



# High prevalence of multi-drug resistant organisms in the gut flora of healthy rural individuals in northern India

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**Background & objectives:** Presence of resistant gut flora in the community is associated with increasing multi-drug resistance (MDR) infections. In this study, the prevalence of MDR organisms in the gut flora of a healthy rural population residing in northern India was determined.

**Methods:** Healthy individuals aged 18-45 yr from Nuh district, Haryana, India were included in this study. Risk factors associated with dysbiosis, diet, lifestyle and exposure to animals was assessed. Qualitative food frequency questionnaire and inflammatory diet score was calculated. Pathogens in stool sample were detected by MALDI-TOF. Evaluation of antimicrobial susceptibility was done by automated Vitek-2 System. The presence of antimicrobial resistance (AMR) genes was evaluated using PCR. An isolate having resistance to at least one antibiotic out of the three or more classes of antibiotics tested was labelled as MDR.

**Results:** Among 153 individuals included in this study (mean age-32.5±8.6 yr, females-58.2%, vegetarian-68.6%), the most frequent organism isolated was *E. coli* (n=137, 89.5%) followed by *K. pneumoniae* (n=19, 12.4%) and *Enterobacter* species (n=23, 15%). Forty seven (30.7%) individuals had sensitive and 42 (27.4%) had MDR organisms. Fifty one (33.3%) were positive for ESBL, 5 (3.3%) were positive for carbapenems, and 18 (11.8%) were positive for both genes. Age, gender, body mass index, diet pattern, or diet score were similar between participants with sensitive and resistant organisms. Resistance against fluoroquinolones was highest [92(48.7%)] among all isolates. Forty nine (25.9%), 25 (13.2%), 24 (12.7%) and 21 (11.1%) isolates, respectively were positive for *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTXM-1</sub> and *OXA-48* genes.

**Interpretation & conclusions:** Overall the study findings suggest that 27 per cent individuals from rural northern India carry MDR organisms in their fecal flora, with an ESBL carriage rate of 44 per cent.

**Key words** Rural India - MDR Gram negative bacteria - gut microflora

Antimicrobial resistance (AMR) is a major public health problem due to infections with multi-drug

resistant (MDR) organisms<sup>1</sup>. Irrational antibiotic use is responsible for increasing MDR infections. According

to a recent report, India has some of the highest antibiotic resistance rates among bacteria that commonly cause infections in the community and healthcare facilities<sup>2</sup>. There is rising resistance to  $\beta$ -Lactams; several studies have reported high prevalence of cephalosporinases, extended spectrum  $\beta$ -lactamases (ESBLs), Amp C and Carbapenemase, amongst the common pathogens<sup>3</sup>. ESBL producing *Enterobacteriales* are now included under the threat pathogen list of Center for disease control (CDC)<sup>4</sup>. Further, community acquired infection with ESBL producing *E. coli* is also quite frequent<sup>5</sup>.

Intestinal colonisation with MDR organisms (MDROs) contributes to the AMR gene reservoir within the gut<sup>6</sup>. This concept of a 'resistome' encompasses the AMR gene profile of the entire resident gut microbiota. Though there are several studies evaluating the burden of resistome in hospitalized individuals and healthy individuals in the community from other parts of globe<sup>7</sup>, there is limited information on the prevalence of AMR genes in healthy individuals from India. The literature on this aspect from India is limited to a single community-based study, and two other studies in healthy neonates and children<sup>8-10</sup>.

A balanced community of inhabitant gut microbiota are crucial for human health and hence have been studied extensively in Indian as well as global populations<sup>11,12</sup>. Alteration in healthy gut flora, or dysbiosis has been extensively studied in many gastrointestinal, metabolic and neurologic disorders. Correction of dysbiosis by fecal microbial transplantation (FMT) can be a promising tool for microbiome manipulation and could be an effective therapy for these disorders<sup>13</sup>. Though FMT is regarded as a safe procedure, in a recent alert by the American Gastroenterology Association, FMT from healthy donors to immunocompromised recipients has reportedly led to sepsis with ESBLs carrying *E. coli* in two patients, of whom one succumbed<sup>14</sup>.

