Detection of *E. coli* O157H7 strains potentially pathogenic to humans in the urine of domestic mice in the town of Daloa (Côte d'Ivoire)

ABSTRACT

House mice, Mus musculus, are classified as one of the most widespread mammals in the world. They harbor and spread many zoonotic pathogens, such as viruses (hantavirus), bacteria (Leptospira interrogans), protozoa (Toxoplasma gondii) and helminths (Hymenolepis spp.). In view of the real public health problems caused by mouse urine in the contamination of domestic foods, this study proposed to contribute to food safety by assessing the sanitary risk of the urinary microbiome of domestic mice. Bacteria were isolated and identified on CHROMAgar^{TM} Orientation, Chromo *E. coli* O157H7 culture media and biochemical tests from urine samples collected from house mice in the city of Daloa. A total of 28 urine samples were tested and three bacterial genera Enterococcus, Stapphylococcus and Escherichia were identified with overall frequencies of occurrence of 60.7 %, 42.9 % and 35.7 % respectively. No significant differences were observed between these frequencies. Within the E. coli strain lineage, the potentially human pathogenic *E. coli* O157:H7 serotype was detected with an overall frequency of 50 %. The presence of *E. coli* O157:H7 in the urinary tract of house mice therefore represent a health risk for the surrounding population. This study therefore recommends through its results, the implementation of good hygiene practices for food safety, which can reduce the risks of transmission of microbial agents.

