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Article

Assessment of Surface-Associated Bacterial Contamination in *Blatta orientalis* from Infested Pig Farms in Bulgaria

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Abstract

Cockroaches are widely recognized as significant vectors for various infectious and parasitic diseases affecting both animals and humans. Cockroaches are more than just common pests; they are among the most medically important insects due to their frequent presence in human habitats. These resilient creatures are major reservoirs and vectors for numerous pathogens, including bacteria, viruses, parasites, and fungi. Recognizing the role of cockroaches in the ecosystem of disease is crucial, and implementing effective control measures is necessary. This study focuses on identifying bacterial contaminants present on the surfaces of Oriental cockroaches (*Blatta orientalis*) collected from pig farms. Using the Vitek 2 Compact system, a modern platform for rapid and automated phenotypic identification, several bacterial species were isolated. The predominant microorganism identified was *E. coli*, followed by *Staphylococcus* spp., *Streptococcus* spp., and *Enterobacter* spp. These findings highlight the potential role of cockroaches in the transmission of harmful infectious diseases to both animal populations and humans.

Keywords: cockroaches; *Blatta orientalis*; Vitek; bacterial pathogens; pig farms

1. Introduction

Cockroaches are widely distributed insects of considerable economic, veterinary, and public health importance. The relationship between insects and humans has evolved over a long history, profoundly affecting human health [1]. Their extensive evolution has equipped them with a remarkable ability to adapt and survive in a wide range of environments, including extreme conditions. Many species are exceptionally well-suited to a synanthropic lifestyle, thriving in human settlements, livestock farms, and food industry facilities [2]. This synanthropic behavior raises critical concerns regarding their role in the transmission of infectious and parasitic pathogens that may affect both humans and animals [3]. Cockroaches are among the most significant sanitary pests that can directly and indirectly harm animals and humans. They are among the few insects that play the role of important vectors in the spread of some of the most devastating diseases affecting humans – dysentery in a maternity ward in the USA in 1950, leprosy in Mexico in 1954 [4,5].

Research has demonstrated that cockroaches are capable of carrying microorganisms and parasites on their surfaces, which positions them as significant vectors in the transmission of various infectious and parasitic diseases affecting both animals and humans [6–12]. Numerous studies indicate that synanthropic species of cockroaches are the primary carriers of food-borne pathogenic microorganisms [13–15]. Although cockroaches do not bite, they contribute to pathogen transmission through surface contamination and fecal excretion [16]. In light of the complex epidemic scenario related to the spread of African swine fever, an increasing number of researchers are raising concerns regarding the potential role of cockroaches and other insects in the dissemination of the causative agent of this disease [17–19].

Pathogens can survive on cockroaches or in their digestive systems for over a month after exposure to contaminated environments or food and water. These pathogens may be released into the environment through their chitinous exoskeletons or feces [20,21]. Given their potential to contribute to the spread of disease, assessing microbial contamination levels in cockroach populations cohabiting with humans and animals is of paramount importance [22]. To evaluate the epidemic risk, specialized methods have been developed for the cultivation and identification of microbial contaminants present on the surface of cockroaches [20; 23-27]. Among contemporary techniques enabling rapid and automated phenotypic identification of microorganisms through colorimetric analysis, the Vitek 2 Compact system (BioMérieux, France) is increasingly utilized in clinical practice [28]. This system typically employs Vitek 2 GP ID and Vitek 2 GN ID cards for the identification of Gram-positive and Gram-negative bacteria, respectively, facilitating the precise differentiation of over 150 distinct pathogenic species. This method is increasingly used as a complementary analytical approach for identifying microbes in various samples from humans and animals, including cockroaches [29–34]. The data collected by many researchers justify and clarify the role of cockroaches in transmitting infectious disease agents, functioning mechanically and serving as one of the three main links in the epidemiological chain, specifically as a spreading factor [23; 27; 35-40].

