Journal of Communicable Diseases
Volume 53, Issue 1 - 2021, Pg. No. 15-22
Peer Reviewed & Open Access Journal

Research Article

Hospital Premises as a Potential Reservoir of Antimicrobial Resistance

Debasish Chattopadhya[1], Leimapokpam Sumitra Devi[2], Rajendra Singh Rautela[3], Shyam Sunder Grover[4], Shobha Broor[5]

[1,2,5]Department of Microbiology, SGT Medical College Hospital and Research Institute, Gurugram, Haryana, India.
[3]Division of Biochemistry, National Centre for Disease Control, New Delhi, India.
[4]Division of Microbiology, National Centre for Disease Control, New Delhi, India.
DOI: https://doi.org/10.24321/0019.5138.202104

I N F O

Corresponding Author:
Debasish Chattopadhya, Department of Microbiology, SGT Medical College Hospital and Research Institute, Gurugram, Haryana, India.
E-mail Id:
dchattopadhya27@gmail.com
Orcid Id:
https://orcid.org/0000-0002-4117-3050
How to cite this article:
Chattopadhya D, Devi LS, Rautela RS, Grover SS, Broor S. Hospital Premises as a Potential Reservoir of Antimicrobial Resistance. *J Commun Dis* 2021; 53(1): 15-22.

Date of Submission: 2021-01-01
Date of Acceptance: 2021-03-21

A B S T R A C T

Soil in hospital premises can be a potential reservoir of organisms with Antimicrobial Resistance (AMR) due to their spread from hospital environment including pre-treated hospital waste. Thus, studying AMR in the soil samples from hospital premises at periodic interval could be helpful in monitoring the trend of its load and spectrum in hospital environment. Prevalence of Extended-Spectrum Beta-Lactamase (ESBL), carbapenemase and New Delhi Metallo-Beta-Lactamase (NDM) varieties of AMR were estimated in *Escherichia coli* and *Klebsiella pneumoniae* as indicator organisms in surface soil samples from hospital premises viz. hospital grounds and pedestrian tracks in relation to the pre-monsoon and post-monsoon seasons at an interval of four years between 2014 and 2018. There was significant increase in the prevalence of ESBL (mainly CTX-M variety), carbapenemase and NDM varieties of AMR in isolates from soil samples collected from hospital premises during post-monsoon season compared to pre-monsoon isolates regardless of the year of sampling although monsoon season did not affect the prevalence of AMR in clinical samples processed during the same period. There was gradual rise in resistance to other antibiotics viz. co-trimoxazole, tetracycline, fluoroquinolones and amoxyclav in soil samples collected from hospital premises during the four years interval. Prevalence of various categories of AMR were higher in samples collected during the post-monsoon season compared to prevalence in clinical isolates from hospital attending population during the corresponding period regardless of the year of sampling. Increasing prevalence of various categories of AMR recorded in hospital premises could indicate inadequate containment measures towards prevention of their spread from hospital environment warranting adaption of requisite measures for prevention.

Keywords: ESBL, Carbapenemase, NDM, Soil, Hospital premises

Introduction

Soil in hospital premises has been reported to be a potential reservoir of organisms with Antimicrobial Resistance (AMR) due to their spread from hospital environment.[1] Thus, monitoring of AMR in the hospital premises could be an important measure for reviewing the existing biosafety measures towards containment of infection in the hospital environment. Contaminated soil from hospital premises could also pose a risk for acquisition of AMR by healthy human subjects visiting indoor patients or accompanying patients to outpatient department for treatment. The situation is likely to be aggravated during monsoon season in India due to frequent flooding of hospital premises by rain water facilitating spread of AMR microbes from any breach in hospital waste disposal system.[1-4]

Several decades of therapeutic use of antibiotics for treatment of infections in India has witnessed increasing prevalence of Extended-Spectrum Beta-Lactamase (ESBL) producing and subsequently carbapenemase producing bacteria belonging to Enterobacteriaceae family resulting in gradual narrowing of the therapeutic options. New Delhi Metallo-Beta-Lactamase (NDM) production by enteric organisms, a relatively recent addition to the problem, has now become a global concern.[5,6] Two organisms of normal gut flora belonging to Enterobacteriaceae family viz. *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) have been commonly selected as indicator organisms to monitor spread of AMR in hospital environment although studies have mostly been limited to single point assessment.[7-9]

A study was undertaken to monitor the change in the prevalence of ESBL, carbapenemase and NDM varieties of AMR in hospital premises over a four years period between 2014 and 2018 and study the effect of monsoon on their load using *E. coli* and *K. pneumoniae* as indicator organisms.