Key words: House mice, uropathogens, *E. coli* O157 : H7, Côte d'Ivoire

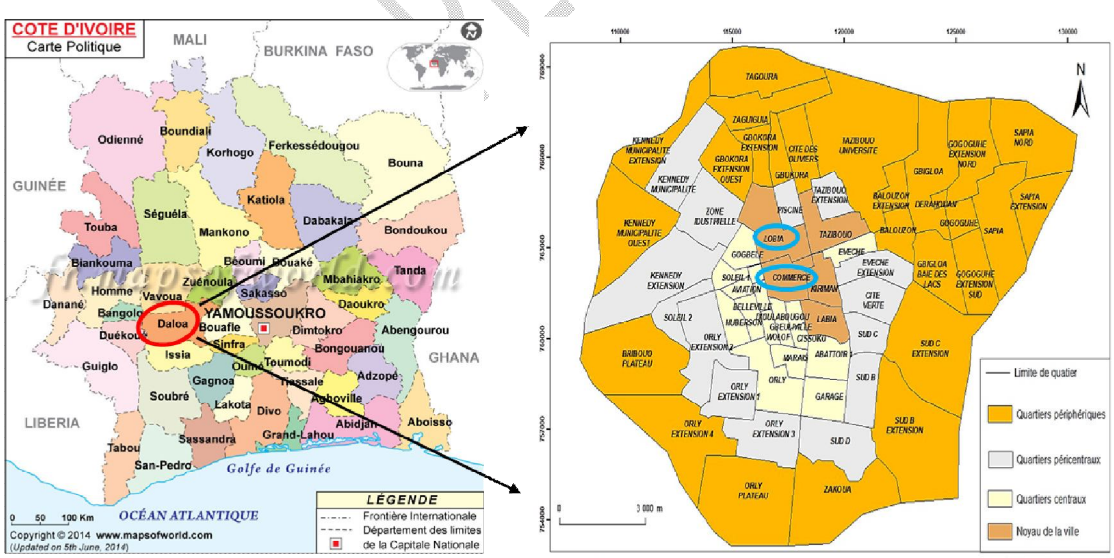
1. INTRODUCTION

House mice, *Mus musculus*, are classified among the most widespread mammals in the world [1]. They are serious ravagors in urban and rural areas and cause important economic damage to cultures, stored food, farms, industries and households [2]. Also, house mice populations harbor and spread zoonotic pathogens, such as viruses (hantavirus), bacteria (*Leptospira interrogans*), protozoa (*Toxoplasma gondii*) and helminths (*Hymenolepis spp*.) [3]. Exposure to water or food contaminated by urine of infected mice is the common source of human infection, commonly known as zoonosis. In households of low socioeconomic status with high numbers of house mice, the risk of transmission of zoonotic infections is higher in various epidemiological settings [4]. Also, house mice are a major source of infection to multidrug-resistant bacteria (MDRB) with zoonotic potential such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus pseudintermedius* (MRPS), and extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* [5,6]. Despite the existence of all this information, little attention is paid to research on the urinary microbiome [7] of these pests that defecate and urinate at any time in most household cooking utensils, on supermarket foods and grains in warehouses, causing serious public health and food safety problems [4]. Indeed, the use of cooking utensils and the consumption of foodstuffs soiled by the urine of infested mice can cause renal and hepatic syndromes with often fatal outcomes without often being able to trace the source of the infection. In view of the real public health problems caused by mouse urine in the contamination of domestic food, this study therefore proposes to contribute to food safety by evaluating the health risk of the urinary microbiome of house mice.

2. MATERIAL AND METHODS

2.1. Study sites

The work was carried out in the Haut- Sassandra region precisely in the town of Daloa, a town in the center-west of Côte d'Ivoire (Figure 1a). This city is located 141 km from Yamoussoukro, the political capital and 383 km from Abidjan, the economic capital of the country. It had 261,789 inhabitants in 2021 with an area of 5,305 km² [8]. Located at 6°53 North latitude and 6°27 West longitude, Daloa is the third most populated city in Côte d'Ivoire after Abidjan and Bouaké [8]. It is delimited to the north by the Vavoua, to the south by the department of Issia, to the east by the department of Bouafle and to the west by the department of Zoukougbeu. Two sampling sites were selected based on well defined criteria for carrying out this study. Indeed, after an investigation, we found a significant presence of mice in the sewers located near some restaurants in the town of Daloa. These are the restaurant located in the Lobia sector (Figure 1b) opposite the UBA Bank (Site 1) and the restaurant located in the Commerce sector around the UTB bus station (Site 2).



a- *Location of the city of Daloa*   b- *Catography of the city of Daloa*

Figure 1 : Mapping of the study area and sample collection sites [8].

2.2. Study design and sample collection

The biological material was consisted of collected mice urine. The sampling points (Site 1 and Site 2) were searched every day from 6:30 a.m. to 10:30 a.m. in the morning and from 6:30 p.m. to 9:30 p.m. at night. On the sites, the different mice traps are activated and placed in the sewers. The mice captured in the traps were seized then a dissection is performed to extract the bladder most often containing urine. Each bladder collected is placed in a cryotube and transported with a cooler at the laboratory of the Research Unit in Genetics and Molecular Epidemiology (URGEM) located at University Jean Lorougnon Guédé (UJLoG). About 50 µL of urine needed for the microbiological and molecular analyzes were extracted from the bladder of the mice using a sterile 1 mL syringe and transferred into 1.5 mL Eppendorf tubes. A total of 28 mice urine samples were collected in the two sites surveyed, including 06 for site 1 and 22 for site 2.

2.3. Isolation and identification of bacterial strains

Each urine sample was cultured on CHROMAgar^{TM} orientation medium using single-use 10 µL loops [9], then incubated under aerobic conditions at 37°C for 24 h. The identification of bacterial species was made on the basis of Gram stain, morphology and biochemical characteristics using the reduced gallery of LEMINOR (Urea; Indol; Simmons citrate agar; Kligler Hajna agar; iron agar and lysine), oxidase and catalase tests. Then, the pink-colored bacterial colonies that look like Escherichia coli were subcultured on the *E. coli* O157H7 medium and then incubated again at 37°C for 24 hours in order to detect the *E. coli* O157H7 variant potentially pathogenic for the human.

2.4. Statistical analysis of data

The frequency of occurrence (F) of the identified bacteria species was calculated by the following formula :

$$F(\%) = \frac{ni}{Nt} \times 100$$

ni: number of urine samples containing bacterium i; Nt: total number of urine samples analyzed.

The Sample equality of proportions test according to the chi-square approximation was carried out using R software version 4.12 to compare the frequencies of occurrence of the identified bacterial species. The differences are statistically significant when the value of the probability p obtained is strictly less than 0.05.

