# Genome Assessment of Antibiotic Resistance Genes in Probiotic Bacteria and a Literature Review

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# Abstract

Having various clinical applications, probiotic bacteria are currently used in the diet. There are reports of antibiotic resistance genes (*ARGs*) in these bacteria that can be transferred to other microflora and pathogenic bacteria. The aim of the study is to examine whole-genome sequence analysis in bacteria with probiotic properties. Moreover, this study follows existing issues about the importance and presence of *ARGs* in these bacteria the dangers of which may affect human health in the years to come. In the present study, 126 complete probiotic bacterial genomes were collected and analysed for *ARGs*. The results of the study shows there are various antibiotic resistant genes of in these bacteria some of which can be transmitted to other bacteria. We propose microorganisms be applied as a probiotic element in various types of products, antibiogram be conducted for a large number of antibiotics and analysis of complete genome sequence for ARGs prediction.


# Introduction

The Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO) (1, 2) and, the International Scientific Association for Probiotics and Prebiotics (ISAPP)(3) describe microorganisms with probiotic properties are described as live microbes the appropriate use of which can be beneficial for host health. European Food Safety Authority (EFSA) or the U.S. Food and Drug Administration, however, do not agree with this definition (4, 5). Some of the various methods such as phenotypic properties and molecular techniques have been used for probiotic bacteria identification at the genus and species level (2, 3). Health benefits of probiotic micro-organisms have been reported in the literature and some bacteria genera have probiotic properties include: *Aeromonas* (6), *Akkermansia* (7), *Arthrobacter (6), Bacillus* (3), *Bifidobacterium* (3), *Enterobacter* (6), *Enterococcus (3)*, *Escherichia* (3), *Lactobacillus* (3), *Leuconostoc* (3), *Lactococcus* (8), *Micrococcus* (6), *Paenibacillus* (9), *Pediococcus (3), Propionibacterium* (10), *Rhodopseudomonas*, *Roseobacter*, *Streptococcus* (3) *Shewanella* (11) and, etc. There are the various application of probiotics species including cancer prevention (12), treatment of diarrhea such as rotavirus diarrhea, anti-hypersensitivity to food (13), immunomodulatory effects (14), antagonistic activity (14), effect on gastrointestinal diseases (15), prevention of gingivitis (15), vitamin production properties (16), antimicrobial properties (16) and, etc. Antibiotic resistance mechanisms in bacteria are include 1- intrinsic, naturally occurring with four mechanisms such as beta-lactamases, efflux pumps, alteration in the antibacterial target site, metabolic pathway inhibition, and 2- acquired, occuring with mutations or acquisition of foreign DNA (14, 17). One of the important ways of gene transfer among bacteria is horizontal gene transfer (HGT)(18) with different mechanisms including conjugation, transduction and, transformation (19). LAB and bifidobacteria have acquired numerous ARGs by conjugation mechanism (20). Conjugative plasmids have been reported in *Bifidobacterium*, *Lactobacillus*, *lactococci*, *Leuconostoc*, *pediococci* and, *Streptococcus thermophilus* (20-23). One of the safety aspects for probiotics is that they fail to carry any transferable antibiotic resistant genes (14). Strains of bacteria harbouring jumping *genes or* TEs carrying ARGs are not suitable and acceptable as probiotic (14). A broad range of antibiotic resistance reported in Lactobacilli (24) are not often transmissible (14).

Resistance to vancomycin has been reported in *Lactobacillus*, *Leuconostoc*, *Pediococcus* and, *Enterococcus* (25, 26). Also, plasmid-dependent antibiotic resistance is uncommon among *Lactobacillus* species (14). Antibiotic resistance to glycopeptides such as vancomycin may be encoded by genomic or extra-genomic DNA. Often, these resistant genes are located within transposons (jumping genes) such as Tn*1546* or other *transposable* elements (TEs), having the capacity to serve as a reservoir for the transmission of resistant genes to other bacteria (27-29). Probiotic strains may naturally contain ARGs or harbor transferable ARGs and should not be used for animal and human consumption (30). Therefore, due to the presence and transmission of such genes to other organisms, they should be monitored carefully in the case of commercial use (31). The aim of the this study was to survey the ARGs based on the whole genome sequence analysis in the probiotic bacteria and to answers four questions concerning these bacteria: I- Are there ARGs in these bacteria? II- Are these antibiotic resistant genes transferable to other bacteria? III- Are the present ARGs in these bacteria always bad? IIII- How many of them are on transferable elements or chromosome?

