academicJournals

Vol. 11(11), pp. 433-439, 21 March, 2017
DOI: 10.5897/AJMR16-8324
Article Number: CDE59D463265
ISSN 1996-0808
Copyright © 2017
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antibiotic resistance patterns of lactic acid bacteria isolated from Nigerian grown salad vegetables

Tajudeen Akanji Bamidele[1*], Bolanle Alake Adeniyi[2] and Muinah Junaid Fowora[1]

[1]Molecular Biology and Biotechnology Division, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria.
[2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Oyo State, Nigeria.

Received 3 October 2016, Accepted 1 December, 2016.

The reports on some foodborne lactic acid bacteria (LAB) possessing antibiotic resistance (AR) genes on mobile genetic elements are on the increase. In Nigeria, such information is rare. This study was therefore designed to determine the presence and locations of AR genes in LAB isolated from locally grown salad vegetables. The LAB used in this study were previously isolated from Nigerian grown cabbage, carrot, cucumber and lettuce and identified by partial sequencing of their 16S rRNA gene. The AR and integrons (*intI*1, 2, 3) genes were detected using polymerase chain reaction after phenotypic agar disc diffusion assay of 20 antibiotics. Extraction and curing of plasmids were performed using standard methods. Univariate analysis was performed to determine resistance to ≥ 2 antibiotics, while multinomial logistic regression was conducted to determine association of resistance patterns with vegetable sources/ types and LAB strains at 95% confidence interval (CI). The entire LAB were phenotypically resistant to ≥2 antibiotics, while uncultured Solibacillus clone RBL-135 was resistant to all and possessed the 454 bp vancomycin (*van*X) gene on chromosomes. Three others, *Lactobacillus plantarum* YML 007 (lettuce), *Lactobacillus plantarum* TCP 008 (cabbage) and *Weissella cibaria* PON 10339 (carrot), also amplified this gene while *Weissella confusa* SJL 602 (lettuce) amplified resistance gene for beta lactam (*bla*Z). This gene (*bla*Z) was also detected in three other LAB but with size corresponding to 500 bp. None of the tetracycline ribosomal protection protein, *tet*(M), (S), (W), efflux *tet*(K), (L), aminoglycoside acetyltransferase and phosphotransferase encoding gene, *aac*(6^1)- *aph*(2^1), integrons (*intI* 1,2,3) were detected. The plasmid cured LAB exhibited same resistance patterns as their wild derivatives. The naturally occurring LAB in the study vegetables are phenotypically multidrug resistant, a few possessing *van*X and *bla*Z resistance genes on chromosomes. Hence, they lack potentials to transfer AR through plasmids and integrons.

Key words: Antibiotic resistance, lactic acid bacteria, salad vegetables, polymerase chain reaction, 16S rRNA, plasmids.

INTRODUCTION

Lactic acid bacteria (LAB) are a group of beneficial microbes that are Generally Regarded As Safe (GRAS)

*Corresponding author. E-mail: deletaju@yahoo.co.uk.

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International LicenseF

and this has qualified them suitable as probiotics and starter cultures (Strom et al., 2005; Gerez et al. 2009). They are residents of different niches such as fermented foods/dairy products, vegetables, meat, gastrointestinal tracts of humans and animals.

