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Antibiogram of Lactobacilli Isolated from Four Different Niches

Keywords: Lactobacilli; Antibiotic resistance; Cell wall inhibitors; Protein synthesis inhibitors

Abstract

Presence of antibiotic resistance in lactobacilli, invites special attention from the public health point of view as they are widely used as starter cultures and probiotics. *Lactobacillus,* a ubiquitous genus, is expected to have an antibiogram that vary remarkably with their source. In the present work antibiotic resistance/sensitivity of *Lactobacillus* species isolated from four different sources: carrot, idli batter, curd and duck faeces were assessed by the Disc diffusion assay. Contrary to the earlier reports of general sensitivity of lactobacilli to the inhibitors of cell wall synthesis, all the four *Lactobacillus* isolates were found to be resistant against the cell wall synthesis inhibitors ampicillin and cephalosporins (cefalexin and cefixime) irrespective of their source of isolation. However the isolates were found to be sensitive to protein synthesis inhibitors like azithromycin, tetracycline, gentamicin, clindamycin and chloramphenicol. In general the isolates were found to be more resistant to those antibiotics that act by inhibiting cell wall synthesis than those which act by inhibiting the protein synthesis. This observation of multidrug resistance by the lactobacilli isolated from different niches reemphasize the significance of considering the antibiogram study as a critical criteria while screening lactobacilli isolates for use in food formulations. Molecular level studies are indicated to have an in-depth understanding of the basis of resistance.

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Introduction

Lactic acid bacteria (LAB) are Gram-positive, catalase negative, acid -tolerant and non-spore forming cocci and rods. The common feature of the ability of LAB to produce lactic acid as a major end product of fermentation of hexoses makes them the potential agents for most of the food fermentations. The LAB are present in a number of natural habitats and are widely utilized as starter cultures and probiotics. Among LAB, The genus *Lactobacillus* is the largest group comprising around 140 species and 30 subspecies [1,2]. Though Lactobacilli have a long history of safe use in the production of fermented foods and beverages, recent reports that indicate that the commensal bacteria including lactic acid bacteria (LAB) could act as reservoirs of antibiotic resistance genes raises concern about the safety of their use [3,4]. Considering the reports of possibility of horizontal transfer of resistance genes between bacterial species, research on the antibiotic resistance of lactobacilli has gained much momentum nowadays. Lactobacilli are intentionally added to food for carrying out desirable fermentations and in the case of probiotic products they should essentially retain their viability during passage through the GIT (Gastrointestinal Tract). So the chances are high that they get ample opportunities to act as potential reservoirs for the transfer of antibiotic resistance genes to pathogenic bacteria. Hence safety evaluation of *Lactobacillus* isolates is highly critical to ensure consumer well-being. Such an assessment also helps in identifying lactobacilli that could be advised for replenishing gastrointestinal microflora during antibiotic therapy. Information in this regard will also be helpful in designing media with selective properties for the isolation of specific lactobacilli from mixed bacterial populations. Also considering the possibility that their natural habitats might be having a profound influence on their antibiotic resistance pattern, this study was designed to conduct the safety assessment of four lactobacilli isolates from different sources and maintained in the culture collection of Dairy Microbiology Department, College of Dairy Science and Technology, Mannuthy, Thrissur, Kerala, through antibiotic-susceptibility assays.

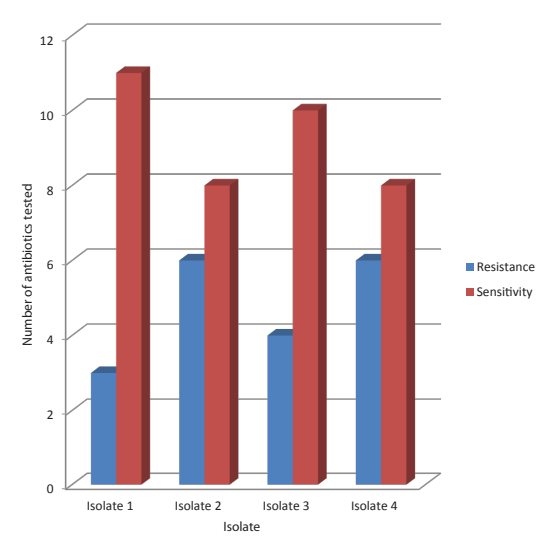

Figure 1: Antibiotic resistance/Sensitivity exhibited by the lactobacilli isolates.

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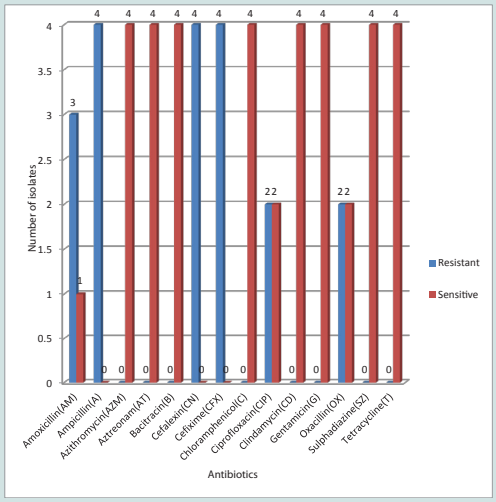

Figure 2: Antibiotic wise resistance/Sensitivity pattern exhibited by the isolates.

