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A Comparative Assessment of the Antibiogram and Extended Spectrum Beta-Lactamase Production By *Klebsiella* Species Isolated In Houseflies From Hospital, Restaurant And Fruit Market Environments

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Abstract
Houseflies have been identified as reservoirs and mechanical vectors of disease-causing agents that can cause serious life threatening diseases in both human and animal populations. This study was designed to assess the antibiogram and extended spectrum beta-lactamase production by *Klebsiella* species isolated in houseflies from hospital, restaurant and fruit market environments. A total of 150 samples (50 each from hospital, restaurant and fruit market environments) were analyzed using Standard Microbiological Methods. Antibiotic susceptibility test of the isolates was determined using disc diffusion method. The isolates were screened for ESBL production using Double Disc Synergy Test (DDST). Results obtained from this study showed that out of the 150 samples analyzed, a total of 82 representing 54.67 % isolates of *Klebsiella* species were obtained. Of these, 33(40.24 %), 38(46.34 %) and 11(13.41 %) were respectively obtained from hospital, fruit market and restaurant environments. Antibiotic susceptibility testing showed that *Klebsiella* species were all susceptible to meropenem, except those from the hospital which were 87 % susceptible. The susceptibility of the isolates to other antibiotics including nalixidic acid, ciprofloxacin, cefoxitin, ceftaxidime, gentamicin, ceftriaxone, sulfamethoxazole-trimetoprim, cefotaxime and ampicillin-sulbactam ranged from 3.51-50 % for hospital; 27.30-90.90 % for restaurant; and 15.80-81.60 % for fruit market environments. Result of extended spectrum beta-lactamase screening showed that of the 82 *Klebsiella* species screened, only 1(3.03 %) from hospital environment was ESBL positive while the rest were ESBL negative. In summary, the study revealed that houseflies from hospital, fruit market and restaurant environments harbor multidrug resistant *Klebsiella* species of which majority of them were not ESBL mediated though a small proportion were ESBL mediated. This study revealed that houseflies are potential agent of spread of multidrug resistant organisms like *Klebsiella* species which is a serious threat to human and animal health.

Keywords: *Klebsiella*, houseflies, antibiotic resistance, ESBLs, hospitals, restaurant, fruit market

I. Introduction

Houseflies also known as *Musca domestica* are cosmopolitan insects well-known for their presence and adaptability to various environmental settings. They measure naturally between 6 to 7 millimeters in length and are characterized by a gray thorax adorned with four longitudinal stripes and a little hairy abdomen. They have transparent wings which permit nimble/agile flight that make them move swiftly over short distances (Boyer *et al.*, 2018; Nazni *et al.*, 2005). They are structurally adapted for picking up pathogens. The proboscis is provided with abundance of fine hairs that readily collect environmental debris. Additionally, each of the six legs of the fly is built-in with hairy structures and pads that secrete a sticky material, which adds to its pathogen transmission potential (Nazni *et al.*, 2005).

They are often found in close association with humans and animals. They are notoriously known for their unprincipled feeding habits such as the consumption of a wide range of organic matter including decaying food, animal feces, and other organic materials (Gehad and Eman, 2011; Adebayo *et al.*, 2012; Khobdel *et al.*, 2008; Pandian and Asumtha, 2001). This multiplicity in their feeding pattern play very vital role as possible vectors of

several pathogens, which they can pick up from dirty/contaminated exteriors and then transmit to foods and living spaces (Boyer *et al.,* 2018; Nazni *et al.,* 2005).

In spite of their ecological roles in recycling and breakdown of nutrient, houseflies pose substantial public health concerns because of their ability to spread diseases such as dysentery, typhoid fever, and cholera (Khobdel *et al.,* 2008; Pandian and Asumtha, 2001; Yang *et al.,* 2019; Ahmed *et al.,* 2013).

Increases in the occurrence of antimicrobial resistance among microbial pathogens have posed a serious health challenge globally. This is worsened by the extensive dissemination of resistant bacteria through various vectors, including houseflies (Graezyk *et al.,* 2011). *Klebsiella* species, notorious for their ability to acquire resistance mechanisms such as extended spectrum beta-Lactamase (ESBL) production, represent a critical concern in healthcare settings due to limited treatment options. They contribute meaningfully to nosocomial and community-acquired infections all over the world (Livermore, 2005). ESBLs, enzymes capable of hydrolyzing a broad range of beta-lactam antibiotics therefore pose a substantial challenge to treatment efficacy, limiting therapeutic options and ever increasing morbidity and mortality rates (Paterson and Bonomo, 2005).

