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Synanthropic rodents and shrews are reservoirs of zoonotic bacterial pathogens and act as sentinels for antimicrobial resistance spillover in the environment: A study from Puducherry, India

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ABSTRACT

Antimicrobial resistance (AMR) is a global public health concern and needs to be monitored for control. In this study, synanthropic rodents trapped from humans and animal habitats in Puducherry, India, were screened as sentinels for bacterial pathogens of public health importance and antimicrobial resistance spillover. From the trapped rodents and shrews ($n = 100$) pathogens viz., *Staphylococcus sp, E. coli* and *Salmonella sp* were isolated from oropharyngeal and rectal swabs on Mannitol salt, Mac Conkey and Xylose lysine deoxycholate media respectively. The AMR genes in these isolates were screened by PCR. A total of 76, *S. aureus* and 19, *Staphylococcus* non *aureus* were isolated. *E. coli* was isolated in 89 samples and among the *Salmonella sp* ($n = 59$), 16, were *S. enteritidis* and 29, were *S. typhimurium.* A total of 46 MRSA isolates with *mec A* ($n = 40$) *and mec C* ($n = 6$) were detected. Also, 36.84% and 5.3% *Staphylococcus* non *aureus* isolates were tested to have *mec A* and *mec C* genes. AMR genes encoding ESBL [bla_{TEM} in 21, bla_{SHV} in 45 and bla_{CTX-M} in 11] was tested positive in 77 *E. coli* isolates. Among, *Salmonella* isolates 44/45 were screened to have AMR genes [*tet* in 13, *sul3 & sul4* in 20 and *qnrA* in 11]. Antibiotic sensitivity test confirmed the antimicrobial resistance. Isolation of pathogens of public health importance and demonstration of genetic elements conferring antimicrobial resistance in the synanthropic rodents confirms that they act as reservoirs and appropriate sentinels to monitor AMR spillover in the environment.

1. Introduction

Rodents make up around 42% of all known mammalian species with high diversity and they are proven reservoirs for pathogens of public health and animal importance [1]. The faeces, saliva, urine and vectors infesting them act as potential sources of infections to human and animals by contaminating the water, food and feed supplies intended for human and animal use [2]. Rodents, by virtue of colonising the sewerage around the human habitats, feed on human and animal excreta and acquire the multidrug resistant pathogens [2,3]. Increased antibiotic resistance due to antibiotic overuse, may have a great impact on human health especially in an environment where animals and humans share the same ecosystem. As a result, the rodents have become a potent reservoir for hosting, maintaining and dispersing multidrug-resistant bacteria to humans and animals [3].

The *mec* genes found on the staphylococcal cassette chromosome *mec* (SCCmec), is a diverse and vast transmissible genetic element that contributes to antimicrobial resistance. There are 14 such *SCCmec* elements reported. *Staphylococcus aureus* Penicillin-binding protein (PBP 2a) with a poor affinity for β-lactams, encoded by *mec A* gene, was the major variant reported to confer methicillin resistance in *S. aureus* [4]. Later in 2011, *mecC*, a homologue to *mecA*, was reported in MRSA in

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human samples from Ireland. Later, several reports of *mecC* arose globally from humans, animals and the environment [5]. The global spread of *mecC* was attributed to maritime travel and dispersal by commensal rodents [6].

World Health Organization (WHO report, 2014) has designated *E. coli* as a critical antimicrobial-resistant bacteria that can cause fatal illnesses such as pneumonia and septicaemia [7]. Extended-spectrumβ-lactamases (ESBL) producing *E. coli* strains are potentially known pathogens that are resistant to the majority of β-lactam antibiotics [8]. ESBL are plasmid encoded enzymes, which hydrolyses the β – lactam antibiotics including penicillin, cephalosporins and aztreonam. ESBL are further clustered into main three types, bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ [9]. *E. coli* with ESBL is acquired from the environment and has been reported in free-roaming rodents, shrews, bats, and wild birds [10].

The genus *Salmonella* has been associated with enteric diseases in humans, which have been classified into typhoidal illness and non-typhoidal salmonellosis (NTS). NTS, is one of the major aetiologies of enteritis globally, causing 93 million enteric infections with a mortality of 155,000 deaths due to diarrhoea every year [11,12]. Isolates of NTS with genes conferring antimicrobial resistance have been reported against antibiotics such as tetracyclines (*tet*), sulphonamides (*sul3 & sul4*), and fluoroquinolones (*qnrA*), nalidixic acid (*nal*) and third generation cephalosporins (*CEP*) [13].

To date, there are no reports on the spectrum of antimicrobial resistance in bacterial pathogens isolated from synanthropic rodents and shrews in India. Hence, the present work was undertaken to understand the dynamics of the spillover of MDR bacterial pathogens into the environment due to overuse of antibiotics in human and animal treatments.

2. Materials and methods

2.1. Collection and processing of samples

This study was carried out in the Puducherry district, UT of Puducherry, India, which lies between 11'x55'x 48" Northern latitude and 79'x49'x 48″ Eastern longitude with an altitude of 27 ft above the mean sea level. The district is a plain land with almost no mountains and forest. Trapping of rodents was carried out in randomly selected villages of Puducherry district for a period of six months from July to December 2022 (Fig. 1). The institutional Animal Ethics Committee approved the study (IAEC/ICMR-VCRC/2018/2). A total of 100 animals were trapped alive in and around Puducherry. The trapped animals were euthanized by exposing them to a chamber saturated with carbon dioxide. The euthanized animals were identified according to their morphological characteristics using standard taxonomical keys [14]. A single oropharyngeal and two anal swabs were collected from each animal using sterile swabs (Himedia, India). The oropharyngeal swabs were transferred individually into the tubes with Luria broth enriched with 7.5% sodium chloride. Each anal swab was individually placed in tubes with Luria broth and selenite F broth respectively. These samples were transported to the Department of Veterinary Microbiology, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry under the cold chain, for the isolation and detection of the bacterial pathogens and AMR genes by PCR.

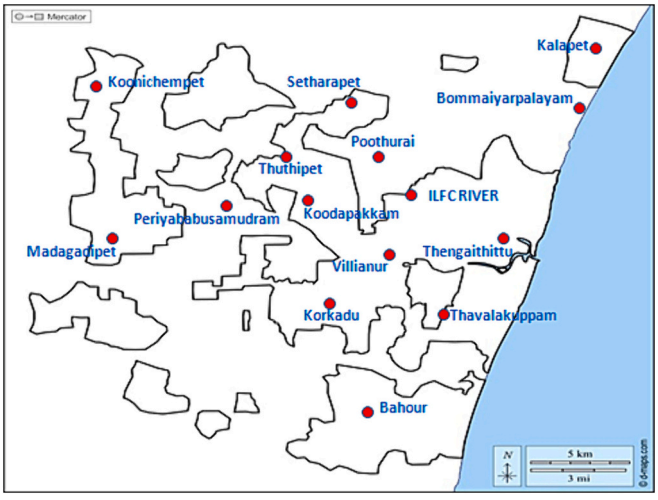

Fig. 1. The map representing the sites of rodents trapping in Puducherry. The red dot indicates the villages, where the rodents were trapped. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Isolation of the bacterial pathogens

All the 100 oropharyngeal samples collected in Luria broth enriched with 7.5% sodium chloride were incubated at 37 °C for 16 to 18 h. Individual inoculum from the above was streaked onto Mannitol salt agar to obtain the mannitol fermenting golden yellow colonies. Similarly, 100 anal swabs collected in Luria broth were incubated and individual inoculum was streaked on the MacConkey agar to obtain the lactose fermenting colonies. The anal swabs collected in Selenite F broth were streaked on Brillant Green (BG) agar to isolate pink-coloured colonies. The pink-coloured colonies obtained in BG agar were propagated on XLD agar to obtain growth of the black-centred red-coloured colonies, specific for *Salmonella* sp.

