

Urban Migrant Labourers as Potential Source for Transfer of Antimicrobial Resistance to Rural Community

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Abstract

The aim of the study was to investigate potential evidence for spread of antimicrobial resistance (AMR) from urban migrant labourers to labourers from local resident rural community due to sharing of common residential premises and other civic amenities. Two groups of unskilled labourers, enrolled for civil construction in a peri-urban area viz. (i) labourers having migrated from urban zone and (ii) labourers from local resident village community were compared in terms of demographic profile, history of various risk factors towards acquisition of AMR and prevalence of extended-spectrum beta-lactamase (ESBL), carbapenemase and New Delhi metallo-beta-lactamase type 1 (NDM-1) categories of AMR at enrolment and after one year of sharing of common residential premises with associated sanitation facilities using *Escherichia coli* as indicator organism. Higher percentage of urban migrant labourers were characterised by low literacy level, history of inhabitation in makeshift shelters without dedicated access to drinking water or cooking space, practice of defecation in open, episodes of illness suggestive of bacterial infections, faulty treatment seeking behaviour and intestinal carriage rate of *E. coli* with various categories of AMR compared to the non-migrant labourers from rural community. The later group showed an increase in the prevalence of carbapenem resistance with NDM-1 production during the duration of co-inhabitation with urban migrant labourers. The present study provided potential evidence for transfer of AMR by urban migrant labourers to non-migrant labourers from rural community that may serve as vehicle for further transmission of AMR to the rural community hitherto unexposed or less exposed to the problem.

Keywords: Migrant labourer, ESBL, Carbapenemase, NDM-1, *Escherichia coli*

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INTRODUCTION

Considerable concern has been expressed globally about the contribution of international human migration in the spread of antimicrobial resistance (AMR)¹⁻³. However, not much attention has been paid to the possible spread of AMR due to domestic migration within a country. Peri-urban area provides employment for both urban and rural community and thus serves as an important common platform for transmission of infections from urban to rural population, unexposed or less exposed to the problem⁴⁻⁶.

The labourer population working in urban cities frequently acquire organisms with AMR as a consequence of inhabitation in poor sanitary conditions, lack of awareness about hygienic practice, inappropriate use of antibiotics due to economic constraints, poor literacy and faulty treatment seeking behaviour^{7,8}.

The present study was carried out to assess the magnitude of AMR based on intestinal carriage rate of extended spectrum beta-lactamase (ESBL), carbapenemase and New Delhi metallo-beta-lactamase type-1 (NDM-1) in *Escherichia coli* as indicator organism in a group of migrant labourers from major urban cities in northern India employed as unskilled labourers for civil constructions in the peri-urban belt of North India and investigate potential evidence for transmission of AMR from the urban migrant labourers to labourers from local resident rural community employed for same job, sharing the same residential premises and civil facilities as that of urban migrant labourers during the employment period.

MATERIALS AND METHODS

The protocol of the study was approved by institutional ethics committee (SGTU/FMHS/MICRO/341). Information sheet was provided to each of the participants and prior consent was obtained from them for the study.

Study location

The study was carried out in three clusters of temporary brick made huts (60 -70 huts per cluster) constructed to provide temporary residence to the unskilled manual labourers (as detailed below), located within 0.5 km distance from the campus of SGT University. Each cluster had one common toilet facility in the form of a

single rectangular block of 5 brick made cubicles adjacent to each other with common walls. Each cluster was provided with one common piped water supply from the reservoir in the university campus with one common tap within residential premises of the labourers to be used by them for the purpose of drinking, cooking, washing clothes and bathing.

Study population

The study population included following two subgroups of labourers, adult males and females (mostly as family members) recruited in contractual capacity for one year as unskilled manual labourers (referred subsequently as labourers) for civil constructions at the SGT University campus located in the peri-urban belt of Haryana state in North India adjoining two major urban cities viz. Delhi and Gurgaon.

The migrant labourer (ML) subgroup

This subgroup included adults engaged in the profession as labourers in urban cities in states other than the state of Haryana for an uninterrupted period of at least 2 years preceding the present employment.

The non-migrant labourers (NML) subgroup

This subgroup included labourers from adjoining rural villages recruited at the same point of time for similar jobs as that of migrant labourers. This category of labourers, originally part time agriculture labourers, were provided residential facilities in the same clusters as that of MLs. Only those labourers who had history of uninterrupted stay in their villages during preceding two years were included in this subgroup.

