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Research Article

Antimicrobial susceptibility patterns and molecular phylogenetics of *Proteus mirabilis* isolated from domestic rats: An environmental driver to antimicrobial resistance in public health in Arusha Tanzania

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Abstract

Proteus mirabilis (*P. mirabilis*) is a bacterial pathogen contributing to opportunistic infections, nosocomial outbreaks, and mostly hematogenous ascending urinary tract infections. It has repeatedly been found in rats. Due to rat-human interaction, rats are likely responsible for spreading these bacteria and their antimicrobial-resistant. This study was performed to genetically characterize and assess the antimicrobial susceptibility patterns of *P. mirabilis* isolated from rats cohabiting with humans in Arusha municipality, Tanzania. A total of 139 rats were trapped from March to May 2021 and identified at the species level using morphological and morphometric features. Deep-intestinal swabs were obtained and pre-enriched in buffered peptone water. *P. mirabilis* was isolated by conventional culture and biochemical methods and confirmed by 16S rRNA polymerase chain reaction and sequencing. Phylogenetics was used to assess the similarities of the isolates. Antimicrobial susceptibility test was done by disk diffusion method using seven antibiotics, including tetracycline, ciprofloxacin, gentamicin, cefotaxime, trimethoprim-sulfamethoxazole, azithromycin, and ampicillin. Resistance genes bla_{TEM}, *tet*A, *tet*B, *mph*A, bla_{SHV}, $bla_{\text{CTX-M}}$, *sul1*, and *sul2* were traced in each isolate using PCR. Mixed rat species, *Rattus rattus* (55.4%), *Mus musculus* (15.8%), and *Mastomys natalensis* (28.8%), were captured. *P. mirabilis* was isolated from four (2.9%) *Rattus rattus* samples. By PCR and sequencing, all were confirmed as *P. mirabilis* and 100% similar to strains from GenBank. Three isolates showed multidrug resistance (MDR) against trimethoprim-sulfamethoxazole, azithromycin, and ampicillin, while all isolates were resistant to azithromycin and ampicillin, and susceptible to ciprofloxacin, gentamicin, and cefotaxime. Three were resistance to trimethoprim-sulfamethoxazole and intermediate to tetracycline. PCR analysis detected *tet*A, bla_{TEM}, *sul1*, and *sul2* resistance genes. Constructed phylogenetic tree showed that all isolates from this study were closely related to isolates from Tunisia. The study has discovered the first *P. mirabilis* isolates from rats in Tanzania with antimicrobial resistance traits that could be of public health concern.

Keywords: *P. mirabilis*, Rats, Antimicrobial resistance, Resistance genes, 16S rRNA

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Introduction

Proteus mirabilis (*P. mirabilis*) is a Gram-negative, facultatively anaerobic, and zoonotic bacterial pathogen of major medical and veterinary importance (Jemilehin et al., 2016). It contributes largely to opportunistic infections, nosocomial outbreaks, and mostly hematogenous and ascending urinary tract infections in humans (Nagano et al., 2003; Hamilton et al., 2018;

Mirzaei et al., 2019). *P. mirabilis* is reported as the third most prevalent etiological agent after *Escherichia coli* and *Klebsiella pneumoniae* in cumbersome urinary tract infections (UTI) and the second after *Providencia stuartii* in catheter-associated bacteriuria in the group of long-term catheterized patients (Warren, 1996; Mirzaei et al., 2019). Other complications include diarrheal diseases, bacteremia, Crohn's disease, respiratory infections, neonatal meningoencephalitis, rheumatoid arthritis, and wound sepsis (O'Hara et al., 2000; Zhang et al., 2021).

P. mirabilis-associated complications are normally treated with commercial drugs. However, antimicrobial resistance (AMR) has emerged as one of the most serious public health concerns of the 21st century (Murray et al., 2022). According to the UK Government-commissioned Review on Antimicrobial Resistance, AMR could kill 10 million people annually by 2050 (O'Neill, 2016). The WHO, FAO and WOAH, and several other researchers agree that the spread of AMR is an urgent issue that requires a multisectoral and globally coordinated action plan to address (Prestinaci et al., 2015; WHO, FAO, OIE, UNEP, 2022).

The emergence and widespread of multidrug-resistant (MDR) *P. mirabilis* have been reported (Girlich et al., 2020; Zhang et al., 2021). The detection of *Salmonella* genome island one variant SGI1-0, extended-spectrum Beta-lactamase (ESBL), *Amp*C-type cephalosporinase, and carbapenemases producers, which are responsible for MDR in *P. mirabilis* indicates that this trait can be transferred to other bacteria of public health importance (Doublet et al., 2010; Girlich et al., 2020). This is due to the bacterial acquisition of plasmids, integrons, insertion sequences, and transposons mediated by AMR traits in their cells, facilitating horizontal gene transfer of resistant plasmids (Doublet et al., 2010; D'Andrea et al., 2011; Iredell et al., 2016).

P. mirabilis can be isolated from a wide range of environments like soil, polluted water sources, sewage systems, humans, poultry, meat, banknotes, bats, and rodents (Mukhtar et al., 2018; Ayyal et al., 2019; Lakshmi et al., 2020). Due to its ecological diversity, *P. mirabilis*-resistant infections can spread across different hosts sharing the same environment and increase public health concerns.

