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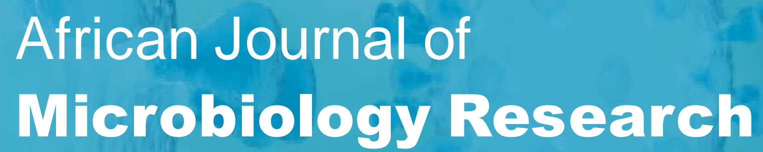

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African Journal of Microbiology Research

Full Length Research Paper

Prevalence of *Bartonella* spp. in rodent and shrew species trapped in Kigoma and Morogoro Regions, Tanzania: A public health concern

Ginethon G. Mhamphi[1,2]*, Abdul S. Katakweba[2], Apia W. Massawe[2], Rhodes H. Makundi[2], Robert S. Machang'u[3], Erick V. G. Komba[4] and Ladslaus L. Mnyone[2,5]

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Bartonella **spp. bacteria are responsible for bartonellosis in humans and animals for which rodents are the main natural reservoirs. Common bartonellosis symptoms include fever, chills, weakness, and headache. This study aimed to determine the prevalence of** ***Bartonella*** **spp. infection in rodents and shrews of the genus** ***Crocidura*** **in the Kigoma and Morogoro regions of Tanzania. Blood culture and conventional PCR targeting a portion of the** ***gltA*** **gene were used to screen and confirm presence** ***Bartonella*** **spp. Among the 1036 small mammals tested, 999 were rodents and 37 were shrew species. The overall prevalence of** ***Bartonella*** **spp. in small mammals was 22.5%.** ***Bartonella*** **spp. was found in 13 rodent species and one** ***Crocidura*** **species. Prevalence varied significantly among host species (p <0.0001) and habitats.** ***Bartonella*** **spp. was found to be widespread in rodent species inhabiting indoor, peridomestic, farm and forest habitats. This study highlights rodents and** ***Crocidura*** **spp. as potential reservoirs of** ***Bartonella*** **spp., likely contributing to the spread of human bartonellosis due to their inevitable interactions in suitable habitats. Further research is needed to characterize zoonotic** ***Bartonella*** **spp., determine their genetic diversity, and assess ecological factors influencing the transmission cycle.**

Key words: Bartonellosis, Habitats, humans, interactions, small mammals.

INTRODUCTION

Bartonella spp. is pleomorphic rod-shaped bacteria (Minnick and Anderson, 2015) that are microaerophilic fastidious (Okaro et al., 2017). A number of species and sub species of the genus *Bartonella* isolated from small mammals have been described including *Bartonella elizabethae, Bartonella tribocorum, Bartonella phoceensis, Bartonella coopersplainsensis, Bartonella rattimassiliensis, Bartonella queenslandensis,* (Klangthong et al., 2015); *Bartonella grahamii, Bartonella taylorii and Bartonella doshiae* (Obiegala et al., 2021).

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Worldwide, studies on bartonellosis have shown that small mammals, especially rodents, serve as the primary natural reservoirs of a diverse range of *Bartonella* spp. (Yu et al., 2022). Bartonellosis in animals and humans is transmitted through bite from blood-sucking arthropod vectors including fleas, ticks, lice, and biting flies (Cheslock and Embers, 2019). Other ways of transmission for *Bartonella* spp. infections include contaminated abrasions, direct contact with infected animals (Okaro et al., 2017), and through blood transfusion or organ transplantation from infected donors (Noden et al., 2014). In mammalian hosts, *Bartonella* spp. attack erythrocytes, endothelial cells, and dendritic cells, which are important in the body's immune response (Birtles, 2005). Infections caused by *Bartonella* spp. result in various clinical manifestations, such as fever, anemia, and inflammation in organs including lymph nodes, heart, liver, spleen, and eyes in humans (Lins et al., 2019). In companion animals, clinical signs of *Bartonella* spp. infection include fever, cardiac murmurs, cough, tachypnea, lameness, and neurological symptoms in dogs, as well as endocarditis and myocarditis in cats (Sykes and Chomel, 2014).

Only a few studies have been conducted on bartonellosis in Tanzania (Gundi et al., 2012; Theonest et al., 2019). Despite being limited in number, these studies, mostly carried out in northern part of Tanzania, have been able to provide a rough picture of the disease situation in Tanzania, especially their likelihood to contribute to febrile illnesses of "unknown origin''. In the areas characterized by scattered agriculture fields with various crops, grazing, and increased human-wildlife interactions (Kimaro, 2014). This, coupled with a high diversity of synanthropic small mammals, poses a higher risk of zoonotic diseases to humans (Shilereyo et al., 2021). However, the information from these studies is too limited to fully explain the epidemiology and risk associated with bartonellosis. Therefore, this study will generate additional information which will considerably contribute towards further understanding of the epidemiology of bartonellosis in Tanzania, particularly with regards to interactions between wildlife, livestock and humans in different habitats. Consequently, this study aimed to determine the prevalence of *Bartonella* spp. in rodents and shrews from different habitats in Kigoma and Morogoro regions. This study provides insight into the presence of bartonellsis to the farmers and livestock keepers particularly in the areas with high interactions with small mammals that serve as a reservoir host for zoonotic pathogens. Understanding these implications can help raise awareness, inform preventive measures and promote early detection of the disease.

MATERIALS AND METHODS

Study sites

This study was conducted in two regions of Tanzania namely; Kigoma and Morogoro. Two districts were purposively selected from each region based on reserved natural land (game reserve/natural forest). In Kigoma region, the selected districts were Kibondo (4.1938° S, 31.0794° E) and Kakonko (3.2469° S, 30.9417° E) (Figure1). In Morogoro region, the selected districts were Kilosa (6.8343° S, 36.9917° E) and Morogoro rural (7.2009° S, 37.8511° E) (Figure 2).

Kibondo and Kankoko districts are bordered by Moyowosi game reserve. One village was selected from each district based on its closeness to the game reserve. These villages were Kigendeka (3°46'24.0"S 30°41'33.2"E) in Kibondo district and Itumbiko (3°17'16.7"S 30°58'38.9"E) in Kakonko district. The main economic activities in these villages include subsistence farming, grazing and poaching for some people from both villages (Pers communication). The other two villages are not bordered by a game reserve. The main economic activities in Kumuhama village (3°35'16.4"S 30°40'40.6"E) are subsistence farming, with all of the land being used for agriculture. In Kihomoka village (3°11'46.5"S 31°2'24.5" E), the economic activities are subsistence farming and grazing.

In the Morogoro region, Kilosa district, Mamboya village (6°18'11.5"S 37°06'23.9"E) is located near the Mamboya mount village reserve. The main economic activities in this village are subsistence farming and grazing. Another study village in Kilosa district was Magubike (6°14'52.884"S 37°9'48.084"E), situated along the Dodoma road. The main activities in this village are subsistence farming, entrepreneurship and grazing. There is no reserved land in this village. Kibuko village (6°57'12.4236" S 37°50'46.98492" E) in Morogoro rural district is engaged in subsistence farming, especially maize, rice and orchard. This village is not bordered by the Kimboza forest reserve. The other study village was Mwarazi (7°0'44.94276" S 37°48'51.27084" E). This village is closely bordered to Kimboza forest reserve on the way to Nyerere National Park. The main economic activities in Mwarazi village are subsistence farming especially maize, rice and orchard farming.

Rodents and Shrews trapping

Rodents and shrews were live trapped in eight villages from both regions. Four of them have a game reserve or reserved land where the traps were set including indoor, peridomestic, farms/fallow and the natural land (game reserve/ village reserved land). The other four villages had traps set only in indoor, peridomestic and farm/fallow land as those villages had no natural reserved land.

Rodents and shrews were trapped during the wet and dry seasons in order to capture seasonal variation. These animals were captured live indoors using modified wire cage traps and outdoors using Sherman® traps. All traps were baited with approximately 5g of peanut butter mixed with maize flour at the ratio of 2:1 (1000g of peanut butter mixed with 500g of maize flour). The indoor traps were augmented with a piece of tomato to increase their attraction to *Rattus rattus*.

In each study village, 20 to 25 houses were purposefully selected based on presence of rats and minimum recommended number of traps. Each house was provided with 2 to 3 modified local wire cage traps, depending on size of the house. These traps were used indoors purposefully because of the neophobic behavior of *Rattus rattus.* For outdoor habitats, a maximum of 100 Sherman traps were used in five trap-lines of 20 traps each. The traps were set at an interval of five meters apart from each trap station and trap lines, for a maximum of three consecutive nights in each village. Traps were checked and re-baited once per day.

