Antimicrobial Drug Resistance of *Escherichia coli* Isolated from Piglets in North East India

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Authors' contributions

This work was carried out in collaboration among all authors. Authors HK and TKD designed and planned the research work. Authors HK and RM collected the samples. Author HK executed the isolation, biochemical and molecular characterization work. Authors HK and LK carried out the antibiotic sensitivity assay. Authors HK, PR and PKS monitored the results and assay. Authors HK and TCT analyzed the data. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The research is aimed to study the antimicrobial drug resistance (AMR) of *Escherichia coli* from fecal samples of pre-weaned piglets in North Eastern states (NE) of India.
Materials and Methods: A total of 457 fresh fecal samples were collected from pre-weaned piglets of organized (n=225) and unorganized (n=232) farms of 4 North Eastern states of India, namely, Manipur, Meghalaya, Mizoram and Nagaland state. Samples were collected from diarrheic (n=339) and non-diarrheic (n=118) piglets in different seasons during the study period. The samples were processed for isolation of *E. coli* and detection of their putative virulence genes by polymerase chain reaction (PCR).
Results: A total of 1286 *E. coli* were isolated. Forty-two isolates (3.26%) were found to be atypical

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enteropathogenic *E. coli* (aEPEC) and thirty isolates (2.33%) belongs to shigatoxigenic *E. coli* (STEC) in which 28 isolates were positive for *stx2* gene and two isolates possessed *hlyA* gene. All the 1286 isolates showed wide variation (0.15-78.69%) in resistance pattern against the 15 antimicrobial agents; of which there is higher resistance against cefalexin (78.69%), amoxycillin (77.13%), ampicillin (72.31%) and enrofloxacin (60.73%). The isolates showed low resistance to imipenem (0.15%), ceftriaxone (8.32%), ciprofloxacin (8.39%) and streptomycin (8.94%). Higher prevalence of AMR to numerous antimicrobials in this study was observed in isolates of organized farm compared to unorganized farming system.

Conclusion: The present study exhibited variation in AMR in different NE states of India as well as in different farming system which indicate that drug consumption and resistance are closely related.

1. INTRODUCTION

Increasing Antimicrobial resistance (AMR) amongst enteric bacteria in production animals and their potential transmission to humans represent a major threat to public health and has been a growing problem worldwide [1]. Bacteria from livestock are believed to be a major source of AMR in the environment, and resistant bacteria and resistance genes may be acquired by the human gut microbiome [2]. In a meeting of the World Health Organization on antimicrobial resistance many low and middle income countries were predicted to have the extensive obstacles in managing AMR which leads to subsequent burden of diseases, in which problem also lies in limited data available on current resistance patterns of common pathogenic bacteria in these countries [3].

The emergence of AMR is a natural phenomenon in all species of microorganisms, but this resistance is also accelerated throughout the world due to overuse of antimicrobial drugs in both humans and animals [4, 5]. Almost 50% of prescribed antimicrobial drugs in modern medicine are considered unnecessary, hence, this overuse and misuse of antimicrobial agents is a major factor leading toward AMR [6]. AMR is a multi-dimensional problem and its containment requires multi-pronged approach. The One Health concept being prioritize around the globe highlights the inter-relatedness among human health, animal health, food and environment and fosters collaborative efforts on the part of the health authorities dealing with these spheres [7]. From human perspective, multidrug resistance bacteria results in more death than cancer and road traffic accidents; with another 10 million projected to die from consequences of AMR by the year 2050, [8]. Economic projections suggest that by 2050, AMR would decrease gross domestic product (GDP) by 2 to 3.5 per cent with a fall in livestock productivity by 3-8 per cent, costing USD100 trillion to the world [9].

