JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Presence of *bla*$_{CTX-M}$ antibiotic resistance gene in *Lactobacillus* spp. isolated from Hirschsprung diseased infants with stoma

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Abstract

Introduction: Although antibiotics have revolutionized health care by saving lives, the evolution of both pathogenic and commensal antibiotic-resistant bacteria are emerging as a threat in the health sector. As for *Lactobacillus* spp., it is usually a non-pathogenic bacteria. However, it can cause infection in immunocompromised condition. In this study, *Lactobacillus* spp. has been isolated from the faeces of infants with Hirschsprung disease (HD), which is congenital aganglionosis of intestine, where surgical approach and antibiotics are frequently used as medical intervention. The aim of this study is to assess the antibiotic resistance pattern and determine the presence of resistance genes, if any, in *Lactobacillus* spp. isolated from HD infants with ileostomy.

Methodology: Six *Lactobacillus* spp. were isolated from faeces of six HD infants and confirmed using both conventional and molecular methods. Antibiotic resistance pattern was checked through disc diffusion method and was further investigated for the presence of antibiotic resistance genes (*bla*$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{OXA-2}$, *bla*$_{IMP}$, *bla*$_{VIM-2}$, *bla*$_{NDM-1}$ and *mcr-1)*.

Results: Antibiotic susceptibility of the isolates showed high level of resistance towards cephalosporins, oxacillin, aztreonam, meropenem and polymyxin group. However, four of the isolates showed the presence of *bla*$_{CTX-M}$ gene after PCR amplification.

Conclusions: To our knowledge, this is the first report on the presence of antibiotic resistance gene *bla*$_{CTX-M}$ in *Lactobacillus* spp. and this presence may pose a serious threat in treatment regimen. As not much is known regarding the presence of *bla*$_{CTX-M}$ in *Lactobacillus* spp., this finding may provide new light to research on antibiotic resistance in gut microflora.

Key words: Hirschsprung disease; antibiotic resistance; *Lactobacillus* spp.; *bla*$_{CTX-M}$ gene.

J Infect Dev Ctries 2019; 13(5):426-433. doi:10.3855/jidc.10968

(Received 17 October 2018 – Accepted 25 February 2019)

Introduction

Hirschsprung disease (HD) is a congenital anomaly in an infant represented with parasympathetic aganglionosis in variable length of the intestine [1]. A fatal complication of this congenital condition is associated with enterocolitis, which is mostly manifested by severe diarrhoea, abdominal pain with distension as well as shock and may cause the death of the infant [2]. To manage this condition, different preventive and therapeutic measures have been reported earlier, which include antibiotics and probiotics as prophylactic interventions as well as different surgical approaches [3].

On the other hand, *Lactobacillus* spp. are common residents of the human gastrointestinal tract from starting of human life along with other resident gut microbes [4]. It is established that *Lactobacillus* spp. has a probiotic effect and play beneficial roles in the gut by executing antimicrobial, anti-infective, anti-inflammatory as well as immunomodulatory activities [5,6]. However, an altered scenario also exists, where it might cause infection in immunocompetent patients. In several studies, *Lactobacillus* spp. had been identified as a causative agent for infective endocarditis, intraabdominal abscess as well as meningitis [7,8]. It may likewise cause bacteremia during pregnancy or other immunosuppressive condition and driving towards death in some cases [9,7].

To check for antibiotic susceptibility pattern and resistance gene in *Lactobacillus* spp. isolated both from food and human is a comparably recent approach [10]. High resistance level towards different antibiotics observed in *Lactobacillus* spp. is getting importance as it can act as a reservoir of antibiotic resistance genes and can be transferred to other bacteria residing in the human gut horizontally by conjugation, natural transfer and transduction [11,12]. Up to now, the presence of several antibiotic resistance genes, both intrinsic and

Table 1. The oligonucleotide primers to identify *Lactobacillus* spp. along with annealing temperature and amplicon size.

Primer sequence (5'-3')	Annealing temperature (^{0}C)	Amplicon size (bp)	References
Forward primer: 5'-GAGGCAGCAGTAGGGAATCTTC-3' Reverse primer: 5'-GGCCAGTTACTACCTCTATCCTTCTTC-3'	58	126	[33]

acquired, have been reported to be found in *Lactobacillus* spp. As for example, tetracycline resistance genes *tet*M, *tet*S, *tet*W, *tet*O, *tet*L as well as erythromycin resistance genes *erm*A, *erm*B were reported as prevalent, among which *tet*M, *tet*S and *erm*B are suggestive to be acquired genes in *Lactobacillus* spp. [12,13].