Sample collection

Each trapped animal was euthanized in a container containing

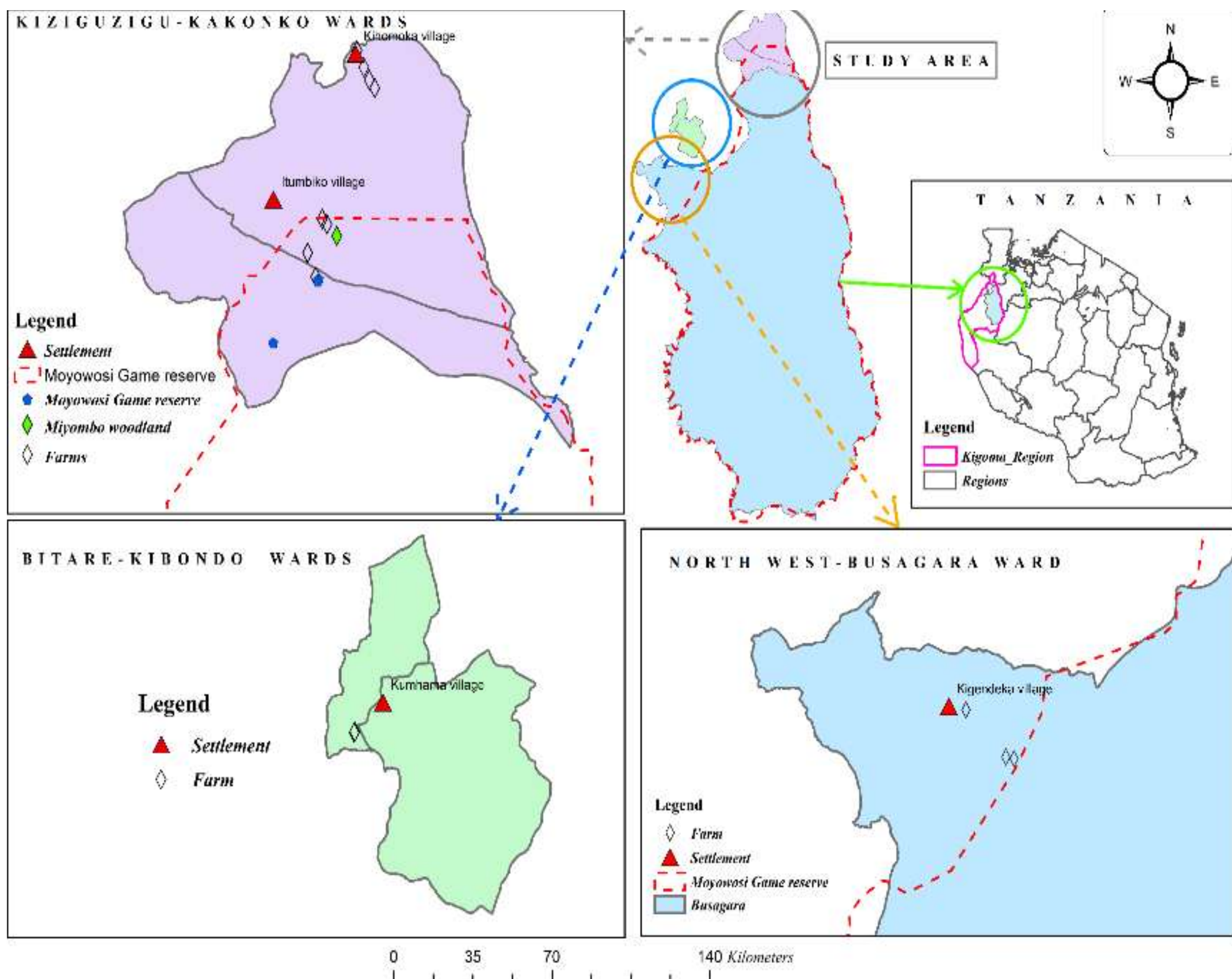

Figure 1. Kakonko and Kibondo Districts in Kigoma region.
Source: Authors

cotton wool soaked in halothane. A cardiac puncture was aseptically performed on each anesthetized animal after treating it with 70% ethyl ethanol. A minimum of 250µl of blood was drawn from the heart and placed in a sterile Ethylenediaminetetraacetic acid (EDTA) micro-vial and kept at -20˚C before being transported under cold conditions using a polystyrene box containing ice packs to the Institute of Pest Management of Sokoine University of Agriculture for laboratory analysis. For all trapped animals, standard body measurements (weight, head and body length, ear length, hind feet length and tail length), sex and sexual condition were recorded for morphological identification at the genus or species level, according to Happold (2013).

Blood culture

Blood culture was conducted according to Tratarис et al. (2012), with some modifications. Briefly, frozen EDTA blood was thawed at room temperature, and then 50µl of the blood was pipetted and smeared onto Columbia Blood Agar (CBA) enriched with 5% horse blood. The plates were left unturned for at least 30 min until the wet smear vaporized. Then, the plates were streaked from the dot inoculum. Plates were then placed in a candle jar and incubated at 35˚C. Fast-growing bacteria were observed after 24 h. Plates showing no growth were re-incubated and observed twice a week for up to three weeks. Purification was done for the mixed growth cultures, by picking suspected colonies and sub culturing them in another CBA medium. Presumptive *Bartonella* positive cultures were identified based on the slow-growth of different colony morphologies including small to medium in size, smooth or rough, moist and dry, self-adhesive, or easy to scrape off the surface of the medium. Other colonies that tended to pit into the medium were difficult to scrape off. Some colonies were clear or displayed faint metallic sheen. Furthermore, Gram and/ or Giemsa stain, catalase and oxidase tests were performed for the bacterial cell morphology. From the purified cultures 3 to 5 colonies were harvested and placed into a micro vial containing 95% ethanol and stored at -20˚C before confirmation by PCR. All procedures were aseptically done under the safety cabinet.

DNA extraction and PCR verification of *Bartonella* suspected cultures

Sub samples of 100 culture positive samples were further confirmed for *Bartonella* DNA using PCR. The DNA was extracted using Zymol

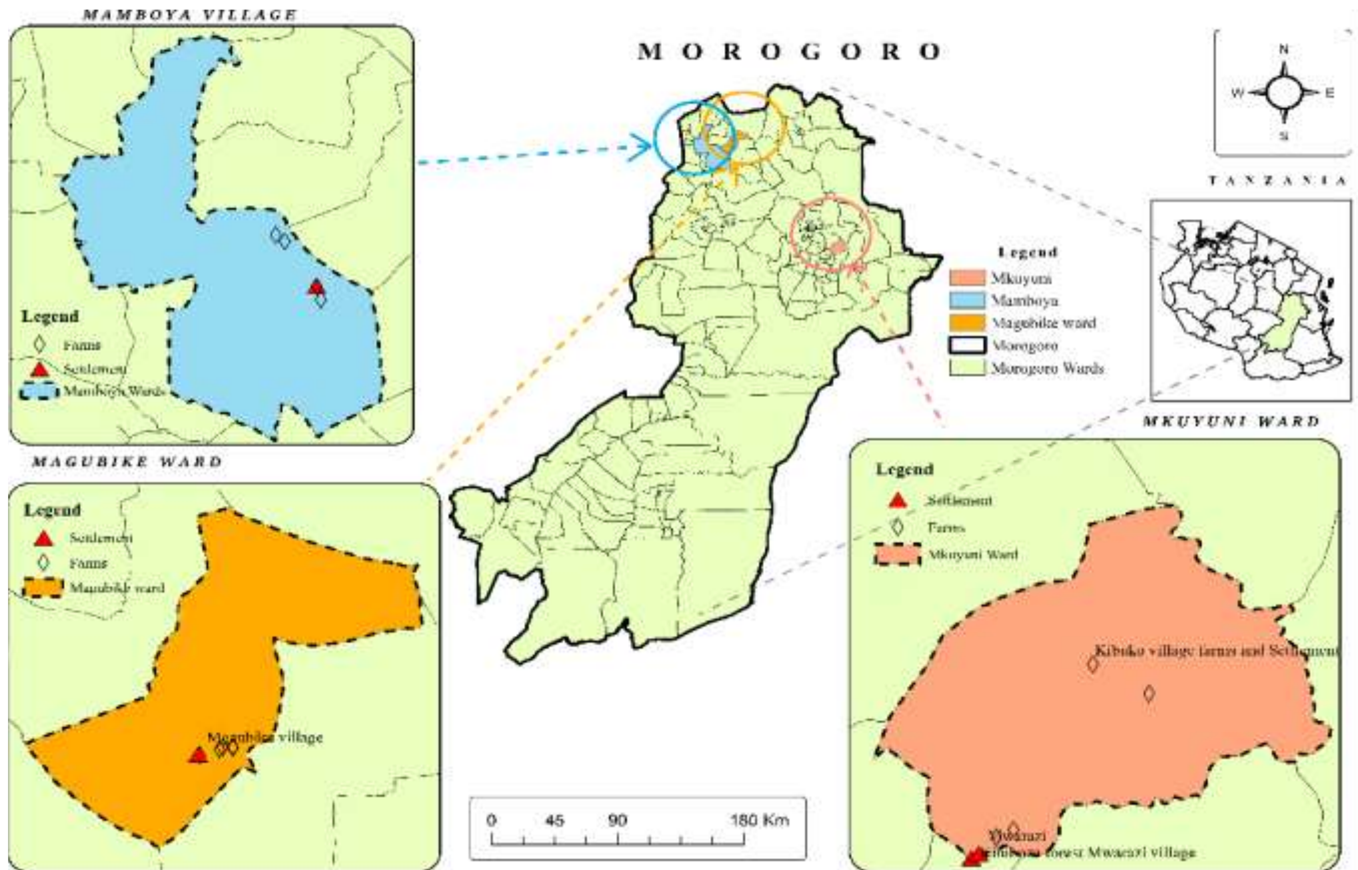

Figure 2. Kilosa and Morogoro Rural Districts in Morogoro region.
Source: Authors

kit (Quick-DNA™ Miniprep Plus Kit), according to manufacturers' instructions and PCR was done according to Norman et al. (1995), with some modifications. Briefly, forward primer BhCS871.p (5'-GGGGACCAGCTCATGGTGG-3') and reverse BhCS1137.n (5'-AATGCAAAAAGAACAGTAAACA-3') targeting a 379 bp of the genus- specific *gltA* gene were used. Three microliters of DNA templates were added into a PCR reaction tube containing 12.5µl of 2x master mix, 0.5µl of 10µM of each primer, and 8.5µl of nuclease-free water. Amplification was done by a thermal cycler by the following parameters: an initial denaturing at 95˚C for five minutes, and 35 cycles of denaturation at 95˚C for one minute, annealing at 56˚C for one minute, and elongation at 72˚C for one minute.

Amplification was finalized by holding the reaction mixture at 72˚C for 10 min. The amplified product was confirmed for the proper size of amplicon by electrophoresis in 1.5% agarose gel.

Statistical data analysis

The overall prevalence of *Bartonella* was estimated by the number of positive samples over the total number of samples subjected to culture. The frequencies of captured individuals were summarized and counted for each genus/species using Microsoft excel and verified in SAS software version 9.1. The prevalence from different variables including regions, habitats, land use category, species, sex and sex condition were calculated as the proportion of positives out of the total number of individuals tested. Chi-square was used to compare prevalence rates between the above variables. Differences of compared small samples sizes (n < 30) were tested with Fisher's exact test. All tests were done using SAS software; $p < 0.05$ was considered significant.

RESULTS

Captured animals

In total, 1147 small mammals were captured during this study, out of which 616 (53.7%) were captured in Kigoma and 531 (46.3%) were captured in Morogoro. The captured animals belonged to different species including *Acomys* spp., *Arvicanthis nairobae, Arvicanthis neumanii, Dendromus* spp., *Aethomys kaiseri, Aethomys chrysophilus, Dasmys* spp., *Tatera,*spp,, *Grammomys* spp,, *Graphuris* spp,, *Lophuromys sikapus, Lophuromys laticeps, Lemniscomys rosalia, Lemniscomys striatus, Lemniscomys zebra, Mastomys natalensis, Mus* spp., *Praomys* spp., *Rattus rattus,* and *Crocidura* spp. These species were examined for prevalence of *Bartonella* spp. Samples collected from 1036 animals (566 from Kigoma and 470 from Morogoro) representing 90.2% of the total

Table 1. Prevalence (%) of *Bartonella* spp. in different rodent and shrew species collected from different habitats across the study villages in Kigoma region.

