

Antibiogram Profile and 16S rDNA RFLP Analysis of Enteric Bacteria from Farm Animals of Andaman Islands

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A total of 63 bacterial isolates were isolated from faecal swab of cattle, goat and poultry from different areas of Andaman. The majority of the isolates were E.coli (37.93%), however, other bacteria viz. Salmonella, Enterobacter, Klebsiella, Pantoea, Serratia, Yersinia spp. were also isolated. The antibiotic sensitivity profiling revealed great variation in the sensitivity pattern. The RFLP pattern of the microorganisms revealed different restriction profile with the restriction enzyme. The result indicated that bacterial isolates from the gut faecal swab were genetically diverse. PCR-RFLP could be used as a rapid and accurate tool for the diversity analysis of bacterial isolates from faecal swab.

KEYWORDS

Antibiotic sensitivity, bacterial isolates, farm animals and genetic characterization, faecal swab.

INTRODUCTION

Faecal swab provides a substrate for studying the dominant microbial flora and dynamics of colonization of microbial flora of the gastrointestinal tract in a large number of animals without sacrificing the animal to obtain samples. The amplification of a universal gene, as the gene that encodes for the 16 S ribosomal-RNA (16S r-DNA), could be a useful method to systematically analyzing the profile of microorganisms present in the gut of the animals with intestinal disorders. The combination of DNA amplification of the

16S r-DNA, digestion with restriction nucleases of amplified DNA, and analysis by agarose gel electrophoresis offers an excellent system to study the composition of microbial gut components without the necessity of in vitro cultures. The present study was conducted to isolate, identify and characterize the bacterial isolates from farm animals by 16 S rDNA profiling and to study the antibiotic resistance pattern.

MATERIALS AND METHODS

Faecal swabs from 23 calves, 10 poultry birds and 17 goat collected from different areas of South Andaman were inoculated in Nutrient broth and incubated at 37°C for 12 h. The bacterial cultures were re-inoculated on to Eosin methylene blue (EMB) agar, McConkey's agar and Hichrome ECC selective agar. The pure cultures were re-streaked and the colonies were isolated. Various biochemical tests were carried out according to Shirling & Gottlieb (1966) as outlined in the Bergy's manual of systematic bacteriology (Williams & Wilkins, 1994) for the identification of the isolates. Biochemical characterizations were performed by using Himedia biochemical kit.

The antibiotic sensitivity test was carried by employing Bauer-Kirby method (Bauer et al, 1966). The standard antibiotics disc (octadisc) supplied by Himedia were used against different isolates. The zone of inhibition was measured with Himeida scale.

Genomic DNA was extracted using the method described by Chen & Kuo (1993) with minor modification. The PCR reaction was performed in a total volume of 50 µl by mixing of the template

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DNA with 2.5 mM concentration of each deoxynucleotide triphosphate (dATP, dGTP, dTTP, dCTP). One μ l concentration of each primer of pA (5'AGAGTTTGATCCTGGCTCAG-3) and pH (5'AAGGAGGTGATCCAGCCGCA -3') described by Edwards et al, 1989 and 3 unit of Taq DNA polymerase in 10 X Taq buffer A were used to amplify the 16S rDNA genes yielding an amplification product of 1500 bp. These reaction were subjected to initial denaturation of 92°C for 2 min followed by 35 cycles of 92°C for 1 min, 48°C for 30 S and 72°C for 2 min and 10 S and a final extension step of 72°C for 6 min and 10 S using Gene Amp® PCR system 9700 (Applied Biosystem, USA). The products were observed using a 1 % agarose gel photographed under trans-illumination.

A total of 33 bacterial isolates were selected for the restriction analysis. Ten μ l of the 16S rDNA PCR product was digested with 5 U of restriction enzyme Hae III and Eco RI in a total volume of 25 μ l for 2 hours at 37°C. The restriction fragments were separated on a 3% agarose gel for 3 hours and visualized using a gel documentation system. A 100 bp ladder was used as a DNA marker. For the analysis of ARDRA molecular weight and Rf value of each band was determined by using molecular analyst software (version 1.5). Similarity coefficient and cluster analysis was performed by the un-weighted pair group method arithmetic average of NTSYS pc 2.02 e.

RESULTS AND DISCUSSION

A total of 63 bacterial isolates were isolated from the faecal swab of calf, poultry and goat. Out of these 29(46.03%) isolates were from calf origin, 19 (30.15%) from goat origin and 15(23.80%) from poultry origin. The Gram staining of these organisms showed that all belonged to Gram -ve type. The isolates were identified up to species level by using various conventional, biochemical and molecular characterization.

