Molecular Insights into Antimicrobial Resistance Traits of Multi Drug-Resistant Commensal Human Gut Microbiota

Satyabrata Bag, Tarini Shankar Ghosh, Jyoti Verma , Ojasvi Mehta,
Thandavarayan Ramamurthy, Bhabatosh Das

Centre for Human Microbial Ecology, Translational Health Science and Technology Institute, Faridabad, India

INTRODUCTION

Antimicrobial resistance (AMR) is a serious concern for public health authorities at global level. Irrational use of antibiotics in healthcare and livestock provides evolutionary advantage to the resistant variants to dominate the ecosystem. Resistance phenotype is very common in enteric bacteria. The most common mechanisms of resistance to the antimicrobials are enzymatic modifications to the antimicrobials or their target molecules. AMR determinants are generally linked with mobile genetic elements and could rapidly disseminate to the bacterial pathogens through horizontal gene transfer. Prevalence of AMR genes among pathogenic bacteria is widely studied but the resistance profile and the genetic traits that encode resistance to the commensal microbiota living in the gut of healthy humans are not well-studied.

In the present study, we have isolated five dominant commensal anaerobic bacteria from the gut of healthy Indians and revealed the genotype and phenotypes of antimicrobial resistance of all the isolates. Antibiogram profile of all the five bacteria was determined. Our study revealed that all the five enteric commensals are multidrug resistant. The genes encoding antibiotic resistance are physically linked with mobile genetic elements and could disseminate vertically to the progeny and laterally to the distantly related microbial species. Hence commensals microbiota could be a potential source of resistance genes to the enteric pathogens.

METHODS

- ***Isolation of commensal human gut microbiota***

Five dominant commensal bacteria (*Faecalibacterium prausnitzii* Indica, *Megasphaera elsdenii* Indica, *Prevotella copri* Indica, *Collinsella aerofaciens* Indica, *Bifidobacterium longum* Indica) were isolated from fresh faecal samples of healthy subjects using an anaerobic workstation (**Whitley DG250**) filled with 80 % N_2, 10% CO_2 and 10 % H_2 and their identity was confirmed by 16S rRNA gene sequencing.

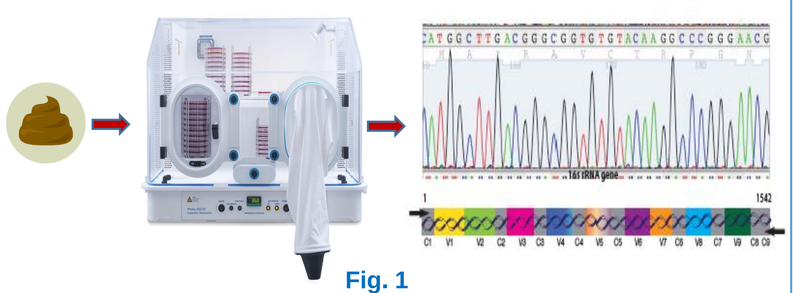

Fig. 1

- ***Antimicrobial susceptibility testing***

Minimal inhibitory concentration of 21 different antibiotics (Table 1) for all the five gut commensals were determined using commercially available E-test strip.

Fig. 2

- ***Whole genome sequencing***

Whole genome sequencing (WGS) of all the five commensal gut bacteria was performed either in Oxford nanopore, Illumina or GS FLX+ platforms and their genomes were annotated by Rapid Annotations using Subsystems Technology (RAST) server.

RESULTS

- ***Gut microbiome of healthy Indians are reservoir of several antibiotic resistance genes***