These findings underscore the urgent need for in-depth studies on AMR pattern in the healthy Indian population. This can help us understand the role and transmission dynamics of AMR in communities and can also aid in the selection of healthy donors for enhancing the safety and efficacy of FMT. Therefore, the present study was undertaken to determine the prevalence of MDROs and ascertain the genotypic profile of fecal AMR bacterial isolates in the gut flora of healthy rural population residing in a rural district of Northern India.

### Material & Methods

This study was conducted at the department of Gastroenterology in collaboration with the departments

of Community Medicine and Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, after obtaining approval from the Institute Ethics Committee. Access to the village households was conducted through an existing tripartite MoU with the State Health Society, Haryana. Before undertaking the actual survey a formal permission was obtained from the head of the requisite village. A written informed consent was obtained from all the study participants before administering study procedures such as questionnaire and collection of stool samples.

*Sample collection:* Samples were collected from healthy individuals in Nuh district, Haryana, in northern India. The district has a population of 1.1 million, with predominant rural inhabitation (89%). The biggest block Nuh with 70 villages was identified of which 15 villages were randomly selected (Fig. 1). The median population size (range) of the selected villages was 2,203 (661-6,597), with median (range) households per villages as 414 (117-1,024), respectively. Random lanes from center of village was approached for sample, the household members were briefed about the study.

Healthy adults aged 18-45 yr with no history of concurrent acute medical illness or hospitalization in past one year, symptoms pertaining to acute gastrointestinal (GI) disease, history of chronic illness, antibiotics/antifungals/antivirals/painkillers intake over the past three months, and other risk factors which could influence the gut microbiome were recruited (Supplementary Table I). A detailed questionnaire was administered (Supplementary Material). Stool samples were collected in sterile containers containing Copan E-swabs and were transported at 4°C to the laboratory. The sample was processed for pathogen isolation, antimicrobial susceptibility testing (AST) and genetic profile of AMR genes.

*Pathogen detection:* All collected samples were cultured on blood agar, MacConkey agar (BD Dickinson, Biosciences, San Jose, CA), and Chrome ColAPSE (Himedia) screening agar plates and incubated for 16-18 hours at 37°C. After incubation, plates were inspected for type of growth. In case of pure growth, the identification of microbes was done by Matrix-Assisted-Laser-Desorption-ionization-Time-of-Flight (MALDI-TOF Biomerieux, Marcy-l'Etoile, France). In the case of multiple colonies, isolation was done as per standard microbiological techniques, followed by MALDI TOF. The confirmed isolates were then cryopreserved for further use.

