



RESEARCH

Open Access

Prevalence of antibiotic resistance in lactic acid bacteria isolated from the faeces of broiler chicken in Malaysia

Nurhazirah Shazali¹, Hooi Ling Foo^{2,4*}, Teck Chwen Loh^{1,5}, Di Wei Choe¹ and Raha Abdul Rahim^{3,4}

Abstract

Background: Probiotics are commonly used as feed additive to substitute antibiotic as growth promoter in animal farming. Probiotic consists of lactic acid bacteria (LAB), which enhance the growth and health of the animal. Probiotic also have higher possibility to become pathogenic bacteria that may carry antibiotic resistant gene that can be transmitted to other LAB species. The aim of this study was to identify the LAB species in the faeces of broiler chicken and to determine the prevalence of antibiotic resistant in LAB of broiler chicken.

Methods: Sixty faeces samples were collected from wet markets located in Klang Valley of Malaysia for the isolation of LAB using de-Mann Rogosa Sharpe medium. Thirteen species of LAB were obtained in this study and the identification of LAB was performed by using API test kit on the basis of carbohydrate fermentation profile. Antibiotic susceptibility assay was then carried out to determine the prevalence of LAB antibiotic resistance.

Results: *Lactococcus lactis* subsp *lactis* was found in nine out of sixty faecal samples. *Lactobacillus paracasei* was the second common LAB species isolated from chicken faecal. No significant difference ($P > 0.05$) was found between the occurrence of *Lactobacillus brevis*, *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Leuconostoc lactis mesenteroides* subsp *mesenteroides/dectranium* and *Pediococcus pentosaceus* isolated from 5 different locations. Most of the isolated LAB was resistant to antibiotic and high variability of the antibiotic resistance was observed among the LAB against 15 types of antibiotics. Penicillin, amoxicillin, chloramphenicol, and ampicillin had significant higher ($P < 0.05$) inhibitory zone than nalidixic acid, gentamycin, sulphamethoxazole, kanamycin, and streptomycin.

Conclusions: Many species of LAB were isolated from the faecal samples of broiler chicken that resistance to the common antibiotics used in the farm. The development of resistant against antibiotics in LAB can be attributed to the long term exposure of antibiotic as growth promoter and therapeutic agents. Thus, it is essential to advise farmer the safety measure of antibiotic application in animal farming. Additionally, the supplementation of probiotic in animal feeding also needs more attention and close monitoring.

Keywords: Lactic acid bacteria, Antibiotic resistance, Broiler chicken

Background

Antibiotic are normally used to treat microbial diseases since 50 years ago. However, excessive use of antibiotic may lead to the development of antibiotic resistance in pathogenic bacteria. The report of antibiotic resistance is

significantly increased due to the overuse and misuse of antibiotics, which has created an enormous selective pressure on the recessive bacteria [1]. Antibiotic resistant bacteria have the ability to resist toward the actions of naturally occurring or synthetically produced compounds inimical to their survival [2].

Scott [3] reported the identical resistance gene present in bacterial species isolated from different hosts. Antibiotic resistant may acquires through the *in-vivo* gene transfer between normal flora of gastrointestinal and antibiotic resistant pathogenic bacteria [4]. In fact, the antibiotic resistant pathogenic bacteria pose a great potential

* Correspondence: hlfoo@upm.edu.my

²Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, UPM Serdang Selangor, Malaysia

⁴Institute of Bioscience, Universiti Putra Malaysia, 43400, UPM Serdang Selangor, Malaysia

Full list of author information is available at the end of the article

threat to human health, especially when the immunity system is not functioning well. Most of the developed countries have prohibited the use of antibiotic as animal growth promoter. Thus, various alternatives have been explored to replace antibiotic as growth promoter. One of the most common and popular alternatives is the application of probiotic as growth promoter in livestock animals. In addition, the probiotic effects of postbiotic metabolites produced by probiotic strains have been shown in various animal species, such as rats [5,6], broilers [7,8], laying hens [9] and post weaning piglets [10], suggesting they have great potential to be used as growth promoter for livestock animals.

Probiotic comprises of beneficial bacteria such as lactic acid bacteria (LAB). LAB is a group of gram-positive anaerobic bacteria which produce predominantly lactic acid from carbohydrate fermentation. Many farmers use probiotic extensively and those bacteria have potential to serve as a host of antibiotic resistance genes with a risk of transferring those genes into many LAB and other pathogens [11].