The aim of this study was to investigate surface bacterial carriage in field populations of cockroaches inhabiting pig farms in the Republic of Bulgaria, with a view to identifying health and epidemic risks.

2. Materials and Methods

A total of 350 Oriental cockroaches, collected from four field populations, were examined in this study, following the methodology of Solomon [24] and Davari [27]. Specimens were collected between June and July 2023 from four industrial-scale pig farms in Bulgaria using monitoring traps. Following euthanasia by supercooling, groups of ten cockroaches were placed in sterile plastic containers containing 5 mL of sterile saline to create pooled samples. Surface contamination was extracted from each pool by vigorous vortexing at low speed (600-800 rpm) for 120 seconds, following the protocols of Alikhani et al. [25] and Haile et al. [26]. From the supernatant, 1 mL was pipetted into two sterile Eppendorf tubes and transported under refrigeration to a specialized microbiological laboratory for densitometric identification of Gram-positive and Gram-negative microorganisms using the VITEK 2 Compact System (BioMérieux, France). Samples were inoculated onto blood agar (Blood Agar Base, HiMedia Laboratories, India) and MacConkey agar (HiMedia Laboratories, India), and incubated aerobically at 37 °C for 24 hours, following a similar procedure described by Mariam [41].

Data Analysis

The statistical package IBM® SPSS® Statistics 26.0 was used to process the data.

3. Results

Microbiological analysis aimed at evaluating the epidemiological potential of synanthropic cockroaches as mechanical vectors of pathogens of sanitary-hygienic and veterinary relevance revealed a substantial bacterial load on their external surfaces (Table 1).

Table 1. Surface bacterial contamination in *Blatta orientalis* collected from pig farms.

Pathogen	Criteria	Farm 1 (n of PS = 10)	Farm 2 (n of PS = 8)	Farm 3 (n of PS = 10)	Farm 4 (n of PS = 7)	Total (n of PS = 35)
Gram-negative bacteria						
<i>E. coli</i>	presens	(6) 60%	(5) 62.5%	(7) 70%	(5) 71.4%	(23) 65.7%
	absence	(4) 40%	(3) 37.5%	(3) 30%	(2) 28.6%	(12) 34.3%

Cramer's V = 0.102; Sig. (p) = 0.047						
<i>Klebsiella pneumoniae</i>	presens	(2) 20%	(2) 25%	(3) 30%	(2) 28.6%	(9) 25.7%
	absence	(8) 80%	(6) 75%	(7) 70%	(5) 71.4%	(26) 74.3%
Cramer's V = 0.092; Sig. (p) = 0.046						
<i>Serratia marcescens</i>	presens	(0) 0%	(0) 0%	(2) 20%	(1) 14.3%	(3) 8.6%
	absence	(10) 100%	(8) 100%	(8) 80%	(6) 85.7%	(32) 91.4%
Cramer's V = 0.323; Sig. (p) = 0.030						
<i>Enterobacter</i> spp.	presens	(3) 30%	(5) 62.5%	(2) 20%	(3) 42.9%	(13) 37.1%
	absence	(7) 70%	(3) 37.5%	(8) 80%	(4) 57.1%	(22) 62.9%
Cramer's V = 0.329; Sig. (p) = 0.028						
<i>Pseudomonas</i> spp.	presens	(0) 0%	(2) 25%	(4) 40%	(2) 28.6%	(8) 22.9%
	absence	(10) 100%	(6) 75%	(6) 60%	(5) 71.4%	(27) 77.1%
Cramer's V = 0.370; Sig. (p) = 0.018						
<i>Proteus mirabilis</i>	presens	(1) 10%	(0) 0%	(0) 0%	(0) 0%	(1) 2.9%
	absence	(9) 90%	(8) 100%	(10) 100%	(7) 100%	(34) 97.1%
Cramer's V = 0.271; Sig. (p) = 0.046						
Gram-positive bacteria						
<i>Staphylococcus</i> spp.	presens	(5) 50%	(3) 37.5%	(2) 20%	(4) 57.1%	(14) 40%
	absence	(5) 50%	(5) 62.5%	(8) 80%	(3) 42.9%	(21) 60%
Cramer's V = 0.291; Sig. (p) = 0.039						
<i>Streptococcus</i> spp.	presens	(2) 20%	(4) 50%	(4) 40%	(3) 42.9%	(13) 37.1%
	absence	(8) 80%	(4) 50%	(6) 60%	(4) 57.1%	(22) 62.9%
Cramer's V = 0.237; Sig. (p) = 0.051						
<i>Enterococcus</i> spp.	presens	(0) 0%	(0) 0%	(3) 30%	(3) 42.9%	(6) 17.1%
	absence	(10) 100%	(8) 100%	(7) 70%	(4) 57.1%	(29) 82.9%
Cramer's V = 0.482; Sig. (p) = 0.043						

