Surveillance and antibacterial activity of commercial antibiotics against Vibrio sp. isolated from Cattle (Bos indicus) farms of Tamil Nadu, India

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Abstract

Total population of Vibrio sp. isolates were estimated from fecal samples of lactating dairy cows (Bos indicus), collected from five different places such as Siruvatthi, Devakottai, Arenthangi, Kundrakudi and Karaikudi of Sivagangai district of Tamil Nadu. The maximum Vibrio sp. of 34 x103 was noticed in cattle fecal sample collected from Siruvatthi area. Five morphologically differed Vibrio colonies were selected and characterized by Random amplification of polymorphic DNA (RAPD) profile analysis to conform distinctive between the five Vibrio sp. Three primers (TW-3, RBA-5 and OPA-2) were used, among the three, TW-3 produced greater fragment patterns range from 750 bp-2000 bp between five different Vibrio sp. isolates and the other two primers RBA-5, OPA-2 showed fewer fragment patterns range from 750 bp-1000 bp. To find out, the control measures of commercially available antibiotics such as amikacin, ampicillin, chloramphenical, ciprofloxacin, ceftazidime, erythromycin, gentamycin, vancomycin, ofloxcin and oxacilin were tested against Vibrio sp. isolates. The zone of inhibition was measured by a ruler after 16-18 hrs and ciprofloxacin showed the maximum zone of inhibition (36 mm) against Vibrio sp. (V-5).

Keywords: Vibrio isolates, antimicrobial activity, antibiotics, zone of inhibition, RAPD

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Introduction

Animal products such as meat, milk, provide daily cash income to agricultural families and also provide much required nutrition to rural population. Disease is the major constraints in live stock industry which is caused by pathogenic organisms like bacteria, virus, fungi and parasites. Vibrios are an infectious bacterial disease of the gut and genital tract causing stomach disorders and infertility and occasional abortions (Grant, 1955). Extensive management practices in cattle farms have led to an increase use of antibiotics, drugs, vaccines and chemicals for the disease management. To have a sustainable growth pattern and increase the economy of livestock production, health management studies can support the industry. Nowadays for the use of antibiotics as prophylactics and growth promoters in food-producing animals and its consequence on the development of antibiotic-resistant bacteria is one area that has become topical in current period. Antibiotics used in both human and veterinary medicine are aminoglycosides, penicillins, cephalosporins, chloramphenicol, tetracyclines, lincosamide, macrolides, nitrofuranes, nitroimidazoles, spectinomycin, sulfonamides, trimethoprim, polymyxins, uinolones, etc (Prescott et al., 2000; Teuber, 2001). More than one million tons of antibiotics released into the biosphere in the last 50 years (Mazel and Davies, 1999) and approximately 50% are estimated to flow into the agriculture and veterinary field (Levy, 1992). The significant of antibiotics at sub therapeutical stage were used for increased growth, feed efficiencies in farm animals like cattle, chicken, pigs and turkey (Animal Health Research Council). Hence, the usage of antibiotic in disease control has been proposed for effective management in cattle. However, the effectiveness of antibiotics and chemicals for certain bacterial disease is still lacking in the cattle farms. Sympathetic and supervision fecal pathogen pollution is a challenging and difficult task. A number of the bacterial pathogens can cause diarrhea in humans and animals as well as reducing growth performance and milk production in adult cattle (Anderson, 1998). Vibrio sp also the majority common bacterial pathogens found in cattle which can pose health risk to the animal (Ademola et al., 2011). Keeping the above facts in mind, the present study was carried out to
find out the *Vibrio* population in the cattle fecal samples of various farms. The solidity *Vibrio* colonies were characterized by Random Amplification of Polymorphic DNA (RAPD) profile and to evaluate effective control, commercial antibiotics against *Vibrio* sp were tested.

