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Original Research Article

Correlation of the presence of multi-resistant bacteria on domestic litter bins handles with the litter bin location

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This study was conducted in order to evaluate the sanitary situation of handles of domestic litter bins in Patras city in the south west of Greece, as litter bins may be a matter of public health importance. Throughout the involved evaluation process we could assess the prevalence of bacteria on the handles of common public litter bins and in consequence, making a correlation of the percentages presence of microbes according to the location of the litter bin. Microbiological analyses were performed according to ISO methods. The study showed that 44% of the handles of litter bins were thriving with bacteria with more than 50cfu/10cm² area. Some of these different bacterial types were potential pathogenic, such as Staphylococcus spp., Enterobacteria spp., Aeromonas spp., Clostridium spp.. Also, in 10.1% of the samples, multi-resistant (>3antibiotics) bacteria were isolated. In addition, there is a significant correlation of the bin's location with respect to the type of the isolated microorganism. We conducted this study because we could not find practical investigations about the number or types of microorganisms existed in the litter bins. Furthermore it reflects the possible risks for public health. We suggest that litter bins should be cleaned up and disinfected properly and systematically with the appropriate disinfectants to minimize the negative impacts on public health.

Key words: Litter bin, garbage, public health, bacteria, antibiotic resistance

INTRODUCTION

The terms "litter" and "solid waste" are both used to describe material that is disposed by people and is neither liquid nor gas. "Waste handling" is considered to be the procedure, which includes the collection, transport and final disposal of litter. A complete solid waste handling system includes source separation, collection, transportation and treatment in the form of the neutralization of harmful substances and even the recovery of some useful products and/or energy (Andreadakis et al., 2000). The handling of solid waste aims at the minimization of the harmful consequences. That can be derived from the improper disposal of that waste causing a pollution to the environment (Panagiotakopoulos, 2002). The increase of population and the change in consumer habits resulted in a huge increase in the waste production which makes their handling a severe environmental problem for the local community but also constitutes a great national and international issue (Zafeiri and Magoulas, 2008). The first problems related to waste disposal appeared when humans started to live in large communities. Scattered garbage in medieval towns caused an abrupt increase of rats populations and the consequent plague known as "black death" was spread throughout flies and became epidemic that killed half of Europe's population in the fourteenth century (Mousiopoulos and Karagiannidis, 2002). In the last few years, special interest has been given to the limitation of the taxing of the environment, aiming at the protection of public health.

Garbage is rich in nutrients that lead to the grow of micro-organisms such as Flavobacterium *peregrinum*, Escherichia *coli*, Streptococcus *faecalis* and Lactobacillus *acidophilus* (Shimoyama et al., 2002). Other microbes that

could be detected in garbage include epidermal or oral microbes of canines or cats. *Salmonella spp*. could be found, because of the residues which are disposed in the litter bins (Kozak et al., 2002; Prado et al., 2006)

The placement of litter bins in urban cities in Greece is subjected to several municipal rules. The litter bins are not to be placed on street corners, under traffic lights, at pedestrian crossings, on ramps for the disabled persons, at bus stops, schools, churches, sports facilities, playgrounds, and places where it is legally prohibited for public security reasons. Certain criteria are in place, which determine the placement of litter bins in front of each building. The number of litter bins used is determined by the annual disposal program. Also, the kind of shops that are active in a certain area is taken into consideration. For example, it is important if there are any shops of sanitary interest (such as grocery shops, restaurants, butcher shop, etc.), which usually produce large amounts of garbage. Also in the case of super markets, the litter bin is placed at the storefront after a previous arrangement with the manager of the shop (Cleaning Regulations, 2009).

The aim of the study is to assess the general condition as well as the microbiological quality of the handles of litter bins used in an urban city such as Patras. The antibiotic resistance of the isolated bacteria was also evaluated. The public health impact of the microbiological findings is discussed.

MATERIALS AND METHODS

The examined litter bins were located in Patras, an urban city in Northern Peloponnesos, SouthWestern Greece. The city has a population of about 165.000 inhabitants and is divided into four districts: the Arctic (27.000 inhabitants), the Eastern (28.000 inhabitants), the Southern (38.000 inhabitants) and the Central (72.000 inhabitants).

