Devi & Chattopadhya | J Pure Appl Microbiol | 16(2):1084-1095 | June 2022
Article 7170 | https://doi.org/10.22207/JPAM.16.2.33
Published Online May 27, 2022
Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Possible Acquisition of ESBL-mediated Antimicrobial Resistance by Farmers from Aquatic Reservoir used for Bathing and Cleaning of Water Buffalos (*Bubalus bubalis*) with Intestinal Carriage of ESBL Producing *Escherichia coli*

Leimapokpam Sumitra Devi and Debasish Chattopadhya*

Department of Microbiology, Faculty of Medicine and Health Sciences, SGT University, Gurugram - 122 505, Haryana, India.

Abstract

Little information is available on the risk of human subjects for acquisition of antimicrobial resistance (AMR) from aquatic environment other than those treated with antimicrobials for aquaculture. Carriage of extended-spectrum beta-lactamase (ESBL) and carbapenemase categories of AMR by enteric bacteria in livestock have been frequently reported. Dissemination of these categories of AMR to the environment thus poses a threat for their transmission to farmers engaged in livestock care posing a severe public health hazard. A study on the prevalence of ESBL- and carbapenemase-mediated AMR among *Escherichia coli* isolated from earth pond environment used for bathing and cleaning of buffalos (*Bubalus bubalis*) and from human subjects engaged in such activity revealed isolation rate of ESBL positivity to be higher in human subjects engaged in washing and bathing of buffalos (37.5%) compared to those without engagement in such activities (20.7%) with CTX-M type ESBL, a group of class A ESBL, as the predominant molecular type (97.4%). While no carbapenemase positivity could be detected among *E. coli* isolated from pond environment or buffalos, small percentage of carbapenemase could be detected among the *E. coli* isolated from human subjects although the risk was not higher than those not associated with bathing and cleaning of buffalos. Bathing and cleaning of buffalos could potentially facilitate transmission of ESBL resistance from livestock to human subjects in pond environment.

Keywords: Ponds, buffalos, ESBL, CTX-M, carbapenemase

***Correspondence:** dchattopadhya27@gmail.com; +91 9811256697

(Received: July 9, 2021; accepted: March 14, 2022)

Citation: Devi LS, Chattopadhya D. Possible Acquisition of ESBL-mediated Antimicrobial Resistance by Farmers from Aquatic Reservoir used for Bathing and Cleaning of Water Buffalos (Bubalus bubalis) with Intestinal Carriage of ESBL Producing *Escherichia coli*. *J Pure Appl Microbiol.* 2022;16(2):1084-1095. doi: 10.22207/JPAM.16.2.33

INTRODUCTION

Most of the small-scale dairy farmers in the rural belt of Haryana state of northern India earn their livelihood by rearing water buffalos (*Bubalus bubalis*) for production of milk.[1] These buffalos are taken to earth ponds frequently for bathing and cleaning which also helps to increase their production of milk (Fig. 1).[2,3] The earth ponds get contaminated with enteric pathogens from the fecal matter of buffalos during the process of cleaning and bathing and thus may serve as source for acquisition of these pathogens by human subjects engaged in such activity.

While antibiotics are not commonly used as growth promoters in animals in India since they are not used as food animals in the country, there has been a perceptible increase in antibiotic resistance in the livestock in the country due to their indiscriminate use in unregulated veterinary sector for treatment of various infections.[4,5]

Escherichia coli, being a member of normal enteric flora of both human and animals, is commonly utilized as an indicator organism to determine the fecal carriage rate of antimicrobial resistance (AMR).[6,7] Misuse of third-generation cephalosporins in both human and livestock created a selection pressure that led to the establishment and spread of Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* in both the sectors.[8] Among the ESBL genes, TEM- and SHV-ESBLs dominated the ESBL landscape in the 1980s and 1990s of the previous centuries, mostly associated with *E. coli* and *Klebsiella pneumoniae* outbreaks in hospitals. However, since 2000s, the CTX-M type of ESBL gene has almost replaced the other variants and it has been identified as the most common among community strains, and evidence of CTX-M-producing isolates being isolated from livestock and domestic pets is increasing, which is concerning because they could serve as a reservoir for resistant organisms' acquisition.[9] Carbapenems are antibiotics used as a last option against drug-resistant bacteria, such as *E. coli* that produces ESBL. Although carbapenem resistance has been observed in isolates obtained from livestock, these antibiotics are not licenced for use in livestock. Thus, different AMR *E. coli* carried by livestock could contaminate the environment and pose a serious AMR threats to public health especially among farmers.[10] A study was taken up to find out prevalence of AMR in ESBL- and carbapenemase producing *E. coli* and their molecular variants in samples collected from pond environment, buffalos and from human subjects exposed to pond environment.

MATERIALS AND METHODS

The protocol of the study was approved by both independent institutional research and ethical committees of SGT University (SGTU/FMHS/MICRO/341). Information sheet was provided to the owners of the buffalos included in the study and informed consent was obtained for collection of samples from them as well as from the buffalos owned by them.

Selection of ponds, human subjects and buffalos

Ponds

The study was limited to two earth ponds, one each located in two villages in the rural belt of Haryana state, India used by small scale dairy farmers for bathing and cleaning of buffalos. These ponds were shallow (knee deep), surrounded by agricultural fields and were never used for aquaculture. The selected ponds were located away from human inhabitation without any healthcare center within 2 km distance. The pond edges were not known to be used for defecation by human subjects and did not have connection with any sewage effluent from the community.