# Methods

Predicated on what was searched in Google Scholar, PubMed, Scoupus, Embase and, Science Direct databases, a total number of 126 probiotic bacteria exist in various genera, including *Akkermansia*, *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Carnobacterium*, *Enterococcus*, *Escherichia*, *Lactococcus*, *Leuconostoc*, *Paenibacillus*, *Pediococcus*, *Propionibacterium*, *Streptococcus*, *Shewanella* and, *Weissella*. Their genomes were downloaded from National Center for Biotechnology Information (NCBI) microbial genomes repository for analysis. The whole genome sequences of probiotic strains listed in Table 1 and contigs and scaffolds were discarded. Whole genome sequences were analysed for the presence of ARGs using the antibiotic resistance gene-annotation (ARG-ANNOT) (32) and Resfinder (33). Expectation value (E) with 1.0E-100 and matrix with BLOSUM62 was used for ARG-ANNOT. The last update was July 2019 for ARG-ANNOT. The threshold for %ID, minimum length and, database update for Resfinder were 90%, 60% and, 01-Oct-2019 respectively.

# Results

Whole-genome sequences of one hundred twenty-six of probiotic bacteria were analysed for ARGs with ARG-ANNOT and Resfinder. The data are shown in Table 1. ARGs have been identified in the *Bacillus subtilis* subsp. *spizizenii*, *Bacillus subtilis* subsp. *inaquosorum*, *Bacillus amyloliquefaciens*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus subtilis*, *Bacillus* sp., *Bifidobacterium animalis*, *Bifidobacterium animalis* subsp *lactis*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Enterococcus faecalis*, *Enterococcus durans*, *Escherichia coli*, *Lactobacillus heilongjiangensis*, *Lactobacillus reuteri* and, *Shewanella* sp. ARG-ANNOT identify 25 ARGs in 30 strains include *cfr*(B), *ant4*-Ib, *erm*(34), *bcl-1*, *cat, msr*(D), *mef*(A), *Lmr*(B), *bsu-1*, *bla-1*, *bla2*, *fosBx1*, *tetW*, *tetO*, *lnu*(C), *lsa*(A), *mph*(D), *dfrE*, Penicillin_Binding_Protein, *ampC2*, *ampH*, *vat*(E), *tetL*, *dfrG* and, *dfrK*. Resfinder identified 18 ARGs in 27 strains include *aadK*, *blaOXA*-548, *mph*(K), *fosB1*, *ant4*-Ib, *erm*(34), *cat*, *msr*(D), *mef*(A), *tetW*, *tetO*, *lnu*(C),

*lsa*(A), *aac*(6')-Iih, *vat*(E), *tetL*, *mdf*(A) and, *dfrG.* Analysis of the results revealed the prevalence of *tetW* gene being more than other ARGs; the gene was found in *Bifidobacterium* and *Lactobacillus*. The data analysis of the results are shown in Table 2, 3. Genes *lsa*(A) and *mph*(D) were identified in *E. faecalis* Symbioflor 1 with ARG-ANNOT but Resfinder just identified *lsa*(A) gene. Resfinder identified one beta-lactamase gene (*blaOXA*-548 gene) but ARG-ANNOT identified 6 beta-lactamase [*bcl-1*, *bsu-1*, *bla-1*, *bla2*, *ampC2*, *ampH* genes] genes.