The total number of litter bins in the city is about 6000. Their distribution per district is: 1300 (Arctic), 1200 (Eastern), 1500 (Southern), and 2000 (Central). The litter bins are of green color and made of plastic material (polycarbonate). They have a pedal system for opening the lid. The normal frequency of their washing and disinfection is considered to be every 15-20 days (Cleaning Regulations, 2009). The disinfection is made by common disinfectants.

In the same time of the same day, ninety nine (99) swab samples from the handles of litter bins were taken by the 6 members of the personnel of Public Health Department. Sampling was performed by personnel, previously trained for the sampling process. Each of the six samplers followed a specific route and collected samples from the handles of randomly selected litter bins (one out of every five litter bins along the predetermined way of each sampler). In order to evaluate the prevalence of bacteria and also because people usually throw their garbage after 8.30 a.m every day, sampling took place between 7:00 am and 7:30 am in one single day.

Each sampler completed a questionnaire with information concerning the sampled litter bins. This information included the district, the street number as well as the GPS signal of each sampled litter bin, the general condition of litter bin (broken or not), the functionality of the pedal system (working or not), the presence or not of garbage and the general sanitary condition (clean or not) of the litter bin.

Then, a cotton swab was brought into contact with the handle of each litter bin by the sampler and a sample of the surface was collected with smooth movements. The sampling area was about 10cm^2 and the movements were repeated three times towards all directions. After sampling, the cotton swab was inserted in a sterilized plastic tube with transport media (1 ml) and each sample was given a specific code number. Samples were carried immediately to the laboratory for further analysis. The sample from each swab was spread onto a 9cm Petri Dish containing Tryptic Soy Agar medium (TSA) (Merck, Darmstadt, Germany) and incubated at 36°C (±1°C) for 24 hours. After the incubation period, two experienced analysts enumerated bacterial and fungal colonies on each plate based on their morphology and the number was recorded. Following that, 30 (30.3%) out of the 99 Petri dishes were randomly selected (first and last of each sampler, as well as three random Petri dishes between them) for further bacteriological analysis. From these Petri dishes, three characteristic and distinctive bacterial colonies were selected and were sub-cultured into new Petri dishes containing TSA. Subsequently, the sub-cultured ninety (90) bacterial colonies were isolated and stained with the use of the Gram stain (Ellis, 2007).

Twenty-five (27.7%) out of these ninety dishes were further sub-cultured and tested in selective media according to ISO methods. Samples were subjected to standard analysis for coliform bacteria and *E.coli* (ISO 9308-01: 2000), intestinal enterococci (ISO 7899-02: 2000) and total count at 22°C and 27°C (ISO 6222Q 1999). The media used were Trypton Bile X-glucoronide (TBX) (Merck, Darmstadt, Germany), Clostridium Selective medium (Oxoid, Hampshire, England), Salmonella Shigella Agar (SS) (Oxoid, Hampshire, England), Membrane-filter Enterococcus selective agar acc. to Slanetz & Bartley (SB) (Oxoid, Hampshire, England), BBL Manitol Salt Agar (BD, Sparks,USA) tested with Oxoid latex staph test (Essers, 1980) and oxidase reaction (Cooper, 1970). Another thirty-five (35) of the initial ninety bacterial colonies were identified with Microscan WalkAway 96 (Date-Behring). The antibiotic resistance of these strains was determined using their Minimum Inhibitory Concentration (MIC) (Andrews, 2001).

For the statistical analysis, SPSS 16.0 was used while for the mapping Arc-GIS software has been applied (ESRI, USA).

RESULTS

The investigation revealed that eighty-five (85.8%) handles

Table 1. General condition of litter bins per district

	AD	SD	ED	CD	Total
Unclean (%)	71,4	100	100	82,2	88
Broken (%)	31,8	42,8	16,7	28,6	30
Non Functional Pedal System (%)	38,6	38,1	22,2	28,6	32
With Garbage (%)	57,1	50	83,3	77,8	67
Average Bacterial count (cfu)	236	507	171	276	297,5
Average Fungal count (cfu)	180	507	126	319	283

(AD: Arctic District, SD: South District, ED: East District, CD: Central District)

of the litter bins were not clean and thriving of bacteria which probably indicates that their cleaning does not take place at the proper frequency. The handles of 30 (30,3%) litter bins were broken. In 33 (33.3%) litter bins, the pedal mechanism was not functional making mandatory the opening of the litter bin by hand. The number of broken litter bins, the non-functional pedal system as well as the general condition of the litter bins correlated significantly ($p<0.05$) with the district (Table 1).