The introduction of antibiotics in human clinical medicine and animal husbandry is one of the greatest achievements of 20th century (Aarestrup, 2005). This was however doused by different reports of antibiotics resistance (AR) by clinically important bacteria and even commensals. Aquilanti et al. (2007) reported the spread of AR in commensal bacteria, creating large reservoirs of AR genes in nonpathogenic bacteria that are linked to the food chain. Most investigations before now have focused on clinically important bacteria (Gevers et al., 2003a; Rizzotti et al., 2009), while information on LAB AR determinants are largely scarce (Jacobsen et al., 2007). In the past decade, attention is being given to this subject. For instance, there has been increasing evidence that points at a crucial role of foodborne LAB as reservoir of potentially transmissible AR genes and this was as a result of continuous assessment of fermented foods for presence of AR strains by several European countries (Witte, 2000; Teuber, 2001; Hammerum et al., 2007; Toomey et al., 2010). Phenotypic and molecular characterization of 121 strains of *Lactobacillus paracasei* isolated from Italian dairy and meat products demonstrated the presence of tetracycline [tet(M), tet(W)] and/or erythromycin resistance erm(C) in different AR isolates (Comunian et al., 2009). Toomey et al. (2010) also reported transfer of *tet*(M) resistance gene from *L. plantarum* to *Lactococcus lactis* BU-2-60 and *E. faecalis* JH2-2. The resistances of food borne LAB to glycopepetide, vancomycin and the β- lactams have been reported at different times although the reports on the latter are scanty. Mobile elements such as plasmids, transposons and integrons are important drivers in bacterial horizontal gene transfer (HGT) and these constitute AR genes with intra and inter- specie transfer of genetic material (Alekshun and Levy, 2007; van Reenen and Dicks, 2011; Santagati et al., 2012). This is dangerous if it occurs in food borne LAB as the AR genes can easily be transferred to potential pathogens in the gastrointestinal tract of man. In Nigeria, regulation on antibiotics administration both as growth promoter in animal husbandry and in clinical management is lacking. The methods of cultivation of salad vegetables using animal dung/human faeces as manure on one hand and the raw consumption of these vegetables on the other encourage transfer of bacteria which carry AR genes; hence there is need to initiate a sustainable biosafety surveillance in terms of AR determinants of the resident LAB in these vegetables.

This study which to the best of the authors' knowledge, is the first in Nigeria, investigated the linkage of mobile genetic elements, plasmid and integrons with AR in LAB isolated from locally grown salad vegetables.

MATERIALS AND METHODS

Sources and identification of LAB

The lactic acid bacteria used in this study were previously isolated from Nigerian grown salad vegetables, cabbage, carrot, cucumber and lettuce procured from 12 different vegetable markets in Lagos, Nigeria. These markets are located at Agboju, Festac, Idi-Araba, Isolo, Ketu, Mafoluku, Mile 12, Mushin, Oja- Oba, Oke-Odo, Oshodi and Yaba. They were identified with combinations of phenotypic and partial sequencing of 16S rRNA gene (Bamidele et al., 2014). These comprised of *Lactobacillus* spp. (8), *Weissella* spp. (3), *Pediococcus* spp. (3), Uncultured *Solibacillus* (1) and *Enterococcus durans* (1) (Table 1).

Antibiogram of LAB

Twenty antibiotics (Table 2) selected based on the previous works done on LAB and clinical relevance, were employed in the susceptibility assays against the LAB. The antibiotics were aseptically placed with the use of disc dispenser on the Lactobacillus susceptibility test medium (LSM) which was previously seeded with overnight growth of LAB isolates that have been diluted to make 0.5 Mac- Farland standard (10^8 CFU/ml). The LSM is made of the combination of MRS (10%) and Isosensitest (90%) agar (Klare et al., 2007). This was done according to Clinical and Laboratory Standards Institute (CLSI 2007) guidelines. The culture was incubated for 24 h after which zones of inhibition were measured in millimeter (mm) and absence of zones taken as resistance.

PCR detection of resistance genes

The primers for specific genes were synthesized by Inqaba Biotech, South Africa. The annealing temperatures for the reaction were calculated based on the melting temperatures (Tm) of each set of primers (that is, forward, F and reverse, R). The amplification was carried out in thermo cycler (vapo. protect, Eppendoff) using AccuPower PCR premix mastermix (Bioneer, USA) added to 3 µl of template DNA. The mixture was made up to 20 µl with distilled water according to the manufacturer's instructions. The primers, genes and PCR conditions are as shown in Table 4.

Plasmid extraction

This was done according to the modified methods of Klaenhammer (1984). Briefly, overnight culture of LAB was suspended in 200 µl of 25% w/v sucrose, 50 mM Tris HCl, 5 mM EDTA (STE buffer, pH 8.0) containing 5 mg/ml lysozyme (Carlroth, Germany) and 10 Uml^{-1} mutanolysin (Sigma, USA). This was incubated at 37°C for 30 min and 250 µl STE buffer (pH 10.5 adjusted by 10 N NaOH) containing SDS (6% w/v) was added followed by mixing (inverting tubes) 10 times. All these were incubated at 62°C for 30 min and cooled to room temperature. On addition of 300 µl potassium acetate (3 M), the mixture was vortexed and later incubated at -20°C for 10 min. This was followed by centrifugation (10,000 rpm for 15 min) after which the supernatant was transferred into sterile Eppendoff tube. The supernatant was extracted with 500 µl phenol/chloroform/ isoamylalcohol (25:24:1) and centrifuged. After centrifugation, the aqueous phase was transferred into sterile Eppendoff tube and precipitated with isopropanol (equal volume), vortexed and centrifuged for 10 min. The supernatant was decanted and pellet dried in air. The dried pellet was dissolved in 50 µl Tris HCl, EDTA (TE) buffer.