2.3. Preparation of template DNA

The template DNA from the colonies isolated in Mannitol salt agar, MacConkey agar and XLD agar was extracted by boiling a loop full of colonies for ten minutes followed by snap chilling [15].

2.4. Identification of Staphylococcus spp. and genes conferring methicillin resistance by PCR

The bacterial isolates derived from Mannitol salt agar were primarily screened for *Staphylococcus* sp. by amplifying 16*S rDNA* partially [16] and then differentiated into *S. aureus* and *Staphylococcus* non *aureus* by amplifying the partial sequences of *nuc* gene [17] specific for *S. aureus*. Uniplex PCR was followed to screen genes conferring methicillin resistance viz. *mec A* [18] and *mec C* [19] in all the *Staphylococcus* isolates.

2.5. Detection of Escherichia coli and the presence of Extended Spectrum Beta Lactamase genes (ESBL) by PCR

The bacterial DNA extracted from the lactose fermenting colonies on Mac Conkey agar were screened for *E. coli* targeting the *uspA* gene [20]. All the *E. coli* positive isolates were screened for Extended spectrum β-lactamase AMR genes viz. bla_{TEM} [21], bla_{SHV} [22] and $bla_{CTX\text{-}M}$ [23].

2.6. PCR Detection of Salmonella sp and non-typhoidal Salmonella sp. and their AMR genes

The bacterial DNA extracted from the black-centred red-coloured colonies on XLD agar were primarily screened for *Salmonella sp* targeting *invA* gene [24]. Upon confirmation of *Salmonella* sp., screening for *S. enteritidis* and *S. typhimurium* was done by specific amplification of *spvC* [25] and *fliC* [26] genes respectively. AMR genes such as *tet* [27], *sul3 & sul4* [28] and *qnrA* [29] were screened by PCR in all the non-typhoidal Salmonella isolates.The details of the primers used for the identification of the bacterial pathogens, and their AMR genes, thermal cycling conditions, target amplicon sizes and the relevant references are summarised Table 1.

Table 1
Details of the primers and annealing temperatures for the detection of the pathogen and antibiotic resistance genes harboured by them.

Bacterial Pathogens	Targeted gene	Sequences	Annealing temperature	References
Staphylococcus sp	*16S rRNA*	AACTCTGTTATTAGCGAAGAACA CCACCTTCCTCCGGTTTGTCACC	50 °C for 1 min	[16]
S. aureus	*nuc*	GCGATTGATGGTGATACGGT AGCCAAGCCTTGACGAACTAAAGC	55 °C for 30 s	[17]
Methicillin resistance	*mec A*	TCCAGATTACAACTTCACCAGG CCACTTCATATCTTGTAACG		[18]
	mec C	GAAAAAAAGGCTTAGAACGCCC GAAGATCTTTTCCGTTTTCAGC	50 °C for 1 min	[19]
E. coli	*uspA*	CCGATACGCTGCCAATCAGT ACGCAGACCGTAAGGGCCAGAT	60 °C for 1.5 mins	[20]
ESBL-producing *E. coli*	bla_{TEM}	ATGAGTATTCAACATTTCCG CTGACAGTTACCAATGCTTA	56 °C for 1 min	[21]
	bla_{SHV}	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG		[22]
	bla_{CTX-M}	CAATGTGCAGCACCAAGTAA CGCGATATCGTTGGTGGTG	59 °C for 30 s	[23]
Salmonella sp	*invA*	GCCAACCATTGCTAAATTGGCGCA GGTAGAAATTCCCAGCGGGTACTG	55 °C for 45 s	[24]
Salmonella enteritidis	*spvC*	GCCGTACACGAGCTTATAGA ACCTACAGGGGCACAATAAC	57 °C for 45 s	[25]
Salmonella typhimurium	*fliC*	CGGTGTTGCCCAGGTTGGTAAT ACTGGTAAAGATGGCT	55 °C for 45 s	[26]
Tetracycline resistance	*Tet*	GCACTTGTCTCCTGTTTACTCCCC CCTTGTGGTTATGTTTTGGTTCCG	53 °C for 1 min	[27]
Sulphonamides resistance	*sul3 & sul4*	TCAACATAACCTCGGACAGT GATGAAGTCAGCTCCACCT	60 °C for 40 s	[28]
Fluoroquinolone resistance	*qnrA*	TCAGCAAGAGGATTTCTCA GGCAGCACTATGACTCCCA	53 °C for 30 s	[29]

2.7. Sequencing and analysis of the PCR products

The amplified PCR products from the representative bacterial isolates and the AMR genes were gel extracted and subjected to Sanger sequencing (Genetic Analyzer 3130XL, Applied Biosystems, USA). The nucleotide sequences were BLAST analysed to confirm the gene identity (*http://www.ncbi.nlm.nih.gov/BLAST*). The nucleotide sequences were deposited in GenBank.

2.8. Phenotypic confirmation of antibiotic Resistance

Representative samples from the isolates tested to have AMR genes were used to carry out the antibiotic sensitivity test (ABST) using the antibiotic impregnated discs procured from Hi Media Laboratories, India. ABST was carried out following the standard procedure [30]. Methicillin resistance in *S. aureus* was confirmed using Methicillin (M) 5 μg. For ESBL *E. coli*, five indicator antibiotics such as CPD - Cefpodoxime (30 μg), CEC - Cefotaxime + Clavulanic acid (30 μg), CAC -Ceftazidime + Clavulanic acid (30 μg), CTR- ceftriaxone (30 μg), and AT- aztreonam (30 μg) were used. Antibiotic resistance in *Salmonella*, was assessed using Tetracycline 30 μg, Enrofloxacin 5 μg, Sulphamethoxazole + trimethoprim 10 μg were used. Resistance to at least one indicator antibiotic was considered as positive screening test (CLSI, 2021) [31].

3. Results

In our study, a total of 100 animals were trapped, of which 75 were shrews (*Suncus murinus*) and 25 were rodents (*Rattus rattus*). From 95/100 (95%) oropharyngeal swabs, we observed the growth of golden yellow colonies in Mannitol salt agar indicating the presence of *Staphylococcus* sp. Further molecular confirmation was carried out by the successful amplification of *16S rRNA* from all the 95 isolates. However, only 76/95 (80%) isolates were found to harbour the thermonuclease gene (*nuc*), specific for *S. aureus*. The rest 19 isolates (20%) were identified as *Staphylococcus* non *aureus*.