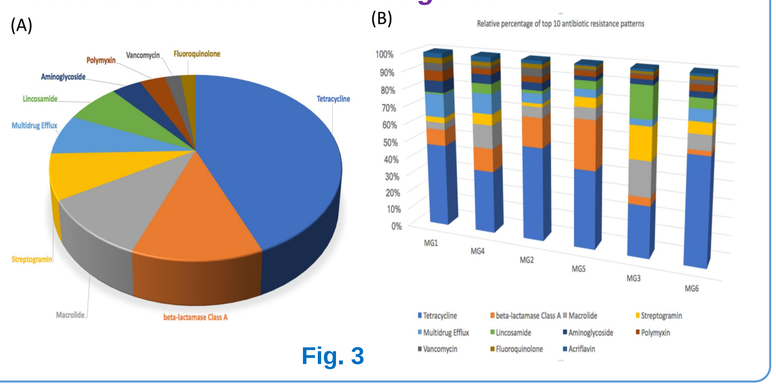

Fig. 3

- ***Most Dominant Microbiota in the Gut of Healthy Indians (n=84)***

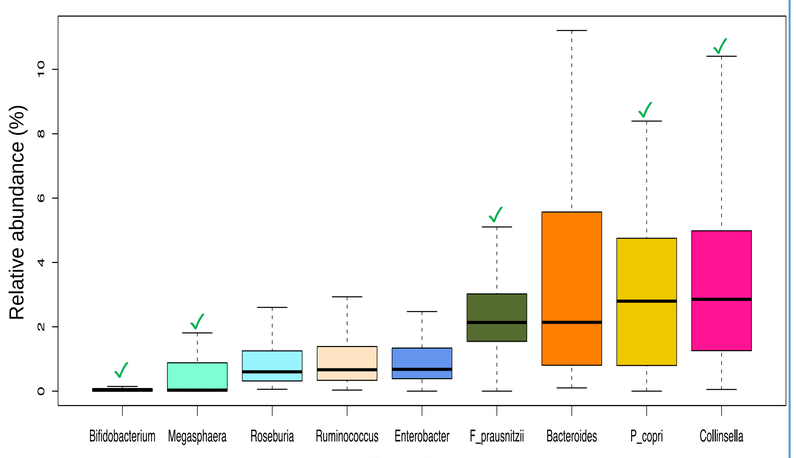

Fig. 4

- ***Dominant commensal gut bacteria are multidrug resistant*** (MIC values in µg/ml are shown in brackets)

Table 1

	M. elsdenii	*F. prausnitzii*	*P. copri*	*B. longum*	*C. aerofaciens*
Ampicillin	R (8)	R (2)	R (>256)	S (0.19)	S (0.38)
Amoxicillin	S (0.023)	S (0.5)	S (0.5)	S (0.125)	S (0.25)
Aztreonam	R (16)	R (>256)	R (24)	R (>256)	R (128)
Cefotaxime	S (4)	R(>32)	R(>32)	S (0.25)	S (1)
Ceftriaxone	S (6)	S (4)	R (>256)	S (0.75)	S (2)
Ciprofloxacin	S (0.047)	R (>32)	R (24)	R (>32)	R (>32)
Clindamycin	S (0.032)	S (<0.016)	S (<0.016)	S (0.016)	S (<0.016)
Colistin	S (0.094)	R (>256)	S (0.023)	R (>256)	R (>256)
Erythromycin	S (1)	R (64)	S (0.064)	S (1.5)	S (2)
Gentamycin	R (24)	S (4)	R (96)	S (3)	S (1.5)
Imipenem	S (0.008)	S (0.19)	S (0.064)	S (0.064)	S (0.032)
Kanamycin	R (32)	R (32)	R (>256)	R (>256)	R (32)
Linezolid	S (0.38)	S (2)	S (1)	S (0.38)	S (0.75)
Meropenem	S (<0.002)	S (0.19)	S (0.047)	S (0.032)	S (0.125)
Nalidixic acid	S (12)	R (192)	R (>256)	R (>256)	R (>256)
Piperacillin	R (>256)	S (1)	I (64)	S (0.25)	S (0.75)
Polymixin	S (0.19)	R (192)	S (<0.064)	R (128)	R (512)
Rifampicin	S (2)	S (0.25)	S (0.032)	S (0.25)	S (0.004)
Sulfamethoxazole	R (256)	R (>1024)	R (>1024)	R (>1024)	R (64)
Tetracycline	I (12)	R (>256)	S (0.064)	R (>256)	R (64)
SXT	R (3)	R (24)	R (1.5)	R (>32)	R (3)

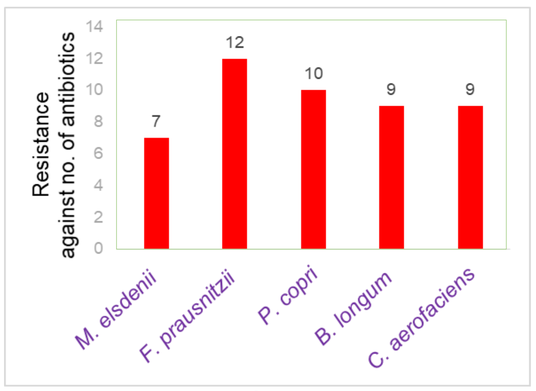

Fig. 5

- ***WGS revealed gut commensals are enriched with multiple AMR genes***

Table 2

	M. elsdenii	*F. prausnitzii*	*P. copri*	*B. longum*	*C. aerofaciens*
Genome Size (Mb)	2.4	2.9	3.9	2.4	2.3
GC content (%)	53.2	56.9	45.4	60.0	60.1
No. of coding sequences	2184	2707	3128	2006	1895
Resistance to Antibiotics & toxic compounds	39	51	44	29	25

