Microbiological hazard for patients caused by bacteria present on synantropic arthropods in hospital environment

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Arthropods in hospital are undesirable elements. However, they invaded hospitals such as other places in urban environment. Arthropods such as cockroaches, houseflies, ants, mosquitoes, spiders could take part in transferring pathogens that might be source of infection. Many authors reported arthropods that occur in hospitals and are important factor in worsening the health of patients (infections, stress, anxiety, allergy, asthma), especially person with decreased immunity.

In the years 2000-2002 German cockroaches were recorded in about 79.2% of hospitals in Poland. In the years 2003-2006 these insects were captured in selected hospitals, in Poland and bacteria present on the surface of the body of cockroaches were isolated. From cockroaches bodies were isolated many strains of bacteria, e.g. *Enterobacter cloacae, Klebsiella pneumoniae, Citrobacter freundii*, *Serratia marcescens, Pseudomonas aeruginosa*, *Staphylococcus epidermidis, Enterococcus faecalis, E. faecium*; and strains of fungi: *Trichosporon beigelii, Fusarium moniliforme i Scopulariopsis brevicaulis.* Many strains of them showed resistance to antibiotics and disinfectants. Several of isolated strains demonstrated important mechanisms of antimicrobial resistance: Gram-negative bacilli - AmpC (+), ESBL (+), some strains of Gram-positive cocci - MLSb and MRCNS.

It was found that some strains *S. marcescens, P. aeruginosa, S. epidermidis* formed a biofilm. The possibility of biofilm formation could explain a resistance to some disinfectants, such as: glucoprotamine, monopotassium persulfate, sodium dichloroisocyjanurate.

Presence of such strains in hospital ward could cause risk for patients; they could be reservoirs of resistance genes which can be transmitted into other bacteria.

The obtained results showed that German cockroaches present in hospital environment should be considered not only as nuisance insects but also as a real source of pathogenic bacteria. Therefore, Pest Management should be an integrated part of nosocomial infection preventive system.

Keywords cockroaches; bacteria; resistance

1. Introduction

Regardless of state and region in the world, hospital's environment is a reservoir of pathogenic microorganisms in a higher or lesser extent. It is estimated that in developing countries, nosocomial infections concern above 25% of hospitalized patients, and in the developed countries from 5 to 10% [1]. The sources of infections can be: patients, medical personnel, visitors or parts of the environment: equipment and hospital items, also arthropods inhabiting hospitals.

Following the literature, hospital buildings can be infested by different insects, both flying and crawling, as well as ectoparasites. Table 1 presents data concerning the occurrence of the insects belonged to different species in the hospitals in Great Britain, Czech Republic, Korea and Poland [2,3,4,5].

Table 1 The occurrence of insect pests in the hospitals in Great Britain, Czech Republic, Korea and Poland

Insects' species	Great Britain	Czech Republic	Korea	Poland
German cockroach *(Blattella germanica L.)*	+	+	+	+
Oriental cockroach *(Blatta orientalis L.)*	+	+	-	+
American cockroach *(Periplaneta americana L.)*	-	-	+	–
(Periplaneta fuliginosa L.)	-	-	+	-
Crickets *(Acheta domesticus L.)*	+	-	-	-
Fleas *(Siphonaptera*)	+	-	-	-
Pharaoh ant *(Monomorium pharaonis L.)*	+	-	-	+
Ants – different species coming from the outside *(Formicidae)*	+	+	-	+
Flies *(Diptera)*	-	+	-	+
Mosquitoes *(Culicidae)*	-	+	-	+

The most common insects in the hospital buildings in these countries were cockroaches: German cockroach *(Blattella germanica L.*), Oriental cockroach *(Blatta orientalis L.*), American cockroach *(Periplaneta americana L.), Periplaneta fuliginosa L.* and ants: Pharaoh ant (*Monomorium pharaonis L.*) and ants from other species coming from the outside to the buldings. Apart from them in hospitals revealed the presence of flies, mosquitoes, fleas and crickets.