Efforts to combat AMR currently rely on reducing the use of antimicrobials in humans and domestic animals (Katakweba et al., 2012a,b; Jemilehin et al., 2016). Despite its efficiency, this may not be effective as AMR carriage has been reported in populations of rats and other wild animals and birds which share the same ecosystem with humans due to the passage of resistant organisms among humans, food and petty animals, fish, and birds (Wakawa and Mohammed, 2015; Gaspary et al., 2016; Kimera et al., 2020; Islam et al., 2021). Carriage of bacterial pathogens in rodents has been widely reported worldwide (Ayyal et al., 2019; Sonola et al., 2021a, 2022), where the most documented species include; *Enterobacteriaceae* (*Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., and *Proteus* spp.) and *Staphylococcaceae* mainly *Staphylococcus aureus* (Osman et al., 2014; Jemilehin et al., 2016; Ogunleye and Carlson, 2016; Ayyal et al., 2019; Sonola et al., 2021a, 2022). This highlights the public health importance of rats in transmitting clinical and veterinary important bacteria like *P. mirabilis* to other organisms. Therefore, screening rats in close association with human homes for their carriage of resistant *P. mirabilis* could help determine their role in transmitting diseases pathogens and AMR traits in humans and animals.

Despite the confirming reports on the involvement of rats as a reservoir of *P. mirabilis* (Ayyal et al., 2019; Ogunleye and Carlson, 2016), little is known about the epidemiological role played by rats as carriers and transmitters of resistant *P. mirabilis* infections. Following the interaction of rats, humans, and food animals in homes, the current study focused on characterizing the antimicrobial susceptibility patterns of *P. mirabilis* isolated from in-house and peridomestic rats in Arusha city, in northern Tanzania. Rats were screened for carriage of resistant *P. mirabilis* to explore the transmission dynamics of the disease among humans and animals under one ecosystem.

Materials and methods

Study area

The study was conducted in Arusha municipality in northern Tanzania. The area has 19 wards composed of urban, peri-urban, and rural areas with a population size of 617,631 (NBS, 2022). This area is surrounded by national parks and game reserves (Ngorongoro Conservation Area, Serengeti National Park, Lake Manyara, Tarangire National Park, and Arusha National Park), indicating the human-animal-wildlife interaction potential facilitating the sharing of bacterial infections. Eight wards were purposively selected for sampling based on high population density and people's complaints about rodent infestations. Sampling was conducted at Unga LTD, Kwawakereketwa, Mita 200, Kilombero, Ngaramtoni, Olasiti, Seliani, and Majengo-Elerai. These study wards were recorded and mapped using a global positioning system (GPS) (Figure 1).

Sampling strategy

A cross-sectional study design was employed. Rats were collected from March to May 2021 by live trapping method using Sherman traps 8×9×23 cm in peridomestic areas and modified wire traps 12×15×20 cm in houses, food stores, and shops (Anthony et al., 2005; Ralaizafisoloarivony et al., 2014). The sample size of rats was estimated based on the prevalence of 10% reported by Ogunleye and Carlson (2016) using the equation described by Hilton et al. (2002);

$$n = Z2P(1 - P)/d2$$

Where: n= sample size, P= prevalence from a reported study= 10%, Z = standard normal deviation at 95% confidence interval =1.96% and D= absolute desired precision at 5% =0.05.

S/N	Wards	# Rats captured
1	Ngaramtoni	57
2	Olasiti	9
3	Majengo-Elerai	18
4	Unga LTD	20
5	Seliani	11
6	Kwawakereketwa	14
7	Mita 200	2
8	Kilombero	8
TOTAL		139

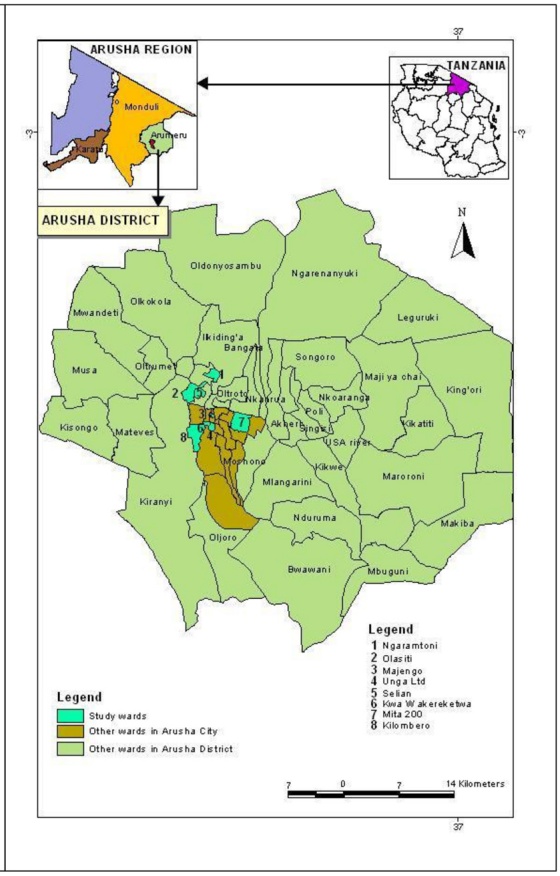

Figure 1: Map of Arusha municipality showing sampling wards (right) and the number of rats captured from each ward (left). Source: Arc GIS 10.3 version (2015).

Table 1: Antimicrobial agent resistant genes, oligonucleotide sequences and annealing temperature used for PCR amplification.