Study village	Habitat	Rodent spp.															Shrew spp.	Prevalence per village/habitat
		Avna	Den	Ak	Ds	Ta	Gr	Lops	Lopl	Lr	Ls	Lz	Mn	Ms	Pr	Rr	Cr	
Itumbiko	Indoor	-	-	-	-	-	-	-	-	-	-	-	1/3 (33.3)	-		1/32 (3.1)	-	2/35 (5.7)
	Peridomestic	1/1 (100)	-	-	-	-	-	-	-	2/2 (100)	1/3 (33.3)	0/1	2/8 (25)	0/1	-	-	0/1	6/17 (35.3)
	Farms	1/2 (50)	-	2/9(22.2)	7/14 (50)	-	0/1	-	-	2/7 (28.6)	9/25(36)	0/5	14/58 24.1)	0/9	-	-	0/2	35/132 (26.5)
	Forest/Bush	1/1 (100)	-	-	1/2 (50)	-	-	-	-	2/3 (66.7)	6/9 (66.7)	-	0/1	1/2 (50)	-	-	-	11/18 (61.1)
	Sub total	3/4 (75)	-	2/9 (22.2)	8/16 (50)	-	0/1 (0)	-	-	6/12 (50)	16/37 43.2)	0/6(0)	17/70 24.3)	1/12(8.3)	-	1/32 (3.1)	0/3 (0)	54/202 (26.7)
Kihomoka	Indoor	-	-	-	-	-	-	-	-	-	0/1	-	0/1	-	-	1/11 (9.1)	-	1/13 (7.7)
	Peridomestic	-	-	2/9 (22.2)	-	-	-	-	-	0/1	1/5 (20)	0/1	7/12 (58.3)	-	-	-	0/2	10/30 (33.3)
	Farm	1/3 (33.3)	-	8/11 (72.7)	1/4 (25)	0/1	-	-	-	0/1	7/13 (53.8)	0/1	15/33 45.5)	0/2	-	-	0/2	32/71 (45)
	Forest/Bush	NA	NA	NA	NA	NA	NA	NA	-	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Sub total	1/3 (33.3)		10/20(50)	1/4 (25)	0/1(0)	-	-	-	0/2 (0)	8/19(42.1)	0/2(0)	22/46(47.8)	0/2 (0)	-	1/11 (9.1)	0/4 (0)	43/114 (37.7)
Kigendeka	Indoor	-	-	-	-	-	-	-	-	-	0/1	-	2/6 (33.3)	-	-	0/5	-	2/12 (16.7)
	Peridomestic	-	-	-	-	-	-	1/1 (100)	-	-	1/3	-	0/2	-	-	-	-	2/6 (33.3)
	Farm/Fallow	2/4 (50)	-	9/17 (52.9)	1/1 (100)	0/1	0/1	-	-	4/5(80)	12/20 (60)	-	18/43 41.9)	0/2	0/1	-	0/3 (0)	46/98 (46.9)
	Forest/bush	-	0/1	2/2 (100)	-	-	-	1/1 (100)	-	1/3 (33.3)	1/4 (25)	-	1/1 (100)	0/3	2/6 (33.3)	-	-	8/21 (38.1)
	Sub total	2/4 (50)	0/1(0)	11/19(58)	1/1 (10)	0/1(0)	0/1 (0)	2/2(100)	-	5/8(62.5)	14/28(50)		21/52(40.4)	0/5(0)	2/7 (28.6)	0/5(0)	0/3 (0)	58/137 (42.3)
Kumhama	Indoor	-	-	-	-	-	0/3	-	-	-	-	-	9/9 (100)	0/1	-	0/6	0/1 (0)	9/20 (45)
	Peridomestic	NA	NA	NA	NA	NA	NA	NA	-	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Farm/Fallow	-	-	3/3 (100)	7/7 (100)	-	0/5	-	0/5	-	12/47 25.5)	-	7/21 (33.3)	1/5 (20)	-	-	-	30/93 (32.3)
	Forest/Bush	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Sub total	-	-	3/3 (100)	7/7 (100)	-	0/8(0)	0/5 (0)	-	-	12/47(25.5)	-	16/30 53.3)	1/6 (16.7)	-	0/6(0)	0/1 (0)	39/113 (34)
Total % per host species		6/11(54.5)	0/1(0)	26/51(51)	17/28(60.7)	0/2(0)	0/10(0)	2/7(28.6)	-	11/22(50)	50/131(38.2)	0/8(0)	76/198(38.4)	2/25(8)	2/7 (28.6)	2/54 (3.7)	0/11 (0)	194/566 (34.3)

Avna-Arvicanthis nairobae, Den-Dendromus, Ak-Aethomys kaiseri, Ds-Dasmys, Ta-Tatera, Gr-Grammomys, Lops-Lophuromys sikapus, Lopl-Lophuromys laticeps, Lr-Lemniscomys rosalia, Lz- Lemniscomys zebra, Ls-Lemniscomys striatus, Pr-Praomys spp., Rr- Rattus rattuss, Ms-Mus spp., Mn-Mastomys natalensis, Cr-Crocidura NA-Not applicable for trapping.
Source: Authors

number of captured animals, were analyzed. Other samples could not be cultured because the animals died in the traps before collection and/or lack of enough blood

The prevalence of *Bartonella* spp

Out of 1036 rodents and shrews tested, 234(22.5%) turned out to be positive for the bacteria on culture. Among them, 231 were rodents (n=999), and 3 were shrews (n=37). For the representative cultures confirmed by PCR, 86 out of 100 cultures were found to be positive. *Bartonella* spp. was detected in 14 host species out of 20 species/ genus of the small mammals investigated. The prevalence of *Bartonella* spp. differed significantly ($p < 0.0001$) among the host species tested, with except for *Gramommys,* spp., *Lemniscomys zebra, Dendromus* spp.*, Graphuris* spp., *Lophuromys sikapus* and *Tatera* spp. whose samples tested negative (Table 1). However, there were no significant differences ($p > 0.05$) in the prevalence of the organisms between seasons, habitats, land category, sex and sexual condition of the hosts from both regions. Furthermore, no significant difference was found in the prevalence of *Bartonella* spp. ($\chi^2 = 24.2297$, df = 11, $p = 0.118$) between hosts in the Morogoro region (Table 2). The prevalence of *Bartonella* spp. was significantly higher in the Kigoma region 34.3%, (n=566) (Table 1) compared to the Morogoro region at 8.5% (n=470) ($\chi^2 = 96.4463$, df = 1, $p <$

Table 2. Prevalence (%) of *Bartonella* spp. in different rodent and shrew species collected from different habitats across the study villages in Morogoro region.

Study village	Habitats	Rodent spp.											Shrew spp	Prevalence per habitat/village
		Ac	Aech	Avneu	Ta	Gr	Graph	Lr	Lz	Mn	Pr	Rr	Cr	
	Indoor	-	-	-	-	-	-	-	-	0/1	-	0/16	-	0/17
	Peridomestic	0/1	-	-	-	-	-	-	-	-	-	-	-	0/1
Mwarazi	Farms	1/4 (0.25)	-	-	0/1	-	-	-	-	14/46 (30.4)	-	0/1	2/14 (14.3)	17/66 (25.8)
	Forest/Bush	1/18 (5.6)	1/1 (100)	-	-	-	0/1	-	-	0/7	1/1	-	0/3	3/31
	Sub total	2/23 (8.7)	1/1 (100)	-	0/1	-	0/1	-	-	14/54 (27)	1/1(100)	0/17	2/17 (11.8)	20/115 (17.4)
	Indoor	-	-	-	-	-	-	-	-	-	-	0/4	-	0/4
	Peridomestic	-	-	-	-	-	-	-	-	1/11(9)	-	-	-	1/11 (9)
Kibuko	Farm	-	-	-	-	-	-	0/2	-	0/68	-	-	1/2 (50)	1/72 (1.4)
	Forest/Bush	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Sub total	-	-	-	-	-	-	0/2	-	1/79 (1.3)	-	0/4	1/2 (50)	2/87(2.3)
	Indoor	-	-	-	-	-	-	-	-	0/1	-	11/36 (30.6)	-	11/37 (30.6)
	peridomestic	0/1	-	-	-	-	-	-	-	0/11	-	-	-	0/12
Mamboya	Farm/Fallow	-	0/1	-	-	-	-	-	-	0/78	-	-	0/2	0/81
	Forest/bush	0/2	0/2	-	-	0/1	-	1/1 (100)	-	-	-	-	-	1/6 (6.25)
	Sub total	0/3	0/3	-	-	0/1	-	1/1 (100)	-	0/90	-	11/36(30.6)	0/2	12/136(8.8)
	Indoor	-	-	-	-	-	-	-	-	0/1	-	1/14 (7.1)	-	1/15 (6.7)
	Peridomestic	NA	NS	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Magubike	Farm/Fallow	0/1	0/7	3/19 (15.8)	0/2	0/1	-	0/1	0/2	2/79 (2.5)	-	-	0/5	5/117 (4.3)
	Forest/Bush	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Sub total	0/1	0/7	3/19 (15.8)	0/2	0/1	-	0/1	0/2	2/80(2.5)	1/1 (100)	1/14 (7.1)	0/5	6/132(4.5)
Total (%) per host species		2/27 (7.4)	1/11 (9.1)	3/19 (15.8)	0/3	0/2	0/1	1/4 (25)	0/2	17/303 (5.6)	1/1 (100)	12/71 (16.9)	3/26(11.5)	40/470 (8.5)

Ac-Acomys, Aech-Aethomys chrysophilus, Avneu-Arvicanthis neumanii, Ta-Tatera, Gr-Gramommys, Graph-Graphuris, Lr-Lemniscomys rosalia, Lz-Lemniscomys zebra, Mn-Mastomys natalensis, Pr-Praomys, Rr-Rattus rattus, Cr-Crocidura. NA-Not applicable for trapping.
Source: Authors

0.0001) (Table 2). Furthermore, significant differences (χ^2=13.1703, df = 3, p = 0.0043) in prevalence were observed among habitats in Kigoma region, where thenatural habitat had a higher prevalence of 48.72% compared to farm/fallow (36.02%), peridomestic (36%), and indoor habitats (16.25%) (Table 1).

