Ann Ist Super Sanità 2021 | Vol. 57, No. 1: 33-36
DOI: 10.4415/ANN_21_01_05

First evidence of *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{OXA-23}}$ carbapenemase genes in human body lice infesting a second-hand T-shirt in a street market in Italy

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Abstract

Background. The spread of carbapenems resistance is a public health concern. The main group of carbapenemases encoding the β-lactamases activity (*bla* genes) is the Metallo-β-lactamases (MBLs).

Methods. The presence of carbapenemase *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-40-like}}$, *bla*$_{\text{OXA-51-like}}$, *bla*$_{\text{OXA-58-like}}$, and *bla*$_{\text{NDM-1}}$ genes was screened by real time PCR in 26 *Pediculus humanus* insects identified from second-hand clothes in a local market in Central Italy. Bacteria diversity was also characterized through shotgun metagenomic amplification for a deep sequencing of the host-associated bacterial microbiomes.

Results. The *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{NDM-1}}$ carbapenemases genes were found and metagenomic analysis showed a great presence of *Acinetobacter* species.

Conclusions. These results suggest a new potential transmission path for carbapenemase gene spread through bacteria ingested by insects infesting humans.

Key words

- *Acinetobacter* spp.
- *Pediculus humanus*
- NDM-1
- OXA
- carbapenem resistance
- Italy

INTRODUCTION

In recent years, infections attributable to carbapenem resistance gram-negative bacteria have increased and, in many cases, have been associated with high morbidity and mortality rates, due to their extensive antibiotic resistance, extremely difficult to treat [1]. The spread of carbapenems resistant Enterobacteriaceae (CRE) and of carbapenem- resistant *Acinetobacter baumannii* (CRAb) has become a major public health concern. In particular, *A. baumannii* may cause healthcare-associated infections worldwide with multidrug-resistant clonal lineage, while community-acquired infections are related to sporadic cases [2, 3]. CRAb and CRE are included in the World Health Organization (WHO) priority list of antibiotic-resistance bacteria to direct future research and development, after an assessment of their importance in both health-care-associated infections and community-acquired infections [1]. Carbapenem resistance was firstly detected in 1990s in *A. baumannii* reporting carbapenemases of class D oxacillinases (OXA), including OXA-23-like, OXA-40-like, OXA-51-like, and OXA-58-like enzymes [4, 5]. One of the main groups of carbapenemases encoding the β-lactamases activity (*bla* genes) is the Metallo-β-lactamases (MBLs), given its unavailability of commercial MBLs inhibitors, and despite the remarkable research in new drugs development [6]. MBLs of clinical importance are IMP (Imipenem MBLs), VIM (Verona integron-encoded MBLs), SPM (São Paulo MBL), and NDM (New-Delhi MBL) families. The *bla*$_{\text{NDM-1}}$ genetic element was identified in 2008 in an Indian patient colonized by *Klebsiella pneumoniae* carrying *bla*$_{\text{NDM-1}}$. The horizontal gene transfer allowed a rapid spread worldwide, among different species circulating in community healthcare and in environmental settings [5, 6]. Several species belonging to the *A. baumannii-A. calcoaceticus* complex have been also identified in arthropods (*Pediculus humanus*, ticks and fleas), that are potential vectors for infection transmission [7, 8]. In Italy, only imported cases of louse borne diseases were described and exclusively, in refugees from endemic countries, while autochthonous body louse vectors were considered ex-

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tinct [9]. Recently, we have searched body lice found on a second-hand T-shirt bought in a street market near Rome (Central Italy) for *Borrelia recurrentis* (relapsing fever), *Rickettsia prowazekii* (epidemic typhus), *Bartonella quintana* (trench fever), *Coxiella burnetii* (Q fever) and *Yersinia pestis* (plague), with negative results [10].

Here, we report the presence of bla_{OXA} and bla_{NDM-1} resistance genes in lice collected from second-hand clothes in Italy. The metagenomic bacterial species investigation has been also performed.

METHODS

In a collection of 26 *Pediculus humanus* insects identified from second-hand clothes in 2018 in a street market, the presence of carbapenemases encoded by $bla_{OXA-23-like}$, $bla_{OXA-40-like}$, $bla_{OXA-51-like}$, $bla_{OXA-58-like}$, and bla_{NDM-1} genes was detected by PCR using primers as previously described and amplicons were fully sequenced [11].

Species diversity was characterized through shotgun metagenomic amplification for a deep sequencing of the host-associated bacterial microbiomes. A metagenomic analysis of V3–V4 region of 16S rRNA gene was performed by Illumina MiSeq platform (Illumina, San Diego, CA) to better understand the relative abundance of the main bacterial phylum and genus in lice containing carbapenemase elements. Tags and taxonomic annotations from obtained sequences, were calculated with a high level of accuracy (99%), using a combination of specific databases. Raw data were trimmed in quality and normalized. The operational taxonomic units (OTUs) were clustered before taxonomic assignment. Taxonomic information was calculated and summarized.

Acinetobacter species was investigated by real time PCR using primes and probe targeting the *rpo*B gene, and amplicons were examined by nucleotide sequence [12].

RESULTS

Carbapenemase genes were identified in 7 out of 26 (24%) different *Pediculus*. The most relevant results were: 2 lice positive for $bla_{OXA-23-like}$, 2 lice for $bla_{OXA-58-like}$, 2 *Pediculus* for bla_{NDM-1}, 1 *Pediculus* showing a combination of $bla_{OXA-23-like}$, $bla_{OXA-58-like}$, bla_{NDM-1}. No positivity was found for $bla_{OXA-40-like}$ and $bla_{OXA-51-like}$ genes. Sequences of PCR generated amplicons displayed a 100% identity with GenBank references: $bla_{OXA-23-like}$ (CP042841), $bla_{OXA-58-like}$ (KF700121), bla_{NDM-1} (CP043053).