On the other hand, very few information was found regarding the presence of the β-lactamase resistance gene in Gram-positive Lactic Acid Bacteria (LAB). Though production of β-lactamase enzyme has been the major mechanism adopted by the bacteria for defence against β lactam, the largest class among all the antibiotics, the resistance is mainly shown by Gram-negative bacteria [14]. Among the four classes of β-lactamase enzymes, as per molecular classification, class A and B has been reported earlier in Gram-positive organisms [15,16]. Besides, a study in recent years has reported the existence of class D β-lactamase in Gram-positive organisms, in which the lack of a conserved arginine residue has been shown to be a "unique substrate binding mode" claiming a cause of high resistance level [17]. In connection to it, a few β-lactam resistance genes naming *bla*Z, *mec*A and bla_{BCL-1} were reported in Gram-positive organisms, among which *bla*Z was found in *Lactobacillus* spp. [18,19]. Regardless the availability of the clinical data about the resistance pattern of different antibiotics in *Lactobacillus* spp.; not much is known about the presence of corresponding some other β-lactamases, carbapenemases and colistin resistance genes such as bla_{TEM}, bla_{CTX-M}, bla_{OXA-2}, bla_{IMP}, bla_{NDM-1}, bla_{VIM-2} as well as *mcr-1* in the same bacterial group [12,20,21]. Therefore, in the present study, antibiotic resistance pattern of *Lactobacillus* spp., isolated from HD infants was investigated through the disc diffusion method as well as molecular methods to check the presence of different antibiotic resistance genes.

Methodology

Subject determination and sample collection

Stool samples were collected from six diagnosed and surgically treated HD infants aged between 40 to 48 weeks with no obtrusive procedure. All the procedures were, undertaken following ethical issues set by the Faculty of Biological Sciences, University of Dhaka, Bangladesh in compliance with the Helsinki Declaration. Informed consents were taken from parents of the infants. As per the history collected from the parents of the infants, they were both breastfed and formula fed and were treated with antibiotics for treatment purpose.

Identification of Lactobacillus *spp.*

About six isolated single colonies were identified by morphological characteristics and biochemical tests as *Lactobacillus* spp. and were further confirmed by PCR amplification [22]. Template DNA was prepared using Qiagen DNA Mini kit and stored in -20^{0}C. The oligonucleotide sequences used as forward and reverse primers' based on 16S rDNA and the annealing temperature that was used for thermal cycler are shown in Table 1.

Antibiotic susceptibility assay

For the antibiotic susceptibility tests, disc diffusion method was performed as described by Rojo-Bezares *et al*. [23] where, MRS agar was used instead of Muller Hinton Media, as the growth of *Lactobacillus* spp. was poor.

Antibiotic resistance gene determination

After selecting the resistant strains, PCR amplification of the template DNA were done using primers, specific for antibiotic resistance genes. Seven primers bla_{TEM}, bla_{CTX-M} bla_{OXA-2}, bla_{IMP}, bla_{NDM-1}, bla_{VIM-2} and *mcr-1* were used to confirm the presence of the antibiotic resistance genes. As a positive control for these PCR amplification, previously identified *Pseudomonas aeruginosa* strains containing those genes were used and for negative control, mastermix for PCR amplification without any DNA sample was used as negative control to check for contamination. Table 2 shows the oligonucleotide sequences of these seven antibiotic resistance genes. Agarose gel electrophoresis was used to detect the gene-specific bands, if any, with the help of a UV transilluminator.

Results

All the six bacteria isolated from stool samples and primarily identified as *Lactobacillus* spp. were confirmed through PCR amplification where the positive control was previously identified *Lactobacillus* spp. in the lab. All these six isolates showed amplicon size of 126 bp and named respectively as HD-a, HD-b, HD-c, HD-d, HD-e and HD-f (Figure 1).

