39 (5.1%), *Streptococcus* spp. 1/39 (2.6%), saprophytic bacilli 1/39 (2.6%), *Klebsiella pneumoniae* 1/39 (2.6%) and fungi 1/39 (2.6%). Overall, 19 cultures (48.7%) developed one species, 6 (15.4%) more than one, while in 14 (35.9%) no bacteria were isolated.

Discussion: To our knowledge, this is the first microbiological study related to public health, which was carried out on the urban transport of Thessaloniki. Impressively, a variety of microbes, belonging to the respiratory tract flora, have been found on the bus handles, most possibly transmitted by infected hands. Similar studies should support the epidemiological surveillance of public places aiming to protect the transmission of pathogenic bacteria and the public health.

Key words
public transport, buses, bacterial contamination

Corresponding author
Konstantinos Tsaknakis
Aiolou 3, 41221, Larisa, Greece
Tel.: +30 6946801395
E-mail: tsaknakis1995@gmail.com

Introduction

The presence of pathogenic microbes in public places is a major risk to the health of the community, because their transmission from ill to healthy people is extremely easy and usual. Public places such as restaurants, public means of transport, schools or hospitals, play a major role in the spread of pathogenic microbes, as other researchers point out too.[1-3] This is eminently worrying, especially when it comes to multi-drug resistant microbes such as *Pseudomonas* spp., *Acinetobacter* spp., *Escherichia coli, Klebsiella pneumoniae,* and *Staphylococcus aureus,* which according to Patil et al. were common causative agents (43.7%) of ventilator-associated pneumonia (VAP), over a period of 1 year.[4] This leads to the question, whether such microbes could infect public transport, and in our case bus lines No 2 and 3, which run in close proximity to more than one central hospitals in the city of Thessaloniki, Greece.

The majority of residents of an urban area use public means of transport, so it is necessary to ensure its hygiene and hence its harmlessness for people. In order to achieve this goal, it is important to study the number and type of microbes occurring in public means of transport and then take measures to limit them if necessary.

In that respect, our research focuses on the type of microbes found in the city buses of Thessaloniki, the only means of public transport that is available in a city of a million inhabitants. These buses carry 167 million passengers per year.[5] Similar research in public means of transport has been conducted in other cities in the USA,[3,6,7] Europe,[8-13] Japan[14] and Africa,[15] suggesting a fairly wide variety of microbes, with a significant presence of *Staphylococcus* spp., and most commonly multi-drug resistant microorganisms. However, no similar research has ever been conducted in Thessaloniki.

Materials and methods

Two representative bus lines were selected because of their long routes, which traverse a very large part of the city, from east to west. These two bus lines traverse areas inhabited by people of various professions and socio-economical levels. Furthermore, both lines pass by and stop at three of the largest general hospitals of the city (AHEPA University General Hospital, Hippokration General Hospital and Agios Pavlos General Hospital).

The sampling took place during December 2016 and the study was conducted in the beginning of 2017. During winter periods the prevalence of respiratory infections is increased. Owing to this fact, their transmission among bus users is more possible and a potential infectious threat through public means of transport should be concerned.

The microbiological study of each selected bus was focused on the stop button located on the central vertical-pole handle. Before the beginning of each bus route, the area of the stop button was cleaned by the use of antibacterial wipes. Thus, the material taken at the end of the route would contain predominantly microbes left by the passengers of that route.

At the bus terminal, a cotton swab dipped in nutrient broth was gently rubbed on the stop button area and then maintained in nutrient broth in sterile conditions until incubation. At most 30 minutes later, swab and broth were incubated at 37ºC for 24h. After this first incubation samples were inoculated on MacConkey and blood agar and re-incubated for 24-48h under the same conditions.

Isolates' identification started by microscopy after Gram-staining and their morphology was studied. Gram-positive isolates were further processed through catalase and coagulase tests while Gram-negative ones were tested upon their biochemical properties using API identification systems (bioMerieux).