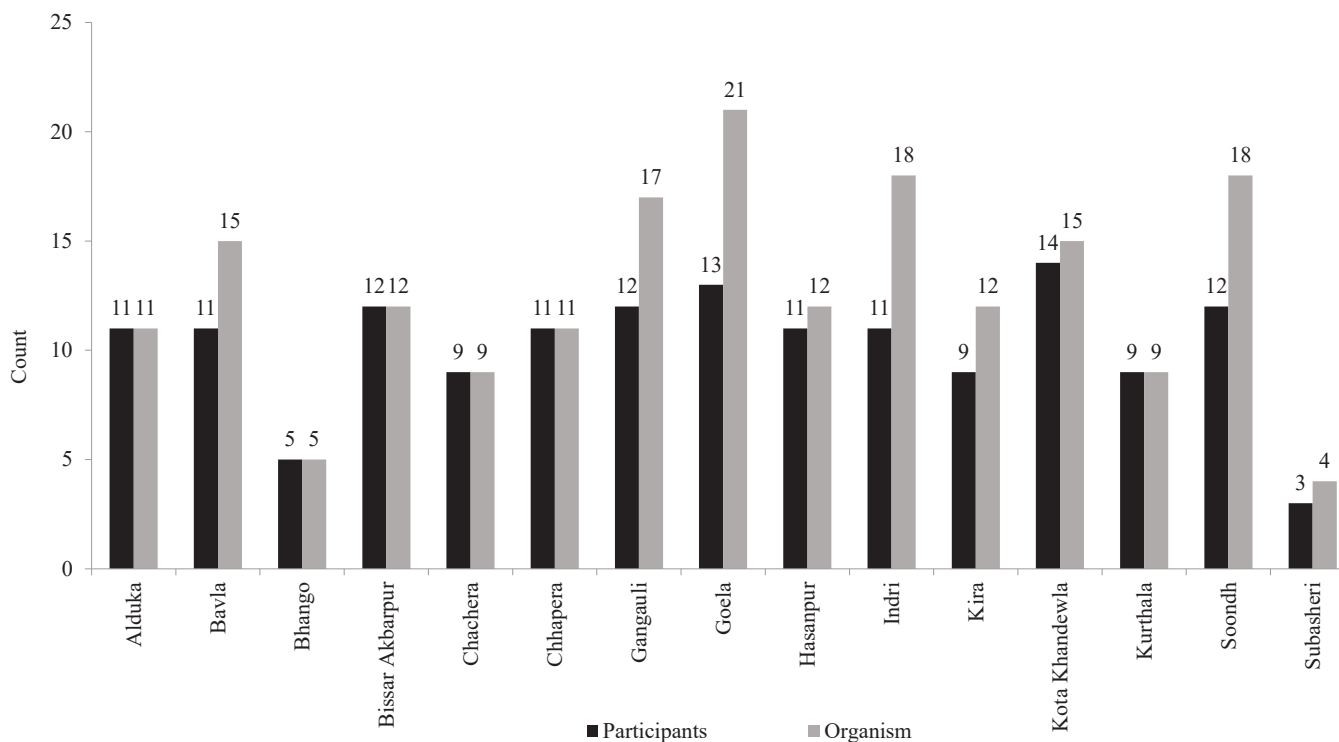


Fig. 1. Number of samples collected from different villages.

**Antimicrobial susceptibility testing:** AST was done by automated AST Vitek 2 System (Biomerieux Marcy-l'Etoile, France) along with disc-diffusion testing and broth-microdilution-testing methods. MIC were defined as per CLSI guidelines<sup>15</sup>. The ESBL and carbapenemase production and colistin resistance was predicted by MIC of ceftazidime, meropenem and colistin respectively. MDR was defined as isolates having resistance to at least one antibiotic out of the three or more classes of antibiotics tested<sup>16</sup>.

**Antimicrobial resistance genomics:** AMR genes for colistin, ESBL and carbapenemase were detected from isolated microbes using PCR as detailed in Supplementary Material. The primer sequence and expected amplicon size for respective genes is shown in Supplementary Table II.

**Diet analysis:** During screening, diet over the past seven days from the date-of-screening was recorded with diet recall method as mentioned in Supplementary Material. The inflammatory component of the diet was assessed by classifying diet into pro-inflammatory (negative score) and anti-inflammatory (positive score) food groups as per the scores given in the Supplementary Table III<sup>17</sup>.

**Statistical analysis:** Categorical variables were presented as numbers (percentage) and continuous as mean±standard deviation or median (IQR). Categorical variables were compared with chi-square test. Continuous variables were compared with Student's *t* test for two comparisons and ANOVA for more than two comparisons. Correlation between resistance profiles of different antibiotic classes was done using Spearman's correlation co-efficient. Analyses were done with SPSS v26 (Stata Corp, College Station, TX).