To compare the results of the antibiotic susceptibility assay, the known standard described in the CLSI guideline (Clinical and Laboratory Standards Institute, 2015) was used for antimicrobial susceptibility testing as has been mentioned previously, where zone diameter > 20 mm was noted as susceptible, 15-19 mm as intermediate and ≤ 14 mm as resistant [20]. Table 3 shows the zone diameter of the six *Lactobacillus* spp. found in disc diffusion method assay for different antibiotics, where numerical scoring system has been used for intermediate susceptible to depict clearly whether the marginal values inclined towards either sensitivity or resistance. This has been avoided for resistant and susceptible ones to reduce clumsiness as it has been used by other workers [20].

While checking the antibiotic resistance genes of the isolated *Lactobacillus* spp. in the agarose gel, four isolates naming HD-a, HD-b, HD-e and HD-f showed bands at desired level for the *bla*$_{CTX-M}$ gene (Figure 2). However, *bla*$_{TEM}$, *bla*$_{OXA-2}$, *bla*$_{IMP}$, *bla*$_{NDM-1}$, *bla*$_{VIM-2}$ and *mcr-1* showed no band after PCR amplification (Figure 3).

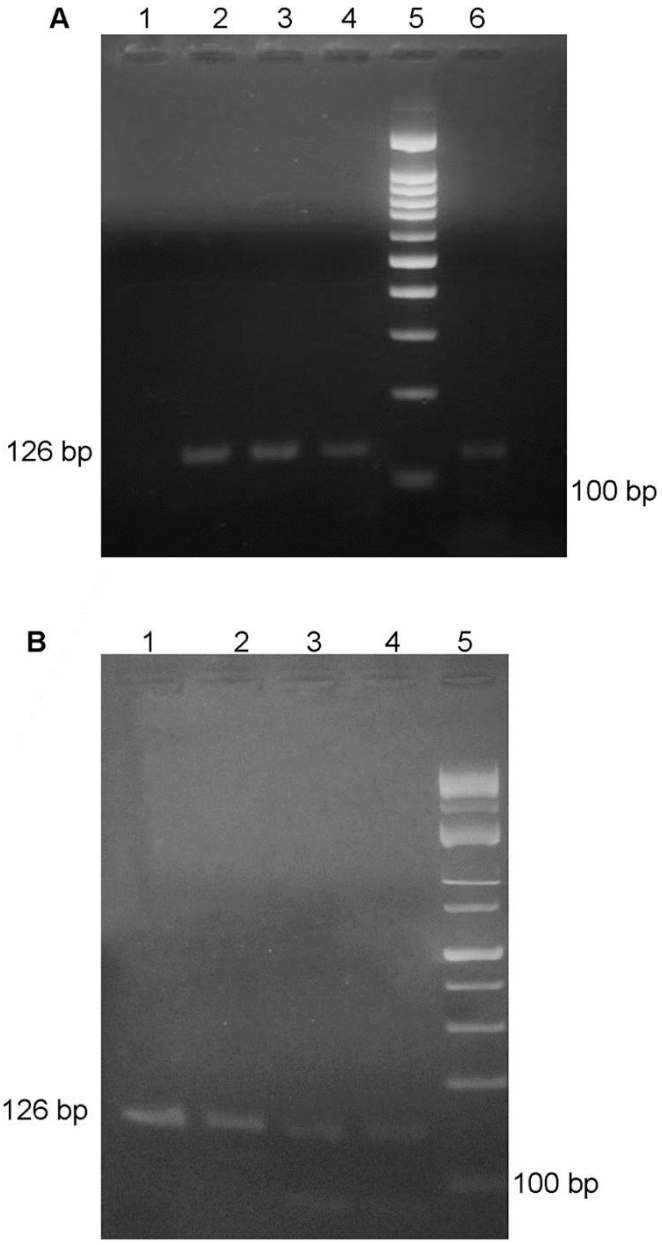
Discussion

Several studies have been performed to check evidence regarding the rise of antibiotic resistance in *Lactobacillus* spp. [12,13,20]. Starting from cell wall inhibitor antibiotics, *Lactobacillus* spp. are the most susceptible to penicillin G, ampicillin and oxacillin, though they show more resistance towards cephalosporins [12]. In contrast, maximum resistance was observed towards oxacillin in the current study, followed by penicillin G and cloxacillin. However, all six isolates were susceptible to amoxiclav (Amoxicillin/clavulanic acid). Among the aminoglycosides, high resistance by *Lactobacillus* spp. has been frequently reported [24]. Likewise, in the present study, most of the isolates from HD infants showed resistance against kanamycin, gentamycin, amikacin, tobramycin and streptomycin. Only two isolates HD-b, HD-c exhibited sensitivity towards kanamycin and amikacin respectively. Regarding quinolones, which inhibits nucleic acid synthesis in bacteria, four among the six isolates showed resistance to ciprofloxacin, norfloxacin and levofloxacin, while they showed susceptibility towards moxifloxacin. Nevertheless, all these isolates were found to be highly resistant to nalidixic acid, as was also described by Sharma *et al*. [25].