Antimicrobial agent	Gene	Oligonucleotide sequence (5'-3')	Size (BP)	Annealing temperature (°C)	Reference
Cephalosporins	*bla*TEM	F-ATG AGT ATT CAA CAT TTC CG R-CCA ATG CTT AAT CAG TGA GG	858	50	Gharrah et al. (2017)
	*bla*SHV	F-ATG CGT TAT ATT CGC CTG TG R-AGC GTT GCC AGT GCT CGA TC	862	58	
	Universal *bla*CTX-M	F-SCS ATG TGC AGY ACC AGT AA R-CCG CRA TAT GRT TGG TGG TG	554	58	
Cefotaxime	*mph*A	F-GTG AGG AGG AGC TTC GCGA G R-TGC CGC AGG ACT CGG AGG TC	403	60	Kim et al. (2004)
Ciprofloxacin	*acr*A	F-CTC TCA GGC AGC TTA GCC CTA A R-TGC AGA GGT TCA GTT TTG ACT GTT	107	60	
Gentamicin	*aac* (3)-I	F-ACC TAC TCC CAA CAT CAG CC R-ATA TAG ATC TCA CTA CGC GC	169	60	
Tetracycline	*tet*A	F-GGT TCA CTC GAA CGA CGT CA R-CTG TCC GAC AAG TTGC ATG A	577	57	Kerrn et al. (2002)
	*tet*B	F-CCT CAG CTT CTC AAC GCG TG R-GCA CCT TGC TGA TGA CTC TT	634	56	
Sulfonamides	*sul1*	F-CGG CGT GGG CTA CCT GAA CG R-GCC GAT CGC GTG AAG TTC CG	433	69	
	sul2	F-GCG CTC AAG GCA GAT GGC ATT R-GCG TTT GAT ACC GGC ACC CGT	293	69	
Universal primer	16S rDNA	27F-AGA GTT TGA TCA TGG CTC AG 1492R-TAC GGY TAC CTT GTT ACG ACT T	1500	58	Lakshmi et al. (2020)

Trapping and processing of rodents and bacterial sample collection and transportation

Live rats were trapped by using Sherman traps 8×9×23 cm (H.B. Sherman Traps Inc., Tallahassee, USA) in peri-domestic areas and modified wire traps 12×15×20 in houses, food stores, and shops (Anthony et al., 2005; Ralaizafisoloarivony et al., 2014). Peanut butter mixed with maize bran was used to bait the traps (Ralaizafisoloarivony et al., 2014). Traps were set in the evening for five consecutive nights and were checked every morning to capture rats to reduce the heat stress experienced by the mammals. Trapped animals were anesthetized with cotton wool soaked in the diethyl ether before the pre-moistened rectal swabs were collected using sterile microbiological swabs (IMPROSWAB®). This was followed by collecting deep intestinal swabs placed into bijou bottles containing sterile transport media (buffered peptone water) and preserved at -20°C pending further laboratory analyses. Animals were cut open, labeled, and fixed in 70% ethanol. Morphometric and anatomical parameters such as body weight, head-body length, tail length, hindfoot length, and ear length were used in genus/species identification together with rodent identification keys (Skinner and Chimimba, 2005). Sex identification was done using morphological features like urogenital distance (distance between the genital papilla area and the anus) to determine the sexual activeness of the rodents (Cunningham and Moors, 1983). Age classification as to whether young, juvenile, or adult, morphometric anatomical parameters such as body weight, body size, and reproductive activeness were used to judge the classification (Klevezal, 2007).

Bacterial isolation, identification, and biochemical characterization

A swab from thawed sampled transport media was inoculated into 10 mL of selective enrichment broth, Muller Hinton broth MH (Oxoid Ltd., Detroit, Michigan, USA), and incubated overnight at 35±2°C. Aseptically loopful of MH culture was streaked onto MacConkey and Blood agar (Oxoid Ltd., Detroit, Michigan, USA). The latter had 15% rabbit blood and was incubated aerobically at 35°C±2 for 24hrs, and differential subculture was done on Xylose Lysine Deoxycholate (XLD) agar media (Oxoid Ltd., Detroit, Michigan, USA). Gram staining was performed for presumptive colonies. The Gram-negative bacilli colonies with wavy concentric circles (swarming pattern) on blood agar, colorless, smooth non-lactose fermenting colonies on MacConkey agar, and yellow colonies with dark centers (H_2S production) on XLD media were subcultured on nutrient agar for further tests as elsewhere (Pearson, 2019). Biochemical tests such as urease, indole, catalase, triple sugar iron test, motility, and oxidase tests were carried out to confirm the identification of isolates. Lastly, the isolates were preserved in nutrient broth with 15% glycerol at -80°C for further analysis.

Antimicrobial susceptibility testing

Kirby–Bauer disc diffusion method on Mueller–Hinton agar was used to determine the resistance patterns of seven antimicrobial agents commonly used in the study area, namely; tetracycline, ciprofloxacin, gentamicin, cefotaxime, trimethoprim-sulfamethoxazole, azithromycin, and ampicillin. This was performed according to the Clinical and Laboratory Standards Institute guidelines of 2021 (CLSI, 2021). The panel of antimicrobials comprised agents commonly used in treating humans and animals; each drug represented a specific class. The panel included; tetracycline (TE, 5 μg), trimethoprim-sulfamethoxazole (SXT, 25 μg) (folate pathway inhibitors), ciprofloxacin (CIP, 5 μg) (Quinolones), cefotaxime (CTX, 5 μg) (cephalosporins), ampicillin (AMP, 10 μg) (Penicillins), azithromycin (AZM, 15 μg) (Macrolides), gentamycin (CN, 10 μg) (Aminoglycosides) (Liofilchem®, Italy). *Escherichia coli* ATCC 25922 strain was used for quality control for its recognized control strain for international susceptibility testing.

