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Antibiotic sensitivity profile of the test isolates of *E. coli* isolated from clinical cases of diarrhea in the kids of goat breeds

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Abstract

The samples were taken using sterile cotton swab tubes, and were labeled and kept in the refrigerator at 4°C until further processing. These samples were collected from less than one month old Jamunapari, Barbari, Jakharana, Sirohi kids during the period from March to June 2010. Some of the commonly used antibiotics like Ampicillin, and Cloxacillin were giving high percentage of resistance at the rate of 75 percent and 100% respectively. This is due to the unscrupulous use of such antibiotics leading to high resistance. About 67 percent of the isolates were resistant to Oxytetracycline, Gentamicin, and Ceftriaxone. Only antibiotic groups like fluoroquinolones and the Chloramphenicol have fared well in effectively inhibiting the in vitro growth of E. coli. Chloramphenicol has given the lowest percentage of resistance at zero and the next best was Enrofloxacin at 34 percent. So, it's a serious issue that out of the commonly used 7 antibiotics, only two was effective, which gives clue about the emerging multi-drug resistant E. coli strains.

Key words: *antibiotic sensitivity, E. coli, kids, goat breeds.*

Introduction

Goat population in the world was 464,323000 in 1980; 591,367000 in 1990; 746,689000 in 2000; 864,394000 in 2008; 1,183 goat breeds; 6,674,801000 human populations in 2008. Goat population in India was 86,900000 in 1980; 113,200000 in 1990; 123,533000 in 2000; 125,732000 in 2008; 39 goat breeds; 1,139,965000 human population in 2008; 1,080 US$ gross national income per capita (Shrestha *et al*., 2011).

Diarrhea is defined as an increased frequency, fluidity, or volume of fecal excretion. The feces may contain blood or mucous and may have foetid odour. The color of the feces may be abnormal. However, it is not possible to definitively determine the infectious organism by looking at the color, consistency, or odor of the feces. A definitive identification requires a sample for microbiological analysis. In livestock, diarrhea is called scours. There are many causes of diarrhea: bacterial, viral, parasites, and diet (Joe Rook 2007)**.** Diarrhoeal diseases are the top listed disease causing death of kids. Colibacillosis and septicemia are generally taken as the most common cause of mortality in juvenile kids in India (Vihan *et al,* 1990) along with other infectious organism like *Cryptosporidium parvum, rota virus, Clostridium perfringens* and *Salmonella* species*.* Morbidity rate (60.8%) and Mortality rate (15-30%) was observed in Colibacillosis in kids in organized farm (Kumar *et al*, 2001). Outbreak of colibacillosis in established farm generally has become endemic.

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Diarrhoea caused by *Escherichia coli* infection is responsible for high rates of mortality in goat kids which causes important economical loss in goat-rearing. Goat kids are especially susceptible to *E. coli* scours during the first week of life. A constant bacteriological finding in enteric colibacillosis is the intense proliferation of certain serotypes of *E. coli* in the small intestine whiles the number of other bacteria in the gut remains normal (Hodgson, 1994). Diseases are the major cause of economic losses and inefficient production of livestock and main constraint in development of livestock particularly of goat industry in country. The risk of endemic diseases contributes significantly, which is considered more obvious under Indian conditions.

Materials and Methods

E. coli was isolated from faeces of Kids suffering from clinical diarrhoea. The Jamunapari, Barbari, Jakhrana, Sirohi goat unit of CIRG Makhdoom, farah, Mathura was selected for collection of sample. The kids showing the symptoms of diarrhoea were selected and screened out. The samples were taken using sterile cotton swab tubes, and were labeled and kept in the refrigerator at 4°C until further processing. These samples were collected from less than one month old Jamunapari, Barbari, Jakharana, Sirohi kids during the period from March to June 2010.

Isolation of *E. coli*

The fecal swabs collected were directly inoculated on to blood agar and MacConkey's agar simultaneously in duplicates for every sample inoculums. Then the plates are incubated at 37ºC for 24 to 48 hours. After growth was observed the similar colonies were stained by the "grams" for examining the size, morphology and staining characteristics. The colony types, which were showing gram negative cocco bacilli, were further used for characterization.