Table 1. Summary of LAB and their source vegetables.

LAB	Cabbage (n)	Carrot (n)	Cucumber (n)	Lettuce (n)	Total (n)
W. confusa	8	2	3	7	20
W. cibaria	4	5	2	2	13
W. paramesenteroides	-	-	1	-	1
Lactobacillus spp.	1	-	-	-	1
L. fermentum	4	1	5	2	12
L. plantarum	7	2	1	5	15
L. reuteri	-	1	-	-	1
L. paralimentarius	-	1	-	-	1
L. brevis	-	2	1	-	3
L. johnsonii	-	-	1	1	2
L. vaginalis	-	-	-	1	1
P. pentosaceus	2	1	6	3	12
P. dextrinicus	1	-	-	-	1
P. acidilactici	-	-	5	2	7
Uncultured solibacillus	-	-	1	-	1
E. durans	-	-	-	1	1

Table 2. The antibiotics used for LAB antibiogram/concentrations.

Antibiotic (code)	Concentration (µg)	Source
Sulphamethoxazole/ Trimethoprim (SXT)	25	Oxoid, UK
Rifampicin (RD)	5	,,
Ciprofloxacin (CIP)	5	,,
Nalidixic acid (NA)	30	,,
Levofloxacin (LEV)	5	,,
Cephalothin (KF)	30	,,
Amoxycillin (AML)	10	,,
Cefocitin (FOX)	30	,,
Ceftriaxone (CRO)	30	,,
Vancomycin (VA)	5	,,
Clindamycin (DA)	5	,,
Imipenem (IPM)	10	,,
Cloxacillin (OB)	5	,,
Cotrimozaxole (COT)	25	Abtek, UK
Chloramphenicol (CHL)	10	,,
Tetracycline (TET)	10	,,
Streptomycin (STR)	10	,,
Augmentin (AUG)	30	,,
Gentamycin (GEN)	10	,,
Erythromycin (ERY)	5	,,

Agarose gel electrophoresis

The PCR products, (5µl) was loaded alongside a 100 bp marker (Solis biodyne, Estonia) on a 1% agarose stained with ethidium bromide. For plasmid, the dissolved pellet (5 µl) was loaded alongside a Lambda DNA/ HindIII marker (Thermo Scientific, USA) on a 0.8% agarose stained with ethidium bromide. This was run for 1 h at 100 V after which they were viewed under UV in a photodocumentation system (Clinix, China).

Plasmid curing experiment

Thirty LAB possessing plasmid DNA, showing phenotypic resistance to at least 2 antibiotics were selected and grown microaerophilically for 72 h at 40°C in sub-lethal dose (75 µg/ml) of acridine orange (BDH) in MRS broth. This dose was arrived at after subjecting them individually to different doses (25, 50, 75 and 100 µg/ml).

The post exposure curing was done by subculturing LAB into

Table 3. Antibiotic susceptibility/ resistance patterns of LAB spp.