Bacterial DNA extraction

Bacterial colonies were obtained from an overnight culture on nutrient agar at 35±2°C. Genomic Deoxyribonucleic Acid (gDNA) (400 μl) was extracted using the Quick-gDNA miniprep extraction kit (Zymo Research, USA) protocol as per the manufacturer's instructions. The supernatant of gDNA was transferred into a clean Eppendorf tube and stored at -20°C for further use. NanoDrop ND-1000 system (NanoDrop Technologies, Inc., Wilmington, DE, USA) was used at spectrophotometer wavelength 260nm (A260/A280) to evaluate the quantity and quality of isolated gDNA. A ratio between 1.8 and 2 indicated a high-quality gDNA.

Polymerase chain reaction (PCR), sequencing and sequence analysis

Primer sequences of 16S rRNA, selected genes from each group of antimicrobials tested, gene name, expected band size of the resistant gene, and primer annealing temperature are listed in Table 1. The optimized PCR was conducted using a total reaction volume of 25 μl containing 12.5 μl of Quick load Taq 2× master mix, 0.5 μl of reverse and forward primers, 6.5 μl of nuclease-free water, and 3 μl of DNA template. The PCR amplification conditions were; 95°C initial denaturation for 5min, followed by 35 cycles, each 94°C denaturation for 30 secs, extension for 72°C for 2min and final extension for 10 min. Uniplex and multiplex PCR to detect 16S rRNA and resistance genes were all conducted with a total reaction volume of 20 μl with *AccuPower*® PCR Premix. The experimental protocol involved 0.5 μl of reverse and forward primers (one pair of primers for uniplex for the genes 16S rRNA, bla_{TEM}, *tet*A, *tet*B, *mph*A, and four pairs of multiplex primers for bla_{SHV}, $bla_{\text{CTX-M}}$, *sul1* and *sul2*), nuclease-free water and 3 μl of DNA template. PCR amplification protocol involved 95°C for 5min of initial denaturation, followed by 35 cycles, each with

Table 2: Frequency and proportions of species, gender, and site of collection of various rodents trapped in Arusha Municipality and *P. mirabilis* prevalence.

Variables		Number of rats	Proportion (%)	Number of rats in a group with *P. mirabilis*	*P. mirabilis* isolation frequency per group (%)
Species	*Rattus rattus*	77	55.4	4	5.2
	Mus musculus	22	15.8	0	0
	Mastomys species	40	28.8	0	0
Gender	Male	76	54.7	2	2.6
	Female	63	45.3	2	3.2
Trap site	Houses	95	68.3	4	4.2
	Peri-domestic	44	31.7	0	0
Total		139	100	4	2.9

94°C for 30 secs of denaturation and annealing temperature 30 sec. This was followed by an extension at 72°C for 2min and a final extension at 72°C for 10 min. Nuclease-free water was added in PCR tubes as a negative control, and GeneAmp® PCR system 9700 (Applied Biosystems, USA) was used as a thermocycler to perform the thermal profiles. PCR products were then separated using an agarose gel with 1.5% Tris EDTA (TBE) buffer with 5μl of PCR product and ladder at 120 Volts for 45 minutes and visualized using gel red staining in an ultraviolet trans-illuminator. This was performed using a gel imaging and documentation system according to the manufacturer's instructions (EZ GelDoc, Bio-Rad, and USA). The post-PCR fragments were purified, cycle-sequenced, and then sequenced by the Sanger sequencing technique at Macrogen (Seoul, Korea).

Outputs of Sanger sequencing were viewed, edited, and trimmed using Sequence Scanner software 2.0 and BioEdit software 7.2 (Hall, 1999). Consensus sequences obtained were compared in the GenBank using Basic Local Alignment Search Tool (BLASTn) on a non-redundant database, whereas a comparison was made between sequences obtained from this study with sequences available at the GenBank database (NCBI). For alignment creation, ten 16S rRNA gene sequences from GenBank with four 16S rRNA genes of *P. mirabilis* isolates from this study were aligned pairwise and multiple-wise using ClustalW (Chenna et al., 2003) in MEGA X software as shown in supplementary figure 1 Figure S1. MEGA X software was used to create a phylogenetic tree using the Maximum Likelihood model (Tamura et al., 2007; Hall, 2013). The phylogeny test to analyze the support strength for each clade was performed using bootstrap confidence analysis at 1000 replications (Hall, 2013).

Statistical analyses

Descriptive statistics were used to get the proportions of rat species, sex, age, and physiological status of the rodents.

Results

Rodents trapped in peri-urban and urban wards in Arusha municipality

A total of 139 rats were captured comprised of three species, namely, *Rattus rattus* (55.4%), *Mus musculus* (15.8%), and *Mastomys natalensis* (28.8%) (Table 2). Among them, 54.7% were females, whereas 45.3% were males.

Isolation of *P. mirabilis*

P. mirabilis was isolated in four (2.9%), out of 139 rat samples. The isolates were collected from both male (50%) and female (50%) rats, three isolates from Ngaramtoni, and one isolate from the Kilombero ward, and all isolates were recovered from *Rattus rattus* species (Table 2). The Gram staining test revealed Gram-negative bacilli colonies which on blood agar had a swarming pattern, colorless with smooth appearance colonies. The colonies were non-lactose fermenting on MacConkey agar and yellow with dark centers (H_2S production) on XLD media, depicting positive culture tests for *Proteus* spp. (Figure 2)

Biochemical properties of *P. mirabilis*

All performed seven biochemical tests, and all four culture-positive samples gave similar results, showing a positive test for *P. mirabilis* (Table 3).

