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Antibiotic-resistant *Enterobacteriaceae* in healthy gut flora: A report from north Indian semiurban community

Madhu Gupta[1], Gunjan Didwal[3], Shruti Bansal[4], Kanica Kaushal[1], Nitya Batra[2], Vikas Gautam[2] & Pallab Ray[2]

[1]Department of Community Medicine, School of Public Health, Department of [2]Medical Microbiology, [3]Advanced Paediatrics Center, Postgraduate Institute of Medical Education & Research, Chandigarh, India & [4]Department of Pathology & Microbiology, University of Nebraska Medical Center, Omaha, NE, USA

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***Background & objectives*: Rampant use of β-lactam antibiotics in both community and hospitals has transformed the human healthy intestinal gut flora into a reservoir of antibiotic-resistant organisms. This study was conducted to find the faecal presence of antibiotic-resistant *Enterobacteriaceae* in faecal samples in the community in north India.**

***Methods*: In this prospective study, 207 stool samples were collected from apparently healthy individuals residing in a semiurban community in Chandigarh, India, from August to October, 2015. Isolates belonging to family *Enterobacteriaceae* were identified using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), and antibiotic susceptibility was determined using Clinical Laboratory Standard Institute disc diffusion method. Detection of extended spectrum β-lactamases (TEM, SHV, OXA-1, CTXM 1, CTXM 2, CTXM 9 and CTXM 8/25), carbapenemases (IMP, VIM and KPC) and New Delhi metallo-β-lactamase was done by multiplex PCR.**

***Results*: Of the population studied, 55.5 per cent were females and 60 per cent were illiterate or had only primary education; 43.4 per cent individuals were aged <20 yr. Overall, 70.5 per cent of stool samples had antibiotic-resistant isolates. Maximum resistance was seen for cephalosporins (60.4%) followed by fluoroquinolones (41.5%). The multidrug-resistant (MDR) isolates were 2.4 per cent. The most commonly detected genes were TEM, SHV, OXA-1, CTXM-1, CTXM-2, CTXM-9 and CTXM-8/25 β-lactamases. *Escherichia coli* was the most common resistant isolate, and TEM was the most common gene detected.**

***Interpretation & conclusions*: Overall, 70.5 per cent members of *Enterobacteriaceae* had antibiotic resistance in the community and 2.4 per cent were MDR. Higher resistance rates were observed for most commonly used drugs such as cephalosporins and fluoroquinolones. High rate of antibiotic-resistant *Enterobacteriaceae* in gut of healthy individuals points towards the need for active screening and prevention of dissemination.**

Key words Antimicrobial resistance - community health - *Enterobacteriaceae* - extended-spectrum β-lactamases - gut resistome - multidrug-resistant organisms

Inappropriate usage of antimicrobials has transformed the human healthy intestinal gut flora into a reservoir of antibiotic-resistant organisms, also called the gut resistome[1]. This selection pressure-driven

disruptive effect on the gut microbiome facilitates these organisms to behave as opportunistic pathogens. Mobile genetic elements-mediated resistance includes extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases and carbapenemases, enabling easy dissemination among bacteria[2,3]. The problem is intensified by increasing prevalence of these resistant bacteria in the community thereby increasing the risk of cross-transmission[4].

Rampant use of β-lactam antibiotics in both community and hospitals is a grave concern, especially in resource-limited countries like India[5]. As a consequence of ineffective antimicrobial stewardship in hospitals and lack of awareness in the community, an increase in resistance in pathogens among hospital-acquired infections escalates the problem compelling the usage of high-end drugs such as carbapenems and polymyxins[6]. To tackle the menace of growing antimicrobial resistance in the country, National Action Plan was recently framed for India[7]. The issue of resistant gut flora at the community level has yet not been addressed. The present study was planned to ascertain antimicrobial resistance among members of *Enterobacteriaceae* from the gut flora of healthy individuals at the community level.

Material & Methods

A prospective, observational study was conducted from August to October 2015 to look for the presence of drug-resistant *Enterobacteriaceae* in 207 stool samples collected randomly in sterile containers from 207 healthy people residing in Burail, a semiurban community in Chandigarh, India. All individuals with any condition with the potential to affect the endogenous flora such as diabetes mellitus, pregnancy, any immunosuppressive disorder, history of recent (within three months) consumption of antibiotics or history of hospitalization in the past one year were excluded. Written informed consent forms were collected from the individuals who participated in the study. The study was conducted after obtaining permission from the Institute Ethics Committee of Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India.