In the context of Abakaliki Metropolis where environmental factors such as sanitation and hygiene practices may influence bacterial transmission dynamics, assessing the antibiogram and extended spectrum beta-lactamase production by *Klebsiella* species isolated in houseflies from hospital, restaurant and fruit market environments is crucial. This is important because houseflies commonly found in urban settings, have been implicated as potential vectors for multidrug-resistant bacteria due to their ability to traverse various environments and come into contact with human habitats (Nayduch *et al.,* 2023).

II. Materials and Methods

Sample collection

One hundred and fifty (150) houseflies (50 each from hospital, restaurant and fruit market environments) were collected using sterilized insect scoop nets. The flies were trapped during their active periods of 10-12 am. Each housefly trapped was aseptically transferred into a sterile culture bottle and properly labeled. All the samples collected were transported to the laboratory section of Applied Microbiology Department for bacteriological analysis.

Bacteriological analysis

Houseflies collected from each environment were put in the refrigerator and were allowed for about 30 minutes for demobilization. A 1 ml of normal saline was added into each container with a fly after demobilization. The mixture of each container was vigorously agitated to dislodge the external content of each fly. A 0.5 ml of each mixture was aseptically transferred into test tubes containing 3 ml of nutrient broth. The tubes were incubated at 37^{o}C for 18-24 hours. Each tube was properly mixed after incubation and a loopfull of it was seeded on aseptically prepared MacConkey agar plates and incubated for 18-24 hours at 37^{O}C. Suspected district colonies of *Klebsiella* species were transferred to a freshly prepared MacConkey agar plates and were incubated for 18-24 hours at 37^{O}C to get the pure colonies. The pure colonies of the isolates were transferred into nutrient agar slants and were incubated for 18-24 hours at 37^{O}C. Slants were stored in the refrigerator between 0-4^{o}C for further identification (Afiukwa *et al.,* 2022; Adebayo *et al.,* 2012; Khobdel *et al.,* 2008; Pandian and Asumtha, 2001).

Gram staining and microscopic identification of isolates

Isolates obtained were Gram stained to determine their gram reactions to the primary stains. A young culture of each isolate was emulsified in a drop of water placed at the center of a clean grease-free slide till a thin smear was made. The smear was heat-fixed by passing the slide through a bunsen burner flame. The heat-fixed smear was flooded with crystal violet and allowed to stand for 30-60 seconds. The stain was washed with slowly running tap water. The smear was flooded again with lugol's iodine and allowed to stand for 30-60 seconds. The slide was then rinsed off with slowly running tap water. The smear was decolorized with 70% ethanol until the color of the crystal violet stops coming out and was also washed off with slowly running tap water. The smear was counter-stained with safranin and allowed to stand for 30-60 seconds, washed off with slowly running tap water. The slide was blotted dry and was then examined under an oil immersion objective lens of a compound microscope. All isolates obtained and Gram stained were Gram-negative (Cheesebrough, 2006).

Biochemical characterization and identification of isolates

All isolates obtained were further identified using conventional biochemical tests such as indole, methyl red, voges prauskauer and citrate tests.

Antibiotic susceptibility test

A young culture (18-24 hour old) of the test organisms were used in this study. Each *Klebsiella* species isolated was standardized to 0.5 MacFaland equivalents and was inoculated on the surface of aseptically prepared

Mueller-Hinton agar plates using sterile swab stick. With sterilized forcepts, discs of ciprofloxacin (30 µg), gentamicin (30 µg), nalixidic acid (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ampicillin-sulbactam (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg), cefoxitin (30 µg), sulfamethoxazole-trimethoprim (30 µg) and meropenem (30 µg) (oxoid Uk) were placed on the surface of the agar plates about 15 mm from the edge of the plate and not close than 25 mm from one disc to another. The antibiotics were allowed to diffuse for about 10 minutes and the plates were incubated at 37^{o}C for 18-24 hours. After 24 hours of incubation, the clear zones of inhibitions were determined using meter rule in millimetre (mm). Any isolate resistant to any of the cefotaxime, ceftaxidime and ceftriaxone were further subjected to ESBL phenotypic screening (Afiukwa *et al.*, 2016; Iroha *et al.*, 2008; NCCLS, 2000; Kirby and Bauer, 1966).

