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Phenotypic characterization and antimicrobial susceptibility testing of *Klebsiella* isolates from *Rattus rattus* captured at university of Abuja metropolis

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The aim of this study is to phenotypically characterize *Klebsiella* isolates from rats. A survey was conducted on 100 swaps samples obtained from captured house rats in University of Abuja main campus from May to August 2021. Isolation and identification of the *Klebsiella* species was done using conventional cultural and biochemical techniques. Of the 100 samples analyzed, 20 (20%) were positive for *Klebsiella*, 12% of the isolates were identified as *Klebsiella Pneumonia* while 8% were identified as *Klebsiella oxytoca*. Isolates were further confirmed by Microbact 24E test kit identification system (Oxiod, UK). *Klebsiella* isolates encountered in this study were further subjected to antimicrobial susceptibility testing using modified single disk diffusion method. Result of the antimicrobial susceptibility testing showed that the *Klebsiella* species were resistant to chloramphenicol (80%), gentamycin (90%), augmentin (80%), Cotrimoxazole (70%) and amoxicillin (70%). However, the Isolates were susceptible to Pefloxacin (100%), Streptomycin (40%), Ofloxacin (100%), Ciprofloxacin (100%) and Sparfloxacin (80%). Conclusively, this study documented the occurrence and existence of multiple resistant strains of *K. Pneumonia* and *K. Oxytoca* in rats in our environment and it is therefore of public health concern.

Key words: *Klebsiella* species, household rat, Rattus *rattus*, antimicrobial susceptibility.

INTRODUCTION

Klebsiella are widely distributed in nature and are part of the normal floral of the gastrointestinal tract of humans and animals (Majumder et al., 2018). They are found in the oropharynx of 1-6% of normal healthy individuals, colonization rate as high as 20% may be seen in hospitalized patients (Arora and Arora, 2012). This colonization may be the source of a good number of lung infections such as severe bronchopneumonia, resulting with chronic destructive and supurative lesions, pleuritis and multiple abscesses in the lungs. High mortality in man and animals is due to septiceamia followed by secondary bacteria invasion (Ernst et al., 2020).

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Klebsiella organisms are often incriminated in most Hospital associated acquired (nosocomial) infections resulting to clinical syndromes such as urinary tract infections, wounds and burns as wounds secondary invaders in other respiratory infections (Majumder et al., 2018). These enteric organisms are also responsible for a significant number of community acquired infections worldwide (Ernst et al., 2020). The organisms are the most frequently encountered gram-negative pathogens causing nosocomial infection of the lower respiratory tract and are second only to *E. coli* as the cause of primary bacteremia associated with gram-negative organisms (Arora and Arora, 2012).

In animals, it is an essential cause of pneumonia, epidemic metritis, cervicitis in mares and septicemia in foals (Wareth and Neubauer, 2021). It has been frequently associated with pneumonia and mastitis in bovine (Piras et al., 2022) leading to high loses in milk production, decreased milk quality and even high mortalities among affected cows (Gorden et al., 2018). Consequently, infection can result in noticeable any monetary involvements in the dairy industry, even in well managed dairy farms (Gorden et al., 2018).

Household rat (*Rattus Rattus*) share living environments with humans and animals, and are predominant in rural areas especially in communities with poor sanitary and environmental conditions (Ogbole et al., 2022). Rodents are infamous for being everywhere since they travel great distances in quest of food between houses and bushes. Domestic rats are omnivores, which makes them susceptible to a number of infectious diseases. Given that rats serve as reservoirs for a variety of bacterial, viral, and parasitic organisms, people are at significant risk of developing virulent diseases linked to *Klebsiella* species (Baidya and Rahman, 2021). Zhong et al. (2020) reported high prevalence of antibiotic resistant *K. pneumonia* from urban rodents and shrews in Southern China. There is paucity of information on the occurrence of *Klebsiella* in rats in the study area. Therefore, this study aimed at the determination of the isolation rate of *Klesiella pneumonia* and *Klebsiella oxytoca* in captured rat in University of Abuja, Federal capital Territory, Nigeria, and the determination of their antimicrobial susceptibilities to commonly used antibiotics.

