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LETTER TO THE EDITOR

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First report of *Raoultella ornithinolytica* carrying *bla*KPC-2 isolated from a dipteran muscoid collected in a garbage from a public hospital in Rio de Janeiro, Brazil

Rio de Janeiro, January 23rd, 2019

Dear Editor

Raoultella is a Gram-negative bacterium frequently isolated from natural environments and has emerged as an important pathogen in recent years[1]. Although this species has been described as susceptible to antibiotics, there are numerous reports of strains presenting a variety of resistance mechanisms, such as ESBL (Extended -spectrum beta- lactamases) and carbapenemases, including $bla_{\text{OXA-48}}$ from bloodstream infections isolates[2], the coexistence of $bla_{\text{KPC-2}}$ and $bla_{\text{IMP-4}}$[3], reported in strains recovered from sediment together with some producers of VIM-1[4]. Importantly, bla_{NDM} positive strains were isolated from clinical specimens[5] highlighting the dissemination of resistance genes from clinical to environmental members of the Enterobacteriaceae family. Flies show the capacity to transfer pathogens and their associated genes between different environments, acting as one of the most important vectors of human diseases worldwide[6].

The purpose of this study was to evaluate the possible role of flies in the dissemination of nosocomial, antibiotic resistant bacteria. Flies were captured within the garbage of a public hospital in Rio de Janeiro, Brazil, and were screened for the presence of mobile resistance determinants against colistin, carbapenems, cephalosporins, aminoglycosides and mobile genetic elements, including integrons and the transposon Tn*4401*. Samples of flies were collected in July 2016 and August 2017. Captures were carried out inside the dumpsters located in the dependencies of the public hospital in Rio de Janeiro, and from other dumpsters at a distance of approximately 100 meters from the hospital. Traps made of plastic bottles were placed at each location and left for 20 h. Trapped flies were taken to the laboratory and identified using dichotomous keys[7].

The flies were individually washed in sterile saline and homogenized with a sterile pestle in 1 mL of saline and vortexed. Aliquots of the diluted homogenates (100 µL) were streaked onto nutrient agar plates supplemented with ceftriaxone (1 mg/L) and incubated at 37 °C overnight. Representative colony types were subcultured on plates of nutrient agar and examined for resistance via the disk-diffusion method[8] towards the antibiotics cefepime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), meropenem (10 µg), gentamicin (10µg), tetracycline (10 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (1.25 + 23.75 µg) and chloramphenicol (30 µg) (Sensifar). Polymerase Chain Reaction (PCR) assays to detect resistance to β-lactams and aminoglycosides were conducted according to the protocol described by Poirel et al.[9]. Plasmid characterization was performed using the PCR-based replicon typing method[10], with plasmids extracted using an alkaline lysis method[11]. Subsequently, gel contents were transferred to nylon membranes, and hybridized with digoxigenin 11-dUTP (Roche) probes[12].

Raoultella ornithinolytica (LEMEF 71) was isolated from a specimen of the *Malacophagomia filamenta* fly. This bacterium was identified by the sequencing of approximately 240 nucleotides belonging to the V5 region of the gene 16S rRNA gene[13] in combination with MALDITOF MS (Bruker LT Microflex). The isolate LEMEF 71 was phenotypically resistant to tetracycline, cefepime, ceftazidime,

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gentamincin and trimethoprim/sulfamethoxazole and was positive by the Carbapenem Inactivation Method (CIM test), presenting a clear carbapenemase activity[14]. PCR-based screening revealed the presence of the bla_{KPC}, bla_{TEM}, *aac(6')-Ib* resistance genes and identified the presence of a plasmid belonging to the *IncK* incompatibility group. The identity of the KPC-2 amplicon was confirmed by its nucleotide sequencing. In addition, the isolate was positive for ISK*pn6*, which belongs to the IS1182 family, and also for ISK*pn7*, a member of the IS21 family, generating a T*np*A target amplicon. Those sequences are components of the transposon Tn*4401*, which is implicated as the origin of $bla_{KPC\text{-}like}$ gene acquisition and is believed to be responsible for its dissemination[15].