Human subjects

All the households of farmers from the two villages owning the buffalos taken regularly to the selected ponds were identified (subgroup I or Sgr I). The residential premises of these households were shared by the human inhabitants as well as by the buffalos reared by them. The following two sub-categories of human subjects from the selected households were enrolled for the study viz. (i) those involved in regular bathing and washing of the buffalos for past one year (all males, subgroup 1a or Sgr 1a) and (ii) age matched males in the same households involved in other activities related to care of buffalos viz. milking, feeding etc. but not involved in bathing and cleaning of buffalos (subgroup 1b or Sgr 1b) for the same duration. The rationale for limiting the history regarding the duration of association with buffalos as preceding one year in the present study was based on the maximum reported duration of colonization by drug resistant *E. coli* (less than

6 months) and the purpose of the study being assessment of the prevalence of recently acquired resistant strains by the human subjects at the time of sampling.[11,12] Those subjects sharing dual responsibilities catered by Sgr Ia and Sgr 1b were not included. Age matched male subjects from an additional randomly selected 60 households, 30 each from the same two villages inhabited by Sgr Ia and Sgr Ib subjects were selected (subgroup II or Sgr II) where the households did not have any livestock in their premises and no member in the household had history of direct exposure to livestock (i.e. they were engaged in different occupations for living e.g. shopkeepers, vegetable vendors, school teachers, etc).

Buffalos

All the buffalos in the selected households inhabited by Sgr I subjects that were taken to the earth ponds regularly by their owners for bathing and cleaning were included in the study for sampling.

Collection of specimens

Identification of sites for sampling of pond and pond environment

This was carried out weekly for 12 months, 4 months (16 weeks) during each season e.g. summer (March-June), monsoon (July-October) and winter (November-February) in the year 2017-2018. Each pond was radially marked in four perpendicular intersecting radial directions. In each direction, three points were identified for sampling viz. pond surface water at 20 feet and at 2 feet distance from the edge of the pond and soil at 6 inches away from the soil-water interface in the same radial direction.

Sampling

Pond water

One hundred ml of surface water sample was collected from each of the selected point in the pond stepwise by submerging pre-sterilized stoppered water bottle up to the depth of approximately 20 cm below the surface of water, opening the stopper, holding the bottle horizontally allowing water to flow freely in the bottle and then closing the mouth of the container by stopper while holding the bottle at the same level.[13] Each week a total of 8 surface water samples were collected from each pond, one each at 2 ft and 20 ft from the edge in 4 radial directions. Thus, a total of 128 surface water samples (8 samples X 16 weeks) were collected from each pond per season totaling to 256 samples from the two ponds.

Fig. 1. Human subject engaged in washing and cleaning of buffalos in earth pond.

Pond bed sludge

Sediment sludge sample was collected from the pond bed at each of the same points from where surface water samples were collected using wide mouth screw capped pre-sterilized glass containers sequentially by opening the lid of the container at the bottom of the pond, dragging the container with mouth open along the pond bed for short distance and recapping the container at the same level before bringing up on the surface.[13] A total of 128 pond bed sludge samples were collected in each season from each pond totaling to 256 samples from the two ponds.

Soil

Soil samples were collected in an area 6 inches away from water soil interface in each of the 4 radial directions selected for sampling of pond water and pond bottom sludge. Sampling was carried out at the selected point covering an approximate area of 10 cm by 10 cm square with five longitudinal, five latitudinal and two diagonal strokes of sterile swabs pre-moistened with nutrient broth, placed in polypropylene tube containing 1.5 ml of nutrient broth and was immediately transported to laboratory in ice pack within 30 minutes.[14]

Buffalos

This was carried out as a cross sectional study among all the buffalos taken to the two ponds for bathing and cleaning. Fresh fecal samples from all the buffalos were sampled within one minute of deposition outside the pond e.g. in the residential premises of their owners. Sterile swabs moistened with nutrient broth were immersed in the fecal material taking care to sample the area not touching the soil, placed in polypropylene tube containing nutrient broth and transported in ice box to the Microbiology laboratory within 30 minutes. Sampling was carried over for several consecutive days to ensure inclusion of all the buffalos.[15]

Human subjects

Each of the study participants was explained regarding collection of feces in sterile leak proof containers provided by the laboratory. Self-collected early morning fecal samples were transported to the Microbiology laboratory of SGTH within one hour of collection in insulated carriers with ice packs provided to the participants.[16]

Bacteriological screening of ESBL producing *E. coli* and identification of the isolates

Ten-fold dilutions of water and sediment sludge samples were prepared in physiological saline. Soil swabs and fecal swabs of buffalos collected in polypropylene tube containing nutrient broth were vortexed for 2 mins. For human sample, approximately 0.5 g of faecal sample was suspended in 1.5 ml of nutrient broth and vortexed for 2 mins.[17,18] One hundred microliter of each sample viz. water, sediment sludge, soil, buffalo feces and human feces was inoculated on two MacConkey agar plates, one supplemented with 2µg of cefotaxime per ml (Mac-CTX) and the other supplemented with 2µg of ceftazidime per ml (Mac-CAZ). The inoculated plates were incubated aerobically at 37°C for 24-48 h. One lactose fermenting colony representing each distinct colonial morphotype suggestive of *E. coli* was regrown in the same selective plate and further identified using Vitek 2 system (BioMerieux, France). One isolate of each morphotype from each sample was selected for ESBL characterization.[18]

