Letter to the Editor

External Bacterial Flora and Antimicrobial Susceptibility Patterns of *Staphylococcus* spp. and *Pseudomonas* spp. Isolated from Two Household Cockroaches, *Blattella germanica* and *Blatta orientalis*

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A study was performed to estimate the prevalence of the external bacterial flora of two domestic cockroaches (*Blattella germanica* and *Blatta orientalis*) collected from households in Tebessa (northeast Algeria). Three major bacterial groups were cultured (total aerobic, enterobacteria, and staphylococci) from 14 specimens of cockroaches, and antibiotic susceptibility was tested for both *Staphylococcus* and *Pseudomonas* isolates. Culturing showed that the total bacterial load of cockroaches from different households were comparable (P<0.001) and enterobacteria were the predominant colonizers of the insect surface, with a bacterial load of (2.1×10^5 CFU/insect), whereas the staphylococci group was the minority. Twenty-eight bacterial species were isolated, and susceptibility patterns showed that most of the staphylococci isolates were highly susceptible to chloramphenicol, gentamycin, pristinamycin, ofloxacin, clindamycin, and vancomycin; however, *Pseudomonas* strains exhibited resistance to amoxicillin/clavulanic acid, imipenem, and the second-generation antibiotic cephalosporin cefuroxime.

Cockroaches are among the most common insects found in industrial and residential environments[1]. Indeed, the species has been highly successful in exploiting niches within human habitation. In addition to being a persistent pest, cockroaches also have the potential to disseminate human pathogens, thereby representing a public health risk[2]. From previous surveys, it has been found the cockroaches harbor a diverse range of bacterial genera including multiple drug-resistant strains, such as *Staphylococcus aureus, Pseudomonas aeruginosa,* Shiga toxin-producing *E. coli*, *Klebsiella* spp., and *Salmonella typhi*, in addition to a range of fungi and viruses[3-4].

In recent years, the incidence of *S. aureus*- and *P. aeruginosa*-linked infections has increased, with highly virulent strains being encountered. In addition, the ability of *Staphylococcus* and *Pseudomonas* species to survive on environmental surfaces increases the probability of persistent contamination in household settings[5]. The aforementioned pathogens can be acquired from hospitals and also increasingly from non-clinical environments (i.e., community-associated infections).

Domestic cockroaches exist in many human habitats, such as hospitals, restaurants, offices, homes, markets, and the urban community, together with the bacteria they harbor. Moreover, the association of cockroaches with the human environment can cause direct food contamination and several health problems, such as allergic responses (skin rashes, watery eyes, and sneezing) particularly in patients who have a lung disease, such as asthma[1]. The problem is possibly exacerbated by the human-made creation of favorable environments (e.g., refuse piles) where cockroaches can breed effectively.

The role of cockroaches in disseminating and increasing the persistence of pathogens, such as *S. aureus* and *P. aeruginosa*, in residential environments remains unknown. Therefore, the present study is directed toward assessing the presence of culturable microflora and pathogens in two household cockroach species (*Blattella germanica* and *Blatta orientalis*) in Tebessa (northeast Algeria) and to determine the antimicrobial resistance patterns of *Staphylococcus* and *Pseudomonas* isolates.

A set of 14 adult (male/female) cockroaches (*Dictyoptera: Blattellidae*) were collected at night on

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each sampling visit in residential homes in Tebessa. The sampling period was performed between March and April 2013 with six visits in total. These 2 months corresponded to the end of winter and mid-spring seasons, when cockroaches exhibit a high rate of growth and development. Individual cockroaches were placed in a sterile tube for transportation to the laboratory, where the species were identified using a dissecting microscope following standards taxonomic keys[1].

Individual cockroaches were transferred to a 0 °C incubator for 5-10 min prior to recovering surface bacteria by submerging in 5 mL of sterile saline solution[6]. Release of microbes from the surface of the cockroaches was facilitated by vortexing at low speed with care being taken not to stimulate regurgitation by the insect.

A dilution series was prepared from the rinse solution with aliquots (0.1 mL) being spread plated onto the appropriate agar, all of which were incubated at 37 °C for 24 h. Specifically, mesophilic aerobic counts were determined using Plate count agar (*Fluka Analytical*), MacConckey (*Fluka Analytical*) for coliforms, Mannitol salt agar (*Biolab*) for enumeration of staphylococci, and Cetrimide agar (*Merck*) for isolation of pseudomonads.