Phenotypic determination of extended spectrum beta-lactamases (ESBL)

Isolates resistant to first and second generation cephalosporins were subjected for extended spectrum beta-lactamase production using Double Disc Synergy Test (DDST). A 0.5 MacFarland equivalent standard of a young culture (18-24 hours old) of each test organism was inoculated on the surface of aseptically prepared Mueller-Hinton agar plates using sterile swab stick (NCCLS, 2000). Discs of ceftazidime (30 µg) and cefotaxime (30 µg) were placed at a distance of 15 mm centre to centre from the centre disc containing Amoxicillin plus Clavulanic acid (20 µg and 10 µg, respectively). The plates were incubated at 37^{O}C for 18-24 hours in an inverted position. Synergistic measurement of 5 mm and above towards the centre disc indicated ESBL positive and are ESBL negative if it measures lower than 5 mm towards the centre disc (Pitout, 2004; Jarlier *et al.*, 1998).

III. Results

Prevalence of *Klebsiella* species Isolated from the body wash of Houseflies from Hospital, Restaurant and Fruit market environments in Abakaliki Metropolis

Results obtained from this study showed that out of the 150 samples (50 each from hospital, restaurant and fruit market environments) analyzed 82 (54.67%) isolates of *Klebsiella* species were obtained. Of the 82 isolates, 33(40.24 %), 38(46.34 %) and 11(13.41 %) were respectively obtained from hospital, fruit market and restaurant environments (Table 1).

Table 1: Percentage Prevalence of *Klebsiella* species Isolated from the body wash of Houseflies from Hospital, Restaurant and Fruit market environments in Abakaliki Metropolis

Sample source	No. of samples analyzed	No. and percentage of *Klebsiella* species isolated
Hospital	50	33(40.24)
Fruit market	50	38(46.34)
Restaurant	50	11(13.41)
Total	150	82(54.67)

Antibiotic Susceptibility and Resistance Pattern of *Klebsiella* species Isolated from the Body wash of Houseflies from Hospital, Restaurant and fruit market environments

Of the 33 isolates of *Klebsiella* species screened from hospital environment, as high as 87.00% were susceptible to meropenem while their susceptibility to ceftaxidime, nalixidic acid and ciprofloxacin ranged from 45.50% - 51.50% (Table 2). The isolates were least susceptible to ceftriaxone, ampicillin-sulbactam, sulfamethoxazole-trimethoprim with their percentage values ranging from 3% - 9.10% (Table 2). The isolates were 100% resistant to cefoxitin while their percentage resistance to ceftazidime, cefotaxime, gentamicin, ampicillin-sulbactam, sulfamethoxazole-trimethoprim and ampicillin-sulbactam ranged from 54.50% - 100% (Table 2).

A total of 11 isolates of *Klebsiella* species were screened from restaurant and there were 100 % susceptible to meropenem, followed by 90.90 % to ciprofloxacin. Their susceptibility to sulfamethozazole-trimethoprim, ceftazidime, cefotaxime, nalidixic acid, gentamicin and ceftriaxone ranged from 27.30 % - 72.70 % (Table 2). However, they were 100% resistant each to ampicillin-sulbactam and cefotixin (Table 2). Their percentage resistance to other antibiotics including ciprofloxacin, ceftriaxone, gentamicin, nalixidic acid, cefotaxime, ceftaxidime and sulfamethozazole-trimethoprim ranged from 9.10 % 72.70 % (Table 2).

All isolates of *Klebsiella* species (38) obtained from fruits market were 100% susceptible to meropenem (Table 2). This was followed by 81.60% each to ciprofloxacin and gentamicin; 38.95% and 71.1% to nalixidic acid and cefotaxime, respectively (Table 2). Their susceptibility to cefoxitin, ceftriaxone, ampicillin-subactam, ceftazidime and sulfamethozazole –trimethoprim ranged from 0.0% - 24.20% (Table 2). In contrast, they were

100% resistant only to ceftriaxone, ampicillin-subactam and cefoxitin. Their resistance to other antibiotics including meropenem, ciprofloxacin, nalixidic acid and cefotaxime ranged from 0.0 % -28.90 % (Table 2).