India has a total of 10.29 million pig population in which the North East Region alone accounted for 3.95 million pigs in the country [10] where pig farming is an integral part of life and provides significant source of income to the farmers. India has a huge potential for livestock related industry, however, overuse of antimicrobials to increase livestock productivity have led to the rising rates of AMR in the past decades in the country [11]. India accounts for 3% of the global consumption of agricultural antibiotics, which is estimated to double by 2030 [12] and is projected by 2020 to be the fourth largest consumer of antibiotics in food animal production after China, USA and Brazil [13]. Lack of sufficient research and paucity of data on antibiotic usage in animals hampers the estimation of an extent of AMR but prevents a nationwide comparison. Absence of uniform regulations on antimicrobial use in animal production poses a serious challenge to the enforcement of rational antibiotic use in the country. Piggery is a promising venture with vast potential in NE India but disease problems including *E. coli* infection along with antimicrobial drug resistance pathogens remain a constant threat to economic viability of swine industry. *Escherichia coli* are usually susceptible to many antimicrobial agents particularly the third generation cephalosporins such as cefotaxime, ceftriaxone and ceftazidime but increasing resistance to many antimicrobials is of serious concern. Keeping in view the importance of AMR in the country, the present research was conducted to study the prevalence of AMR *E. coli* from piglets of organized and unorganized farms in North East India.

2. MATERIALS AND METHODS

2.1 Faecal Samples

A total of 457 fresh faecal samples were collected from piglets under 9 weeks old from organized (n=225) and unorganized (n=232) farms of four North Eastern States of India (Fig.1), namely, Manipur (n=108), Meghalaya (n=124), Mizoram (n=120), and Nagaland (n=105) during 2015. Samples were collected from diarrhoeic (n=339) and apparently healthy (n=118) piglets including cross breed (n=327) and indigenous local (n=130) piglets. For this study, the pig farms were categorized as organized and unorganized farm according to their pig rearing practice. Organized pig farms have been considered to be those rearing more than 50 pigs and followed intensive system of farming, whereas, unorganized farms are backyard farming where farmers reared 5-10 pigs.

2.2 Isolation and Identification

Freshly collected faecal samples were directly streaked on MacConkey's agar (Hi-media, India) plates for *E. coli* isolation and incubated at 37ºC for 20 h. Four pink coloured colonies were randomly picked up from each plate and streaked on Eosin methylene blue (EMB) agar plates (Hi-media, India) followed by 20 h incubation at 37ºC. Colonies with characteristic metallic sheen were studied for their morphological characteristics and biochemical tests such as indole, methyl red, Voges Proskauer test, citrate utilization test, Hydrogen sulphide production on triple sugar iron [14]. Isolates were stored as pure culture on semi-solid Luria Bertani agar at 4ºC and in glycerol stock at -20°C.

2.3 Molecular Detection of EPEC and STEC

Template DNA was prepared by boiling and snap chilling method as per standard procedure. A bacterial suspension of 1 ml was taken in a sterile microtube and pelleted by centrifugation at 8000 rpm for 10 min at 4°C. The bacterial pellet was washed thrice with sterile normal saline solution (0.85% w/v) and finally pelleted at 8000 rpm for 5 min at 4°C. The bacterial pellet was then re-suspended in 300 µl sterile nuclease free water and boiled for 10 min in a boiling water bath followed by immediate chilling for 15 min.

The lysate was centrifuged again at 8000 rpm for 5 min and the supernatant was used as template DNA for PCR assay.

Molecular characterization of *E. coli* isolates was performed by multiplex PCR as described by [15] targeting *eaeA* (384bp), *stx$_1$* (180bp)*, stx$_2$* (253bp) and *hlyA* (534bp) genes. PCR was performed with a total volume of 25µl reaction mixture containing 1X PCR buffer, 100mM dNTP, 1.5mM MgCl$_2$, 1U Taq polymerase, 20pM forward and reverse primers and 4µl of DNA template. The PCR performed in a Mastercycler Gradient (Eppendorf AG, Germany) involved an initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 45 sec, annealing at 61°C for 45 sec and extension at 72°C for 45 sec; and one cycle of final extension at 72°C for 10 min. The PCR products were separated by electrophoresis on 1.5% agarose gels containing ethidium bromide (final concentration of 0.5µg/ml) in Tris-borate buffer and using 100 bp DNA ladder (Fermentas) as a molecular size marker. The amplicons were visualized and photographed by gel documentation system (AlphaImager, USA). All the *eaeA* positive isolates were further subjected by specific PCR for the detection of *bfpA* gene (426 bp) as described by [16] with initial denaturation step of 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, elongation step at 72°C for 1 min and final extension at 72°C for 5 min.