Upon completion of the incubation period, colonies were counted and levels expressed as CFU/cockroach. Suspected *Pseudomonas* and *Staphylococcus* colonies were subcultured onto fresh agar plates then incubated at 37 °C for 24 h prior to performing biological confirmation tests using microscopy in conjunction with Gram staining and screening for catalase and oxidase, in addition to substrate utilization profiles using API Staph and API 20E (Biomérieux). Antibiotic susceptibility was determined using the Disc Diffusion Method on Mueller-Hinton agar (*Fluka Analytical*) according to standards and recommendations of the Antibiogram Committee of the French Society for Microbiology. The challenge panel consisted of 21 antibiotics that were sub-divided into two selected groups: *Pseudomonas* and staphylococci (Table 2). The strains *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 were used for quality control.

After a 24-h incubation, the culture plates with antibiotic discs were examined for the presence of growth inhibition, which is indicated by a clear zone (expressed in mm) surrounding each standards disc; accordingly, the results were recorded for each sample as sensitive (S), intermediate (I), or resistant (R).

Linear models (LMs) were used to model the specific effects of cockroach sex, species, and even location on specific bacterial group abundance (total flora, enterobacteria, and staphylococci). The LM summary was given for each bacterial group traits, including certain statistics outputs, namely residual standard error (RSE) and *t*-value with *P*-value (*P*). An agglomerative hierarchical clustering (AHC) was applied for determining relationships between isolated strains according to their behavior towards the different antimicrobial drugs tested (Antibiogram profile), which were grouped according to similarities based on Pearson's correlation coefficient.

Fourteen adult cockroaches were collected and found to be *B. orientalis* and *B. germanica* (7 cockroach samples/species). From plate counts recovered from the different species, it was found that enterobacteria loading was significantly higher ($P<0.001$) for *B. germanica,* whereas staphylococci was encountered less frequently (Figure 1A, Table 1). For *B. orientalis*, the bacterial loading was significantly lower compared with *B. germanica*, although staphylococci were recovered. The levels of total mesophilic and enterobacteria counts were found to be dependent on the site where the cockroach was captured ($P<0.001$).

The LMs applied for all parameters (cockroach, trapping location, bacterial group) showed a significant positive linkage ($P<0.001$) associated with *B. germanica* adult females, which consisted the model intercept of GLM (Table 1). The LMs revealed that the male captured insects were deemed negatively related to total flora and enterobacteria group, whereas *B. orientalis* exhibited a positive correlation with the two later bacterial enumerated groups.

Trapping location (corridor and kitchen) revealed a very high significant effect ($P<0.001$) associated with the two enumerated groups (enterobacteria and total flora). In general, all GLM terms corresponding to the factors species, sex, and trapping location had no effect ($P>0.05$) on variations of staphylococci abundance and occurrence (Table 1).

Among the 28 bacteria isolated from collected cockroaches, 21 isolates (RA=75%) belonged to the group of gram-positive staphylococci and only seven strains (RA=25%) of *Pseudomonas*, although *P. aeruginosa* were among those isolated. Among the *Staphylococcus* isolates, 46% were identified as *S. aureus* (13 isolates), with the remaining species

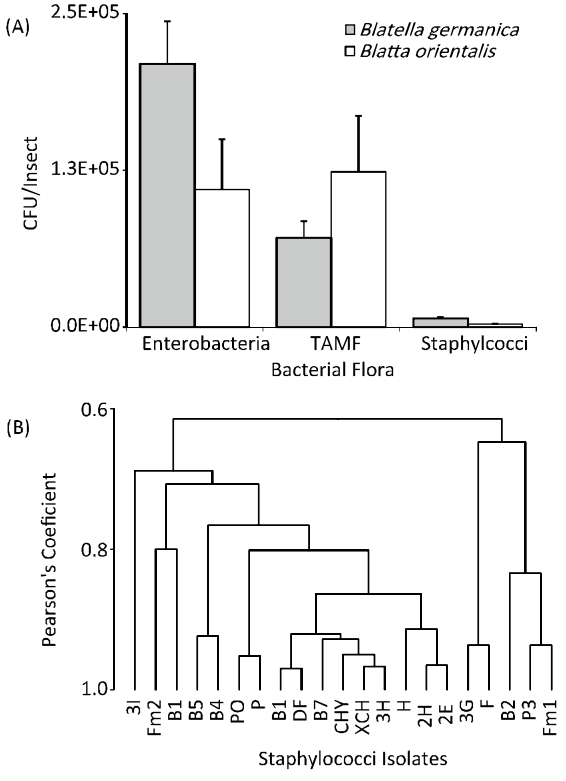

Figure 1. Bacterial flora hosted by two cockroach species (values are the average of two measurements±SD) (A). Agglomerative hierarchical clustering (AHC) illustrating relationships among staphylococci isolates based on antibiotic susceptibility profiles (B).

identified as *S. hemolyticus* (*n*=2, RA=7.1%), *S. warneri* (*n*=2, RA=7.1%), *S. epidermidis* (*n*=1, RA=3.5%), *S. lugdunensis* (*n*=1, RA=3.5%), and *S. sciur* (*n*=1, RA=3.5%).

