

Original Research Article

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Antibiotic Sensitivity Pattern of Different Bacterial Species Isolated from Faecal Samples of Pups Affected with Canine Parvoviral Enteritis

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ABSTRACT

Keywords

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Among the viruses, infection with canine parvovirus 2 (CPV-2) was considered as highly contagious and causes acute haemorrhagic gastroenteritis and myocarditis in dogs. In the present study, faecal samples from fourteen pups affected with canine parvoviral enteritis were subjected to detailed bacteriological examination. It revealed the presence of bacterial species like *E. coli*, *Salmonella spp.*, *Klebsiella spp.*, *Pseudomonas spp.*, *Staphylococcus spp.*, and *Streptococcus spp.* in different combinations along with CPV infection. Invitro antibiotic sensitivity test of whole cultures revealed highest sensitivity to amikacin (85.71 %) followed by other antibiotics such as cefepime, chloramphenicol, cefotaxime, ceftriaxone, levofloxacin, ceftazidime and amoxycillin with clavulanic acid and all the samples exhibited resistance to ampicillin and metronidazole.

Introduction

Canine parvovirus 2(2a, 2b and 2c) is one of the most significant viral causes of acute hemorrhagic gastroenteritis and myocarditis in canine population, which is highly contagious and the disease may be often fatal. Acute enteritis is the most common clinical manifestation of the disease especially in puppies' upto 6 months of age (Goddard and Leisewitz, 2010) whereas myocarditis is very rarely seen unless infection occurs in utero or in puppies less than 8 weeks old born to unvaccinated bitches (Hoskins, 1997). Factors that predispose puppies to parvoviral infection include lack of protective immunity, intestinal

parasites, overcrowding, unhygienic and stressful environmental conditions. CPV-2 spreads rapidly among dogs via faecal route (direct transmission) or through oronasal exposure to fomites contaminated with infective faeces (indirect transmission) (Johnson and Smith., 1983). The main source of infection seems to be the faeces of infected dogs, as 10^9 virus particles can be shed in one gram of faeces during the acute enteric form of the disease. Faecal shedding of virus may continue for a maximum period of 3 to 4 weeks after clinical or subclinical disease. Intestinal tract damage secondary to viral

infection increases the risk of bacterial translocation leading to coliform septicemia and development of systemic inflammatory response syndrome that can progress to septic shock. The purpose of the present study was to determine the various types of bacterial species in faecal samples of pups affected with canine parvoviral enteritis and to study the *in vitro* antibiotic sensitivity pattern of whole cultures to different antibiotics.

Materials and Methods

In the present study, a total of twenty four faecal samples were collected with sterile swabs from pups presented with symptoms suggestive of canine parvoviral enteritis (dullness, pyrexia, vomiting, bloody foul smelling diarrhoea etc.) and subjected to sandwich lateral flow immune chromatography assay (Scan Vet™ PARVO kit).

Isolation and identification of bacteria

Faecal samples from fourteen CPV positive pups were inoculated simultaneously into nutrient broth and selenite broth and incubated at 37°C for 24 hrs aerobically, then a loopful of bacterial culture was streaked on nutrient agar plates. Based on morphology and from Gram's staining, cultures were inoculated into specific or selective media like MacConkey agar, Mannitol salt agar, Eosin- methylene blue agar, Edwards medium and on xylose lysine deoxycholate (XLD) agar medium. Pathogenecity was tested by inoculating on blood agar.

Primary identification of bacteria was done based on colony morphology, type of hemolysis, Gram's staining, biochemical characteristics and pure cultures were identified upto genus level as per the Bergy's Manual of Determinative Bacteriology (Holt *et al.*, 2000).

Antibiotic sensitivity test

The antibiotic sensitivity test was performed on the faecal whole cultures from nutrient broth *in vitro* by Kirby Bauer disc diffusion method on Mueller Hinton Agar (MHA) plates. The antibiotics used in present study were Amikacin (AK), Amoxycylav (AMC), Ampicillin (AMP), Cefepime (CPM), Cefotaxime (CTX), Ceftazidime (CAZ), Ceftriaxone (CTR), Chloramphenicol (C), Levofloxacin (LE) and Metronidazole (MT). The sensitivity pattern was read by measuring the diameter of zone of inhibition corresponding to different antibiotics in millimeter as per the interpretation chart provided by the manufacturer.