On MacConkey's agar only lactose fermenting (LF +) colonies, which were pink in colour were isolated and sub cultured for further characterization. To check whether the bacteria are *E. coli* (because there are other lactose fermenters like *Klebsiella* and *Enterobacter*) the LF+ colonies were re-inoculated on EMB agar for presence of metallic sheen, which was given only by *E. coli.* The organism was identified on the basis of culture, morphological and biochemical characteristics (Karter, 1973). On the basis of microscopic examination, morphology of bacteria was noted as rod, spiral or filament. It was differentiated by Bio-chemical characterization as per Jackie Reynolds (2005).

Initially the Glass wares and other media are sterilized and kept ready. MHA was sterilized by autoclaving at the rate 121ºC in 15 lbs for 15minutes and poured in to Petri plates and checked for sterility. Bacterial Inoculums was prepared from each isolates in normal saline and matched with McFarland Standards as mentioned by Kirby & Bauer (1966). A lawn culture was prepared using the primary inoculums by spreading the inoculums on to the Agar surface nicely by using a sterile glass spreader (sterilized by 70% alcohol). After 15 minutes, the antibiotics discs were applied on to the agar surface by the applicator/ sterile forceps with optimum distance between each antibiotic disc. The Petri plates embedded with antibiotic discs are incubated at 37ºC for 24 hours. After incubation the results are read by measuring the zone of inhibition using a calibrated scale. The results are interpreted for each antibiotic by measuring the zone of inhibition and comparing the standard chart for the particular bacteria of interest.

Results and Discussion

Isolates were further assessed for the antibacterial drug susceptibility by using disc diffusion method. Out of 8 antibiotics, the isolates were found to be resistant for Ampicillin, Enrofloxacin, Oxytetracycline, Gentamicin, Ceftriazone, Cloxocillin with inhibition zone diameter ranging between 10-15 mm. Chloramphenicol were highly sensitive for

E. coli strains to producing the inhibition zone of 23-26 mm and Ampicillin, Oxytetracycline, Gentamicin, Ceftriazone, and Cloxocillin resulted found to be intermediate the inhibition zone of 13-16 mm each.

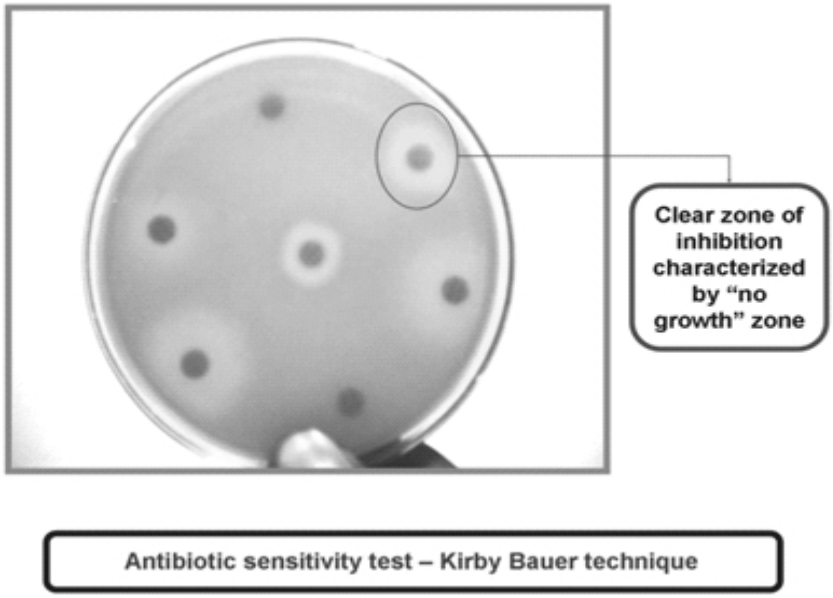

Fig.1.

Table 1: Antibiotic Sensitivity profiles of the field isolates of *E. coli*.