Processing of stool samples: One loopful of each stool sample was suspended in 4 ml sterile saline (0.9% NaCl). From this, 100 µl was spread on Muller Hinton agar (Difco, Becton Dickinson, Gurgaon, India) plates containing break point concentrations (µg/ml) of the following drugs (HiMedia, Mumbai, India): amikacin (16), gentamicin (4), cefotaxime (1), cefepime (8), ceftazidime (4), piperacillin-tazobactam (16), imipenem (1), meropenem (1) and ciprofloxacin (1). Control strains, ATCC *Escherichia coli* 25922 and *Pseudomonas aeruginosa* 27853, were used to ensure the MIC breakpoints. Identification of isolates was done using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltoniks, Bremen, Germany). Susceptibility pattern was determined using Clinical Laboratory Standard Institute disc diffusion method[8] and were characterized as resistant (R), sensitive (S) and intermediate (I). The percentage of resistant *Enterobacteriaceae* was calculated by dividing the number of resistant isolates by the total number of samples screened during that period. An isolate was defined as multidrug resistant (MDR) if it was resistant to two or more classes of antibiotics tested. Isolates resistant to one or more antibiotic class were stored in brain-heart infusion broth with 15 per cent glycerol at −80°C for further use.

Molecular detection of antibiotic resistance genes: Detection of TEM, SHV, OXA-1, CTXM 1, CTXM 2, CTXM 9, CTXM 8/25 (coding for ESBL), IMP, VIM, KPC (coding for carbapenemase) and New Delhi metallo-β-lactamase (NDM) was done by multiplex PCR. PCR conditions, primers and the programme used was as described by Dallenne *et al*[9]. Control strains harbouring these enzymes were used to validate each run. All the control strains were obtained from Christian Medical College, Vellore (TEM, SHV, CTXM2, CTXM9, CTXM 8/25, VIM, IMP and NDM from *E. coli* strains; OXA-1 from *P. aeruginosa*; KPC from *Klebsiella pneumoniae*) except CTX-M1 which was isolated from our *E. coli* strain and confirmed by sequencing. Percentage detection of genes in resistant isolates was calculated.

Statistical analysis: Statistical analysis was done using SPSS Version 16.0 (IBM Corp., NY, USA), Open Epi: Open Source Epidemiologic Statistics for Public Health Version 3.03a and Epi Info™ 7.1.5 (CDC, Atlanta, USA). The presence of resistant phenotypes and resistant genes are presented as percentages. Descriptive statistical analysis was done for the sociodemographic variables.

Results & Discussion

Of the 207 stool samples collected, 55.5 per cent (115/207) were females. More than 60 per cent of the

population was illiterate or had primary education; 43.4 per cent individuals were <21 yr, 45 per cent were between 21 and 40 yr, 8.6 per cent were between 41 and 60 yr and three per cent were between 61 and 80 yr. Three individuals had amoxicillin, amoxicillin-clavulanic acid and cefixime in the past one month and hence were excluded. One of the individuals, administered doripenem at the time of sample collection was also excluded. Ten individuals had a history of hospitalization in the past one year. Two individuals were pregnant at the time of sample collection, one had hypertension and one had a history of kidney stones.

From 207 stool samples, 146 isolates belonging to family *Enterobacteriaceae* were obtained from 139 individuals (7 individuals had 2 different species). All these were resistant to one or more antibiotic class. Overall, 70.5 per cent (146/207) isolates were antibiotic resistant. Results of antibiotic resistance among *E. coli*, *K. pneumoniae*, *E. cloacae* and *Morganella morganii* isolates are shown in Table I. Maximum resistance was seen for cephalosporins (60.4%) followed by fluoroquinolones (41.5%). One likely reason for this could be the irrational use of these antibiotics in the community as India has been reported to be the largest consumer of antimicrobials for human use in the world[10]. Other reasons could be self-prescription by the patients, lack of optimum knowledge about rational use of drugs including fixed-drug combinations among healthcare practitioners[11] and wide availability of illegitimate drugs[12]. Less resistance was observed for carbapenems (1.4%) and aminoglycosides (0.9%), as these antibiotics are mainly limited to hospital settings. The overall percentage of MDR isolates was 2.4 per cent. This reflected a low level of MDR in the community.

