



Isolation of *Klebsiella pneumoniae* strains from *Musca domestica* (houseflies) and their antibiotic sensitivity and resistance patterns, Philippines

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Abstract

The common housefly (*Musca domestica*) is a vector of various microbial and parasitic pathogens. In this study, *Klebsiella pneumoniae* strains were isolated from houseflies caught in the premises of a public market, school canteens and hospital in Tacloban City, province of Leyte, Philippines. Standard cultivation techniques for enteric bacilli were followed while the identification and antibiotic sensitivity testing were done using the Vitek 2 automated system. Results showed that out of 120 houseflies caught, only 16 (13.3%) had isolates of *Klebsiella pneumoniae*. These isolates were sensitive to Cefrofloxacin, Ceftazidime, Ceftriaxone, Gentamicin, Imipenem, Meropenem, Piperacillin/Tazobactam, and Trimethoprim/Sulfamethoxazole, intermediate to Colistin and resistant to Ampicillin. This study proved that in our city, there are houseflies serving as vector of *K. pneumoniae* that are either sensitive or resistant to commonly prescribed antibiotics.

Keywords: Antibiotics, houseflies, *Klebsiella pneumoniae*, Philippines, Vitek 2

Introduction

Klebsiella pneumoniae is a capsulated, atrichous, non-spore-forming Gram-negative rod-shaped bacteria belonging to Family Enterobacteriaceae. In apparently healthy individuals, it is commonly isolated from the nasopharynx and gastrointestinal tract (Tille, 2013) [23]. However, there are increasing reports associating *K. pneumoniae* with bacteremia, urinary tract infection and pneumonia especially in immunocompromised and hospitalized patients (Paczosa & Mecsas, 2016) [17]. It is recognized as a common cause of health-care associated infections and treatment can be problematic because of antibiotic resistant strains (Rawy *et al*, 2020) [21].

Studies have shown that *K. pneumoniae* is present in water, wastewater, sludge, soil, vegetation, insects such as houseflies and animals, (Bahndorff *et al*, 2017; Barati *et al*, 2016) [6-7]. Colonies on solid plate differential medium such as Eosin Methylene Blue Agar (EMB) or McConkey Agar (MAC) appear pink to indicate lactose fermentation and very mucoid that tend to coalesce due to its polysaccharide capsule (Wen & Zang, 2015) [25]. The capsule is considered a primary virulence factor of *K. pneumoniae* aside from lipopolysaccharide, fimbriae, outer membrane proteins and iron-chelating siderophores (Abbas *et al*, 2024) [1].

The main purpose of this study was to isolate *K. pneumoniae* from houseflies (*Musca domestica* L.) caught in the premises of a public market, school canteens and a hospital in Tacloban City, province of Leyte, Philippines and determine their sensitivity and resistance patterns using ten commonly prescribed antibiotics.

Materials and Methods

Study design

This study utilized the descriptive research design (Calmorin and Calmorin, 1995) [9] and it was conducted from January to May 2024.

Collection of houseflies

Purposive sampling technique was used to collect the houseflies for this study following a modified protocol by Ranjbar *et al*. (2016) [20]. Four sampling areas were chosen: a public market, a public elementary school canteen, a public high school canteen, and a public hospital in Tacloban City (approximately 11.2433° N latitude and 125.0047° E longitude), the capital city of the province of Leyte, Philippines with an estimated 250,000 inhabitants. The choice was based on the observation that many people and houseflies are seen on these sites. During a collection day, 10 strands of cotton rope each measuring 20 cms long were boiled for 1 hr in a caldron containing crabs, shrimps and water. This was meant to kill bacteria contaminating the ropes and provide an attractive smell for the houseflies. Then, in the sampling area, a 20 m clothes-line wire was extended and tied to posts about 1.5 m from the ground. Each cotton rope was fastened to the wire about 1.0 m away from each other with paper clips to hang. Once a housefly alighted on a rope, an open and clean 6 x 30 cm plastic bag was slowly raised to contain the rope and catch the insect. Then, the mouth part of the plastic bag was gripped by hand and was pulled off from the rope. The plastic bag was knotted to seal, coded and placed in a styrofoam box until ten houseflies from each sampling area were obtained. This procedure was done in order to avoid cross-contamination of each housefly with bacteria. Collection was done during daytime once a week for three weeks giving a total of 30 houseflies from each sampling site or a grand total of 120 houseflies for this study. The plastic bags with houseflies were placed at -10°C for 1 h to kill the insects.

Identification of houseflies

Houseflies were identified as *M. domestica* based on the descriptions of available manuals (Arroyo & Capinera, 2020; Kirby, 2008; SANBI, 2024) [3, 14, 22]. Each housefly

was removed from the plastic bag with sterile forceps, placed on a sterile Petri dish in a laminar flow and examined using a hand lens to identify the species and sex.