## Results

A total of 153 healthy volunteers were enrolled from 15 villages, the sample distribution is detailed in Figure 1. Mean age of participants was 32.5±8.6 yr with 58.2 per cent being females. Mean body mass index (BMI) was 22.2±4.8 kg/m<sup>2</sup>. Among these 105 (68.6%) participants were vegetarian and 48 (31.4%) members consumed both vegetarian and non-vegetarian (mixed) diet. About 62.1 per cent of the participants were exposed to cattle (Table I) and the mean diet score was 5.2±4.3.

A total of 189 organisms were isolated from 153 participants, of these 123 (80.3%) participants were positive for only one organism, while the remaining were positive for ≥2 organisms (Table I). Village

**Table I.** Characteristics of total number of participants including demographics, diet pattern, and organisms isolated in stool (n=153)

Mean Age yr (Mean±SD)	32.5 ± 8.6
Gender (Females)*	80 (58.2)
Diet Pattern*	
Vegetarian	105 (68.6)
Mixed	48 (31.4)
Body mass index (Kg/m <sup>2</sup> ) (Mean±SD)	22.2 ± 4.8
Cattle in house*	95 (62.1)
Diet Score(Mean ± SD)	5.2 ± 4.3
Number of participants	153
Number of participants with single organisms	123 (80.4)
Number of participants with 2 organisms	24(15.7)
Number of participants with 3 organisms	6 (3.9)
Types of organisms isolated (n=189)	
<i>Escherichia coli</i> *	137
<i>Klebsiella pneumoniae</i> *	19
<i>Enterobacter cloacae</i> *	11
<i>Enterobacter faecium</i> *	9
<i>Enterobacter aerogenes</i> *	3
<i>Klebsiella oxytoca</i> *	2
<i>Citrobacter koseri</i> *	1
<i>Citrobacter Freundii</i> *	1
<i>Escherichia fergusonii</i> *	1
<i>Enterococcus gallinarum</i> *	1
<i>Pseudomonas aeruginosa</i> *	1
<i>Shigella flexneri</i> *	1
<i>Shigella group</i> *	1
<i>Shigella sonnei</i> *	1
*Presented as n(%) or n only (type of organisms); SD, standard deviation	

wise distribution of organisms is shown in Figure 2. Among 153 individuals, 137 had their culture positive for *E. coli* (89.5%). Among all organisms (n=189), the most frequent organism isolated was *E. coli* followed by *K. pneumoniae* and *Enterobacter* species. Three participants who were positive for *Shigella* group bacteria were completely asymptomatic.

**Antibiotic resistance profile of the study participants:** Of all the participants, 24 (15.7%) had isolates that were sensitive to all drugs, without any AMR gene carriage. Phenotypically, 47 (30.7%) participants had isolates sensitive to all drugs, 64 (41.8%) had resistance to single/two drug classes, and 42 (27.4%) had MDR

isolates. Isolates were resistant to a median of 3(IQR: 2-4) classes of drugs. ESBL genes were detected among isolates in 51 (33.3%) participants, only carbapenemases genes in 5(3.3%) and both genes in 18 (11.8%) participants. Among the *E. coli*, 28 (20.4%) were MDR isolates. ESBL genes were detected in 43(31.4%), carbapenemase gene in 5 (3.6%), and both genes in 15 (10.9%) isolates (Table II).

There was no difference in age, gender, BMI, diet pattern, presence of cattle in house or diet score between participants who had phenotypically sensitive organisms, those with single/two drug resistance, and those with MDR organisms (Table III).

All isolates showed highest resistance against fluoroquinolones 92 (48.7%), followed by cotrimoxazole 56 (31.3%). Resistance pattern of isolates for other antibiotics is mentioned in Table IV. Among the *E. coli* also, highest resistance was present against fluoroquinolones 79 (57.2%), minocycline 25 (18.1%), aztreonam 6 (18.8%), and cephalosporin's 15 (10.9%). *Klebsiella* sp. showed least resistance to fluoroquinolones. All the isolates of *Klebsiella* were sensitive to carbapenems and only 10 per cent were resistant to third generation cephalosporins. Only *Enterobacter* sp. [2 (14.3%)] showed resistance to colistin. *Citrobacter/Shigella* revealed highest resistance to aminoglycosides [4 (66.7%)] (Table IV).