Antibiotics/conc (µg)	LAB spp					
	Lactobacillus spp. (n)		*Pediococcus* spp. (n)		*Weissella* spp. (n)	
	R	S	R	S	R	S
Sulphamethoxazole/ Trimethoprim (SXT-25)	14	10	10	4	15	10
Rifampicin (RD- 5)	2	19	2	11	0	22
Ciprofloxacin (CIP- 5)	12	8	4	5	6	14
Nalidixic acid (NA- 30)	19	1	9	0	19	2
Levofloxacin (LEV- 5)	0	7	1	3	2	16
Cephalothin (KF- 30)	0	9	0	4	0	17
Amoxycillin (AML- 10)	9	14	3	12	6	19
Cefoxitin (FOX- 30)	4	15	4	9	3	19
Ceftriaxone (CRO- 30)	22	0	14	1	24	1
Vancomycin (VA- 5)	22	1	14	1	21	2
Clindamycin (DA- 5)	2	20	1	12	4	21
Imipenem (IPM- 10)	0	23	0	15	0	25
Cloxacillin (OB- 5)	22	2	16	0	27	1
Cotrimozaxole (COT- 25)	17	3	11	4	15	9
Chloramphenicol (CHL- 10)	3	17	1	4	1	23
Tetracycline (TET- 10)	10	9	8	7	5	19
Streptomycin (STR- 10)	9	11	8	7	10	14
Augmentin (AUG-30)	12	8	10	5	14	10
Gentamycin (GEN-10)	1	19	0	15	1	23
Erythromycin (ERY- 5)	2	18	0	14	1	23

[R]Resistant, [S]Sensitive. Uncultured *Solibacillus* was resistant to all the tested antibiotics.

Fresh sterile MRS agar, incubated for 24 h and another round of plasmid extraction performed to ascertain the success or otherwise of curing. The LAB that showed no plasmids were finally used for another round of antibiogram using the antibiotics they were resistant to. The absence of resistance thereafter was interpreted as the initial resistance being plasmid mediated.

Statistical analysis

Univariate analysis was performed to determine resistance to ≥ 2 antibiotics. A multinomial logistic regression using IBM SPSS vs 20 was conducted to determine association of LAB resistance patterns with vegetable sources/types and strains at 95% confidence interval (CI).

RESULTS

Antibiogram of LAB

The entire LAB were multi drug resistant, while 97.0% were resistant to cloxacillin, ceftriaxone each and 94.0% resistant to vancomycin. All the LAB except one (uncultured Solibacillus) was sensitive to imipenem. Rifampicin was the next in terms of activity against the LAB after imipenem as 91.4% of the LAB were sensitive to this antibiotic. This was followed by clindamycin (Table 3).

Plasmid analysis

Seventy-four percent (74.0%) of the selected LAB isolates possessed less than 2 kb plasmid while 83.0% carried 23 kb. Forty-two percent (42.0%) carried at least two copies each. LAB from cucumber carried most of the plasmid (38.0%), followed by cabbage (32.0%), lettuce (27.0%) and carrot (3.0%). The pattern of Antibiogram remained basically the same as in pre-curing experiment.

PCR detection of resistance genes

The results of PCR showed four different LAB strains carrying 454 bp vancomycin resistance (*van*X) genes. These LAB isolated mostly from Oke-Odo are as follows, uncultured Solibacillus clone RBL- 135, *L. plantarum* YML 007, *L. plantarum* TCP 008, *W. cibaria* PON 10339. *Weissella confusa* SJL 602 from lettuce amplified 325 bp resistant gene for beta lactam (*bla*Z).

Three other LAB, *L. fermentum* LG1, *L. brevis* BB7 (both from carrot) and *L. fermentum* PBCC11 from lettuce amplified this gene (*bla*Z) but corresponding to a larger size of 500 bp. None of the genes encoding tetracycline ribosomal protection proteins (RPPs) (*tet*S, *tet*W, *tet*M), efflux (*tet*K, *tet*L), aminoglycosides resistance *aac*(6^1)-*aph* (2^1) and also integrase enzyme *intl* 1, 2, 3 was

Table 4. Primers, genes and PCR conditions for antibiotics resistance in LAB.