Molecular screening for methicillin resistance indicated that 40 (52.6%) (10 rodents and 30 shrews) and 6 (7.9%) (2 rodents and 4 shrews) among 76 *Staphylococcus aureus* isolates, were tested to harbour *mec A* and *mec C* genetic elements respectively signifying the presence of MRSA in these synanthropic rodents/shrews. Further screening of the 19 *Staphylococcus* non *aureus* isolates, indicated the occurrence of *mec A* and *mec C* genetic elements in 7 (36.8%) (2 rodents and 5 shrews) and 1 (1 shrew) (5.4%) isolates respectively. A total of five *S. aureus* (1 rodent and 4 shrews) isolates were found to harbour both *mec A* and *mec C* genetic elements conferring resistance to Methicillin.

The growth of characteristic dry, pink lactose fermenting colonies on the Mac Conkey's agar was witnessed in 89/100 (89%) anal swabs, suggesting the possible presence of *E. coli*. Further, confirmation of *E. coli* in all the 89 isolate was done by PCR amplification of the universal stress protein A (*uspA*) gene. Among the 89 *E. coli* isolates,77 (86.5%) were identified as ESBL producers by PCR.

Altogether, 21 (23.6%) (6 rodents and 15 shrews), 45 (50.6%) (8 rodents and 37 shrews) and 11 (12.5%) (2 rodents and 9 shrews) of the isolates were found to carry ESBL genes viz. bla_{TEM}, SHV and bla_{CTX-M}, respectively. The presence of both bla_{TEM} and SHV was witnessed in 13 isolates (16.8%) (2 rodents and 11 shrews), bla_{SHV} and bla_{CTX-M} in7 isolates (9.1%) (1 rodent and 6 shrews) whereas the occurrence of all 3 ESBL genes (bla_{TEM}, bla_{SHV} and bla_{CTX-M}) was found in 2 (2.6%) isolates (1 shrew and 1 rodent).

The culture of anal swabs (n = 100) in XLD agar, resulted in the growth of black-centred red-coloured colonies characteristic of *Salmonella* sp. from 59 (59%) swabs. PCR amplification of the *invA* gene in all the 59 isolates confirmed the presence of *Salmonella* spp. Further the *Salmonella enterica* serovar *enteritidis* and *Salmonella enterica* serovars *typhimurium* present among the 59 isolates were confirmed by the amplification of *spvC* and *fliC* genes in 16 (27.2%) and 29 (49.15%) isolates respectively. Among the 59 isolates, 44 (97.78%) were found positive for the presence of antimicrobial resistance genes. Altogether, 13 (28.8%) (3 rodents and 10 shrews), 20 (44.44%) (5 rodents and 15 shrews) and 11(24.44%) (2 rodents and 9 shrews) isolates were found to carry AMR genes viz. *tet, sul3& sul4* and *qnrA* conferring resistance to tetracyclines, sulphonamides and fluoroquinolones respectively. In addition, the occurrence of a combination of AMR genes was also observed in these isolates, *tet* and *sul3 & sul4* in six isolates (13.63%) (2

rodents and 4 shrews), *tet* and *qnrA* in two isolates (4.5%) (2 shrews), and the presence of both *sul3 & sul4* and *qnrA* was found in one (2.27%) (1 shrew) isolate (Table 2). The nucleotide sequences of the genes amplified for identification and confirmation of bacterial pathogens and the antimicrobial resistance genes were aligned using ClustalW software and deposited in the GenBank, the details of the accession ID assigned to the respective gene are given in Table 3. Antibiotic sensitivity test using selected isolates harbouring the genes associated with antimicrobial resistance confirmed the antibiotic resistance to methicillin in *S. aureus* (Fig. 2A), Cefpodoxime, Cefotaxime + Clavulanic acid, Ceftazidime + Clavulanic acid, Ceftriaxone, and Aztreonam, in *E. coli* (Fig. 2B) and tetracycline, enrofloxacin and sulphamethoxazole+ trimethoprim in *Salmonella* sp. (Fig. 2C).

4. Discussion

AMR is a multifaceted problem and its alarming rise in human and veterinary medicine coupled with its spread to the environment poses a grave challenge containing AMR. Hence, the spread of AMR in the environment is monitored by assessing the presence of AMR pathogens in sentinels such as wild animals [32] and rodents [33]. There is no data on AMR contamination in the environment through rodents and shrews from India. Hence, we assessed the spill over of AMR pathogens in the environment using the synanthropic rodents and shrews as sentinels.

MRSA, being the major nosocomial AMR pathogen, also emerged as a community-associated infection and is the major health care burden in Asia [33]. Hence, we screened the presence of MRSA from the oral swabs collected from the trapped rodents and shrews and found that they harboured *S. aureus* (80%, $n = 76$) and *Staphylococcus* non *aureus* (20%, $n = 19$). The occurrence of *S. aureus* in the trapped rodents and shrews was 30% and 70% respectively. The prevalence of 80% of *S. aureus* in the trapped mammals in our study is much higher than the reports of 7.1% [34] to 41.86% [35] in free-living wild animals and rodents trapped in pig farms in Spain and Netherlands respectively. Among the *S. aureus* isolates, carriage of AMR genetic elements such as *mec A* and *mec C* were evident in 40 and 6 isolates respectively. The presence of both *mec A* and *mec C* genetic elements in the trapped rodents confirms the MRSA contamination in the environment from human and livestock sources [36].

Although, *S. aureus* with *mecC* genetic element in milk was first reported in India [37], we report the isolation of MRSA with *mec C* from rodents and shrews for the first time in India. The prevalence of MRSA in shrews and rodents in our study is 73.9% and 26.3% respectively. Ge et al., 2019 [38] reported a higher prevalence of MRSA in *Rattus norvegicus* (5.6%) than in shrews 0.5%, while we have observed a higher positivity of MRSA in shrews (74%) than in rodents (26%). The difference in the MRSA positivity across species could be attributed to factors such as season, geographical location and sample source [39].

The detection of AMR genetic elements *mec A* and *mec C* in *Staphylococcus* non *aureus* isolates was very interesting. Of the 19 *Staphylococcus* non *aureus* isolates, 7/19 (36.84%) and 1/19 (5.26%) isolates tested positive to harbour *mecA* and *mecC* genes. Phumthanakorn et al., 2022 [40] reported that among the 185 coagulase-negative *Staphylococci* isolated from dogs and cats, 25(13.4%) were tested to harbour the genetic elements conferring resistance to methicillin. Co-habitation of different microbial communities in a common niche leads to the horizontal transfer of AMR genetic elements to the cohabiting microbes [40]. Haaber et al.,2017 [41] reported that the coagulase-negative *Staphylococci* spp. acquired antimicrobial resistance genes by horizontal transfer from *S. aureus*.