Among *Enterobacteriaceae*, *E. coli* harboured the maximum resistance in this study. A study done by Kothari *et al*[13], wherein gut colonization of exclusively breast-fed healthy neonates was studied, showed widespread resistance to ampicillin (87%) and cephalosporins. The authors hypothesized that the acquisition of resistance genes was through breastfeeding, contact with siblings and pets as well as horizontal transfer in the gut microbiota. In a rural-based study from Central India, schoolchildren aged 1-3 yr were seen to harbour MDR bacteria to a tune of 70 per cent, of which 57 per cent were ESBL producers[14]. Furthermore, their environment which included animals, drinking water, common source- and waste-water were studied for resistance and showed 29, 41, 30 and 30 per cent multidrug resistance, respectively[14]. In our study, although the source and factors were not studied, since the community studied was congested mostly inhabited by migrant workers, possible factors for acquisition of resistance could have been contamination of household surfaces, food and water due to overcrowding and poor sanitation. Studies show dissemination of resistant organisms through environmental contamination, especially in such settings[15,16].

Isolates resistant to either of the three cephalosporins (cefotaxime/ceftazidime/cefepime)

Table I. Presence of antibiotic resistance among members of *Enterobacteriaceae* isolated from 207 stool samples

Selected isolates (n)	% presence in stool samples	Cephalosporin resistance (%)	Ciprofloxacin resistance (%)	Carbapenem resistance (%)	Aminoglycoside resistance (%)	β-lactam resistance (%)	MDR (%)
Escherichia coli (131)	63.2	Cefotaxime: 54 Ceftazidime: 39 Cefepime: 42	40	Imipenem, meropenem: 1.4	Amikacin, gentamicin: 0.9	3	2.4
Klebsiella pneumoniae (11)	5.4	Cefotaxime: 5 Ceftazidime: 4 Cefepime: 4	0.5	0	0	0.5	0
Enterobacter cloacae (2)	0.9	Cefotaxime: 0.9 Ceftazidime: 0.9 Cefepime: 0.5	0.5	0	0	0	0
Enterobacter asburiae (1)	0.5	0	0	0	0	0	0
Morganella morganii (1)	0.5	0.5	0.5	0	0	0	0
Total (146)	70.5	60.4	41.5	1.4	0.9	3.5	2.4

MDR, multidrug-resistant

Table II. Percentage of extended spectrum beta-lactamase-encoding genes among cephalosporin-resistant isolates of family *Enterobacteriaceae*

Cephalosporin-resistant isolates	TEM n (%)	SHV n (%)	OXA n (%)	CTXM1 n (%)	CTXM2 n (%)	CTXM9 n (%)	CTXM8/25 n (%)
Escherichia coli (n=115)	50 (24)	9 (4.3)	35 (17)	30 (14.5)	4 (2)	4 (2)	10 (5)
Klebsiella pneumoniae (n=9)	1 (0.5)	1 (0.5)	2 (0.9)	0	0	0	0
Enterobacter cloacae (n=2)	1 (0.5)	1 (0.5)	0	0	0	0	0
Morganella morganii (n=1)	0	0	1 (0.5)	1 (0.5)	0	0	0
Total (127/207)	52 (25.1)	11 (5.3)	38 (18.3)	31 (14.9)	4 (1.9)	4 (1.9)	10 (4.8)

were tested for the presence of seven ESBL-encoding genes (TEM, SHV, OXA-1, CTXM-1, CTXM-2, CTXM-9 and CTXM-8/25) as shown in Table II. The carbapenem-resistant *E. coli* isolates[3] were screened for the presence of carbapenemase-encoding genes IMP, VIM and KPC. VIM was detected in only one *E. coli* isolate (0.5%). The other two did not have any carbapenemase-encoding genes tested. None of the selected antibiotic-resistant isolates demonstrated the presence of NDM gene.

ESBL-producing organisms in the gut behave as opportunistic pathogens and in suitable conditions can translocate the gut barrier and present as bacteremia. Recent reports of community-onset urinary tract infections have been associated with ESBL-producing *E. coli*[17]. In the present study, ESBL production was seen more in *E. coli* with TEM being the most common ESBL. Study by Kothari *et al*[13] showed ESBL, AmpC and coproduction of both in 20.6, 19.9 and 11.2 per cent isolates, respectively.

Worldwide, there has been an increase in the number of carbapenem-hydrolyzing enzymes[18,19]. VIM, an integrin-associated metallo-β-lactamase, was present in only one isolate in this study. This reflected the low level of circulation of carbapenemases in the community. However, more studies are needed to assess the faecal presence of carbapenam-resistant *Enterobacteriaceae* (CRE) in the community.