- ***AMR genes are often physically linked with mobile genetic elements***

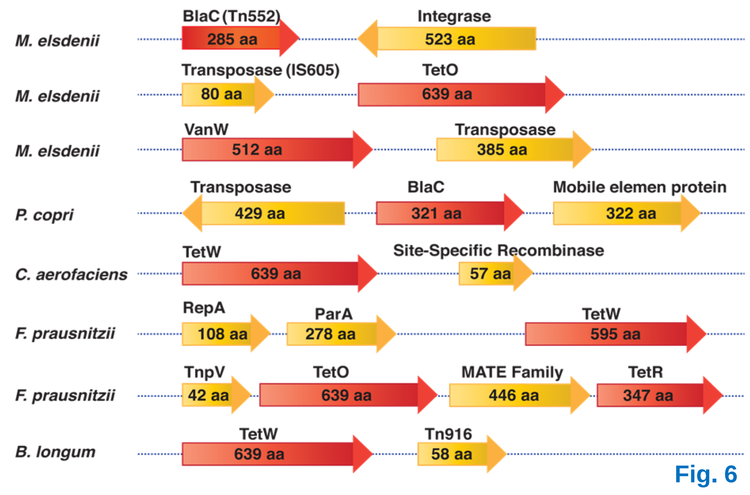

Fig. 6

- ***AMR genes are prevalent both in commensal and pathogenic bacteria***

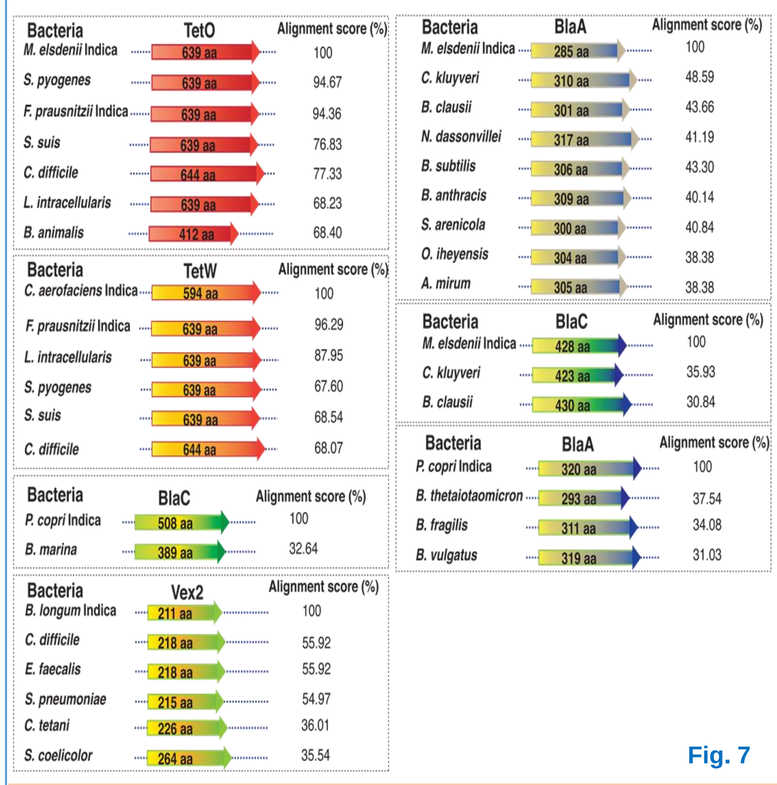

Fig. 7

DISCUSSION

- Commensal human gut microbiota could be a potential source of AMR genes to the enteric pathogens.
- We proposed a model of resistance traits dissemination among commensals, opportunistic pathogens and pathogenic bacterial species.

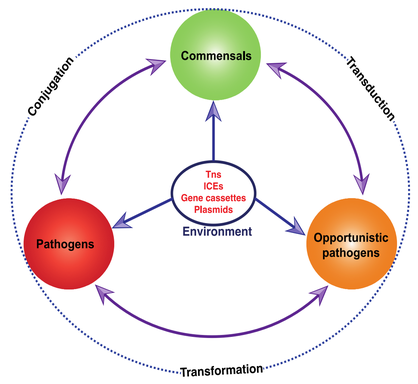

Fig. 8

- This study will be helpful to understand the holistic picture of the prevalence of AMR genes in commensals and pathogens and help in antibiotic therapy and infectious disease management.

CONCLUSION

- Genomes of commensal bacteria encode several AMR functions.
- AMR genes are often linked with mobile genetic elements.
- Dominant gut commensals studied here are multi drug resistant.
- Gut microbiome of healthy Indians are reservoir of several antibiotic resistance genes.

ACKNOWLEDGEMENT

Authors acknowledge Mr Dhiren, Mr Pawan and Ms Mayanka for technical support; Department of Biotechnology, Govt. of India for funding and Don Whitley Scientific, UK for travel grant.