Results and Discussion

Out of twenty four pups in the present study, fourteen pups were found to be positive for CPV infection with sandwich lateral flow immunochromatography assay. Earlier, in many studies immunochromatography based test kits were employed for the detection of parvovirus in faecal samples of dogs, since it is a rapid, simple, reproducible and sensitive diagnostic test (Esfandiari and Klingeborn, 2001, Pillai and Deepa, 2010, Filipov *et al.*, 2011 and Dongre *et al.*, 2013).

In the present study, culturing of faecal samples from fourteen CPV affected dogs revealed the presence of gram negative bacteria such as *E.coli*, *Salmonella spp.*, *Klebsiella spp.*, *Pseudomonas spp.* and gram positive bacteria such as *Staphylococcus spp.* and *Streptococcus spp.* in different combinations along with CPV infection (Table 1). Cultural and biochemical characteristics of different species of bacteria isolated from faecal samples of affected dogs are depicted in Figure 1. Invasion of secondary bacteria in the present study might be due to leukopenia and compromise of

immune system. CPV causes necrosis of gut-associated lymphoid tissue resulting diminished local immunity to infectious agents and predisposing to bacterial infection, as suggested by Turk *et al.*, (1992). Similarly secondary bacterial infection with *E. coli* (Isogai *et al.*, 1989, Zschock *et al.*, 1989 and Basava reddy., 2013), *Clostridium perfringens* (Turk *et al.*, 1992 and Hoskins., 2006), *Campylobacter spp.* (Sandstedt and Wierup., 1981, Hagiwara *et al.*, 1996 and Hoskins., 2006), *Klebsiella spp.* and *Staphylococcus spp.* (Basava reddy., 2013), and *Salmonella spp* (Hoskins., 2006 and Basava reddy., 2013) was reported from isolation studies on faecal samples of dogs with parvoviral enteritis. Koutinas *et al.*, (1998) isolated *E. coli*, *Staphylococcus spp.*, *Enterococcus durans* and *Corynebacterium*

spp. from urine samples of CPV affected dogs, and stated that it might be due to the faecal contamination of genital organs. Other species of bacteria like *Serratia spp.*, *Acinobacter anitratus*, *Citrobacter freundii*, *Staphylococcus intermedius*, *Streptococcus spp.*, *Klebsiella pneumoniae*, *K.oxytoca*, *Escherichia coli* and *Enterobacter spp.* were also isolated from bacteriological culture of swabs obtained from intravenous catheters placed to parvoviral infected dogs (Lobetti *et al.*, 2002 and Greene and Decaro, 2012).

Nivy *et al.*, (2011) isolated an unusually resistant *E. coli* strain from the cerebrospinal fluid of a CPV affected dog and stated that dogs with CPV infection have an increased risk of secondary bacterial infections due to neutropenia and immunosuppression.

Table.1 Mixed infection of CPV with different bacterial species

Type of isolates	No. of positive faecal samples	Percent (%)
CPV + <i>E.coli</i> + <i>Staphylococcus spp.</i>	3	21.43
CPV + <i>E.coli</i> + <i>Streptococcus spp.</i>	2	14.28
CPV + <i>Salmonella spp.</i> + <i>Staphylococcus spp.</i>	3	21.43
CPV + <i>Salmonella spp.</i> + <i>Streptococcus spp.</i>	1	7.14
CPV + <i>Klebsiella spp.</i> + <i>Streptococcus spp.</i>	2	14.28
CPV + <i>Pseudomonas spp.</i> + <i>Staphylococcus spp.</i>	3	21.43

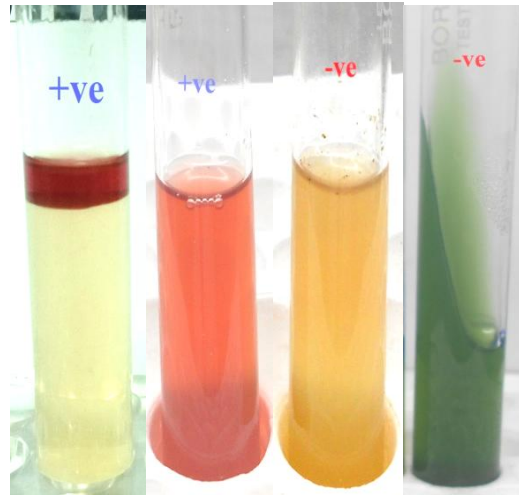
Table.2 Antibigram of the microbial isolates emanating from CPV affected dogs (n=14)