The antibiotic resistant LAB has been detected by using DNA sequences which is responsible for antibiotic resistance traits. Egervärn *et al.* [12] reported the emergence of antibiotic resistant in *Lactobacillus reuteri* and *Lactobacillus plantarum*. Moreover, *Lactobacilli*, *Pediococci* and *Leuconostoc* spp. have been reported to be highly resistant to vancomycin [11,13] and some *Lactobacilli* have high resistance to bacitracin, cefoxitin, ciprofloxacin, fusidic acid, streptomycin, sulphadiazine, teicoplanin and vancomycin [14]. Most of the reported LAB that resistant to antibiotics was isolated from food sources. These include the most commonly used probiotic species such as *Lactobacillus casei*, *Lactobacillus acidophilus*, *L. reuteri*, or *Lactobacillus rhamnosus*, among others, or the yogurt starter bacteria *Lactobacillus delbrueckii* [15-17]. However, the information of antibiotic resistant LAB isolated from the gut of broiler chickens in Malaysia is very limited. Thus, the objective of this study was to investigate the antibiotic resistance profile of LAB isolated from the gut of broiler chickens in Klang Valley, Malaysia.

Materials and methods

Sample origin and collection

A total of 60 faeces samples were collected from chicken purchased from wet markets located nearby Universiti Putra Malaysia: Serdang Jaya, Seri Kembangan, Dengkil, Putrajaya, Kajang, Pantai Dalam, and Jenderam Hilir. The age of the chicken was 42 days. The faeces were collected directly from caeca junction to the end of the cloaca of large intestine. The faeces samples (1 g each) were kept at -20°C until further analysis. The experimental design has obtained approval from the Ethics Committee of Universiti Putra Malaysia.

Enumeration and isolation of lactic acid bacteria

The faeces sample was mixed homogeneously at a ratio of 1 g sample with 9 ml of peptone water in the universal bottle and incubated for one hour at room temperature. The samples were then subjected to 10-fold serial dilution using 0.1% (v/v) peptone water [18] and 0.1 ml of each appropriate diluted sample was then plated onto de Man Rogosa Sharpe (MRS) agar and incubated under anaerobic condition at 30°C for 48 hours [19]. The colony forming unit (CFU) per gram of sample was expressed as logarithm at the base of 10 ($\text{Log}_{10}\text{CFU/g}$). The enumeration of LAB was conducted in triplicates. After 48 hours of incubation, a colony was picked and streaked onto MRS agar and incubated for additional 48 h at 30°C . This process was repeated twice and a pure colony was then transferred to MRS broth and incubated at 30°C for 24 h. The pure LAB culture was then kept in MRS broth supplemented with 20% (v/v) glycerol and incubated at -20°C until further analysis.

Identification of lactic acid bacteria

LAB culture was identified phenotypically on the basis of carbohydrate fermentation profile by using API 50CH kit (BioMerieux, France) according to the instruction of manufacturer. The carbohydrate fermentation profile was then analysed by using APILAB Plus software version 3.3.3 (BioMerieux, France) to identify the species of each isolated LAB culture.

Antibiotic susceptibility assay

The antibiotics used for susceptibility assay were ampicillin (10 μg), clindamycin (2 μg), erythromycin (15 μg), gentamicin (10 μg), streptomycin (25 μg), tetracycline (30 μg), chloramphenicol (30 μg), kanamycin (30 μg), sulphamethoxazole/trimethoprim (25 μg), vancomycin (30 μg), ciprofloxacin (5 μg), amoxicillin (10 μg), bacitracin (10 μg), nalidixic acid (30 μg), and penicillin (10 μg) (Oxoid Ltd, England). The antibiotics were selected due to their common use in local animal farming. A total of 1 ml LAB culture grown in MRS broth was collected by centrifugation at $1000 \times g$ for 5 min. The cell pellet was collected and washed twice using 1 ml of 0.85% (w/v) NaCl, followed by suspending the cell pellet with 0.5 ml of 0.85% (w/v) NaCl. The cell suspension was adjusted to 0.5 Mc Farland by using 2 ml of NaCl 0.85% (w/v) prior to spread plate on MRS agar. The antibiotic disc was then placed on MRS agar plate. The diameter of inhibitory zones was measured after 48 h of incubation at 30°C under anaerobic condition. The assay was conducted in triplicates [20].