Bacterial isolation, identification and antibiotic testing

Each housefly was placed in a screw capped tube containing 5 mL autoclaved Trypticase Soy Broth (TSB), labelled and incubated overnight at 37°C. To check if the plastic bags were contaminated or not with *K. pneumoniae*, 2 plastic bags per batch were added each one with 5 mL TSB, then, pipetted to sterile screw-capped tubes and also incubated overnight at 37°C. The next day, a loopful from each TSB tube was streaked to an EMB Agar plate and incubated at 37°C for 18 hrs. Pink and mucoid colonies were subjected to Gram's and India Ink staining. An isolate that was non-spore forming Gram-negative bacilli and capsulated was sub-cultured on Trypticase Soy Agar (TSA) slant and incubated at 37°C overnight. Culture media were purchased in dehydrated form from HiMedia Laboratories Pty. Ltd. (India) and prepared according to the manufacturer's instructions. Final identification of *K. pneumoniae* was done with Vitek 2 automated system (bioMerieux, USA). Likewise, sensitivity and resistance patterns to ten commonly prescribed antibiotics was done with the same machine following the protocols of the Clinical Laboratory Standards Institute (CLSI, 2024) [10].

Stock cultures

Three similar colonies of *K. pneumoniae* from a plate culture were inoculated in 2 mL TSB and grown overnight at 37°C. The next day, 0.5 mL of the TSB culture was mixed with 0.5 mL autoclaved glycerine in a screw-capped plastic tube and stored at -10°C for at least 3 months. A sub-culture following the same process was done to maintain the bacterial strains for research and instruction purposes.

Results

In this study, 30 houseflies were caught from each sampling area or a total of 120 insects. Cultures on EMB plates gave 33 (27.5%) isolates that were mucoid and lactose fermenters. These isolates were Gram-negative non-spore forming capsulated bacilli. Out of these, 16 (13.3%) were identified as *K. pneumoniae* using Vitek 2 automated system (Table 1). No Gram-negative bacteria was isolated from the plastic bags only.

Houseflies caught from the premises of a high school canteen had the most isolates of *K. pneumoniae* with 6, public market and hospital with 4 isolates each, and only 2 from the elementary school canteen.

Table 1: Distribution of houseflies and characteristics of isolated mucoid colonies

| Location of Sampling Areas | Number of Houseflies Caught | Mucoid and Pink Colonies | Gram (-) Non-spore Forming bacilli | Positive India Ink Staining | <i>Klebsiella pneumoniae</i> Identified by Vitek 2 |
|----------------------------|-----------------------------|--------------------------|------------------------------------|-----------------------------|--|
| Tacloban Public Market | 30 | 9 | 9 | 9 | 4 |
| Elementary school canteen | 30 | 6 | 6 | 6 | 2 |
| High School canteen | 30 | 7 | 7 | 7 | 6 |
| Hospital vicinity | 30 | 11 | 11 | 11 | 4 |
| Total | 120 | 33 (27.5%) | 33 (27.5%) | 33 (27.5%) | 16 (13.3%) |

As shown in Table 2, all 120 flies caught were *M. domestica*. There were 62 (51.7%) females and the rest were males. On the other hand, out of 16 (13.3%) that were

positive for *K. pneumoniae*, 12 (10.0%) were female houseflies and only 4 (3.3%) were males or a ratio of 3:1.

Table 2: Distribution of houseflies by sex and presence or absence of *Klebsiella pneumoniae* isolates

| Sex | Frequency & Percentage | | With <i>K. pneumoniae</i> | | Without <i>K. pneumoniae</i> | |
|---------|------------------------|--------|---------------------------|-------|------------------------------|-------|
| Males | 58 | 48.3% | 4 | 3.3% | 54 | 45.0% |
| Females | 62 | 51.7% | 12 | 10.0% | 50 | 41.7% |
| Total | 120 | 100.0% | 16 | 13.3% | 104 | 86.7% |

As shown in Table 3, *K. pneumoniae* strains isolated from houseflies in Tacloban City were 100% sensitive to Cefotaxime, Ciprofloxacin, Gentamicin, and Trimethoprim/Sulfamethoxazole, 93.8% sensitive to

Ceftriaxone, Imipenem, Meropenem and Piperacillin/Tazobactam, 100% intermediate to Colistin, and 100% resistant to Ampicillin.