Table 2. Oligonucleotide sequence of seven antibiotic resistant genes with annealing temperature and amplicon size.

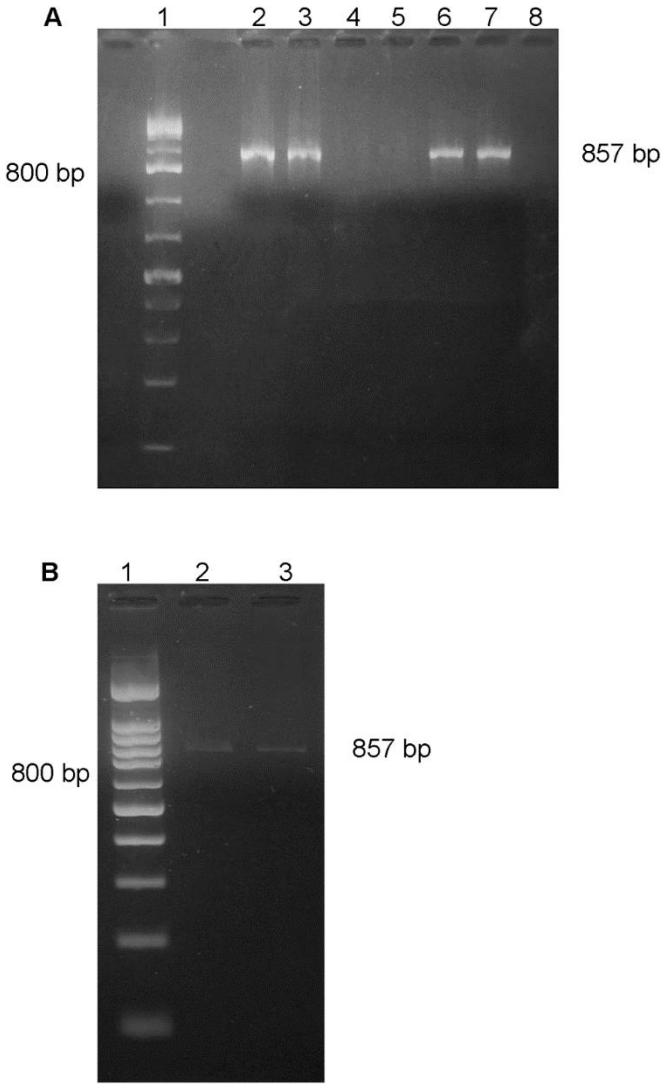
Primer sequence of antibiotic resistant gene (5'-3')	Annealing temperature (^{0}C)	Amplicon size (bp)	References
bla$_{TEM}$ Forward: 5'-AAAATTCTTGAAGACG-3' Reverse: 5' TTACCAATGCTTAATCA-3'	58	768	[34]
bla$_{CTX-M}$ Forward: 5'-ACGCTGTTGTTAGGAAGTG-3' Reverse: 5'-TTGAGGCTGGGTGAAGT-3'	58	857	[35]
bla$_{OXA-2}$ Forward: 5'-TTCAAGCCAAAGGCACGATAG-3' Reverse: 5'-TCCGAGTTGACTGCCGGGTTG-3'	65	702	[36]
bla$_{IMP}$ Forward: 5'- GAAGGCGTTTATGTTCATAC-3' Reverse: 5'-GTATGTTTCAAGAGTGATGC-3'	54	587	[31]
bla$_{NDM-1}$ Forward: 5'-ACCGCCTGGACCGATGACCA-3' Reverse: 5'-GCCAAAGTTGGGCGCGGTTG-3'	63	200	[32]
bla$_{VIM-2}$ Forward: 5'-AAAGTTATGCCGCACTCACC-3' Reverse: 5'-TGCAACTTCATGTTATGCCG-3'	59	864	[32]
mcr-1 Forward: 5'-CGGTCAGTCCGTTTGTTC-3' Reverse: 5'-CTTGGTCGGTCTGTAGGG-3'	45	309	[30]

Figure 1. Molecular identification of *Lactobacillus* spp. by PCR amplification.