Statistical analysis

The data was analysed using one way analysis of variance (ANOVA) with probability level of 0.05 ($P < 0.05$) using SAS statistical software [21].

Table 1 Lactic acid bacteria species isolated from faecal samples collected from various locations

Locations	<i>L. acidophilus</i>	<i>L. brevis</i>	<i>L. curvatus</i>	<i>L. delbrueckii</i> subsp <i>delbrueckii</i>	<i>L. fermentum</i>	<i>L. paracasei</i> subsp <i>paracasei</i>	<i>L. plantarum</i>	<i>L. rhamnosus</i> (<i>L. casei</i> subsp <i>rhamnosus</i>)	<i>L. salivarius</i>	<i>Lc. lactis</i> subsp <i>lactis</i>	<i>Leu. lactis mesenteroides</i> subsp <i>mesenteroides/dextranicum</i>	<i>P. damnosus</i>	<i>P. pentosaceus</i>
Dengkil 1	0	0	1	0	0	3	0	0	0	1	0	0	1
Dengkil 2	0	0	2	0	0	0	0	1	0	1	0	2	0
Jenderam Hilir	0	0	1	3	0	0	0	0	0	1	0	0	1
Kajang	0	2	0	0	0	0	0	0	0	2	2	0	0
Serdang Jaya 1	0	0	1	0	0	0	1	0	0	2	0	2	0
Serdang Jaya 2	0	2	0	2	1	1	0	0	0	0	0	0	0
Seri Kembangan	1	0	0	1	0	3	0	1	0	0	0	0	0
Pantai Dalam	0	0	0	0	1	0	1	0	2	0	2	0	0
Putrajaya	0	1	0	0	0	0	2	0	1	2	0	0	0
UPM	0	0	0	0	0	0	1	0	1	0	1	0	3
Total	1	5	5	6	2	7	5	2	4	9	5	4	5

Note: A total of 6 samples were collected from each location.

Results and discussion

Identification of lactic acid bacteria

All LAB species isolated from faeces of broiler chicken were identified by API Kit. Table 1 shows the identity of LAB species isolated from various wet markets located in Klang Valley of Malaysia. The result demonstrated that different LAB species were isolated from different wet market and the most common LAB species isolated from chicken faecal was *Lactococcus lactis* subsp *lactis* as it was isolated from 9 out of 60 samples. *Lactobacillus paracasei* was the second common LAB species isolated from chicken faecal. However, there was no significant different ($P > 0.05$) between the occurrence of *Lactobacillus brevis*, *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Leuconostoc lactis mesenteroides* subsp *mesenteroides/dextranum* and *Pediococcus pentosaceus* as they were isolated from 5 different locations. *Pediococcus damnosus* was isolated from 4 locations, whereas *Lactobacillus fermentum* and *L. rhamnosus* (*Lactobacillus casei* subsp *rhamnosus*) were isolated from 2 locations.

It has been reported that *L. acidophilus* and *Lactobacillus salivarius* have higher chance to be isolated among the *Lactobacillus* strains colonizing in the crop intestine [22]. However, *L. acidophilus* and *L. salivarius* were only found in faeces samples 1 and 4, respectively; indicating *L. acidophilus* and *L. salivarius* were not a common LAB found in the faeces of chicken sold in Klang valley of Malaysia.

Antibiotic resistance of lactic acid bacteria

Table 2 shows the inhibitory zone of antibiotic susceptibility for nine LAB regardless of species obtained from faecal samples. Penicillin, amoxicillin, chloramphenicol, and ampicillin had significant higher ($P < 0.05$) inhibitory zone than nalidixic acid, gentamycin, sulphamethoxazole, kanamycin, and streptomycin. These results were similar with the previous report, where lactobacilli were most sensitive to penicillin and ampicillin except for amoxicillin and chloramphenicol [23]. However, there was no significantly different ($P > 0.05$) between vancomycin and bacitracin. Similar inhibitory zone was also found in bacitracin, nalidixic acid, gentamycin, sulphamethoxazole, kanamycin, and streptomycin. LAB was sensitive to clindamycin, chloramphenicol, amoxicillin, penicillin, erythromycin, and ampicillin in current study. However, it was resistant against nalidixic acid, bacitracin, gentamycin, ciprofloxacin, sulphamethoxazole, kanamycin, tetracycline, streptomycin and vancomycin. These results indicate that LAB is not sensitive to beta-lactams group of antibiotics due to the absence of peptidoglycan in LAB cell. However, Lavanya et al. [24] reported recently that most of the LAB isolated from fermented milk were resistant to penicillin G and only 10% were susceptible to ampicillin. Beta-lactams antibiotics were the most effective drugs for the

