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ANTIBIOTIC-RESISTANT *ENTEROBACTERIACEAE* IN HEALTHY GUT FLORA: A REPORT FROM COMMUNITY IN SOUTH PUNJAB

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Abstract:
Context and objectives*: the frequent use of β-lactam antibiotics in the population and in hospitals has transformed the healthy human intestinal flora into a reservoir of antibiotic-resistant organisms. This study was conducted to determine the presence of antibiotic-resistant Enterobacteriaceae in faeces in the northern Indian community.*
Methods: *In this prospective study, 207 stool samples of apparently healthy individuals were collected from August to October 2015 . Enterobacteriaceae isolates were identified using the matrix-assisted laser desorption ionization time, while air mass spectrometry (MALDI-TOF MS) and antibiotic susceptibility were determined using the Clinical disc diffusion method Laboratory Standard Institute. The spread spectrum β-lactamase detection (TEM, SHV, OXA-1, CTXM 1, CTXM 2, CTXM 9 and CTXM 8/25), carbapenemase (IMP, VIM and KPC) .*
Results: *of the interviewed population, 55.5 percent were women and 60 percent were illiterate or had only primary education; 43.4 percent of people were under 20 years old. Overall, 70.5 percent of stool samples had antibiotic-resistant isolates. Maximum resistance was observed for cephalosporins (60.4%), followed by fluoroquinolones (41.5%). The multiresistant (MDR) isolates were 2.4 percent. The most frequently detected genes were TEM, SHV, OXA-1, CTXM-1, CTXM-2, CTXM-9 and CTXM-8/25-β-lactamase. Escherichia coli was the most abundant resistant isolate and TEM was the most frequently detected gene.*
Interpretation and conclusions: *Overall, 70.5 percent of Enterobacteriaceae members had antibiotic resistance in the community and 2.4 percent were MDR. Higher resistance rates were observed for the most commonly used drugs such as cephalosporins and fluoroquinolones. A high rate of antibiotic-resistant Enterobacteriaceae in the intestines of healthy individuals indicates the need for active screening and prevention of spread.*
Key words *Antimicrobial resistance - community health - Enterobacteriaceae - extended-spectrum β-lactamases - gut resistome -multidrug-resistant organisms*

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INTRODUCTION:

Misuse of antibiotics has transformed the human healthy intestinal flora intoa reservoir of antibiotic-resistant organisms, also known as intestinal resistance1. This selective effect on the intestinal microbiota, caused by selective pressure, allows these organisms to behave as opportunistic pathogens. Resistance mediated by mobile genetic elements includes extended spectrum β-lactamases (ESBL), β-lactamases (AmpC) and carbapenemases, which facilitate their easy diffusion among bacteria2,3. The problem is compounded by the increasing prevalence of these resistant bacteria in the community and the associated risk of cross-transmission4.

Frequent use of β-lactam antibiotics in the population and in hospitals is a serious problem, especially in countries with limited resources such as India5. Due to the ineffective antimicrobial effect in hospitals and the lack of awareness in the population, an increase in pathogens in hospital-acquired infections aggravates the problem, forcing the use of high-quality drugs such as carbapenems and polymyxins6. The problem of resistant intestinal flora at the community level has not been addressed. The present study was designed to determine the antibiotic resistance of Enterobacteriaceae members from the intestinal flora of healthy individuals at the community level.

MATERIAL & METHODS:

From August to October 2015, a prospective observational study was conducted to study the presence of drug-resistant Enterobacteriaceae in 207 stool samples randomly collected in sterile containers from 207 healthy individuals residing in South punjab . All individuals with a medical condition that can affect the endogenous flora, such as diabetes mellitus, pregnancy, immunosuppressive disorders, history of antibiotics (within 3 months) or history of admission last year were excluded. Forms with written consent were collected by the people who participated in the study.

Processing of stool samples: one cycle of each stool sample was suspended in 4 ml of sterile saline solution (0.9% NaCl). From this 100 microliters was distributed to Mueller-Hinton agar plates (Difco, Becton Dickinson, Gurgaon, India) containing breakpoint concentrations (µg / ml) of the following drugs : amikacin (16), gentamicin (4), cefotaxime (1),Cefepim (8), ceftazidime (4), piperacillin-tazobactam(16), imipenem (1), meropenem (1) and ciprofloxacin (1). The control strains, ATCC Escherichia coli 25922 and Pseudomonas aeruginosa 27853, were used to ensure MIC breakpoints. Isolation was performed by matrix-assisted laser desorption ionization time of air mass spectrometry (MALDI-TOF MS) (Bruker Daltoniks, Bremen, Germany). The susceptibility model was determined using the Disc 8 diffusion method of the Clinical Laboratory Standard Institute and 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. One isolate was called multi-drug resistant (MDR) when resistant to two or more classes of tested antibiotics. Isolates resistant to one or more classes of antibiotics were stored in a broth for cerebral heart infusion containing 15% glycerol -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 (ESBL coding), IMP,VIM, KPC (carbapenemase encoding) and metallo-β-lactamase (NDM) were performed by PCR multiplex. The PCR conditions, the primers and the program used were described by Dallenne et al9. The control strains containing these enzymes were used to validate each series. All the control strains were obtained from the Nishter Medical College (TEM, SHV, CTXM2, CTXM9, CTXM 8/25, VIM, IMP and NDM) from E. coli variety; OXA-1 of P. aeruginosa; KPC from Klebsiella pneumoniae) except for CTX-M1, which was isolated from our E. coli strain and confirmed by sequencing. The percentage detection of genes in resistant isolates was calculated.