The prevalence of 89% of *E. coli* found in the trapped rodents and shrews in our study is slightly higher than the 83.8% reported for rodents at the local zoo in Trinidad and Tobago [41] and 19.88% in rats in the east region of Gabon in Central Africa [42], Vancouver, Canada (41.5%), Berlin, Germany (58.2%), and Nairobi, Kenya (66.7%), [36] [3,43,44]. In contrast, Nhung et al.,2015 [45] reported an incidence of 90.91% of *E. coli* isolates from synanthropic rats and shrews in Vietnam. Among the 89 *E. coli* isolates, 77 (86.51%) were confirmed to harbour genetic elements such as bla_{TEM} (23.59%), bla_{SHV} (50.56%) and $bla_{CTX\text{-}M}$ (12.35%) respectively. Literak et al., 2009 [45] reported that 2.5% of *E. coli* isolated from black rats in Africa were ESBL-producers. However, Burriel et al., 2008 [46] reported that 61.5% of the *E. coli* isolated from *R. norvegicus* in a port from Greece were tested resistant to twelve antimicrobial agents. Gilliver et al., 1999 [47] reported that 90% of the coliforms isolated from wild rodents in northwest England were beta-lactam resistant.

In addition, some isolates were tested to harbour more than one genetic element encoding ESBL. A total of 13 (16.88%) isolates were tested positive for both bla_{TEM} and bla_{SHV} genes, 7 (9.09%) isolates for bla_{SHV} and $bla_{CTX\text{-}M}$ genes, and 2 (2.59%) isolates were tested to carry bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ genes. Out of 77, 22 (28.57%) were found to be positive for more than one AMR gene. A scoping review reported that the order of prevalence of ESBL genotype globally in rats were $bla_{CTX\text{-}M}$ (61%), bla_{TEM} (26%) and bla_{SHV} (13%) [48]. Ho et al., 2015 [48] reported a prevalence of 7.7% and 13.9% of $bla_{CTX\text{-}M}$ producing multidrug-resistant *E. coli* in black and brown rats trapped in 18 districts of Hong Kong. Onanga et al., 2020 [42] reported human-origin *E. coli* carrying $bla_{CTX\text{-}M}$ genes in rats in Gabon, Central Africa.

By $bla_{CTX\text{-}M}$ gene characterization, the wild rodents in Hong Kong were identified to have acquired *E. coli* from humans and livestock [48]. Molecular clonal diversity characterization of the $bla_{CTX\text{-}M}$ gene, would have given an insight into the source of *E. coli*. However, this remains as the major limitation of our study. However, the presence of the genetic elements conferring resistance to beta-lactams in our study is in line

Table 2
Pathogens isolated from rodents/shrews and results of the molecular screening of genetic elements conferring antimicrobial resistance.

The animal reservoir	*Staphylococcus* spp. and the AMR genetic element tested positive in the rodent/shrew screened					
	Staphylococcus aureus		*Staphylococcus* non-*aureus*		*Staphylococcus aureus*	
	mec A	*mec C*	*mec A*	*mec C*	*mec A and mec C*	
Rattus rattus	10	2	2	0	1	
Suncus murinus	30	4	5	1	4	
Total	40	6	7	1	5	
The animal reservoir	***E.coli* and the AMR genetic element tested positive in the rodent/shrew screened**					
	bla_{TEM}	**bla_{SHV}**	**$bla_{CTX\text{-}M}$**	**bla_{TEM} and bla_{SHV}**	**bla_{SHV} and $bla_{CTX\text{-}M}$**	**bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$**
Rattus rattus	6	8	2	2	6	1
Suncus murinus	15	37	9	11	1	1
Total	21	45	11	13	7	2
The animal reservoir	***Salmonella* spp. and the AMR genetic element tested positive in the rodent/shrew screened**					
	tet	***sul 3 and sul 4***	***qnrA***	***tet* and *qnrA***	***tet* and *sul 3 & sul 4***	***sul 3 & sul 4 and qnrA***
Rattus rattus	3	5	2	–	2	–
Suncus murinus	10	15	9	2	4	1
Total	13	20	11	2	6	1

Table 3
Genbank accession ID of the bacterial pathogens and their AMR gene sequences.

Pathogen	*Staphylococcus sp*	*S. aureus*	MRSA	MRSA		
Gene	***(16S rRNA)***	***(nuc)***	***(mec A)***	***(mec C)***		
Genbank accession ID	OQ979122, OQ979123	OQ992661, OQ992662	OQ992663, OQ992664	OQ992664		
Pathogen	***E. coli***					
Gene	***(uspA)***	**bla$_{TEM}$**	**SHV**	**bla$_{CTX-M}$**		
Genbank accession ID	OQ872769	OR066219	OR066218	OR066217		
Pathogen	***Salmonella spp***	***S. enteritidis***	***S. typhimurium***			
Gene	***(invA)***	***(spvC)***	***(fliC)***	***tet***	***sul3 & sul4***	***qnrA***
Genbank accession ID	OR066220, OR066221	OR066224, OR066225	OR066222, OR066223	OQ992668, OQ992669	OQ992665, OQ992666	OQ992667

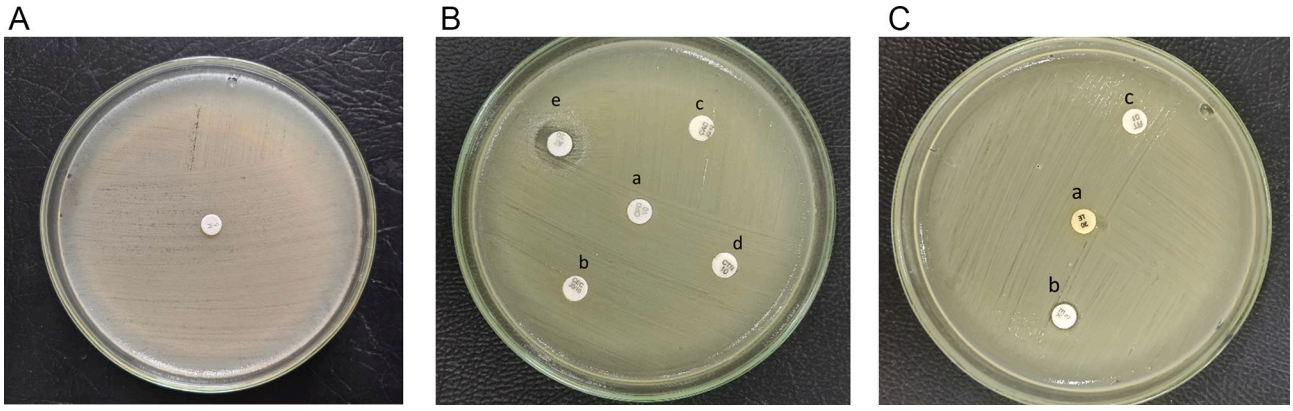

Fig. 2. A. Phenotypic confirmation of methicillin resistance (5 μg) in *Staphylococcus aureus* isolated from a shrew. The isolate was tested by PCR to harbour the AMR genes *mec A* and *mec C*.
B. Demonstration of resistance to (a) Cefpodoxime (30 μg), (b) Cefotaxime + Clavulanic acid (30 μg), (c) Ceftazidime + Clavulanic acid (30 μg), (d) Ceftriaxone (30 μg), and (e) Aztreonam (30 μg) in *E.coli,* harbouring genetic elements (bla$_{TEM}$. bla$_{SHV}$ and bla$_{CTX-M}$).
C. A non typhoidal *Salmonella* isolate tested to harbour genetic elements such as *tet*, *qnrA* and *sul* genes, exhibiting complete resistance to antibiotics such as (a) Tetracycline, (b) Enrofloxacin, (c) Sulphamethoxazole + trimethoprim.

with the spillage of antimicrobial resistance from human and animal pathogens to the environment. Guenther et al., 2012 [44] reported that rodents harbouring multi resistant *E. coli* are regarded as emerging zoonotic threats to humans. Thus highlighting the risk of transmission of these pathogens to human and animals in our study sites.