The results of *Pseudomonas* spp. susceptibility patterns are presented in Table 2. All of the seven strains were susceptible (100%) to the third generation cephalosporin-tested drug ceftazidim, the aminoglycoside gentamicin, and aminoglycoside tobramycin. Although they were all resistant to the amoxicillin+clavulanic acid, carbapenem agent imipenem, and to the second generation cephalosporin cefuroxime. Among *Pseudomonas* strains, six were predominantly sensitive (85.7%) to ciprofloxacin and amikacin, respectively, whereas 14.3% of the strains were categorized as intermediate.

In the present study, all staphylococci isolates were found to be highly susceptible to chloramphenicol, gentamycin, and pristinamycin; their efficacy against the species was recorded as (100%) followed by vancomycin/ofloxacin (95.2%) and clindamycin (85.7%). Similarly, the present investigation indicated that 71.4% of bacterial isolates were sensitive to oxacillin and more than 50% of the strains were susceptible to erythromycin and fusidic acid. Overall, for the antibiotic susceptibility test, 27.3% was recorded as antibiotic resistant, 7.3% as intermediate, and 65.4% as sensitive (Table 2). However, susceptibility patterns of *S. aureus* and coagulase-negative staphylococci isolates showed

Table 1. Results from Linear Models Testing the Effects of Individual Factors (Cockroach Species, Sex, and Provenance) on the Parameters of Bacterial Abundance (Total Flora, Enterobacteria, and Staphyloccci Groups)

Variable	Estimate	Std. Error	*t*-value	*P*	Significance
Total flora					
Intercept	3.94E+05	2.06E+04	19.13	<0.001	***
Sex [Male]	-269	1.60E+04	-0.02	0.987	NS
Species [*B. orientalis*]	5.69E+03	1.84E+04	0.31	0.765	NS
Provenance [Corridor]	-3.94E+05	2.44E+04	-16.14	<0.001	***
Provenance [Kitchen]	-3.91E+05	1.60E+04	-24.51	<0.001	***
Enterobacteria					
Intercept	1.08E+05	1.97E+04	5.47	<0.001	***
Sex [Male]	-70	1.53E+04	-0.01	0.996	NS
Species [*B. orientalis*]	400	1.76E+04	0.02	0.982	NS
Provenance [Corridor]	-1.08E+05	2.33E+04	-4.61	<0.01	**
Provenance [Kitchen]	-1.07E+05	1.53E+04	-7.00	<0.001	***
Staphylococci					
Intercept	-1.44E+03	5.58E+04	-0.03	0.980	NS
Sex [Male]	1.53E+03	4.33E+04	0.04	0.973	NS
Species [*B. orientalis*]	-130	4.99E+04	0.00	0.998	NS
Provenance [Corridor]	7.51E+04	6.61E+04	1.14	0.285	NS
Provenance [Kitchen]	1.57E+03	4.33E+04	0.04	0.972	NS

Note. NS: no significance, ** highly significant, $P<0.01$; *** very highly significant, $P<0.001$.

that the majority of the resistances were exhibited toward the commonly used antimicrobial penicillin G.

Such diversity of expressing susceptibility towards different antibiotics was brightened by Pearson's correlation coefficient (AHC) that allowed spread of all staphylococci isolates to three different classes at 66% similarity coefficient (Figure 1B) distinguished from each other by antibiotic susceptibility profiles of tested antibiotics. The maximum number of strains (16) was represented in Cluster I, whereas two phenotypes formed Cluster II. Cluster III was made up of three strains.

The 28 isolates recovered from cockroaches could be subdivided into members belonging to *Staphylococcus* or *Pseudomonas* genera. Infections caused by multiple drug-resistant *S. aureus* and *P. aeruginosa* are increasing both in hospitals and the general community[4,7]. Several studies have examined the relationship between the environment and bacterial flora carried by cockroaches in residential areas, hospital, or other premises to highlight a possible risk of human contamination, as well as differences in both quantity and quality of isolated germs[3,6].