Template DNA was prepared from all six isolates primarily identified as *Lactobacillus* spp. Positive control used here was previously identified *Lactobacillus* spp. and negative control was the mastermix used for PCR amplification without any DNA sample to check for contamination. After PCR amplification, all the six isolates showed an amplicon size of 126 bp to confirm them as members of *Lactobacillus* spp. and named respectively as HD-a, HD-b, HD-c, HD-d, HD-e and HD-f.
Here, in Figure 1(a): Lane 1- Negative control, Lane 2- HD-a, Lane 3- HD-b, Lane 4- HD-e, Lane 5-100 bp ladder and Lane 6 showing positive control. In Figure 1(b); Lane 1- Positive control, Lane 2- HD-c, Lane 3- HD-d, Lane 4- HD-f, Lane 5- 100 bp ladder.\

Figure 2. Presence of bands at the desired level in agarose gel for antibiotic resistance gene *bla*$_{CTX-M}$ in *Lactobacillus* spp.



After selecting the resistant strains, PCR amplification of the template DNA was done using primers, specific for antibiotic resistance genes. Positive control used here was previously identified *Pseudomonas aeruginosa* strain containing *bla*$_{CTX-M}$ gene and negative control was the mastermix used for PCR amplification without any DNA sample to check for contamination. In the agarose gel, four isolates naming HD-a, HD-b, HD-e and HD-f showed bands in the desired level for the *bla*$_{CTX-M}$ (857 bp) gene. Here, in Figure 2(a); Lane 1-100 bp ladder, Lane 2- HD-a, Lane 3- HD-b, Lane 4- HD-c, Lane 5- HD-d, Lane 6-Positive control, Lane 7- HD-e, Lane 8 showed negative control. In Figure 2(b) Lane 1-100 bp ladder, Lane 2- Positive control, Lane 3- HD-f.

Table 3. Antibiotic sensitivity pattern of *Lactobacillus* spp.