Legend: n – number; PS - pulled sample of ten *Blatta orientalis* individuals; Cramer's V – measure of association strength between categorical variables (range: 0 = no association to 1 = perfect association); Sig. (p) – p-value indicating the probability that the observed association occurred by chance; values less than 0.05 were considered statistically significant. All statistical analyses were performed using IBM® SPSS® Statistics version 26.0.

The quantitative distribution of Gram-negative and Gram-positive microorganisms isolated from the surface of synanthropic cockroaches collected from the surveyed pig farms is shown in Figures 1 and 2. The Cramér's V values, ranging from 0.092 to 0.482, indicate a weak to moderate association between bacterial species variability and farm location.

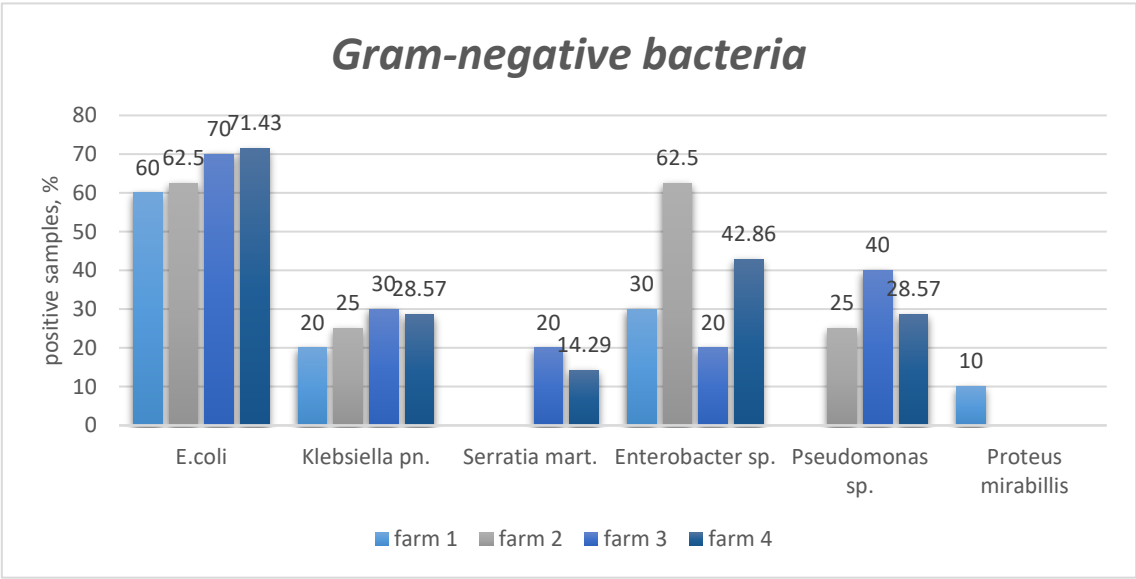


Figure 1. Quantitative distribution of Gram-negative microorganisms isolated from *Blatta orientalis* across different pig farms.