Antimicrobial susceptibility patterns of *P. mirabilis* isolates

All three strains obtained from Ngaramtoni strains (2, 3, and 4) portrayed multidrug resistance (MDR) against trimethoprim-sulfamethoxazole, azithromycin, and ampicillin. All isolates were resistant to azithromycin and ampicillin but susceptible to ciprofloxacin, gentamicin, and cefotaxime, three were resistant to trimethoprim-sulfamethoxazole, and three were intermediate to tetracycline as shown in Table 4 and Figure 3.

PCR results of 16S rRNA gene and resistance genes

All four isolates exhibited 16S rRNA gene with a band size of 1500bp, which is *P. mirabilis*. All tested isolates amplified *tet*A, bla_{TEM}, *sul1*, and *sul2* resistance genes with a prevalence of 100% (Figure 4). Isolate KR64RR (Strain 1) was phenotypically sensitive to tetracycline and sulfamethoxazole-trimethoprim. However, it, respectively, carried *tet*A, as well as *sul1* and *sul2* resistance genes in its genome (Figure 4), and the remaining isolates were intrinsically resistant to tetracycline and trimethoprim-sulfamethoxazole.

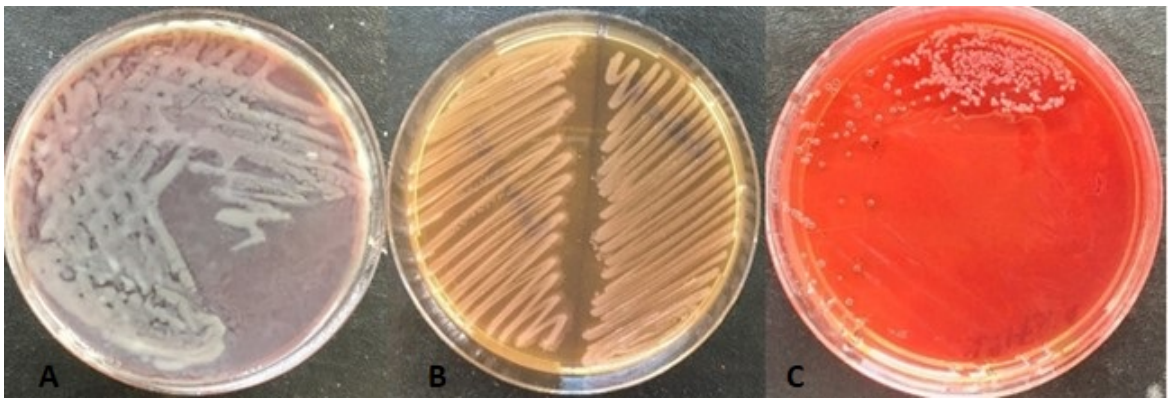

Figure 2: Colony morphology of *Proteus* spp. on XLD (A), MacConkey (B), and blood agar (C).

Table 3: Biochemical properties of *P. mirabilis*.

Serial No.	Biochemical test	Property
1	Catalase	+
2	Gram staining	-
3	H_2S production on TSI	+
4	Indole	-
5	Motility	+
6	Oxidase	-
7	Urease	+

Sequencing, alignment, and phylogenetic tree results

The sequences of the obtained four isolates in the current study were similar to those of *P. mirabilis* in the database with a 100% query cover. This was also depicted in pairwise and multiple alignments of the four isolated sequences with the other ten from GenBank, which was done in MEGA X software using ClustalW, as shown in supplementary figure 1 (Figure S1). Corresponding to the evolutionary tree (Figure 5), all Tanzanian isolates had a similar evolutionary relationship with isolates from Tunisia and Venezuela. They are also closely related to isolates from Iraq and Pakistan, Italy, and the United Kingdom and far different from isolates from the USA, India, and Sudan.

Discussion

Human-animal interaction is associated with a worldwide spread of infectious pathogens and MDR strains among humans and animals, particularly antimicrobial-resistant *P. mirabilis*. Due to rodent-human interactions, especially in the study area, this study aimed to genetically characterize and assess antimicrobial susceptibility patterns of *P. mirabilis* isolated from rats cohabiting/interacting with Humans. To the best of our knowledge, this is the first study in Tanzania to screen and genetically characterize antimicrobial-resistant *P. mirabilis* in rats. From this study, out of 139 rats, four rats, specifically *Rattus rattus*, were found to harbor *P. mirabilis*.