Study design

The study was cross-sectional in nature carried out in two phases one year apart viz. enrolment phase (March, 2017) and follow up phase (February, 2018). Demographic and epidemiological information were collected from the participants following enrolment in 2017. Sampling for the study of AMR was carried out during first 2 weeks following enrolment and during last 2 weeks prior to the end of contract period of one year i.e. in February, 2018.

Collection of information

A pre-designed proforma was employed to collect information on age, sex, educational level and civic facilities availed by the subjects during the 6 months period prior to enrolment viz.

type of accommodation, source of drinking water, use of toilet and space for cooking.

Information was collected regarding frequency and duration of episodes of illness during preceding two years suggestive of bacterial infections viz. chest infection, urinary tract infection, diarrhoea, skin infections like boils or furuncles and purulent discharge per ear. Information was sought on the prevalent practice of treatment seeking behaviour in these episodes of illness i.e. from hospital, registered medical practitioners, pharmacies or by self. Compliance to duration of medication prescribed by registered medical practitioners was also evaluated. History of hospitalization (>3 days duration) in preceding five years was obtained from each labourer.

Collection of samples

Human samples

Morning stool samples were collected for three consecutive days during each phase from both the categories of labourers (ML and NML) who were provided training for self-collection of morning stool samples in sterile containers provided to them. In addition to the labourers group, sampling of morning stool was carried out for three consecutive days during each phase from 200 randomly selected age, sex and economically matched healthy residents of the same nearby villages from which the NMLs were recruited for the present study in order to collect data on the baseline prevalence of the various categories of AMR harboured by *E. coli* in the population. The collected stool samples were transported in insulated carrier box with ice packs to the microbiology laboratory, SGT Hospital within one hour.

Samples from toilet premises in the residential clusters

Soil samples around each of the three blocks of toilets used by the labourers were collected during the same two phases as that of collection of human stool samples for 10 consecutive days (except Saturday and Sunday) in each phase. Samples were collected at 20 approximately equidistant points on a line 2ft away from the edge of the four walls of the rectangular toilet blocks. Samples were collected at each 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 immediately before use⁹. The points of soil sampling during the enrolment phase were identified that were subjected to resampling during the follow-up phase with reasonable accuracy.

Processing of samples for isolation of ESBL positive *Escherichia coli* (ESBL-EC)

Stool samples

Approximately 0.5 g of stool sample was suspended in 1.5 ml of nutrient broth. From each of the suspensions, 0.1 ml was plated on ESBL screening media viz. two MacConkey agar plates, one supplemented with 2 µg of cefotaxime per ml (Mac-CTX) and the other one supplemented with 2 µg of ceftazidime per ml (Mac-CAZ) and incubated aerobically at 37°C for 18-24 h¹⁰. Five randomly selected lactose fermenting colonies with identical morphotype suggestive of *E. coli* on both Mac-CTX and Mac-CAZ were identified using Vitek 2 system (BioMerieux, France).

Soil samples

The swabs of soil samples collected in nutrient broth were vortexed for 2 minutes following which 0.1 ml was plated on ESBL screening media (Mac-CTX and Mac-CAZ) and processed further as in case of stool samples.

Phenotypic confirmation of *E. coli* isolates for ESBL production

ESBL production among the *E. coli* isolates screened by the screening media was confirmed by phenotypic confirmatory tests i.e., double disc synergy test (DDST) as described earlier^{11,12} using two pairs of antibiotic discs, ceftazidime (30 µg) and ceftazidime plus clavulanic acid (30 µg plus 10 µg) discs and cefotaxime (30µg) and cefotaxime plus clavulanic acid (30 µg plus 10 µg) discs. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 strains were used as ESBL-positive and ESBL-negative control strains respectively.