Materials and Methods

Study Location

The present study was conducted in the department of Microbiology, SGT Medical College Hospital, a 600 bedded newly established multi-specialty hospital located in the peri-urban belt of Haryana state in northern India.

Study Period and Periodicity of Sampling

The study was prospective in nature conducted in two phases i.e., in 2014 and in 2018. Sampling was carried out weekly for three months (12 weeks) in each year during each of the two monsoon-related seasons identified on the basis of Indian climatic conditions i.e., pre-monsoon (March-May) and post-monsoon (September-November).[10]

Collection of Samples

Surface soil samples were collected from two categories of locations within hospital premises viz. (i) hospital grounds and (ii) pedestrian tracks used by hospital attending population.

Selection of Sampling Sites and Frequency

Surface Soil from Hospital Grounds

Two parallel longitudinal lines, each at 4 feet and at 10 feet distances, were marked from the four boundary walls of the rectangular hospital block excluding pedestrian tracks. Four equidistant points on the length of each line on the four sides of the hospital block were randomly selected for weekly sampling using computer generated random numbers. Thus, a total of 16 soil samples each were collected at 4 feet and at 10 feet distances from the four sides of the hospital block per week totalling to 192 samples at each of the distances over the period of 3 months (12 weeks) during the pre-monsoon and post-monsoon seasons.

Surface Soil Samples from Pedestrian Tracks

Two locations at 4 feet and at 10 feet away from hospital building edge were selected on two pedestrian tracks used by the hospital attending population. Three random points across the transverse breadth of the track at 4 feet and 10 feet distances from the hospital building were sampled twice in a week i.e., 6 samples per week each at 4 feet and 10 feet distances on each pedestrian track, totalling to 72 samples/ track/ per season of 12 weeks or total of 144 samples per season from the two selected tracks in each year. Repeat sampling of the pedestrian tracks was carried out after an interval of four years in the same manner during pre-monsoon and post-monsoon seasons of the year 2014 and 2018.

Collection of Samples

Surface soil samples were collected from each sample collection point covering an approximate area of 10 cm by 10 cm square with five longitudinal, five latitudinal and two diagonal strokes using sterile swabs pre-moistened with nutrient broth immediately before use.[11] After collection of the sample, the swab was placed in polypropylene tube containing 2 ml nutrient broth and transported to laboratory in ice pack within 30 minutes.

Processing of Samples for Screening of ESBL and Carbapenemase Producing *E. coli* and *K. pneumoniae* Isolates

Surface soil swabs from hospital premises, collected in nutrient broth were vortexed for 2 mins. Each sample was inoculated in 10 volumes of enrichment broth (LB broth, Sigma Aldrich, USA) followed by overnight aerobic incubation at 37°C. The enriched sample was plated on a set of four MacConkey agar plates viz. (i) plain MacConkey for isolation of *E. coli* and *K. pneumoniae* (ii) two MacConkey

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202104

agar plates, one supplemented with 2 µg of cefotaxime per ml (Mac-CTX) and the other supplemented with 2 µg of ceftazidime per ml (Mac-CAZ), both as screening media for isolation of ESBL producing *E. coli* (ESBL-EC) as well as ESBL producing *K. pneumoniae* (ESBL-KP) and (iii) one MacConkey agar plate supplemented with 1mg/ L of ertapenem (Mac-ETP) as screening medium for isolation of carbapenemase producing *E. coli* (CR-EC) and *K. pneumoniae* (CR-KP). All the plates were incubated aerobically overnight at 37°C. Two to three randomly selected lactose fermenting colonies suggestive of *E. coli* or *K. pneumoniae* were picked up from each plate and were subjected to species confirmation using Vitek 2 system (BioMerieux, France) and also to ensure concordance in the species identification as *E. coli* or *K. pneumoniae* in the colonies selected from different plates based on colony morphology. *E. coli* and *K. pneumoniae* strains growing in ESBL screening media and in carbapenemase screening media were identified as potential ESBL and carbapenemase producing strains respectively.[12,13]