Phenotypic confirmatory test for detection of ESBL production

Double disc synergy test (DDST)

E. coli isolates were subjected to DDST, a phenotypic confirmatory test for ESBL detection.[19] Two pairs of antibiotic discs, ceftazidime (30 µg) and ceftazidime plus clavulanic acid (30 µg plus 10 µg) discs as first pair and cefotaxime (30µg) and cefotaxime plus clavulanic acid (30 µg plus 10 µg) discs as second pair were placed on a plate of Mueller Hinton agar inoculated with the suspension (turbidity of 0.5 MacFarland standard) of isolates. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as control strains.

Molecular identification of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes

Polymerase chain reaction (PCR) for detection of bla_{TEM}, bla_{SHV} and bla_{CTX-M} was carried out for strains showing ESBL positivity in DDST using the pre-published sequences viz. TEM primers (TEMF ATGAGTATTCAACATTTCCGTG, TEMR TTACCAATGCTTAATCAGTGAG) amplifying 840-bp fragment, SHV primers (SHVSF ATTTGTCGCTTCTTTACTCGC, SHVSRTTTATGGCGTTACCTTTGACC) amplifying

1051-bp fragment and CTX-M primers (CTX-MF TTTGCGATGTGCAGTACCAGTAA, CTX-MR CGATATCGTTGGTGGTGCCATA) amplifying 544-bp fragment.[20]

Antibiotic susceptibility testing

All the ESBL positive isolates were subjected to antibiotic susceptibility testing (AST) by Kirby-Bauer disc diffusion method on Mueller-Hinton agar and the results were interpreted based on zone of inhibition validated as per CLSI guidelines.[19] Following antibiotics were tested as per disc concentration and strength indicated: ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), piperacillin/tazobactam (100/10 µg), amikacin (30µg), gentamicin (10µg), cefotaxime (30µg), ceftriaxone (30µg), cefepime (30µg), aztreonam (30µg), ciprofloxacin (5µg), co-trimoxazole (25µg), ertapenem (10µg), meropenem (10µg)

Table 1. Positivity and molecular types of ESBL among *E. coli* isolated from pond environment

Season Positivity	ESBL	Location for collection of pond sample				Edge soil (LS)
		2 ft from edge (L1)		20 ft from edge (L2)		
		Sub-surface water (L1a) n= 128 No (%)	Sediment sludge (L1b) n= 128 No (%)	Sub-surface water (L2a) n=128 No (%)	Sediment sludge (L2b) n=128 No (%)	n =128 No (%)
Summer	ESBL	41 (32)@	30 (23.4)	18 (14.1)	17 (13.3)	12 (9.4)
	CTX-M alone*	35 (85.4)	26 (86.7)	17 (94.4)	15 (88.2)	11 (91.7)
	CTX-M with other ESBL genes*	3 (7.3)	2 (6.7)	0	2 (11.8)	0
	Other ESBL genes alone*	3 (7.3)	1 (3.3)	1 (5.6)	0	1 (8.3)
Monsoon	ESBL	56 (43.8)@	46 (35.9)	32 (25.8)	32 (25)	15 (11.7)
	CTX-M alone*	48 (85.7)	39 (84.8)	29 (90.6)	32 (100)	13 (86.7)
	CTX-M with other ESBL genes*	5 (8.9)	5 (10.9)	3 (9.4)	0	2 (13.3)
	Other ESBL genes alone*	3 (5.4)	2 (4.3)	0	0	0
Winter	ESBL	30 (23.4)@	20 (15.6)	10 (7.8)	14 (10.9)	5 (3.9)
	CTX-M alone*	24 (80)	18 (90)	8 (80)	11 (78.3)	4 (80)
	CTX-M with other ESBL genes*	4 (13.3)	2 (10)	0	2 (14.3)	1 (20)
	Other ESBL genes alone*	2 (6.7)	0	2 (20)	1 (7.1)	0

*Calculated as no (%) of the total ESBL positive isolates

Statistical comparisons

@ L1a vs L2a: Summer *P*= 0.001; Monsoon *P*= 0.004: Winter *P*= 0.001

Comparison of seasons	L1a	L1b	L2a	L2b
Monsoon vs Summer	0.05	0.03	0.03	0.02
Monsoon vs Winter	0.001	<0.001	<0.001	0.003
Summer vs Winter	NS (0.1)	NS (0.1)	NS (0.1)	NS (0.6)

Note: (i) None of the samples in any location of pond environment yielded *E. coli* isolate with carbapenem resistance
(ii) There was no statistical difference in rate of ESBL positivity among *E. coli* isolated from sub-surface water and sediment sludge at both the locations in pond (L1 andL2) regardless of season.