Target gene	Primer	Sequence (5^1- 3^1)	Size (bp)	Annealing temperature (^oC)	PCR conditions	Reference
tet (M)	tetM- F tetM- R	GTGGACAAAGGTACAACGAG CGGTAAAGTTCGTCACACAC	406	58	94°C for 5 min, 30 cycles 94°C for 1 min, annealing temp for 1 min, 72°C for 2 min, 72°C for 10 min.	Ng et al., 2001
tet (S)	tetS- F tetS- R	ATCAAGATATTAAGGAC TTCTCTATGTGGTAATC	573	50		Gevers et al., 2003
tet (W)	tetW- F tetW- R	GAGAGCCTGCTATATGCCAGC GGGCGTATCCACAATGTTAAC	168	60		Aminov et al., 2001
tet (K)	tetK- F tetK- R	TTAGGTGAAGGGTTAGGTCC GCAAACTCATTCCAGAAGCA	697	58		Aarestrup et al., 2000
tet (L)	tetL- F tetL- R	CATTTGGTCTTATTGGATCG ATTACACTTCCGATTTCGG	456	56		Aarestrup et al., 2000
aac(6')-*aph*(2')	aac- F aac- R	CCAAGAGCAATAAGGGCATA CACTATCATAACCACTACCG	230	58	95°C for 30 s, 58°C 45 s, 72°C 2 min; 30×	Rojo-Bezares et al., 2006
bla(Z)	blaZ- F blaZ- R	TACTTCAACACCTGCTGCTTTCG CATTACACTCTTGGCGGTTTCAC	325	61	95°C for 30 s, 61°C 45 s, 72°C 2 min; 30×	Liu et al., 2009
van(X)	vanX- F vanX- R	TCGCGGTAGTCCCACCATTCGTT AAATCATCGTTGACCTGCGTTAT	454	60	95°C for 30 s, 60°C 45 s, 72°C 2 min; 30×	Liu et al., 2009
Intl-1,2,3	hep 35 hep 36	TGCGGGTCAAGGATTTGGATTT CAGCACATGCGTATAAAT	491	54	94°C for 2 min, 35 cycles of 94°C for 30s, 54°C for 45 s, 72°C for 45s, 72°C for 7 min	White et al., 2000

tet- Tetracycline resistant gene, aac (61) - aph (21) - aminoglycoside acetyltransferase and phosphotransferase encoding gene, bla- beta lactamase gene, van- vancomycin resistance gene, intl- integrase encoding gene.

amplified by any of the LAB.

Statistical analysis

The vegetable sources/types, LAB strains have no statistical significance with resistance patterns ($p>0.05$).

DISCUSSION

The identities of resident LAB in cabbage, carrot, cucumber and lettuce employed in this study were confirmed by partial sequencing of their 16S rRNA genes. This according to European Food Safety Authority is the first approach in the study concerning AR gene/safety of foodborne bacteria (EFSA, 2012). The occurrence of *Lactobacillus* spp. in largest number is in tandem with various reports (Tamang et al., 2005; Gomes et al., 2010; Gad et al., 2014). Salad vegetables are served and eaten raw in Nigeria and indeed many other climes as such, any AR borne on conjugative mobile genetic elements poses a great danger to the populace. Our investigation of linkage of mobile genetic elements, plasmids and integrons to AR in LAB is the first in the locally grown salad vegetables. The Antibiogram of LAB is still interpreted with a great deal of inconsistencies (Franz et al., 2005; Hummel et al., 2007) as the phenomenon of break-point/cut off values are yet to be duly validated in many LAB strains. This is partly due to the fact that LAB AR issues emerged barely 2 decades ago as they have ever been generally regarded as safe and the growth medium for LAB (MRS) renders the activities of test antibiotics sub optimal. In this study, the LSM, as demonstrated by Klare et al. (2007), was employed and susceptibilities were interpreted based on CLSI (2007) guidelines. Majority of the LAB were phenotypically multi drug resistant but this did not correlate with their possession of respective AR genes. For instance, *tet* gene for tetracycline frequently reported in food borne LAB was not detected in this study. The tetracycline ribosomal protection proteins (RPPs) and efflux pump were not detected. This may be associated with the phenomenon of 'silent' gene or inducible AR genes often reported in LAB. For instance, Hummel et al. (2007) reported some LAB strains possessing silent chloramphenicol acetyltransferase (*cat*) gene under inducing and non-inducing conditions. In phenotypic terms, most of the LAB were susceptible to erythromycin. The resistance of LAB to glycopeptides, vancomycin is infrequently reported especially in *Weissella* spp. unlike the findings in this study where the resistance gene (*van*X) was detected in *W. cibaria* PON 10339 alongside other LAB strains. The resistance to this antibiotic is

reported to be intrinsic in *Lactobacillus* spp. All the LAB carrying *van*(X) gene were phenotypically resistant to vancomycin. This innate resistance of LAB to vancomycin has been demonstrated to be due to replacement of D-ala- D- ala precursor of muramyl pentapeptide in peptidoglycan in LAB with D-lact or D-ser (Nelson, 1999; Mathur and Singh, 2005; Ammor et al., 2007). It must be mentioned here that about 9 other vancomycin resistance genes other than *van*(X) have been reported to date and *van*(X) gene is uncommonly detected in LAB in general and *Weissella* spp. in particular. All the vancomycin genes are not usually borne on mobile genetic elements (Klein et al., 2000). Uncultured Solibacillus clone RBL-135 was resistant to all tested antibiotics in this study including imipenem but was greatly inhibited by ofloxacin (data not shown).