2.4 Antimicrobial Susceptibility Assay

All the isolates were subjected to *in vitro* antimicrobial susceptibility test by disk diffusion method which was performed on Mueller-Hinton agar plates as per recommendation of Clinical Laboratory Standard Institute [17] using 15 commercially available antibiotic disks, viz., ampicillin (10 mcg), amoxycillin (30 mcg), aztreonam (30 mcg), cefalexin (30 mcg), ceftazidime (30 mcg), cefixime (5 mcg), ceftriaxone (30 mcg), cefotaxime (30 mcg), ciprofloxacin (5 mcg), enrofloxacin (10 mcg), gentamicin (10 mcg), imipenem (10 mcg), nalidixic acid (30 mcg), piperacillin (10 mcg) and streptomycin (10 mcg). A pure colony of each isolate was inoculated into 5 ml of sterile Luria Bertani broth under constant shaking at 37°C overnight and a consistent density of each suspension of 2 ml is spread uniformly over prepared agar plates using sterile L-spreader, allowed to dry for 5 min before the

antibiotic disks were applied. The plates were incubated at 37°C for 20 h and zones of inhibition were measured using zone size interpretative chart. *Escherichia coli* ATCC 25922 was used as control organism in the present study.

2.5 Statistical Analysis

The difference among the *E. coli* isolates resistance patterns in the 4 NE states was evaluated by chi square test with the level of significance set at five percent.

3. RESULTS AND DISCUSSION

A total of 1286 *E. coli* were isolated from 457 fecal samples, with higher rate of isolation from piglets of organized (n=654) than unorganized farms (n=632). Higher number of isolates (n=1042) were recovered from diarrhoeic samples compared to samples of apparently healthy piglets (n=244). Of the 1286 *E. coli* screened by multiplex PCR assay for EPEC (*eaeA*) and STEC (*stx1, stx2,* and *hlyA*); forty two isolates (42/1286; 3.26%) from diarrhoeic piglets

were found to possess *eaeA* gene (EPEC). All 42 were found to be negative for the *bfpA* gene and classified as atypical EPEC. Thirty isolates (30/1286; 2.33%) were found to be STEC including 28 isolates positive for *stx2* gene and 2 isolates possessed *hlyA* gene.

All the 1286 *E. coli* isolates were subjected to antimicrobial susceptibility test against 15 selected antimicrobial agents. All of them including atypical EPEC and STEC showed resistance to at least three antimicrobials of which, cefalexin (78.69%) exhibited highest level of resistance followed by amoxycillin (77.13%), ampicillin (72.31%), enrofloxacin (60.73%), piperacillin (50.46%), nalidixic acid (29.70%), cefixime (16.32%), gentamicin (11.97%), ceftazidime (11.50%), aztreonam (11.50%), cefotaxime (9.25%), streptomycin (8.94%), ciprofloxacin (8.39%), ceftriaxone (8.32%) and imipenem (0.15%). Conversely, higher susceptibility of the isolates was recorded against imipenem (99.84%) followed by ceftriaxone (91.67%), ciprofloxacin (91.60%), streptomycin (91.05%), cefotaxime (90.74%), aztreonam (88.49%) and ceftazidime (88.49%).

Fig. 1. A map representing the four Northeast states of India in which representative sampling were collected for the study. (Source: http:/www.mea.gov.in/india-at-glance.htm)

Results of comparison of the antimicrobial resistance pattern of *E. coli* isolates in the 4 NE states of India is depicted in Table 1. and statistical analysis with significant different set at $\leq$ 0.05 is depicted in Table 2. In case of resistance to ampicillin, there was no significant difference between isolates of Mizoram and Manipur but there was significant difference between Meghalaya and Nagaland. Highest (85.53%) and lowest (53.87%) resistance was observed among the isolates from Mizoram and Nagaland, respectively. In case of amoxycillin, all the isolates of the other 3 states showed significant difference from Nagaland with highest (84.3%) and lowest (64.51%) resistance recorded from isolates of Mizoram and Nagaland, respectively. In case of resistance to aztreonam, there was no significant difference between isolates of the 3 states except Mizoram with highest (24.0%) and lowest (4.56%) resistance was observed from Mizoram and Manipur, respectively. In case of resistance to cefalexin, there was no significant difference between isolates of Meghalaya and Manipur or between Manipur, Nagaland and Mizoram but there was significant difference of Meghalaya from Mizoram and Nagaland with highest (83.05%) and lowest (74.15%) resistance from Meghalaya and Mizoram, respectively.