There was significant resistance correlation (or a trend towards correlation) between resistance to Beta Lactam/ Beta-lactamase inhibitor combination and all other classes of antibiotics, except colistin. Similarly, resistance to beta-lactam/beta-lactamase inhibitor combination, cephalosporins, aztreonam and carbapenems had significant correlation between each other (Supplementary Table IV).

**Antimicrobial resistance gene profile:** The proportion of ESBL (43.9%) gene was almost three times the proportion of carbapenemase gene (14.3%) among all isolates. Highest ESBL and carbapenemase positivity was seen for *Klebsiella* species (57.1 and 19%, respectively; Table V. Twenty eight out of 40 MDR isolates (70%), 29/80 (36.5%) single/two drug resistant isolates, and 26/69 (37.7%) phenotypically sensitive isolates, respectively, were ESBL positive. The genotypic resistance profile of all isolates and individual organisms is mentioned in Table V. None of the isolates were positive for *Mcr1* and *Mcr5* genes.

The phenotypic and genotypic profiling showed that ~50 per cent of the isolates with resistance to

Village	Organism														Total
	<i>C. koseri</i>	<i>C. Freundii</i>	<i>E. aerogenes</i>	<i>E. cloacae</i>	<i>E. coli</i>	<i>E. faecium</i>	<i>E. fergusonii</i>	<i>E. gallinarum</i>	<i>K. oxytoca</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>S. flexneri</i>	Shigella group	Shigella sonnei	
Alduka					11										11
Bavla			1	1	10				1	2					15
Bhango					5										5
Bissar Akbarpur	1			1	10										12
Chachera					8					1					9
Chhapera				2	7		1				1				11
Gangauli				1	11	3				1				1	17
Goela			1		11	1				7		1			21
Hasanpur				1	9					1			1		12
Indri				1	11	2		1	1	2					18
Kira			1		9	1				1					12
Kota Khandewla				1	14										15
Kurthla				2	7										9
Soondh		1			11	2				4					18
Subasheri				1	3										4

Fig. 2. Organisms isolated from different villages.

**Table II.** Resistance profile (drug resistance and AMR gene profile) of the isolates from fecal samples according to overall isolates (n=189), number of participants (n=153), and for *E. coli* (n=137)

Types of resistance	All Isolates (n=189)	All Participants (n=153)	Participants with <i>E. coli</i> (n=137)
Overall phenotypic resistance	120 (63.5)	106 (69.3)	91 (66.4)
Phenotypic resistance profile in fecal samples			
-No resistance	69 (36.5)	47 (30.7)	46 (33.5)
-Single/ two drug resistance	80 (42.3)	64 (41.8)	63 (45.9)
-MDR	40 (21.1)	42 (27.4)	28 (20.4)
Genotypic resistance profile in fecal samples			
-No AMR Gene	100 (52.9)	79 (51.6)	74 (54.1)
-Only ESBL gene	106 (32.8)	51 (33.3)	43 (31.1)
-Only carbapenemases gene	6 (3.2)	4 (2.6)	5 (3.6)
-Both ESBL and carbapenemases genes	21 (11.1)	18 (11.1)	14 (10.1)

Data is presented as n(%). MDR, multi-drug resistant; AMR, antimicrobial resistance gene; ESBL, extended spectrum beta lactamase; *E. coli*, *Escherichia coli*