Antibiotic susceptibility testing

Antibiotic susceptibility testing (AST) of all the confirmed ESBL-EC isolates was carried out by disc diffusion method on Mueller-Hinton agar and the AST was performed using antibiotic discs viz. ampicillin (10 µg), amoxicillin-clavulanic acid (20/10µg), piperacillin/tazobactam (100/10 µg), amikacin (30 µg), gentamicin (10 µg), six beta-lactam antibiotics viz. cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ceftiofur (30 µg), cefepime (30 µg) and aztreonam (30

µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), tetracycline (10 µg), tigecycline (15 µg), and three carbapenem group of antibiotics viz. ertapenem (10 µg), meropenem (10 µg) and imipenem (10 µg). Results were interpreted as per CLSI guidelines (CLSI 2019)¹².

Phenotypic confirmation of carbapenemase production among ESBL-EC

In-house Carba NP test was performed for phenotypic confirmation of carbapenemase production among the *E. coli* isolates (CR-EC) showing resistance to one or more carbapenems used in AST¹². *K. pneumoniae* ATCC BAA 1705 and *E. coli* ATCC 25922 were used as positive and negative control strains respectively.

Molecular identification of *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} and *bla*_{NDM} genes

Molecular characterization of all the isolated *E. coli* strains was carried out in 2018. Phenotypically confirmed ESBL-EC were subjected to PCR assay as described earlier to detect the presence of *bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX-M} genes using pre-published primer sequences, viz. TEM primers (TEM forward ATGAGTATTCAACATTTCGGTG, TEM reverse TTACCAATGCTTAATCAGTGAG) amplifying 840-bp fragment, SHV primers (SHV forward ATTTGTGCTTCTTTACTCGC, SHV reverse TTTATGGCGTTACCTTTGACC) amplifying 1051-bp fragment and CTX-M primers (CTX-M forward TTTGCGATGTGCAGTACCAGTAA, CTX-M reverse CGATATCGTTGGTGGTGCCATA) amplifying 544-bp fragment^{11,13}.

PCR for detection of *bla*_{NDM} was carried out for carbapenemase producing stains using the pre-published sequences viz. forward 5'-ACCGCTGGACCGATGACCA-3' and reverse 5'-GCCAAAGTTGGGCGCGGTTG-3', which amplified 264 bp fragment of the *bla*_{NDM} gene¹⁴. Gene sequencing of the amplicons was done for all the *bla*_{NDM} genes with amplicons purified by PCR purification kit (QIAGEN, Hidden, Germany). The purified products were sequenced on ABI PRISM 3130XL sequencer using Big Dye Terminator cycle sequencing kit (Perkin Elmer). The sequences obtained were checked using BIOEDIT software followed by blast at the National Centre for Biotechnology Information website search (<https://blast.ncbi.nlm.nih.gov/blast.cgi>). The derived sequences were aligned with reference sequences from the database of GenBank.

Statistical analysis

The demographic and epidemiological parameters were analysed for all the subjects initially enrolled in the study while the microbiological parameters between the two phases i.e. enrolment phase and follow-up phase were compared only for those human subjects available for sampling at both the phases.

Comparison of categorical variables viz. various risk factors associated with acquisition of AMR and prevalence of various categories of AMR between the two subgroups as well as between the two phases of the study i.e. enrolment and follow up phases were analyzed by univariate analysis using χ^2 test. P value < 0.05 was considered as statistically significant and it was adjusted for multiple comparisons. Fisher's exact test was used if any of the cell frequency was less than five.

RESULTS

A total of 136 and 124 labourers belonging to ML and NML subgroups respectively were enrolled initially in the present study for assessment of all baseline demographic and epidemiological parameters. A total of 8 labourers from 4 families (as husbands and their wives), four each from ML and NML subgroups left the job in between and thus were not available for evaluation of changes in AMR prevalence over the follow-up period. Thus, a total of 132 and 120 subjects from ML and NML subgroups respectively were considered for comparison of AMR prevalence between enrolment and follow-up phase.

The labourers in the ML subgroup were in the profession as unskilled labourers for a median duration of 5.4 years (95% CI 3.2-8.6) in three cities viz. Delhi, Jaipur and Gurgaon of north India with uninterrupted stay in one of the three cities for at least 6 months (mean duration 9 ± 1.5 months) prior to enrolment in the present study.