Southern hybridization assays revealed weak signals with bands considered to represent plasmid DNA, suggesting that the bla_{KPC} gene was present, but the number of copies of the plasmide was low (Figure 1). Attempts to transfer the plasmid by *in vitro* conjugation were unsuccessful, suggesting that the plasmid is most likely of non-conjugative type. Previous studies of clinical isolates of *Raoultella* spp. showed that bla_{KPC} was carried on by an 11-kb plasmid located within a Tn*4401* integration structure[16]. In addition, a wastewater strain has been documented and contained a novel IncP-6 promiscuous plasmid containing a Tn*3* transposon composed of ISK*pn6*/$bla_{KPC\text{-}2}$/$bla_{TEM\text{-}1}$/ISK*pn27*[17]. More recently, the emergence of *mcr*-1, encoding resistance to colistin, within an IncI2 plasmid of *R. planticola* isolated from flies, has been reported in China[18].

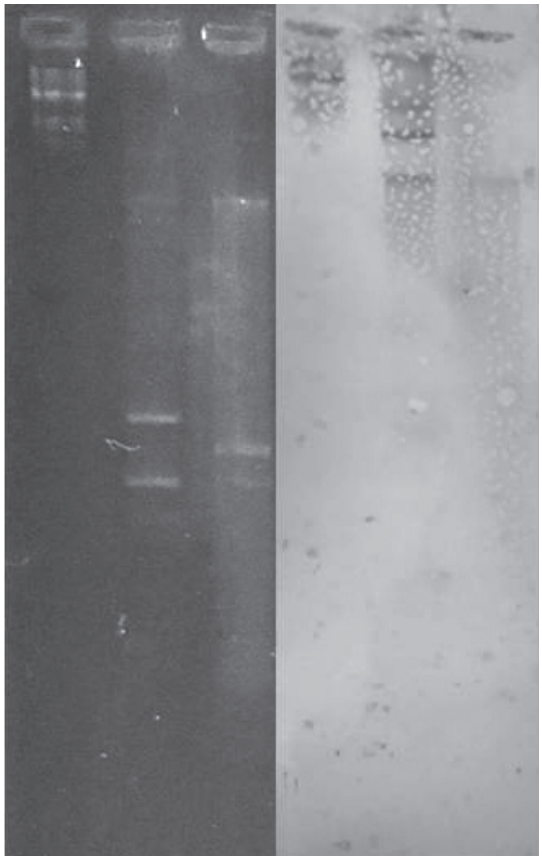

Figure 1 - A) Plasmid extractions from cultures of Klebsiella pneumoniae isolates and Raoultella ornithinilytica isolate that produce β-lactamase blaKPC-2 gene; B) Southern hybridization of a transferred plasmid extraction, conducted with an internal probe for blaKPC-2. Lane 1, DNA molecular-weight marker III digoxigenin- labeled; Lane 2, Klebsiella pneumoniae (positive control); Lane 3 Raoultella ornithinilytica (LEMEF 71).

Due to their reproductive and trophic link to microbe-rich substrates, flies have long been implicated as reservoirs and potential vectors of resistant bacteria[19]. It is not possible to definitively determine whether the isolate LEMEF 7 was originated from within the hospital or if it was resident in the extended environment. However, it should be noted that previous studies have demonstrated that samples collected from a range of hospital environments contained carbapenemase-producing organisms, including non-pathogenic ones that may serve as resilient reservoirs of resistance genes and plasmids[20].

In conclusion, the findings presented in this study, to the best of our knowledge, represent the first detection of KPC-2 producing *R. ornithinolytica* isolated from flies. This study reinforces the hypothesis that this resistance mechanism is rapidly disseminating in environmental isolates of Enterobacteriaceae. Moreover, insects are numerous and diverse in many environments, therefore our finding suggests their potential role in the dissemination of antibiotic resistance. Our study is of great concern due to its epidemic potential, since the emergence of KPC represents a severe threat to human health around the globe.

AUTHORS' CONTRIBUTIONS

INC: flies collection, bacterial isolation, molecular methods, data interpretation, article writing. EARC, MMCQ, MHSVB, KRC: design, data interpretation. JAL: phenotypic methods. VZ: design, data interpretation, article writing, approval of the review to be published and laboratory supervision.

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