Antibiotic Sensitivity Testing (AST) of the *E. coli* and *K. pneumoniae* Isolates

Antibiotic Sensitivity Testing (AST) of the *E. coli* and *K. pneumoniae* isolated on plain MacConkey plates was performed by disc diffusion method and the results were interpreted as per CLSI guidelines.[14] The following antibiotic discs were used: ampicillin (10µg), amoxicillin-clavulanic acid (20/10µg), piperacillin/tazobactam (100/10µg), amikacin (30 µg), gentamicin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), chloramphenicol (30 µg), co-trimoxazole (25 µg), ertapenem (10 µg), meropenem (10 µg), imipenem (10 µg), tetracycline (30 µg) and tigecycline (15 µg).

Phenotypic Confirmatory Tests for ESBL and Carbapenemase Production

ESBL Production

E. coli and *K. pneumoniae* isolates from ESBL screening media i.e., Mac-CTX and Mac-CAZ were subjected to Double Disc Synergy Test (DDST) as phenotypic confirmatory test for ESBL production using ceftazidime (30 µg) and ceftazidime plus clavulanic acid (30 µg plus 10 µg) discs as first pair and cefotaxime (30µg) and cefotaxime plus clavulanic acid (30 µg plus 10 µg) discs as second pair as described earlier.[12] *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as ESBL positive and ESBL negative control strains respectively.

Carbapenemase Production

E. coli and *K. pneumoniae* isolates from CR screening medium i.e., Mac-ETP were initially subjected to modified Hodge test (MHT) as described earlier.[13] However, all the *E. coli* and *K. pneumoniae* isolated from the Mac-ETP plate were also subjected to revalidation of carbapenemase production on the basis of Carba-NP test in accordance with new CLSI guidelines. Both the methods employed *K. pneumoniae* ATCC BAA 1705 and *E. coli* ATCC 25922 as positive and negative control strains respectively. Only those isolates showing evidence of carbapenemase production by Carba-NP test were considered for further evaluation.[14]

Polymerase Chain Reaction (PCR) for Detection of ESBL Genes and NDM Gene

ESBL Genes

PCR assay was done to detect the presence of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes using specific primers with pre-published sequences viz. ATGAGTATTCAACATTTCCGTG (forward) and TTACCAATGCTTAATCAGTGAG (reverse) for bla_{TEM}, ATTTGTCGCTTCTTTACTCGC (forward) and TTTATGGCGTTACCTTTGACC (reverse) for *blaSHV* and TTTGCGATGTGCAGTACCAGTAA (forward) and CGATATCGTTGGTGGTGCCATA (backward) for bla_{CTX-M}. Three previously confirmed isolates of *E. coli* from the laboratory producing bla_{TEM}, bla_{SHV}, bla_{CTX-M} were used as positive controls. Nuclease-free water without DNA template was included in every PCR assay as negative control.[12,15]

NDM Gene

PCR for detection of bla_{NDM} was carried out for carbapenemase producing (Carba-NP positive) stains using primer sequences, forward 5'-ACCGCCTGGACCGATGACCA-3' and reverse 5'-GCCAAAGTTGGGCGCGGTTG-3', which amplified 264 bp fragment of the *blaNDM* gene as desribed earlier.[12] The PCR products were purified by PCR purification kit (QIAGEN, Hidden, Germany) and run on gel electrophoresis followed by ethidium staining to confirm specificity of NDM gene by matching with the molecular weight markers.

Collection of Information on Clinical Samples from Hospital Attending Population

Information on positivity rate of ESBL, CRE, CTX-M and blaNDM genes among *E. coli* and *K. pneumoniae* isolated from clinical specimens processed by the hospital laboratory during the two seasons and years corresponding to the present study i.e., pre-monsoon and post-monsoon seasons in the years 2014 and 2018 along with their antibiotic resistance pattern was collected from the records of a separate ongoing hospital-based surveillance.

Statistical Analysis

Chi square test was employed to undertake bivariate analysis of categorical variables with Yate's correction for cell values less than 5. P-value < 0.05 was considered statistically significant.