DISCUSSION

This study reports the presence of *Bartonella* spp. from rodents and shrews from different habitats in the study regions. An overall prevalence of 22.5% of *Bartonella* spp. was found in rodents and shrews collected from indoor, peridomestic, farm/fallow and natural forests. This occurrence could be explained by the widespread nature of *Bartonella* bacteria among rodents and shrews in all sampled habitats, as previously described by Divari et al. (2021). The presence of *Bartonella* spp. in different rodents and shrews from various habitats could be attributed to the abundance and distribution of each host species and vectors in a particular habitat, as suggested by Assefa and Chelmala (2019). This could be further explained by the presence of suitable habitats in the study areas that support the breeding of various small mammals, as highlighted by Mayamba et al. (2019).

However, it is important to note that the habitat features were not the same across all regions. For instance, the Kigoma region observed large natural bushes around farms and houses, which are more favorable for different species of rodents, as noted by Nunn et al. (2021).

Nevertheless, typical field rodents, including Aethomys spp., Lemniscomys spp. and Mastomys spp. were trapped indoors in some villages, possibly due to the presence ofnatural bushes surrounding certain houses. This interaction between field rodents and indoor environments could potentially increase the rate and risk of pathogen transmission from outdoor to indoor and vice versa.

The occurrence of the Bartonella spp. in rodents and shrews from indoors and agricultural fields holds public health importance because bartonellosis is a zoonotic disease and more than 10 rodents related Bartonella spp. are known to be zoonotic (Demoncheaux et al., 2022).

In the current study, prevalence of Bartonella spp. in rodents varied substantially among the genus/species detected. This variation could be explained by the observed differences in the abundance and diversity of rodents from different sampled habitats, as earlier explained by Mardosaitė et al. (2021). Additionally, close contacts between rodent species, humans and other animals in human-related activities may contribute to the variation in prevalence (Islam et al., 2021).

Furthermore, the variation in ectoparasite types among rodent species, as observed by Peterson et al. (2017), along with the ecological behaviors such as burrowing and nesting in different rodent species, create favorable microhabitats for ectoparasites to breed (Böge et al., 2021). Grooming behavior is also important in the spread of *Bartonella* spp. within the same host species, which may influence the prevalence of *Bartonella* spp. infection in rodent communities (Bordes et al., 2007).

The presence of *Bartonella* spp. found in this study was comparable to some previous studies. Kamani et al. (2013), reported prevalence of 26% in Nigeria, while Theonest et al. (2019), and Diarra et al. (2020) detected prevalence of 17% and 17.7% in Tanzania and Mali respectively. This similarity in prevalence could be attributed to the high prevalence of *Bartonella* spp. in small mammals from various countries, as explained by several authors globally (Krügel et al., 2022).

On the contrary, zero prevalence was reported in some sylvatic rodent species including *Lemniscomys zebra*, *Tatera* spp. and *Gramommys* spp. Failure to detect *Bartonella* spp. from these rodents could be attributed to the host specificity of *Bartonella* species (Vayssier-Taussat et al., 2009) and the likelihood of host genotypes differing in their vulnerability and ability to support the replication of certain pathogens (Ostfeld and Keesing, 2012). It is worth noting that these rodent species were trapped in the same locations and habitats where other rodent species tested positive.

Nevertheless, small sample size of these rodent species investigated may have influenced the prevalence of *Bartonella* spp. in them. Further studies are encouraged to investigate the status of immunity against *Bartonella* spp. in these rodent species.

Moreover, this study detected *Bartonella* spp. from small mammals inhabiting habitats characterized by human activities including in houses, agricultural farms, and the human-wildlife interface habitats. Therefore, it implies that small mammals could carry the pathogens to human habitations, thereby increasing the risk of *Batonella* spp. infection in humans and domestic animals. However, due to unclear clinical signs and unfamiliar, bartonellosis could be underestimated risk factor contributing to some medically important diseases.

Conclusions

The study identified considerable prevalence of *Bartonella* spp. in rodents and shrews collected from different habitats across all studied districts. This implies considerable distribution of health risks to humans and domestic animals due to close contact and interactions with those small mammals particularly around the agricultural fields and human settlements. The findings of this study raise awareness of the disease among communities living in close proximity to small mammals. From these findings upcoming studies should focus on characterizing the zoonotic *Bartonella* spp., their genetic diversity and ecological factors that influence the transmission cycle. Additionally, investigations on the occurrence of *Bartonella* spp. infections in humans and the identification of risk factors for such infections should be conducted. This information will be crucial in developing control strategies to protect public health.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Use of bacterial biocontrol agents for the control of *Fusarium oxysporum f.sp. Lycopersici* (Fusarium wilt) in tomatoes

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***Fusarium oxysporum f.sp Lycopersici* (Fusarium wilt) is a serious plant pathogen that can cause yield losses >80% in tomatoes. The disease is controlled mainly by use of synthetic pesticides, which may be harmful to both humans and the environment. In this study, the potential use of antagonistic bacteria was reported for the control of fusarium wilt. First, we isolated and characterized fungi from tomato plants with wilt symptoms. Analysis of the ITS gene indicated fungi from the genus *Fusarium, Alternaria, Plectosphaerella*, *Aspergillus*, *Gibellulopsis*, *Trichoderma*, *Papiliotrema*, *Rhodotorula*, *Mucor*, *Ustilago*, *Sporothrix* and *Cumuliphoma*. 20 bacterial isolates were then screened from soils collected around the tomato plants for antagonistic activity against *F.oxysporum* using soft agar overlay method. Bacterial isolates showing inhibition zone diameters >3mm and consistent in reducing mycelial growth of the pathogen were further characterized and subsequently used for field experiments which were conducted during the short rain season in 2021 and 2022. The isolates grew at an optimum salt concentration of 5 - 10 % (w/v), pH range of 5 - 8 and a temperature of 30°C. Phylogenetically, they were affiliated to *Bacillus subtilis*, *Paenibacillus polymyxa*, *Brevibacillus laterosporus*, *Bacillus velezensis*, *Bacillus amyloliquefaciens* and *Paenibacillus poriae* whereas one of the isolates was identified as *Myroides odoratimimus*. Results from the field experiments showed that all selected strains significantly reduced average disease incidence (mean ± SE) for both years on day 14 (14.4% ± 3.49), day 28 (15.9% ± 3.44) and day 42 (15.2% ± 2.825) respectively. Therefore, these isolates are good candidates for the development of effective biological control programs against *F. oxysporum f.sp Lycopersici* affecting tomatoes.**

Key words: Pathogenic fungi, antagonistic bacteria, screening, assessment, disease incidence, disease severity.

INTRODUCTION

Tomato, *Lycopersicon esculentum Miller* (Solanales: Solanaceae), is one of the most farmed tropical foods on the planet (Ouedraogo et al., 2021). It is one of the major vegetable in Kenya's agricultural farming systems (Ochilo et al., 2019).

It accounts for 38.1% of the total vegetable production

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and 6.72% of the entire horticultural produce (Karuri, 2023). Kenya is ranked 6th in Africa with a production capacity of 397,007 tones (Nakhungu et al., 2021). In Kenya, the main tomatoes-producing counties are Kirinyaga (14%), Kajiado (9%), and Taita Taveta (7%), where farming is predominantly done using the open field (95%) and greenhouse systems (5%) (Mwangi et al., 2020).

Tomato production is hampered by a variety of soil-borne pathogens predominated by *Fusarium oxysporum* that causes Fusarium wilt of tomatoes leading to yield losses >80% (Devi et al., 2022). Fusarium wilt management has yielded few results over the years, with the most popular method being the application of fungicides, which has also been deemed unworkable due to *F. oxysporum's* soil-borne nature (Venkataramanamma et al., 2022). The chemical fungicides Prochloraz and Carbendazium are effective against tomato wilt but despite their low toxicity to the environment, the potential impacts of these fungicides on animal and human health have not been taken into consideration (Yang et al., 2021). Carbendazim toxic traits to the environment are detectable in food, soil and water and its continuous use has negative impacts on humans, invertebrates, aquatic life forms and soil microorganisms (Zhou et al., 2022). Carbendazim is employed as a soil drencher, however, drenching is not viable for large-scale use (Mbeyagala et al., 2022). On the other hand, Prochloraz has been extensively used to control the Fusarium wilt of tomatoes (Gomaa et al., 2022). Prochloraz elicits *in vitro* effect by agonizing the *Ah* receptor and inhibiting aromatase activity while *in vivo,* it modulates the androgen-regulated gene expressions in the prostate of mammals (Bonefeld-Jorgensen and Long, 2020). Globally, pesticides are toxic to human and animal health as well as the environment and the majority of the regulatory agencies are attentive to reduce the use of hazardous pesticides(Pesticides Action Network Europe, 2020).

Due to the difficulties in controlling Fusarium wilt, an alternative disease management strategy for Fusarium wilt in tomatoes is necessary. Studies by Ayantola and Fagbohun (2021) have previously demonstrated fungal and bacteria species for example, *Trichoderma* species, non-pathogenic *Fusarium* species, and Bacillus that have antagonistic activity against Fusarium wilt. In this study, the authors isolated and characterized fungi from tomato plants with wilt symptoms, screened bacterial strains in the laboratory and tested promising bacterial strains for their biocontrol ability against the Fusarium wilt of tomatoes under field conditions.

MATERIALS AND METHODS

Enrichment and Isolation of fungal pathogen and bacteria biocontrol isolates

Samples used in this study were collected from an F1 Rambo tomato variety plot at the University of Embu farm (0.5156 ⁰S and 37.456 ⁰E) in Kenya. The region is characterized by a warm and temperate climate with significant rainfall throughout the year. The soils are mostly humic nitisol, which are deep, well-weathered with moderate to high inherent fertility (Serafim et al., 2013). Ten tomato plants with wilt symptoms were randomly sampled for the isolation of *Fusarium oxysporum* pathogen. Isolation was carried out from the stems, roots and the collar of the samples showing wilt symptoms. Putative biocontrol agents were isolated from one gram of soil randomly collected from three different F1 Rambo tomato plots. Selective media for isolation of *Fusarium* spp was prepared by incorporating 50µg/ml ampicillin to inhibit the growth of bacteria. The root, stem and collar root samples were surface sterilized with 1.5% bleach for 10 seconds followed by 70% ethanol for 30 s and rinsed with sterile water for 30 s, freshly infected tissues were selected for isolation. The sterile samples were then inoculated on Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Cornmeal Agar (CMA) and Sabouraud Dextrose Agar (SDA) selective media and incubated at 28 ⁰C for five days.