Table 2: Percentage Susceptibility and Resistance Pattern of *Klebsiella* species Isolated from Body Wash of Houseflies from Hospital, restaurant and Fruit market environments

Antibiotics	**Hospital**		**Restaurant**		**Fruit market**	
	***Klebsiella* spp**		***Klebsiella* spp**		***Klebsiella* spp**	
	% Susceptible	**% Resistance**	**% Susceptible**	**% Resistance**	**% Susceptible**	**% Resistant**
Meropenem	29(87.00)	4(13.00)	11(100)	0(0.00)	38(100)	0(0.00)
Nalixidic acid	16(48.50)	17(51.50)	7(63.60)	4(36.40)	30(78.95)	8(21.05)
Ceftriaxone	1(3.00)	32(97.00)	9(81.80)	2(18.20)	0(0.00)	38(100)
Ciprofloxacin	17(51.50)	16(48.50)	10(90.90)	1(9.10)	31(81.60)	7(18.40)
Sulfamethozazole-trimethoprim	3(9.10)	30(90.90)	3(27.30)	8(72.70)	13(24.20)	25(65.80)
Cefotaxime	11(33.33)	22(66.67)	7(63.60)	4(36.40)	27(71.10)	11(28.90)
Ampicillin-subactam	3(9.10)	30(90.90)	0(0.00)	11(0.00)	0(0.00)	38(100)
Ceftazidime	15(45.50)	18(54.50)	7(63.60)	4(36.40)	6(15.80)	32(84.20)
Cefoxitin	0(0.00)	33(100)	0(0.00)	11(0.00)	0(0.00)	38(100)
Gentamicin	9(27.27)	24(72.73)	8(72.70)	3(27.30)	31(81.60)	**7**(18.40)

Occurrence of ESBL Producing *Klebsiella* species Isolated from the Body wash of Houseflies from Hospital, Restaurant and Fruit market Environments.

In this study, out of the 82 *Klebsiella* isolates (33 from hospital, 38 from fruit market and 11 from restaurant) screened for the presence of ESBLs, only 1(3.03%) isolate of *Klebsiella* species from hospital environment tested positive for ESBL production (Table 3). All other isolates including those from restaurant and fruit market environments tested negative (Table 3).

Table 3: Percentage Occurrence of ESBLs in *Klebsiella* species Isolated from the body wash of Houseflies from the Hospital, Restaurant and fruit market environment.

Sample source	**Isolates obtained**	**Number of isolates screened for ESBL production**	**Percentage ESBL positive**	**Percentage ESBL negative**
Hospital	*Klebsiella*	33	1(3.03)	32(96.63)
Restaurant	*Klebsiella*	11	(0.0)	11(100)
Fruit market	*Klebsiella*	38	(0.0)	38(100)
Total		**82**	1	81

Plate 1: ESBL positive Isolate of *Klebsiella* species from housefly

IV. Discussion

Klebsiella species is one of the opportunistic pathogens well-known for their capacity to cause series of infections, often exhibiting resistance to multiple antibiotics, including those crucial for treatment (Dong *et al.,* 2022). Flies that transmit infectious agents are frequently found around human and animal waste and landfills, from where they disperse to areas of human habitation and activity (Nazni *et al.,* 2005 Nayduch *et al.,* 2023). *M. domestica* therefore is a very good mechanical vector of human and animal pathogens because of it biology and ecology. They reproduce in areas like cattle barns, poultry houses, slaughter houses and hospitals (Holt *et al.,* 2007; Nayduch *et al.,* 2023). Their external organs (legs, wings and mouthparts) have been scientifically reported to constitute a large source of bacteria (Graczyk, 1999).