**Materials and Methods**

**Collection of fecal samples**

Fecal samples of lactating dairy cows (*Bos indicus*) were collected from various cattle farms of five different places (Arenthangi, Karaikudi, Kundrakudi, Siruvatthi and Devakottai) in Tamilnadu, India. Fresh fecal sample was scooped with sterile spatula from each farms and packed in sterile polythene bags. Immediately after the collection, the fecal samples were transported to the lab under controlled condition for microbial examinations.

**Total plate count of Vibrio sp.**

The total *Vibrio* population of five different places was enumerated by adopting spread plate method. One gram of cattle fecal (*Bos indicus*) sample was diluted (10% wt/vol) in buffered peptone water (9 ml). Samples were serially diluted (upto 10<sup>-5</sup>) using peptone water. 89 grams of TCBS was suspended in 1000 ml of distilled water and heated up to boiling point to dissolve the medium completely. Then it was cooled and poured into the sterilized petriplates and allowed to solidify. The sample was spread on a petriplate using “L” shaped spreader and the inoculated plates were incubated at 37°C. After 24 hrs, the colonies were counted and population density was expressed as Colony Forming Units (CFU) per gram of samples.

**Selection of Vibrio sp. isolates**

Morphologically differed colonies were picked and streaked again in TCBS medium plates to confirm the specific *Vibrio* spp. isolates and incubated at 37°C. Five morphologically different isolates were selected to analyse the genetic variation of *Vibrio* spp. using RAPD analysis. All the purified strain was maintained in Luria-Bertani (LB) broth at -20°C with 15% glycerol for further studies.

**Isolation of DNA and RAPD analysis**

Bacterial genomic DNA was extracted by the following method of Sambrook et al. (1989). Bacterial isolates were cultured in 10ml of LB broth at 29°C in agitation for 18 hrs. The bacterial cultures were centrifuged at 5000 rpm for 5 min at 4°C separately. The pellets were suspended with sucrose TE buffer, lysozyme was added and incubated for 30 min. After incubation, 100 µl of 0.5 M EDTA (pH 8) and 60 µl of 10% SDS were added. Subsequently, 250 µl of Tris equilibrated phenol and 250 µl of chloroform: isoamyl alcohol (25:24:1) were mixed gently. The mixtures were centrifuged and an equal volume of chloroform and isoamyl alcohol (24:1) mixture was added to the upper aqueous layer. The aqueous phases in sterile tube were collected and precipitated with double the volume of 100% ice-cold ethanol and 3 M sodium acetate. The samples were centrifuged at 12000 rpm and DNA pellets were washed twice with 70% ethanol and air dried. The DNA pellets were suspended with 30 µl of TE buffer (pH 8.0). The DNA pellets and the random primers, TW-3 (5’TTCCCGTTCG3’) RBA-5 (5’TTCCCCCGAC3’) OPA-2 (5’AGTCAGCCAC3’) were used for RAPD analysis by a thermal cycler (Eppendorf-Germany). The each template DNA was amplified by PCR with cocktail of standard PCR buffer 2.5 µl, 10 mM dNTP 0.5 µl, primer 2.0 µl, template DNA 2.0 µl, Taq polymerase 0.2 µl. The amplification conditions were 1 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 32°C and 1 min at 72°C and a final extension at 72°C for 10 min. The PCR amplified products were analysed in 1.5% agarose gel electrophoresis using ethidium bromide and patterns were analysed using gel documentation system.

**Antimicrobial activity of antibiotics against Vibrio sp. isolates**

*Vibrio* sp. isolates were tested by disc diffusion method for antimicrobial susceptibility. Commercially available antibiotic disc (HIMEDIA, Mumbai), amikacin, ampicillin, chloramphenicol, ciprofloxacin, ceftazidime, erythromycin, gentamycin, vancomycin, ofloxacin and oxacillin were used and the zone of inhibition was measured by after 16-18 hrs. The zone of inhibition was expressed as resistant, intermediate and susceptible. The results were recorded and interpreted.