Fungal mycelia were picked based on their morphological features such as color and the hyphal tips aseptically transferred to new PDA plates to obtain axenic cultures. Pure cultures were maintained on PDA slants. Soil samples for bacterial isolation were serially diluted six-fold in sterile PBS and 100 µl aliquots from the fourth and sixth dilution spread plated on Trypticase Soy Agar (TSA) basal media supplemented with 50 µg/ml cyclohexamidine to inhibit fungal growth. The plates were sealed and incubated at 30 ⁰C until colonies appeared. Single colonies were selected based on morphological features such as pigments, elevation, and shape and sub-cultured on fresh media.

Morphological, physiological and molecular characterization of isolates

Fungal and bacterial isolates morphological characteristics (color, elevation, shape, surface) were described using standard microbiological techniques (Cappuccino and Sherman, 2014). All the bacterial isolates were physiologically screened on different salt concentrations ranging (0%-15%), pH levels ranging (pH 4-8) and temperature ranges ranging (25 -40 °C) and data were recorded by measuring the OD_{600} at a four hour interval starting from 0 h to 24 h. The bacterial isolates were screened for their ability to produce extracellular enzymes by first spotting the isolates separately on the TSA basal media supplemented with either 0.2 % of soluble starch (g/L), 0.5 % of Birchwood xylan, 0.5 % casein, 0.1% of tributyrate or 1.5g/l of carboxymethylcellulose. Phosphate utilization was described by inoculating the isolates on phosphate media supplemented with bromothymol blue and incubated for 48 h at 30 ⁰C. Phosphate utilization was indicated by the color change of the media from green to orange and the formation of halo zones around the bacteria. Un-inoculated plates and tubes acted as the negative control in all the tests.

Genomic DNA was extracted from fungal isolates as described by Sambrook (1989) and used as a template for a polymerase chain reaction. The 18S rRNA gene was amplified using universal fungal primers pair forward primer ITS-1 F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and the reverse primer ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') (Gardes and Bruns, 1993). Amplicons were generated on a Sure Cycle 8800 (Agilent Technologies) in a total reaction volume of 50 µl (34.0 µl PCR water, 10.0 µl polymerase buffer, 2.5 µl of each primer, 0.3 µl Taq polymerase and 1.0 µl of genomic DNA). The PCR reaction conditions were as follows: 5 min at 95 °C followed by 35 cycles (1 min of denaturing at 96 °C, 1 min of annealing at 45 °C, 1 min of extension at 72 °C) followed by a final extension step of 5 min at 72 °C. The 16S rRNA gene was amplified using universal bacterial primers pair 8F (5'-AGGTTTGATCCTGGCT-3') and 1492R (5'-CGGCTACCTTGTTACGACTT-3') as described (Galkiewicz and

Kellogg, 2008). Amplicons were generated on a Sure Cycle 8800 (Agilent Technologies) in a total reaction volume of 50 µl (34.0 µl PCR water, 10.0 µl polymerase buffer, 2.5 µl of each primer, 0.3 µl Taq polymerase and 1.0 µl of genomic DNA). The PCR reaction conditions were as follows: 5 minutes at 95 °C followed by 36 cycles (1 min of denaturing at 96 °C, 1 min of annealing at 53 °C, 1 min of extension at 72 °C) followed by a final extension step of 5 min at 72 °C respectively. Amplified products were separated on a 1 % agarose gel in TAE buffer and visualized under UV light after staining with a fluorescent dye (Sambrook et al., 1989).

The amplified fragments were purified using the QIAquick PCR purification Kit (Qiagen, Germany) following the manufacturer's protocol. PCR products were sequenced using the same universal primers at Inqaba biotech, South Africa. The raw sequences were edited using Chromas Lite (https://technelysium.com.au/wp/ chromas) and compared to the sequences in the public databases using the basic local alignment search tool (BLAST) on the National Center for Biotechnology Information (NCBI) website (htt://www.ncbi.nih.gov). The clean sequences were aligned using Clustal W software; while phylogenetic analyses were carried out using molecular evolutionary genetics analysis MEGA 11 (Filipski et al., 2015) using the neighbour-joining method (Kumar et al., 2016).

Bacterial biocontrol antagonism assays

Twenty bacterial isolates were evaluated for their potential antagonistic activity against the mycelial growth of the *Fusariu*m *oxysporum* pathogen using the soft agar overlay technique (Hockett and Baltrus, 2017). Bacteria suspension of sole bacteria biocontrol agent with a cell density of 1 x 10^6 CFU/ml was prepared and spread plated on TSA and allowed to dry for 10 mins. After drying, 50 µl of the fungal isolates with a cell density of 1 x 10^6 spores/ml were equidistantly positioned on each plate and incubated at 28 °C for 48 h. Three replicates were made per isolate with 3 plates each and maintained in the incubator at 28ºC. The antagonistic activity was indicated by clear zones of inhibition measured in millimetres and the degree of inhibition of each isolate was then rated on a scale of 0-4 where 0=no visible inhibition,1=both organisms stopped growing on contact, 2=inhibition zone <2mmin width, 3=the inhibition zone was between 2mm and 3mm in width, 4=the inhibition zone was >4mm in width. For further assessment and molecular characterisation, ten isolates exhibiting the greatest inhibitory activity with an inhibition zone greater than 3 mm were selected. Isolates with inhibition zones less than 3mm were deemed less effective and were eliminated from the study.

Assessment of biocontrol activities of the putative bacterial biocontrols On *F. Oxysporum* under field conditions

Field trials were conducted from June to September 2021 and June to September 2022 at the University of Embu farm in Embu County, Kenya using the F1 Rambo tomato variety. Experimental blocks comprised of groups of four seedlings planted in a randomized complete block design with three blocks of twelve treatments each with two acting as the positive and negative controls. The plots in the blocks measured 110cm long with a spacing of 100cm along the paths in each row. A bacteria biocontrol isolates suspension with a cell density of 1 x 10^6 CFU/ml was applied four times to a run-off after the first inoculation using a pump sprayer. Sterile distilled water was used as the negative control and Carbendazium chemical fungicide was used as the positive control. Pathogen inoculum was prepared by inoculating 10 µl of the pathogen with a concentration of 1 x 10^6 spores/ml. Two hours after spraying the biocontrol isolates, the pathogen inoculum containing 1 x 10^6 spores/ml was spotted at the first, second, third and fourth leaves from the top of the plant, using sterile cotton buds. On the same day, the negative control plants were sprayed with sterile distilled water while the positive control plants were sprayed with Carbendazium chemical fungicides. Disease incidence was recorded as the percentage of diseased leaflets in a 20- leaflet sample. Six 20-leaflet samples were collected randomly per replicate row, a total of 24 samples. Foliar disease severity was rated in the field as a percentage of necrotic foliage (0–100%) in each replicate row or by counting lesions on each of 20–50 sampled leaflets per replicate row. Fruit disease incidence and fruit numbers were determined at the end of the field experiment. To assess the colonization efficiency of the pathogen and the biocontrol, the fungal pathogen and biocontrol agent were re-isolated using standard microbiological procedures as described above and characterized.

Data analysis

The statistical analyses were conducted using SAS 9.2 (SAS Institute Inc.), and linear regression analysis used to evaluate the in vitro conidial germination and mycelia growth inhibition data.

For the field experiments, data were analyzed for each year separately due to significant effects observed in a preliminary analysis. For each date in each year, disease incidence and severity were analyzed with a two-way ANOVA (PROC GLM, SAS software) and average ratings were compared among treatments with Duncan's multiple range test at P=0.05. Any subsequent reference to significant effects of factors or differences among means denotes significance at a level of $P < 0.05$, unless otherwise specified.

RESULTS

Characterization of fungi pathogens

A total of 27 isolates with typical fungal characteristics were recovered from diseased F1 Rambo tomato plants. PDA media supported growth of 20 isolates, MEA supported growth of 3 isolates, SDA supported growth of 2 isolates and CMA supported growth of 2 isolates. The morphological characteristics of select fungal isolates are shown in Figure 1. Comparative analysis of 18S rRNA against the NCBI database showed that the fungal isolates were distributed in 12 genera affiliated to the phylum *Ascomycota*, *Basidiomycota* and *Mucoromycota* (Table 1). Among the isolates, 7 were affiliated to the genus *Fusarium,* 5 to the genus *Alternaria*, while the other genera represented were *Plectosphaerella* (2), *Aspergillus* (2), *Gibellulopsis* (2), *Trichoderma* (1), *Papiliotrema* (1), *Rhodotorula* (1), *Mucor* (1), *Ustilago* (1), *Sporothrix* (1) and *Cumuliphoma* (1), out of the 27 isolated fungal isolates, only 24 were blasted.A total of 20 bacteria isolates were recovered, and 10 isolates showed antagonistic activity against the Fusarium pathogen, which were then further identified. The identified bacteria isolates were affiliated to *Bacillus subtilis*, *Paenibacillus polymyxa*, *Brevibacillus laterosporus*, *Bacillus velezensis*, *Bacillus amyloliquefaciens* and *Paenibacillus poriae* whereas one of the isolates was identified as *Myroides odoratimimus*, two isolates were mixed during the sequence analysis and were not blasted (Table 2).

Ten isolates that showed inhibitory activity against the

Figure 1. Morphological characteristics of isolated fungal isolates, plates 1a and 1d shows the colony frontal view with pink colonies and white brown colonies respectively, 1b, 1c, 1f, 1g and 1e shows the colonies opposite view with dark -brown, pink- white, black-brown, black-white and brown-white colonies respectively.
Source: Authors

Table 1. Fungal isolates and their closest neighbors in the NCBI database.