A significantly higher proportion of subjects in ML subgroup had no formal education or had education only upto primary (4th standard) level compared to NML subgroup (81 out of 136 i.e. 59.6% with no formal education and 48 out of 136 i.e. 35.3% with education upto primary level in ML subgroup versus 14 out of out of 124 i.e. 11.3% with no formal education and 13 out of 124 i.e. 10.5% with education upto primary level in NML

subgroup; $P < 0.01$ for both levels of education, data not shown in the table). Higher proportion of the ML subgroup had resided in make-shift road side shelters covered with polythene sheets before enrolment while the labourers belonging to NML subgroup had their stay in stable residences (brick made) registered in their names. While the practice of defecation in the open was prevalent among the labourers in both the subgroups, the proportion was significantly higher in the ML subgroup with majority resorting to the practice of defecating in the open, mostly on the edges of open drainage lines or boundary walls while higher proportion of subjects in the NML subgroup reported to have had the facility of dedicated toilets. Most of the labourers in the ML subgroup revealed history of using public roadside taps for drinking water and had to cook in open space adjacent to their shelters compared to the labourers in NML subgroup who enjoyed the privilege of having dedicated piped water supply for drinking and self-owned closed space for cooking (Table1).

Episodes of illness prior to enrolment viz. chest infection and diarrhoea were significantly more frequent in the ML subgroup compared to NML subgroup although none of the episodes required hospitalisation or parenteral medication in either of the two subgroups (data not shown in table). However, treatment seeking behaviour for these episodes was different between the subgroups. While higher proportion of illness in the ML subgroup were attended by registered medical practitioners compared to NML subgroup, the same attended by pharmacists was more

common in the later subgroup compared to the former. Practice of self-medication was more prevalent in the ML subgroup compared to NML subgroup while left over medicines were used in small percentages of illness by both the subgroups. Non-completion of the duration of treatment recommended by registered medical practitioners was more commonly reported by the ML subgroup of labourers compared to NML subgroup (Table 2).

The fecal carriage rate of ESBL-EC was significantly higher in the ML subgroup compared to those belonging to NML subgroup at the time of enrolment for the employment. However, on follow-up, there was no significant difference in the fecal carriage rate of ESBL-EC between both the subgroups due to decrease in their carriage rate in the former subgroup with concomitant increase in similar carriage rate in the later subgroup during the follow-up. CTX-M was the predominant molecular variety of ESBL detected among the ESBL-EC isolates from both ML and NML subgroups. The trend in the resistance rates of carbapenemase and NDM-1 variety of AMR during the follow-up period was similar to that observed in case of ESBL-EC positivity i.e. decline in rates in the ML subgroup with increase in the NML subgroup, during the follow-up period. On relative terms, the increase in the prevalence of NDM-1 during the follow-up period was more apparent in the NML subgroup when the same subgroup, detected to be free from carriage of NDM-1 at enrolment, acquired the same category of resistance in 8.7% of subjects during follow-up. There was significant increase in the isolation rates

Table 1. Comparison of risk factors towards acquisition of AMR between migrant labourers (ML) and non-migrant labourers (NML)

Characteristics of the study population		ML (n=136) No. (%)	NML (n=124) No. (%)
Accommodation in makeshift shelters*		82 (60.3) ¹	2 (1.6)
Defecation practice in open		134 (98.5) ^{a,1}	84 (67.7) ^b
Source of drinking water	Separate piped supply	2 (1.5)	70 (56.5) ²
	Public tap	132 (97) ¹	40 (32.6)
	Tube well	2 (1.5)	10 (8.0) ²
	Brick well	0 (0)	4 (3.2)
Cooking in open space		134 (98.5) ¹	2 (1.6)

* Mostly polythene sheet tents in open space near construction site
a = Open space, adjacent to boundary walls, open drainages; b = Agricultural fields
Statistical comparisons: 1 = ML > NML ($p < 0.01$); 2 = NML > ML ($p < 0.01$)

of ESBL, carbapenemase and NDM-1 producing *E. coli* from the soil samples of toilet premises during the follow-up period (Table 3). The prevalence of ESBL-EC and ESBL-CR at enrolment in the NML subgroup was comparable to that of healthy non-labour rural community in their native villages with prevalence of ESBL-EC and ESBL-CR as 22.5% (45 out of 200) and 2.5% (5 out of 200) respectively at enrolment and 23.5% (47 out of 200) and 3% (6 out of 200) respectively at follow-up without any statistical difference between the two phases (data not shown in table). No NDM-1 could be detected in enrolment or follow-up samples from the healthy non-labour rural population.