The resistances to β-lactams (*bla*Z) antibiotics were pronounced in this study as 97.0% of the LAB were phenotypically resistant to cloxacillin while more than 90.0% were resistant to other β-lactams including cephalosporins. This indicates intrinsic or natural factors. The *bla*(Z) gene detected in *W. confusa* SJL 602 is also uncommonly reported in this LAB. In fact, this search in literature for this gene in this LAB yielded no positive reports as most of the carriers were *Lactobacillus* spp. This gene was amplified in 3 other LAB (*L. fermentum* LG1, *L. brevis* BB7 both from carrot and *L. fermentum* PBCC11 from lettuce) in this study corresponding to unexpected size of 500 bp. This may be due to non-specific binding or mutation. DNA sequencing or DNA-DNA hybridization study should be done to confirm the identity of this gene.

Majority of the LAB possessed plasmids and in fact all the detected LAB carrying AR genes did. The presence of this mobile genetic element is in line with the work of Olukoya et al. (1993) where 80.0% of the LAB from Nigerian fermented foods were carriers of plasmids some up to five with size ranging from 1.8 to 45.0 kb. In this study, the size range was from less than 1.0 to 23.1 kb. Seventy-four percent (74.0%) of the LAB carried plasmids while 42.0 and 39.0% carried 2 and 3 copies, respectively. In this study also, the method of Klaehammer (1984) was modified to isolate the plasmid; briefly, the combination of enzymes, lysozyme (5 mg/ml) and mutanolysin (10 U/ml) was employed and the incubation extended to 30 min at 37°C. The authors hope to investigate the types of plasmid (in terms of their biotechnological/functional qualities) borne by these LAB in future.

The non-linkage of plasmid to resistances seen in this study prompted us to assay for integrase enzyme carrying gene cassettes with resistance to up to 10 antibiotics at the same time (Clementi and Aquilanti, 2011). None of the LAB in this study possessed integrons.

The main gap in this study was the non-inclusion of assay for conjugative transposons Tn 916-1545 and insertion sequences (IS) elements which have also been implicated in HGT of AR genes in food borne LAB (Clewell et al., 1995; Churchward, 2002; Hummel et al., 2007; Devirgiliis et al., 2009).

In conclusion, the identified LAB in the study cabbage, carrot, cucumber and lettuce grown locally were phenotypically multidrug resistant, most possessing plasmid DNA which is not linked to the AR based on post curing outcomes. A few were detected to possess vancomycin, *van*(X) and β-lactams, *bla*(Z) resistance genes and none possessed the multi-drug resistant carriers, integrase enzyme. The LAB therefore can be said to be safe in terms of their ability to transfer AR genes through plasmid and integrons based on this study. There is also the need to scale up this study to other regions of the country and initiate biosafety surveillance of LAB in salad vegetables.

CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest whatsoever.

REFERENCES

Aarestrup FM (2005). Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic Clin. Pharmacol. Toxicol. 96:271-281.

Aarestrup FM, Agerso Y, Gerner- Smidt P, Madsen M, Jensen LB (2000). Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark.Diagn. Microbiol. Infect. Dis. 37: 127- 137.

Alekshun MN, Levy SB (2007). Molecular mechanisms of antibacterial multidrug resistance. Cell 128:1037- 1050.

Aminov RI, Garrigues-Jeanjean N, Mackie RI (2001). Molecular ecology of tetracycline resistance: Development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. Appl. Environ. Microbiol. 67:22-32.

Ammor MS, Florez AB, Mayo B (2007). Antibiotic resistance in nonenterococcal lactic acid bacteria and bifidobacteria. Food Microbiol. 24:559-570.