MATERIALS AND METHODS

Sample collection and preparation

Rat capture

The traditional method for rat captured used was the snap trap method placed in their routes with attractive bait such as roasted fish to attract the rats to the area (Christie et al., 2017). The traps are placed inside closet, under any furniture or bushy passages or other routes that the rats can possibility pass through. This cleverly arrangement lure some of the rats into the traps and have them captured. The captured rats are transferred into a shoebox and transported to the laboratory for further analysis.

Sample collection

The rat was properly restrained by grasping firmly at the base of the tail, by applying tension so that the surface of the rat was grasped using the free hand. The rat was firmly grasped over the shoulder and close to the base of the skull between the thumb and the forefinger (Christie et al., 2017). The vent of the rat was squeezed to remove fecal and urinary materials. A sterilized swap stick was used to aseptically absorbed the material (fecal and urine) and then dipped in peptone water for 24 h for non-selective enrichment. A total of 100 rectal swabs were collected from captured rat using sterile swab sticks in ten different locations within University of Abuja for a period of ten weeks. The rats were captured with local traps. All samples were appropriately labeled, placed in cool thermos and immediately transported to Veterinary Microbiology Laboratory, University of Abuja for laboratory analysis.

Isolation and identification of *Klebsiella*

This study was conducted according to the method adopted by Mailafia et al. (2003). Each swab sticks containing the samples were dipped in test tubes containing 10ml of prepared nutrient broth, and then incubated at 37^0C for 24 h. A loopful of the inoculum was streaked onto already prepared plates of Eosine methylene blue agar (Oxoid, UK) incubated at 37^0C for 24 h. *Klebsiella* species produces large, mucoid, pink to purple colonies with no metallic green sheen on EMB agar. The lactose fermented colonies were picked and sub-culture on nutrient agar plates, incubated at 37^OC for 24 h to obtained pure culture and subsequently inoculated on nutrient agar slant for further characterization.

Presumptive isolates were characterized microscopically using Gram staining and biochemically using catalase, oxidase, triple sugar iron, indole, citrate, methy red, voges proskauer and urea tests as described by Cheesbrough (2006).

Characterization of *Klebsiella* isolates using Microbact 24E Test kits

Using the commercial test kits described by the manufacturers, Microbact™ 24E (Oxoid, UK) isolates were fully biochemically characterized. These biochemical test reactions are included in the Microbact™ identification kits (Oxiod): oxidase, catalase, coagulase, Gram staining, H2S, glucose, mannitol, hylose, indole, urease, VP, citrate, gelatin, inositol, sorbitol, rhamnose, sucrose, lactose, arabinose, adonitol, raffinose, salicin, arginine and nitrate. The results obtained were interpreted to identify the isolates using the Microbact™ computer aided identification package (Oxiod) supplied along with the kits in combination with the Cowan and Steel's Manual for the Identification of Medical Bacteria (1974). A suspension of the overnight culture of the organism was emulsified in 5ml sterile saline solution and then adjusted to 0.5 McFarland turbidity standards (approximately equal to 1.5×10^8 CFU/ ml of the bacterial suspension).

The wells of the individual substrate were exposed by cutting the end tag of the sealing strip and slowly peeling it backward. The strip was placed in a holding tray, using a sterile Pasteur pipette, 4 drops (approximately 100µm) of the bacterial suspension was inoculated into each well and overlaid with sterile mineral oil. The inoculated rows were sealed with adhesive seal and the specimen identification number was written on the end tag with a marker pen, and then incubated at 37^0C for 18 to 24 h. Following incubation, the reactions were evaluated as positive or negative by comparing it with the color chart. Reactions involving different colours (yellow, red, tan, green, blue) as shown in Figure 1 were observed. The interpretation of the result was based on an octal coding system