Antibiotic sensitivity pattern of the ESBL-EC isolates from the two subgroups showed comparably high degree of co-resistance to the beta-lactam group of antibiotics except newer beta-lactams viz. cefepime and aztreonam in both the subgroups. Resistance to ceftiofur, a third-generation cephalosporin, known to be used mainly for livestock ailment rather than human use, was recorded only among the *E. coli* isolates from NML subgroup at enrolment without any

positivity detected in ML subgroup at the same point. Moderate degree of co-resistance was also recorded for carbapenem group of antibiotics in both subgroups although prevalence was higher in ML subgroup compared to NML subgroup. Moderate degree of co-resistance was also noted for three antibiotics other than beta-lactam and carbapenem groups i.e. fluoroquinolone (ciprofloxacin), co-trimoxazole and tetracycline tested at enrolment with comparable prevalence between the two subgroups. On follow-up, significant change in prevalence of co-resistance were recorded in both the subgroups for those antibiotics with difference in prevalence recorded at enrolment. Prevalence of resistance were found to decrease for all the three carbapenem group of antibiotics tested in the ML subgroup with concomitant increase in the NML subgroup during the follow-up period. On the other hand, the ML subgroup was found to acquire resistance to ceftiofur during the follow-up period with concomitant reduction on follow-up in the NML subgroup for this antibiotic (Table 4).

Table 2. Magnitude of selected illness suspected to be of bacterial origin requiring intake of medicine and treatment seeking behaviour among the labourers

Characteristics		Study population		P-value
		ML (n= 136)	NML (n= 124)	
Incidence of SBI requiring intake of medication (episodes per person year)	Chest infection	0.46	0.18	<0.001 ¹
	Urinary tract infection	0.31	0.29	NS (0.8)
	Diarrhoea	0.24	0.1	0.0041
	Suppurative infections (boils, ear discharge, eye infection)	0.03	0.02	NS (0.5)
Treatment seeking behaviour, No(%) out of total SBI episodes	From hospital	12 (8.5)	8 (6.5)	NS (0.5)
	From RMP	32 (22.7) ¹	11 (8.9)	0.001 ¹
	From pharmacist	54 (39.7)	87 (70.2) ²	<0.001 ²
	Self-medication	26 (19.1) ¹	7 (5.6)	0.001 ¹
	Use of stored medicine	12 (8.8)	11 (8.9)	NS (0.9)
	Completion of recommended duration of treatment*	5 (15.6)	6 (54.5) ²	NS (0.6)

SBI = Suspected bacterial infections; RMP = Registered Medical Practitioner

*Calculated out of the episodes attended by registered medical practitioners.

Note: Only two subjects in migrant labourers subgroup and one subject in non-migrant labourers subgroup had single episode of hospitalization in preceding 5 years

Statistical comparisons:1= ML >NML (p<0.05); 2= NML >ML(p<0.05); NS = Not significant

DISCUSSION

There is no internationally recognized definition of migrants. According to the International Federation of Red Cross and Red Crescent Societies' policy on migration, migrants are described as people who leave or flee their places of habitual residence to go to a new place, across international borders or within their own state, to seek better or safer perspectives and thus includes labour migrants, stateless migrants, migrants deemed irregular by public authorities, migrants displaced within their own country, refugees and asylum-seekers⁶. While the focus has mainly been directed so far towards AMR hazards associated with international migration, problem of internal migration within a country has been underestimated in this regard. According to a report by United Nations Development Programme (UNDP), the number of those who moved across the major zonal demarcations within their countries was nearly four times larger (740 million) than those who moved internationally (214 million)¹⁵. The National Sample Survey Organisation (NSSO) of India estimated that in 2007-08 there were 326 million internal migrants (28.5% of the population)¹⁶. In India about 59% of growth in the urban population is due to natural increase with rural to urban migration contributing to about 21% of increase¹⁷. Migration

between states in India is mostly an outcome of economic need, poor people living in rural India frequently migrate to urban cities in search of employment⁴. Migration of labourers in search of jobs within short period following birth of their children coupled with frequent change of workplace within or outside the state may explain lack of education beyond primary level among majority of labourers in the ML subgroup. In a nationwide study undertaken in 13 Indian cities on migrant labourers, the overall analysis showed 43.4% of the migrants reportedly living in non-notified slums and 11.7% in dwellings at work sites while the corresponding figures for the subsection of those in the city of Delhi were found be 48.5% and 42.7% respectively reflecting the rapid expansion of civil infrastructures in the city. In the same study only 4.7% of migrant labourers in the city of Delhi were reported to be living in open spaces including footpaths, market place, under the bridges etc. This is in contrast to our study that demonstrated more than 60% of the migrant labourers living in open spaces with polythene sheet covered shelters. This could be due to the difference in the nature of labour jobs for which the labourers were recruited since the former study subjects were surveyed from building construction sites that usually provide closed brick shelters within the building under

