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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 is a major public health concern. Rodents and shrews live in closer proximity to humans and animals and serve as reservoirs for the transmission of several diseases, including AMR pathogens. There is no data available from India on surveillance of AMR bacteria in rodents and shrews. In this study, bacterial pathogens viz, *Staphylococcus sp, Escherichia coli* and *Salmonella sp* were isolated from rodents and shrews and genetic elements conferring antimicrobial resistance in the pathogens were screened by PCR. Bacterial pathogens in the oropharyngeal and anal swabs collected from the rodents and shrews trapped (n=100) were cultured on selective media such as Mannitol salt, Mac Conkey and Xylose lysine deoxycholate media to isolate *Staphylococcus sp., E. coli* and *Salmonella sp.* respectively. A total of 76 (80%) *S. aureus* and 19 (20%) *Staphylococcus* non *aureus* were isolated. *E. coli* was isolated in 89 samples and among the *Salmonella sp* (n=59), 16 (27%) were *S. enteritidis* and 29 (49.1%) were *S. typhimurium.* In *S. aureus* isolates, *mec A and mec C* (MRSA) were detected in 40 (52.6%) and 6 (7.9%) isolates respectively. Also, 7 (36.84%) and 1 (5.3%) *Staphylococcus* non *aureus* isolates were tested to harbour *mec A* and *mec C* genes. A total of 77 (86.5%) *E. coli* isolates had genes encoding ESBL [TEM in 21 (23.59%), SHV in 45 (50.6%) and CTXM in 11 (12.4%)]. Among the 45 pathogenic *Salmonella sp,* 44 (97.78%) harboured AMR genes [*tet* in 13 (28.9%), *sul3 & sul4* in 20 (44.4%) and *qnrA* in 11 (24.4%)]. Thirty-eight animals harboured more than one zoonotic pathogen and 21 with multi-drug resistant genes. Our findings signify that these rodents and shrews may serve as sentinels to screen environment spillover of AMR pathogens and also act as reservoir to transmit MRSA, ESBL, *Salmonella sp.* human/animals. Also these animals aid in the transfer of antimicrobial resistance genetic elements to non-pathogenic organisms.

Keywords: rodents and shrews; MRSA; ESBL; *Salmonella* sp; AMR; sentinels; drug resistance

Short title: Zoonotic bacterial pathogens and AMR in synanthropic rodents

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 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, TEM, SHV and CTX-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.

Materials and Methods

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 feet 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 (Figure 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 (Figures 2 and 3). 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.
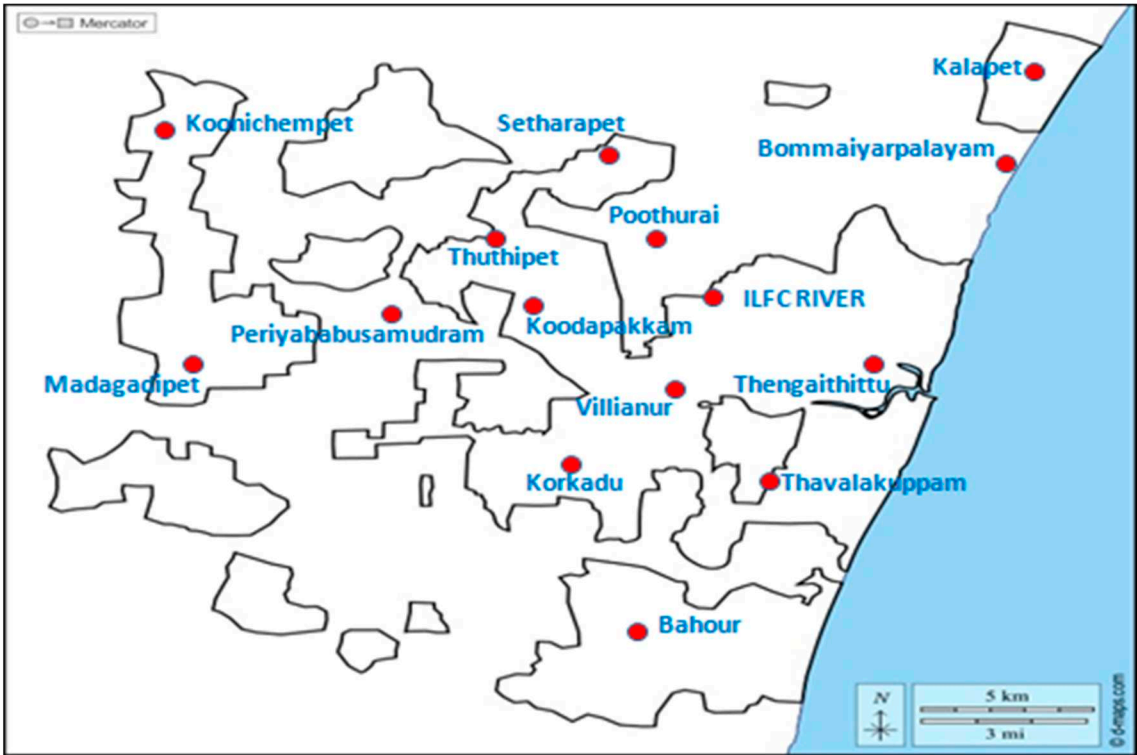
Figure 1. The map representing the sites of rodents trapping in Puducherry. The red dot indicates the villages, where the rodents were trapped.