Statistical analysis: statistical analysis was performed with SPSS version 16.0 (IBM Corp., NY, USA), Open Epi: open source epidemiological statistics for public health version 3.03a and Epi Info ™ 7.1.5 (CDC, Atlanta, USA), The presence of resistant phenotypes and resistant genes is expressed as a percentage. A descriptive statistical analysis was performed for the socio-demographic variables.

RESULTS & DISCUSSION:

Of the 207 stool samples collected, 55.5 percent (115/207) were females. More than 60 percent of the population was illiterate or had primary education.

43.4% of the population was <21 years, 45% were between 21 and 40 years, 8.6% were between 41 and 60 years and three percent were between 61 and 80 years old. Three people had amoxicillin, amoxicillin-clavulanic acid and cefixime in the last month and were therefore excluded. One of the people receiving doripenem at the time of sampling was also excluded. Ten people had a medical history in the last year.

Two people were pregnant at the time of sampling, one with hypertension and one with a history of kidney stones.

From 207 stool samples, 146 isolates of the Enterobacteriaceae family were obtained from 139 individuals (7 individuals had 2 different species). All of these were resistant to one or more classes of antibiotics. Overall, 70.5 percent (146/207) of the isolates were resistant to antibiotics. Results of antibiotic resistance in E. coli,

Isolates of K. pneumoniae, E. cloacae and Morganella morganii are shown in Table I. Maximum resistance was observed for cephalosporins (60.4%) followed by fluoroquinolones (41.5%). A probable reason for this could be the irrational use of these antibiotics in the community, as Pakistan is considered the largest consumer of antimicrobial agents for human use in the world10.

Other reasons could include patients' self-prescribing, lack of optimal knowledge on the rational use of drugs, including combinations of fixed drugs among health professionals11 and the wide availability of illegitimate drugs12. Carbapenems (1.4%) and aminoglycosides (0.9%) showed less resistance. because these antibiotics are mainly limited to hospital settings. The total percentage of MDR isolates was 2.4 percent. This reflected a low level of MDR in the EU Community.

Among the Enterobacteriaceae, E. coli showed the highest resistance in this study. A study conducted by Kothari et al.13, which studied intestinal colonization of healthy and exclusively breastfed newborns, showed widespread resistance to ampicillin (87%) and cephalosporins. The authors hypothesized that the acquisition of resistance genes occurred through breastfeeding, contact with siblings and domestic animals and horizontal transfer to the intestinal microbiota. A rural study in central punjab found that 70% of pupils aged 1 to 3 were infected with MDR, of which 57% were ESBL producers14. Furthermore, their environment, which included animals, drinking water, common sources and effluents, was tested for resistance and showed a multi-drug resistance of 29, 41, 30 and 30%, respectively14. In our study, the source and factors have not been studied. Since the interviewed community was mainly inhabited by migrant workers, the possible factors for the acquisition of resistance could have been the contamination of domestic surfaces, food and water due to overcrowding and lack of toilets. Studies show the spread of resistant organisms through environmental contamination, especially in such environments15,16.

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

Selected isolates in (n) samples	% presence in stool	Cephalosporin resistance (%)	Ciprofloxacin resistance (%)	Carbapenem resistance (%)	minoglycoside 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)

Isolates resistant to any of the three cephalosporins (cefotaxime / ceftazidime / cefepime) were tested for the presence of seven ESBL coding genes (TEM, SHV, OXA-1, CTXM-1, CTXM-2,CTXM-9 and CTXM-8/25) as shown in Table II. Carbapenem-resistant E. coli isolates were screened for the presence of genes encoding carbapenemase IMP, VIM and KPC. VIM was recognized only in one Isolated E. coli (0.5%). The other two had not tested the carbapenemase coding genes. None of the selected antibiotic-resistant isolates showed the presence of the NDM gene.

Intestinal ESBL producing organisms behave as opportunistic pathogens and, under appropriate conditions, can move the intestinal barrier and be present as bacteremia. Recent reports of urinary tract infections occurring in the community have been linked to the appearance of ESBL E. coli17. In the present study, increased ESBL production was observed in E. coli, with TEM being the most abundant ESBL. A study by Kothari et al13 showed ESBL, AmpC and co-production respectively of 20.6, 19.9 and 11.2 percent isolates. Globally, the number of carbapenem hydrolysing enzymes has increased18,19. VIM, a integrin-associated metal-β-lactamase, was present in only one isolate in this study. This reflected the low prevalence of carbapenemase in the community. However, further studies are needed to study the presence of carbapenam-resistant Enterobacteriaceae (CRE) in stool in the community.

CONCLUSION:

A major limitation of the study was that stool samples were taken from random individuals and the entire population of the community was not screened. Furthermore, it would have been interesting to assess the current presence of antimicrobial resistance in foods consumed by this community and to relate them to the stool microbiota. Active monitoring is important to find the truth Size of intestinal resistant colonizers in the community. The screening will help not only to assess the real scenario, but also to formulate a policy of controlling hospital infections, highlighting the prudent use of antibiotics in the community. It also helps to take preventive measures to prevent the resistance gene pool from being enriched with these resistant organisms.

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