Table 3. Prevalence of fecal carriage of ESBL, types of ESBL, carbapenemase and NDM-1 types of AMR in *E. coli* in human subjects and in soil samples from toilet premises of labourers

Type of resistance	Human subjects				Toilet premises (n=600)	
	ML (n=132)		NML (n=120)		En No. (%)	Fu No. (%)
	En No. (%)	Fu No. (%)	En No. (%)	Fu No. (%)		
ESBL	63 (47.7)	45 (34.1) [#]	28 (23.3)	46 (38.3) [@]	72 (12)	128 (21.3) [@]
CTX-M ESBL alone*	45 (71.4)	34 (75.6)	23 (82.2)	39 (84.8)	59 (81.9)	101 (77.7)
CTX-M with other ESBL genes*	7 (11.1)	4 (8.8)	3 (10.7)	5 (10.9)	5 (6.9)	17 (13.1)
Other ESBL genes *	11 (17.5)	7 (15.6)	2 (7.1)	2 (4.3)	8 (11.1)	7 (5.4)
Carbapenemase*	11 (17.5)	4 (8.9) [#]	1 (3.6)	6 (13) [@]	1 (1.4)	8 (6.3) [@]
NDM-1*	8 (12.7)	3 (6.7) [#]	0	4 (8.7)	0	3 (2.3)

ML= Migrant labourers; NML= Non-migrant labourers; En = Enrolment; Fu = Follow-up.

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

[#] Significant decrease in prevalence on follow-up (P < 0.05) compared to enrolment in the same subgroup.

[@]Significant increase in prevalence on follow-up (P<0.05) compared to enrolment in the same subgroup.

construction till the construction was completed. The migrant labourers at construction sites in the country reportedly have access to public tap as source of potable water and majority of them are reported to be practicing defecation in open which is similar to our observation⁸. Public health related facilities such as provision of safe drinking water, improved sewage disposal and housing have been considered to be the basic pre-requisites for containing the incidence of communicable diseases of major public health importance not only in developing countries^{18,19} but also in a developed country⁷. Significant differences in availability of these basic facilities could explain higher incidence of episodes of illness suspected to be of bacterial origin in ML subgroup compared to NML subgroup in the present study.

Shortage of available registered medical practitioners in rural India is well recognised²⁰ that could be the reason for the majority of the rural population resorting to help from pharmacies for medication. Self-medication is a globally

recognized concern with the prevalence range in India and other developing countries reported to be as high as 78% - 92.8%²¹⁻²³, although limited studies have focussed on antibiotic related self-medication. One comparative study on antibiotic related self-medication between urban and rural population within same zone in India has shown the prevalence of such practice to be more common in the former sector compared to later, although the study was based on survey conducted in pharmacy outlet²⁴. As opposed to this pharmacy outlet-based study that may not be representative of the problem in the general community, the present study on antibiotic usage was based on survey in two distinct subpopulations from urban and rural community. Considering the overall low literacy rate in the two study subpopulations to clearly recognise medications as antibiotics and non-availability of prescriptions, the present study relied on usage of drugs in ailments suggestive of bacterial infection as correlate of antibiotic usage which showed self-medication of probable

Table 4. Antibiotic resistance profile of ESBL-EC isolated from faecal samples of human study subjects and soil samples from toilet premises of the labourers