Figure 2. Collection of the oro-pharyngeal sample for the isolation of *Staphylococcus spp.* from a shrew.

Figure 3. Collection of the anal swab from a shrew for the isolation of *E. coli* and *Salmonella spp.*

Isolation of the bacterial pathogens:

All the 100 oropharyngeal samples collected in Luria broth enriched with 7.5% sodium chloride were incubated at 37⁰C for 16 to 18 hrs. 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. TEM[21], SHV [22] and CTX-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 sec	17
Methicillin resistance	*mec A*	TCCAGATTACAACTTCACCAGG CCACTTCATATCTTGTAACG	50°C for 1 min	18
	mec C	GAAAAAAAGGCTTAGAACGCCC GAAGATCTTTTCCGTTTTCAGC		19
E. coli	*uspA*	CCGATACGCTGCCAATCAGT ACGCAGACCGTAAGGGCCAGAT	60°C for 1.5 mins	20
ESBL-producing *E. coli*	TEM	ATGAGTATTCAACATTTCCG CTGACAGTTACCAATGCTTA	56°C for 1 min	21
	SHV	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG		22
	CTX-M	CAATGTGCAGCACCAAGTAA CGCGATATCGTTGGTGGTG	59°C for 30 sec	23
Salmonella sp	*invA*	GCCAACCATTGCTAAATTGGCGCA GGTAGAAATTCCCAGCGGGTACTG	55°C for 45 sec	24
Salmonella enteritidis	*spvC*	GCCGTACACGAGCTTATAGA ACCTACAGGGGCACAATAAC	57°C for 45 sec	25
Salmonella typhimurium	*fliC*	CGGTGTTGCCCAGGTTGGTAAT ACTGGTAAAGATGGCT	55°C for 45 sec	26
Tetracycline resistance	*Tet*	GCACTTGTCTCCTGTTTACTCCCC CCTTGTGGTTATGTTTTGGTTCCG	53°C for 1min	27
Sulphonamides resistance	*sul3 & sul4*	TCAACATAACCTCGGACAGT GATGAAGTCAGCTCCACCT	60°C for 40 sec	28
Fluoroquinolone resistance	*qnrA*	TCAGCAAGAGGATTTCTCA GGCAGCACTATGACTCCCA	53°C for 30 sec	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 sequenceswere BLAST analysed to confirm the gene identity*(http://www.ncbi.nlm.nih.gov/BLAST).* The nucleotide sequences were deposited in GenBank.