Isolate code	Closest neighbor	Acession	Similarity %
F86	*Papiliotrema flavescens*	AB085796	73.50
F87	*Plectosphaerella cucumerina*	AF176951	100.00
F88	*Fusarium oxysporum*	AB110910	99.80
F89	*Fusarium oxysporum*	KX823406.1	100.00
F91	*Plectosphaerella cucumerina*	AF176951	100.00
F92	*Fusarium chlamydosporum*	MK212931.1	99.80
F94	*Aspergillus niger*	AM270051	100.00
F95	*Rhodotorula mucilaginosa*	AB042787	99.63
F96	*Alternaria sp.*	KP872520	100.00
F97	*Trichoderma atroviride*	JN941691	99.82
F98	*Mucor nidicola*	MK281565.1	84.17
F99	*Gibellulopsis nigrescens*	GU180613	99.61
F100	*Ustilago sphaerogena*	MH860258.1	99.82
F101	*Sporothrix sp.*	JX192795	99.44
F102	*Verticillium dahliae*	AF104926	99.81
F103	*Fusarium chlamydosporum*	MK212931.1	99.80
F104	*Fusarium chlamydosporum*	KF494035.1	90.91
F106	*Alternaria sp.*	MT626575.1	100.00
F107	*Aspergillus niger*	MK841442.1	99.64
F108	*Alternaria alternata*	MN249500.1	99.62
F109	*Cumuliphoma omnivirens*	MH861962.1	100.00
F110	*Fusarium oxysporum*	LT746252.1	99.41
F111	*Fusarium equiseti*	MTPY01000045	99.80
F112	*Alternaria alternata*	AF218791	92.15

Source: Authors

Table 2. Bacterial isolates and their closest neighbors in the NCBI database.

Code	closest strain	Accession	Identity%
B1	*Myroides odoratimimus*	BCMQ01000053	98.74
B2	*Bacillus subtilis*	CYHS01000026	99.21
B3	*Paenibacillus polymyxa*	HG324073	98.30
B4	*Brevibacillus laterosporus*	CP011074	99.65
B5	*Bacillus velezensis*	MPHE01000006	97.20
B6	*Brevibacillus laterosporus*	DQ371288.2	98.63
B7	*Bacillus amyloliquefaciens*	HQ021420	99.32
B9	*Paenibacillus peoriae*	MRTU01000002	85.14

Source: Authors

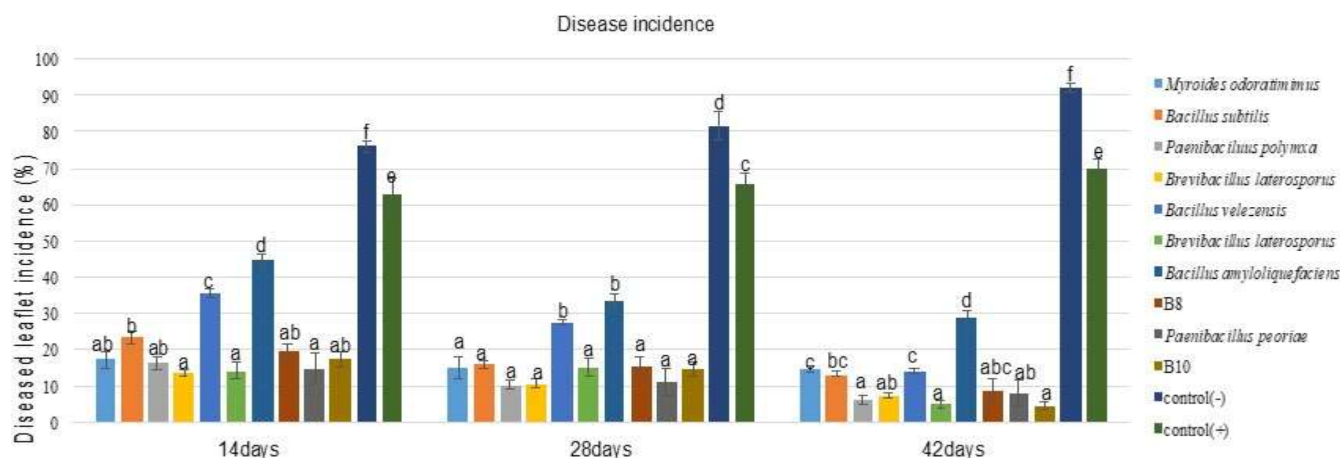

Figure 2. Disease incidence of Fusarium wilt in F1 Rambo tomato variety plants treated with bacterial biocontrols at a 14, 28 and 42-day interval after treatment. Bars headed with the same letter are not significantly different within the same day (p=0.05) by DMRT.
Source: Authors

isolated fungal pathogens were tested for their ability to grow at various physiological conditions. Results showed that the bacteria isolates grew at varying salinities ranging from 0 % to 15 % and the optimum growth of all isolates recorded at a 5% NaCl concentration (Supplementary Figure 1). Optimum pH for growth was observed between pH 5 and pH 6 and the least growth at pH 4 and pH 8 (Supplementary Figure 2).

The temperature range for growth ranged between 25 and 35 °C (Supplementary Figure 3). The isolates were able to use various substrates for growth as shown in Table 3. Isolate B9 grew best at 25 ºC and showed slight amylase activity. The rest of the isolates showed moderate to high protease activity. Seven isolates (B1, B2, B4, B7, B8, B9 and B10) had moderate to high phosphate utilization ability whereas isolates B3 and B9 had high lipase enzyme activity and moderate cellulose enzyme activity (Table 3).

Antagonism assays

The bacterial isolates B1, B2, B3, B4, B5, B6, B7 and B9 inhibited the growth of the three 18S rRNA confirmed *F. oxysporum* pathogens compared to the controls (Table 4).

The noted that isolates B2, B3, B4, B5, B6, B7 and B9 exhibited the greatest inhibitory activity with inhibition zones exceeding 3 mm. The selected isolates also inhibited the growth of the other isolated fungal pathogens, namely *Fusarium chlamydosporum*, *Alternaria alternata*, *Plectosphaerella cucumerina*, *Aspergillus niger*, *Gibellulopsis nigrescens*, *Trichoderma atroviride*, *Papiliotrema flavescens*, *Rhodotorula mucilaginosa*, *Mucor nidicola*, *Ustilago sphaerogena*, *Sporothrix* and *Cumuliphoma ominivirens* isolated from the F1 Rambo diseased tomato leaves to a moderate extent.

Assessment of biocontrol activities of the putative bacterial biocontrols On *F. Oxysporum* under field conditions

Isolates B1, B2, B3, B4, B5, B6, B7, B9 with the most promising in vitro agar plate antagonistic effect against F. oxysporum were effective in the reducing foliar disease

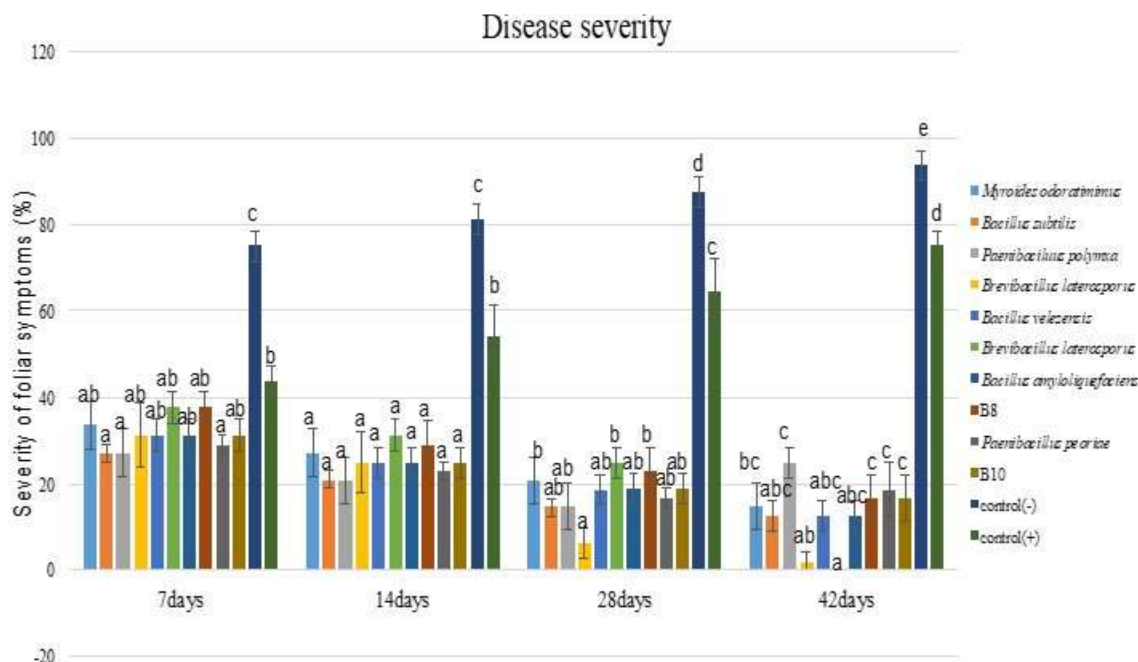

Figure 3. Foliar severity of Fusarium wilt in F1 Rambo tomato variety plants treated with bacterial biocontrols in field conditions. Bars headed with the same letter are not significantly different within the same day (p=0.05) by DMRT.
Source: Authors

Table 3. Activity screening for extracellular enzyme production on different substrates.

Isolate code	Starch	Xylan	Casein	Tributyrate	CMC	Citrate	Phosphate	Catalase
B1	-	-	+++	+++	++	++	++++	++++
B2	-	++++	+++	+++	-	++	++++	++++
B3	-	+++	-	++++	+++	-	-	++++
B4	-	-	++++	+++	+++	++	++++	++++
B5	-	-	+++	-	-	+++	-	++++
B6	-	-	++++	++	-	-	-	++++
B7	-	-	+++	-	-	++	++	++++
B8	-	-	++++	-	-	+++	+++	++++
B9	+++	-	+++	++++	+++	-	+++	++++
B10	-	-	+++	++++	-	+++	++++	++++

(**++**) indicates low activity (growth took ≥ 4 days), (**+++**) denotes moderate activity (growth took 2-3 days), (**++++**) indicates high activity (growth took ≤ 1 day) and **(–)** sign indicates no visible enzyme activity.
Source: Authors

incidence in F1 Rambo tomato plants (p< 0.001) under field conditions in 2021. The performance of the strains on the field experiment was used to select the strains to be used in 2022 field experiment. The isolates significantly reduced average disease incidence (mean ± SE) for both years on day 14 (14.4% ± 3.49), day 28 (15.9% ± 3.44) and day 42 (15.2% ± 2.825) respectively with analysis being done differently on both years. The highest disease incidences in both years were recorded in the negative control plants (Figure 2).