In case of resistance to ceftazidime, ciprofloxacin and streptomycin, there was no significant difference between the *E. coli* isolates with highest (18.76% and 16.92%) and lowest (5.16% and 1.61%) resistance to ceftazidime and streptomycin was observed from isolates in Mizoram and Nagaland, respectively, whereas highest (15.69%) and lowest (1.30%) resistance to ciprofloxacin was observed from isolates in Mizoram and Manipur, respectively. However, there was significant difference in resistance to cefixime of the isolates of Mizoram from the other three states with highest (26.46%) and lowest (8.38%) resistance from Mizoram and Nagaland, respectively. Antimicrobial drug resistance to ceftriaxone, cefotaxime and nalidixic acid was significantly different among the four NE states. Highest resistance to ceftriaxone and cefotaxime (16.3% and 17.53%) was observed from the isolates of Mizoram and lowest resistance (1.61% and 2.58%) from Nagaland state, respectively, whereas highest (48.3%) and lowest (7.1%) drug resistance to nalidixic acid was observed in *E. coli* isolates of Mizoram and Manipur, respectively. In case of resistance to enrofloxacin, there was significant difference in

resistance between the isolates in Meghalaya from the other NE states with highest (69.76%) and lowest (52.25%) AMR recorded from isolates in Meghalaya and Nagaland, respectively. In case of resistance to gentamicin, there was significant difference in resistance between isolates in Mizoram and Meghalaya with highest (14.76%) and lowest (9.01%) resistance recorded in isolates of Mizoram and Meghalaya, respectively. In case of resistance to piperacillin, there was significant difference in AMR from isolates of Manipur and Nagaland with highest (66.44%) and lowest (34.83%) resistance observed from isolates of Manipur and Nagaland, respectively. Except for imipenem, resistance for each antibiotic was significantly different at $P \leq 0.05$ from all the NE states.
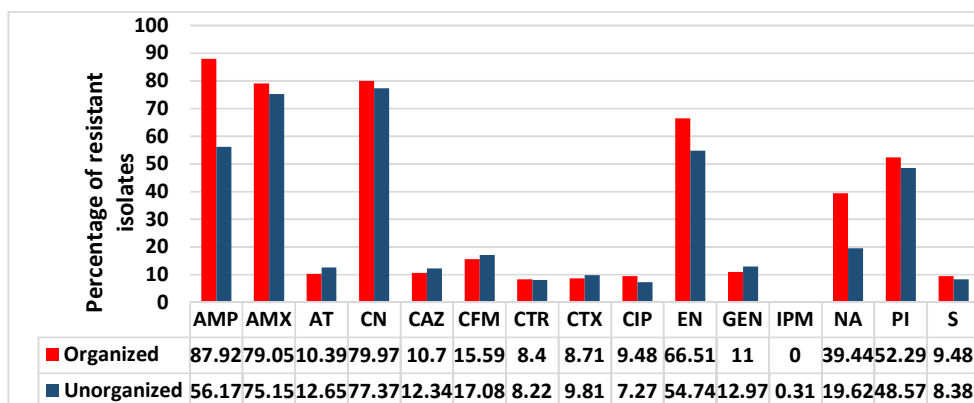
Comparison of drug resistance profile of *E. coli* isolates exhibited higher prevalence of AMR to numerous antimicrobials originated in organized farm practice compared to unorganized farming (Fig. 2). Of the 654 isolates obtained from organized farms, ampicillin (87.92%) showed highest level of resistance followed by cefalexin (79.97%), amoxycillin (79.05%) and enrofloxacin (66.61%). No isolate was found resistant to imipenem (0.0%) and antimicrobials such as ceftriaxone (8.40%), cefotaxime (8.71%), ciprofloxacin (9.48%) and streptomycin (9.48%) were also found to be the least resistant antibiotics in organized farming system. Except for imipenem, resistance for each antimicrobial was significantly different at $P \leq 0.05$ from all the 4 states. In case of isolates (n=632) in unorganized farms, cefalexin (77.37%) showed highest level of resistance followed by amoxycillin (75.15%) and ampicillin (56.17%), whereas AMR of *E. coli* against imipenem (0.31%), ciprofloxacin (7.27%) and ceftriaxone (8.22%) were recorded the lowest comparatively. Resistance to enrofloxacin was non-significant among the isolates in all the 4 NE states with highest (57.32%) and lowest (47.76%) resistance recorded from isolates of Mizoram and Nagaland, respectively. Except for imipenem and enrofloxacin, drug resistance for each antimicrobial was significantly difference at $P \leq 0.05$ from all the 4 states. Comparison of AMR pattern between organized and unorganized farm of the NE states of India, revealed that there was highly significant difference in AMR to ampicillin, nalidixic acid and enrofloxacin, whereas, non-significant difference in AMR was observed against imipenem and ceftriaxone.