**Results**

**Isolation, identification and population analysis of Vibrio sp. isolates**

Cattle fecal samples were collected from various farms of southern parts of Tamilnadu (Siruvatthi, Devakottai, Arenthangi, Kundrakudi and Karaikudi). Each isolates in the plates showed different
The phenotypic characteristics of isolated bacteria from fecal sample of *Bos indicus* are shown in the Table 1. The total number of *Vibrio* pathogen counted in TCBS medium of cattle fecal sample with the dilutions (10^-5) varied between 3x10^2 to 34x10^-5 CFU/g. The maximum *Vibrio* spp of 34 x 10^-5 was noticed in cattle fecal sample collected from cattle farm of Siruvatthi cattle farm and followed by 21 x 10^-5 from Devakottai cattle farm, 6 x 10^-5 from Arenthangi cattle farm, 4 x 10^-5 from Kundrakudi cattle farm and 3 x 10^-5 from Karaikudi cattle farm.

**Table 1.** Phenotypic characteristics of bacteria collected from *Bos indicus*

<table>
<thead>
<tr>
<th>Medium</th>
<th>Colour</th>
<th>Shape</th>
<th>Consistency</th>
<th>Elevation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCBS</td>
<td>Green</td>
<td>Round</td>
<td>Round pinpoint colony</td>
<td>Concave</td>
</tr>
<tr>
<td>TCBS</td>
<td>Yellow</td>
<td>Irregular</td>
<td>Curl pinpoint colony</td>
<td>Concave</td>
</tr>
<tr>
<td>TCBS</td>
<td>Yellow</td>
<td>Round</td>
<td>Mucoid colony</td>
<td>Concave</td>
</tr>
<tr>
<td>TCBS</td>
<td>Yellow</td>
<td>Round</td>
<td>Flat, pinpoint colony</td>
<td>Convex</td>
</tr>
<tr>
<td>TCBS</td>
<td>Yellow</td>
<td>Round</td>
<td>Mucoid, undulated colony</td>
<td>Concave</td>
</tr>
</tbody>
</table>

**Antibiotic sensitivity of Vibrio sp. isolates from cattle fecal samples**

All *Vibrio* isolates were sensitive to antibiotics such as ampicillin, amikacin, chloramphenical, ciprofloxacine, celtazidime, erythromycin, gentamycin, vancomycin, ofloxacin, except oxacilin (Fig.1). Among the selected ten antibiotics ciprofloxacine showed the maximum zone of inhibition (36 mm) against V5 and vancomycin showed the minimal zone of inhibition (6 mm) against V5. Ampicillin showed the zone of inhibition (10 mm) against V2. Oxacilin did not show activity against all *Vibrio* (V1-V5) sp. (Table 2). The results were plotted in a graph to compare the antibiotic sensitivity of bacterial strains isolated from cattle fecal samples (Fig. 2).

**Molecular analysis of the Vibrio sp.**

The molecular analysis was carried out only five different *Vibrio* spp isolates. The genomic DNA of selected *Vibrio* sp. were isolated from cattle *Bos indicus* fecal matter (Fig. 3). The molecular size of the genomic DNA (*Vibrio* sp) was around 23 kb. No difference was observed at the molecular size of five different genomic DNA. Selected *Vibrio* sp were further studied for the molecular analysis to differentiate at the genus level by RAPD technique using three universal random primers. TW-3 showed the amplified fragment patterns from 750 bp-2000 bp. RBA-5 primer produced only one amplified fragment around 750 bp in the V1 and V5 isolates. OPA-10 primer showed amplified fragment patterns from 750 bp -1000 bp. TW-3 produced greater banding patterns between *Vibrio* sp.