2. Microorganisms carried by arthropods present in the hospital environment.

According to the literature, from cockroaches, flies and ants were isolated microorganisms which are often pathogenic hospital strains or alert pathogens [6,7,8]. Because of this, arthropods are generally considered as the possible sources of the pathogenic microorganisms and their transmitters in this environment. In the studies performed on the microflora of the arthropods caught in the hospital environment in Prague, Sramova et al. [2] found that from among 116 bacterial isolates obtained from the bodies of the arthropods, 59% were extracted from: flies, cockroaches, bees and *Tenebrionid* beetles; 16% from ants and spiders; 6% from mosquitoes and 19% were from accidentally caught insects, such as moths and beetles. Among bacterial strains isolated from hospital arthropods, Gram negative rods were dominant (88% from all isolates). Coagulase – negative cocci including *Staphylococcus hominis* and multi-drug-resistant *Enterococcus sp.* established 13% of all isolations [2].

In the studies on the bacterial flora from the American cockroaches *Periplaneta americana* provided in large health care institution in Brasil, 74.6% were Gram – negative rods and 25.40% were Gram – positive cocci [9]. From the German cockroaches *Blattella germanica L.* caught in the hospitals in Hamadan in Iran about 60% of bacterial isolates were Gram – negative rods; 17,5% were Gram – positive cocci respectively [10]. Studies carried out in Poland showed significantly higher species diversity among the isolated bacteria from the external layers of the cockroaches: in Gram – positive cocci strains (20 species) than among Gram – negative rods (10 species). Among the isolates 8.8% of the Gram (+) catalase (+) cocci, 34.2% of the Gram (+) catalase (-) cocci, 39.5% of the Gram (-) fermenting rods, 1.7% of the Gram (-) non-fermenting rods, 15.8% of the Gram (+) cocci were obtained [8].

Most often isolated from the arthropods Gram – negative rods belonged to the following genera: Klebsiella spp, Escherichia spp, Enterobacter spp, Citrobacter spp, Proteus spp, Seratia spp, Pseudomonas spp, Acinetobacter spp [6,7,8,9]. However, isolated Gram – positive cocci most often belonged to the following genera: Staphylococcus spp, Enterococcus spp, Micrococcus spp, Streptococcus spp. From the arthropods caught in the hospitals were isolated also yeast and fungi: Candida spp, Aspergillus spp, Rhizopus spp, Trichosporon spp, Fusarium spp, Penicillinum spp and other [6,7,8,9].

Aspergillus flavous, *Aspergillus niger*, *Penicillium spp*., *Fusarium spp*., *Microsporum gypseum*, *Alternaria spp.* strains were separated from the houseflies caught in the hospital in Sanadaj in Iran [11]. Fungi of *Aspergillus* type, including *A. niger*, were also isolated from *Caliphoridae* flies caught from close to the hospital's areas and in the hospitals [12].

Microorganisms, which were extracted from arthropods inhabiting the hospital environment and nearby areas, belonged to the species which are most often present in the environment: in soil, on the surface of water, on plants, on organic remains, and what is more, they can be the part of natural flora of human.

It is suggested, that many bacterial strains among those isolated from arthropods, can be an important etiological factor for nosocomial infections. Most of these strains are common in the environment and opportunistic – they cause

infection only for immunodeficient patients, who are hospitalized and who suffer from such diseases as diabetes or AIDS.

3. The characteristic of selected microorganisms isolated from the arthropods caught in the hospital environment.

3.1. Resistance to antibiotics and chemotherapeutics.

Many bacterial strains isolated from arthropods caught in the hospitals or nearby areas, were resistant to antibiotics and chemotherapeutics. They also could have the following mechanisms of resistance [8].
- ESBL (extended spectrum of beta lactamases) and AmpC (AmpC beta lactamases) – (Gram – negative rods),
- methicillin resistance (MRSA – methicillin resistant *Staphylococcus aureus*,
- MRSE – methicillin resistant *Staphylococcus epidermidis,*
- MRCNS – methicillin resistant coagulase – negative Staphylococci),
- MLSb (resistance to macrolides, lincosamides, streptogramin B) – (Staphylococci).