3. RESULTS

3.1. Differentiation of bacteria on CHROMAgarTM orientation medium

Culture on CHROMAgar^{TM} Orientation medium made it possible to isolate and identify several bacterial colonies on the basis of their color, appearance and sizeb (Figure 2). Bacteria infestations of the urinary tract of house mice are usually polybacterial infestations (Figure 2). The bacterial genera encountered in all of the two surveyed sites are *Enterococcus*, *Staphylococcus* and the species *Escherichia coli*. Indeed, *Enterococcus* are Gram-positive bacteria. They are distinguished on the CHROMAgar^{TM} Orientation chromogenic medium by their green-turquoise color and small size (Figure 2). *Staphylococcus* are also Gram-positive bacteria. The colonies of this bacterial genus are characterized by the golden color and an opaque appearance on the chromogenic medium. (Figure 2). However, *Escherichia coli* is a Gram-negative bacterium belonging to the Enterobacteriaceae family. The colonies of this species are characterized by a pink or reddish coloration with the appearance of a halo around the colony (Figue 2).

Figure 2: Growth of bacteria from the urinary tract of house mice on ChromAgar™ chromogenic differential medium

3.2. Occurrence of bacterial species isolated from the urinary tract of dosematic mice

A total of 28 urine samples were analyzed and three (03) bacterial genera were identified for both study sites. Table 1 presents the frequency of occurrence of bacteria isolated from the urinary tract of house mice. Bacteria of the genus Enterococcus, Staphylococcus, and Escherichia coli were isolated for an overall frequency of occurrence of 60.7 %, 42.9 %, and 35.7%, respectively. According to the equality of proportions test, the differences observed between these frequencies (X2 = 3.733; P = 0.155) were not significant. At the scale of the study sites, these three bacterial genria were isolated at identical frequencies of 66 % at site 1. However, at site 2, Enterococcus, Staphylococcus and Escherichia coli had frequencies of 59 %, 36.4 % and 27.3 % respectively. Although no statistically significant difference was obtained between these frequencies in this study, the genus *Enterococcus* was the most frequent followed by *Staphylococcus* and finally *Escherichia coli*.

Table 1: Frequency of occurrence of bacterial species isolated from the urinary tract of mice in the different sites surveyed

Sites	**N**	**Isolated Bacteria**			***P-value***
		***Enterococcus* spp**	***Staphylococcus* spp**	***Escherichia coli***	
Site 1	06	04 (66 %)	04 (66 %)	04 (66 %)	1
Site 2	22	13 (59 %)	08 (36.4 %)	06 (27.3 %)	0.08677
Total	**28**	**17 (60.7%)**	**12 (42.9 %)**	**10 (35.7 %)**	**0.155**

N: Number of urine samples collected and analyzed

3.3. Detection of the pathogenic strain *E. coli* O157: H7 and health risk

Escherichia coli is a ubiquitous bacterial species highly diverse that forms an important part of the normal intestinal flora of humans and arm-blooded animals. In order to test for the probable presence of the human pathogenic E. coli O157: H7 variant in the urinary tract of house mice, Escherichia coli colonies isolated on ChromAgar^{TM} orientation medium were transferred to *E. coli* O157: H7-specific chromogenic medium. The *E. coli* O157 : H7 variant is characterized by large, light pink colonies with a halo (Figure 3). Among the 10 *Escherichia coli* strains detected, 5 strains were able to grow on the Chrom *E. coli* O157:H7 medium, i.e. an overall frequency of 50 %. At the site level, the occurrence of this variant is 75 % for site 1 and 33.3 % for site 2. Although mice in site 1 are 6 times more likely to be contaminated by this human strain than in site 2, the frequencies obtained are not significantly different ($OR_{1/2}$= 6.00 ; 95 % CI (0.35, 101.57) ; P = 0.524).