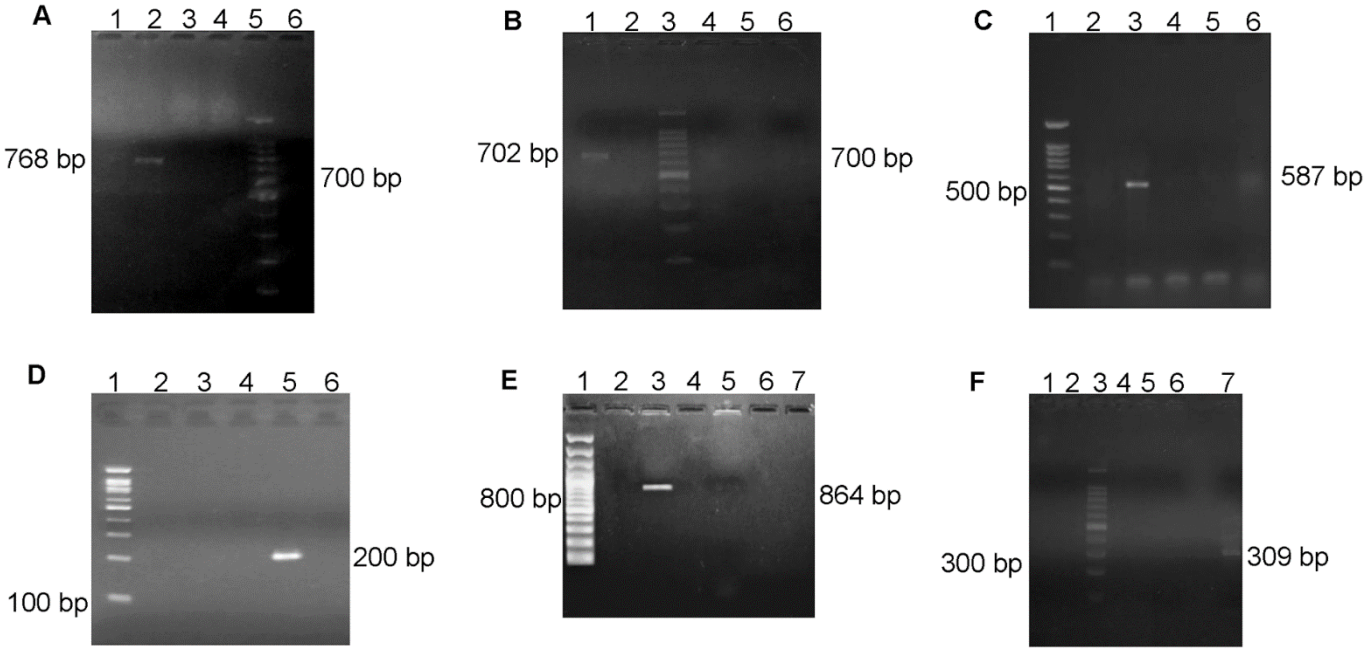
Group	Name of antibiotic	Concentration	Antibiotic resistance pattern					
			HD-a	HD-b	HD-c	HD-d	HD-e	HD-f
β-lactams	Penicillin G	10 IU	S	R	S	S	R	S
	Ampicillin	10 µg	S	S	S	S	R	S
	Amoxicillin	10 µg	S	S	S	S	R	R
	Amoxiclav (Amoxicillin/ Clavulanic acid)	20µg/ 10 µg	S	S	S	S	S	S
	Cloxacillin	5 µg	S	S	18 ± .35	18 ± 0	R	R
	Oxacillin	1 µg	R	R	15 ± .71	S	R	R
Aminoglycosides	Kanamycin	30 µg	R	S	R	R	R	R
	Gentamycin	10 µg	R	19 ± .71	R	15 ± .71	R	R
	Amikacin	30 µg	R	R	S	15 ± .71	R	R
	Tobramycin	10 µg	R	19 ± .71	R	R	R	R
	Streptomycin	10 µg	R	R	R	R	R	R
	Netilmicin	30 µg	15 ± .35	16 ± .71	16 ± .71	18 ± .71	R	R
Quinolones	Ciprofloxacin	5 µg	S	18 ± 0	R	R	R	R
	Norfloxacin	10 µg	S	18 ± .35	R	R	R	R
	Ofloxacin	5 µg	S	16 ± .35	R	18 ± .35	R	R
	Nalidixic acid	30 µg	R	R	R	R	R	R
	Levofloxacin	5 µg	S	18 ± .71	R	R	R	R
	Moxifloxacin	5 µg	S	S	S	17 ± .35	S	S
	Pefloxacin	5 µg	18 ± .35	16 ± .35	R	R	R	15 ± .35
Cephalosporins	Ceftriaxone	30 µg	19 ± .35	S	S	S	S	S
	Cefuroxime	30 µg	S	R	S	S	R	R
	Cefepime	30 µg	S	R	S	S	R	R
	Ceftazidime	30 µg	R	R	15 ± .35	17 ± .71	R	R
	Cefexime	5 µg	R	R	18 ± 0	R	R	R
	Cefoxitin	30 µg	R	R	R	18 ± .35	R	R
	Cephalexin	30 µg	R	R	S	S	R	R
Tetracycline	Tetracycline	30 µg	S	R	S	S	R	S
	Doxycycline	30 µg	S	18 ± .35	S	S	S	S
	Minocycline	30 µg	S	18 ± .35	S	S	S	S
	Tigecycline	15 µg	S	S	S	S	R	S
Macrolides	Azithromycin	15 µg	S	R	S	S	S	R
	Erythromycin	15 µg	S	S	S	S	S	S
Glycopeptides	Vancomycin	5 µg	R	R	R	R	R	R
Monobactam	Aztreonam	30 µg	R	R	R	R	R	R
Carbapenem	Imipenem	10 µg	S	S	S	S	15 ± .35	14 ± .71
	Meropenem	10 µg	R	R	S	S	R	R
Polymyxin	Polymyxin B	300 IU	R	R	R	R	R	R
	Colistin Sulphate	10 µg	R	R	R	R	R	R
Oxazolidinone	Linezolid	30 µg	S	S	S	S	R	S
Aminocoumarin	Novobiocin	30 µg	S	S	18 ± .35	19 ± 0	S	S
Sulfonamides	Cotrimoxazole (trimethoprim /suiphamethoxazole)	85 µg	R	R	R	R	R	R
Azolidione	Nitrofurantoin	300 µg	S	S	S	S	S	S
Lincosamides	Clindamycin	2 µg	S	15 ± .35	S	S	R	18 ± 0
Others	Chloramphenicol	30 µg	S	S	S	S	S	14 ± .71
	Piperacillin/Tazobactam	100µg /10µg	S	R	S	S	R	R
	Rifampicin	5 µg	S	S	S	S	R	R
	Metronidazole	50 µg	R	R	R	R	R	R