Table 2. Prevalence of AMR in human subjects with or without companion animals (water buffalos)

Type of resistance	Samples from human subjects			Statistical comparisons		
	With companion animals		Without companion animals			
	SgrIa (n=104)	SgrIb (n=82)	Sgr II (n=196)	SgrIa Vs Sgr II	SgrIa Vs Sgr II	SgrIb Vs Sgr II
ESBL	39(37.5)	17 (20.7)	44 (22.4)	0.01	0.006	NS, 0.8
CTX-M alone*	31(79.5)	13 (76.4)	37 (84.1)	NS (0.6)	NS (0.9)	NS (0.5)
CTX-M with other ESBL genes*	7 (17.9)	2 (11.8)	5 (11.4)	NS (0.8)	NS (0.7)	NS (1)
Other ESBL genes alone*	1 (2.6)	2 (11.8)	2 (4.5)	NS (0.2)	NS (0.7)	NS (0.3)
Carbapenemase	3 (7.7)	2 (11.8)	4 (9.1)	NS (0.6)	NS (0.8)	NS (0.8)

*Calculated as no. (%) of the total ESBL positive isolates

Note: Prevalence of ESBL-EC in samples from companion animals (buffalos) was found to be 31.1% (89 out of 286), of which 80.9% (72 out of 89), 12.4% (11 out of 89) and 6.7% (6 out of 89) were found to harbour CTX-M alone, CTX-M with other ESBL genes and other ESBL genes alone respectively. None of the isolates from pond environment or companion animal showed evidence of carbapenemase production.

and imipenem (10µg) and tigecycline (15µg). In addition, a third-generation cephalosporin viz. ceftiofur known to be used only in veterinary sector, was also included, (EFT disc, 30µg, Oxoid Ltd., United Kingdom) included and the results were interpreted as per CLSI guidelines for drugs used for veterinary use.[21]

Statistical analysis

Prevalence of ESBL, CTX-M and other ESBL types and carbapenem resistance were expressed in terms of percentage resistant and were compared by chi-square test as categorical variables. P-value less than 0.05 was considered to be statistically significant.

RESULTS

During each of the three seasons, a total of 256 pond surface water samples, comprising of 128 collected from each pond and 256 pond bed sludge samples collected from same locations were analyzed in the present study. Fecal specimens from 104, 82 and 196 human subjects belonging to categories Sgr 1a, Sgr Ib and Sgr II respectively and a total of 286 buffalos sharing residential premises with Sgr Ia and Sgr Ib human subjects were subjected to similar analysis.

Prevalence of ESBL producing *E. coli* was detected to be maximum during rainy season followed by summer and winter in all locations of pond environment i.e. surface water, pond bed sludge and soil. Out of the three sites in each radial direction chosen for sampling of pond environment, samples collected at 2 ft distance from water edge showed higher prevalence of ESBL producing *E. coli* in surface water as well as in pond sludge compared to other two sites regardless of the season. However, there was no statistical difference in the prevalence of ESBL producing *E. coli* between samples collected from surface water and that from sludge at the same point of collection in the pond. Analysis of molecular types of the ESBL positive isolates revealed CTX-M variety alone to be the predominant type regardless of the point of collection from pond environment with very few isolates found to harbor CTX-M plus other ESBL genes and other ESBL genes alone (Table 1).

Prevalence of ESBL producing *E. coli* along with its CTX-M variety was more in the Sgr Ia human subjects compared to those belonging to Sgr Ib and Sgr II (Table 2). There was high prevalence of ESBL producing *E. coli* among the isolates from buffalos (89 out of 286 i.e. 31.1% of buffalos) that belonged predominantly to CTX-M type alone (72 out of 89 i.e. 80.9 %) (data shown in footnote of Table 2). Carbapenem resistance could be detected in human subjects belonging to various subgroups with comparable prevalence without being detected in any sample from pond

Table 3. Co-resistance pattern of the ESBL producing *E. coli* isolated from human subjects, companion animals (buffalos) and pond environment

	Human subjects			Companion animals n=89 No (%)	Pond environment n =378 No (%)	Statistical comparison between various categories of human subjects			
	Sgr Ia n=39 No (%)	Sgr Ib n=17 No (%)	Sgr II n=44 No (%)			Overall	Sgr Ia Vs Sgr Ib	Sgr Ia Vs Sgr II	Sgr Ib Vs Sgr II
AMC (20/10 µg)	14(35.9)	6 (35.3)	18 (40.9)	21 (23.6)	73 (19.3)	NS (0.7)	NS (1)	NS (0.6)	NS (0.7)
GEN (10 µg)	7 (20.5)	1 (5.9)	3 (6.8)	21 (23.6)	27 (7.1)	NS (0.07)	NS (0.2)	NS (0.1)	NS (0.9)
CTR (30 µg)	39(100)	17 (100)	42 (95.5)	84 (94.4)	378 (100)	NS (0.2)	NS (1)	NS (0.3)	NS (0.6)
EFT (30 µg)	13(33.3)	3 (17.6)	0	50 (56.2)	168 (44.4)	<0.001	NS (0.2)	0.001	0.01
CPM (30 µg)	11(28.2)	4 (23.5)	12 (27.3)	16 (18)	43 (11.4)	NS (0.9)	NS (0.7)	NS (0.9)	NS (0.8)
AT (30 µg)	39 (100)	17 (100)	44 (100)	89 (100)	360 (95.2)	-	-	-	-
CIP (5 µg)	10 (25.6)	3 (17.6)	8 (18.2)	17 (19.1)	32 (8.5)	NS (0.4)	NS (0.5)	NS (0.4)	NS (1)
COT (25 µg)	12 (30.8)	2 (11.8)	2(4.5)	21 (23.6)	60 (15.9)	0.004	NS (0.1)	0.001	NS (0.3)
ETP (10 µg)	3 (7.7)	2 (11.8)	4 (9.1)	0 (0)	0 (0)	NS (0.8)	NS (0.6)	NS (0.8)	NS (0.9)
TE (10 µg)	17 (43.6)	4 (23.5)	7 (15.9)	41 (46.1)	135 (35.7)	0.002	NS (0.2)	0.006	NS (0.5)