In 56 samples (56.6%), 0 up to 50 total bacterial colonies (cfu) were counted whereas in 43 samples (43.4%) more than 50 cfu were recorded. As for fungi, in 49 samples (49.5%) 0-50 colonies were enumerated, while in 50 samples (50.5%) > 50 colonies were recorded. The average bacterial and fungal counts in each district are shown (Table I). According to statistical analysis, the bacterial numbers had a significant correlation to the district of the litter bin ($p<0.05$). The majority of the isolated bacteria were Gram positive (76%). Based on their microscopic image, the microorganisms were predominately characterized as bacilli (76%) and les often as cocci (24%). The bacterial numbers on the handle of each litter bin are shown in Figure 1.

From the isolated bacteria, 6 (6.6%) were identified as Escherichia *coli,* 2 (2.2%) as Clostridium *perfringens,* 3 (3.3%) as Clostridium spp., 3 (3.3%) as Salmonella spp., 2 (2.2%) as Shigella spp., 3 (3.3%) as Enterococcus *faecalis,* 1 (1.1%) as Enterobacter *clocae,* 1 (1.1%) as Chryseobacterium (F.) *indologenes,* 1 (1.1%) as Aeromonas *hydrophila*, 2 (2.2%) as Rothia *mucilaginosa,* 1 (1.1%) as Kokuria *kristinae,* 8 (8.9%) as Micrococcus or related species, 7 (7.8%) as Staphylococcus *aureus,* 9 (9.9%) as Staphylococcus *auricularis*, 2 (2.2%) as Staphylococcus *haemoliticus*, 1 (1.1%) as Staphylococcus *hominis*-novo, 2 (2.2%) as Staphylococcus *haemoliticus*, 1 (1.1%) as Staphylococcus *lugdunensis* and 1 (1.1%) as Staphylococcus *capitis.* Because the number of colonies in each petri dish was very high, it was not possible to identify every characteristic colony.

DISCUSSION

Although the litter bins should be frequently disinfected, there are cases that these guidelines are not followed. Also, it is common, that litter bins are used by people susceptible to infections such as elderly or children. Although a pedal system exists, in several cases this system does not function making the contact with the handles unavoidable.

To our knowledge, this is the first study, concerning the prevalence of microorganisms (potentially pathogenic) on the handles of litter bins. The microbiological quality of these handles could pose an important public health issue (especially in cities) because of the possible use of litter bins by children or elderly people, especially if the litter bin pedal mechanisms are broken necessitating the manual use of the handle.

In our study, several different types of microorganisms have been isolated. Of note, Staphylococcus spp., Aeromonas spp. Staphylococci are frequently isolated from bovine milk, body sites, surfaces and cavities and occasionally from the environment. Coagulase-negative staphylococci (CNS) such as Staphylococcus *hominis - novo*are currently recognized as some of the most important causes of nosocomial infections worldwide, principally related to bloodstream infections in humans (D'Azevedo et al., 2008). Staphylococcus *aureus* is a common aetiological agent of foodborne intoxication and is a significant marker of food quality and surface cleanliness. Poultry meat is a common vehicle of foodborne illness, with *S. aureus* usually being one of the causes of outbreaks involving large numbers of people (Losito, 2005).

