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Molecular detection and antimicrobial resistance of *Klebsiella pneumoniae* from house flies (*Musca domestica*) in kitchens, farms, hospitals and slaughterhouses

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Summary Identifying disease vectors and pathogens is one of the key steps in controlling vector-borne diseases. This study investigated the possible role of house flies (*Musca domestica*) as vectors in the transmission of *Klebsiella pneumoniae* in Chaharmahal VA Bakhtiari and Isfahan provinces of Iran. House flies were captured from household kitchens, cattle farms, chicken farms, animal hospitals, human hospitals and slaughterhouses. Isolation of *K. pneumoniae* from external surfaces and guts of the flies was performed using MacConkey agar (MA) and thioglycollate broth (TGB). Identification of the isolates was performed with phenotypic techniques and polymerase chain reaction (PCR). A total of 600 house flies were sampled during the study period from different locations in four different seasons. Overall, 11.3% of

Abbreviations: *K. pneumoniae*, *Klebsiella pneumoniae*; MA, MacConkey agar; TGB, thioglycollate broth; PCR, polymerase chain reaction; *M. domestica*, *Musca domestica*; TSB, tryptic soy broth; CLSI, National Committee for Clinical Laboratory Standards.

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the captured house flies were positive for *K. pneumoniae*. In Chaharmahal VA Bakhtiari province, the prevalence was 12.7%, while in Isfahan province, 10.0% of the sampled house flies were infected with *K. pneumoniae*. Season-wise, the highest prevalence of infections among the house flies was in summer. The organisms were highly resistant to ampicillin, amoxicillin, cefotaxime and piperacillin. A lowest level of resistance was observed for imipenem/cilastatin. The findings of this study demonstrated that house flies are potential vectors of antibiotic-resistant *K. pneumoniae* in Isfahan and Chaharmahal provinces, Iran. Control efforts for infections caused by this particular bacterium should take *M. domestica* into account.

Introduction

The house fly, *Musca domestica*, is considered to be an important insect pest of both human and domestic animals that disseminate infectious diseases [1,2]. The fly occurs in the same environment with human and animals, and its maggots (larvae) are highly rich in organic matter and have high microbial flora [3,4]. Regular contact of the fly with wastes and animals provide an opportunity to transmit pathogens to both humans and animals [5—7]. Among other pathogens, house flies are known to transmit *Pseudomonas* spp., Enterobacteriaceae, *Staphylococcus aureus*, *Vibrio cholera, Chlamydia trachoma*, *Salmonella* spp. and *Klebsiella* spp. [5,8—15]. These pathogens are carried on the fly's legs and other body parts [16]. There are essentially four different ways in which house flies may transmit infectious microorganisms [1]: (i) on the hairs and surface of its body, (ii) on the glandular hairs on its feet, (iii) by regurgitation of vomitus, and (iv) by passage through the alimentary tract. Therefore, the fly may function as a temporary mechanical vector, or the pathogen concerned may survive for a longer period of time within the fly's body, in many instances with no adverse effect upon the carrier host. This latter possibility provides an opportunity for multiplication of the pathogen.

Klebsiella species are known to be responsible for more than 10% of in-hospital nosocomial infections [9,10,17,18]. Reports indicate that some of these infections, which at times involve antimicrobial-resistant strains, are vectored by insect pests, including house flies and cockroaches [5,7,10,17]. In Iran, such reports on the isolation of antimicrobial-resistant *Klebsiella* species from house flies are lacking. Therefore, the aim of this study was to ascertain the role of house flies in the carriage of antimicrobial-resistant strains of *Klebsiella* species in different locations in Chaharmahal VA Bakhtiari and Isfahan provinces of Iran.

Materials and methods

Study area and sample collection

The present longitudinal study was conducted in Isfahan (32.6333°N, 51.6500° E) and Chaharmahal VA Bakhtiari (32.3256° N, 50.8644° E) provinces located in the central and southwest areas of Iran, respectively (Fig. 1). A total of 600 randomly selected house flies were collected from four house kitchens, four cattle farms, two animal hospitals, four human hospitals, two slaughterhouses and two chicken farms, selected at random in the two provinces. The flies were either captured manually or by sticky trap methods. The fly samples were then transported to the laboratory at the Biotechnology Research Center using separate sterile tubes to prevent cross-contamination between samples. In the laboratory, flies were identified and killed by refrigeration at −20 °C in a cold chamber. They were then placed in 5 ml peptone water and left at room temperature for 5 h before being processed.

Isolation of *Klebsiella* spp. from external surfaces of house flies

Each housefly was transferred to a sterile test tube containing 2 ml of sterile normal saline and shaken thoroughly for 2 min. A fixed volume of these washings were then cultured on to MacConkey agar (MA) (Merck, Germany) plates for primary isolation. The washings were also inoculated into thioglycollate broth (TGB) and both media were incubated overnight at 37 °C. Subcultures were made from TGB onto MacConkey agar plates and incubated overnight at 37 °C. Colonies of *Klebsiella* spp. recovered on the MacConkey agar were identified according to a method described earlier [19]. Briefly, lactose-fermenting colonies were identified