From the external body surface of the German cockroaches *Blattella germanica L.* were isolated *E. cloacae* strains, and some of them showed phenotypically ESBL activity besides AmpC beta-lactamases [13]. The presence of plasmids with ESBL enzymes in the bacteria meant resistance to the penicillins, cephalosporins and monobactams. On the same plasmid can be present genes, which determine resistance to other groups of antibiotics, for example amino - glycosides or sulfonamides. ESBL enzymes, which are currently detected in *Enterobacter cloacae, Serratia marcescens, Klebsiella oxytoca*, in the past were typicall for the strains of *K. pneumoniae* and *E. coli* only [14].

The strains of *Klebsiella sp.* were extracted from *Tapinoma melanocephalum (Fabricius), Paratrechina longicornis (Latraille), Monomorium pharaonis (L.)* ants, which had infested ICU and Infirmaries in Campos Dos Goytacazes in Brazil. The strain of K. *oxytoca* isolated from the pediatric ward has shown the resistance to 10 of 16 tested antibiotics [6]. Fotedar et al. [15] had shown that houseflies *Musca domestica L.* caught in the surgical ward could be the potential hospital's vector of multi-resistant strains of *Klebsiella spp.* What is more, 82% of *Klebsiella spp* strains isolated from flies caught in the hospital and 96.3% of *Klebsiella spp* isolated from patients' wounds were resistant to four antibiotics at least. Additionally, strains of *Klebsiella spp,* isolated from flies caught in households located 5 km away from the hospital, were sensitive to used antibiotics [15].

The strains of *Klebsiella spp* were also obtained from cockroaches caught in the hospitals [16]. In those studies it was stated, that more than 85% of these isolates were resistant to more than four types of antibiotics, whereas all strains of *Klebsiella spp* isolated from the control cockroaches were sensitive. This difference was statistically significant. [16]

Generally, *Klebsiella sp* strains are characterized typically by presence of chromosomally encoded beta-lactamases (class A), which give them resistance to penicillins, and what is more, many of them have beta-lactamases with a wide range of activity. The latter gives them the resistance to carbenicillin, ampicillin and quinolones. It is also documented that the strains of *Klebsiella pneumoniae* and *E. coli*, (especially resistant - for example those which produce ESBL), have a high ability to spreading in the hospital environment [14].

Prado et al. [9], found that 13% of the strains of *Serratia marcescens* isolated from *Periplaneta americana* which were caught in hospitals, were resistant to gentamicin. The strains of *Serratia liquefaciens* isolated from the cockroaches in hospitals in Poland showed insensivity to commonly used antibiotics or chemotherapeutics; they were characterized by presence of AmpC and ESBL mechanisms of resistance [8].

The strains of *Citrobacter freundii* with AmpC and ESBL mechanisms of resistance were also isolated from cockroaches caught in the hospital environment. The expression of AmpC beta-lactamases (C1 class) was an induced type and they are active to penicillins, cephalosporins and monobactams. Strains with AmpC (+) phenotype are resistant *in vivo* to cefotaxime and azteronam, despite the exhibited sensitivity *in vitro*[8]. Davari et al. [17] isolated *C. freundii* from *Musca domestica L.* flies caught in hospitals; they constitute 28.4% of all isolates and they showed high resistance to the I generation of the cephalosporins.

From ants *T. melanocephalum (Fabricius)* and *P. longicornis (Latraille)* present in the hospital environment in Campos dos Goytacazes were separated *S. aureus* strains MRSA type. [6]. Such strains were isolated also from *Calliphoridae* flies caught around buildings of the hospital in Warsaw [12]. This strain was methicillin- resistant (it was resistant to all of the beta-lactam antibiotics) and additionally was insensitive to amino- glycosides and doxycyclin [12].

Resistance to methicillin was also found in strains of coagulazo-negative Staphylococcus (CoNS) isolated from ants caught in the hospitals in Brazil. Two of them *(Staphylococcus saprophiticus*, *Staphylococcus equorum)* were also resistant to vancomycin [6].

Presence of MRSE – methicillin resistant *Staphylococcus epidermidis* in the hospital environment is undesirable, because there is the probability of the transmission of *mecA* DNA genes, which are responsible for resistance to methicillin, from one species of staphylococci to the other (e.g. on *Staphylococcus aureus*) [18,19].