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202104

Result

There was significant increase in the prevalence of various categories of AMR in indicator organisms i.e., *E. coli* and/or *K. pneumoniae* isolated from soil samples in hospital grounds and pedestrian tracks over the four years period between 2014 and 2018 (Tables 1 and 2). In both the years, prevalence of AMR was higher in samples collected during post-monsoon season compared to pre-monsoon season and in samples collected at closer distance from hospital block i.e., 4 ft. compared to samples collected at greater distance i.e., 10 ft. on hospital grounds as well as on pedestrian tracks. In both the years the prevalence of various categories of AMR in soil samples collected from pedestrian tracks were higher than samples collected from hospital ground at corresponding distances regardless of the season although the difference was not statistically significant. The CTX-M was the predominant type of ESBL regardless of the location, season or year of isolation. However, while isolates in 2014 did not demonstrate any evidence of NDM production, samples collected during 2018 showed emergence of NDM production among the isolates (Table 1 and 2).

The prevalence of ESBL, CRE, CTX-M and NDM gene were recorded in *E. coli* and/or *K. pneumoniae* isolates from clinical samples during pre-monsoon as well as post-monsoon seasons were noted to be higher in 2018 compared to the corresponding seasons in the year 2014. However, unlike the soil samples collected from hospital premises including pedestrian tracks, prevalence of various categories of AMR in clinical specimens did not vary between the pre-monsoon and post-monsoon samples in both 2014 and in 2018 (Table 3).

On comparison, prevalence of various categories of AMR in samples collected from hospital premises were lower than the prevalence of similar categories of AMR in clinical isolates during pre-monsoon period but were higher in post-monsoon period in both the years (Table 1-3).

Table 1.Prevalence of various categories of AMR in *E. coli* and/or *K. pneumoniae* isolated from surface soil samples in the hospital ground

Location	Type of AMR	Prevalence (%) of AMR in *E. coli* and/or *K. pneumoniae* isolates			
		2014		2018	
		Pre-mon (n= 192)**	Post-mon (n= 192)**	Pre-mon (n= 192)**	Post-mon (n= 192)**
4 ft*	No of isolates	18 (9.4)	32 (16.7)[a]	39 (20.3)[b]	52 (27.1)[a,b]
	ESBL	06 (33.3)	15 (46.9)[a]	18 (46.2)[b]	35 (67.3)[a,b]
	CTX-M alone[@]	04 (66.7)	11 (73.3)	13 (72.2)	28 (80)
	CTX-M with other ESBL genes[@]	01 (16.7)	03 (20)	03 (16.7)	05 (14.3)
	Other ESBL genes[@]	01 (16.7)	01 (6.7)	02 (11.1)	02 (5.7)
	CRE	01 (5.6)	06 (18.8)[a]	07 (17.9)[b]	17 (32.7)[a,b]
	NDM	00	00	01 (2.7)	04 (7.7)[a]
10 ft* (n=192)	No. of isolates	13 (6.8)	26 (13.5)[a]	22(11.5)[b]	38 (19.8)[a,b]
	ESBL	04 (30.8)	11 (42.9)[a]	09 (40.9)[b]	19 (50)[a,b]
	CTX-M alone[@]	03 (75)	8 (72.7)	07 (77.8)	13 (68.4)
	CTX-M with other ESBL genes[@]	01 (25)	01 (9.1)	01 (11.1)	04 (21.1)
	Other ESBL genes[@]	00	01 (9.1)	01 (11.1)	02 (10.5)
	CRE	00	00	01 (4.5)	03 (7.9)[a]
	NDM	00	00	00	02 (5.3)

Pre-mon= Pre-monsoon, Post-mon = Post-monsoon.

*Indicates distance from the edge of the hospital building.

**No of samples processed.

[@]Calculated out of total ESBL producing *E. coli* and/or *K. pneumoniae* isolates.

Statistical comparisons:

a=Significant increase in prevalence, post-monsoon vs. pre-monsoon.

b=Significant increase in prevalence between years of study in corresponding seasons, 2018 vs. 2014.