In EU/EEA, the salmonellosis was reported to rank second among the zoonoses with a report of 91,857 cases, during 2018 [49]. Interestingly, we observed that 59% of the rodents/shrews in our study to harbour *Salmonella* spp. However, there are reports of nil [50], and high [43] prevalence of *Salmonella* spp. in rodents. Further molecular typing indicated the presence of NTS pathogens *Salmonella enterica serovar enteritidis* (27.7%) and *Salmonella enterica serovar typhimurium* (49.15%). The major mode of transmission of NTS is through contaminated food [13]. *Salmonella enterica serovar enteritidis* (28.57%) and *Salmonella enterica serovar typhimurium* (63.49%) were detected in rats inhabiting poultry farms in South Africa [51]. *Salmonella enterica* serovars *enteritidis* and *Salmonella enterica* serovar *typhimurium* are the common pathogenic *Salmonella* spp. reported in 5 developing countries like South Africa, Egypt, Indonesia, India and Romania and the major source of NTS infection in human is the contamination of food with human/ animal wastes [52]. NTS are of public health importance as it is associated with non-invasive enteritis and fatal bloodstream infections in children and adults [53].

We first documented the antimicrobial resistance in *Salmonella* sp. isolated from rodents and shrews in Puducherry, India. In that, 44/45 (97.78%) were found to carry antimicrobial resistance genes. Altogether, 13/45 (28.88%), 20/45 (44.44%), and 11/45 (24.44%) of the isolates were found to carry AMR genes viz. *tet*, *sul3 & sul4* and *qnrA* respectively. Himsworth et al., 2018 [43] studied the prevalence of AMR in *Salmonella* sp. isolated from wild urban Norway and black rats from Canada. Out of 633 rodents screened, 13 were positive for *Salmonella sp*, out of which 15.38% ($n = 2$) were tested resistant to sulphonamides and 7.69% ($n = 1$) to tetracycline.

Sulfonamides are widely used as antibiotics for the treatment of infectious diseases in human, animals. It is also commonly used for water treatment in poultry houses, thus making sulfonamides as a major environment pollutant in the poultry house effluents [54]. Balakrishnan et al., 2016 [30] isolated fluoroquinolones resistant *E. coli* isolates of animal origin from Puducherry. In contrast, Ramatla et al., 2022 [55] found a higher percentage of *qnrA* and *tet* gene resistance in rodents in South Africa. These quinolones are being used to treat human Salmonellosis for over 4 decades. Fluoroquinolones have been considered as one of the last options for the treatment of *Salmonella sp* [56]. The misuse and overuse of quinolone antibiotics in both veterinary and human medicine have led to the emergence of resistance.

The presence of AMR genes and confirmation of its phenotypic expression by ABST in the pathogens isolated from rodents and shrews clearly indicates the irrational use of antibiotics in veterinary and human treatments. Which eventually lead to the spillover of MDR pathogens in the environment. As rodents and shrews, mostly populate in the sewage in and around human habitations, they may have picked up the multi-resistant strains from the human and animal excreta [44]. Also, they may act as surrogate indicators of the environmental spillover of AMR pathogens.

In our previous studies at Puducherry, we observed that the rodents and shrews lives in closer proximity with the human habitats and animal sheds. The synanthropic adaptation of these rodents with human and livestock is mainly facilitated by the availability of food from the human habitation, animal sheds and shelter from the sewage system and vegetative environment. Though shrews are insectivores, they get trapped due to their inquisitive exploration to the aroma of the bait used in the traps. Rodents and shrews has been incriminated as animal

reservoirs for scrub typhus, leptospirosis, salmonellosis, cryptosporidiosis etc. [57]. Human cases of Scrub typhus [58], Leptospirosis [59] and Salmonellosis [60] are commonly reported in Puducherry. Our molecular surveys have also confirmed the presence of *O. tsutsugamushi* in rodents/shrews and their ectoparasitic mite vectors [61–63], Leptospirosis (unpublished data) and Salmonellosis (current study). Therefore, the enzootic maintenance of pathogens of public health importance and AMR pathogens (derived from human and animals) in these rodents/shrews poses a great risk of transmission through their secretions and excretions to human and livestock. Hence integrated rodent control using rodenticides, implementation of rodent proofing measures and eco-system modifications around human residences and animal's sheds could largely contribute to the break in chain of disease transmission to human and animals. To curtail the development of superbugs, necessary stewardship measures have to be undertaken for judicious use and disposal of antibiotics.

5. Conclusions

In conclusion, we report that 89% of the rodents and shrews were tested to harbour more than one zoonotic bacterial pathogens, whereas 55% of them were found to carry multiple AMR genes posing a potential health risk to human, livestock and pet animals. This study emphasizes the screening of rodents/shrews as sentinels to test the spillover of antimicrobial resistance in the environment. The natural prevalence of the other zoonotic pathogens and the current findings of AMR pathogens in these animals underscores the potential risk to human and animal health. Hence, we strongly recommend, implementation of preventive measures encompassing "One Health" approach to curtail the rodent/shrew transmitted pathogens.

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CRediT authorship contribution statement

Nivedha Devanathan: Methodology, Writing – original draft. **Hirak Kumar Mukhopadhyay:** Conceptualization, Supervision, Writing – original draft. **Krishan Kumar Sihag:** Methodology, Writing – original draft. **A. Terence Nathan:** Methodology, Writing – original draft. **Aravindasamy Chakkaravarthi:** Methodology. **Lakshmy Srinivasan:** Methodology. **Mouttou Vivek Srinivas:** Methodology. **Jayalakshmi Vasu:** Methodology. **Venkatesa Perumal Shanmugam:** Supervision. **Manju Rahi:** Conceptualization, Supervision. **Panneer Devaraju:** Conceptualization, Funding acquisition, Supervision, Writing – original draft.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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References