Antibiotics (Disc potency)	ML		NML		Toilet premises	
	En (n=63)	Fu (n=45)	En (n=28)	Fu (n=46)	En (n=72)	Fu (n=128)
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
AMC (20/10 µg)	22 (34.9)	14 (31.1)	11(39.3)	20 (43.5)	18 (25)	37 (28.5)
PIT (100/10 µg)	5 (7.9)	2 (4.4) [#]	2 (7.1)	4 (8.7)	0	2 (1.5)
AK (30 µg)	3 (4.8)	2 (4.4)	0	2 (4.3)	0	0
GEN (10 µg)	5 (7.9)	4 (8.9)	2 (7.1)	5 (10.9)	2 (2.8)	6 (4.6)
CAZ (30 µg)	63 (100)	43 (95.6)	27 (96.4)	46 (100)	67(93.1)	124 (95.4)
EFT (30 µg)	0	8 (17.8) [®]	9 (32.1)	6 (13) [#]	4 (5.5)	17 (13.1) [#]
CPM (30 µg)	24 (38.1)	15 (33.3)	7 (25)	20 (43.5)	3 (4.2)	9 (6.9)
AT (30 µg)	63 (100)	45 (100)	27 (96.4)	43 (97.8)	69 (95.8)	128 (98.5)
ETP (10 µg)	13 (20.6)	6 (13.3) [#]	2 (7.1)	6 (13) [®]	2 (2.8)	11 (8.6) [®]
IMP (10 µg)	15 (23.8)	8 (17.8) [#]	2 (7.1)	9 (19.6) [®]	4 (5.6)	12 (9.4) [®]
MRP (10 µg)	14 (22.2)	6 (13.3) [#]	3 (10.7)	7 (15.2) [®]	2 (2.8)	11 (8.6) [®]
CIP (5 µg)	28 (44.4)	22 (48.9)	11(39.3)	16 (34.8)	8 (11.1)	20 (15.6)
COT (25 µg)	26 (41.3)	18 (35.6)	11(39.2)	19 (41.3)	9 (12.5)	14(10.9)
TE (10 µg)	22 (34.9)	13 (28.9)	12 (42.9)	20 (43.5)	10 (13.9)	16 (12.5)

ML= Migrant labourers; NML= Non-migrant labourers; En = Enrolment; Fu = Follow-up; AMC= Amoxyclov; PIT= Piperacillin/Tazobactam; AK= Amikacin; GEN= Gentamicin; CAZ= Ceftazidime; EFT= Ceftiofur; CPM= Cefepime; AT= Aztreonam; ETP= Ertapenem; IPM= Imipenem; MRP= Meropenem; CIP= Ciprofloxacin; COT= Co-trimoxazole; TE= Tetracycline

Statistical comparisons:

[#]Significant decrease on follow-up (P<0.05) compared to enrolment in the same subgroup.

[®]Significant increase on follow-up (P <0.05) compared to enrolment in the same subgroup.

Note: All the isolates were resistant to ampicillin, ceftriaxone and cefotaxime and sensitive to tigecycline

antibiotics to be more prevalent in ML subgroup compared to NML subgroup similar to that observed in pharmacy based survey.

While there are few reports on the intestinal carriage rate of ESBL mediated AMR among healthy rural community in India, ranging from 15.4% to 33.8%^{25,26}, there is hardly any information available on carriage rate of carbapenem and NDM-1 mediated AMR in the healthy rural community, most of the reports originating from studies on hospitalized patients from rural community¹⁴. Laxminarayan and Choudhary reported that non-prescription sales of carbapenems in India is among the highest in the world contributing to growing problem of carbapenem resistance²⁷. To the best of our knowledge, there is very little information available on the carriage rate of carbapenemase and NDM-1 mediated resistance in asymptomatic rural community. High resistance rate among NML subgroup to ceftiofur, a third-generation cephalosporins mainly used for ailments in the livestock population²⁸ could be due to their association with livestock care where prevalence of resistance to this antibiotic has been reported to be as high as > 50%²⁹. Such possibility may be of serious concern since resistance to ceftiofur and cephalosporins e.g. ceftriaxone are shared by common genes viz *bla*_{CMY-2} and *bla*_{CTX-M}³⁰ and reported emergence of resistance to third generation cephalosporins of human importance in *E. coli* after ceftiofur use³¹. Due to the cheaper cost of some antibiotics, especially ciprofloxacin, co-trimoxazole and tetracycline³², these antibiotics are indiscriminately used in India by population with low socioeconomic status explaining moderate resistance to these antibiotics among the *E. coli* isolates obtained from both the subgroups at enrolment³². A study on rural community from central India reported multidrug resistant *E. coli* with high resistance to these antibiotics ranging from 28-69%²⁶.