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202104

Table 2.Prevalence of various categories of AMR in *E. coli* and/or *K. pneumoniae* isolated from surface soil samples on the pedestrian tracks

Location	Type of AMR	Prevalence of AMR in *E. coli* and/or *K. pneumoniae* No. (%)			
		2014		2018	
		Pre-mon (n=144)**	Post-mon (n=144)**	Pre-mon (n=144)**	Post-mon (n=144)**
4 ft*	No of isolates	19 (13.2)	31 (21.5)	37 (25.7)[b]	45 (31.3)
	ESBL	08 (42.1)	16 (51.6)	21 (56.8)[b]	32 (71.1)[b]
	CTX-M alone[@]	04 (50)	11 (68.8)[a]	16 (76.2)	26 (81.3)
	CTX-M with other ESBL genes[@]	03 (37.5)	05 (31.2)	04 (9.5)	05 (15.6)
	Other ESBL genes[@]	01 (12.5)	00	01 (4.7)	01 (3.1)
	CRE	01 (5.2)	04 (12.9)	08 (21.6)[b]	16 (35.6)[b]
	NDM	00	00	01 (2.7)	06 (13.3)[a]
10 ft*	No. of isolates	14 (9.7)	20 (17.4)	29 (20.1)[b]	35 (24.3)[b]
	ESBL	06 (42.9)	12 (48)	15 (51.7)[b]	20 (57.1)
	CTX-M alone[@]	04 (66.7)	9 (75)	12 (80)	15 (75)
	CTX-M with other ESBL genes[@]	01 (16.7)	2 (16.7)	01 (6.7)	05 (25)
	Other ESBL genes[@]	01 (16.7)	01 (8.3)	02 (13.3)	00
	CRE	01 (7.1)	03 (15)	03 (10.3)	09 (25.7)[b]
	NDM	00	00	01 (3.4)	03 (8.6)[a]

Pre-mon = Pre-monsoon; Post-mon = Post-monsoon.

Notes:

* Indicates distance from the edge of the building towards pedestrian tracks.

[@]Calculated out of total ESBL producing *E. coli* and/or *K. pneumoniae* isolates.

Statistical comparisons:

a=Significant increase in prevalence, post-monsoon vs. pre-monsoon.

b=Significant increase in prevalence between years of study in corresponding seasons, 2018 vs. 2014.

Table 3.Prevalence of various categories of AMR in *E. coli* and/or *K. pneumoniae* isolated from clinical specimens

Source of samples	Type of AMR	Prevalence (%) of AMR in *E. coli* and/or *K. pneumoniae*			
		2014		2018	
		Pre-mon (n=1652)*	Post-mon (n=1857)*	Pre-mon (n=2456)*	Post-mon (2789)*
Clinical specimens	No. of isolates	106 (6.4)	132 (7.1)	325 (13.2)[a]	392 (14.1)[a]
	ESBL	45 (42.5)	61 (46.2)	181 (55.7)[a]	225 (57.4)[a]
	CTX-M alone[@]	33 (73.3)	47 (77)	147 (81.2)	186 (82.7)[a]
	CTX-M with other ESBL genes[@]	07 (15.6)	09 (14.8)	34 (18.8)	24 (10.7)
	Other ESBL genes[@]	05 (11.1)	05 (8.2)	00	15 (6.7)
	CRE	03 (2.8)	05 (3.8)	29 (8.9)[a]	43 (10.9)[a]
	NDM	00	00	04 (1.2)	06 (1.5)

Pre-mon = Pre-monsoon; Post-mon = Post-monsoon

Notes:

*Indicates number of clinical specimens processed

[@]Calculated out of total ESBL producing *E. coli* and/or *K. pneumoniae* isolates

Statistical comparisons:

There was no statistical difference in prevalence, post-monsoon vs. pre-monsoon samples.

a=Significant increase in prevalence between years of study in corresponding seasons, 2018 vs. 2014.

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202104

Antibiotic sensitivity pattern of the *E. coli* and/or *K. pneumoniae* isolated from hospital premises including pedestrian tracks showed an increase in the prevalence of resistance to cephalosporin and carbapenem group of antibiotics over the study period. An increasing prevalence of resistance was also noted for other antibiotics viz. co-trimoxazole, tetracycline, fluoroquinolones and amoxyclav during the same period. Pattern of resistance to various antibiotics among the in *E. coli* and/or *K. pneumoniae* isolates from hospital premises including pedestrian tracks were similar to that observed in clinical isolates (Table 4). prevalence of various categories of AMR in soil samples collected at 4 ft distance compared to that collected at 10 ft distance, regardless of the year of collection, reflects dissemination of AMR from hospital environment.