S. No	Drug	Sensitivity		Intermediate		Resistant	
		No.	%	No.	%	No.	%
1.	Ampicillin	0	0.00	0	0.00	14	100.00
2.	Amikacin	12	85.71	1	7.14	1	7.14
3.	Amoxyclav	1	7.14	3	21.43	10	71.43
4.	Cefepime	7	50.00	4	28.57	3	21.43
5.	Cefotaxim	5	35.71	3	21.43	6	42.86
6.	Ceftazidime	2	14.28	6	42.86	6	42.86
7.	Ceftriaxone	5	35.71	5	35.71	4	28.57
8.	Chloramphenicol	6	42.86	3	21.43	5	35.71
9.	Levofloxacin	5	35.71	3	21.43	6	42.86
10.	Metronidazole	0	0.00	0	0.00	14	100.00

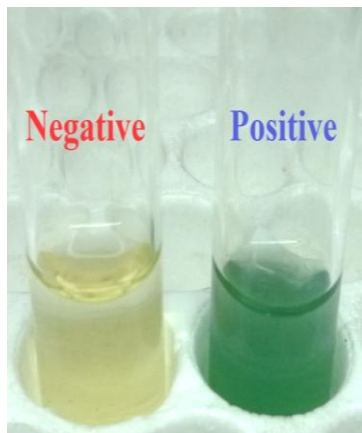
Fig.1 Cultural and biochemical characters of different bacterial species



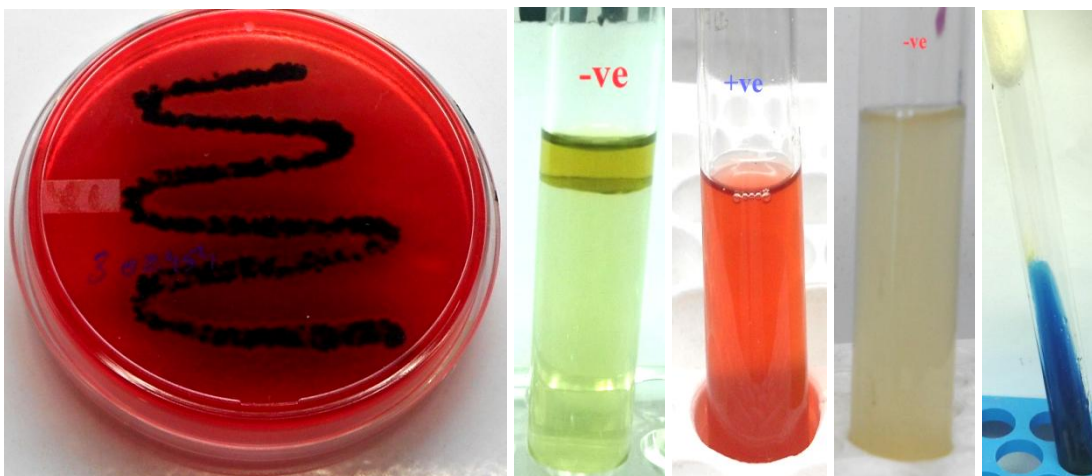
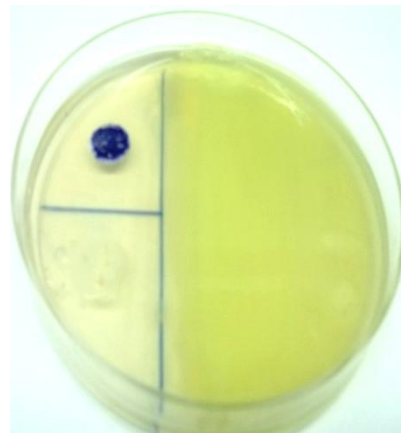
E. coli on EMB agar (metallic sheen)