In this present study, out of the 150 samples (50 each from hospital, restaurant and fruit market environments) analyzed 82 (54.67%) isolates of *Klebsiella* species were obtained. Of the 82 isolates, 33(40.24 %), 38(46.34 %) and 11(13.41 %) were respectively obtained from hospital, fruit market and restaurant environments. This indicates that the body wash of houseflies from fruit market had the highest number of *Klebsiella species* and that was followed by those from the hospital and the least were obtained from restaurants. Knowing that sugar is a very important substrate that encourages the growth and multiplication of microorganisms, the prevalence of the *Klebsiella species* in fruit market environment could be associated with the high sugar content in the fruits which attracts more flies (potential pathogen carriers) than other sites investigated. Our outcomes are in line with other research findings which highlighted the importance of houseflies in carrying various pathogenic bacteria particularly *K. pneumoniae* being the most important at USA and Iran (Thaddeus *et al.* 2001, Khalil *et al.* 1994). It also agreed with the study by Gashaw *et al.* 2024; Davari *et al.,* 2010; Vazirianzadeh *et al.* 2008; Fotedar *et al.,* 1992) who isolated *Klebsiella* species from the external body wash of houseflies from different environments. Davari *et al.,* 2010 reported more pathogenic bacteria from houseflies caught in hospitals than those caught from slaughter house which is not in line with our findings where we isolated more *Klebsiella* species from the fruit market when compared with those from the hospital. Gashaw *et al.,* 2024; Davari *et al.,* 2010; Vazirianzadeh *et al.,* 2008 and Mawak and Olukose, 2006 isolated both *E. coli* and *Klebsiella* species from houseflies which is not out of place from our study. It is also in tandem with the study carried out in Umahia, Abia State by Nwankwo *et al.* (2019) who isolated *Klebsiella* species in hoseflies from different sources including clinic, restaurant, refuse, etc. Our results therefore conformed with the highlight on the importance of houseflies in the spread of disease-causing pathogenic bacteria especially *K. pneumonia* (Nayduch *et al.,* 2023).

The antibiogram profile demonstrated high rate of resistance among *Klebsiella* isolates, particularly against commonly used antibiotics such as cefoxitin, ampicillin-subactam, sulfamethoxazole-trimethoprim and ceftriaxone. These antibiotics are often recommended for both community-acquired and hospital-acquired infections in Nigeria. Antibiotic resistance has persistently posed a severe global health problem especially in developing countries where bacteria that cause human disease are also those in which emerging antibiotic resistance is mostly apparent (Huemer *et al.,* 2020). Moreso, flies have been reported to carry multi-drug resistant

bacteria in hospital environments and may play vital roles in the transmission of human pathogens within hospitals (Davari *et al.*, 2010).

In the present study, antibiotic susceptibility and resistance patterns of *Klebsiella* species to different antibiotics showed that meropenem had the highest activity against them while cefoxitin had the least activity. Of the 33 isolates of *Klebsiella* species screened from hospital, only 87.00% was susceptible to meropenem. Their susceptibility to ceftaxidime, nalixidic acid and ciprofloxacin ranged from 45.50% - 51.50% and were least susceptible to ceftriaxone, ampicillin-sulbactam, sulfamethoxazole-trimethoprim with the percentage range of 3% - 9.10 %. The isolates were 100% resistant to cefoxitin while their percentage resistance to ceftazidime, cefotaxime, gentamicin, ampicillin-sulbactam, sulfamethoxazole-trimethoprim and ampicillin-sulbactam ranged from 54.50% - 100%. Eleven (11) isolates of *Klebsiella* species screened from restaurant were 100 % susceptible to meropenem, followed by 90.90% to ciprofloxacin. Their susceptibility to sulfamethozazole-trimethoprim, ceftazidime, cefotaxime, nalidixic acid, gentamicin and ceftriaxone ranged from 27.30% - 72.70%. However, they were 100% resistant each to ampicillin-sulbactam and cefotixin; but their percentage resistance to other antibiotics including ciprofloxacin, ceftriaxone, gentamicin, nalixidic acid, cefotaxime, ceftaxidime and sulfamethozazole-trimethoprim ranged from 9.10% -72.70%. From the fruits market, 38 isolates of *Klebsiella* species screened were 100 % susceptible to meropenem. Their susceptibility to nalixidic acid, cefotaxime, ciprofloxacin and gentamicin ranged from 38.95 % - 81.60 % while their susceptibility to cefoxitin, ceftriaxone, ampicillin-subactam, ceftazidime and sulfamethozazole-trimethoprim ranged from 0.0% - 24.20%. The isolates were 100% resistant only to ceftriaxone, ampicillin-subactam and cefoxitin. Our findings showed that meropenem had the highest activity against the isolates from the three sites while cefoxitin had the least activity. Also, *Klebsiella* species from hospital were more resistant to the antibiotics used than isolates from restaurant and fruit market environments. The observed resistance in this study suggests that *Klebsiella* species associated with houseflies may serve as prospective reservoirs for dissemination of antibiotic resistance genes in both human and animal settings. Their resistance to various antibiotics can be as a result of presence of some drug resistant gene, additional gain of other genes through horizontal gene transfer or by physiology dependent resistance (Mitchell *et al.* 2004, Rangrez *et al.* 2006). The *K. pneumonia* isolated from hospital houseflies according to the report by Davari *et al.*, 2010 are more resistant to the antibiotics used than those from slaughterhouse, which corroborates with our findings. Nwankwo *et al.* (2019); Fotedar *et al.* (1992) and Sramova *et al.* (1992) reported similar multiple-resistance to antibiotics in *Klebsiella* spp. from houseflies in hospitals environments which also align with our study. In tandem with our findings too is the work by Macovei and Zurek (2006) who detected antibiotic-resistant and potentially virulent pathogen in houseflies from food settings. Also, no resistance to meropenem was recorded except in *Klebsiella* species from hospital which slightly disagrees with the report by Yang *et al.* (2013) who reported no resistance of the isolates at all to meropenem and imipenem. The presence of these pathogens indicates that houseflies (*M. domestica*) are good vectors of pathogens which pose serious health risks to humans and animals.