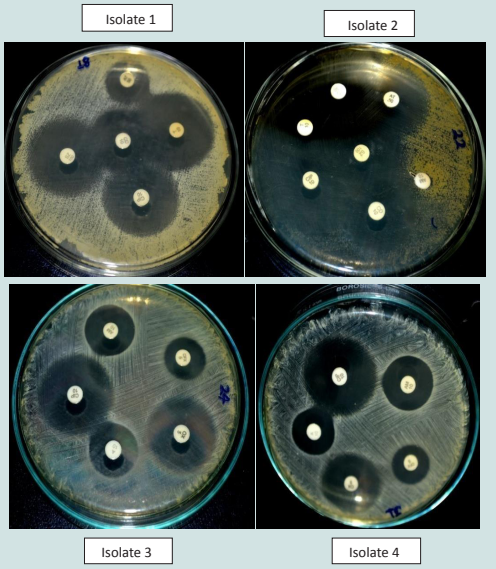

Figure 3: Mueller-Hinton agar plates showing the zone of inhibition by various antibiotics against the isolates.

Materials and Methods

Lactobacilli cultures

Four *Lactobacillus* isolates maintained in the stock culture collection of Department of Diary Microbiology, College of Dairy Science and Technology were used for this study. The isolates (1,2,3 and 4) were from carrot, idli batter, curd and duck faeces respectively. The cultures preserved as glycerol stock were activated by growing in sterile MRS (deMan, Rogosa, Sharpe, Himedia, Mumbai) broth at 37 °C for 24 h. The cultures thus activated were stored under refrigeration with fortnightly activation in sterilized MRS broth. Working cultures were prepared by inoculating 0.1 ml of the stock culture in sterilized MRS broth followed by incubation at 37 °C for 48 hours.

Antibiogram of isolate

The antibiotic susceptibility of the isolate was determined as per the standard method [5]. Activated culture of each isolate was swabbed over the surface of Mueller Hinton agar (Himedia) agar plates. Fourteen different antibiotic discs (Amoxicillin (AM, 10 mcg), Ampicillin (A, 10 mcg), Azithromycin (AZM, 15 mcg), Aztreonam (AT, 50 mcg), Bacitracin (B, 10 units), Cefalexin (CN, 30 mcg), Cefixime (CFX, 5 mcg), Chloramphenicol (C, 30 mcg), Ciprofloxacin (CIP, 10 mcg), Clindamycin (CD, 10 mcg), Gentamicin (G, 50 mcg), Oxacillin (OX, 5 mcg), Sulphadiazine (SZ, 100 mcg), Tetracycline (T, 10 mcg), HiMedia Laboratories Pvt. Ltd, India) were placed over the surface of the inoculated plate. The plates were incubated at 37 °C for 24 h. The diameter of the Zone of inhibition around each disc was measured and expressed in mm. Results were interpreted as sensitive, S (≥ 21 mm); intermediate, I (16-20 mm) or resistant, R (≤ 15 mm) [6].

Results and Discussion

Irrespective of the source of isolation all the isolates exhibited remarkable resistance to a number of antibiotics revealing multidrug resistance (Figure 1). Among the isolates, those from idli batter and duck faeces were resistant against highest number of antibiotics (6/14).

Of the 14 antibiotics tested eight antibiotics namely azithromycin, aztreonam, bacitracin, chloramphenicol, clindamycin, gentamycin, sulphadiazine and tetracycline were found to inhibit all the isolates used in this study (Figure 2). Mueller Hinton agars showing the zone of inhibition of different antibiotics to the tested isolates are shown in Figure 3. In general antibiotics elicit their action by inhibiting synthesis of bacterial cell wall, protein, folate or by inhibiting the action of DNA gyrase. Contrary to the earlier reports of sensitivity of lactobacilli to cell wall inhibitors all the four *Lactobacillus* isolates tested in this study were found to be resistant to half of the cell wall synthesis inhibiting antibiotics tested (Ampicillin, Cefalexin and Cefixime, Table 1 and Figure 4) [7]. Widespread sensitivity toward penicillins has already been reported in lactobacilli used as starter cultures [8]. Contrary to this, a pattern of resistance against Beta-lactams (Amoxicillin, Ampicillin and Oxacillin) was exhibited by the

Table 1: Antibiotic resistance/Sensitivity of the isolates.