AMC: Amoxyclav, GEN: Gentamicin, CTR: Ceftriaxone, EFT: Ceftiofur, CPM: Cefepime, AT: Aztreonam, CIP: Ciprofloxacin, COT: Co-trimoxazole, ETP: Ertapenem, TE: Tetracycline, TGC: Tigecycline
Note: All the isolates were sensitive to piperacillin/tazobactam, amikacin and tigecycline and resistant to ampicillin, and cefotaxime.

environment or from animal source i.e. from buffalos.

Prevalence of the co-resistance pattern among the ESBL resistant *E. coli* isolated from the three subgroups of human subjects showed identical pattern for all the antibiotics tested except that for gentamicin, ciprofloxacin and ceftiofur that were found to be more prevalent among the Sgr Ia i.e. the subgroup of human subjects engaged in bathing the buffalos in the pond compared to Sgr Ib i.e.. the human subjects in the same residential premises involved in other types of care of buffalos as well as to Sgr II human subjects that were not associated with direct contact with any livestock. Such difference was more marked for Ceftiofur compared to other two antibiotics i.e. gentamicin and ciprofloxacin (Table 3).

DISCUSSION

Numerous studies on ESBL-producing *Enterobacteriaceae* isolated from aquatic environments have been focused on reservoirs fed by antimicrobials in aquaculture.[22-24] There is an isolated report on ESBL-producing *Enterobacteriaceae* in water reservoir serving as source of drinking water in rural area.[25] To the best of our knowledge there is hardly any study on assessment of risk for acquisition of ESBL producing Enterobacteriaceae from earth pond used for bathing and cleaning of livestock by humans.

In the present study, prevalence of AMR in pond environment was found to be maximum during the rainy season compared to other seasons regardless of the type of sample i.e. pond edge soil or sediment sludge or surface water and location of sampling in the pond i.e. 2ft or 20 ft away from pond edge. In a study by Hoa, et al. in Vietnam higher prevalence of Tetracycline and Ampicillin resistant bacteria were recorded in the rainy season than other seasons.[26] Heavy rains during the monsoon season leads to considerable flow of run-off water from soil and anthropogenic sources into aquatic environments carrying bacteria and accompanying resistant genes.[27,28] The load of different varieties of AMR was found to be maximum in both surface water and sediment samples at the point closer to the edge of the pond (at 2 ft from edge) compared to the similar samples collected further away from the edge (20 ft away from the edge) and least from the dry soil on the edge. While the ponds selected in the present study were away from any health care setting (> 2 km distance), we could not rule out the possible contribution of runoff water from surrounding agricultural soils that are frequently irrigated with canal water receiving untreated community sewage.[29] Moreover, local birds e.g. common egrets, pigeons visiting the pond may contribute to ESBL producing *E. coli* as reported in elsewhere.[30] These factors may explain highest prevalence of ESBL producing *E. coli* detected in surface water and sludge samples drawn from the area closer to the edge of the pond compared to the location further away from the edge and the pond edge soil samples that is exposed to sunlight and dry atmosphere providing the least favorable environment for persistence or growth of bacteria. In the present study *E. coli* with ESBL positivity were isolated from the pond bottom sludge samples with same frequency as that of surface samples at the same site. This appears to be a paradoxical observation considering that pond sediment is known to be rich in organic matters[31] and coliforms are facultative anaerobes.[32] Probable explanation for such observation could be shallowness of pond insignificant enough to result in difference at the two levels and vigorous mixing of water due to movement of buffalos and humans.[27]

ESBL producing isolates, especially those producing CTX-M type enzymes, are able to colonize into almost any kind of setting and are now being increasingly acquired by the human community.[33] As a consequence, the CTX-Ms have emerged to be the dominant type of ESBL over the years replacing the TEM and SHV types in both human,[34] veterinary sector[35] as well as in environment[36] which is also reflected in the present study showing the CTX-M variety being the predominant molecular variety among the ESBL isolates from all sources.

Present study showed that the activities like bathing and cleaning of the livestock in earth ponds could serve as important risk factors for transmission of AMR from the livestock to human since prevalence of various categories of AMR studied were significantly higher in the subjects engaged in bathing and cleaning of livestock (sgr Ia) compared to the age and sex matched members

in same families without such engagement (sgr Ib) as well as to the group of villagers without having direct exposure to livestock (sgr II). Considering that the ponds selected in the present study in Northern India were not used for any activity like aquaculture or had any access to effluents from health care establishment, the apparent source of AMR demonstrated in *E. coli* isolated in pond water and pond bed sludge could be the fecal contamination from buffalos that showed high prevalence of intestinal carriage of similar organisms in the present study. In contrast to developing countries, livestock are not used as food animals in India and thus application of antibiotics as growth promoters does not seem to account for such high prevalence of AMR in them. However, in contrast to the use of antibiotics in human, there are no well-defined regulatory guidelines on veterinary use of antibiotics in India resulting in their indiscriminate use.[37] Thus, unregulated use of antibiotics for various ailments in livestock could account for high AMR in them encountered in the present study. A survey published in 2005 by the OIE (World Organization for Animal Health) revealed that lack of regulatory guidelines controlling the use of antimicrobial agents in livestock is not unique for India.[38] Even in developed countries like United States, many antibiotics are readily available to the livestock farmers without any prescription.[39] Transmission of resistant bacteria from animals to humans through oral route have been documented in numerous reports among subjects in close association with livestock.[40,41] Our observation adds another scenario for transmission of antibiotic resistant bacteria through oral route hitherto unreported.