**Table III.** Characteristics of participants on the basis of resistance profile in stool samples

Parameters	Sensitive (n=47)	Single/ two drug resistant (n=64)	Multi-drug resistant (n=42)	P value
Age (yr)	33.8 ± 9.2	31.9 ± 8.7	31.9 ± 7.5	0.43
Gender, (females); n(%)	27 (57.5%)	35 (54.7%)	27 (64.3%)	0.61
Body Mass Index	22.2 ± 3.1	22.6 ± 4.9	22.3 ± 3.7	0.83
Diet pattern; n(%)				0.42
Veg	32 (68.1%)	41 (64.1%)	32 (76.2%)	
Mixed	15 (31.9%)	23 (35.9%)	10 (23.8%)	
Cattle in house; n(%)	31 (66%)	39 (60.9%)	25 (59.5%)	0.79
Diet score	5.3 ± 4.7	5.3 ± 4.1	4.9 ± 4.2	0.89

various drugs carried ESBL genes: beta-lactam (64%), aztreonam (56.6%), aminoglycoside (40%) and

minocycline (61.2%), while 16.3 to 50 per cent isolates carried carbapenemase gene Table IV.

**Table IV.** Antibiotic resistance profile for organisms isolated from healthy individuals

Organisms	Colistin (n=169)	Beta-lactam + beta-lactamase inhibitor (n=179)	Third generation cephalon-sporin* (n=179)	Fourth generation cephalon-sporin* (n=179)	Aztreonam (n=178)	Carbapenem (n=179)	Aminoglycoside (n=179)	Fluroquinolone (n=189)	Mincycline (n=178)	Tigecycline (n=188)	Cotrimoxazole (n=179)
For all isolates	2 (1.2)	14 (7.8)	18 (10.1)	7 (3.9)	30 (16.9)	2 (1.1)	10 (5.6)	92 (48.7)	31 (17.4)	2 (1.1)	56 (31.3)
Per individual organisms											
<i>E. Coli</i> (n=137)	0	13 (9.4)	15 (10.9)	7 (5.0%)	26 (18.8)	2 (1.4)	6 (4.3)	79 (57.2)	25 (18.1)	1 (0.7)	49 (35.5)
<i>Enterococcus</i> (n=10)	0	0	0	0	0	0	0	4 (40)	0	0	0
<i>Enterobacter</i> (n=14)	2 (14.3)	0	1 (7.1)	0	0	0	0	3 (21.4)	2 (14.3)	0	3 (21.4)
<i>Klebsiella</i> (n=21)	0	0	2 (9.5)	0	3 (14.3)	0	0	4 (19)	2 (9.5)	0	2 (9.5)
<i>Pseudomonas/ Citrobacter/ Shigella</i> (n=6)	0	1 (16.7)	0	0	1 (16.7)	0	4 (66.7)	2 (33.3)	2 (33.3)	1 (16.7)	2 (33.3)
ESBL genes present	1 (0.5)	4 (0.22)	2 (0.11)	2 (0.11)	19 (10.6)	1 (0.5)	6 (0.33)	30 (15.8)	23 (12.9)	1 (0.5)	33 (18.4)
Carbapenemase genes present	0 (0)	3 (0.16)	2 (0.11)	3 (0.16)	10 (5.6)	1 (0.5)	2 (0.11)	10 (5.2)	6 (3.1)	0 (0)	3 (1.6)

Data is presented as n(%).  
\*3rd and 4th generation cephalosporins include cefoperazone sulbactam, ceftazidime and ceftipime, respectively