Table 2 Diameter inhibitory zone (Mean \pm SE), cm of antibiotic susceptibility test for LAB regardless of species from chicken faecal sample

Antibiotics	Diameter of inhibitory zone (cm)
Nalidixic acid	0.02 ^e \pm 0.02
Clindamycin	1.22 ^b \pm 0.16
Bacitracin	0.33 ^{de} \pm 0.07
Chloramphenicol	1.89 ^a \pm 0.17
Amoxicillin	1.93 ^a \pm 0.16
Gentamycin	0.07 ^e \pm 0.04
Ciprofloxacin	0.21 ^d \pm 0.06
Penicillin	1.99 ^a \pm 0.17
Sulphamethoxazole	0.11 ^e \pm 0.05
Kanamycin	0.05 ^e \pm 0.04
Tetracycline	0.75 ^c \pm 0.13
Erythromycin	1.17 ^b \pm 0.15
Ampicillin	1.84 ^a \pm 0.16
Streptomycin	0.05 ^e \pm 0.04
Vancomycin	0.51 ^{cd} \pm 0.12

Note: ^{a-e}Means with different superscripts are significantly different ($P < 0.05$).

treatment of *Staphylococci* infections. However, Tyler et al., [25] reported that beta-lactams antibiotics have no longer effective to treat *Staphylococcus aureus* infections such as methicillin-resistant *S. aureus* and vancomycin-resistant *S. aureus*.

The development of antibiotic resistance in LAB has revealed the fact that the common use of antibiotic as growth promoter in the farm has allowed LAB to develop resistance against antibiotics [26]. The antibiotic resistance in LAB may also acquire through horizontal transferring of resistance gene to the normal LAB by conjugative transposons [1]. As reported by Gilchrist et al. [26], antibiotic resistance in LAB was partly due to poor control of antibiotic as therapeutic and growth promoter in animal feeding. This may contribute to the build-up reservoir of antibiotic resistant bacteria in the gut. Hence, the current result suggests that antibiotic resistance screening is essential before any LAB strains are selected as feed additive for animal feeding.

Profile of lactic acid bacteria antibiotic resistance

Table 3 shows the diameter of inhibitory zone of antibiotic susceptibility among the LAB species. The inhibitory zone for *Leu. lactis mesenteroides* subsp *mesenteroides/dextranicum* was significantly higher ($P < 0.05$) than other LAB species for the tested antibiotics. Bassam [27] reported that *Leu. lactis mesenteroides* was resistant to 100% vancomycin, 75% teicoplanin, 87.5% clindamycin, 75% chloramphenicol, erythromycin, gentamicin and streptomycin, 62.5% ampicillin, penicillin G and tetracycline, 50% kanamycin and trimethoprim. *L. acidophilus* was

Table 3 Diameter, cm (Mean ± SE) of inhibitory zone of lactic acid bacteria species