# Discussion

To date, due to the emergence of antibiotic resistance, the safety of probiotic strains is essential for use in humans and animals (17). Bacterial strains used in foods should not carry any transferable ARGs (34). It has been proposed the ARGs reservoir in advantageous bacterial populations could presumably play a role in the transfer of resistance genes to pathogenic and opportunistic bacteria (35, 36). Probiotic strains may bear the potential to transmit ARGs into pathogenic or commensal bacteria (microbiota) (17). There are concerns about antibiotic resistance in the lactic acid bacteria (LAB) present in our diet. In addition, LAB acquires ARGs from environmental bacteria and the genes might be transferred to the endogenous microbiota (17). Antibiotic resistance is not always transferable in Lactic acid bacteria (LAB) but genes carried on plasmid may transfer to other pathogenic bacteria (37). Some bacteria, such as *Carnobacterium* spp., *Enterococcus* spp., *Lactococcus* spp., *Leuconostoc* spp. and, *Streptococcus thermophilus* found in various food products and supplements contain ARGs and are capable of transferring genes to others microorganisms (38, 39). One of the reasons that *Bacillus* strains are not suitable as probiotics is their potential to transfer (16). In some LABs, there is a conjugation system and the system is presumably capable to transmit ARGs (40). *Bifidobacterium* species are intrinsically resistant to gentamycin, kanamycin, metronidazole, nalidixic acid, neomycin, polymyxin B and, streptomycin (14). Werner et al. reported that there is no transfer of *vanA*-containing plasmids among *Enterococcus* species, *Lactococcus* species and, *Bifidobacterium* species (29). Chang et al. reported *vanX* gene in *Lactobacillus plantarum* in 2009 (41). Moreover, a study by Mater et al. reported transfer *vanA* gene from enterococci to a probiotic *Lactobacillus* acidophilus strain in mice (42). In our study, vancomycin resistance genes were identified in none of the strains. Additionally, Penicillin_Binding_Protein, *ampC2* and, *ampH* genes were identified in *E. coli* Nissle 1917. Resistance gene *ermB* has also been reported in *L. plantarum* DG507 (43), *Lactobacillus lactis* (44), *Lactobacillus crispatus* (45), *L. reuteri* (46), *L. reuteri* CH2-2, *Lactobacillus curvatus*, *Lactobacillus sakei*, *Lactobacillus paracasei*, *Lactobacillus brevis*, *Lactobacillus salivarius* CHS1-E,CH7-1E (46, 47), *Lactobacillus vaginalis* NWL35 (48), *L. salivarius* BFE 7441(49) in commercial probiotic products. The *bla*OXA-548 gene (this gene is in class D beta-lactamase) is variant of the *bla*OXA-48 gene (carried by plasmid)(50, 51) and has been reported in *Shewanella hafniensis* strain Sh29 (51). In literature, there is no information about its transmission in other bacteria although Ceccarelli et al. and Zou et al. reported variants of *bla*OXA-48-like gene in *Shewanella* species (52, 53)**.** The most common tetracycline resistance genes is *tet*(W) which first reported in *Butyrivibrio fibrisolvens* (54, 55) and this gene is carried on a conjugative transposon TnB1230 (56) or mobile genetic element (mob) gene (57). Moreover,Kazimierczak et al. reported *tet*(W) gene