Table 2. Antibiotic Susceptibility Patterns of Staphylococci (*n*=21) and *Pseudomonas* (*n*=7) Isolates

Isolates	Antibiotic Susceptibility (%)		
	Resistant	Intermediate	Sensitive
Staphylococcus spp.			
Penicillin (P)	100	0	0
Oxacillin (OX)	28.6	0	71.4
Ofloxacin (OF)	4.8	0	95.2
Vancomycin (V)	4.8	0	95.2
Fusidic Acid (AF)	47.6	0	52.4
Lincomycin (L)	57.1	23.8	19.0
Clindamycin (CL)	9.5	4.8	85.7
Pristinamycin (PR)	0	0	100
Spiramycin (SP)	47.6	42.9	9.5
Erythromycin (E)	28.6	19.0	52.4
Chloramphenicol (C)	0	0	100
Gentamicin (G)	0	0	100
Pseudomonas spp.			
Cefuroxime (CXM)	100	0	0
Amoxicillin/clavulanic acid (AMC)	100	0	0
Imipenem (IMI)	100	0	0
Ceftazidime (CAZ)	0	0	100
Ciprofloxacin (CIP)	0	14.3	85.7
Tobramycin (TOB)	0	0	100
Gentamycin (CN)	0	0	100
Amikacin (AK)	0	14.3	87.7

Our findings showed much difference in the positive rate for bacterial abundance in the cockroaches' outer surface, which can be explained by the fact that there are differences between the environments and occupying niches of the insects (i.e., a homogeneous contamination of the bacterial flora of the external environment)[1]. However, the occurrence of gram-negative species (enterobacteria) on the surface was higher compared with gram-positive species that showed low density. These results are similar to those reported in the literature[8]. In fact, variation of bacterial richness among habitations have a direct impact on insect density according to their sanitation conditions, food dispersal, and availability for insects. It is known that sites hygienically improved carry fewer pathogens and synanthropic organisms. However, it has been reported that the dominant species in homes as well in hospitals and food establishments are gram-negative *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp.[1,8].

Indeed, with their lifestyle, cockroaches can readily move from contaminated zones (sanitation, garbage) and may create the opportunity to spread disease-causing organisms on food and food preparation surfaces[2]. Many studies highlighted a possible and potential risk of human contamination through bacteria carried by cockroaches in connection with human habitats[6].

The high prevalence of bacteria harbored on the body of cockroaches portends public health risks, in particular transmission of community-acquired infections. Cockroaches always carry a great variety of bacterial species collected from the environment in which they live, as shown in previous data for hospital environments[1] and human dwellings in the present study. Several authors described cockroaches collected from hospitals to have more bacterial counts than cockroaches found in residential areas[3,9] due to their permanent contact with infested sites.

In this study, the majority of bacterial species tested showed resistance. Other studies reported that multiple drug-resistant bacteria of medical importance, such as *P. aeruginosa*, *S. aureus*, *Salmonella* spp., and *Klebsiella* spp., have been isolated from cockroaches in hospitals and urban environments[8]. Furthermore, the tendency of

cockroaches to move freely and to inhabit toilets and sewers may aggravate the problem.

The development of antibiotic resistance is not the same in all bacteria as it is not the same either for the same bacterium. The gram-negative bacilli isolated in this study showed high resistance to most antibiotics tested, including the second generation cephalosporin (cefuroxime), the two β-lactam antibiotics amoxicillin+clavulanic acid, imipenem, and even the aminoglycoside tobramycin. Indeed, the study of susceptibility revealed high frequencies of staphylococci resistant to penicillin, spiramycin, lincomycin, fusidic acid, and oxacillin. The bacterial strains are likely subjected to pressure of a specific antibiotic in their environment. Recent studies have reported an increased prevalence of vancomycin-resistant *S. aureus* (VRSA) as well as strains with heterogeneous resistance to vancomycin (HSARIV)[10]. The high rate of resistance of *S. aureus* to antibiotics, such as aminoglycosides, macrolides, or fluoroquinolones, justifies the importance of careful monitoring of the strains' dissemination in household environments, especially when it is considered as a potential point of exchange for large staphylococci, including meticillin-resistant and coagulase-negative groups. Hence, our findings clearly indicate that all our collected cockroaches in residential areas harbor the two potential pathogenic species and the high prevalence of microorganisms harbored on the insects' body may portend public health risks. By significantly decreasing the sanitation level, *B. germanica* and *B. orientalis* may play an important role as mechanical vectors of a wide range of pathogenic bacteria, including several antibiotic resistant species.

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