Aquilanti L, Garofalo C, Osimani A, Silvestri G, Vignaroli C, Clementi F (2007). Isolation and molecular characterization of antibiotic-resistant lactic acid bacteria from poultry and swine meat products. J. Food Prot. 70:557-565.

Bamidele TA., Adeniyi BA., Ayeni FA, Akinsinde KA. (2014). Identification, Antagonistic activities against *Vibrio cholerae* 01 of lactic acid bacteria isolated from Nigerian grown salad vegetables. Int. J. Pharm. Res. Biosc. 3:56-65.

Churchward G (2002). "Conjugative transposons and related mobile elements." In: Mobile DNAII (ed) N. Craig,R.Craigie,and M.Gellert (Washington,DC: ASM Press), Pp.177-191.

Clementi F, Aquilanti L (2011). Recent Investigations and Updated Criteria for the Assessment of Antibiotic Resistance in Food Lactic Acid Bacteria. Anaerobe 17:394-398.

Clewell DB, Flannagan SE, Jaworski DD (1995). Unconstrained bacterial promiscuity: the Tn916-Tn1545 family of conjugative transposons. Trends. Microbiol. 3:229-236.

Clinical and Laboratory Standards Institute (CLSI) (2007). Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria: approved guideline. CLSI document M45- A (ISBN 1-56238-607-7). CLSI, Wayne, Pennsylvania.

Comunian R, Daga E, Dupre I, Paba A, Devirgiliis C, Piccioni V, Perozzi G, Zonenschain D, Rebecchi A, Morelli L, De Lorentiis A, Giraffa G