transfer at low frequencies between *Bifidobacterium longum* F8 and *Bifidobacterium adolescentis* L2-32R in laboratory tests (54). *tet*(L) gene has also been reported in *Lactobacillus sakei* Rits 9 carried on a plasmid (58). Wilcks et al. reported *tet*(L) gene being more readily transferable than *tet*(M) gene in *Enterococcus faecalis* isolated from raw food (59). Also, Toomey et al. in 2010 reported that *tet*(M) gene can transferre *L. plantarum* to *Lactococcus lactis* BU-2-60 and to *Enterococcus faecalis* JH2-2 (60). *tet*(M) gene is carried on Tn*916* family and Tn6086 (61, 62). HGT can occur in foodborne *Lactobacillus* and *Lactococcus* species including *Lactobacillus brevis*, *L. paracasei*, *Lactobacillus plantarum*, *Lactococcus lactis*, *Lactococcus garviae* for *tet*(M) gene (62). HGT *erm*(B) gene has also been reported in *Lactobacillus fermentum*, *Lactobacillus plantarum* and, *Lactobacillus salivarius* (62). Rosander et al. reported *tet*(W) and *lnu*(A)[ lincosamide resistance gene] genes carried on plasmids in *Lactobacillus reuteri ATCC 55730* (63). Additionally, Jacobsen et al. reported horizontal transfer of *tet*(M) and *erm*(B) genes from *Lactobacillus plantarum* to *E. faecalis* JH2-2 carried on plasmid (64). Also a laboratory study reported that *Lactobacillus reuteri* 12002 harboring *erm*(B) gene can be transferred to enterococci (37). Some of transposons such as TnFO1 (identified in Enterococcus faecalis strain FO1 and isolated from cheese) are transferred by the conjugation mechanism of *Enterococcus faecalis* into other organisms including *Lactococcus lactis* subsp.*lactis* biovar.*diacetylactis*, *Leuconostoc mesenteroides* and, etc.*(65).* Nawas et al. reported *erm*(B) and *tet*(S) genes in *Lactobacillus* and *Streptococcus thermophiles* in Chinese fermented foods (48). In another study, *tet*(M) and *erm*(B) genes were reported in *Lactobacillus gasseri*, *Lactobacillus casei*, *L. acidophilus* (66) and, *L. paracasei* (67). Pan et al. (2011) also reported *tet*(M), *erm*(B) and, *aphA3* genes in *L. plantarum*, *Lactobacillus fermentum*, *Lactobacillus helveticus* and, *Enterococcus faecium* in Chinese fermented foods-pickles and, sausages (68). Ledina et al. detected *tet*(M) gene in *L. paracasei* isolated from Serbian raw milk cheeses (69). In the study by Zonenschain et al. *erm*(B) and *erm*(C) genes in *L. plantarum* were reported in fermented dry sausage (46). Furthermore, resistance genes *erm*(B), *tet*(M) and, *tet*(S) were reported in *Lactobacillus fermentum* NWL24, *L. salivarius* NWL33, *L. sakei* NWL22, *L. brevis* NWL59, *L. brevis*, *Lactobacillus kefiri* (48). *erm*(B) and *msr*(C) genes were also reported in *E. faecium*, *E. durans* and, *Pediococcus pentosaceus* in traditional fermented foods and curd (47). In our study, none of the strains possessed the *ermB*, *tet*(S), *aph A3*, *erm*(C), *tet*(S) and, *tet*(M) genes but *tetO* and *msr*(D) genes were identified in *B. breve* BR03 and *B. coagulans* DSM 2314 respectively. There are any ARGs in *S. thermophilus* APC151, CNRZ1066, KLDS 3.1003 and, LMD-9. Tetracycline resistance genes such as *tet*(O), *tet*(Q), *tet*(M), *tet*(S), *tet*(W), *tet*(36), *tet*(Z), *tet*(O/W/32/O/W/O), *tet*(W/O), *tet*(K), *tet*(L) and aminoglycoside resistance genes such as *aph*(3)-IIIa, *aac*(6), *aph*(2), *ant*(6), *aaa*(60), *aph*(200), *lnu*(A) have also been reported in *Lactobacillus* (17, 70). In literature, *erm*(X), *tet*(L), *tet*(M), *tet*(O), *tet*(W), *tet*(O/W) and, *tet*(W/32/O) genes have been reported in *Bifidobacterium* (71). In another study, Perreten et al. reported *str*, *tet*(S) and, *cat* genes in *Lactococcus lactis* strain K214 of raw milk soft cheese (72). In the current study, 3 whole genome sequences of *Lactococcus lactis* subsp. *lactis* with various strains were analysed in which no ARGs was identified. Tetracycline resistance gene *tet*(W) was also reported in *B. longum* B36 (73) and *Bifidobacterium* spp. (20, 74). In our study, this gene was identified in *B. animalis* BL3, RH; *Bifidobacterium animalis* subsp *lactis* I-2494, AD011, B420, BB-12, Bi-07, Bl-04, BLC1, HN019, V9, DSM10140; *B. longum* BORI, BBMN68, DJO10A; *Bifidobacterium longum* subsp. *longum* JDM301; and *L. reuteri* ZLR003. Further, Kastner et al.