However, the surfaces of equipment can also be a source of *S. aureus* contamination. Upon re-investigation of the microflora of human ears, it has been found that the predominant gram positive, catalase-positive cocci inhabiting this specific site belong to the new species Staphylococcus *auricularis* and Staphylococcus *capitis* (Kloos and Schleifer, 1983). Aeromonas *hydrophila* and other motile aeromonads are among the most common bacteria in freshwater habitats throughout the world, and these bacteria frequently cause disease among cultured and feral fish (Cipriano et al., 1984). Aeromonas spp. have also been isolated from vegetables and a variety of animal products including meat, poultry, seafood, and milk (Janda et al., 1999). Only in recent years has the clinical importance of motile Aeromonas isolates been recognized. These pathogens have been associated with several categories of human infections, such as gastroenteritis, peritonitis, endocarditis, meningitis, septicaemia, and

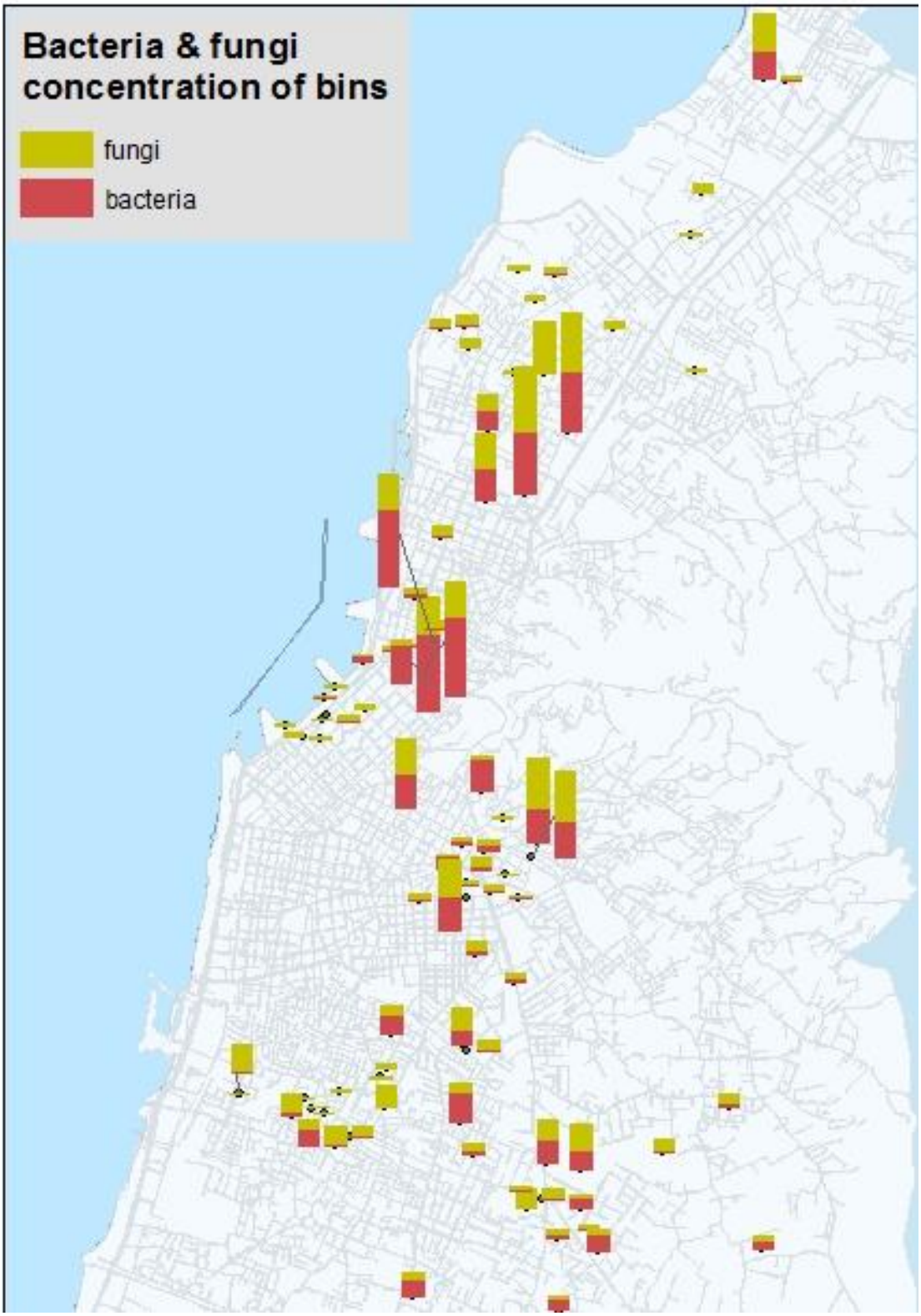

Figure 1: Sampling locations and bacterial colony counts of handles of each litter bin sampled

urinary tract and wound infections (Krovacek et al., 1994). Documented cases of infections due to Kocuria *spp.* are limited. *K. kristinae* (previously known as Micrococcus *kristinae*), was first described in 1974 . This organism is an aerobic, gram-positive coccus occurring in tetrads, and the majority of strains are non-pathogenic. Clinically similar to