Most of the data on carbapenem resistance in rural community from this part of the country, although limited, are hospital based showing prevalence rate as 7.8 to 22.1%.[42-44] Observation in our laboratory indicated a steady rise in the prevalence of carbapenemase producing *E. coli* and *K. pneumoniae* in clinical specimens among patients from the local rural community attending hospitals over the period between 2015 and 2018.[45] However, there is hardly any report on estimation of carriage rate of carbapenem resistance in rural community other than those seeking health care that may be an important determinant of the burden in the population.

Comparable prevalence of carbapenem resistance in various subgroups of human subjects i.e. those involved in the activity of bathing and cleaning of buffalos, those involved in other activities related to other types of care of buffalos and those without any direct exposure to the buffalos suggest lack of association with livestock as the primary cause of such resistance. In an earlier study on surveillance of carbapenem resistance in a subpopulation of hospital attending patients (mostly farmers with livestock) from the same rural community in which the ponds in the present study are located the prevalence of carbapenem resistant among *E. coli* was found to be (7.8%) that could be epidemiologically linked with history of prior exposure to hospital environment rather than association with livestock.[42] Carbapenems are not permitted for animal use globally.[46] To the best of knowledge, there has not been any report from India on carbapenem resistance among animals that supports lack of any isolation of carbapenem resistant *E. coli* in samples from buffalos in the present study.

Fluoroquinolones, third-generation cephalosporins and tetracyclines are the most commonly used antibiotics in veterinary sector in many developing countries, resulting in injudicious use of these antibiotics.[47] Moreover, use of penicillins, tetracyclines, macrolides and aminoglycosides are pronounced in veterinary medicine, and these have been used for more than 50 years in livestock for treatment of ailments like mastitis, pneumonia and metritis.[48] An interesting observation of the present study was the finding of resistance to ceftiofur exclusively among human subjects having companion livestock, in significantly higher proportion among those engaged in bathing and cleaning of buffalos compared to those without such engagement. Ceftiofur, a third-generation cephalosporin is known to be used exclusively for veterinary ailments and is not considered for human use due to its toxicity.[49] However, ceftiofur has been reported to be capable of transferring resistance to other members of third generation Cephalosporins of human therapeutic importance.[50]

CONCLUSION

Higher carriage rate of antibiotic resistance detected in the human population

engaged in the bathing and cleaning of buffalos, especially for the antibiotics used in veterinary therapy suggests the activities associated with bathing and cleaning of buffalos as definite risk factors towards acquisition of such resistance.

AKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

None.

AUTHORS' CONTRIBUTION

LSD contributed in specimen collection, conducting laboratory work, data analysis and manuscript writing. DC contributed in study conception and designing the protocol, supervised the overall work and manuscript writing. Both the authors read and approved the final manuscript for publication.

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, SGT University under the protocol no. SGTU/FMHS/MICRO/341.