reported *tet*(W) gene in *L. reuteri* SD 2112 with PCR amplification (75) but we not identified this gene in the whole genome sequence (NC_015697) of this bacterium with ARG-ANNOT and Resfinder. Tetracycline resistance genes *tet*(M) and *tet*(L) were reported in *L. sakei* Rits 9 (58) but merely *tet*(L) gene was identified in *L. reuteri* ZLR003 in our study. Lincomycin resistance gene *lnu*(A) was detected in *L. reuteri* ATCC 55730 (63). In our study, *lnu*(C) gene was found in *B. longum* BBMN68 but the gene not identified with Resfinder. Gene *rpsL* was reported in *B. breve* of yakult (76); however, it could not be found in our study. *dfrA* gene was reported in *S. thermophilus* and *L. lactis* (41) but in our the study, *dfrG* and *dfrK* genes were identified in *L. reuteri* ZLR003 and *dfrE* gene in *E. faecalis* Symbioflor 1. In Resfinder only *dfrG* gene was identified in *L. reuteri* ZLR003. Resistance genes *erm*(B), *tet*(W) and, *tet*(M) were identified in *Lactobacillus*, *Pediococcus* and, *Lactococcus* (77) but the genes could not be found in *Lactococcus lactis* subsp. *lactis* NCDO 2118 and *Pediococcus pentosaceus* LP28. Genes *tet*(M), *ant6* and, *aph* 3'-IIIa were also reported in *S. thermophilus* and *Lactobacillus delbruekii* ssp. *Bulgaric* in Chinese yoghurts (78). Jaimee et al. reported presence of aminoglycoside-resistance genes including *aac*(6')Ie, *aph*(2")Ia and, *aph*(3')IIIa in *Lactobacillus plantaram* (18). Ouoba et al. detected aminoglycoside resistance genes including *aph*(3')-III, *aadA* , in *Lactobacillus casei* and *L. paracasei* (79). In our study, *ant4*-Ib was identified in *B. clausii* KSM-K16. Bozdogan et al. and Girlich et al. reported *aaD2, erm*(34), *bcl*-1 and, *cat*(Bcl) genes in *Bacillus* spp. (71) and we identified *erm*(34), *cat* and, *bcl*-1 genes in *B. clausii* KSM-K16. Girlich et al. (2007) reported *bcl*-1 gene in *B. clausii* NR (80). Gene *bcl*-1 was not identified with Resfinder in this bacterium. Genes *erm*(B), *tet*(M), *erm*(LF), *vat*(E-1), *mdt*(A), *cat*, *str* were reported in *L. gasseri* dairy products (20). In the current study, *vat*(E) gene was identified in *L. heilongjiangensis* DSM 28069. Chloramphenicol acetyl transferases (*cat* genes) were also reported in *L. acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus johnsonii*, *L. reuteri* and, *L. plantarum* (17). In the current study, phosfomycin resistance gene (*fosBx1*) was identified in *Bacillus* sp. DU-106 and *Lmr*(B) gene was identified in *Bacillus subtilis* PY79, *Bacillus subtilis* TO-A JPC, *Bacillus subtilis* subsp. *inaquosorum* DE111 and, *Bacillus subtilis* subsp. *spizizenii* ATCC 6633. Murata et al. reported *Lmr*(B) gene in *Bacillus subtilis* strain 168 (81). Liu et al. reported *msrC* and *vanX* genes in *E. faecium* and *L. plantarum* isolated from marketed foods and drugs, respectively. Moreover, they identified *dfrA* gene in *S. thermophilus* and *Lactococcus lactis* (41). Campedelli et al. assessed 182 whole genome sequences for *ARGs* in *Lactobacillus* spp. with Comprehensive Antibiotic Resistance Database (CARD). They reported penicillin binding proteins (PBPs) and d-alanine d-alanine ligase (Ddl) in all *Lactobacillus* genomes investigated. Also othere *ARGs* including *ant(6)*, *ant(9)*, *cmlA*, *cat*, *lsa*, *tet*(M), *tet*(S), *tet*(Q), and *tet*(W), *tet*(L) and *tet*(P), *erm*(B), *mef*(E), and *mef*(B) were identified in their study (82).

# Conclusion

In many studies, it has been reported that LAB acquires various *ARGs*, especially tetracycline and erythromycin agents; therefore, fermented foods and dairy probiotic products may be a source of antibiotic resistance. For the use of a bacterium as a probiotic, we recommend that the minimum inhibitory concentration (MIC) be determined for a large number of antibiotics and analysis of whole genome sequences for ARGs prediction. In the review of the literature, probiotic bacteria are considered to

resistance genes pool and transfer these to microflora and pathogenic bacteria. Our results revealed that there are various *ARGs* in probiotic bacteria including *Lactobacillus*,*Bifidobacterium* and, *Bacillus*; therefore, designing new guidelines seems to be highly necessary.

# Declarations


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### Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

### Conflict of Interest

The authors declare no conflicts of interest.

### Statement of Informed Consent

The article does not contain any studies in patients by any of the authors.

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## Tables

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