Lice carrying bla_{NDM-1} element were considered for microbiome bacterial composition. The analysis was assessed at phylum and genus levels and the taxa composed of more than 3% were considered, with a total of usable sequences classified into 8 phyla.

As presented in *Table 1*, the majority of the obtained operational taxonomic units (OTUs) belonged to Moraxellaceae (30%-60%, *Acinetobacter*), Burkholderiaceae (9%-15%, *Delftia*), Rhizobiaceae (4%-11%), Enterobacteriaceae (3%-13%), Xanthomonadaceae (3%-6%), Flavobacteriaceae (3%-4%), and Pseudomonadaceae (0%-14%).

Acinetobacter spp. were the most represented species and were detected in all lice containing bla_{NDM-1} genes and in 24 out of 26 (92%) of collected insects. Nucleotide sequences of *rpo*B (zone1) genes were compared with the GenBank database sequences, revealing the existence of different species of *Acinetobacter* non-*baumannii*, as *A. ursingii* (accession number EF611406 with 100 % identity), *A. pittii* (accession number CP014477 with 100% identity), and *A. johnsonii* (accession number CP037424 with 100% identity).

DISCUSSION

Carbapenem antimicrobial resistance is a public-health problem of global dimension, especially because it is largely found in Gram-negative pathogens. The characteristic gene transfer pathway confers a rapid spreading, causing serious outbreaks and a limited treatment option. To date, in healthcare facilities and within the community, the exact prevalence of carbapenemase-producing Enterobacteriaceae and of carbapenem-resistant *A. baumannii*, is still unknown [1, 4].

Head louse infestation is very common worldwide, with a close head-to-head contact transmission, while the body louse infestation is less frequent and related to poor hygiene. Lice may carry a broad spectrum of pathogens, including Enterobacteriaceae and *Acinetobacter* spp., and acquire these bacteria during blood meals from infected patients. The transmission of these

Table 1
Metagenomic analysis of 3 body louse carrying bla_{NDM-1} element

Family/genus	Body louse carrying bla_{NDM-1}	Body louse carrying bla_{NDM-1}	Body louse carrying $bla_{OXA23-like}$, $bla_{OXA58-like}$, bla_{NDM-1}
Moraxellaceae/*Acinetobacter*	60%	39%	55%
Burkholderiaceae/*Delftia*	11%	9.0%	15%
Xanthomonadaceae/*Stenotrophomonas*	5.0%	3.0%	6.0%
Flavobacteriaceae/*Flavobacterium*	3.0%	4.0%	4.0%
Rhizobiaceae/*Ochrobactrum*	0.0%	11%	4.0%
Other Rhizobiaceae	6.0%	0.0%	4.0%
Enterobacteriaceae/Candidatus *Riesia*	5.0%	13%	3.0%
Pseudomonadaceae/*Pseudomonas*	0.0%	14%	0.0%
Others	10%	7.0%	9.0%

microorganisms within the community could occurs through the insect feces or by scratching of the skin [13].

The prevention for reducing person-to-person transmission of CRE is a WHO priority [1].

In this study we found different bacterial species in lice implicated in carbapenem antimicrobial resistance. The large presence of *Acinetobacter* leads to speculate an association of these species with the carbapenemase resistance determinants. The detection of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-58-like}}$ and $bla_{\text{NDM-1}}$ genes in body lice, containing a broad range of bacterial species, suggest a relationship with all identified bacteria, especially with Enterobacteriaceae, found in metagenomic analysis. As described by several authors, *Acinetobacter* strains were found in body and head lice, but their clinical implication is still unknown and the vector competence for the maintenance and transmission of those pathogens is not established [8, 13-15].

A high prevalence of *Acinetobacter* species DNA carriage (40.8%), mostly *A. baumannii* (32.9%), in clothes lice collected in 2013-2018 period was observed, tending to increase over time [16]. Moreover, our presented data showed an uncommon finding of different bacteria species, possibly associated to carbapenemase resistance in lice. In particular we found $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-58-like}}$ and $bla_{\text{NDM-1}}$ carbapenemase elements. The $bla_{\text{OXA-23-like}}$ gene encoding the OXA-23 carbapenemase is widespread in clinical isolates and derives from the chromosome of *Acinetobacter radioresistens,* representing an intrinsic gene [17, 18]. The $bla_{\text{OXA-58-like}}$ and $bla_{\text{NDM-1}}$ are the most frequently variants found in *Acinetobacter* species [6, 17, 19]. Further studies are required to better understand the potential transmission of pathogens carrying carbapenemase genes from vectors to humans, including the possibilities to grow bacteria present in these insects for direct association of these resistance determinants and bacterial specie. Greater attention is due to extra-hospital reservoirs of these opportunistic drug-resistant bacteria and their potential involvement in emerging human community-acquired infections.

Acknowledgements

The authors wish to thank Giuseppina Mandarino for the language revision.

Funding

Authors have no funding source to disclose for the present study.

Conflict of interest statement

Authors have no competing interests to disclose for the present study.

Authors' contributions

FM: performed molecular methods and data analyses; LV and AC: analyzed genomic data and revised the manuscript; MM, MDL LT, CL, AM and FR: provided samples and insect data and gave input into the study design; AC: conceived the study, coordinate the study and wrote the manuscript. All authors read and approved the manuscript.

Received on 25 September 2020.
Accepted on 25 January 2021.

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