Lactic acid bacteria	Sulphamethoxazole	Kanamycin	Tetracycline	Erytromycin	Ampicillin	Streptomycin	Vancomycin
<i>L. acidophilus</i>	0.00 ^a ±0.00	0.00 ^a ±0.00	1.28 ^a ±0.16	1.22 ^a ±0.16	1.22 ^a ±0.16	0.00 ^a ±0.00	0.00 ^a ±0.00
<i>L. brevis</i>	0.00 ^c ±0.00	0.00 ^c ±0.00	0.60 ^{abc} ±0.08	1.12 ^{abc} ±0.15	2.02 ^{ab} ±0.26	0.00 ^c ±0.00	0.50 ^{bc} ±0.06
<i>L. curvatus</i>	0.00 ^a ±0.00	0.00 ^a ±0.00	0.00 ^a ±0.00	0.60 ^a ±0.08	1.40 ^a ±0.18	0.00 ^a ±0.00	0.52 ^a ±0.07
<i>L. delbrueckii</i> subsp <i>delbrueckii</i>	19.75 ^a ±2.54	22.00 ^a ±2.84	24.25 ^a ±3.13	26.50 ^a ±3.42	29.30 ^a ±3.78	31.00 ^a ±4.00	33.78 ^a ±4.36
<i>L. fermentum</i>	2.25 ^a ±0.29	2.50 ^a ±0.03	3.08 ^a ±39	3.62 ^a ±0.47	4.68 ^a ±0.60	3.50 ^a ±0.45	4.40 ^a ±0.57
<i>L. paracasei</i> subsp <i>paracasei</i>	0.00 ^a ±0.00	0.28 ^{fg} ±0.04	1.30 ^{cdef} ±0.17	1.88 ^{abcd} ±0.24	2.60 ^{ab} ±0.34	0.22 ^{fg} ±0.03	1.00 ^{defg} ±0.13
<i>L. plantarum</i>	0.00 ^f ±0.00	0.00 ^f ±0.00	1.80 ^{abcd} ±0.23	1.58 ^{abcd} ±0.20	2.50 ^{ab} ±0.32	0.00 ^f ±0.00	1.15 ^{cdef} ±0.15
<i>L. rhamnosus</i> (<i>L. casei</i> subsp <i>rhamnosus</i>)	20.00 ^a ±2.58	22.25 ^a ±2.87	25.15 ^a ±3.25	27.38 ^a ±3.53	30.02 ^a ±3.88	31.25 ^a ±4.03	33.50 ^a ±4.33
<i>L. salivarius</i>	2.25 ^a ±0.29	3.00 ^a ±0.39	2.75 ^a ±0.36	3.45 ^a ±0.45	4.22 ^a ±0.55	3.50 ^a ±0.45	4.25 ^a ±0.55
<i>Lc. Lactis</i> subsp <i>lactis</i>	0.25 ^b ±0.03	0.00 ^b ±0.00	0.78 ^b ±0.10	1.92 ^a ±0.25	2.35 ^a ±0.30	0.00 ^b ±0.00	0.48 ^b ±0.06
<i>Leu. lactis mesenteroides</i> subsp <i>mesenteroides/ dextranicum</i>	39.5 ^a ±5.10	44.00 ^a ±5.68	48.50 ^a ±6.26	53.65 ^a ±6.93	57.90 ^a ±7.48	62.00 ^a ±8.01	66.50 ^a ±8.59
<i>P. damnosus</i>	4.50 ^a ±0.58	5.00 ^a ±0.65	5.88 ^a ±0.76	6.55 ^a ±0.85	6.90 ^a ±0.89	7.00 ^a ±0.90	7.50 ^a ±0.97
<i>P. pentosaceus</i>	19.05 ^a ±2.46	21.00 ^a ±2.71	23.65 ^a ±3.05	26.65 ^a ±3.44	30.10 ^a ±3.89	30.00 ^a ±3.87	32.25 ^a ±4.16

Note: ^{a-g} Means with different superscripts within row are significantly different (P< 0.05).

resistant ($P < 0.05$) against nalidixic acid, gentamycin, ciprofloxacin, sulphamethoxazole, kanamycin, streptomycin and vancomycin. However, Sieo *et al.* [28] claimed that *L. acidophilus* isolated from chicken gastrointestinal tract was resistant to 100% chloramphenicol ($\geq 200 \mu\text{g/ml}$), 58% to erythromycin ($\geq 200 \mu\text{g/ml}$) and 17% to tetracycline ($\geq 200 \mu\text{g/ml}$). *L. brevis* and *L. curvatus* had similar trend of resistance against the antibiotics. However, *L. brevis* was more resistant to ciprofloxacin (a fluoroquinolone), tetracycline, and vancomycin as found in the study of Fukao *et al.* [29]. *L. delbrueckii* subsp *delbrueckii*, *L. fermentum*, *L. rhaminosus*, *L. salivarius*, *Leu. mesenteroides*, *P. damnosus*, *P. pentosaceus* were sensitive to all the tested antibiotics.