The isolates were tested using conventional biochemical methods and PCR; after that, all isolates were sequenced and, with the help of BLAST and multiple sequence alignment of sequenced isolates, all isolates were 100% similar to *P. mirabilis* in the GenBank. Using 16S rRNA partial sequences, the evolutionary tree was constructed and showed that all isolates from the current study were almost similar as they clustered together. This could be due to the presence of conserved and variable regions in their genomes (Mukhtar et al., 2018) and also due to geographical and ecological similarities, as all isolate was obtained from rats of the same species and the same environment (Kishony and Leibler, 2003; Ferenci, 2019). From the phylogenetic tree constructed, these isolates were closely related to isolates from Tunisia (Figure 5). Our findings showed a prevalence of 2.9% (4/139) for *P. mirabilis*, all of which were isolated from *Rattus rattus*. These findings are consistent with the results from Iraq and Nigeria, which reported a prevalence of 2-3% (Jemilehin et al., 2016; Ogunleye and Carlson, 2016; Ayyal et al., 2019). However, our findings were lower than 22.2% from Sudan (Mukhtar et al., 2018), which indicates that the prevalence of *P. mirabilis* may vary from country to country, which could be attributed to the differences in study design, geographic location, differences in transportation media used, temperature, and humidity differences that could affect bacterial growth and multiplication. The presence of *P. mirabilis* in rats, especially *Rattus rattus*, which are house rats, is an indicator of the role of rats in the transmission of

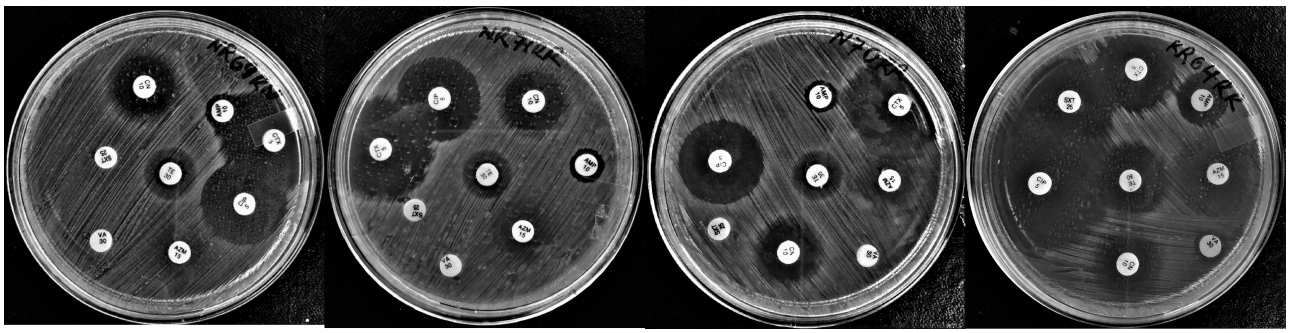

Figure 3: Muller Hinton Agar plates showing the drug resistance patterns of the four isolates of *P. mirabilis* from the study: From left to right is NR69RR (Strain 2 from Ngaramtoni), NR71RR (Strain 4 from Ngaramtoni), NR70RR (Strain 3 from Ngaramtoni), KR64RR (Strain 1 from Kilombero). TET= tetracycline; SXT= trimethoprim-sulfamethoxazole; CIP= ciprofloxacin; CTX= cefotaxime; AMP= ampicillin; AZM= azithromycin; GN= gentamicin.

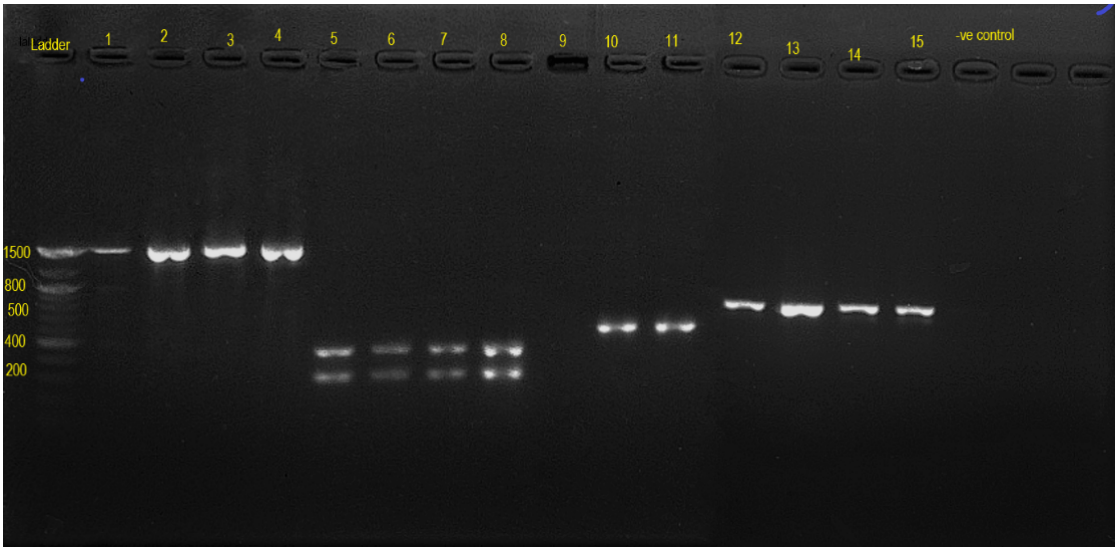

Figure 4: Gel picture of PCR amplification of 16S rRNA and detection of resistance genes in *P. mirabilis*. Lanes 1-4 represent 16S rRNA at an expected size of 1500bp. Lanes 5-15 show the band sizes of resistant genes; lanes 5-8 are for *sul1* and *sul2* of the expected size of 433 and 293, respectively; lanes 10-11 contains *tet*A of the expected size of 577bp; lane 12-15 holds bla_{TEM} of expected band size of 858bp and lastly the negative control.

the bacteria between humans and animals, and therefore pose a risk of transmission of this bacteria and their resistant strains to other animals in the ecosystem, human beings and the environment. In regard to this reality, it is crucial, therefore, to include rodent control in integrated zoonotic disease management.