A major limitation of the study was that stool samples were collected from random individuals and the whole community population was not screened. Further, it would have been interesting to evaluate the contemporary presence of antibiotic resistance in food products consumed by this community and correlate the same with faecal microbiota.

Active surveillance is important to find the true magnitude of gut-resistant colonizers in the community. Screening will not only help assess the actual scenario but also help formulate an infection control policy for hospitals and hence emphasize on the judicious use of antimicrobials in the community. It will also help in taking preventive measures to stop the resistance gene pool from getting enriched with these resistant organisms.

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***Conflicts of Interest*:** None.

References

1. van Schaik W. The human gut resistome. *Philos Trans R Soc Lond B Biol Sci* 2015; *370* : 20140087.
2. Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi J Biol Sci* 2015; *22* : 90-101.
3. Jacoby GA. AmpC beta-lactamases. *Clin Microbiol Rev* 2009; *22* : 161-82.
4. van Duin D, Paterson DL. Multidrug-resistant bacteria in the community: Trends and lessons learned. *Infect Dis Clin North Am* 2016; *30* : 377-90.
5. Tamhankar AJ, Karnik SS, Stålsby Lundborg C. Determinants of antibiotic consumption - development of a model using partial least squares regression based on data from India. *Sci Rep* 2018; *8* : 6421.
6. Swaminathan S, Prasad J, Dhariwal AC, Guleria R, Misra MC, Malhotra R, *et al.* Strengthening infection prevention and control and systematic surveillance of healthcare associated infections in India. *BMJ* 2017; *358* : j3768.
7. Government of India. *National action plan on antimicrobial resistance (NAP-AMR) 2017 - 2021*. Government of India; 2017. Available from: *http://www.searo.who.int/india/topics/antimicrobial_resistance/nap_amr.pdf,* accessed on August 15, 2018.
8. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*; *23rd informational supplement.* CLSI Document M100-S23. Wayne, PA: CLSI; 2013.
9. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the

detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. *J Antimicrob Chemother* 2010; *65* : 490-5.

10. VanBoeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, *et al*. Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. *Lancet Infect Dis* 2014; *3099* : 1-9.
11. Goswami N, Gandhi A, Patel P, Dikshit R. An evaluation of knowledge, attitude and practices about prescribing fixed dose combinations among resident doctors. *Perspect Clin Res* 2013; *4* : 130-5.
12. Bate R, Tren R, Mooney L, Hess K, Mitra B, Debroy B. Pilot study of essential drug quality in two major cities in India. *PLoS One* 2009; *4* : e6003.
13. Kothari C, Gaind R, Singh LC, Sinha A, Kumari V, Arya S, *et al.* Community acquisition of β-lactamase producing *Enterobacteriaceae* in neonatal gut. *BMC Microbiol* 2013; *13* : 136.
14. Purohit MR, Chandran S, Shah H, Diwan V, Tamhankar AJ, Stålsby Lundborg C, *et al.* Antibiotic resistance in an Indian rural community: A 'one-health' observational study on commensal coliform from humans, animals, and water. *Int J Environ Res Public Health* 2017; *14* : pii: E386.
15. Stålsby Lundborg C, Diwan V, Pathak A, Purohit MR, Shah H, Sharma M, *et al.* Protocol: A 'one health' two year follow-up, mixed methods study on antibiotic resistance, focusing children under 5 and their environment in rural India. *BMC Public Health* 2015; *15* : 1321.
16. Wright J, Gundry S, Conroy RM. Household drinking water in developing countries: A systematic review of microbiological contamination between source and point-of-use. *Trop Med Int Health* 2004; *9* : 106-17.
17. Picozzi SC, Casellato S, Rossini M, Paola G, Tejada M, Costa E, *et al.* Extended-spectrum beta-lactamase-positive *Escherichia coli* causing complicated upper urinary tract infection: Urologist should act in time. *Urol Ann* 2014; *6* : 107-12.
18. Schwaber MJ, Carmeli Y. Carbapenem-resistant *Enterobacteriaceae*: A potential threat. *JAMA* 2008; *300* : 2911-3.
19. Rai S, Das D, Niranjan DK, Singh NP, Kaur IR. Carriage prevalence of carbapenem-resistant *Enterobacteriaceae* in stool samples: A surveillance study. *Australas Med J* 2014; *7* : 64-7.

For correspondence: Dr Pallab Ray, Department of Medical Microbiology, Postgraduate Institute of Medical Education & Research, Chandigarh 160 012, India
e-mail: drpallabray@gmail.com