However, the salient observation in the present study was higher prevalence of various categories of AMR in indicator organisms in soil samples from hospital premises during the post-monsoon period compared to similar isolates from corresponding locations during pre-monsoon period in both the years of sampling suggesting additional contribution of

Table 4.Profile of resistance to various antibiotics in *E. coli* and/or *K. pneumoniae* isolates from various sources

Antibiotic	Antibiotic resistance profile of *E. coli* and/or *K. pneumoniae* isolates from various samples					
	Clinical samples		Soil samples from hospital premises		Soil samples from the pedestrian tracks	
	2014 (n= 238)*	2018 (n=717)*	2014 (n= 89)*	2018 (n= 151)*	2014 (n= 84)*	2018 (n= 146)*
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
AMP	193 (81.1)	631 (88.1)	74 (83.1)	135 (89.4)	72 (85.7)	133 (91.1)
AMC	108 (45.4)	467 (65.1)[a]	50 (56.2)	103 (68.2)[a]	50 (59.5)	105 (71.9)[a]
PIT	58 (24.4)	163 (22.7)	22 (24.7)	39 (25.8)	24 (28.6)	35 (24)
AK	45 (18.9)	120 (16.7)	20 (22.5)	31 (20.5)	24 (28.6)	32 (21.9)
GEN	63 (26.5)	149 (20.8)	28 (31.5)	46 (30.5)	30 (35.7)	49 (33.6)
CTX	109 (45.8)	411 (57.3)[a]	37 (41.6)	83 (55)[a]	36 (42.9)	85 (58.2)[a]
CAZ	105 (44.1)	404 (56.3)[a]	35 (39.3)	83 (54.9)[a]	36 (42.9)	84 (57.5)[a]
AT	108 (45.4)	407 (56.8)	35 (39.3)	82 (54.3)	35 (41.7)	85 (58.2)
ETP	11 (4.6)	72 (10)[a]	07 (7.9)	28 (18.5)[a]	07 (8.3)	36 (24.7)[a]
IPM	13 (5.5)	76 (10.6)[a]	10 (11.2)	30 (19.9)[a]	07 (8.3)	38 (26)[a]
MRP	14 (5.9)	76 (10.6)[a]	09 (10.1)	28 (18.5)[a]	08 (9.5)	38 (26)[a]
COT	238 (60.5)	608 (84.8)[a]	58 (65.2)	132 (87.4)[a]	53 (63.1)	131 (89.7)[a]
C	124 (52.1)	535 (74.6)[a]	39 (43.8)	76 (50.3)	38 (45.2)	79 (54.1)
CIP	117 (49.2)	525 (73.2)[a]	56 (62.9)	119 (78.8)[a]	54 (64.3)	118 (80.8)[a]
OF	108 (45.4)	408 (56.9)[a]	41 (46.1)	114 (75.5)[a]	40 (47.6)	118 (80.8)[a]
TE	138 (58)	467 (65.1)[a]	60 (67.4)	117 (77.5)[a]	55 (65.5)	115 (78.8)[a]

* Total isolates in the year (pre-monsoon and post-monsoon combined)

'a' indicates significant rise ($p< 0.05$) in 2018 compared to 2014

AMP = Ampicillin, AMC = Amoxyclav, PIT = Piperacillin/tazobactam, AK= Amikacin, GEN = Gentamicin, CTX = Cefotaxime, CAZ = Ceftazidime, AT = Aztreonam, ETP = Ertapanem, IPM = Imipenem, MRP = Meropenem, C = Chloramphenicol, COT = Co-trimoxazole, CIP = Ciprofloxacin, OF = Ofloxacin, TE = Tetracycline

Note: All the isolates were susceptible to tigecycline.