REFERENCES

1. Freed SA, Freed RS. Cattle in a North Indian Village. *Ethnol.* 1972;11(4):399-408. doi: 10.2307/3773071
2. Cruz-Cruz LADL, Guerrero-Legarreta I, Ramirez-Necoechea R, et al. The behavior and productivity of water buffalo in different breeding systems: a review. *Vet Med-Czech.* 2014;59(4):181-193. doi: 10.17221/7479-VETMED
3. Presicce GA. The Buffalo (*Bubalus bubalis*) - Production and Research. Benthem Science Publishers; 2017. doi: 10.2174/9781681084176117010l
4. Holmes AH, Moore LSP, Sundsfjord A, et al. Understanding the mechanism and drivers of antimicrobial resistance. *The Lancet.* 2016;387(10014):176-187. doi: 10.1016/S0140-6736(15)00473-0
5. Marshall BM, Levy SB. Food animals and antimicrobial: impact on human health. *Clin Microbiol Rev.* 2011;24(4):718-733. doi: 10.1128/CMR.00002-11
6. Gao L, Hu J, Zhang X, et al. Dissemination of ESBL-producing *Escherichia coli* of chicken origin to the nearby river water. *J Mol Microbiol Biotechnol.* 2014;24(4):279-285. doi: 10.1159/000365786
7. Gao L, Hu J, Zhang X, et al. Application of swine manure on agricultural fields contributes to extended-spectrum β-lactamase-producing *Escherichia coli* spread in Tai'an, China. *Front Microbiol.* 2015;6:313. doi: 10.3389/fmicb.2015.00313
8. Benavides JA, Salgado-Coxito M, Opazo-Capurro A, et al. ESBL-producing *Escherichia coli* carrying CTX-M genes circulating among livestock, dogs, and wild mammals in small-scale farms of Central Chile. *Antibiotics.* 2021;10(5):510. doi: 10.3390/antibiotics10050510
9. Pitout JDD, Nordmann P, Laupland KB, Poirel L. Emergence of *Enterobacteriaceae*-producing extended-spectrum beta-lactamases (ESBLs) in the community. *J Antimicrob Chemother.* 2005;56(1):52-59. doi: 10.1093/jac/dki166
10. Ghazali MF, Chai MH, Sukiman MF, Mohamad MN, Ariffin SMZ. Prevalence of carbapenem-resistant *Escherichia coli* (CREC) within farm animals in Malaysia. *Int J Infect Dis.* 2021;101(S1):534-535. doi: 10.1016/j.ijid.2020.09.1386
11. Lubbert C, Straube L, Stein C, et al. Colonization with extended-spectrum beta-lactamase-producing and carbapenemase-producing *Enterobacteriaceae* in international travellers returning to Germany. *Int J Med Microbiol.* 2015;305(1):148-156. doi: 10.1016/j.ijmm.2014.12.001
12. Ruppe E, Armand-Lefevre L, Estellat C, et al. High rate of acquisition but short duration of carriage of multi-drug resistant *Enterobacteriaceae* after travel to the tropics. *Clin Infect Dis.* 2015;61(4):593-600. doi: 10.1093/cid/civ333
13. Batram J, Balance R. UNEP/WHO publication: Water quality monitoring - a practical guide to the design and implementation of freshwater quality studies and monitoring programs. United Nations Environment Programme and the World Health Organization, 1996.
14. Freeman JC, Nimmo J, Gregory E, et al. Predictors of hospital surface contamination with ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*: patient and organism factors. *Antimicrob Resist Infect Control.* 2014;3:5. doi: 10.1186/2047-2994-3-5
15. Mercat M, Clermont O, Massot M, et al. *Escherichia coli* population structure and antibiotic resistance at a buffalo/cattle interface in Southern Africa. *Appl Environ Microbiol.* 2016;82(5):1459-1467. doi: 10.1128/AEM.03771-15
16. Luvsansharav UO, Hirai I, Nakata A, et al. Prevalence of and risk factors associated with faecal carriageof CTX-M b-lactamase-producing *Enterobacteriaceae* in rural Thai communities. *J Antimicrob Chemother.* 2012;67(7):1769-1774. doi: 10.1093/jac/dks118
17. Schmidt AS, Bruun MS, Dalsgaard I, Pedersen K, Larsen JL. Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with four Danish Rainbow trout Farms. *Appl Environ Microbiol.* 2000;66(11):4908-4915. doi: 10.1128/