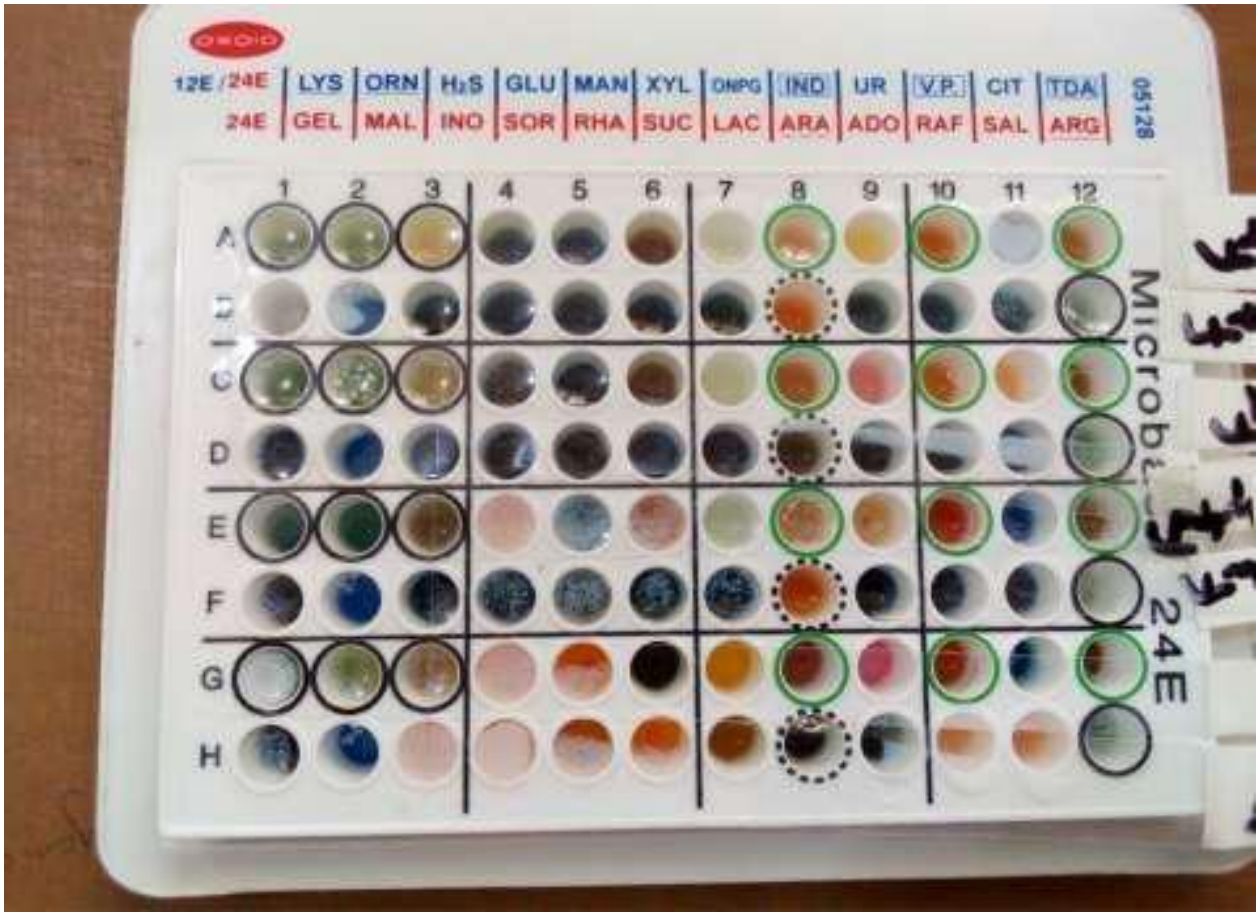

Figure 1. Pictorial presentation of Microbact^{TM} identifying the isolates to specie level.
Source: Authors

which was adopted for Microbact. Each group of 3 reactions produces a single digit of the code. Using the result obtained, the indices of the positive reactions were circled. The sum of these indices in each group of the three reactions formed shown against the organism name was the percentage share of the probability for that organism (Sinanjung et al., 2020).