**Table V.** Antimicrobial resistance gene profile of organism isolated from healthy individuals (n=189)

Organisms	ESBL				Overall ESBL*	Carbapenemase			
	<i>TEM</i>	<i>SHV</i>	<i>CTXM</i>	<i>OXA-1</i> (n=180)		<i>KPC</i>	<i>NDM</i>	<i>OXA-48</i>	Overall carbapenemases*
For all isolates	49 (25.9)	25 (13.2)	24 (12.7)	11 (6.1)	83 (43.9)	7 (3.7)	1 (0.5)	21 (11.1)	27 (14.3)
Per individual organisms									
<i>E. Coli</i> (n=137)	36(26.3)	12 (8.7)	21 (15.3)	11 (8)	72 (52.6)	2 (1.5)	1 (0.7)	20 (14.6)	21 (15.3)
<i>Enterococcus</i> (n=10)	4 (40)	0	0	0	4 (40)	0	0	0	0
<i>Enterobacter</i> (n=14)	3 (21.4)	1 (7.1)	1 (7.1)	0	5 (35.7)	1 (7.1)	0	0	1 (7.1)
<i>K. pneumoniae</i> (n=21)	4 (19)	12 (57.1)	1 (4.8)	0	12 (57.1)	4 (19)	0	1 (4.8)	4 (19)
<i>Pseudomonas/Citrobacter/ Shigella</i> (n=6)	2 (33.3)	0	1 (16.7)	0	3 (50)	0	0	0	0

\*Isolate positive for two genes is considered as one

## Discussion

We report a high proportion of healthy individuals with fecal carriage of MDR organisms in this study. Healthy individuals from Nuh district were selected through an extensive questionnaire to rule out possible factors which could affect the AMR carriage, and only 31 per cent of the study participants had no fecal carriage for drug resistant organisms. Approximately 42 per cent individuals carried isolates with resistance against single/two drug classes, and 27 per cent carried MDRO. As per a *Niti Aayog* report<sup>18</sup>, Nuh is the most backward district in India with poor health and education infrastructure. We had previously shown that, among healthy individuals, the microbiome of rural individuals was healthier than urban<sup>12</sup>. Though we did not have a comparative group of urban healthy adults, the prevalence of MDR in rural individuals was more than expected. Use of antibiotics in deprived areas like Nuh is increasing, possibly due to the increasing rate of irrational antibiotics prescription by informal practitioners in these villages that might be contacted first for simple illnesses. Although, human antibiotic use is the most important factor, other factors such as animal to human transfer (due to indiscriminate antibiotic use in animals and food industry), effect of diet, presence of AMR genes in waste and effluents could also be responsible for increasing MDR burden in healthy individuals<sup>19</sup>.

In a similar study from Chandigarh, India, overall prevalence of antibiotic resistance in healthy gut flora was found to be 70.5 per cent, similar to the phenotypic resistance in this study (69.3%). However, the prevalence of MDR was significantly high in this study, 27 per cent, as compared to only 2.4 per cent in Chandigarh<sup>8</sup>, which could be ascribed to differences in

population (rural vs. semi-urban), exposure of residents to livestock (62% in this study), and antibiotic use patterns. In other rural-based studies from central India, 70 per cent of the MDR was seen in fecal flora in children of 1-3 yr age<sup>10</sup>. Similar heterogeneity has also been observed in other studies, and prevalence of MDR positivity varied from 51 per cent to as high as 93 per cent<sup>9,10,20-23</sup>. Possible reasons for this heterogeneity could be the nature of participants, definition of MDR, method of detection, and similar reasons as described above. As an extension of the healthy gut microbiome study described above<sup>12</sup>, the AMR profiling of five dominant commensals revealed that all the dominant gut bacteria were MDR, being resistant to at least seven different antimicrobials<sup>20</sup>. Therefore, multi-drug resistance is also being reported in commensals, possibly due to their inadvertent exposure to antibiotics and toxic compounds.

The predictors of MDR organism carriage include higher socio-economic status, exposure to antibiotics or hospitalization over the past one year, and recent proton pump inhibitor and non-steroidal anti-inflammatory drug (NSAID) use<sup>21-23</sup>. We selected individuals from the same geography having a similar lifestyle to control for confounders which can affect the fecal resistome. Among the possible predictors which were measured in this study age, gender, diet, and BMI did not have any influence. Diet is one of the major determinants of gut microbiome<sup>24</sup>, although the effect of diet on gut resistome and AMR gene profile has been less explored<sup>25,26</sup>. Possible reasons for lack of association between diet and AMR profile could be rather uniform diet in the same geography, and bias associated with recall method of diet assessment.