Table 1 Microbes isolated from surfaces of public buses

Microbes	Isolates
S. aureus	9
S. non-*aureus*	9
Diphtheroids	5
Moraxella	3
Gram (-) rods	2
Streptococcus spp	1
Klebsiella spp	1
Saprophytic bacilli	1
Fungi	1

Results

In total, 39 samples collected from equal number of buses were cultured; 28 (72%) of the samples came from line 2 and 11 (28%) from line 3. Nineteen of the 28 samples (67.9%) of line 2 proved positive and 9 (32.2%) negative, while the 11 line-3 samples resulted to 6 (54.5%) positive cultures and 5 (45.5%) to negative.

Eighteen of 39 samples (46.2%) developed Gram(+) cocci; all of them proved to be *Staphylococcus* spp. Half of them, (9 out of 18, 50%), had positive coagulase tests, and were thus characterized as *Staphylococcus aureus*. Five of the cultures contained not only staphylococci but other microbes as well, such as *Moraxella* spp. (2), saprophytic bacillus (1), Gram (-) rod (1), fungus (1).

Diphtheroids were isolated in five cultures, Gram (-) rods in one, *Klebsiella* spp. in one, while one culture resulted to the simultaneous growth of moraxella, diphtheroid and streptococcus species. The microorganisms isolated and their frequencies are presented on Table 1.

Thus, in total, a single species of microorganism grew in 19 (48.7%) cultures, in 6 (15.4%) more than one, and 14 (35.9%) cultures proved to be negative, as presented in Figure 1. The combinations of bacteria found in some cultures, as well as their frequencies, are shown in Table 2.

Discussion

Microorganisms are an integral part of the environment and, therefore, also colonize the means of tran-

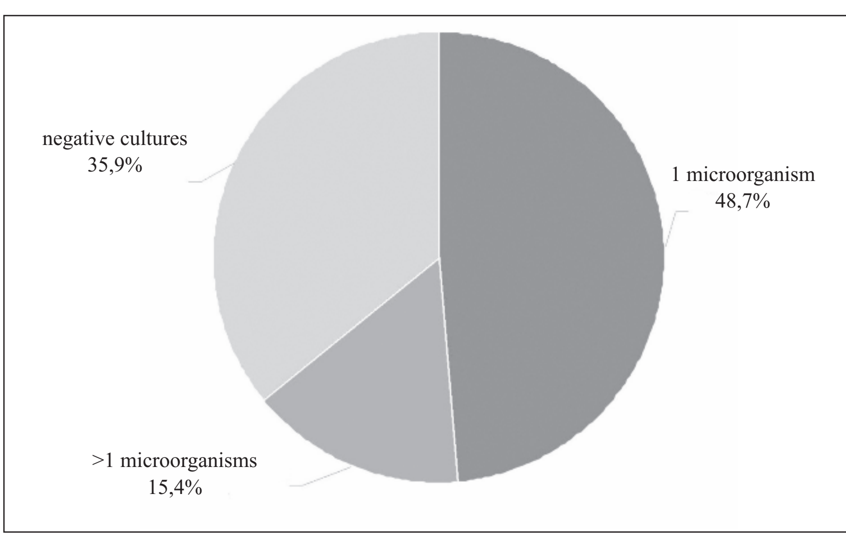

Figure 1

Isolates in bus samples.

Table 2 Samples where co-existence of microbes was noticed

Microbial combinations	No of Samples
S. aureus + *Moraxella*	1
S. non-*aureus* + Moraxella	1
S. aureus + Saprophytic bacilli	1
S. non-*aureus* + Gram (-) rods	1
S. non-*aureus* + Fungus	1
Streptococcus + Moraxella + Diphtheroids	1

sport in a city. The detection of microbes in the city buses of Thessaloniki, is not surprising; other studies, such as the recent work of Hsu et al. conducted in the subway system of Boston, Massachusetts,[6] have shown the presence of microbes in the means of public transport of many cities worldwide.[1,3,7-16]