(2009). Susceptibility to tetracycline and erythromycin of *Lactobacillus paracasei* strains isolated from traditional Italian fermented foods. Int. J. Food Microbiol. 138:151-156.
Devirgiliis C, Coppola D, Barile S, Colonna B, Perozzi G (2009). Characterization of the Tn916 conjugative transposons in a food-borne strain of *Lactobacillus paracasei*. Appl. Environ. Microbiol. 75: 3866-3871.
European Food Safety Authority (EFSA) (2012). Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2012 update). EFSA J. 10:13-30.
Franz CMAP, Hummel A, Holzapfel WH (2005). Problems related to the safety assessment of lactic acid bacteria starter cultures and probiotics. Mitt. Lebensm Hyg. 96:39-65.
Gad GFM, Abdel- Hamid AM, Faray ZSH (2014). Antibiotic resistance in lactic acid bacteria isolated from some pharmaceutical and dairy products. Braz. J. Microbiol. 45:25-33.
Gerez CI, Torino IM, Rolian G, de Valdez FG (2009). Prevention of bread mould spoilage by using lactic acid bacteria with antifungal properties. Food Control 20:144-148
Gevers D, Danielsen M, Huys G, Swings J (2003). Molecular characterization of *tet*(M) genes in *Lactobacillus* isolates from different types of fermented dry sausage. Appl. Environ. Microbiol. 69: 1270-1275.
Gevers D, Masco L, Baert L, Huys G, Debevere J, Swings J (2003a). Prevalence and diversity of tetracycline resistant lactic acid bacteria and their tet genes along the process line of fermented dry sausages. Syst. Appl. Microbiol. 26:277-283.
Gomes FCO, Silva CLC, Vianna CR, Lacerda ICA., Borelli BM, Nunes AC, Franco GR, Mourao MM, Carlos RA (2010). Identification of lactic acid bacteria associated with traditional Cachaca fermentations. Braz. J. Microbiol. 41:486-492.
Hammerum AM, Heuer OE, Emborg HD, Bagger-Skjot L, Jensen VF, Rogues AM, Skov RL, Agerso Y, Brandt CT, Seyfarth AM, Muller A, Hovgaard K, Ajufo J, Bager F, Aarestrup FM, Frimodt-Moller N, Wegener HC, Monnet DL (2007). Danish integrated antimicrobial resistance monitoring and research program. Emerg. Infect. Dis. 13:1632-1639.
Hummel AS, Hertel C, Holzapfel WH, Franz CM (2007). Antibiotic resistances of starter and probiotic strains of lactic acid bacteria. Appl. Environ. Microbiol. 73:730-739.
Jacobsen L, Wilcks A, Hammer K, Huys G, Gevers D, Andersen SR (2007). Horizontal transfer of tet(M) and erm(B) resistance plasmids from food strains of *Lactobacillus plantarum* to *Enterococcus faecalis* JH2-2 in the gastrointestinal tract of gnotobiotic rats. FEMS Microbiol. Ecol. 59:158-166.
Klaenhammer TR (1984). A general method for plasmid isolation in lactobacilli. Curr. Microbiol. 10:23-28.
Klare I, Konstabel C, Werner G, Huys G, Vankerckhoven V, Kahlmeter G, Hildebrandt B, Müller-Bertling S, Witte W, Goossens H (2007). Antimicrobial susceptibilities of *Lactobacillus*, *Pediococcus* and *Lactococcus* human isolates and cultures intended for probiotic or nutritional use. J. Antimicrob. Chemother. 59: 900-912.
Klein G, Hallmann C, Casas IA, AbadJLouwers J, Reuter G (2000). Exclusion of vanA, vanB and vanC type glycopeptides resistance in strains of *Lactobacillus reuteri* and *Lactobacillus rhamnosus* used as probiotics by polymerase chain reaction and hybridization methods. J. Appl. Microbiol. 89:815-824.
Liu C, Zhang ZY, Dong K, Yuan JP, Guo XK (2009). Antibiotic resistance of probiotic strains of lactic acid bacteria isolated from marketed foods and drugs. Biomed. Environ. Sci. 22:401-412.
Mathur S, Singh R (2005). Antibiotic resistance in food lactic acid bacteria – a review. Int. J. Food Microbiol. 105:281-295.
Nelson RR (1999). Intrinsically vancomycin-resistant gram-positive organisms: clinical relevance and implications for infection control. J. Hosp. Infect. 42:275-282.
Ng LK, Martin I, Alfa M, Mulvey M (2001). Multiplex PCR for the detection of tetracycline resistant genes. Mol. Cell Probes 15:209-215.
Olukoya DK, Ebigwei SI, Adebawo OO, Osiyemi FO (1993). Plasmid profiles and antibiotics susceptibility patterns of *Lactobacillus* isolated from fermented foods in Nigeria. Food Microbiol. 10:279-285.
Rojo-Bezares B, Sáenz Y, Poeta P, Zarazaga M, Ruiz-Larrea F, Torres C (2006). Assessment of antibiotic susceptibility within lactic acid bacteria strains isolated from wine. Int. J. Food Microbiol. 111: 234–240.
Rizzotti L, LaGioia F, Dellaglio F, Torriani S (2009). Molecular diversity and transferability of the tetracycline resistance gene *tet*(M), carried on Tn916-1545 family transposons, in enterococci from a total food chain. Antonie Van Leeuwenhoek 96:43-52.
Santagati M, Campanile F, Stefani S (2012). Genomic diversification of enterococci in hosts: the role of the mobilome. Front. Microbiol. 3:1-9.
Strom K, Schnurer J, Melin P (2005). Co- cultivation of antifungal *Lactobacillus plantarum* MiLAB 393 and *Aspergillus nidulans*, evaluation of effects on fungal growth and protein expression. FEMS Microbiol. Lett. 246: 119- 124.
Tamang JP, Tamang B, Schillinger U, Franz CMAP, Gores M, Holzapfel WH (2005). Identification of predominant lactic acid bacteria isolated from traditionally fermented vegetable products of the Eastern Himalayas. Int. J. Food Microbiol. 105:347-356.
Teuber M (2001). Veterinary use and antibiotic resistance. Curr. Opin. Microbiol. 4:493-499.
Toomey N, Bolton D, Fanning S (2010). Characterisation and transferability of antibiotic resistance genes from lactic acid bacteria isolated from Irish pork and beef abattoirs. Res. Microbiol. 161:127-135.
Van Reenen CA, Dicks LM (2011). Horizontal gene transfer amongst probiotic lactic acid bacteria and other intestinal microbiota: what are the possibilities? A review Arch. Microbiol. 193:157-168.
White PA, Mciver CI, Deng YM, Rawlinson WD (2000). Characterisation of two new gene cassettes, *aad*A5 and *dfr*A17. FEMS Microbiol. Lett. 182: 265-269.
Witte W (2000). Selective pressure by antibiotic use in livestock. Int. J. Antimicrob Agents 16(Suppl 1):S19-S24.