According to Ouoba *et al.* [30], *L. acidophilus*, *L. fermentum*, and *L. rhaminosus* contained positive amplicons of resistance genes encoding aminoglycoside (aph (3')-III, aadA, aadE) and tetracycline tet (S), and hence they had higher prevalence of phenotypic resistance for aminoglycoside. This can be attributed to intrinsic resistance of different species since none of these bacteria were resistant to penicillin (first generation β -lactam). These organisms have the potential to act as reservoir of antimicrobial resistant genes and which have potential to be transferred to other bacteria. Moreover, intrinsic resistance to vancomycin was confirmed for *L. paracasei*, *L. salivarius* and *L. plantarum*, and *L. salivarius* was also typically resistant to erythromycin [31]. In other study, *L. salivarius* had the highest resistance to kanamycin and neomycin, but demonstrated the lowest resistance to penicillin [32]. *L. paracasei* subsp *paracasei* was usually used as probiotic in lactic acid fermented food for human consumption [33].

Vankerckhoven *et al.* [34] reported that *L. paracasei* 8700:2 strain was not resistant to antibiotic. However, *L. paracasei* subsp *paracasei* was found to be more resistant against nalidixic acid and sulphamethoxazole as compared to other antibiotics as found in this study. Furthermore, *L. plantarum* was sensitive to all the tested antibiotics except nalidixic acid, gentamycin, sulphamethoxazole, kanamycin and streptomycin. In contrast, Toomey *et al.* [35] claimed that *L. plantarum* harbored erm (B) and msrA/B genes, and tet (M) gene that resistant to erythromycin and tetracycline, respectively. Additionally, it is also intrinsically resistant to vancomycin. Similar trend was also found in *Lc. lactis* except for sulphamethoxazole in current study. Tetracycline and erythromycin-resistance genes were found in *Lc. lactis* and this microbe is normally representing the fermenting microflora of typical Italian traditional cheese Mozzarella di Bufala Campana [36]. It has been reported that *L. fermentum* 1, *Lc. lactis* subsp. *lactis* 1, *L. paracasei* subsp. *paracasei* 1, *L. rhamnosus* and *Lc. lactis* subsp. *lactis* 2 isolated from raw poultry meat were resistant to polymyxin B (PB 100), trimethoprim (TM 5),

tetracycline (TE 30), oxacillin (OX 1), kanamycin (K 30), erythromycin (E 15), gentamycin (CN 10), ciprofloxacin (CIP 5) and cephalothin (CL 30) [37].

Conclusions

In conclusions, many species of LAB were found in the faecal samples of broiler chicken. Each species of LAB has different inhibitory zone related to antibiotic susceptibility. These results indicate that LAB may develop resistance against antibiotic that may result from the horizontal transferring of resistant gene to other microflora in the gut. The development of resistance to antibiotic can be attributed to long term usage of antibiotic as therapeutics and growth promoter. Thus, it is important and essential to advise the farmer a proper way of antibiotic use as therapeutic and growth promoter agents. Additionally, supplementation of unknown source of probiotic as feed additive needs to be monitored closely in animal feeding.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NS and DWC conceived the idea and carried out the antibiotic resistance studies and initial analyses on collected data. NS has drafted the manuscript. HLF involved in experimental design, data acquisition, analyses and interpretation and revising the manuscript. TCL involved in conceptual the experimental design, data acquisition, analyses and interpretation of data, and revising the manuscript. RAR involved in acquisition of data and revising the manuscript. All authors read and approved the manuscript.

Acknowledgement

This project was supported by Long-Term Research Grant Scheme (LRGS) from Ministry of Education Malaysia.

Author details

¹Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400, UPM Serdang Selangor, Malaysia. ²Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, UPM Serdang Selangor, Malaysia. ³Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, UPM Serdang Selangor, Malaysia. ⁴Institute of Bioscience, Universiti Putra Malaysia, 43400, UPM Serdang Selangor, Malaysia. ⁵Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400, UPM Serdang Selangor, Malaysia.

Received: 25 September 2013 Accepted: 15 January 2014

Published: 22 January 2014

References

1. Madden D: Antibiotic resistance in *Escherichia coli* A practical investigation of bacteria conjugation. *National Centre for Biotechnology Education (NCBE): the University of Reading* 2009.
2. WHO ISDA: Antimicrobial resistance Bad bug, no drugs as antibiotic discovery stagnates a public health crisis advance a review. *Official journal of the Australian Society for Microbiology Inc* 2007, **28**:152.
3. Scott KP: The role of conjugative transposons in spreading antibiotic resistance between bacteria that inhabit the gastrointestinal tract. *Cell Mol Life Sci* 2002, **59**:2071–2082.
4. Schjorring S, Krogfelt KA: Assessment of bacterial antibiotic resistance transfers in the gut. *Int J Microbiol* 2011. doi:10.1155/2011/312956.
5. Loh TC, Lee TM, Foo HL, Law FL, Rajion MA: Effects on growth performance, faecal microflora and plasma cholesterol after supplementation of spray-dried metabolite to postweaning rats. *Czech J Anim Sci* 2008, **54**:10–16.