Strains of staphylococci *(S. epidermidis, S. xylosus* and *S. equorum*) which were isolated from the cockroaches caught in the hospitals in Warsaw, were resistant to methicillin, and what is more, those *S. epidermidis* i *S. equorum*

were resistant to erythromycin and clindamycin (MLSb mechanism - induced mechanism of resistance to macrolides, lincosamides and streptogramin B). Extracted *Staphylococcus hominis* strain was resistant to at least three commonly used antibiotics, including gentamicin [8].

German cockroaches from hospitals were also source of *Enterococcus faecalis, Enterococcus faecium* and *Enterococcus casseliflavous, Enterococcus durans* and *Enterococcus avium* strains [8]. All those bacteria were sensitive to antibiotics. Isolated *E. casseliflavous* strain was medium-sensitive to vancomycin, (it is connected with v*anC* phenotype of this species, which have resistance to low concentrations of vancomycin) but medium sensitivity to teicoplanin [8]. In this work authors found that strains of enterococci carried by *Diptera* were resistant to some aminoglycosid chemotherapeutics, but sensitive to vancomycin [12]. It is known, that enterococci enquire and transmit genes determining resistance to many antibiotics which are used in hospitals against various types of bacteria, e.g. *Streptococcus spp, Staphylococcus spp*. An example is transmission of resistance gene vancomycin *vanA* to *S. aureus* [20].

Strains of *Pseudomonas sp.* were often isolated from insects caught in different environments, including also hospital areas. From hospitals' cockroaches strains of *P. aeruginosa* and *P. putida*, - resistant to penicillins, cotrimoxazole and trimethoprim were obtained. They didn't produced beta - lactamase of extended ESBL substrate spectrum (-) nor induced beta-lactamase IBL (-) as well [8].

3.2. The sensitivity of bacterial strains isolated from the arthropods caught in the hospitals on disinfectant agents.

Pancer et al. [13,21] determined the biocide effectiveness of several disinfectants against the microorganisms isolated from arthropods caught in the hospital. Carrier method and MIC designation (the lowest concentration inhibiting the growth of bacteria) were used. The ability of several biocides to kill microorganisms which had shown ability to creating biofilm was also assessed.

In the study with the carrier method was used, it was found, that preparations contained 21.5% potassium peroxymonosulphate and 99% sodium dichloroisocyanurate respectively were effective in 100%, and preparation with 26% glukoprotamin was ineffective to *S. marcescens, S. epidermidis* strain (Table 2) [22].

Those authors specified also MIC values for the active substances, i.e. glukoprotamin and potassium peroxymonosulphate, which are the component of popular disinfectants using in hospitals. It was also shown that for some strains of *E. cloacae* isolated from cockroaches, MIC for glukoprotein was very high - 125 $mg.L^{-1}$. All bacteria strains isolated from cockroaches had high MIC value for potassium peroxymonosulphate of 1000 $mg.L^{-1}$ as well. In this work was also stressed that the effectiveness of the preparations used in working concentrations against *E. cloacae* (originated from insects) incubated for 5 days on a catheter was low: glukoprotamin was ineffective against 75% of strains [13].

In the studies of effectiveness of disinfectants against rods isolated from the hospital environment is was found that *S. marcescens* strain isolated from German cockroaches caught in the hospital had very low sensitivity (MIC value 500 $mg.L^{-1}$) to the glukoprotamin used in the working concentration 5200mg/L. This strain had shown an ability to growth as a biofilm. In this case working concentrations of the potassium peroxymonosulphate (4300mg/L) and sodium dichloroisocyanurate (1795,2 mg/L) were ineffective. It was found also, that *P. aeruginosa* multi drug resistant strains (MRD) were insensitive to the working concentrations of the glukoprotamin (5200mg/L) and the potassium peroxymonosulphate of 4300 mg/L when they had formed biofilm on the drain [21].

Table 2 shows some data on sensivity/insensivity of selected multidrug resistant microorganisms isolated from German cockroaches in Polish hospitals.

Table 2 Effectiveness of selected disinfectant agents on MDR microorganisms isolated from German cockroaches caught in hospital environment.