Biochemical characterization and sugar fermentation test revealed that, overall, the

majority of the bacterial isolates (37.93%), detected from poultry, goat and calf faecal swabs belonged to *Escherichia coli* group. Altogether, a total of 11 bacterial genera were identified which included *Escherichia*, *Salmonella*, *Enterobacter*, *Serratia*, *Cedeceae*, *Yersinia*, *Klebsiella*, *Hafnia*, *Pseudomonas*, *Bacillus* and *Pantoea*. The bacterial isolates of poultry origin mainly *Salmonella* (21.42%), *Serratia* (14.28%). The isolates from the goat origin mainly belonged to *E. coli* (57.89%) and *Enterobacter* (15.78%).

The in-vitro antibiotic sensitivity pattern revealed great variation in bacterial isolates (Table 1). Overall sensitivity pattern of the isolates revealed 100% sensitivity to Penicillin G followed by Gentamicin (96.65 %), Co-trimoxazole and Chloramphenicol (83.09%), ofloxacin (80%), ciprofloxacin (69.66%) and tetracycline (26.6%). The isolates from poultry origin showed 100% sensitivity to gentamicin, 84.66 % to chloramphenicol, 76.42% to tetracycline, 69.23% to streptomycin, 53.34% to co-trimoxazole, 46.15% to ampicillin and 28.27% sensitivity to ciprofloxacin.

The bacterial isolates from goat origin showed 100% sensitivity to tetracycline and gentamicin, 94.73% to chloramphenicol, 89.47% to ciprofloxacin, 88.88% to ceftriaxone, 84.2% to co-trimoxazole, 90% to streptomycin and 63.15% to clindamycin.

Overall, the antibiotic sensitivity profile of the isolates showed great variation to different antibiotics. Except *Pseudomonas* spp, all the other bacterial isolates showed sensitivity to antibiotics. *Klebsiella* spp. showed 100% sensitivity to all the antibiotics followed by *Enterobacter* spp (93.6%), *Escherichia* spp (90.83%), *Cedecea* spp (86.6%), *Pantoea* spp (80%), *Bacillus* spp (75%), *Serratia* spp. (76.6%), *Yersinia* spp. (75%) and *Hafnia* spp (20%). Highest activity was produced by gentamicin which showed 98.8% activity followed by chloramphenicol (75.26%), ciprofloxacin (78.02%), tetracycline (69.29%) and co-trimoxazole (57.62%) respectively.

The 16S rDNA of the 33 isolates amplified with primer PA & PH. Gel electrophoresis of

undigested PCR product revealed that all the isolates produced a single band of about 1500 bp (Fig 1). The restriction pattern obtained after digestion of the amplified 16SrDNA fragment with Eco RI revealed 25 restriction patterns (Fig 2). Digestion of 16SrDNA product with Hae III produced 20 restriction patterns (Fig 3). Different patterns were obtained for each enzyme. The result indicated that bacterial isolates from the gut faecal swab were genetically diverse. PCR-RFLP could be used as a rapid and accurate tool for the diversity analysis of bacterial isolates from faecal swab. A similarity test was then performed based on composite ARDRA profile by using Eco RI and Hae III which showed high similarity index around 80%.

The amplification of a universal gene, as the gene that encodes for the 16 S ribosomal RNA is could be a useful method to systematically analyze the profile of microorganisms present in the gut of the animals and birds with intestinal disorder. The combination of DNA amplification of the 16SrDNA, digested with restriction nucleases of amplified DNA and analysis by agarose gel electrophoresis offers an excellent system to study the composition of microbial gut component without the necessity of in-vitro cultures.

RFLP profiles could be a valuable system to analyze the microbial components involved in gut disorders of animals. The reproducibility of RFLP profile is very high and is possible to construct a database of RFLP profiles which could be useful at diagnostic laboratories.

The antimicrobial susceptibility data from the present study showed great variation to different antibiotics. The potential for transfer of antimicrobial resistance from enteric bacteria of food animals is a cause of concern. Contact with food animals or their excreta or consumption of foods of animal origin has been suggested to be the main route of dissemination of resistance from food-producing animals into human populations (Barnes et al, 1997). The variations in the antibiotic sensitivity of the isolate may be accounted for by difference in the genes encoding resistance to the various antimicrobials since

resistance phenotypes may arise from many different genetic determinants (Bensink et al, 1983). The major influences on the amplification and spread of antimicrobial resistant bacteria are the use of antimicrobial agents in human medicine and their use in livestock for therapy, metaphylaxis, prophylaxis and growth promotion. Resistant bacteria from domestic animals can be transmitted to man indirectly via the food chain or directly from the animal, and potentially result in food-borne illness in humans that is less responsive to treatment with conventional antimicrobial drugs.