AEM.66.11.4908-4915.2000
18. Valverde A, Coque TM, Sanchez-Moreno MP, Rollan A, Baquero F, Canton R. Dramatic increase in prevalence of fecal carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae during non-outbreak situations in Spain. *J Clin Microbiol.* 2004;42(10):4769-4775. doi: 10.1128/JCM.42.10.4769-4775.2004
19. Clinical Laboratory Standard Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing; 29th Informational Supplement. M100-S27, CLSI, Wayne, Pennsylvania, USA, 2019.
20. Sidjabat HE, Paterson DL, Adams-Haduch JM, et al. Molecular epidemiology of CTX-M-producing *Escherichia coli* isolates at a tertiary medical center in Western Pennsylvania. *Antimicrob Agents Chemother.* 2009;53(11):4733-4739. doi: 10.1128/AAC.00533-09
21. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard. 3rd ed. CLSI document M31-A3. Wayne, Pennsylvania, USA, 2007.
22. FAO. The state of world fisheries and aquaculture 2018 - Meeting the sustainable development goals. Rome, Licence: CC BY-NC-SA 3.0 IGO, 2018.
23. Cabello FC, Godfrey HP, Tomova A, et al. Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. *Environ Microbiol.* 2013;15(7):1917-1942. doi: 10.1111/1462-2920.12134
24. Kerry J, Hiney M, Coyne R, Cazabon D, NicGabhainn S, Smith P. Frequency and distribution of resistance to oxytetracycline in microorganisms isolated from marine fish farm sediments following therapeutic use of oxytetracycline. *Aquaculture.* 1994;123(1-2):43-54. doi: 10.1016/0044-8486(94)90118-X
25. Zhang H, Zhou Y, Guo S, Chang W. Multidrug resistance found in extended-spectrum beta-lactamase-producing *Enterobacteriaceae* from rural water reservoirs in Guantao, China. *Front Microbiol.* 2015;6:267. doi: 10.3389/fmicb.2015.00267
26. Hoa PTP, Managaki S, Nakada N, et al. Antibiotic contamination and occurrence of antibiotic resistant bacteria aquatic environments of northern Vietnam. *Sci Total Environ.* 2011;409(15):2894-2901. doi: 10.1016/j.scitotenv.2011.04.030
27. Qureshi AA, Dutka BJ. Microbiological studies on the quality of urban storm water runoff in southern Ontario, Canada. *Water Res.* 1979;13(10):977-985. doi: 10.1016/0043-1354(79)90191-X
28. Kistemann T, ClaBen T, Koch C, et al. Microbial load of drinking water reservoir tributaries during extreme rainfall and runoff. *Appl Environ Microbiol.* 2002;68(5):2188-2197. doi: 10.1128/AEM.68.5.2188-2197.2002
29. EPA document: Protecting water quality from agricultural run-off. United States Environmental Protection Agency (4503T), document no. EPA 841-F-05-001. Washington DC, 2005.
30. Radimersky T, Frolkova P, Janoszowska D, et al. Antibiotic resistance in faecal bacteria (*Escherichia coli*, *Enterococcus* spp.) in feral pigeons. *J Appl Microbiol.* 2010;109(5):1687-1695. doi: 10.1111/j.1365-2672.2010.04797.x
31. Sahuquillo M, Miracle MR, Morata SM, Vincente E. Nutrient dynamics in water and sediment of Mediterranean ponds across a wide hydroperiod gradient. *Limnologica*. 2012;42(4):282-290. doi: 10.1016/j.limno.2012.08.007
32. Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney Practical Medical Microbiology. 14th ed. London: Churchill Livingstone 1996:361-381.
33. Canton R, Coque TM. The CTX-M lactamase pandemic. *Curr Opin Microbiol.* 2006;9(5):466-475. doi: 10.1016/j.mib.2006.08.011
34. Livermore DM, Canton R, Gniadkowski M, et al. CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother.* 2007;59(2):165-174. doi: 10.1093/jac/dkl483
35. Carattoli A. Animal reservoirs for extended spectrum beta-lactamase producers. *Clin Microbiol Infect*. 2008;14(Suppl 1):117-123. doi: 10.1111/j.1469-0691.2007.01851.x
36. Taneja N, Sharma M. Antimicrobial resistance in the environment: The Indian scenario. *Indian J Med Res.* 2019;149(2):119-128. doi: 10.4103/ijmr.IJMR_331_18
37. Kumar R, Kalaiselvan V, Verma R, Kaur I, Kumar P, Singh GN. Veterinary pharmacovigilance in India: A need of the hour. *Indian J Pharmacol.* 2017;49(1):2-3. doi: 10.4103/0253-7613.201035
38. OIE annual report on antimicrobial agents intended for use in animals: Better understanding of the global situation. World Organization for Animal Health (OIE), Paris, France. 2017.
39. Green AL, Carpenter LR, Edmisson DE, et al. Producers attitudes and practices related to antimicrobial use in beef cattle in Tenesse. *J Am Vet Med Assoc.* 2010;237(11):1292-1298. doi: 10.2460/javma.237.11.1292
40. Levy SB, FitzGerald GB, Macone AB. Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. *N Engl J Med.* 1976;295(11):583-588. doi: 10.1056/NEJM197609092951103
41. van den Bogaard AE, London N, Driessen C, Stobberingh EE. Antibiotic resistance of fecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. *J Antimicrob Chemother.* 2001;47(6):763-771. doi: 10.1093/jac/47.6.763
42. Devi LS, Grover SS, Khare S, Chattopadhya D. Carbapenemase and NDM-1 production by *Escherichia coli* and *Klebsiella pneumoniae* from patients belonging to a rural community in North India hospitalized with community- acquired infections. *J Commun Dis*. 2018;50(2):5-10. doi: 10.24321/0019.5138.201808
43. Sekar R, Srivani S, Amudhan M, Mythreyee M. Carbapenem resistance in a rural part of southern India: *Escherichia coli* versus *Klebsiella* spp. *Indian J Med Res.* 2016;144(5):781-783. doi: 10.4103/ijmr.IJMR_1035_15
44. Sekar R, Mythreyee M, Srivani S, Sivakumaran D, Lallitha S, Saranya S. Carbapenem-resistant Enterobacteriaceae in pediatric blood stream infections in rural Southern India. *Indian Pediatr.* 2017;54(12):1021-1024. doi: 10.1007/s13312-017-

1204-1

45. Devi LS, Broor S, Rautela RS, Grover SS, Chakravarti A, Chattopadhya D. Increasing prevalence of *Escherichia coli* and *Klebsiella pneumoniae* producing CTX-M type extended-spectrum beta-lactamase (CTX-M-ESBL), carbapenemase and NDM-1 in patients from a rural community with community acquired infections: A three years study. *Int J Appl Basic Med Res.* 2020;10(3):156-163. doi: 10.4103/ijabmr.IJABMR_360_19
46. World Organization for Animal Health (OIE). OIE list of antimicrobial agents of veterinary medicine 2015.
47. Ajayi A, Oluyege A, Olowe A, famurewa A. Antibiotic resistance among commensal *E. coli* isolated from faeces from cattle Ado-Ekiti Nigeria. *J Anim Vet Adv.* 2011;10(2):174-179. doi: 10.3923/javaa.2011.174.179
48. Pagel SW, Gautier P. Use of antimicrobial agents in livestock. *Rev Sci Tech.* 2012;31(1):145-188. doi: 10.20506/rst.31.1.2106
49. Hornish RE, Kotarski SF. Cephalosporins in veterinary medicine-ceftiofur use in food animals. *Curr Trop Med Chem.* 2002;2(7):717-731. doi: 10.2174/1568026023393679
50. Wittum TE. The challenge of regulating agricultural ceftiofur use to slow the emergence of resistance to extended-spectrum cephalosporins. *Appl Environ Microbiol.* 2012;78(22):7819-7821. doi: 10.1128/AEM.01967-12