	Strain of bacteria	Hospital	Drug resistance; resistance mechanism present	Antibacterial activity of surface disinfectants: (+) growth of microorganism; (-) no growth obs.		
				26% glucoprotamin	21.5% potassium peroxymonosulphate	99% sodium dichloroizocyanurate
Gram (-) rods	*Pseudomonas aeruginosa*	B	AMP, AMC, SXT, W	-	-	-
	Pseudomonas putida	B	AMP, AMC, SXT, W	-	-	-
	Serratia marcescens	B	S	+	-	-
	Serratia liquefaciens	E	AmpC (+), ESBL(+)	-	-	-
	Enterobacter cloacae	A	AMP, AMC,	-	-	-
	Enterobacter cloacae, 2 strain	F	AmpC (+), ESBL(+)	-	-	-
	Klebsiella pneumoniae	F	AMP	-	-	-
Gram (+) cocci	*Staphylococcus hominis*	A	MRCNS, SXT, W, RL, FD, GM, AMP	-	-	-
	Staphyloccocus equorum	A	MRCNS, W, MLSb(+), RL	-	-	-
	Staphyloccocus epidermidis	A	MLSb(+), AMP, TE, RL	+	-	-

3.3. The ability to form a biofilm

Microbes in the natural environment are rarely found as plankton. They have tendency to absorption for creating a biofilm, which adheres to the solid surfaces or the surfaces of the cells of other organisms. The growth of bacteria in this form significantly reduces the effectiveness of antibiotics and disinfectant agents.

Pancer et al. [21] found, that selected strains of Gram (-) negative rods, e.g. *S. marcescens, S. rubidea, S. liquefaciens, E. cloacae, C. freundii, P. putida, P. aeruginosa* isolated from the cockroaches have an ability to form a biofilm on the drain.

None of the isolated *E. cloacae* strains from the cockroaches' shields did have an A type adherence, whereas most of them showed the distributed adhesion (C type); 7 out of 12 tested strains isolated from cockroaches showed adherence which was sensitive to the presence of 1% of the mannose [13].

The strains of *S. epidermidis* isolated from the cockroaches' shields, expressed the virulence factors: they produced biofilms in microplates and they possessed an ica AD gene responsible for the ability to form a biofilm [23].

4. The assessment of the infection risk for the patients.

In the project 3P05D10624 which was hold in Poland in the years 2003 - 2006 studies concerning the assessment of the role of the synantropic cockroaches as the possible source of the infections in the hospital environment were carried out.

For this purpose the model was elaborated, which allowed numerical assessment of the microbial infection risk for patients in the surveyed hospitals. Microbiological risk's evaluation was based on the properties of the isolated microorganisms. With using statistical methods, the connection between biological factors spreading by infected cockroaches (*Blattella germanica* L.) and the risk of the occurrence of the infection in patients hospitalized in the hospital, was searched.

The data used for the assessment of the microbiological risk, were as follows:

1. Identified bacterial strains isolated during researchers from the shields of the insects caught in the hospitals. As a risk was taken the presence of the strains relatively pathogenic, drug-resistant, more often as an etiologic factor in the hospital's infections, for example *Enterobacter cloacae*, *Serratia marcescens*, *Enterococcus feacalis* and others.

2. The sensitivity to antibiotics and chemotherapeutics of the selected bacteria. Most of the Gram-negative bacteria were resistant to the antibiotics, and some of them have produced beta-lactamase of AmpC and ESBL type. The resistance to methicillin (MRCNS) and the induced mechanism of the resistance to macrolides, lincosamides and streptogramin B (MLSb) were found among the coagulase-negative staphylococcus. Some strains of enterococci had reduced sensitivity to ciprofloxacin, and *E. faecalis* was resistant also to tetracycline.

3. Activity and effectiveness (in the working concentration) of the 3 disinfectants using in the hospitals (active ingredients: glukoprotamin, sodium dichloroisocyanurate and potassium peroxymonosulphate) against isolated hospital strains of bacteria selected by authors. The preparation containing glukoprotamin was the least effective against the bacteria isolated from the hospital environment (60% of effectiveness); the most effective (100%) was the preparation containing the potassium peroxymonosulphate. All of the preparations showed low effectiveness against bacteria growing as a biofilm.

4. Other characteristics indicative for the virulence of the bacterial strains, such as the ability to grow as a biofilm on the drain, the adhesion of bacteria to human cells, the cell line Hep-2 ect..