Table 1. Antimicrobial resistance pattern of *E. coli* isolates in the 4 NE states of India

Antimicrobial		Manipur (n=307)	Meghalaya (n=344)	Mizoram (n=325)	Nagaland (n=310)	Total (n=1286)
AMP	S	56 (18.24)	110 (31.97)	47 (14.46)	143 (46.12)	356 (27.68)
	R	251 (81.75)	234 (68.02)	278 (85.53)	167 (53.87)	930 (72.31)
AMX	S	68 (22.14)	65 (18.89)	51 (15.69)	110 (35.48)	294 (22.86)
	R	239 (77.85)	279 (81.1)	274 (84.30)	200 (64.51)	992 (77.13)
AT	S	293 (95.43)	310 (90.11)	247 (76.0)	288 (92.90)	1138 (88.49)
	R	14 (4.56)	34 (9.88)	78 (24.0)	22 (7.09)	148 (11.50)
CN	S	62 (20.19)	60 (16.94)	84 (25.84)	78 (25.16)	274 (21.30)
	R	245 (79.8)	294 (83.05)	241 (74.15)	232 (74.83)	1012 (78.69)
CAZ	S	287 (93.48)	293 (85.17)	264 (81.23)	294 (94.83)	1138 (88.49)
	R	20 (6.51)	51 (14.82)	61 (18.76)	16 (5.16)	148 (11.50)
CFM	S	267 (86.97)	286 (83.13)	239 (73.53)	284 (91.61)	1076 (83.67)
	R	40 (13.02)	58 (16.86)	86 (26.46)	26 (8.38)	210 (16.32)
CTR	S	291 (94.78)	311 (90.40)	272 (83.69)	305 (98.38)	1179 (91.67)
	R	16 (5.21)	33 (9.59)	53 (16.30)	5 (1.61)	107 (8.32)
CTX	S	289 (94.13)	308 (89.53)	268 (82.46)	302 (97.41)	1167 (90.74)
	R	18 (5.86)	36 (10.46)	57 (17.53)	8 (2.58)	119 (9.25)
CIP	S	303 (98.69)	299 (86.91)	274 (84.30)	302 (97.41)	1178 (91.60)
	R	4 (1.30)	45 (13.08)	51 (15.69)	8 (2.58)	108 (8.39)
EN	S	120 (39.08)	104 (30.23)	133 (40.92)	148 (47.74)	505 (39.26)
	R	187 (60.91)	240 (69.76)	192 (59.07)	162 (52.25)	781 (60.73)
GEN	S	266 (86.64)	313 (90.98)	277 (85.23)	276 (89.03)	1132 (88.02)
	R	41 (13.35)	31 (9.01)	48 (14.76)	34 (10.96)	154 (11.97)
IPM	S	307 (100)	342 (99.41)	325 (100)	310 (100)	1284 (99.84)
	R	0 (0.0)	2 (0.58)	0 (0.0)	0 (0.0)	2 (0.15)
NA	S	285 (92.83)	218 (63.37)	168 (51.69)	233 (75.16)	904 (70.29)
	R	22 (7.1)	126 (36.62)	157 (48.30)	77 (24.83)	382 (29.70)
PI	S	103 (33.55	171 (49.7)	161 (49.53)	202 (65.16)	637 (49.53)
	R	204 (66.44)	173 (50.29)	164 (50.46)	108 (34.83)	649 (50.46)
S	S	297 (96.74)	299 (86.91)	270 (83.07)	305 (98.38)	1171 (91.05)
	R	10 (3.2)	45 (13.08)	55 (16.92)	5 (1.61)	115 (8.94)