In the present study the majority of the isolates belonged to *E. coli*. Although most strains of *E. coli* are nonpathogenic but they are considered as indicator of fecal contamination in food and about 10-15 % of intestinal coliforms are opportunistic and pathogenic serotypes (Barnes et al, 1997) and cause a variety of lesions in immune-compromised hosts. Many intestinal disorders are characterized by a microbial colibacillosis, with an increment or a reduction of bacterial counts. Introduction of new molecular techniques, and specially the amplification of DNA by PCR (polymerase chain reaction), allowed the possibility of cutting off the use of in vitro cultures and to broaden the study of the intestinal changes to uncultured microbiota or to microbiota difficult to culture. The combination of DNA amplification of the 16S r-DNA, digestion with restriction nucleases of amplified DNA, and analysis by agarose gel electrophoresis offers an excellent system to study the composition of microbial gut components without the necessity of in vitro cultures.

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TABLES

Table1. Antibiotic sensitivity pattern (%) of bacterial isolates from calf, poultry and goat

Bacteria	Gentamicin (30 µg)	Chloramphenicol (25 µg)	Co-Trimoxazole (25 µg)	Ciprofloxacin (10 µg)	Tetracycline (30 µg)
<i>Escherichia spp.</i>	100	97.05	88.23	90.32	78.57
<i>Salmonella spp</i>	88.88	55.55	66.66	76.58	56.46
<i>Enterobacter spp</i>	100	100	88.00	80.00	100
<i>Serratia spp</i>	100	100	66.66	66.66	50.00
<i>Cedeca spp</i>	100	100	66.66	66.66	100
<i>Yersinia spp</i>	100	0	100	100	0
<i>Klebsiella spp</i>	100	100	100	100	0
<i>Hafnia spp</i>	100	0	0	0	0
<i>Pseudomonas spp</i>	0	0	0	0	0
<i>Bacillus spp</i>	100	100	0	100	0
<i>Pantoea spp</i>	100	100	0	100	100

FIGURES

Fig. 1: Amplification of 16S rDNA (1500 bp) of the bacterial isolates

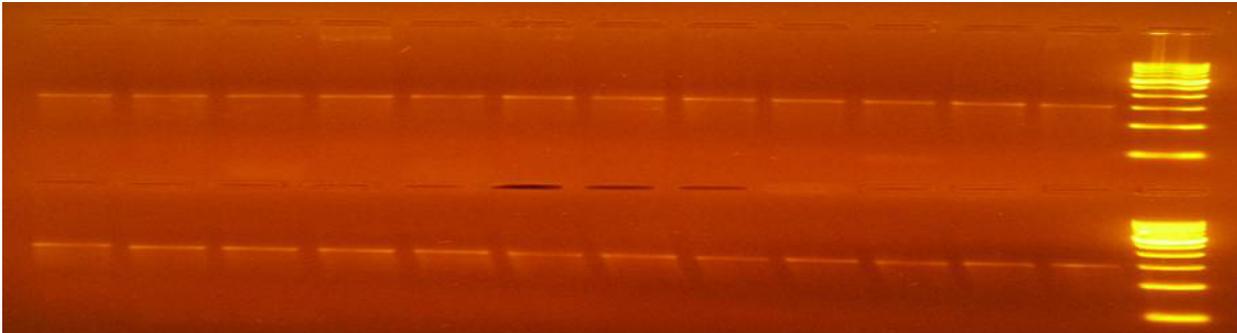


Fig. 2: Digestion of the amplified 16S rDNA fragment with Eco R1

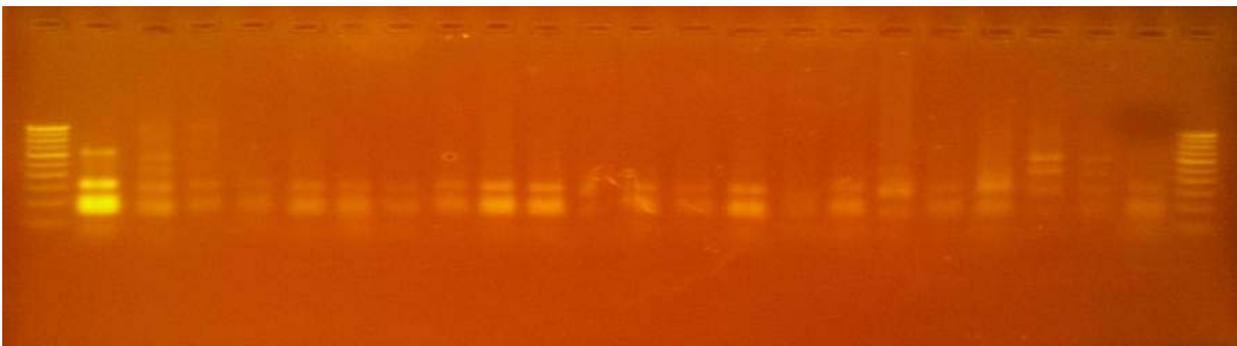


Fig. 3: Digestion of the amplified 16S rDNA fragment with Hae III