Table 3: Antibiotic sensitivity and resistance patterns of *Klebsiella pneumoniae* from houseflies

| Antibiotic | Number of Isolates | Sensitive | Intermediate | Resistant |
|-------------------------------|--------------------|-------------|--------------|-------------|
| Ampicillin | 16 | 0 (0.0%) | 0 (0.0%) | 16 (100.0%) |
| Ceftazidime | 16 | 16 (100.0%) | 0 (0.0%) | 0 (0.0%) |
| Ceftriaxone | 16 | 15 (93.8%) | 0 (0.0%) | 1 (6.3%) |
| Ciprofloxacin | 16 | 16 (100.0%) | 0 (0.0%) | 0 (0.0%) |
| Colistin | 16 | 0 (0.0%) | 16 (100.0%) | 0 (0.0%) |
| Gentamicin | 16 | 16 (100.0%) | 0 (0.0%) | 0 (0.0%) |
| Imipenem | 16 | 15 (93.8%) | 1 (6.3%) | 0 (0.0%) |
| Meropenem | 16 | 15 (93.8%) | 0 (0.0%) | 1 (6.3%) |
| Piperacillin/Tazobactam | 16 | 15 (93.8%) | 1 (6.3%) | 0 (0.0%) |
| Trimethoprim/Sulfamethoxazole | 16 | 16 (100.0%) | 0 (0.0%) | 0 (0.0%) |

Discussion

This study was conducted to isolate *K. pneumoniae* strains from houseflies (*M. domestica*) caught in the premises of a public market, elementary and high school canteens and a government hospital in Tacloban City, the capital city of the province of Leyte, Philippines. Using standard cultivation techniques and Vitek 2 identification system, there were 33 (27.5%) houseflies that harbored lactose fermenting, mucoid and Gram-negative non-spore forming bacilli. However, only 16 (13.3%) were positive for *K. pneumoniae*, indicating that the rest of the isolates were either species related to this bacterium or other enteric bacteria. Our result of 13.3% was higher than the 11.3% positivity rate based on the report of Ranjbar *et al.* (2015) [20] but lower than the 14.5% rate of Bashir *et al.* (2022) [8]. Perhaps the strict policies to maintain cleanliness and sanitation in the areas covered in this study could explain the result we obtained. Nevertheless, this finding proved that some houseflies in this city can serve as vector for *K. pneumoniae* which agreed with studies conducted in other countries (Bashir *et al.*, 2022; Ghalehnoo, 2015; Nazari *et al.*, 2017; Ranjbar *et al.*, 2016) [8, 11, 16, 20]. Houseflies have been known to serve as vector of potentially harmful microorganisms (Issa, 2019) [12]. Detailed studies on housefly biology have shown that a mother can also transmit bacterial pathogens through her eggs to the next generation of houseflies (Pava-Ripoll *et al.*, 2015) [18]. In our study, a sex ratio of 3:1 meant that female houseflies had three times more possibility of transmitting *K. pneumoniae* strains than male houseflies. Perhaps, in its effort to search for food to nourish her eggs, female houseflies would seek for more organic and decomposing matter as compared to the males and in the process would acquire more strains of the said bacteria.

In our study, the identification of *K. pneumoniae* and antibiotic sensitivity testing were done using Vitek 2 system. This automated machine has revolutionized the work in the microbiology laboratory and has proven to be fast, effective, reliable and accurate (Ling *et al.*, 2001; Quesada *et al.*, 2010) [15, 19].

K. pneumoniae is considered a very important bacterial pathogen in humans with significant morbidity and mortality (Karande 2026) [13]. It contains an extracellular polysaccharide capsule which is a hallmark of virulence that enables the bacteria to evade phagocytosis and enhance resistance to intracellular killing (Wen *et al.*, 2015; Zhu *et al.*, 2021) [25, 26]. Biofilm formation, presence of pili and expression of siderophores also contribute to the virulence of this bacteria (Wang *et al.*, 2020) [24].

Resistance to one or more antibiotics have been reported in *K. pneumoniae* isolated from patients (Alanezi *et al.*, 2022; Awoke *et al.*, 2012; Ayatollahi *et al.*, 2020) [2, 4, 5] and houseflies caught from various places (Bahndorff *et al.*, 2017; Nazari *et al.*, 2017; Ranjabr *et al.*, 2016) [6, 16, 20]. However, in our study, all 16 (100.0%) strains of *K. pneumoniae* were sensitive to Ceftriaxime, Ciprofloxacin, Gentamicin and Trimethoprim/Sulfamethoxazole; 93.8% sensitive to Ceftriaxone, Imipenem, Meropenem and Piperacillin/Tazobactam. Whereas, all isolates were intermediate to Colistin and resistant to Ampicillin. These findings could be considered a good indicator of rational prescription practice for commonly used antibiotics. However, the presence of all isolates of *K. pneumoniae* that were Ampicillin resistant reflected also the common practice of prescribing Ampicillin first since it is a cheap

antibiotic. In addition, there are stores that allow clients to buy the antibiotic over-the-counter. These practices could eventually lead to the emergence of Ampicillin resistant bacteria.

Conclusion

This study has proven that houseflies can serve as a vector of *K. pneumoniae* strains in Tacloban City, Philippines. These isolates were mostly sensitive to commonly prescribed antibiotics except for Ampicillin where all strains showed antibiotic resistance.

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