6. Loh TC, Chong SW, Foo HL, Law FL: Growth performance and fecal microflora of rats offered metabolites from lactic acid bacteria. *J Appl Anim Res* 2009, **34**:61–64.
7. Thanh NT, Loh TC, Foo HL, Hair-Bejo M, Azhar BK: Effects of feeding metabolite combinations produced by *Lactobacillus plantarum* growth performance, faecal microbial population, small intestine villus height and faecal volatile fatty acids in broilers. *Br Poult Sci* 2009, **50**:298–306.
8. Loh TC, Thanh NT, Foo HL, Hair-Bejo M, Azhar BK: Feeding of different levels of metabolite combinations produced by *Lactobacillus plantarum* growth performance, faecal microflora, volatile fatty acids and villi height in broilers. *Anim Sci J* 2010, **81**:205–214.
9. Choe DW, Loh TC, Foo HL, Hair-Bejo M, Awis QS: Egg production, faecal pH and microbial population, small intestine morphology, and plasma and yolk cholesterol in laying hens given liquid metabolites produced by *Lactobacillus plantarum* strains. *Br Poult Sci* 2012, **53**:106–115.
10. Thu TV, Loh TC, Foo HL, Yaakub H, Hair-Bejo M: Effects of liquid metabolite combinations produced by *Lactobacillus plantarum* growth performance, faeces characteristics, intestinal morphology and diarrhoea incidence in postweaning piglets. *Trop Anim Health Prod* 2011, **43**:69–75.
11. Gueimonde M, Sánchez B, de Los Reyes-Gavilán CG, Margolles A: Antibiotic resistance in probiotic bacteria. *Front Microbiol* 2013, **4**:202. doi:10.3389/fmicb.2013.00202.
12. Egervärn M, Roos S, Lindmark H: Identification and characterization of antibiotic resistance genes in *Lactobacillus reuteri* and *Lactobacillus plantarum*. *J Appl Microbiol* 2009, **17**:1658–1668.
13. Hamilton-miller JMT, Shah S: Vancomycin susceptibility as an aid to the identification of lactobacilli. *Let Appl Microbiol* 1998, **26**:153–154.
14. Danielsen M, Wind A: Susceptibility of *Lactobacillus* spp. to antimicrobial agents. *Int J Food Microbiol* 2003, **82**:1–11.
15. Ammor MS, Flórez AB, Van Hoek AHAM, de Los Reyes-Gavilán CG, Aarts HJM, Margolles A, Mayo B: Molecular characterization of intrinsic and acquired antibiotic resistance in lactic acid bacteria and bifidobacteria. *J Mol Microbiol Biotechnol* 2008, **14**:6–15.
16. Korhonen JM, Danielsen M, Mayo B, Egervärn H, Axelsson L, Huys G: Antimicrobial susceptibility and proposed microbiological cut-off values of lactobacilli by phenotypic determination. *Int J Probiot Prebiot* 2008, **3**:257–268.
17. Mayrhofer S, van Hoek AHAM, Mair C, Huys G, Arts HJM, Kneifel W: Antibiotic susceptibility of members of the *Lactobacillus acidophilus* group using broth microdilution and molecular identification of their resistance determinants. *Int J Food Microbiol* 2010, **144**:81–87.
18. Foo HL, Loh TC, Law FL, Lim YZ, Kufli CN, Gulam R: Effects of feeding *Lactobacillus plantarum* I-UL4 isolated from Malaysian Tempeh on growth performance, faecal flora and lactic acid bacteria and plasma cholesterol concentrations in postweaning rats. *Food Sci Biotechnol* 2003, **4**:403–408.
19. Foo HL, Loh TC, Lim YS, Syukriyah MH, Kufli CN, Law FL: Effect of fermented fruits on growth performance, shedding of Enterobacteriaceae and Lactic Acid Bacteria and plasma cholesterol on rats. *Pakistan J Nut* 2003, **2**:228–233.
20. Bauer AW, Kirby WMM, Sherris JC, Turck M: Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966, **45**:493–496.
21. SAS Institute: *SAS/STAT User's Guide, Version 6*. 5th edition. Cary, NC: SAS Institute Inc; 1999.