Antibiotics susceptibility test

Antimicrobial susceptibility of *Klebsiella* isolates were tested using the disk diffusion method prescribed by Kirby- Bauer et al. (1966) and in accordance with the guidelines of the Clinical Laboratory Standard Institute (CLSI, 2018). The antibiotics used were cotrimoxazole (30µg), chloramphenicol (30µg), gentamycin (30µg), augmentin (10µg), amoxicillin (30µ), pefloxacin (30µg), streptomycin (30µg), ofloxacin (10µg), ciprofloxacin (30µg) and sparfloxacin (10µg) (Hi media, India). An overnight culture of each isolate was prepared in nutrient broth and incubated at 37 °C for 18 h. The turbidity of the broth was adjusted to McFarland standard of 0.5. The inoculum was then spread on already prepared plates of Mueller Hinton's agar (Oxoid, UK) and left standing for 1-2 min. Using forcep, antibiotics multi-discs (Hi media, India) were aseptically placed on the inoculated plates and then incubated at 37 °C for 24 h. After incubation, the zones of inhibition were measured to the nearest millimeter using a transparent ruler and the values were recorded and interpreted as sensitive, intermediate and resistant according to CLSI, 2018 guidelines.

Data analysis

Statistical Package for Social Sciences (SPSS version 2.6) was used for data analysis. Simple descriptive statistics such as frequency, percentages and tables were used to express the rate of occurrence of *Klebsiella* isolates.

RESULTS

Table 1 shows the biochemical characterization of 20 suspected isolated was carried out as exhibited by *K. pneumonia* and *K. oxytoca* which include Citrate Utilization test Triple Sugar Iron test, Urea test, Methyl Red test, Indole test, and Voges Proskauer test respectively. Table 2 shows that out of the 100 samples analyzed, 20 (20%) yielded growth for *Klebsiella*, 12% of the 20% *Klebsiella* isolates were identified as *K. Pneumonia* while 8% as *K. Oxytoca.* The prevalence and distribution of *Klebsiella* species isolates from rat in

Table 1. Results of Biochemical reactions of 20 suspected isolates of *Klebsiella pneumonia* and *Klebsiella oxytoca* from household Rats (*Rattus Rattus*) in University of Abuja, FCT.

Biochemical test	Number of tested organisms	Number of positive organisms	Number of negative organisms
GR	20	0	20
C	20	20	0
O	20	0	20
TSI	20	20	0
M	20	0	20
U	20	20	0
CI	20	20	0
I	20	8	12
MR	20	0	20
VP	20	20	0

GR – Gram reaction, C- Catalase, O- Oxidase, TSI- Triple Sugar Iron test, M- Motility test, I-Indole test, CI- Citrate test, U- Urease test, MR- Methyl red test, VP- Voges Proskauer
Source: Authors

different location within the University of Abuja Main campus with Faculties of Veterinary medicine and Agriculture showing the highest percentage prevalence while faculties of Health science and Management sciences shows the least percentage prevalence. The prevalence and distribution of confirmed *K. pneumonia* and *K. oxytoca* isolates from rats swab in the University of Abuja Main campus using Microbact 24E identification test kit as seen in Table 3. The microbact test identified the following biochemical test in percentage such as Lysine (100%), Malonate (100%) , Glucose (80%), Xylose (80%), Citrate (80%), and Arginine has the highest percentage that was positive in the five sample tested while Urease (60%), V-P (60%), Gelatin (60%), Lactose (60%), Arabinose (60%) and Raffinose have moderate percentage that was positive. Mannitol (40%), ONPG (40%), TDA (40%), Sorbitol (40%), and Rhamnose (40%) which are slightly moderate in percentage while Ornithine (20%), Indole (20%), and Adonitol (20%) have lower percentage and Salicin (0%) which shows the lowest percentage that was positive of the isolates.