To date, the rampant crisis of antimicrobial response, especially MDR among many genera of the family *Enterobacteriaceae*, indicates a major public health risk (Mirzaei et al., 2019). Past studies reported that isolates of *P. mirabilis* were sensitive to most antimicrobial classes, opposing recent findings from different countries across the globe and Tanzania in particular, reporting increased resistance to *P. mirabilis* (Mnyambwa et al., 2021). The current study showed that all *P. mirabilis* isolates were 75% resistant to tetracycline and sulfamethoxazole. This varies from results in Ibadan, Nigeria, which reported 100% resistance to tetracycline for *P. mirabilis* isolates from rats (Ogunleye and Carlson, 2016). Tetracyclines and sulfamethoxazole-trimethoprim antimicrobials have been frequently used in animals (Katakweba et al., 2012a). This could be the reason behind the development of resistance against them, as reported in the current study.

Furthermore, this study reveals 100% resistance to azithromycin and ampicillin. Ampicillin resistance in our study concurs with reports from Tanzania, Iran, Nigeria, and Sudan which have reported similar patterns of resistance to ampicillin in clinical rats and currency isolates (Ogunleye and Carlson, 2016; Mukhtar et al., 2018; Mirzaei et al., 2019; Mnyambwa et al., 2021). The 100% resistance found in azithromycin in this study matches with results from India and Babylon (Al-khafaji et al., 2013). The observed high resistance to these antimicrobials may be contributed to the frequent use and misuse of antimicrobials during the treatment and prevention of both livestock and human diseases (Kissinga et al., 2018); this is because most of the antimicrobials are readily available, cheap and ob-

Table 4: Antimicrobial resistance patterns of *P. mirabilis* isolates from domestic rats in Arusha Municipality.

Strain ID	Antimicrobial class*						
	Tetracycline	Folate pathway inhibitors	Quinolones	Cephalosporins	Penicillins	Macrolides	Aminoglycosides
	TET	SXT	CIP	CTX	AMP	AZM	GN
1	S	S	S	S	R	R	S
2	I	R	S	S	R	R	S
3	I	R	S	S	R	R	S
4	I	R	S	S	R	R	S

*TET= tetracycline; SXT= trimethoprim- sulfamethoxazole; CIP= ciprofloxacin; CTX= cefotaxime; AMP= ampicillin; AZM= azithromycin; GN= gentamicin; R= resistant; I= intermediate; S= susceptible.

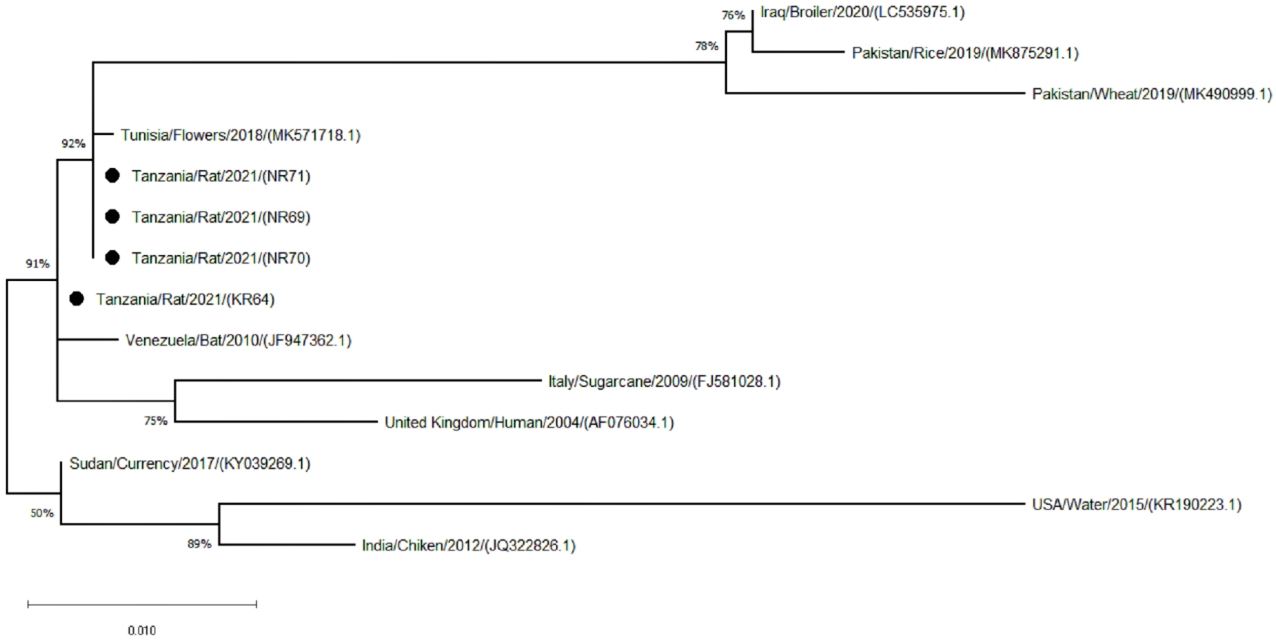

Figure 5: Phylogenetic tree of *P. mirabilis* from this study. The sequences indicate the country, source, isolation year, and accession number. The phylogeny was inferred with 1000 bootstrap support values (numbers with %). Bullets indicate the study isolates.