Note: Percentage (%) is given in parentheses

S = Sensitive; R = Resistant; Amp = Ampicillin, AMX = Amoxycillin, AT= Aztreonam, CN = Cephalexin, CAZ = Ceftazidime; CFM = Cefixime, CTR = Ceftriaxone, CTX = Cefotaxime, CIP = Ciprofloxacin, EN = Enrofloxacin, GEN = Gentamicin, IPM = Imipenem, NA = Nalidixic acid, PI = Piperacillin, S = Streptomycin

	AMP	AMX	AT	CN	CAZ	CFM	CTR	CTX	CIP	EN	GEN	IPM	NA	PI	S
■ Organized	87.92	79.05	10.39	79.97	10.7	15.59	8.4	8.71	9.48	66.51	11	0	39.44	52.29	9.48
■ Unorganized	56.17	75.15	12.65	77.37	12.34	17.08	8.22	9.81	7.27	54.74	12.97	0.31	19.62	48.57	8.38

Fig. 2. Comparative analysis of antimicrobial resistance profile of *E. coli* isolates of organized and unorganized farms in North East India

Table 2. Statistical analysis of antimicrobial resistance pattern of *E. coli* isolates in the 4 NE states of India

Antimi crobial	Manipur (n=307)	Meghalaya (n=344)	Mizoram (n=325)	Nagaland (n=310)	Total (n=1286)
AMP	251 (81.75) [a]	234 (68.02) [b]	278 (85.53) [a]	167 (53.87) [c]	930 (72.31) [*]
AMX	239 (77.85) [b]	279 (81.1) [ab]	274 (84.30) [a]	200 (64.51) [c]	992 (77.13) [*]
AT	14 (4.56) [c]	34 (9.88) [b]	78 (24.0) [a]	22 (7.09) [bc]	148 (11.50) [*]
CN	245 (79.8) [ab]	294 (83.05) [a]	241 (74.15) [b]	232 (74.83) [b]	1012 (78.69) [*]
CAZ	20 (6.51) [b]	51 (14.82) [a]	61 (18.76) [a]	16 (5.16) [b]	148 (11.50) [*]
CFM	40 (13.02) [bc]	58 (16.86) [b]	86 (26.46) [a]	26 (8.38) [c]	210 (16.32) [*]
CTR	16 (5.21) [c]	33 (9.59) [c]	53 (16.30) [a]	5 (1.61) [d]	107 (8.32) [*]
CTX	18 (5.86) [c]	36 (10.46) [b]	57 (17.53) [a]	8 (2.58) [d]	119 (9.25) [*]
CIP	4 (1.30) [b]	45 (13.08) [a]	51 (15.69) [a]	8 (2.58) [b]	108 (8.39) [*]
EN	187 (60.91) [b]	240 (69.76) [a]	192 (59.07) [bc]	162 (52.25) [c]	781 (60.73) [*]
GEN	41 (13.35) [ab]	31 (9.01) [b]	48 (14.76) [a]	34 (10.96) [ab]	154 (11.97) [*]
IPM	0 (0.0)	2 (0.58)	0 (0.0)	0 (0.0)	2 (0.15)
NA	22 (7.1) [d]	126 (36.62) [b]	157 (48.30) [a]	77 (24.83) [c]	382 (29.70) [*]
PI	204 (66.44) [a]	173 (50.29) [b]	164 (50.46) [b]	108 (34.83) [c]	649 (50.46) [*]
S	10 (3.2) [b]	45 (13.08) [a]	55 (16.92) [a]	5 (1.61) [b]	115 (8.94) [*]