**Discussion**

Cattles are the most important reared traditionally and extensively cultured in India. Extensive management practices in cattle farms has led to an increase use of hormones, antibiotics, drugs, vaccines, chemicals for maximizing the production and disease management. Disease problem arising in live stock and poultry can be attributing mainly to poor environment condition and climatic changes. Successful production achieved by scientific planning and good management in farming environment. Disease causing agents are controlled by massive use of antibiotic in the system may affect the animal and surrounding environment. However, antibiotic resistance is a complex problem worldwide in animal production industry. Many countries are devoted to minimizing public health risks by safeguarding the efficiency of antimicrobial therapy and applying systemic monitoring of zoonotic bacteria of animal origin (Martel et al., 2000). Bacteria susceptibility to antimicrobial agents was performed by the disk diffusion method using guidelines established (Razvykh et al., 1990; Bauer et al., 1996). In turkey eighty two *Vibrion* sp were treated for their sensitivity to eight antibiotics, and found majority of the strains were highly sensitive to all antibiotics.

The beef and cattle often have a very low prevalence of fecal pathogens, molecular epidemiology suggests (Hoar et al., 2001). A number of the bacterial pathogens can cause diarrhea in humans and animals as well as reducing growth performance and milk production in adult cattle (Anderson, 1998). But sufficient data is not available to antibiotic against *Vibrios* from cattle fecal sample.
Fig. 1. Inhibitory zone of different antibacterial discs (Ampicillin (A), Amikacin (AK), Chloramphenical (C), Ciprofloxacin (CF), Ceftazidamie (CA), Erythromycin (E), Gentamycin (G), Ofloxacin (OF), Oxacilin (OX) and Vancomycin (VN) against *Vibrio* spp. isolates from *Bos indicus*.

![Image of inhibitory zones for different antibacterial discs against *Vibrio* spp. isolates from *Bos indicus*.](image)

Fig. 2. Inhibitory activity of antibiotics against different *Vibrio* sp. isolated from *Bos indicus*.

![Graph showing inhibitory activity of antibiotics against different *Vibrio* sp. isolated from *Bos indicus*.](image)

Fig. 3. RAPD profile with random primer (A:TW-3,B:RBA-5,C:OPA-10) of selected five *Vibrio* sp (V1-V5) from cattle fecal (*Bos indicus*).

![Image of RAPD profiles for selected five *Vibrio* sp (V1-V5) from cattle fecal (*Bos indicus*).](image)
The diseases caused by bacteria may be controlled by proper sanitation, good management practices, providing quality feed, manipulating the bacterial population (beneficial bacteria) with the proper feed additives, medicines and disinfectant schedules. In the present study, shows the minimum bacterial colonies of the group was noticed in cattle fecal samples collected from Karaikudi (3x10^5), indicates the good health of the cattle and hygienic condition of the cattle farms. The maximum colony forming unit (CFU) values of Vibrio sp. isolates noticed in Siruvatthi (34x10^5) and (21x10^5) values in Devakottai clearly indicates poor sanitation of farms yard and health statue of the cattle.

The RAPD method has been widely used in the development of molecular diagnostic techniques for bacteria, because it is a powerful tool that allows comparative analysis of genomes between different isolates of the same species by establishing distinct molecular markers (Akopyanz et al., 1992; Schierwater et al., 1993; Cocconcelli et al., 1995). In the present study, RAPD profiles obtained using a universal random primer for the Vibrio sp (V1-V5). RAPD profile results showed the different between the bandy patterns of each strain. In the present study, ten antibiotics were tested against the isolated Vibrio sp. All the five isolates were tested for their antibiotic sensitivity test with the selected commercially available antibiotics like chlorphenicol, ciprofloxacin, erythromycin, ampicillin, amikacin, ofloxacin, gentamycin, oxacillin, vancomycin, and cefazidime. Among the selected ten antibiotics, ciprofloxacin showed the maximum zone of inhibition (36 mm) against V5 and vancomycin showed the minimal zone of inhibition (6 mm) against V5.

Ampicillin showed the zone of inhibition (10 mm) against V2 and did not show any activity with antibiotics. Oxacilin did not show activity against all Vibrio (V1-V5) sp. The result indicates that only selected antibiotics are effective against the isolated Vibrio sp. It is very much essential to find out the effectiveness of antibiotic before going for treatment for the betterment of livestock population.

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References