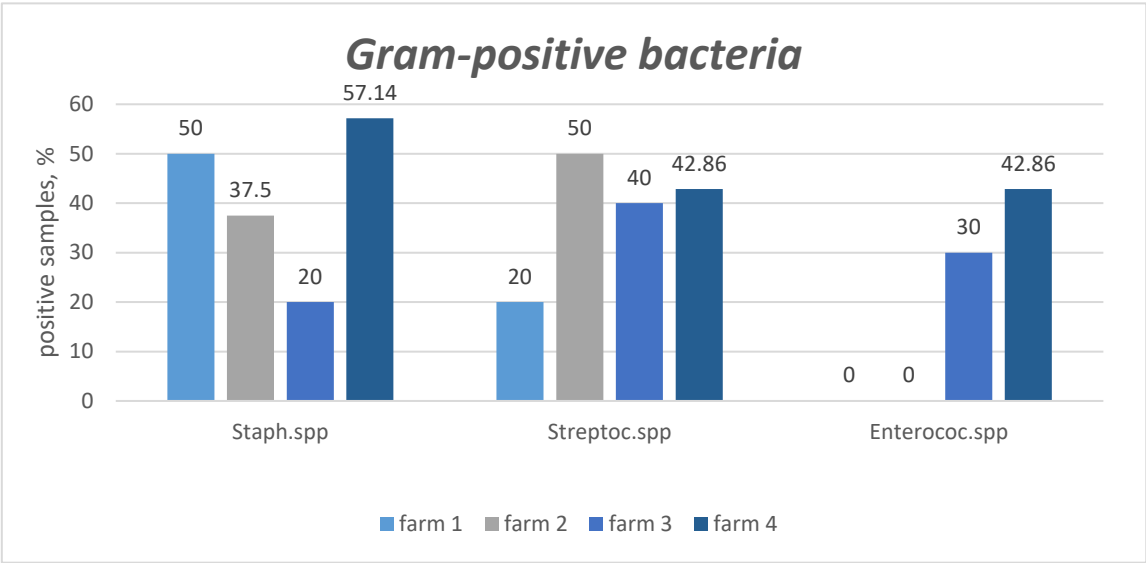


Figure 2. Quantitative distribution of Gram-positive microorganisms isolated from *Blatta orientalis* across different pig farms.

As shown in the summarized data in Figure 3, *Escherichia coli* was the predominant microbial isolate recovered from synanthropic cockroaches across all pig farms, followed by *Staphylococcus* spp., *Streptococcus* spp., and *Enterobacter* spp.