Approximately 44 per cent isolates were ESBL gene positive, and 14 per cent were positive for *Carbapenems* gene, similar proportions being observed among *E. coli*. In a recent meta-analysis, globally, 21.1 per cent of inpatients in healthcare setting, and 17.6 per cent healthy individuals had the presence of ESBL *E. coli* in fecal samples<sup>7</sup>. However, the prevalence in healthy Southeast Asians in this meta-analysis was 35.3 per cent, similar to our study. In the study from Chandigarh, prevalence of ESBL genes among cephalosporin resistant isolates was 1.9-25 per cent (different genes)<sup>8</sup>. In the present study, among 18 cephalosporin resistant isolates, 10 were ESBL positive. In another study of 115 healthy volunteers, ESBL-producing *E. coli* had a prevalence of 19 per cent, with CTX-M being the predominant type followed by TEM, unlike our study, where TEM was most common followed by CTX-M<sup>27</sup>.

Among 40 MDR isolates, about 70 per cent were ESBL positive, while 1/3<sup>rd</sup> of phenotypically sensitive isolates also carried ESBL genes. This phenotypic-genotypic discordance could be explained by the presence of silent/cryptic or proto resistance genes, which can encode AMR but may not be expressed or may undergo modifications after expression<sup>19</sup>.

Similar to other prevalence studies, *E. coli* was the most common organism isolated followed by *Klebsiella* species. Overall *E. coli* carriage among individual participants was 89.5 per cent (137/153), similar to that reported by Martinson *et al*<sup>28</sup>, (~90%). Carriage rate for *K. pneumoniae* (12.4%), was also similar to the review by Podschum R *et al*<sup>29</sup> (*K. pneumoniae* fecal carriage in Indian population: 5-38%). Surprisingly, Shigella was detected in three individuals, though all were asymptomatic. Asymptomatic Shigella carriage has also been reported in other studies<sup>30</sup>. Highest antibiotic resistance was seen for fluoroquinolones followed by cotrimoxazole. Least resistant antibiotics were colistin, tigecycline and carbapenems. Like our study, 41.5 per cent fluoroquinolones resistance was seen in Chandigarh study, although unlike ours, maximum resistance was seen for cephalosporins. Carbapenem resistance was also similar between the two studies. Heterogeneity on this aspect has also been reported in other studies. In a study from rural Thailand, maximum resistance was seen against tetracycline, and fluoroquinolones resistance was seen in only 19.7 per cent<sup>21</sup>. Studies from Vietnam have reported high frequency of colistin resistance and *mcr* gene carriage, possibly due to significant colistin use in livestock<sup>31,32</sup>. In the present study, only two isolates were colistin resistant and none carried *mcr* gene.

Though the study was strengthened by detailed dietary evaluation and estimation of phenotypic and genotypic resistance profile, but reported predictors of MDR and ESBL carriage in stool like history of antibiotic use and hospitalization beyond three months was not obtained. However, the study sample was well characterized with the questionnaire and we ruled out maximum factors that could affect the gut microbiome in short-term. The sample size was reasonable enough to characterize the AMR profile but a multicentric large cohort study should be conducted in order to evaluate the effect of diet and lifestyle on gut microbiome. Also, with advancement in the techniques, gut resistome through metagenomics approach should also be evaluated in further studies. A more detailed demographic data on socioeconomic status would also help us to understand the spread of resistance in healthy population.

To conclude we report that 27 per cent individuals from rural northern India are positive for MDR organisms in their fecal flora, with an ESBL carriage rate of ~40 per cent. These findings provide important insights on burden of MDR in the community, and a roadmap for urgent antibiotic stewardship measures to control the imminent threat of community associated MDR infections.

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