The present study demonstrated comparably high prevalence of co-resistance to common beta-lactam group of antibiotics in ESBL-EC isolated at enrolment from both the subgroups, a finding that was expected since the isolates selected for study of co-resistance were the ones screened as ESBL positives (vide materials and methods). While no change in prevalence of

co-resistance to beta-lactam group of antibiotics was demonstrable in both the subgroups on follow-up, a decline in the prevalence of co-resistance to carbapenem group of antibiotics could be observed during the follow-up in the ML subgroup with concomitant increase in prevalence of similar categories of resistance in NML subgroup during the same period. In absence of history of hospitalisation or parenteral therapy in the study population such resistance to carbapenems could be due to colonisation of carbapenem resistant organisms acquired through contaminated food, water and environment rather than any antibiotic pressure³³. This is further supported by higher prevalence of ESBL and carbapenem mediated resistance observed in the ML subgroup that was characterised by unhygienic living conditions compared to NML subgroup. Resistance in *Enterobacteriaceae* group of bacteria acquired through international travel are mostly reported to disappear by six months on return to home country^{34,35}. In a longitudinal study with monthly monitoring of a cohort of 245 travellers with travel-acquired multi-drug resistant *Enterobacteriaceae*, the resistant strains were found to persist in only 33% after 4 weeks and in only 5% after 6 months³⁵. Thus it may be assumed that reduction in prevalence of ESBL and carbapenem resistance in the ML subgroup was due to shedding of the organisms at a rate higher relative to the rate of new acquisition while the increase in the NML subgroup was due to higher rate of acquisition of same organisms relative to their shedding in the process of sharing of environment e.g. toilets, washing and bathing area. This was further substantiated by the finding that prevalence of resistance to beta-lactam and carbapenem group of antimicrobials at enrolment in the NML subgroup was comparable to that of healthy rural community in their native villages. In the study by Haverkate et al. the estimated probability of transfer of ESBL producing *Enterobacteriaceae* from an index patient to the household contact was 67% with person-to-person transmission occurring at a rate of 0.0053/ person/day and decolonization at 0.0026/ person/day for index patients³⁶.

Concomitant increase in the prevalence of co-resistance to carbapenem group of antibiotics among ESBL positive isolates from soil

in toilet premises during the follow-up period, commensurate with the observed increase in carbapenem resistance suggest that contaminated soil environment in toilet premises could be a potential source for acquisition of AMR by the NML subgroup of labourers. Increase in the prevalence of ESBL, carbapenemase and NDM-1 varieties of AMR among *E. coli* in the soil samples from toilet premises during the follow-up period indicate that toilet premises could be a potentially conducive platform for acquisition of these varieties of AMR. Acquisition of high rate of AMR by the labourers recruited from local villages is a matter of concern keeping in mind possible carriage of such resistance by them to their native villages on their return from employment and consequent spread to the local rural community hitherto unexposed or minimally exposed to the problem.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

DC contributed to the study conception and designing the protocol of the study, carried out data analysis and contributed in manuscript writing; LSD contributed in samples collection, data collection, conducting laboratory work and manuscript writing; SSG contributed in data collection and conducting laboratory work; SB supervised the overall work and undertook revision of the manuscript. All authors read and approved the final manuscript.

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DATA AVAILABILITY

GenBank accession numbers for *bla*_{NDM-1} are given below: ML subgroup (Enrolment): NPS_1 JX292120.1, NPS_2 JX292121.1, NPS-3 JX292122.1, NPS_4 JX292123.1, NPS_5 KJ410404.1, NPS_6 KJ410405.1, NPS_7 KJ410406.1, NPS_8 KU158096.1 ML subgroup (Follow-up): NPS_9 KU158097.1, NPS_10 KU158098.1, NPS_11 KU158099.1

NML subgroup (Follow up): NPS_12

KU158100.1, NPS_13 KU158101.1, NPS_14 KU158102.1, NPS_15 KU158103.1

Toilet premises (Follow-up): MN433632, MN433631, MN433630

ETHICS STATEMENT

This study was approved by both independent institutional research and ethical committees of SGT University, approval no. SGTU/FMHS/MICRO/341.

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