Discussion

Increasing prevalence of AMR in the soil samples collected in 2018 compared to that collected in 2014, regardless of the sampling location indicate gradual rise in the prevalence of AMR in hospital environment over the years. Higher monsoon to the load of AMR in soil samples. In northern part of India, rainfall is reasonably heavy during monsoon season resulting in frequent flooding of ground soil. An upcoming health care set up as the present one with numerous subsurface reservoirs in the hospital premises receiving untreated hospital sewage with improperly

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202104

covered lids, or breach in the integrity of the sewage collection channel may provide opportunity for overflow of untreated sewage into surrounding areas. Magnitude of AMR in pre-treated hospital sewage is a reflection of its load in hospitalised patients and in hospital environment.[16] However, there are several factors that may amplify the prevalence of AMR in sewage. Firstly, the hospital sewage contains various antibiotics from hospital usage that can create pressure towards emergence and amplification of resistant bacteria.[17] Secondly, various categories of AMR evaluated in the present study i.e., ESBL, carbapenemase and NDM-1 are commonly carried on plasmids.[18] In-vitro conjugation experiments have shown that plasmid mediated resistance to these antibiotics can easily be transferred to *E. coli* as recipient from sewage isolates e.g. *Acineotbacter baumannii* as donor.[19] Moreover, presence of biofilm in sewage has been shown to be a favourable environment for such transfer[20] although transfer has been shown even in environmental surface waters.[9] Higher prevalence of AMR in pedestrian tracks compared to the samples from hospital premises at corresponding distances could be due to additional contribution by the movement of people visiting the hospital.

One observation in our study was inability to record any seasonal difference in the prevalence of AMR in the isolates from hospital attending population in both the years. Admittedly, this could be due to difference in profile of hospital attending population in terms of many variables like age, sex, clinical diagnosis and nature of specimens that were not taken into account in the present study. There are reports suggesting lack of perceptible difference in magnitude of communicable diseases in rural population. While acute respiratory infections and pneumonia are reported to be common in pre-monsoon summer days, diarrhoeal diseases are reported to be more common during monsoon seasons in rural India.[21,22] However, hospital attending population in the present study represented a small fraction of the disease burden in the rural community known to seek treatment from non-health care sources that could be another variable responsible for lack of perceptible seasonal difference in the isolation rates of the two organisms observed in the present study.

The significant rise in the prevalence of resistance to some of the non-beta-lactam antibiotics among the *E. coli* and/or *K. pneumoniae* isolates over the study period reflects continued increase in the usage of these antibiotics, driven by cost factors and over the counter availability.[23-25] Marginal but statistically insignificant increase in the prevalence of resistance to carbapenem group of antibiotics detected in AST compared to that detected by Carba-NP test could be due to resistance mechanisms other than carbapenemase production e.g. efflux pump or chromosomal porin mutation by the carbapenem resistant by additional isolates.[26] Comparable resistance pattern in the indicator organisms between the hospital isolates and the isolates from the hospital premises strengthens the possibility of spread of AMR from hospital environment. To the best of our knowledge, this is the first longitudinal study from India attempting to monitor the trend of AMR in hospital premises highlighting the need for periodic monitoring of sewage disposal system and biosafety measures.

Conclusion

The present study provides evidence in favor of spread of AMR from hospital environment including sewage effluents to the soil in the premises of the hospital following monsoon season. Periodic monitoring of AMR in hospital premises may be helpful in adapting necessary measures to prevent leakage or overflow as well as adherence to safe disposal of hospital wastes.

Funding

This research project received no financial grant from any funding agency.

Conflict of Interest: None

References

1. Modi G, Mishra SK, Modi BS et al. Production and characterization of multiple drug resistant cultures isolated from hospital premises. *Indian J Life Sci* 2013; 3: 7-14.
2. Lamba M, Graham DW, Ahammad SZ. Hospital waste water releases carbapenem resistance pathogens and genes in urban India. *Environ Sci Technol* 2017; 51: 13906-12.
3. Taneja N, Sharma M. Antimicrobial resistance in the environment: The Indian scenario. *Indian J Med Res* 2019; 149: 119-28.
4. Nicolle LW. Infection control programmes to contain antimicrobial resistance. WHO/CDS/CSR/DRS/2001.7, World Health Organisation, 2001.
5. Kumaraswamy KK, Toleman MA, Walsh TR et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan,and the UK: a molecular, biological and epidemiological study. *Lancet Infect Dis* 2010; 10: 597-602.
6. Kumar SG, Adithan C, Harish BN, et al. Antimicrobial resistance in India: A review. *J Nat Sci Biol Med* 2013; 4: 286-91.
7. Jarvis WR, Munn VP, Highsmith AK et al. The epidemiology of nosocomial infections caused by Klebsiella pneumoniae. *Infect Cont* 1985; 6: 68-74.
8. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods and pathogenicity factors. *Clin Microbiol Rev* 1998; 11: 589-603.
9. Haberacht HB, Nealon NJ, Gilliand JR et al. Antimicrobial-