Mechanism of Action	Antibiotics	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Cell wall inhibitors	Amoxicillin	S	R	R	R
	Ampicillin	R	R	R	R
	Aztreonam	S	S	S	S
	Bacitracin	S	S	S	S
	Cefalexin	R	R	R	R
	Cefixime	R	R	R	R
	Oxacillin	S	R	S	R
Protein synthesis inhibitors	Azithromycin	S	S	S	S
	Clindamycin	S	S	S	S
	Chloramphenicol	S	S	S	S
	Gentamicin	S	S	S	S
	Tetracyclin	S	S	S	S
DNA gyrase inhibitor	Ciprofloxacin	S	R	S	R
Inhibition of folic acid synthesis	Sulphadiazine	S	S	S	S

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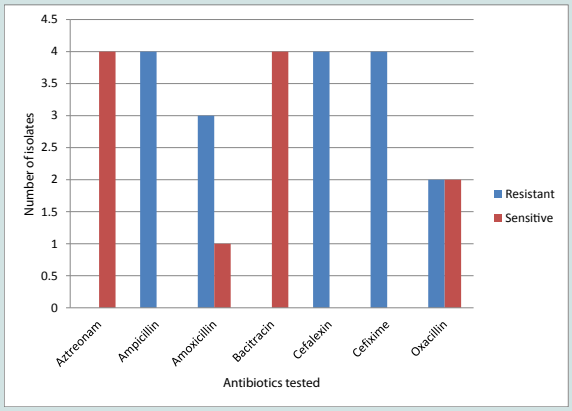

Figure 4: Resistance/Sensitivity of the isolates against cell wall synthesis inhibitors.

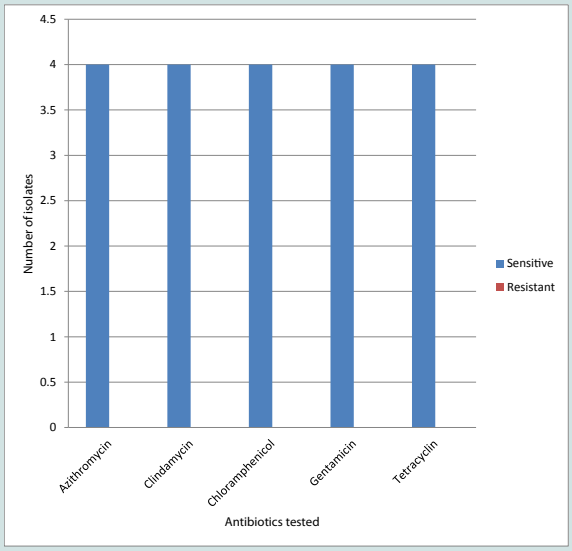

Figure 5: Resistance/Sensitivity of the isolates against protein synthesis inhibitors.

Lactobacillus isolates in the present study. Resistance exhibited by all the isolates against cephalosporin antibiotics (cefalexin and cefixime) is in agreement with the earlier reports of resistance of lactobacilli to cephalosporins [7]. Sensitivity was shown by all the isolates to the cell wall synthesis inhibitors, Bacitracin (targets cell membrane) and Aztreonam. Variations were observed among the isolates in their response to the other two cell wall inhibitors tested namely, amoxicillin and oxacillin. Isolate 1 was sensitive to amoxicillin whereas all other isolates were resistant. In the case of oxacillin, isolates 2 and 4 were resistant to it and others were sensitive. Similar pattern was observed in the case of the DNA gyrase inhibitor, Ciprofloxacin with isolates 2 and 4 being resistant and others sensitive.

All the isolates irrespective of their source of isolation were found to be sensitive to the protein synthesis inhibitors (azithromycin, chloramphenicol, clindamycin, gentamicin and tetracycline, Table 1 and Figure 5). Lactobacilli are reported to be highly resistant to aminoglycosides (gentamycin, kanamycin, streptomycin) that act by inhibiting protein synthesis [9]. Interestingly in this work, contrary to this report all the tested isolates were found to be sensitive to gentamycin, an aminoglycoside. Sensitivity exhibited by the isolates against the antibiotics, chloramphenicol, tetracycline and clindamycin is in agreement with earlier reports of susceptibility of lactobacilli to even low concentrations of many protein synthesis inhibitors including these antibiotics [9]. Sensitivity of the lactobacilli isolates to tetracycline is reported by other reseachers also [7,10]. However contradictory to this, resistance to tetracycline is reported as the most frequent among lactobacilli [11,12]. Earlier reports by a number of authors [8,13-15] ranking the resistance genes, *tet*(M) for tetracycline resistance as most common in LAB also supports the prevalence of tetracycline resistance in LAB. However in the present study all the isolates were found to be sensitive to this particular antibiotic.

Conclusion

Multiple antibiotic resistance in lactobacilli do raise an alarm about their safety aspects. However the fact that many LAB exhibits intrinsic resistance to antibiotics and that in many cases the resistance is of non- transmissible type reduces the severity/ intensity of this problem to some extent. Yet it is necessary to subject these phenotypically identified antibiotic resistant isolates to further molecular level studies to characterize the resistance as either transmissible or non-transmissible. As presence of intrinsic resistance as well as resistance due to chromosomal mutations poses a low risk of horizontal dissemination, the food industry always prefers to use strains with such attributes than those harbor highly transmissible antibiotic resistance genes. So it is always preferable to include the antibiotic resistance assessment as a selection criterion while screening for potential starter cultures and probiotics.

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