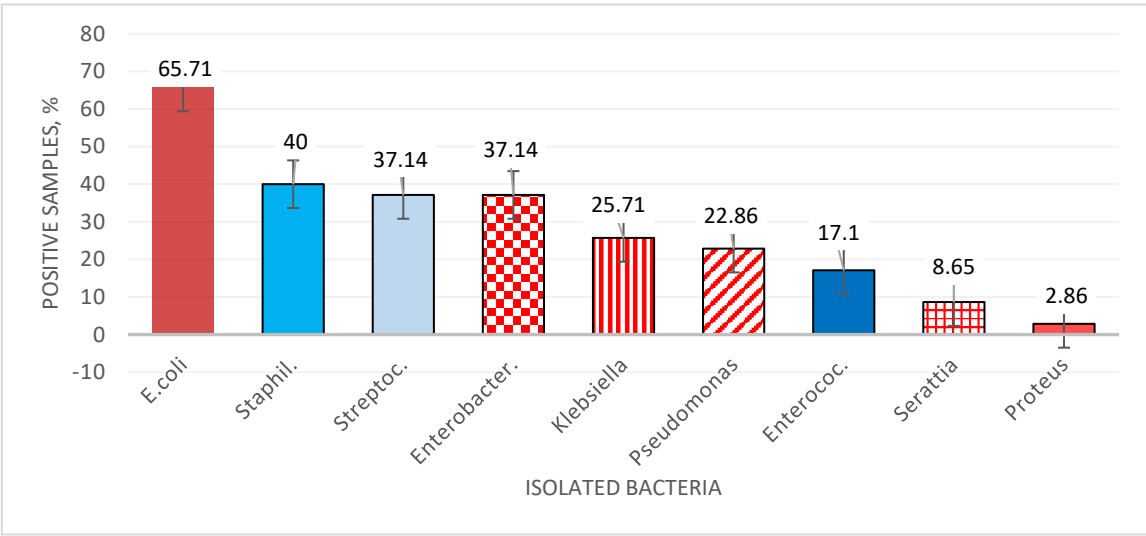


Figure 3. Summary of the quantitative distribution of microorganisms isolated from *Blatta orientalis* across all pig farms.

4. Discussion

Cockroaches are recognized as sanitary and hygienic pests of high epidemiological importance, acting as vectors for numerous pathogenic agents. When inhabiting contaminated environments or consuming infected food and water, various viruses, bacteria, fungi, and protozoa may persist on their exoskeletons or within their digestive tracts for extended periods and be subsequently released into the environment [20,21].

Fang et al. [22] emphasize the critical need to assess microbial contamination in cockroach populations, particularly those cohabiting with humans and animals. Globally, most studies in this area have focused on German cockroaches and other synanthropic or exotic species. In Bulgaria, one of the few studies was conducted by Popova et al. [42] also on an exotic cockroach species. However, there remains a substantial knowledge gap regarding the vectorial role of the black oriental cockroach, especially considering its frequent and severe infestations in pig farming facilities documented in our field observations.

In our investigation, significant surface bacterial contamination was detected in oriental cockroaches from pig farms (Table 1). The isolates included nine microbial taxa: *Escherichia coli*, *Staphylococcus* spp., *Streptococcus* spp., *Enterobacter* spp., *Klebsiella pneumoniae*, *Pseudomonas* spp., *Enterococcus* spp., *Serratia marcescens*, and *Proteus mirabilis*. These microorganisms are considered conditionally pathogenic, forming part of the normal or transient flora of the skin, mucous membranes, and intestines of mammals and birds, yet capable of causing disease under specific conditions in humans and animals.

Our findings are consistent with previous studies, such as those by Zarchi et al. [43], who identified 19 bacterial species from cockroach surfaces and digestive tracts, including *E. coli*, Group D *Streptococcus*, *Bacillus* spp., *Klebsiella pneumoniae*, and *Proteus vulgaris*. Similarly, Holakouei et al. [44] reported a dominant presence of *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp., and *Serratia* spp. in German cockroaches.

A pronounced prevalence of Gram-negative bacteria, particularly from the *Enterobacteriaceae* family, was observed in our samples. This aligns with studies by Fotedar et al. [23], Cloarec et al. [35], Rivault et al. [45], and Vythilingam et al. [46], which reported similar bacterial profiles in German and American cockroach species. These bacteria pose serious health threats, including urinary tract infections, sepsis, and gastroenteritis [47].

Controlling synanthropic cockroach populations is essential, particularly in livestock environments, where their presence increases the risk of zoonotic transmission. Our findings support previous research indicating the role of American cockroaches in spreading enteropathogens [45; 48–51].

Experimental studies by Zurek & Schal [52] demonstrated that cockroaches shed large amounts of viable, virulent *E. coli* in their feces after initial exposure to contaminated material. Similarly, our study identified *E. coli* as the predominant contaminant on the surface of oriental cockroaches across all sampled farms. Pathogenic strains of *E. coli* are among the most frequently isolated pathogens in swine in veterinary practice [53].

Enterotoxigenic and verotoxigenic strains of *E. coli* are the main cause of colenteritis and colienterotoxiosis in newborn and weaned pigs, as well as edematous disease in postweaned pigs, often with high mortality [54–56].