S.No	Ampicillin	Enrofloxacin	OTC	Gentamicin	Ceftriazone	Cloxacillin	Chloram-phenicol
3b1	7mm R	22mm I	14mm I	11mm R	13mm R	No Zone	18mm I
3b2	13mm I	22mm I	No Zone	09mm R	15mm I	No Zone	19mm I
4a	08mm R	16mm I	No Zone	15mm I	07mm R	06mm R	23mm S
5a	16mm I	15mm R	15 I	12mm R	15mm I	07mm R	18mm I
5b	11mm R	23mm I	13 I	11mm R	14mm I	07mm R	16mm I
6b	09mm R	20mm I	No Zone	16mm I	13mm I	09mm R	23mm S
8'	13mm I	24mm I	No Zone	11mm R	18mm I	10mm R	26mm S
9'	06mm R	06mm R	No Zone	12mm R	12mm R	07mm R	18mm I
13b	No Zone	08mm R	No Zone	13mm I	No Zone	07mm R	23mm S
2a	No Zone	09mm R	No Zone	08mm R	No Zone	No Zone	21mm S
1b	08mm R	19mm I	No Zone	12mm R	10mm R	No Zone	21mm S
3a	09mm R	24mm I	16mm I	15mm I	11m R	08mm R	22mm S

R =Resistant, I= Intermediate, S = Sensitive

Table 2: Effect of Antibiotics on different isolates of *E. coli*

S. No	Antibiotic	Resistant	Intermediate	Sensitive
1	Ampicillin (A)	9	3	-
2	Enrofloxacin (Ex)	4	8	-
3	Oxytetracycline (O)	8	4	-
4	Gentamycin (G)	8	4	-
5	Ceftrioxone (Cs)	8	4	-
6	Cloxacillin (Cx)	12	-	-
7	Chloramphenicol (C)	-	5	7

R =Resistant, I= Intermediate, S = Sensitive

Table 3: Percentage of Resistance of the total isolates shown to different antibiotics

S. No	Antibiotic	Percentage
1.	Ampicillin (A)7	5%
2.	Enrofloxacin (Ex)	34%
3	Oxytetracycline (O)	67%
4	Gentamycin (G)	67%
5	Ceftrioxone (Cs)	67%
6	Cloxacillin (Cx)	100%
7	Chloramphenicol (C)	00%

Antibiotic sensitivity test were done to study the trend in susceptibility to commonly used antibiotics in the field. Only those screened using *bfp*A gene were subjected to antibiotic sensitivity test. Some of the commonly used antibiotics like Ampicillin, and Cloxacillin were giving high percentage of resistance at the rate of 75 percent and 100% respectively. This is due to the unscrupulous use of such antibiotics leading to high resistance. About 67 percent of the isolates were resistant to Oxytetracycline, Gentamicin, and Ceftriaxone. Only antibiotic groups like fluoroquinolones and the Chloramphenicol have fared well in effectively inhibiting the *in vitro* growth of *E. coli.* Chloramphenicol has given the lowest percentage of resistance at zero and the next best was Enrofloxacin at 34 percent. So, it's a serious issue that out of the commonly used 7 antibiotics, only two was effective, which gives clue about the emerging multi-drug resistant *E. coli* strains.

Gupta *et al* (2000) subjected drug sensitive test for isolates of faecal sample of effective kids, with the range of modern antibiotics and samples were sensitive to Amikacin, Cefotaxime, Ceftazidime, Ceftrioxone, Cefuroxime, Cefadroxyl, Kanamycin and Netilmycin.

Jose *et al*, (2001) isolated quinolone resistance pathogenic and non-pathogenic *Escherichia coli* strains from healthy ruminants. In cattle, 5.9% of the strains were resistant to nalidixic acid and 4.9% were resistant to enrofloxacin and ciprofloxacin, whereas in sheep and goats only 0.5% and 1.4%, respectively, of the strains were resistant to nalidixic acid and none to fluoroquinolones. Most of the strains resistant to quinolones were non-pathogenic strains isolated from cattle. But in the current study the resistance to fluoroquinolones was as high as 34 percent, making it a concern, for the future use of these antibiotics.

Kumar *et al,* (2003) isolated nineteen different serotype of *E. coli* from faecal sample of diarrheic cow and calves. Multiple drug resistance was a common feature belonging to isolates from all categories of animals. Highest resistance was against ampicillin and oxytetracycline.

Conclusion

Biochemical studies like IMViC, TSI, Oxidase, Catalase and Nitrate tests identified different strains of *E. coli* from clinical cases of diarrhea. Only Chloramphenicol showed good percentage of affectiveness against the pathogenic *E. coli* isolates, Ampicillin, Enrofloxacin, Oxytetracycline, Gentamycin, Ceftrioxone, and Cloxacillin antibiotics showed alarmingly low level of effectiveness in inhibiting the growth of pathogenic *E. coli* in vitro.

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