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. TEM, SHV and CTX-M, respectively. The presence of both TEM and SHV was witnessed in 13 isolates (16.8%) (2 rodents and 11 shrews), SHV and CTX-M in7 isolates (9.1%) (1 rodent and 6 shrews) whereas the occurrence of all 3 ESBL genes (TEM, SHV and 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.

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 | | | | | |
	TEM	SHV	CTX-M	TEM and SHV	SHV and CTX-M	TEM, SHV, and CTX-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*)	TEM	SHV	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

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 [30] and rodents [31]. 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 [31]. 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% [32] to 41.86% [33] 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 [34].

Although, *S. aureus* with *mecC* genetic element in milk was first reported in India [35], 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 [36] 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 [37].

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   *mec A* and *mec C* genes. Phumthanakorn *et al.,* 2022 [38] 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 [38]. Haaber *et al.,*2017 [39] 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 [39] and 19.88% in rats in the east region of Gabon in Central Africa [40], Vancouver, Canada (41.5%), Berlin, Germany (58.2%), and Nairobi, Kenya (66.7%), [3,36,41,42]. In contrast, Nhung *et al.,*2015 [43] 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 TEM (23.59%), SHV (50.56%) and CTX-M (12.35%) respectively. Literak *et al.,* 2009 [43] reported that 2.5% of *E. coli* isolated from black rats in Africa were ESBL-producers. However, Burriel *et al.,* 2008[44] 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 [45] 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 TEM and SHV genes, 7 (9.09%) isolates for SHV and CTX-M genes, and 2 (2.59%) isolates were tested to carry TEM, SHV, and CTX-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 CTX-M (61%), TEM (26%) and SHV (13%)[46]. Ho *et al.,* 2015 [46] reported a prevalence of 7.7% and 13.9% of CTX-M-producing multidrug-resistant *E. coli* in black and brown rats trapped in 18 districts of Hong Kong. Onanga *et al.,* 2020 [40] reported human-origin *E. coli* carrying CTX-M genes in rats in Gabon, Central Africa.

By CTX-M gene characterization, the wild rodents in Hong Kong were identified to have acquired *E. coli* from humans and livestock [46]. Molecular clonal diversity characterization of the CTX-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 with the spillage of antimicrobial resistance from human and animal pathogens to the environment. Guenther *et al.,* 2012 [42] 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 [47]. Interestingly, we observed that 59% of the rodents/shrews in our study to harbour *Salmonella spp.* However, there are reports of nil [48], and high [41] 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[49]. *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[50]. NTS are of public

health importance as it   is associated with   non-invasive enteritis and fatal bloodstream infections in children and adults [51].

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 [41] 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 2 isolates (15.38%) were tested   resistant to sulphonamides and 1 (7.69%)   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 [52]. Balakrishnan *et al.,* 2016 [53] isolated fluoroquinolones resistant *E. coli* isolates of animal origin from Puducherry. In contrast, Ramatla *et al.,* 2022[54]   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* [55]. 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 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 [42]. Also, they may act as surrogate indicators of the environmentalspillover of AMR pathogens. To curtail the development of superbugs, necessary intervention measures have to be undertaken for judicious use and disposal of antibiotics.

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, livestockand pet animals. This study underscores the screening of rodents/shrews as sentinels to test the spillover of antimicrobial resistance in the environment.

Author Contributions: HKM, PD & MR contributed to conception and design of the study. PD acquired funds. AC and TNA carried out trapping, morphological identification of rodents and extraction of nucleic acid.   ND, KKS, LS, VMS, JV carried out the isolation of the pathogens, PCR detection of pathogens and AMR genetic elements, nucleotide sequencing and analysis. DP, HKM, MR and VP supervised the experiments. PD, ND, HKM, KKS and TNA organized the database and wrote the first draft of the manuscript. All the authors contributed to manuscript revision and approved the final version.

Conflict of Interests: Nil

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