Table 4 shows the antimicrobial susceptibility and resistance of ten antibiotics used in this study. The resistant antibiotics include; Chloramphenicol (CH 30µg), Gentamycin (CN 30µg), Augmentin (AU 10µg), Cotrimoxazole (SXT 30µg) and Amoxicillin (AM 30µg) while the susceptible antibiotics include; Pefloxacin (PEF 30µg), Streptomycin (S 30µg), Ofloxacin (OFX 10µg), Ciprofloxacin (CPX 10µg) and Sparfloxacin (SP 10µg).

Table 5 shows that from the ten antibiotics tested for susceptibility test to the *Klebsiellae* species, it was observed that four isolates dissipated the longest pattern of resistance to seven antibiotics (AM, AU, CH,CN,SP,S and SXT). The next eight isolates dissipated the second longest pattern of resistance to six antibiotics (AM, AU, CH, CN, S, and SXT).

Furthermore, the next two isolates each showed varying resistance patterns namely; AM, AU, CH, CN and SXT displaying five antibiotics, three antibiotics were for AU, CH and CN and one antibiotic was for CN respectively.

DISCUSSIONS

The result of this study conducted at the University of Abuja Campus, Airport road document the occurrence of *K. pneumonia* and *K. oxytoca* from household rats in the school environment. The observed morphological characteristics of the isolates showed typical *Klebsiella* species with circular, dome shaped, mucoid and greyish white colony on nutrient agar. While in EMB agar it gives circular, mucoid and pink purple colonies due glucose fermentation as previously documented by paramedics (2021). The mucoid nature of the colonies is due to the presence of capsular material produced by the organisms (Khaertynov et al., 2018). The overall prevalence of *Klebsiella* species was 20% which indicate the existence of *Klebsiella* species from fecal materials of captured house rats from the University of Abuja campus. This result is closely similar to the study carried out in Ilorin (Fadeyi et al., 2016) and Enugu (Ejikeugwu et al., 2013) all in Nigeria, where they recorded the prevalence of 26.7 and 26.0% respectively. This finding is also lower than the finding of Leangapichart et al. (2021) with a prevalence of 39% from pigs. The distribution of *Klebsiella* organisms in captured house rats shows *K. pneumonia* (12%) to be higher than *K. oxytoca* (8%).These differences could be due to environmental factors that exposes the animal to the organisms and differences in sample size based on the availability of captured house rat in those locations. The presence of *Klebsiella* species should be a source of concern to

Table 2. Prevalence and distribution of *Klebsiella pneumonia* and *Klebsiella oxytoca* by location.

Locations	Number of collected samples	Number of positive organisms	% *K. pneumonia*	Number of positive organisms	% *K. oxytoca*
Faculty of Veterinary medicine	15	2	16.7	1	12.5
Old boys hostel	10	1	8.3	1	12.5
New boys hostel	10	0	0	0	0
Old girls hostel	10	2	16.7	1	12.5
New girls hostel	10	0	0	0	0
Faculty of Agriculture	15	4	33.3	2	0
Faculty of Health Sciences	5	0	0	0	0
Faculty of management sciences	5	0	0	0	0
Faculty of Art	10	2	16.7	2	25
Faculty of Sciences	10	1	8.3	1	12.5
Total	100	12	100(12)	8	100(8)

Source: Authors

public health since rats serve as a reservoir and it accounts for a significant proportion of urinary tract infections, pneumonia, septicemias and soft tissue infections (Massinga et al., 2021).

In this study, the ratio of *K. pneumonia* to *K. oxytoca* was approximately 2:1. This agrees with Fideyi et al. (2016) who had stated that Extended Spectrum Beta Lactam producing *Klebsiella* are mainly caused by the two species *K. pneumonia* and *K. oxytoca* in the ratio of 3:1. This finding may be related to the pathogenicity in association with bacteriophages and hence medical importance of these of *Klebsiella* compared with others (Karumidze et al., 2012).