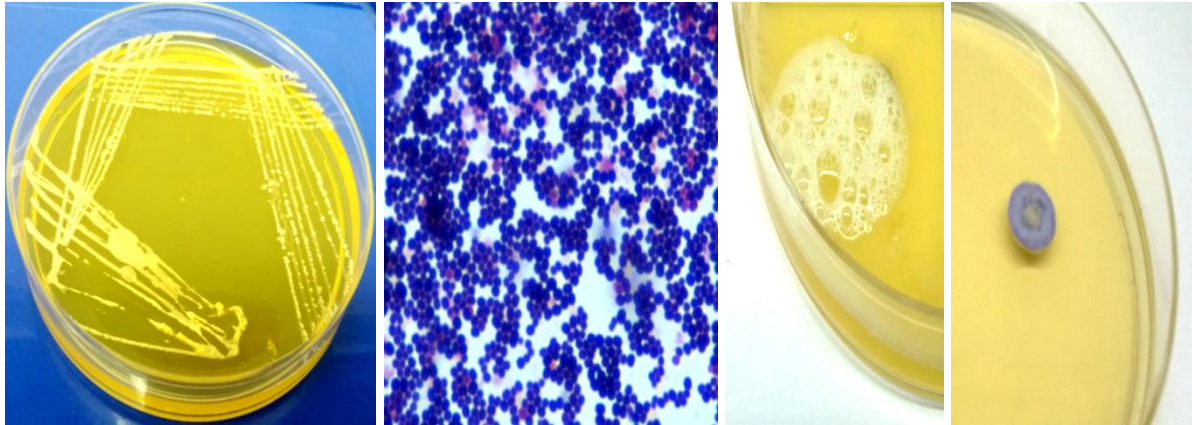
IMViC +, +, -, - of *E. coli*



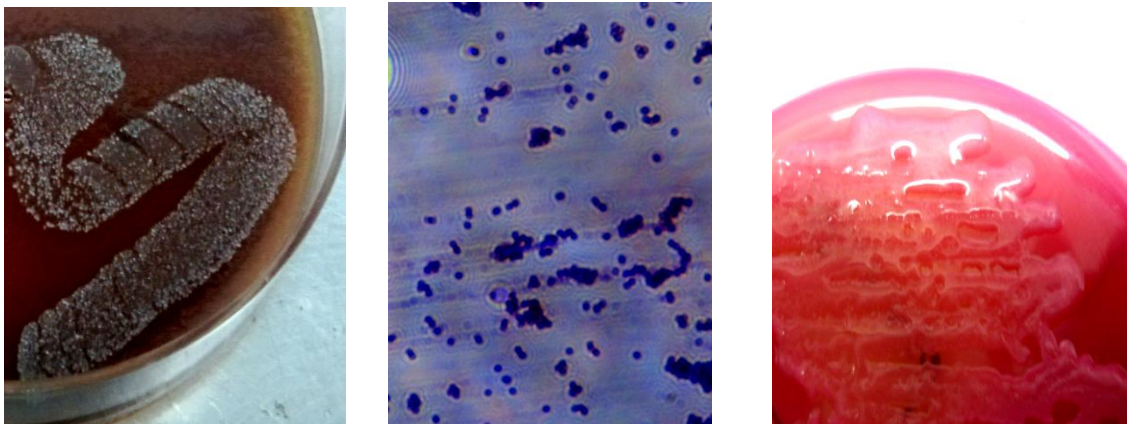
Pseudomonas spp. in nutrient broth and Catalase +ve; Oxidase +ve



Salmonella spp. on XLD agar and IMViC: -, +, -, +



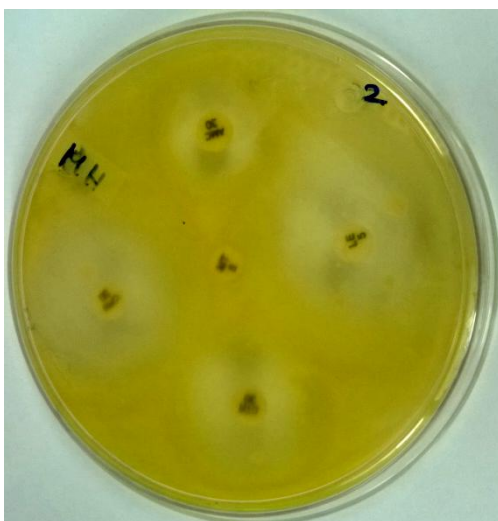
Staphylococcus spp. on Mannitol salt agar; Gram's staining; Catalase and Oxidase +ve



Streptococcus spp. colonies on Edward's and Gram's staining

Klebsiella spp. on MacConkey agar medium

Fig.2 ABST (Antibiotic sensitivity pattern of bacteria to different antibiotics)