22. Hillmi HTA, Surakka A, Apajalahti J, Saris PEJ: Identification of the most abundant *Lactobacillus* species in the crop of 1- and 5-week-old broiler chickens. *Appl Environ Microbiol* 2007, **73**:67–73.
23. Kaktcham PM, Zambou NF, Tchouanguep FM, El-Soda M, Choudhary ML: Antimicrobial and safety properties of lactobacilli isolated from two Cameroonian traditional fermented foods. *Sci Pharm* 2012, **80**:189–203.
24. Lavanya B, Sowmiya S, Balaji S, Muthuvelan B: Screening and characterization of lactic acid bacteria from fermented milk. *Br J D Sci* 2011, **2**:5–10.
25. Tyler LH, Roberta JW, Christian M: Potent small molecule suppression of oxacillin resistance in methicillin resistant *Staphylococcus aureus*. *Angew Chem Int Ed* 2012, **51**:11254–11257.
26. Gilchrist MJ, Greko C, Wallinga DB, Beran GW, Riley DG, Thorne PS: The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. *Environ Health Perspect* 2007, **115**:313–316.
27. Bassam YK: *Leuconostoc mesenteroides* cause Nosocomial UTI at a tertiary care center in North India. *J Thi-Qar Univer* 2011, **4**:23–34.
28. Sieo CC, Norhani A, Tan WS, Ho YW: Plasmid profiling and curing of *Lactobacillus* strains isolated from the gastrointestinal tract of chicken. *J Microbiol* 2005, **43**:251–256.
29. Fukao M, Tomita H, Yakabe T, Nomura T, Ike Y, Yajima N: Assessment of antibiotic resistance in probiotic strain *Lactobacillus brevis* KB290. *J Food Protect* 2009, **72**:1923–1929.
30. Ouoba LI, Lei V, Jensen LB: Resistance of potential probiotic lactic acid bacteria and bifidobacteria of African and European origin to antimicrobials: Determination and transferability of the resistance genes to other bacteria. *Int J Food Microbiol* 2008, **121**:217–224.
31. Blandino G, Milazzo I, Fazio D: Antibiotic susceptibility of bacterial isolates from probiotic products available in Italy. *Microbiol Ecol Health Dis* 2008, **20**:199–203.
32. Majhenič AC, Matijašič BB: Antibiotics influence on lactic acid bacteria inhabiting gastrointestinal tract. *Mljekarstvo* 2001, **51**:119–134.
33. Sigrid M, Angela HAM, Christiane M, Geert H, Henk JM, Wolfgang K, Konrad JD: Antibiotic susceptibility of members of the *Lactobacillus acidophilus* group using broth micro dilution and molecular identification of their resistance determinants. *Int J Food Microbiol* 2010, **144**:81–87.
34. Vankerckhoven V, Geert HH, Vancanneyt M, Klared CVI, Romonde MB, Entenzaf JM, Moreillon P, Windg RD, Knolg J, Wiertz E, Poti B, Vaughanj EE, Kahlmeterl KG, Gossensa H: Biosafety assessment of probiotics used for human consumption: recommendations from the EU-PROSAFE project. *Tren Food Sci Technol* 2008, **19**:102–114.
35. Toomey N, Bolton D, Fanning S: Characterization and transferability of antibiotic resistance genes from lactic acid bacteria isolated from Irish pork and beef abattoirs. *Res Microbiol* 2010, **161**:127–135.
36. Devirgiliis C, Barile S, Caravelli A, Coppola D, Perozzi G: Identification of tetracycline and erythromycin resistant Gram-positive cocci within the fermenting microflora of an Italian dairy food product. *J Appl Microbiol* 2010, **109**:313–323.
37. Lengkey HAW, Balia RL, Togoe I, Taşbac BA, Ludong M: Isolation and identification of lactic acid bacteria from raw poultry meat. *Biotechnol Anim Hus* 2009, **25**:1071–1077.

doi:10.1186/1757-4749-6-1

Cite this article as: Shazali et al.: Prevalence of antibiotic resistance in lactic acid bacteria isolated from the faeces of broiler chicken in Malaysia. *Gut Pathogens* 2014 **6**:1.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