Note: Percentage (%) is given in parentheses
*Values bearing different superscripts ([a, b, c, ab, bc]) differ significantly between the states; * Denote significantly different at ≤ 0.05; Amp = Ampicillin, AMX = Amoxycillin, AT= Aztreonam, CN = Cefalexin, CAZ = Ceftazidime, CFM = Cefixime, CTR = Ceftriaxone, CTX = Cefotaxime, CIP = Ciprofloxacin, EN = Enrofloxacin, GEN = Gentamicin, IPM = Imipenem, NA = Nalidixic acid, PI = Piperacillin, S = Streptomycin*

Antimicrobial resistance in enteric bacteria is a serious problem globally in both human and veterinary medicine. Food animals such as pigs may act as a reservoir and potential source for dissemination of resistant bacterial population in the environment. The most common mechanism of resistance to beta-lactam antibiotics among gram negative bacteria including *E. coli* is the production of extended-spectrum-betalactamases which hydrolyze most penicillins, extended spectrum cephalosporins and aztreonam; which have been increasing worldwide [18]. Pork is the major protein source for human consumption in North East India and people in rural countryside reared 5 to 10 pigs near their houses and in most cases share common source of water, hence there is probably a high risk of transmission of infection as well as antimicrobial resistant pathogens in the region.

Antimicrobial resistance was observed at a range of 0.15-78.69%, which shows extreme variation in the susceptibility of *E. coli* to various antimicrobial agents. Our findings show maximum resistance against cefalexin, amoxycillin, ampicillin and enrofloxacin; and high degree of susceptibility to imipenem, ceftriaxone, ciprofloxacin, streptomycin and cefotaxime. High resistance rate to cefalexin, ampicillin,

amoxycillin and enrofloxacin in the North East India is alarming, as most of the veterinarians use the above drugs commonly for treatment of gastroenteritis and other infection. In India, there are reports of 92.9% resistance of *E. coli* isolates to ampicillin in children [19] and resistance of 80.43% in poultry in Eastern India [20] which also were particularly true with our finding; however, their finding of high resistance to cefotaxime (47.5%) disagree with our report in which we could detect only 9.25% resistance against it. Also, Pandey et al., [21] reported 100% resistance of *E. coli* isolates of pigs to cephalexin and ampicillin. We reported high susceptibility of the isolates to ciprofloxacin (91.6%) and gentamicin (88.02%) which agreed with reports of other scientists [21,20] where ciprofloxacin was the drug of choice as far as susceptibility is concerned and high susceptibility to ciprofloxacin that has been observed is consistent with the finding of other authors [22]. There is a variation in resistance to streptomycin, ampicillin, cefotaxime, ceftriaxone and ceftazidime as compared to findings of Sasirekha et al., [23]. Resistance to gentamicin in sub-Saharan African countries has been reported to vary up to 35% [24] for *E. coli* and 55.6% in India [19] which disagree with our study where we could detect only low percentage of AMR to gentamicin. A high degree of AMR was also reported in India

against cephalexin and enrofloxacin in *E. coli* infection of poultry [25,26] which were in accordance with our report. This pattern of susceptibility was also observed by other scientists [27,28,29]. Considering these resistance percentages, cephalexin and ampicillin as an affordable antibiotic for infections will likely become less efficacious in clinical treatment, leaving third-generation cephalosporins and fluoroquinolones as drugs of choice for empirical treatment.

Carbapenems such as imipenem are the new β-lactams having broad spectrum of antimicrobial activity and are usually reserved for treating infections caused by multidrug-resistant agents and wide variety of infections, particularly in cases of infections caused by cephalosporin-resistant bacteria [30,31,32]. Any decrease in susceptibility to imipenem should be reported and taken seriously. In our study, only 0.15% of the isolates were found resistant to imipenem which is in corroboration with findings of other scientists [33, 34]. Our report of low resistance against ceftriaxone agrees with report on ceftriaxone resistant *E. coli* from chicken meat [35]. Imipenem is not in use in North East India and other drugs such as ceftriaxone, cefotaxime, ciprofloxacin because of their high cost have limited usage among the farmers. This may probably explain the higher susceptibility of these drugs to *E. coli* infection in the region.