Sensitivity to Ceftriaxone



Sensitivity to Cefepime and Cefotaxime



Sensitivity to Amikacin

The antibiogram of whole cultures present in the faecal samples of fourteen CPV affected dogs is presented in Table 2 and Figure 2. In the present study, amikacin, an aminoglycoside showed highest sensitivity of 85.71 percent due to its predominant activity against Gram-negative aerobic bacteria and *Staphylococcus spp.*, are usually susceptible to aminoglycosides but most other Gram-positive aerobes are not. Amikacin is particularly important in treating serious *Pseudomonas spp.* and other Gram-negative infections in immunosuppressed patients (Maddison *et al.*, 2008).

Earlier, Dutta *et al.*, (2008) reported 72.22 percent of sensitivity of amikacin to *Salmonella spp.* isolated from diarrhoeic dogs. Cefepime in the present study showed 50 percent sensitivity, is a fourth generation cephalosporin which had increased activity against both Gram-positive and Gram-negative bacteria, moderate activity against *Pseudomonas spp.* and enhanced activity against *Staphylococcus spp.* (Maddison *et al.*, 2008). In the present study, chloramphenicol had 42.86 % sensitivity. Earlier, *Salmonella* organisms isolated from diarrhoeic dogs exhibited 72.22 percent of sensitivity to chloramphenicol, as was reported by Dutta *et al.*, (2008). Sharma (2003) reported 87.50 percent of sensitivity of chloramphenicol

against bacterial isolates from diarrhoeic dogs. Chloramphenicol is bacteriostatic for most Gram-positive and many Gram-negative aerobic bacteria but can be bactericidal against some very sensitive bacteria. Acquired resistance occurs in many species, especially where chloramphenicol is in common use. *Pseudomonas aeruginosa*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Salmonella* and *Proteus* show patterns of plasmid-mediated resistance (Maddison *et al.*, 2008). The *in vitro* drug sensitivity of both ceftriaxone and cefotaxime was found to be 35.71 percent in the present study.

Both included under third generation cephalosporin group, had decreased Gram-positive but increased Gram-negative activity (susceptible Gram-negative bacteria include *Escherichia coli*, *Klebsiella spp.*, *Proteus spp.*, *Haemophilus spp.*, *Actinobacillus spp.*, *Salmonella spp.*) as stated by Maddison *et al.*, (2008). But the less sensitivity in the present study could be due to the involvement of *Pseudomonas spp.* against which the sensitivity is variable (Maddison *et al.*, 2008) and presence of Gram-positive organisms and wide spread use of these antibiotics since quite a long period. Earlier Dutta *et al.*, (2007) reported 55.56 percent of sensitivity of cefotaxime to *Salmonella* isolates from diarrhoeic dogs. Cefotaxime exhibited 85.03

percent of sensitivity to bacteria isolated from diarrhoeic dogs (Singh, 2008). In the present study, the *in vitro* drug sensitivity of amoxicillin with clavulanic acid was found to be 7.14 percent. Amoxicillin-clavulanate, in contrast to amoxicillin alone, has activity against penicillinase-producing *Staphylococcus* and has enhanced activity against Gram-negative pathogens. However, *Enterobacter*, *Citrobacter*, *Pseudomonas aeruginosa*, *Serratia* and methicillin-resistant *Staphylococcus* are resistant (Maddison *et al.*, 2008). Emergence of many resistant strains of Gram-negative bacteria and increasing prevalence of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter spp* as pathogens might be responsible for decreased spectrum of amoxicillin as they are intrinsically nonsusceptible to aminopenicillins (Maddison *et al.*, 2008).

Ampicillin and metronidazole exhibited 100 percent resistance, which might be attributed to the frequent and indiscriminate use of these antimicrobial agents. Previously Dutta *et al.*, (2007) reported 100 percent of resistance of ampicillin to Salmonella isolates and Sharma (2003) reported cent percent resistance of metronidazole against bacteria isolated from diarrhoeic dogs.

The present study concluded that, the most common bacteria involved were of Gram-negative species (*E. coli*, *Salmonella spp.*, *Klebsiella spp.*, and *Pseudomonas spp.*) and few Gram-positive species (*Staphylococcus spp.*, and *Streptococcus spp.*). Antibiogram studies revealed 85.71 percent of sensitivity to amikacin and 35.71 percent to ceftriaxone.

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