[1] The Encyclopedia of Mammals, 3rd ed, Oxford University Press, 2006 (ISBN 978-0-19-920608-7.).
[2] B.G. Meerburg, G.R. Singleton, A. Kijlstra, Rodent-borne diseases and their risks for public health, Crit. Rev. Microbiol. 35 (2009) 221–270, https://doi.org/10.1080/10408410902989837.
[3] F.M. Gakuya, M.N. Kyule, P.B. Gathura, S. Kariuki, Antimicrobial Resistance of bacterial organisms isolated from rats, East Afr. Med. J. 78 (2001) 646–649, https://doi.org/10.4314/eamj.v78i12.8934.
[4] S.J. Peacock, G.K. Paterson, Mechanisms of methicillin Resistance in Staphylococcus aureus, Annu. Rev. Biochem. 84 (2015) 577–601, https://doi.org/10.1146/annurev-biochem-060614-034516.
[5] V. Silva, S.I. Gabriel, S.B. Borrego, M.T. Tejedor-Junco, V. Manageiro, E. Ferreira, L. Reis, M. Caniça, J.L. Capelo, G. Igrejas, et al., Antimicrobial Resistance and Genetic Lineages of *Staphylococcus Aureus* from Wild Rodents: First Report of mecC-Positive Methicillin-Resistant *S. aureus* (MRSA) in Portugal, Animals (Basel) 11 (2021) 1537, https://doi.org/10.3390/ani11061537.
[6] T.A. Hansen, T. Joshi, A.R. Larsen, P.S. Andersen, K. Harms, S. Mollerup, E. Willerslev, K. Fuursted, L.P. Nielsen, A.J. Hansen, Vancomycin gene selection in the microbiome of urban Rattus Norvegicus from hospital environment, Evol Med Public Health 2016 (2016) 219–226, https://doi.org/10.1093/emph/eow021.
[7] WHO Antimicrobial Resistance, Global Report on Surveillance, 2014.
[8] S.M. Masoud, R.M. Abd El-Baky, S.A. Aly, R.A. Ibrahem, Co-existence of certain ESBLs, MBLs and plasmid mediated quinolone Resistance genes among MDR E. Coli isolated from different clinical specimens in Egypt, Antibiotics (Basel) 10 (2021) 835, https://doi.org/10.3390/antibiotics10070835.
[9] V.S. Sonola, A. Katakweba, G. Misinzo, M.I. Matee, Molecular epidemiology of antibiotic Resistance genes and virulence factors in multidrug-resistant Escherichia Coli isolated from rodents, humans, chicken, and household soils in Karatu, northern Tanzania, Int. J. Environ. Res. Public Health 19 (2022) 5388, https://doi.org/10.3390/ijerph19095388.
[10] K.H. Ong, W.C. Khor, J.Y. Quek, Z.X. Low, S. Arivalan, M. Humaidi, C. Chua, K.L. G. Seow, S. Guo, M.Y.F. Tay, et al., Occurrence and antimicrobial Resistance traits of Escherichia Coli from wild birds and rodents in Singapore, Int. J. Environ. Res. Public Health 17 (2020) 5606, https://doi.org/10.3390/ijerph17155606.
[11] T.T. Ao, N.A. Feasey, M.A. Gordon, K.H. Keddy, F.J. Angulo, J.A. Crump, Global burden of invasive Nontyphoidal Salmonella disease, 2010(1), Emerg. Infect. Dis. 21 (2015) 941–949, https://doi.org/10.3201/eid2106.140999.
[12] S.E. Majowicz, J. Musto, E. Scallan, F.J. Angulo, M. Kirk, S.J. O'Brien, T.F. Jones, A. Fazil, R.M. Hoekstra, International collaboration on enteric disease "burden of illness" studies the global burden of Nontyphoidal Salmonella gastroenteritis, Clin. Infect. Dis. 50 (2010) 882–889, https://doi.org/10.1086/650733.
[13] P.F. McDermott, S. Zhao, H. Tate, Antimicrobial Resistance in Nontyphoidal Salmonella, Microbiol Spectr 6 (2018), https://doi.org/10.1128/microbiolspec.ARBA-0014-2017.
[14] V.C. Agrawal, *Taxonomic Studies on Indian Muridae and Hystricidae: Mammalia, Rodentia*; Records of the Zoological Survey of India; Zoological Survey of India: Calcutta, 2000 (ISBN 978-81-85874-31-9.).
[15] M.P. Soumya, R.M. Pillai, P.X. Antony, H.K. Mukhopadhyay, V.N. Rao, Comparison of Faecal culture and IS900 PCR assay for the detection of mycobacterium Avium Subsp. Paratuberculosis in bovine Faecal samples, Vet. Res. Commun. 33 (2009) 781–791, https://doi.org/10.1007/s11259-009-9226-3.
[16] K. Zhang, J. Sparling, B.L. Chow, S. Elsayed, Z. Hussain, D.L. Church, D.B. Gregson, T. Louie, J.M. Conly, New Quadriplex PCR assay for detection of methicillin and mupirocin Resistance and simultaneous discrimination of Staphylococcus aureus from coagulase-negative staphylococci, J. Clin. Microbiol. 42 (2004) 4947–4955, https://doi.org/10.1128/JCM.42.11.4947-4955.2004.
[17] O.G. Brakstad, K. Aasbakk, J.A. Maeland, Detection of Staphylococcus aureus by polymerase chain reaction amplification of the Nuc gene, J. Clin. Microbiol. 30 (1992) 1654–1660, https://doi.org/10.1128/jcm.30.7.1654-1660.1992.
[18] D.C. Oliveira, H. de Lencastre, Multiplex PCR strategy for rapid identification of structural types and variants of the Mec element in methicillin-resistant Staphylococcus aureus, Antimicrob. Agents Chemother. 46 (2002) 2155–2161, https://doi.org/10.1128/AAC.46.7.2155-2161.2002.
[19] M. Stegger, P.S. Andersen, A. Kearns, B. Pichon, M.A. Holmes, G. Edwards, F. Laurent, C. Teale, R. Skov, A.R. Larsen, Rapid detection, differentiation and typing of methicillin-resistant Staphylococcus aureus Harbouring either mecA or the new mecA homologue mecA(LGA251), Clin. Microbiol. Infect. 18 (2012) 395–400, https://doi.org/10.1111/j.1469-0691.2011.03715.x.
[20] J. Chen, M.W. Griffiths, PCR differentiation of Escherichia Coli from other gram-negative Bacteria using primers derived from the nucleotide sequences flanking the gene encoding the universal stress protein, Lett. Appl. Microbiol. 27 (1998) 369–371, https://doi.org/10.1046/j.1472-765x.1998.00445.x.
[21] K. Colom, J. Pérez, R. Alonso, A. Fernández-Aranguiz, E. Lariño, R. Cisterna, Simple and reliable multiplex PCR assay for detection of blaTEM, Bla(SHV) and blaOXA-1 genes in Enterobacteriaceae, FEMS Microbiol. Lett. 223 (2003) 147–151, https://doi.org/10.1016/S0378-1097(03)00306-9.
[22] J.K. Rasheed, C. Jay, B. Metchock, F. Berkowitz, L. Weigel, J. Crellin, C. Steward, B. Hill, A.A. Medeiros, F.C. Tenover, Evolution of extended-Spectrum Beta-lactam Resistance (SHV-8) in a strain of Escherichia Coli during multiple episodes of bacteremia, Antimicrob. Agents Chemother. 41 (1997) 647–653, https://doi.org/10.1128/AAC.41.3.647.
[23] T.K. Dutta, I. Warjri, P. Roychoudhury, H. Lalzampuia, I. Samanta, S.N. Joardar, S. Bandyopadhyay, R. Chandra, Extended-Spectrum-β-lactamase-producing Escherichia Coli isolate possessing the Shiga toxin gene (Stx1) belonging to the O64