Here, the experiment was done in triplicate. Zone diameter for being intermediate resistant are given in numerical values with standard deviation (SD) to depict clearly whether the marginal values inclined towards sensitivity or resistance. This has been avoided for resistant and susceptible one to reduce clumsiness. The unit is mm (millimeter). For others, to depict if they are Susceptible (S) or Resistant (R) notation are included (HD-a, HD-b, HD-c, HD-d, HD-e, HD-f are the name of the isolates).

Among the cephalosporins, ceftriaxone was found to be most active against the *Lactobacillus* spp. The isolates described in this study were found to be highly resistant towards ceftazidime, cefixime and cefoxitin and only two isolates (HD-c, HD-d) showed intermediate susceptibility. In several other previous studies regarding the susceptibility pattern of the cephalosporins on *Lactobacillus* spp. both susceptibility and resistance had been reported [12,25]. However, functional reasons for this susceptibility or resistance are yet to explore in detail. Furthermore, all the study isolates were also resistant to aztreonam. Again, some previous studies have exhibited that, most *Lactobacillus* spp. are susceptible to antibiotics like erythromycin, tetracycline and chloramphenicol, although a few were found to be resistant towards azithromycin [24,25]. Similar to this, all six *Lactobacillus* spp. of this study, expressed intermediate to high susceptibility towards chloramphenicol and erythromycin. However, for azithromycin, except for HD-b and HD-f, other four isolates showed susceptibility. In terms of the tetracycline group, the isolates showed intermediate to high susceptibility towards doxycycline and minocycline, while two of them naming HD-b and HD-e were resistant to tetracycline; and HD-e to tigecycline. Additionally, a number of different studies suggested *Lactobacillus* spp. being intrinsically resistant to vancomycin, though it is effective against other Gram-positive bacteria [26,12]. Accordingly, in this study, all six isolates were found to be highly resistant to vancomycin.

In recent years, researchers have also isolated carbapenem-resistant *Lactobacillus* spp. [21]. In this study, among the carbapenem group, imipenem was found to be most effective against the isolates, while they exerted resistance towards meropenem, other than the isolate HD-c and HD-d, which were found to be susceptible as described by other workers [20,21]. Moreover, all the isolates of the present study showed high resistance to antibiotics like polymyxins (polymyxin B, colistin sulphate), metronidazole, co-trimoxazole, and susceptibility towards nitrofurantoin

Figure 3. PCR results of antibiotic resistance genes (*bla*$_{TEM}$, *bla*$_{OXA-2}$, *bla*$_{IMP}$, *bla*$_{NDM-1}$, *bla*$_{VIM-2}$ and *mcr-1*) in *Lactobacillus* spp. respectively.



The resistant strains were evaluated for the presence of antibiotic resistance genes *(bla*$_{TEM}$, *bla*$_{OXA-2}$, *bla*$_{IMP}$, *bla*$_{NDM-1}$, *bla*$_{VIM-2}$ *and mcr-1)* by PCR amplification with appropriate control. Positive control used here was previously identified *Pseudomonas aeruginosa* strain containing those genes accordingly and negative control was the mastermix used for PCR amplification without any DNA sample to check for contamination. However, none of the strains (HD-a, HD-b, HD-c, HD-d, HD-e, and HD-f) revealed bands at the desired level for these genes. Here, for antibiotic resistance gene *bla*$_{TEM}$ (768 bp) in Figure 3(a); Lane 1- HD-a, Lane 2- Positive control, Lane 3- HD-b, Lane 4- HD-e, Lane 5-100 bp ladder and Lane 6- Negative control. For *bla*$_{OXA-2}$ (702 bp) in Figure 3(b); Lane 1- Positive control, Lane 2- HD-a, Lane 3- 100 bp ladder, Lane 4- HD-e, Lane 5- Negative control and Lane 6- HD-f. For *bla*$_{IMP}$ (587 bp) in Figure 3(c); Lane 1-100 bp ladder, Lane 2- Negative control, Lane 3- Positive control, Lane 4- HD-c, Lane 5- HD-d, Lane 6- HD-f. For *bla*$_{NDM-1}$ (200 bp) in Figure 3(d); Lane 1-100 bp ladder, Lane 2- HD-a, Lane 3- HD-c, Lane 4- HD-e, Lane 5- Positive control, Lane 6- Negative control. For *bla*$_{VIM-2}$ (864 bp) in Figure 3(e); Lane 1-100 bp ladder, Lane 2- HD-a, Lane 3- Positive control, Lane 4- HD-b, Lane 5- HD-c, Lane 6- HD-e, Lane 7- Negative control. For *mcr-1* (309 bp) in Figure 3(f); Lane 1- HD-b, Lane 2- HD-d, Lane 3-100 bp ladder, Lane 4- HD-e, Lane 5- HD-f, Lane 6- Negative control, Lane 7- Positive control. Agarose gel image for negative results of all strains was not included to avoid redundancy.