The prevalence of *Klebsiella* organisms shows Faculty of Agriculture and its environs had highest (33%) while new boys' hostel, new girls' hostel, Faculties of Health and Management sciences had no prevalence (0%). The highest frequency may be due to immunocompromised individual or host and differences in sample size. To a much lesser degree, *K. Oxytoca* has been isolated from rat specimens. Uzoamaka et al. (2017), in Nigeria who confirmed that *K. Pneumoniae* was among the most common causes of lower respiratory tract infections, neonatal septicemias and bacteremia in children. More importantly, *K. pneumonia* is the leading cause of mastitis, metritis and pneumonia in man (Marques et al., 2019). Due to their widespread presence and shared living space with humans and other animals, house rats have been highlighted in this study as important potential carriers of *Klebsiella* infection. Further biochemical test was done to characterize the isolate and differentiate it from other closely related organism and enteric bacteria.

This research also shows that the samples of rectal swab that tested positive for *Klebsiella* species were isolated from immune competent and immune compromised host. This study connotes with the report of Paterson et al. (2004) who stated that *Klebsiella* species are involved in various infections affecting immunocompetent and immunocompromised hosts with a wide spectrum involving urinary, respiratory and gastrointestinal tracts. In addition, they cause bacteremia, septicemia and various organs and soft tissue infections.

During the cause of the study, antimicrobial susceptibility test was performed following the disk diffusion method protocol M27 reference method of National Committee for Clinical Labouratory Standards (NCCLS) with slight modification. It was discovered that *Klebsiella* species are highly resistant to Cotrimoxazole, Chloramphenicol, Gentamycin, Augmentin and Amoxicillin. Massinga et al. (2021) also documented that *Klebsiella pneumonia* is resistant to Amoxicillin-clavulanic acid Gentamicin. They are susceptible to Pefloxacin, Streptomycin, Ofloxacin, Ciprofloxacin and Sparfloxacin. Generally speaking, some of the organisms isolated in this study are resistant to some commonly used antimicrobial drugs. Resistance observed in these studies could be plasmid

Table 3. Identification of the isolate using microbact 24E.

Biochemical test	Number of tested organisms	Number of positive organisms	Number of negative organisms	% of positive organisms	% of negative organisms
Lysine	20	20	0	100	0
Ornithine	20	4	16	20	80
H_2S	20	0	20	0	100
Glucose	20	16	4	80	20
Mannitol	20	14	6	40	60
Xylose	20	16	4	80	20
ONPG	20	6	14	40	60
Indole	20	4	16	20	80
Urease	20	14	6	60	40
V-P	20	14	6	60	40
Citrate	20	18	2	80	20
TDA	20	6	14	40	60
Gelatin	20	14	6	60	40
Malonate	20	20	0	100	0
Inositol	20	4	16	40	60
Sorbitol	20	4	16	40	60
Rhamnose	20	4	16	40	60
Sucrose	20	4	16	40	60
Lactose	20	16	4	60	40
Arabinose	20	16	4	60	40
Adonitol	20	2	18	20	80
Raffinose	20	16	4	60	40
Salicin	20	2	18	20	80
Arginine	20	18	2	80	20

Source: Authors

mediated by which resistance genes are transferred via transposons from one generation to another. Resistance could also be due to rampart use, misuse or abuse of these essential antimicrobial agents.

Since rats are disease reservoirs, humans should make sure that all food is securely covered or stored to stop rodents from contaminating the food. If correct management and hygienic conditions are not taken, *Klebsiella* infections will continue to rise. House rats consume our food products in their struggle to survive, including rice, garri, beverages, vegetables, etc. Because they carry pathogenic microorganisms that can infect humans negatively, proper precautions should be taken to reduce, if not completely eliminate, their interaction with environment. Rats are an important source of zoonotic infections that cause illness and mortality in humans. This is particularly problematic in the school environment with regard to rat-associated health risks because the

Table 4. Antimicrobial susceptibility and resistance of *Klebsiella* isolates commonly use Antimicrobial agents.