Similarly, the isolates significantly reduced (p<0.001) the foliar disease severity from 14th to 42nd-day post-infection and after treatment in all the treated plants compared to the non-treated plants and the Carbendazium treated plants (Figure 3).

Bacillus. Laterosporus (B6 and B4) were the most effective in the reduction of foliar disease severity in both years (Figure 3). *Bacillus subtilis* and *Bacillus laterosporus* were the most effective in the reduction of disease incidence on fruits and improvement of marketable yields compared to other bacterial biocontrol treatments and the controls (Figure 4). There was a

Table 4. Activity screening against *Fusarium oxysporum* pathogens.

Bacteria antagonists	***Fusarium oxysporum* pathogens**		
Isolate code	**Isolate F88**	**Isolate F89**	**Isolate F110**
B1	++++	+++	++++
B2	++++	++++	+++
B3	+++	++++	++++
B4	++++	++++	++++
B5	++++	++++	+++
B6	+++	++++	++++
B7	++++	+++	++++
B8	++++	++++	++++
B9	+++	++++	++++
B10	++++	++++	+++
B11	-	++	-
B12	+	+	-
B13	++	-	+
B14	+	-	-
B15	+	++	-
B16	++	+	+
B17	+	-	-
B18	++	+	++
B19	-	+	-
B20	++	+	-

(-) no visible inhibition, (+) both organisms stopped growing on contact, (++) the inhibition zone between the pathogen and the antagonist was < 2mm in width, (+++) the inhibition zone was between 2mm and 4mm, (++++) the inhibition zone was > 4mm.
Source: Authors

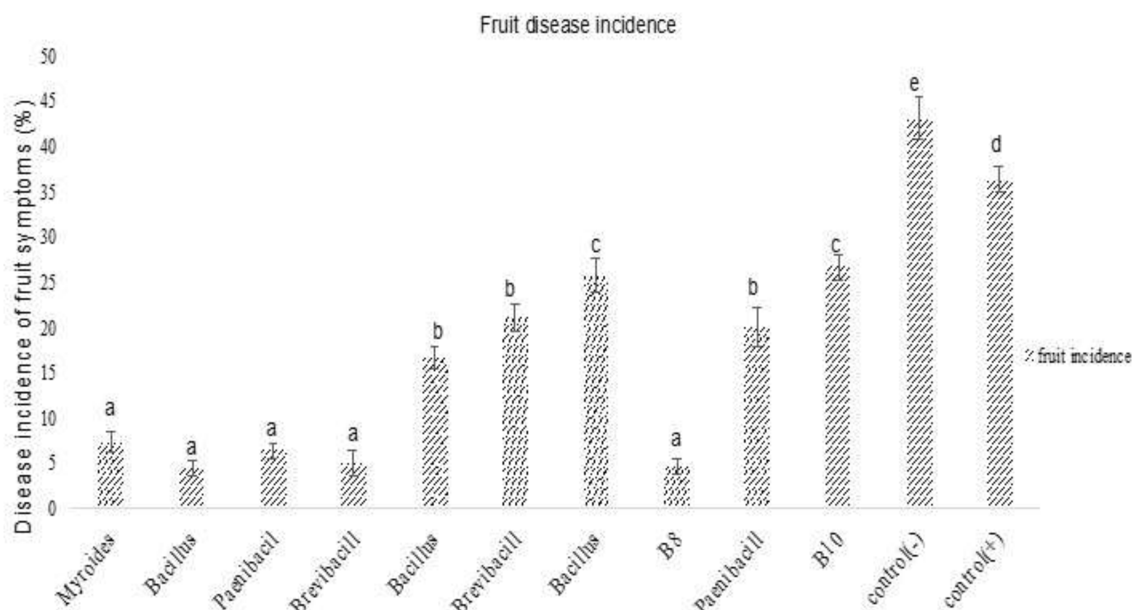

Figure 4. Fruit disease incidence of Fusarium wilt in F1 Rambo tomato variety plants treated with bacterial biocontrols in field conditions. Bars headed with the same letter are not significantly different within the same day (p=0.05) by DMRT.
Source: Authors

significant difference (p<0.001) in disease incidence on fruits among the bacterial biocontrol treatments and the controls in both years (4).

DISCUSSION

The aim of the study was to isolate biocontrol agents (BCAs) effective against the *Fusarium oxysporum* pathogen from F1 Rambo tomato-growing soil. According to Collinge et al. (2022), disease-suppressive soils are an interesting source of BCAs with potential ability against soil-borne pathogens. Fusarium-suppressive soils have not yet been thoroughly studied. Fungal pathogens and bacterial biocontrol isolates were isolated from the same tomato-growing fields previously affected by Fusarium wilt. According to Hashemi et al. (2022), isolating the BCAs from the same ecological niche, as the targeted pathogen is very promising since the organisms are used to the same space, making them very active and effective in competing for space and nutrients. This leads to niche overlap, limiting the growth of the pathogen and protecting the plants.

Morphological characterization for both fungal and bacterial isolates was an important parameter in the preliminary identification of the isolated strains. For the bacterial strains, colony characterization showed wide variability in terms of their color and elevation. Many bacterial isolates were represented by white-pigmented colonies. The type of media used for culturing, stress, and other unfavorable circumstances all affect the formation of pigment in bacterial isolates (Ahmad et al., 2022). Production of pigments by bacterial isolates indicates that the isolates have the ability to produce secondary metabolites (Ahmad et al., 2022). The colony morphology of the fungal isolates was diverse, with pinkish-white colonies depicted by the majority of the isolates. Pigmentation in fungi is greatly influenced by unfavorable conditions, culture media, and other stress factors (Verde-Yáñez et al., 2023). Fungal pigments are reported to be important biological compounds with antimicrobial, antioxidant, and immune-modulating properties (Abo Nahas et al., 2021).

pH 5.0-8.0, sodium chloride concentrations ranging from 0 to 5%, and temperatures ranging from 25-30 °C were the optimum conditions for all the bacterial biocontrol isolates (supplementary Figures 1, 2 and 3). Soil bacteria have been reported to survive in saline agricultural soils where high salinity results from the application of chemical fertilizers and irrigation practices (Numan et al., 2018). In this study, the phylogenetic analysis of the selected bacteria suggests that the majority were affiliated with the genus Bacillus. According to Howell et al. (2022), some of the soil halophilic bacteria that have been reported include the genus Bacillus. Halophilic soil bacteria have been observed to play important roles in the soil, such as promoting plant growth directly and indirectly through the production of phytohormones (Etesami and Glick, 2020). They also act as biocontrol agents of host plant diseases and improve plant nutritional status. Halophilic soil bacteria also possess the ability to solubilize plant phosphates (Bonaventure et al., 2023). Reports from Numan et al. (2018) have also stated that salt-tolerant bacteria isolated from soil promote seedling growth and increase plant biomass by balancing the salinity stress in the soil. The importance of salt-tolerant bacteria makes them sole candidates for research as biocontrol agents.

The growth of the isolates was observed at a pH range of 5-8 (supplementary Figure 2). The results suggest that the selected bacterial isolates were tolerant to a wide range of pH. According to Guo et al. (2019), most soil bacteria work best around neutral pH, although Msimbira and Smith (2020) reported that soil organisms can develop mechanisms to survive environmental variations, thus explaining why some of the selected bacteria did well at pH levels slightly above and slightly below neutral pH.

From the bacterial antagonism in vitro assays, ten bacteria were selected. According to Al-Rashdi et al. (2023), the inhibitory effect observed from the assays could be attributed to diffusible metabolites produced by the bacterial strains. When in contact with the fungi, these metabolites prevent normal radial growth (Wang et al., 2019). Antagonistic bacteria have been reported to produce enzymes that degrade pathogen cells and inhibit their growth (Peral-Aranega et al., 2020). To our knowledge, this is the first study to investigate the antagonistic activity of Bacillus and Myroides genera against *F. oxysporum* in the rhizospheres of F1 Rambo tomatoes grown in humic nitisol soil. The results obtained from this study showed that the bacterial biocontrol isolates had different antagonistic behavior against the *F. oxysporum* pathogen despite being isolated from the same ecological niche. Their ability to produce different hydrolytic enzymes and metabolites could explain the diverse differences among the Bacillus and Myroides isolates from the same ecological niche. The high in vitro antifungal activity shown by the isolates in this study may be attributed to the effect of multiple antifungal metabolites, including lytic enzymes like amylases, proteases, and cellulases (Sunera et al., 2020).

The in vitro results in this study show that Bacillus and Myroides genera can inhibit the growth of *F. oxysporum* isolated from diseased tomato plants.

Ten selected biocontrol agents were moderately effective against Fusarium wilt. The lower leaves of the untreated negative F1 Rambo tomato plants showed severe yellowing and wilting symptoms seven days after Fusarium pathogen infection, eventually leading to death by necrosis. The treated groups, on the other hand, showed varied wilting symptoms, most likely due to bacterial biocontrol agents' competition for nutrients, lowering the prevalence of Fusarium wilt. This implies

that the application of bacterial biocontrol treatments significantly lowered ($p < 0.001$) the wilt severity of the plants (Figure 2). Disease incidence was highest in the untreated negative control plants inoculated with the Fusarium pathogen only and sprayed with sterile distilled water. According to Karthika et al. (2020), biocontrol agents reduce disease incidence by inducing systematic resistance to pathogens and by producing antibiotics that compete with pathogens for ecological niches and nutrients. The ten bacterial biocontrol treatments in this study significantly reduced the foliar disease severity (Figure 3). Brevibacillus laterosporus [B4, B6] had the highest mean efficacy in reducing disease severity with 2.08ab and 0a mean on the 42nd day after treatment, respectively. The reduction efficacy of all treatments was consistent from the 7th to the 42nd day post-*F. oxysporum* infection. Brevibacillus laterosporus [B6] and P. peoriae biocontrols [B9] showed the highest mean reduction in foliar disease incidence as shown in (Figure 2). All bacterial biocontrol treatments showed moderate effectiveness against Fusarium wilt when applied singly. The data demonstrate that bacterial biocontrol antagonists isolated from F1 Rambo tomato-growing soil enhanced the F1 Rambo tomato plant health and yields. Majeed et al. (2018) reported that antagonists used as biocontrol agents suppress the disease-causing soil pathogens by competing at the active sites, thus reducing the intensity of disease development and subsequently stimulating plant growth and yields. Concerning the fruit Fusarium wilt disease incidence, it was unclear why the significant reduction ($p < 0.001$) in disease severity of the plants did not result in a significant reduction in disease incidence in fruits (Figure 4).