serogroup associated with human disease in India, J. Clin. Microbiol. 51 (2013) 2008–2009, https://doi.org/10.1128/JCM.00575-13.
[24] S. Aabo, O.F. Rasmussen, L. Rossen, P.D. Sørensen, J.E. Olsen, Salmonella identification by the polymerase chain reaction, Mol. Cell. Probes 7 (1993) 171–178, https://doi.org/10.1006/mcpr.1993.1026.
[25] M.W. Wood, J. Mahon, A.J. Lax, Development of a probe and PCR primers specific to the virulence plasmid of Salmonella Enteritidis, Mol. Cell. Probes 8 (1994) 473–479, https://doi.org/10.1006/mcpr.1994.1068.
[26] C. Soumet, G. Ermel, N. Rose, V. Rose, P. Drouin, G. Salvat, P. Colin, Evaluation of a multiplex PCR assay for simultaneous identification of Salmonella Sp., Salmonella Enteritidis and Salmonella typhimurium from environmental swabs of poultry houses, Lett. Appl. Microbiol. 28 (1999) 113–117, https://doi.org/10.1046/j.1365-2672.1999.00488.x.
[27] R.Y. Olobatoke, S.D. Mulugeta, Incidence of non-Typhoidal Salmonella in poultry products in the north West Province, South Africa, S. Afr. J. Sci. 111 (2015) 7, https://doi.org/10.17159/sajs.2015/20140233.
[28] C. Chu, C.H. Chiu, W.Y. Wu, C.H. Chu, T.P. Liu, J.T. Ou, Large drug Resistance virulence plasmids of clinical isolates of Salmonella Enterica Serovar Choleraesuis, Antimicrob. Agents Chemother. 45 (2001) 2299–2303, https://doi.org/10.1128/AAC.45.8.2299-2303.2001.
[29] C. Kehrenberg, S. Friederichs, A. de Jong, G.B. Michael, S. Schwarz, Identification of the plasmid-borne quinolone Resistance gene qnrS in Salmonella Enterica Serovar Infantis, J. Antimicrob. Chemother. 58 (2006) 18–22, https://doi.org/10.1093/jac/dkl213.
[30] S. Balakrishnan, P.X. Antony, H.K. Mukhopadhyay, R.M. Pillai, J. Thanislass, V. Padmanaban, M.V. Srinivas, Genetic characterization of fluoroquinolone-resistant Escherichia Coli associated with bovine mastitis in India, Vet World 9 (2016) 705–709, https://doi.org/10.14202/vetworld.2016.705-709.
[31] M.P. Weinstein, Performance standards for antimicrobial susceptibility testing: vol. Supplement M100, in: Clinical and Laboratory Standards Institute: Wayne, Pa, 30th edition, 2020 (ISBN 978-1-68440-066-9.).
[32] M. Vittecoq, S. Godreuil, F. Prugnolle, P. Durand, L. Brazier, N. Renaud, A. Arnal, S. Aberkane, H. Jean-Pierre, M. Gauthier-Clerc, et al., Antimicrobial Resistance in wildlife, J. Appl. Ecol. 53 (2016) 519–529, https://doi.org/10.1111/1365-2664.12596.
[33] C.-J. Chen, Y.-C. Huang, New epidemiology of Staphylococcus aureus infection in Asia, Clin. Microbiol. Infect. 20 (2014) 605–623, https://doi.org/10.1111/1469-0691.12705.
[34] M.C. Porrero, G. Mentaberre, S. Sánchez, P. Fernández-Llario, S. Gómez-Barrero, N. Navarro-Gonzalez, E. Serrano, E. Casas-Díaz, I. Marco, J.-F. Fernández-Garayzabal, et al., Methicillin resistant Staphylococcus aureus (MRSA) carriage in different free-living wild animal species in Spain, Vet. J. 198 (2013) 127–130, https://doi.org/10.1016/j.tvjl.2013.06.004.
[35] A.W. Van De Giessen, M.G. Van Santen-Verheuvel, P.D. Hengeveld, T. Bosch, E. M. Broens, C.B.E.M. Reusken, Occurrence of methicillin-resistant Staphylococcus aureus in rats living on pig farms, Prev. Vet. Med. 91 (2009) 270–273, https://doi.org/10.1016/j.prevetmed.2009.05.016.
[36] L. García-Álvarez, M.T.G. Holden, H. Lindsay, C.R. Webb, D.F.J. Brown, M. D. Curran, E. Walpole, K. Brooks, D.J. Pickard, C. Teale, et al., Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study, Lancet Infect. Dis. 11 (2011) 595–603, https://doi.org/10.1016/S1473-3099(11)70126-8.
[37] T.K. Dey, B.R. Shome, S. Bandyopadhyay, N.K. Goyal, Å. Lundkvist, R.P. Deka, R. Shome, N. Venugopal, D. Grace, G. Sharma, et al., Molecular characterization of methicillin-resistant staphylococci from the dairy value chain in two Indian states, Pathogens 12 (2023) 344, https://doi.org/10.3390/pathogens12020344.
[38] J. Ge, X. Zhong, Y. Xiong, M. Qiu, S. Huo, X. Chen, Y. Mo, M. Cheng, Q. Chen, Methicillin-resistant Staphylococcus aureus among urban rodents, House shrews, and patients in Guangzhou, Southern China, BMC Vet. Res. 15 (2019) 260, https://doi.org/10.1186/s12917-019-2012-8.
[39] M.C. Erickson, Overview: Foodborne Pathogens in Wildlife Populations, in: M. Jay-Russell, M.P. Doyle (Eds.), Food Safety Risks from Wildlife, Springer International Publishing, Cham, 2016, pp. 1–30. ISBN 978–3–319-24440-2.
[40] F. Baquero, T.M. Coque, J.-L. Martínez, S. Aracil-Gisbert, V.F. Lanza, Gene transmission in the one health microbiosphere and the channels of antimicrobial Resistance, Front. Microbiol. 10 (2019) 2892, https://doi.org/10.3389/fmicb.2019.02892.
[41] C. Nkogwe, J. Raletobana, A. Stewart-Johnson, S. Suepaul, A. Adesiyun, Frequency of detection of Escherichia Coli, Salmonella Spp., and Campylobacter Spp. in the Faeces of wild rats (Rattus Spp.) in Trinidad and Tobago, Vet. Med. Int. 2011 (2011) 686923, https://doi.org/10.4061/2011/686923.
[42] R. Onanga, P.P. Mbehang Nguema, G.R. Ndong Atome, A. Mabika Mabika, B. Ngoubangoye, W.L. Komba Tonda, J.C. Obague Mbeang, J. Lebibi, Prevalence of extended-Spectrum β-lactamases in *E. coli* of Rats in the Region North East of Gabon, Vet Med Int 2020 (2020) 5163493, https://doi.org/10.1155/2020/5163493.
[43] C.G. Himsworth, E. Zabek, A. Desruisseau, E.J. Parmley, R. Reid-Smith, C. M. Jardine, P. Tang, D.M. Patrick, Prevalence and Characterization of E.Coli and Salmonella Spp in the feces of wild urban Norway and black rats (Rattus Norvegius and Rattus Ratttus) from an Inner City Neighbourhood of Vancouver, J. Wildl. Dis. 51 (2015) 589–600, https://doi.org/10.7589/2014-09-242.