S/N	Antibiotics	Drug conc (µg)	Number of susceptible organisms (%)	Number of resistant organisms (%)
1	SXT	30	6(30)	14(70)
2	CH	30	4(20)	16(80)
3	SP	10	16 (80)	4(20)
4	CPX	30	20(100)	0(0)
5	AM	30	6(30)	14(70)
6	AU	10	4(20)	16(80)
7	CN	30	2(10)	18(90)
8	PEF	30	20(100)	0(0)
9	OFX	10	20(100)	0(0)
10	S	30	8(40)	12(60)

SXT- Cotrimoxazole , CH-Chloramphenicol , SP- Sparfloxacin , AM- Amoxacillin , AU-Augmentin , CN- Gentamycin , PEF- Pefloxacin , OFX- Ofloxacin , S- Sreptomycin, CPX-Ciprofloxacin.
Source: Authors

Table 5. Resistant pattern of *Klebsilla* Isolates.

Isolate number	Resistant pattern	Number of resistant antibiotic
1	AM, AU, CH, CN, SP, S, SXT	7
2	AM, AU, CH, CN, SP, S, SXT	7
3	AM, AU, CH, CN, SP, S, SXT	7
4	AM, AU, CH, CN, SP, S, SXT	7
5	AM, AU, CH, CN, S, SXT	6
6	AM, AU, CH, CN, S, SXT	6
7	AM, AU, CH, CN, S, SXT	6
8	AM, AU, CH, CN, S, SXT	6
9	AM, AU, CH, CN, S, SXT	6
10	AM, AU, CH, CN, S, SXT	6
11	AM, AU, CH, CN, S, SXT	6
12	AM, AU, CH, CN, S, SXT	6
13	AM, AU, CH, CN,SXT	5
14	AM, AU, CH, CN,SXT	5
15	AU, CH, CN	3
16	AU, CH, CN	3
17	CN	1
18	CN	1

AM-Amoxacillin, AU-Augumentin, CH-Chloramphenicol, CN-Gentamycin, SP-Sperfloxacin, S-Sreptomycin, SXT- Cotrimoxazole
Source: Authors

University community provides a suitable habitat for rats, leading to close contact between rat and people and potentially zoonotic disease transmission. Some species of house rat like *Mastomys natalensis* has been associated with dangerous viral zoonotic infection such as *Lassa* fever.

Klebsiella infection remains among those infectious diseases of potential serious threat to public health because they attack the host when the immune system is compromised (Ogbole et al., 2022).

Zhong et al. (2020) reported in a study conducted in China that capsular serotyping of *K. pneumonia* have shown to depict important hyper virulent serotypes (K1, K2, K5, K20 and K57) of the bacteria. The hyper virulent seroptypes are the overlapping serotypes observed in both animals and humans (Zhong et al., 2020).

Therefore, capsular serotyping or genotyping is further required to establish the particular serotypes isolated or phenotypically characterize in this study. Hence, molecular study is required to detect resistance genes in these isolates. Finally, this study might have shown zoonotic importance of *K. pneumonia* and *K. Oxytoca* further study on serotyping should be carried out which is one of the limitation of our study for proper characterization of the organism within the study area.

Conclusion

This study shows that *Klebsiella* species exist in rats in the main Campus of University of Abuja. Out of 100 samples analyzed 20(20%) were tested positive to *Klebsiella* and among them, some species were found to be *K*. *pneumoniae* and *K*. *oxytoca* and the percentage of the occurrence of *Klebsiella* specie found are 12(12%) for *K. pneumonia* and 8(8%) for *K. oxytoca*. This prevalence is a thing of concern to public health because rodent has been part of our environment daily. The increasing trend of antibiotic resistance should be of immense public health concern. Proper legislation is needed to control *Klebsiella* infection within the environment.

RECOMMENDATION

More research should be done on animals that live with rats such as giant African rat, mouse, guinea pigs, squirrels, hares and shrews because they are carriers of diseases using advance technology. More drugs should also be used for antimicrobial susceptibility testing because of their increasing growth and resistance of the organism like *Klebsiella* species. Studies should include other microorganisms found in rats in other geographic location. There is need for serious public health awareness of this bacterial infection and its zoonotic signs.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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