The occurrence of extended spectrum beta-lactamases (ESBLs) in microbial isolates from environmental samples such as houseflies climaxes the potential for these insects (houseflies) to add to the dissemination of resistant bacteria beyond clinical settings (Gashaw *et al.*, 2024). Of the 82 isolates (33 *Klebsiella* spp. from hospital; 38 *Klebsiella* spp. from fruit market and 11 *Klebsiella* spp from restaurant) screened for the presence of ESBLs, only 1(3.03%) isolate of *Klebsiella* species from hospital environment tested positive while all other isolates including those from restaurant and fruit market environments tested negative. The presence of ESBLs among *Klebsiella* isolates from houseflies in Abakaliki Metropolis suggests a troublesome situation where environmental factors may contribute to the spread of ESBL genes. This finding aligns with global concerns regarding the environmental dissemination of antibiotic resistance, emphasizing the need for comprehensive surveillance and control measures (Aljeldah, 2022). This result is not out of place because houseflies from the hospital environment may have come in contact with patients or hospital wastes harboring multidrug resistant organisms including ESBL producers. ESBL-producing *Enterobacteriaceae* have been reported in flies from Asia, Africa and Europe with colonization rates of up to 17% which is in line with our findings, though the percentage value is high when compared to ours. Our study however disagrees with the report by Solà-Ginés *et al.* (2015) who isolated beta-lactamase-producing *Escherichia coli* from houseflies in Spanish broiler, stating the possible contribution of houseflies to the rise and spread of virulence and resistance genes into different ecological niches. This finding therefore bring into line with global concerns about the environmental dissemination of antibiotic resistance, emphasizing the need for comprehensive surveillance and control measures (Aljeldah, 2022).

In conclusion, this study provides valuable insights into the impact of housefly environments on its potential to spread multidrug resistance. We therefore recommend that appropriate steps should be taken to control flies especially in hospital environments where they can easily pick up antibiotic resistant bacteria to areas they were not before. Food and fruit vendors should also be educated and monitored to protect their products from houseflies which are vectors of microbial pathogens.

Declarations

Authors' contributions

AFN and ACA conceptualize the research, joined in sample collection, microbial isolation and antimicrobial sensitivity testing; OB was involved in antimicrobial sensitivity screening**,** while NNP and AVO prepared the first draft of the manuscript. All authors were involved in the final manuscript preparation.

Declaration of Conflict of Interest:

None

Ethical Approval and Informed Consent

This study did not use human subjects or animal models. Hence, ethical consideration was not applicable.

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