These data showed that drug use and resistance are closely related. Lack of scientific literatures and data regarding AMR of *E. coli* in organized and unorganized farming system did not allow us to compare our results. The single main factor contributing for the increase in the antibiotics resistance is irrational use of antibiotics [36]. Amoxycillin, cefalexin, enrofloxacin and ampicillin are commonly used in veterinary practices in North East India since they are easily available and high resistance of the isolates to these drugs in the region might be the result of the indiscriminate use of these antibiotics in piggery industry which may contribute to the selection of resistant variants. Other explanations include transmissible or plasmid mediated drug resistance and mutational changes in the genes that are crucial to the development of resistance in *E. coli*.

Higher prevalence of AMR in organized compared to unorganized farming system might be due to direct correlation with managemental practices in the region as farmers of small private holdings are unable to support regular medication at their own and hence, decrease utilization of antimicrobials. However, in case of many organized farms usage of regular antibiotics for many years for treatment and probably sub-therapeutic doses being administered for prevention or control of infection may contribute to higher AMR. History of any illness in pigs and their treatment could be a risk factor affecting the distribution of antimicrobial-resistant genes. In North East, several public organized pig farms usually administered many types of antimicrobials without appropriate veterinary supervision. This is likely one of the consequence of the antimicrobial abuse which leads to higher AMR. Practice management of animals was found to affect different type of antimicrobial-resistant which showed the type of practice and premises as a major risk factor for antimicrobial-resistance in *E. coli* in pigs. This also represent an increased opportunity for acquisition of multi-drug resistant bacteria carrying the antimicrobial-resistant genes. Other potential risk factors such as type of feed also may lead to higher AMR where pigs in many organized farming system are fed manufactured or processed feed mostly supplemented with antimicrobials. Animals that has received antimicrobial treatment was at higher risk for fecal shedding of antimicrobial-resistant *E. coli*, compared to the animals that didn't receive antimicrobial treatment. Many AMR genes were significantly associated with the administration of antibiotics, which many studies has also been suggested before that inappropriate and overuse use of antimicrobials are the principal causes of widespread antimicrobial resistance, which consequently may have clinical health consequences on both humans and animals [37].

The co-existence of virulent and resistant genes in a species may result in the emergence of hybrid plasmids of both resistance and virulence characteristics among *E. coli* strains which in turn, poses an increased public health risk [38]. Our study revealed that all the *E. coli* isolates including aEPEC and STEC strains exhibited resistance against three or more antimicrobial agents, suggesting the presence of MDR strains as well as *E. coli* with virulence genes [25,38]. The finding is worrisome with potential clinical implications, since the dissemination of resistance trait will hamper the therapeutic possibilities for treatment of *E. coli* infection and may further complicate any future drug therapy. The prevalence of such a large number of MDR *E. coli* indicates the widespread and extensive

use of antibiotics. Taking into account the huge pork consumption and closer contact between human populations and porcine in the region, the research findings warrant a more critical appraisal of diarrhoegenic *E. coli* with particular attention to antimicrobial drug resistance. Detection of *E. coli* strains with simultaneous combination of virulence and MDR resistance traits is a cause of public health concern, and it indicate the nature of continuous selective pressure which promote combinations of virulent and resistant strains. Hence, regular monitoring of such strains in food animals is very much necessary which must include adequate antimicrobial susceptibility testing and molecular characterization.

4. CONCLUSIONS

Escherichia coli with AMR traits were common in porcine population of organized and unorganized farms in different North Eastern states of India with higher prevalence of AMR in organized farming system. Occurrence of high levels of resistance against most antimicrobials and prevalence of multidrug resistant aEPEC and STEC strains call for further attention regarding usage of antibiotics in piggery sector and maintenance of strict hygienic measures. In India, too little is known about antibiotic use in food animals particularly related to piggery, and a nationwide surveillance system is required to determine antibiotic consumption and resistance patterns. A multi-sectoral and multi-disciplinary approach with combined efforts and supervision of antimicrobial administration with proper laboratory tests is required to tackle the problem of antimicrobial drug resistant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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