The isolated microbes, were those anticipated , because they mostly belong to the main natural human flora.[17–19]

Hsu et al., as mentioned above, carried out a similar research concerning public transport in 2016, which revealed that *Propionobacterium, Corynebacterium, Staphylococcus* and *Streptococcus* predominated on the Boston subway surfaces.[6] Other microbes, which were isolated from vertical poles, were: *Pseudomonas, Micrococci, Moraxellae, Tissierelae, Actinomycetes, Enterobacteria, Lactobacilli, Neisseria* etc.[6] In accordance with the results of this study, Hsu et al. detected microbes that are human skin and oral commensals.[6]

Moreover, since some of the isolated microbes in both studies belong to the human upper respiratory flora, the only ways of depositing the above mentioned microbes on the vertical poles were either airborne (e.g. sneeze) or via infected hands. Considering the season of the samples' collection (winter), this suggestion largely justifies the transmission and spread of respiratory infections. Therefore, the responsibility for the microbial spreading is attributed to the users of public devices and means, in this case the bus handles, as it is clearly stated by Dixon B. and Yeh et al. too.[3,16] Consequently, basic standards of prevention and hygiene should be respected, as indicated by health organisms.[20,21]

Regarding the selection of the bus routes, it has been mentioned that lines 3 and 2 travel in close proximity to two and three large hospitals of the city, respectively.[22,23] It was noticed that, though without any statistical significance (p=0.4355), more of the line's 2 samples (67.9%) gave positive cultures, compared with the positivity for line 3 (54.5%). Apparently, there is a possibility for hospital-originated microbes to contaminate bus surfaces, posing thus a potential risk to the health of the community.

An extensive study comparing the levels of bacterial contamination of public places and facilities around a hospital (automatic teller machines, public toilets and public transport commercial motorcycle crash helmets) with the ones inside it, was carried out in 2014 in Kigali city, Rwanda, by Nigatu et al.[15] *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* spp., *Escherichia coli, Salmonella* spp., *Klebsiella* spp., *Enterobacter aerogenes* and *Pseudomonas* spp., were found on out-of-hospital surfaces, while some of these bacteria were also previously detected inside the local hospital.[15] The conclusion was that improvement and compliance with hygiene rules in hospitals, as well as in the population, is equally important to improve and ensure the safety of commonly approached surfaces.[15] Similar bacteria were isolated from the samples of this study, belonging to human natural flora.

Interestingly, according to Stepanovic et al., who investigated the presence of MRSA in the public transport system of Serbia, public transport can serve as reservoir of drug-resistant staphylococci for human infection.[12] In addition, Mendes et al., in their research concluded that the spread of EMRSA-15, a common hospital-associated lineage, among different public transports and due to that it is a common nasal colonizer, is of concern and warrants adequate public health control measures.[13] That being said, public transports might serve as a reservoir for other microorganisms including possibly multi-drug resistant bacteria of nosocomial origin.

Disagreements among various researchers can be attributed to the fact that the bacterial populations found depend on many factors. They are related to the materials that the surfaces are made of, like porous or non-porous, and where were these situated, thus allowing some researchers to find anaerobic microorganisms,[3,6] while the current climatic conditions and the location where each study is conducted, is of high importance too. This can be explained by the fact that the temperatures prevailing during the sampling period might not have favored the proliferation of all bacteria, while passengers' gloves might have mechanically removed microorganisms, resulting to false negative or low diversity cultures.

It is assumed that urban means of transport in Thessaloniki do not pose significant microbial risks to citizens, as most microbes seem to be part of the normal human flora. However, on line 2, 80% of the total number of the bacteria identified, were *S. aureus* and *Klebsiella*, hence potentially pathogenic. Furthermore, the presence of respiratory tract bacteria on bus handles possibly indicates inadequate hygiene practices among citizens and therefore suggests that initiatives should be undertaken in order to inform population about infection-protecting behaviors.