[44] S. Guenther, A. Bethe, A. Fruth, T. Semmler, R.G. Ulrich, L.H. Wieler, C. Ewers, Frequent combination of antimicrobial multiresistance and Extraintestinal pathogenicity in Escherichia Coli isolates from urban rats (Rattus Norvegicus) in Berlin, Germany, PLoS One 7 (2012) e50331, https://doi.org/10.1371/journal.pone.0050331.
[45] I. Literák, M. Dolejska, A. Cizek, C.A.T. Djigo, Reservoirs of Antibiotic-Resistant Enterobacteriaceae among Animals Sympatric to Humans in Senegal: Extended-Spectrum Beta-Lactamases in Bacteria in a Black Rat (*Rattus Rattus*), Afr. J. Microbiol. Res. 3 (2024) 751–754.
[46] A.R. Burriel, S.K. Kritas, V. Kontos, Some microbiological aspects of rats captured alive at the Port City of Piraeus, Greece, Int. J. Environ. Health Res. 18 (2008) 159–164, https://doi.org/10.1080/09603120701358432.
[47] M.A. Gilliver, M. Bennett, M. Begon, S.M. Hazel, C.A. Hart, Antibiotic Resistance found in wild rodents, Nature 401 (1999) 233–234, https://doi.org/10.1038/45724.
[48] P.-L. Ho, W.-U. Lo, E.L. Lai, P.Y. Law, S.M. Leung, Y. Wang, K.-H. Chow, Clonal diversity of CTX-M-producing, multidrug-resistant Escherichia Coli from rodents, J. Med. Microbiol. 64 (2015) 185–190, https://doi.org/10.1099/jmm.0.000001.
[49] European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC) The European Union One Health 2018 Zoonoses Report, EFSA J. (17) (2019) e05926, https://doi.org/10.2903/j.efsa.2019.5926.
[50] H. El-Sharkawy, A. Tahoun, A.E.-G.A. El-Gohary, M. El-Abasy, F. El-Khayat, T. Gillespie, Y. Kitade, H.M. Hafez, H. Neubauer, H. El-Adawy, Epidemiological, molecular characterization and antibiotic Resistance of Salmonella Enterica Serovars isolated from chicken farms in Egypt, Gut Pathog 9 (2017) 8, https://doi.org/10.1186/s13099-017-0157-1.
[51] M.O. Taioe, O.M.M. Thekisoe, M. Syakalima, T. Ramatla, Confirmation of Antimicrobial Resistance by Using Resistance Genes of Isolated Salmonella Spp. in Chicken Houses of North West, South Africa, JWPR 9 (2019) 158–165, https://doi.org/10.36380/scil.2019.wvj20.
[52] E.K. Barbour, D.B. Ayyash, W. Alturkistni, A. Alyahiby, S. Yaghmoor, A. Iyer, J. Yousef, T. Kumosani, S. Harakeh, Impact of sporadic reporting of poultry Salmonella Serovars from selected developing countries, J. Infect. Dev. Ctries. 9 (2015) 1–7, https://doi.org/10.3855/jidc.5065.
[53] N.A. Feasey, G. Dougan, R.A. Kingsley, R.S. Heyderman, M.A. Gordon, Invasive non-Typhoidal Salmonella disease: an emerging and neglected tropical disease in Africa, Lancet 379 (2012) 2489–2499, https://doi.org/10.1016/S0140-6736(11)61752-2.
[54] F. Bertelloni, G. Tosi, P. Massi, L. Fiorentini, M. Parigi, D. Cerri, V.V. Ebani, Some pathogenic characters of paratyphoid Salmonella Enterica strains isolated from poultry, Asian Pac J Trop Med 10 (2017) 1161–1166, https://doi.org/10.1016/j.apjtm.2017.10.023.
[55] T. Ramatla, M. Tawana, K.E. Lekota, O. Thekisoe, Antimicrobial Resistance genes of Escherichia Coli, a bacterium of "one health" importance in South Africa: systematic review and Meta-analysis, AIMS Microbiol 9 (2023) 75–89, https://doi.org/10.3934/microbiol.2023005.
[56] M. Abdel-Maksoud, R. Abdel-Khalek, A. El-Gendy, R.F. Gamal, H.M. Abdelhady, B. L. House, Genetic characterisation of multidrug-resistant *Salmonella Enterica* serotypes isolated from poultry in Cairo, Egypt. *Afr*, J Lab Med 4 (2015) 7, https://doi.org/10.4102/ajlm.v4i1.158.
[57] H. Dahmana, L. Granjon, C. Diagne, B. Davoust, F. Fenollar, O. Mediannikov, Rodents as hosts of pathogens and related zoonotic disease risk, Pathogens 9 (2020) 202, https://doi.org/10.3390/pathogens9030202.
[58] M. Vivekanandan, A. Mani, Y.S. Priya, A.P. Singh, S. Jayakumar, S. Purty, Outbreak of scrub typhus in Pondicherry, J. Assoc. Physicians India 58 (2010) 24–28.
[59] S.B. Shekatkar, B.N. Harish, G.A. Menezes, S.C. Parija, Clinical and serological evaluation of leptospirosis in Puducherry, India, J Infect Dev Ctries 4 (2010) 139–143, https://doi.org/10.3855/jidc.384.
[60] R.S. Rao, S.K. Amarnath, S. Sujatha, An outbreak of typhoid due to multidrug resistant Salmonella Typhi in Pondicherry, Trans. R. Soc. Trop. Med. Hyg. 86 (1992) 204–205, https://doi.org/10.1016/0035-9203(92)90573-U.
[61] P. Devaraju, B. Arumugam, I. Mohan, M. Paraman, M. Ashokkumar, G. Kasinathan, J. Purushothaman, Evidence of natural infection of Orientia Tsutsugamushi in vectors and animal hosts – risk of scrub typhus transmission to humans in Puducherry, South India, Indian J. Public Health 64 (2020) 27, https://doi.org/10.4103/ijph.IJPH_130_19.
[62] T. Balasubramanian, U. Sambath, R.D. Radja, G. Thangaraj, P. Devaraju, L. Srinivasan, P. Srinivasan, M.G. Nair, K. Raja, A.W. Lakkawar, et al., Pathological responses in Asian House shrews (Suncus Murinus) to the naturally acquired Orientia Tsutsugamushi infection, Microorganisms 12 (2024) 748, https://doi.org/10.3390/microorganisms12040748.
[63] G.P. Ritu, W. Arif, K.K. Sihag, A. Chakravarthi, T.N. Anthony, L. Srinivasan, V. Balakrishnan, A. Kumar, E. Ayanar, P. Devaraju, Comparative evaluation of different tissues and molecular techniques for the zoonotic surveillance of scrub typhus, Vector-Borne and Zoonotic Diseases vbz.2023.0069 (2024), https://doi.org/10.1089/vbz.2023.0069.