Conclusion

The study revealed that the bacteria biocontrol agents isolated were quite diverse based on partial sequence analysis. The isolates exhibited the ability to produce extracellular enzymes, which could aid in understanding their antagonistic activity and effectiveness in inhibiting the growth Fusarium wilt in tomatoes. When employed as treatments in a field experiment, all bacteria isolates significantly decreased the incidence and severity of the disease in tomatoes, leading to an increase in yield as compared to the control group. Therefore, a more comprehensive approach such as combining biocontrol agents in integrated management systems could enhance their efficacy, leading to their exclusive use as biocontrol agents in tomato production.

DESCRIPTION OF SUPPLEMENTARY MATERIAL FILE

The supplementary material file depicts the physiological conditions of the biocontrol isolates. The isolates grew at varying salinities ranging from 0 % to 10 % and with the optimum growth of all isolates recorded at a 5% NaCl concentration (Supplementary Figure 1, Isolates growth rate at different salt concentration). Optimum pH for growth was observed between pH 5 and pH 6 and the least growth at pH 4 and pH 12 (Supplementary Figure 2, Isolates growth rate at different pH levels). The temperature range for growth ranged between 25 and 35°C (Supplementary Figure 3 Isolates growth rate at different temperature ranges).

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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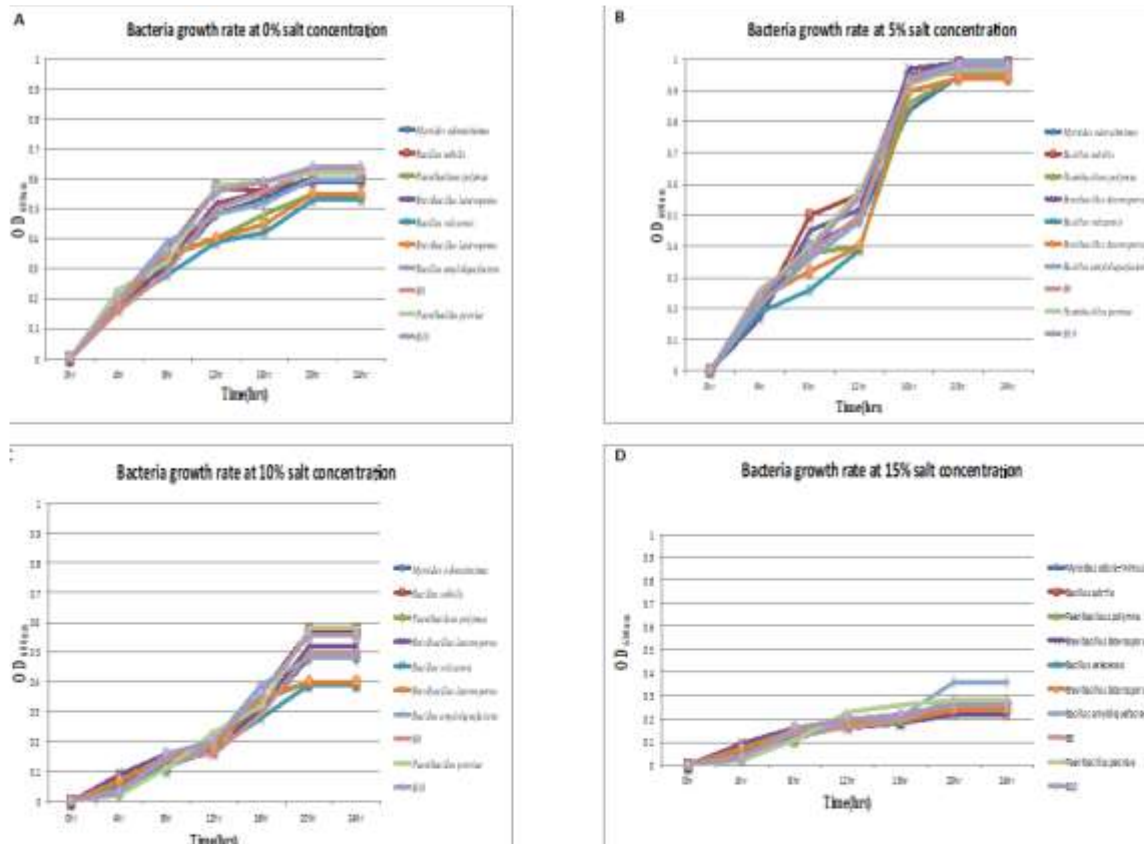
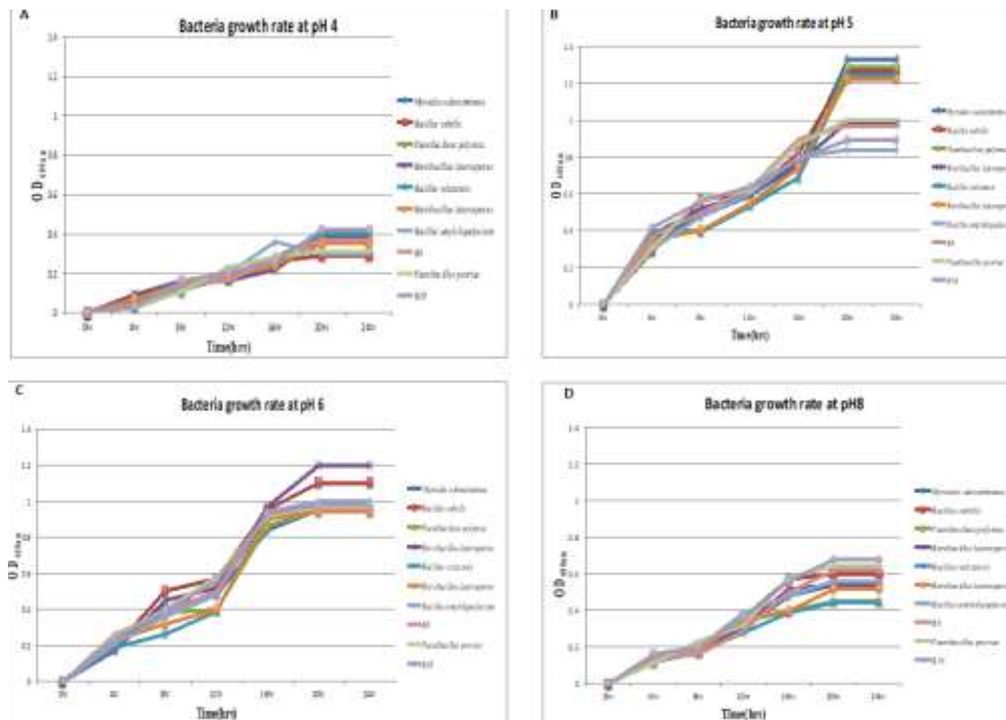
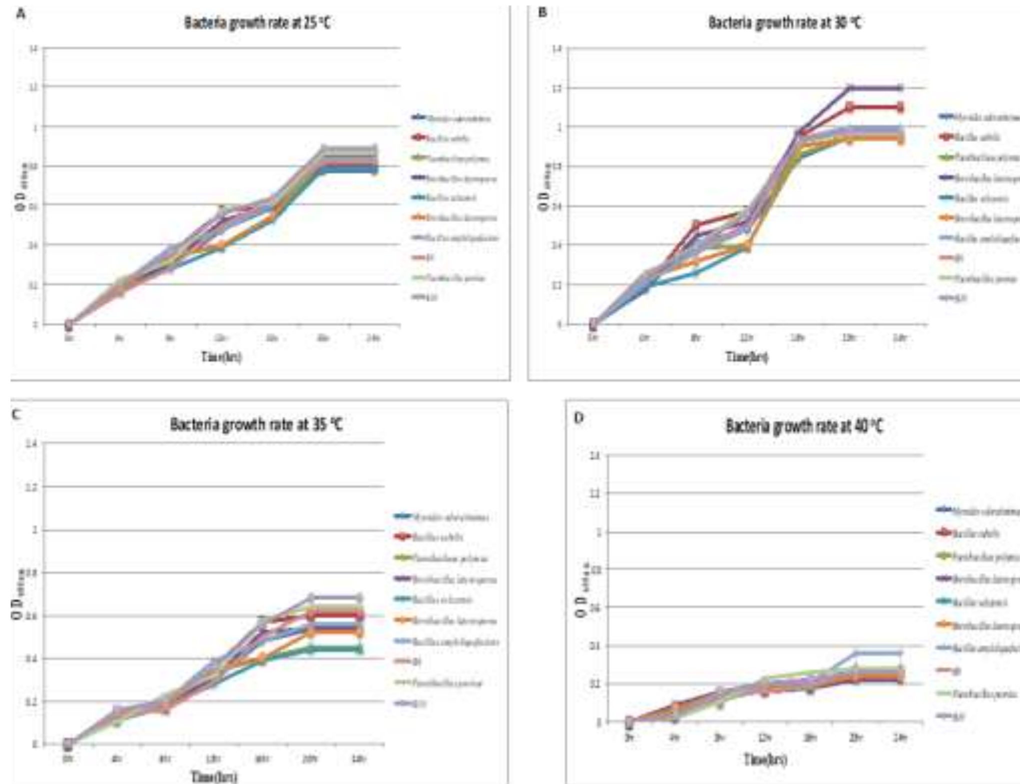


Supplementary Figure 1. Isolates at different salt concentrations. Growth at 0% salt concentration (A); Growth at 5% salt concentration (B); Growth at 10% salt concentration (C); Growth at 15% salt concentration (D).



Supplementary Figure 2. Growth at different pH ranges, Growth at pH 4(A); Growth at pH 5(B); Growth at pH 6(C); Growth at pH 8(D).



Supplementary Figure 3. Growth at different temperature ranges. Growth at 25ºC (A); Growth at 30ºC (B); Growth at 35ºC (C); Growth at 40 ºC (D).

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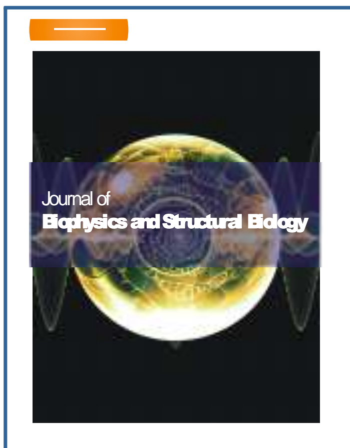

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