In conclusion, buses traveling through an entire city can disseminate any kind of microbes, while the transport of passengers to and from hospitals creates a potential risk of transmitting pathogenic, drug-resistant, nosocomial strains to the community. To assess and prevent this risk, a more detailed and focused future research would be very important.

Conflict of Interest

There is no conflict of interest for anyone of the four authors of the present study.

Περίληψη

Μικροβιακοί πληθυσμοί στα αστικά λεωφορεία της Θεσσαλονίκης, Ελλάδα

Αντώνιος Μόρσι Γερογιάννης, Αναστασία Στοϊμένη, Κωνσταντίνος Τσακνάκης, Μαρία Εξηντάρη
Εργαστήριο Μικροβιολογίας, Σχολή Επιστημών Υγείας, Ιατρικό Τμήμα,
Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης

Σκοπός: Οι δημόσιοι χώροι, συμπεριλαμβανομένων και των δημοσίων λεωφορείων, από βιολογική άποψη χαρακτηρίζονται από πολλούς και ποικίλους μικροοργανισμούς. Σκοπός της παρούσας έρευνας είναι η μελέτη των μικροβίων που υπάρχουν μέσα στα αστικά λεωφορεία μιας πόλης ενός εκατομμυρίου κατοίκων, όπως η Θεσσαλονίκη.
Υλικό - Μέθοδοι: Τα δείγματα προήλθαν από την περιοχή του κομβίου στάσης που βρίσκεται πάνω σε ορθοστάτες-χειρολαβές των λεωφορείων. Τα λεωφορεία που διερευνήθηκαν ήταν των διαδρομών 2 και 3, που διασχίζουν ολόκληρη την πόλη από ανατολικά προς δυτικά. Τα δείγματα ελήφθησαν στο τέλος κάθε διαδρομής σε ώρες αιχμής (10 π.μ. έως 2 μ.μ.) και καλλιεργήθηκαν σε άγαρ McConkey και αιματούχο. Την απομόνωση των μικροβίων ακολούθησε ταυτοποίηση και τυποποίηση βάσει της μορφολογίας και των βιολογικών και βιοχημικών ιδιοτήτων τους.
Αποτελέσματα: Σε 18 από τις 39 καλλιέργειες (46,15%) βρέθηκαν στελέχη σταφυλοκόκκου, με το 50% από αυτά να είναι χρυσίζοντες. Επίσης απομονώθηκαν 5/39 (12,82%) διφθεροειδή, 3/39 (7,69%) μοραξέλλα, 2/39 (5,12%) Gram(-) βακτηρίδια, 1/39 (2,56%) στρεπτόκοκκος, 1/39 (2,56%) σαπροφυτικός βάκιλλος, 1/39 (2,56%) κλεμπσιέλλα πνευμονίας και 1/39 (2,56%) μύκητας. Συνολικά 19 καλλιέργειες (48,71%) ανέπτυξαν ένα μικροβιακό είδος, 6 (15,38%) πάνω από ένα, ενώ σε 14 δεν αναπτύχθηκε κανένα μικρόβιο.

Συζήτηση: Σύμφωνα με την υπάρχουσα βιβλιογραφία, αυτή είναι η πρώτη μικροβιολογική έρευνα σχετική με τη δημόσια υγεία, που έλαβε χώρα στις δημόσιες συγκοινωνίες της Θεσσαλονίκης. Εντύπωση προκαλεί το γεγονός ότι το κοινό προφανώς δεν ακολουθεί βασικούς κανόνες υγιεινής καθώς στις χειρολαβές βρέθηκε ποικιλία μικροβίων, ιδίως της αναπνευστικής χλωρίδας, προφανώς λόγω επαφής μολυσμένων χειρών. Παρόμοιες μελέτες θα πρέπει να υποστηρίζουν την επιδημιολογική επιτήρηση δημοσίων χώρων, προστατεύοντας έτσι τη δημόσια υγεία.

Λέξεις κλειδιά
δημόσια συγκοινωνία, λεωφορεία, μικροβιακή μόλυνση

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