tained over-the-counter without a description (Katakweba et al., 2018). Moreover, Sonola et al. (2021b) suggested that the human-rats-environment interphase facilitates the crossover of resistance genes among different pathogens from the hosts. *P. mirabilis* isolates were more susceptible to cephalosporins, quinolones, and aminoglycosides (cefotaxime, ciprofloxacin, and gentamicin), respectively, with 100% activity. These findings concur with several studies on rats and poultry isolates (Ogunleye and Carlson, 2016; Mirzaei et al., 2019; Owoseni et al., 2021). This indicates that these antimicrobial groups are the remaining potential drugs that should be wisely used to prevent resistance development against them. This finding indicates that the presence of *P. mirabilis* among rats trapped in houses and around peri-domestic areas may be due to environmental interactions and commonalities in the food chain, which further contributes to the spread of antimicrobial resistance traits among various species sharing the ecosystem with humans (Hamilton et al., 2018). This affects small mammals that share the environment with humans and animals, as the Arusha region is the potential to keep livestock like cattle, poultry, sheep, goats, and pigs (Katakweba et al., 2018).

The emanation and expansion role of MDR *P. mirabilis*, particularly those producing ESBLs, is a setback in the medical and veterinary fields. To our understanding, this is the first report on *P. mirabilis* isolates from rats in Tanzania. In this study, isolates from rats showed intrinsic acquisition of resistance genes; *tet*A, *sul1*, *sul2*, and bla_{TEM}. Detection of bla_{TEM}, narrow spectrum B- lactamase gene that accounts for resistance to narrow-spectrum cephalosporins and penicillin, explains why all isolates showed phenotypic resistance to ampicillin. The results are similar to a study in Japan (Ahmed et al., 2007), from zoo animal isolates. Occurrence of *tet*A, *sul1*, and *sul2* in all isolates resistant and susceptible to tetracyclines and sulfamethoxazole/trimethoprim suggests that some antimicrobial agents in bacteria are "silent" and agreeing to a report in India (Deekshit et al., 2012). This indicates a threat as silent genes can be expressed *in-vivo* under antimicrobial utilization pressure and transferred to other intestinal and environmental microflora

(Wang et al., 2011). In Tanzania, these findings can be supported through a study by Katakweba et al. (2018); Sonola et al. (2021a) and Sonola et al. (2022) who revealed the *tet* and *sul* genes from fecal samples collected from livestock and intestinal contents from rodents.

This study has some limitations; rodent trapping was conducted on five consecutive nights; therefore, lacking seasonality and a small sample size has been used. Thus, we urge that the study should be extended to include the dry and wet seasons. Accounting for seasonality enables more in-depth studies to understand how much if any, season contributes to bacterial prevalence in rodents. Despite this limitation, the study gives useful insight into how rodents act as a reservoir for bacterial-resistant strains and their risk of transmission to other organisms.

Conclusions

The presence of MDR *P. mirabilis* isolates in house rats implicate the possibility of widespread transmission of resistance genes and bacteria in the studied area, with the possibility of causing infections that are difficult to treat. The antimicrobials used in this study are the ones that are commonly used in the area for treating both human and animal infections, implying that they have limited success in their intended use. Moreover, dissemination of MDR *P. mirabilis* from rat fecal and urine droppings could result in environmental contamination that can lead to the acquisition of the trait by other pathogenic bacteria like *Salmonella* and other unintended bacteria and hence a serious public health concern.

Therefore, comprehensive interventions using a one-health approach would be required to control the situation. Such measures should include improving awareness of the community on the proper use of antimicrobial drugs in humans and animals, good house conditions and hygienic waste management, integrated rodent control measures, and improving treatment regimens for humans, and other veterinary animals by microbial susceptibility testing before drug prescription.

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Authors contributions. FPN, AM, AASK: study conceptualization and design, data collection, analysis and interpretation of data; FPN, MEB: preparation of manuscript draft; AM, AASK: supervision; All authors reviewed the results and approved the final version of the manuscript.

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Supplementary Data

Ndakidemi, F. P., Baravuga, M. E., Mzula, A. and Katakweba, A. S. Antimicrobial susceptibility patterns and molecular phylogenetics of *Proteus mirabilis* isolated from domestic rats: an environmental driver to antimicrobial resistance in public health in Arusha Tanzania. 3(1): 13-23.

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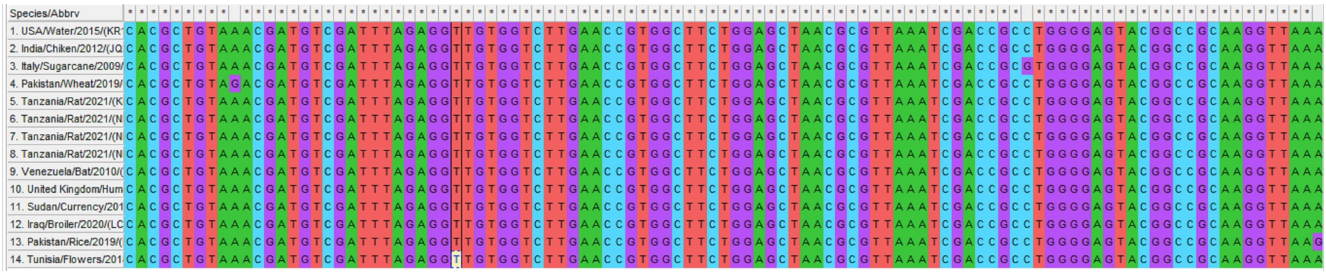

Figure S1: A multiple alignment mold of nucleotides. Stars at the top resemble reserved regions in each column. The first column on the left side shows the list of sample identities from the study and GenBank.