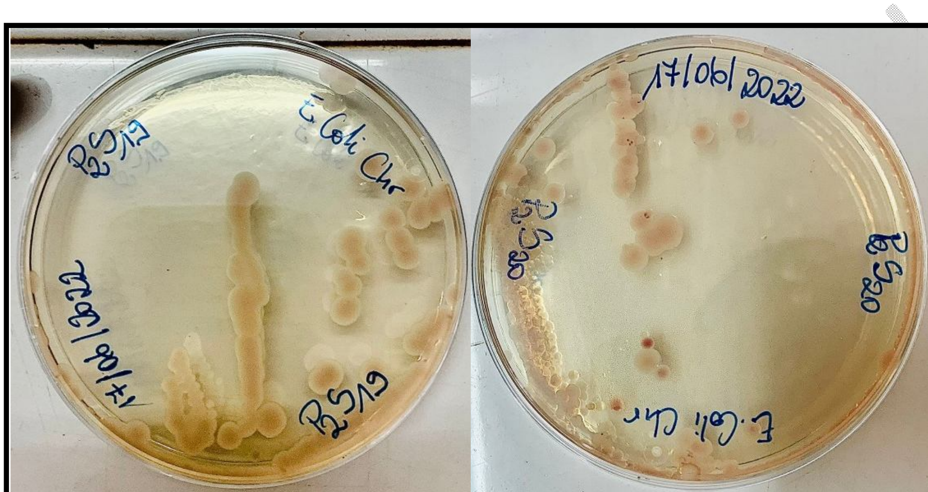


Figure 3: *Escherichia coli* colonies on chromogenic medium specific *E. Coli* O157: H7

Table 2: Frequency of occurrence of the variant *E. Coli* O157: H7

		E. Coli O157 : H7	
	N	Positif	F (%)
Site 1	4	3	75
Site 2	6	2	33.3
Total	10	5	50
Fisher's Exact Test	$OR_{1/2}$= 6.00	95% CI (0.35, 101.57)	P = 0.524

N: Number of E. coli strains detected per site, F: Frequency of occurrence

4. DISCUSSION

Bacteria belonging to the genus *Enterococcus*, *Staphylococcu*s and the species *Escherichia coli* were the contaminating agents, encountered in the urinary tract of the domestic mice studied. These results indicate that urine from asymptomatic mice is not sterile. Indeed, mice, being classified as pets in some communities, could be a source of transmission of pathogens to humans [10]. In contrast to this study, Forster et al. [11] found eight (08) bacteria in the urinary microbiome of mice including betaproteobacter, acetobacter, Escherichia, Kaistobacter, Roseococcus, Rubellimicrobium and Sphingomonas. This difference in abundance and type of species would be related to the environment or habitat of the mice and the method used. Indeed, for the study of the mouse urinary microbiome, Forster et al. [11] proceeded by targeted sequencing of the V3 region of the 16S rRNA gene. This technique is more resolutive and discriminating than the isolation technique on bacterial culture medium and allows to highlight a great specific diversity of the microbiome.

E. coli is one component of the natural microflora of the gastrointestinal tract of animals and humans, but pathogenic strains such as O157:H7 can cause a variety of diseases through different virulence determinants [12]. *E. coli* O157:H7 infection in humans has been well documented, but infection of pets such as house mice is still poorly known or not documented. This study suggests the presence of *E. coli* O157:H7 in the urinary tract of house mice. Its presence in the urine of these mice demonstrates its zoonotic nature, which represents a potential danger for humans. The zoonotic nature of this serotype has also been demonstrated by several studies [13,14]. Ruminants are considered the primary reservoir of *E. coli* O157:H7, although it has been isolated from other animal species such as pigs, billed gulls, geese and compagny animals [15]. This strain is potentially pathogenic for humans and can lead to serious complications such as acute renal failure and neurological damage leading to death [16]. Thus, the use of cooking utensils and the consumption of foodstuffs contaminated with urine from infested mice can cause hemolytic uremic syndromes in humans [16].

Escherichia coli O157:H7 is food and waterborne [13]. The contamination of mice by this serotype could be explained by the fact that the sites from which the mice are collected are sewage drainage sites from the Daloa prison that are constantly subjected to urine and fecal discharges from the population. Located close to the food court, these sites are therefore health risk areas for people frequenting these food courts [4].

5. CONCLUSION

This study has shown that the urine of house mice is not sterile and constitutes a reservoir of pathogens. The detection of *E. coli* 0157 : H7 bacterial strains producing Shigatoxins, potentially pathogenic for humans, in the urinary tract of house mice, represents a health risk for the surrounding population. This study recommends through its results, the implementation of good hygiene practices for food safety, which can reduce the risks of transmission of microbial agents.

ETHICAL APPROVAL

No ethical statement is required by local authorities for domestic animal sampling such as house mice.

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