Waldvogel et al. [57] reported the persistence of viable *E. coli* F18 strains in cockroach feces for up to 8 days post-exposure, with high bacterial loads (4.4×10^5 CFU g⁻¹) comparable to those found in infected pigs (1.9×10^6 CFU g⁻¹). This underscores the vector potential of cockroaches for these pathogens. Multiple studies have confirmed the transmission capacity of German cockroaches for verotoxigenic *E. coli* F18, an important pathogen of pigs [57,58]. Therefore, targeted cockroach density control is recommended as an integral part of disease prevention and control programs in pig farming [57].

The demonstrated vector competence of cockroaches for *E. coli* F18, along with the high concentrations of fecal coliforms in their excreta, represents a significant epidemiological concern [57]. The ability of cockroaches to migrate into infected farms poses a considerable risk for pathogen dissemination, potentially undermining disinfection protocols and compromising biosecurity measures. This threat is particularly pronounced during disease outbreaks, when control interventions—such as animal removal, cleaning of feeders and drinkers, and intensive disinfection using strong-smelling agents—often provoke mass migration of cockroaches to adjacent, unaffected facilities. Given that *E. coli* F18 cells remain viable and virulent after passage through the cockroach

digestive tract and can be excreted in large numbers for over a week, the potential for disease spread via migrating cockroaches is both evident and alarming [52].

Staphylococcus spp. and *Streptococcus* spp. were the next most frequently isolated genera in our study. Numerous investigations have highlighted the potential role of cockroaches as vectors of bacterial pathogens, particularly these two genera. Their frequent isolation from cockroach populations suggests a possible route for transmission to humans and animals. Studies by Fotedar et al. [23] in Taiwan and India, Salehzadeh et al. [59] in Iran, and Vazirianzadeh et al. [60] have reported significant associations between cockroach infestations and the presence of pathogenic bacteria. Additionally, Schauer et al. [61,62] and Lampert et al. [63] identified high concentrations of staphylococci in various cockroach species, especially in the hindgut of *Shelfordella lateralis*. Of particular concern is *Staphylococcus aureus*, a bacterium colonizing the skin and mucosal surfaces of approximately 30% of the human population, known for its ability to cause serious infections [64]. The increasing detection of antibiotic-resistant *S. aureus* strains in cockroaches, as documented by Menasria et al. [38], Islam et al. [65] and Abdolmaleki et al. [66], underscores their growing importance as a public health threat.

Our investigation revealed concerning levels of *Klebsiella pneumoniae* contamination across all surveyed pig farms. These findings are consistent with those of Cotton et al. [67], who reported a high prevalence of *Klebsiella* in German cockroaches. Their work highlights the critical role of cockroaches as vectors in the transmission of nosocomial infections, emphasizing the urgent need for comprehensive pest control strategies in both agricultural and healthcare environments.

In one of the studied farms, *Proteus mirabilis* was detected on the surfaces of examined cockroaches. This bacterium has also been identified in the gastrointestinal tracts of German and American cockroaches [68–70], as well as in exotic species [42]. Additionally, the presence of *Pseudomonas aeruginosa* was confirmed—an opportunistic pathogen frequently isolated from various cockroach species, including those implicated in hospital infestations [71]. *P. aeruginosa* is particularly notable for its high resistance to disinfectants and its prominent role in healthcare-associated infections.

5. Conclusions

The findings of this study highlight significant sanitary and epidemiological risks associated with cockroach infestations in livestock production and food processing environments. In particular, the detection of *Blatta orientalis* in swine facilities was strongly associated with high levels of bacterial surface contamination. *Escherichia coli* was identified as the predominant microbial isolate, followed by *Staphylococcus* spp., *Streptococcus* spp., *Enterobacter* spp., *Klebsiella pneumoniae*, *Pseudomonas* spp., *Enterococcus* spp., *Serratia marcescens*, and *Proteus mirabilis*, reflecting a diverse and complex microbial profile. These results underscore the necessity of implementing strict hygiene and biosecurity measures to mitigate the risk of pathogen transmission in such settings.

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Abbreviations

The following abbreviations are used in this manuscript:

GP ID	Gramm positive identification card
GN ID	Gramm negative identification card
PS	Pulled samples
n	number

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