5. The cockroaches'' resistance to insecticides used for their control agents in hospitals: bendiocarb, deltamethrin, chlorpyrifos, fipronil. The resistance to 2 from 4 at least was found in every hospital. The degree of the settlement by the cockroaches in the hospitals was different: from 0 to 100 caught insects, the bigger the more resistant insects in the hospital.

Risk for patients of being infected by bacteria transmitted by cockroaches in every of the researching hospitals was assessed with the point scale, taking into consideration 5 factors: the number of the caught cockroaches (from 0 to 100) and their resistance to insecticides (from 0 to 11), the number of the bacterial strains isolated from insects and their allegiance to the pathogenic species in the hospital infections (from 0 to 56), the number of the bacterial strains in the hospital, which were resistant to one or more antibacterial drugs (from 0 to 48), the occurrence of the features responsible for the virulence of strains, which was specified in the laboratory test and decreasing the sensitivity for disinfectants of the selected hospital strains.

Total scores (in the table 3) were calculated as a product of all intermediate components, corrected by the environmental factors (detais – in Gliniewicz et al. and Stypułkowska – Misiurewicz et al.) [24,25]

Table 3 The microbiological risk connected with the cockroaches' presence in the hospitals.

Hospitals	Resistance (scaled)	The No of the isolated strains	Antibiotic-resistance	The resistance to the disinfectants	The point risk scale
A	1.43	26	32	2	9508.6
B	1.79	16	20	1,5	3428.6
C	1.64	16	14	1	1472.0
D	1.64	45	32	1,5	3548.6
E	1.64	43	40	2	5651.4

As shown in the table 3, the highest resistance of cockroaches to the control agents was in the hospital B. The cockroaches occurring there were insensitive for 3 of 4 researching insecticides: chlorpiryfos, deltamethrin and fipronil. The most sensitive to the tested insecticides were cockroaches caught in the hospital A: they were sensitive to 2 active substances (bendiocarb and chloropiryfos). In th hospitals C, D and E the cockroaches were only sensitive to 1 of 4 testing insecticidal compounds.

Most bacteria were isolated from caught insects in D and E locations (hospital kitchens), which is shown in the tab. 2 values of the risk (points 45 and 43). But taking into consideration bigger danger connected with the presence of the pathogens in the hospital wards, after making the correction, the biggest risk was for the hospital A.

In case of the risk caused by the bacterial strains' resistance to the antibiotics, the highest point value was calculated for the object E, and the lowest – for the hospital C. In the last hospital the presence of the resistance to 3 tested disinfectants among the bacteria strains haven't been found (Table 3). In the hospitals A and E testing bacterial strains were resistant to 2 of tested disinfectants, in hospitals B and D – to one.

The cockroaches in the hospital C were resistant to 3 active substances, like in the hospitals C and E (Table 3).

According to the data, the biggest risk (by the using point scale) was estimated for the hospital A (9508.6 points). The risk was made by the presence of many pathogenic and resistant to antibiotics bacterial strains. As studies have shown, some of them were insensitive to surface disinfectants (Table 2). In this case, cockroaches were the most sensitive to the insecticides among 5 studied populations. The lowest risk (with the conducted estimates) was in the hospital C (1472.0 points).

Developed model can be an attempt of the evaluation for the microbiological risk, when the synantrophic insects carrying on their bodies microorganisms, are in the hospital. Despite the existing threat of the potentially pathogenic

microorganisms, which show virulent features, and what is more, they are resistant to antibiotics and disinfectants, probably implemented procedures of preventing the hospital infections, including the proper disinfection and insect control, greatly reduce the risk of the occurrence of the hospital infection, as was shown in the testing hospitals in Warsaw.

Based on the results of the presenting studies we can conclude that the cockroaches' role (and also other arthropods) in the spreading bacteria in the hospital environment should not be omitted. Permanent supervision, monitoring and preventive measures for the prevention of the hospital infections should be complex. They should include the monitoring of the strains isolated from the widely understood hospital environment for the assessment of their resistance to the antibacterial drugs, agents using for the disinfection in the hospital, the assessment of the insect control agents effectiveness and arthropods' resistance to insecticides.

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