Table 2. % Resistance of isolated bacteria in each tested antibiotic

Antibiotics	Resistant		Total tested bacteria
	Number	Percentage	Number
Amikacin	0	0,0%	3
Amox/K Clav	9	42,8%	21
Amp/Sulbactam	0	0,0%	2
Ampicillin	4	26,7%	15
Aztreonam	3	100%	3
Cefazolin	9	42,8%	21
Cefepime	11	61,1%	18
Cefotaxime	10	45,5%	22
Cefoxitin	0	0,0%	2
Ceftazidime	2	66,7%	3
Ceftriaxone	1	33,3%	3
Cefuroxime	6	42,8%	14
Cephalothin	9	42,8%	21
Chloramphenicol	1	5,5%	18
Ciprofloxacin	1	4,7%	21
Clindamycin	9	47,4%	19
Ertapenem	0	0,0%	2
Erythromycin	3	15,8%	19
Fusidic Acid	1	5,5%	18
GentamIcin	1	4,7%	21
Imipenem	8	36,3%	22
Levofloxacin	0	0,0%	2
Meropenem	8	36,3%	22
Moxifloxacin	0	0,0%	1
Ofloxacin	2	10,5%	19
Oxacilin	8	44,4%	18
Penicillin	4	28,5%	14
Pip/Tazo	0	0,0%	3
Rifampin	1	5,2%	19
Synercid	1	5,2%	19
Teicoplanin	1	5,2%	19
Tetracycline	1	4,5%	22
Ticar/K Clav	8	38,1%	21
Tobramycin	0	0,0%	1
Trimeth/Sulfa	0	0,0%	22
Vancomycin	1	5,2%	19

K. rosea, a single case of catheter-related bacteraemia due to *K. kristinae* has been reported in a patient with ovarian cancer (Ma et al., 2005).

As it concerns their antibiotic resistance (Table 2), 10 (10.1%) isolated bacterial strains were found to be resistant in more than 3 antibiotics. Specifically, Staphylococcus *hominis*-novo was resistant to 22 different antibiotics. 7 bacterial strains [Staphylococcus *auricularis* (3), Staphylococcus *haemoliticus* (2), Staphylococcus *lugdunensis* (1), Staphylococcus *capitis*-ureo (1)] were resistant to 10 or more antibiotics. The Enterobacter *clocae* was found resistant to 5 antibiotics and Chrysobacterium (F.) *indologenes* was resistant to 3 antibiotics. The rest of the isolated bacterial strains were found resistant in less than 3 antibiotics (data not shown).

Litter bins are an essential part of everyday life for urban residents. When the handles are not clean and bear an abundance of potential pathogenic bacteria and fungi, the risk for the public health is increased. The prevalence of bacteria in the handles of the litter bins as well as their antibiotic multi resistance could be a very important consideration for public health in the local community. The risk may pertain not only to the municipality's employees who are responsible for garbage disposal but also to anyone who throws his garbage, and especially characteristic population groups such as children or pregnant women who may come into contact with the handles of litter bins.

Conclusions

Litter bins' placement is highly correlated to the type of microorganisms isolated in them. Litter bins' situation can pose a threat for public health. For this reason, they should be cleaned up and strelilized thoroughly and consistently

to limit the negative effects on public health.

Recommendations

In order to protect the public health, all municipal litter bins should be cleaned and disinfected on a regular basis by trained personnel and they should also be in excellent condition. Moreover the pedal systems must be functional so that people avoid the use of their hands to open the lids. The city's residents should be informed about the risks and take necessary precautions if they notice that a litter bin is not in a good condition.

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