resistant *Escherichia coli* from environmental waters in northern Colorado. *J Environ Public Health* 2019.
10. India Meteorological Department, Ministry of Earth Sciences, Government of India. Available from: https://mausam.imd.gov.in/imd_latest/contents/monsoon.php,
11. Freeman JC, Nimmo J, Gregory E et al. Predictors of hospital surface contamination with ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*: patient and organism factors. *Antimicrob Resist Infect Cont* 2014; 3: 5.
12. Devi LS, Broor S, Rautela RS et al. Increasing prevalence of *Escherichia coli* and *Klebsiella pneumoniae* producing CTX-M type extended-spectrum beta-lactamase (CTX-M-ESBL), carbapenemase and NDM-1 in patients from a rural community with community acquired infections: A three years study. *Int J Appl Basic Med Res* 2020; 10(3): 156-163.
13. Devi LS, Broor S, Chakravarti A et al. Livestock manure as potential reservoir of CTX-M type extended-spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* associated with carbapenemase production. *J Pure Appl Microbiol* 2020; 14: 171-181.
14. Clinical Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing, 29th Informational Supplement. M100-S27, 2019. Pennsylvania, Wayne, USA.
15. Sidjabat HE, Paterson DL, Adams-Haduch JM et al. Molecular Epidemiology of CTX-M-Producing *Escherichia coli* Isolates at a Tertiary Medical Centre in Western Pennsylvania. *Antimicrob Agents Chemother* 2009; 53: 4733-9.
16. Prado T, Pereira WC, Silva DM et al. Detection of extended spectrum beta-lactamase producing *Klebsiella pneumoniae* in effluents and sludge of a hospital sewage treatment plant. *Lett Appl Microbiol* 2008; 46: 136-41.
17. Bengtsson-Palme J, Larsson DJ. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. *Environ Int* 2016; 86: 140-149.
18. Kazemian H, Heideri H, Ghanavati R et al. Phenotypic and genotypic characterisation of ESBL, Amp-C and carbapenamase producing *Klebsiella pneumoniae* and Escherichia coli isolates. *Med Princ Pract* 2019; 28: 547-54.
19. Zhang C, Qiu S, Wang Y et al. Higher isolation of NDM-1 producing Acinetobacter baumannii from the sewage of the hospitals in Beijing. *PLoS ONE* 2013; 8: e64857.
20. Tanner WD, Atkinson RM, Goel RK et al. Horizontal transfer of the blaNDM-1 gene to *Pseudomonas aeruginosa* and Acinetobacter baumannii in biofilms. FEMS Microb Lett 2017; 364: fnx048.
21. Sharma MK, Bhatnagar T, Goel NK et al. Operationalization of surveillance of communicable diseases in Chandigarh. *J Commun Dis* 2005; 37: 197-202.
22. Kumari R, Nath B, Midha T et al. Morbidity profile and seasonal variation of diseases in a primary health center in Kanpur district: a tool for the health planners. *J Family Med Prim Care* 2012; 1: 86-91.
23. Ahmad A, Patel I, Mohanta G et al. Evaluation of Self-medication practices in rural area of town Sahaswan at Northern India. *Ann Med Health Sci Res* 2014; 4 (Suppl 2): S73-8.
24. Nafade V, Huddart S, Sulis G et al. Over-the-counter antibiotic dispensing by pharmacies: a standardized patient study in Udupi district, India. *BMJ Glob Health* 2019; 4: e001869.
25. Alvarez-Uria G, Zacharia S, Thomas D. High prescription of antimicrobials in a rural district hospital in India. *Pharm Pract* 2014; 12: 384.
26. Lutgring JD, Limbago BM. The problem of carbapenamase-producing carbapenem-resistant Enterobacteriaceae detection. *J Clin Microbiol* 2016; 54: 529-34.

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202104