as well as rifampicin, other than HD-e and HD-f, as has been reported by others [27].

In terms of avoiding antibiotic actions, different antibiotic-inactivating enzymes are produced by bacteria such as β-lactamase, which is a crucial one [17]. Various studies have shown β-lactamase production evidence in Gram-negative bacteria, in which multidrug-resistant Gram-negative bacteria, producing extended spectrum β-lactamase (ESBL) has become a recent health concern [28,29,14]. The major ESBL enzymes are TEM, SHV and CTX-M and among these three, multiple variants of CTX-M producing isolates are increasing rapidly across the world [28,29]. The dissemination of *bla*$_{CTX-M}$ gene has been enormous due to the presence of transposons within the multidrug resistance region of the ESBL producing bacteria [29]. However, one the most effective therapeutic drug for the ESBL producing bacteria has been carbapenems, though carbapenemase enzymes (KPC, VIM, IMP, NDM-1, and OXA) production in bacteria had also been reported [28,29]. Another therapeutic option for such multidrug-resistant bacteria can be colistin, which may also become resistant due to the presence of *mcr* genes [30].

Despite showing antibiotic resistance when identified by disc diffusion, none of the six isolates showed the presence of antibiotic resistance genes (*bla*$_{TEM}$, *bla*$_{OXA-2}$, *bla*$_{IMP}$, *bla*$_{NDM-1}$, *bla*$_{VIM-2}$ and *mcr-1*) after PCR amplification, where the positive control was previously identified *Pseudomonas aeruginosa* strain containing those genes accordingly. However, when the isolates were further investigated for the *bla*$_{CTX-M}$ gene, four of the isolates (HD-a, HD-b, HD-e and HD-f) showed band at the desired level after amplification by PCR in which the positive control was molecular class C ESBL producing *Pseudomonas aeruginosa* strain containing *bla*$_{CTX-M}$ gene in the lab. In all the cases *Pseudomonas aeruginosa* has been used as control strain as there are several studies claiming the presence of those genes in it along with functional studies[31,32].

Previous studies have shown that there has been a huge rise in the number of organisms that produce multiple variants of β-lactamase and are mostly reported in Gram-negative bacteria [14]. However, not much has been known about the *bla*$_{CTX-M}$ gene in Gram-positive bacteria, especially *Lactobacillus* spp. The presence of antibiotic-resistant *Lactobacillus* spp. in HD infants may be able to transfer the resistance gene to other pathogenic bacteria, which will make the treatment procedure more complicated.

Conclusion

In the present study, the presence of *bla*$_{CTX-M}$ in *Lactobacillus* spp. isolates provide evidence of the resistance gene in Gram-positive bacteria. As CTX-M enzymes have multiple variants, further investigation is required for the detection of any alteration or mutation in the gene found from the *Lactobacillus* spp. isolated from HD infants, which will help us to improve the treatment regimen in enterocolitis like complications.

Acknowledgements

The authors thank the Mathematics and Natural Sciences Department of BRAC University (Dhaka, Bangladesh) for supporting this work. The authors would like to thank the study participants. The authors also acknowledge Dr Hossain Shahid Kamrul Alam and Asma Binte Afzal for their kind support.

Author Contributions

The study was conceived by CRA and UK. Sample collection, experiments, result interpretation was done by UK, SA, MK and MH. The manuscript was prepared by CRA and NC and all authors contributed to the final version.

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Conflict of interests: No conflict of interests is declared.